Live birth rates in different subgroups of poor ovarian responders according to Bologna and POSEIDON group classification criteria

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Abstract

Background: Live birth rates (LBRs) have been separately evaluated in two poor ovarian responder (POR) classification methods. We designed the present study to compare the LBRs according to Bologna criteria or Patient-Oriented Strategies Encompassing IndividualizeD Oocyte Number (POSEIDON) group classifications to determine the important predictive factors for LBR in patients with POR.

Methods: In this cross-sectional study, we evaluated a database that contained clinical and laboratory information on infertility treatment cycles of all patients with at least one POR after standard controlled ovarian stimulation (COH) during in vitro fertilization/intracytoplasmic sperm injection (IVF/ICSI) cycles from December 2015 to December 2017. The eligible data were collected and the subjects divided into five groups according to Bologna criteria and four groups according to POSEIDON group classification.

Results: We assessed 812 patients diagnosed with POR; overall, 517 underwent embryo transfer (ET) during the last treatment cycle (63.6%). The results indicated a total clinical pregnancy rate of 19.3% (100 cases) and LBR of 16.1% (86 cases). According to Bologna criteria, 41 patients were not included in any group. Analysis of treatment cycle outcome showed that patients classified as Bologna group II had a higher LBR than the other groups. In terms of POSEIDON classification, all of the patients could be classified into groups. Patients in POSEIDON group 3 had the highest LBR. According to multivariable regression analysis, the significant independent variables that remained in the model as important predictive factors for live births were the number and quality (good and excellent) of the embryos transferred, and POSEIDON group 3 classification.

Conclusion: The results indicated that the POSEIDON group classification could be more comprehensive and practical than Bologna criteria for diagnosing and categorizing POR patients. In addition, we noted that the number and quality of transferred embryos were the most important prognostic factors for live births in POR patients. Therefore, we suggest that clinicians consider COH protocols that have a freezing embryo strategy and the collection of more good quality embryos to improve the probability of a live birth.

Plain English Summary

The present study was designed to evaluate the in vitro fertilization/intracytoplasmic sperm injection (IVF/ICSI) outcomes among various subgroups of poor ovarian (POR) patients according to the Patient-Oriented Strategies Encompassing IndividualizeD Oocyte Number (POSEIDON) group classification and the Bologna criteria in an attempt to determine the most important predictive values for success after treatment cycles in these patients.

The database of infertility treatment cycles in all of the patients with at least one POR after standard controlled ovarian stimulation (COH) during IVF/ICSI cycles was evaluated from December 2015 to December 2017.

Totally, we investigated 812 patients diagnosed with POR; overall, 517 of them underwent embryo transfer (ET) in the last treatment cycle (63.6%), with a total clinical pregnancy rate of 19.3% (100 cases) and live birth rate (LBR) of 16.1% (86 cases). According to the Bologna criteria, 41 of these patients were not included in any group. On the other hand, all of the patients were grouped according to the POSEIDON classification. According to multivariable regression analysis, the significant independent variables that remained in the model as important predictive factors for live births in the study population were the number and quality (good and excellent) of the transferred embryos, and POSEIDON group 3 classification.

In conclusion, the POSEIDON group classification could be more comprehensive and practical than the Bologna criteria for diagnosing and categorizing POR patients. In addition, the number and quality of transferred embryos were the most important prognostic factors for LBR in POR patients.

Background

Poor ovarian response (POR) to gonadotropin stimulation is associated with cycle cancellation and reduced chance of a live birth in an assisted reproductive technology (ART) program, and remains a challenging and frustrating subject for clinicians and patients [1]. The definition of POR was established under the auspices of the European Society of Human Reproduction and Embryology (ESHRE), and recorded as the Bologna criteria [2]. Prior to this consensus, tremendous heterogeneity existed in the study populations for classification of POR patients; approximately 41 different definitions were applied to describe POR and resulted in a poor predictive value for meta-analyses [3]. Although the Bologna criteria provide a very important step in reducing heterogeneity; however, in daily clinical practice, the relevant data that support this definition are limited [4]. In a realistic approach, the term POR should refer to the ovarian response. Therefore, one stimulated cycle is considered essential for the diagnosis of POR. In this regard, some researchers have debated that a significant degree of heterogeneity exists with the Bologna criteria [5-7]; they have concluded that the minimal criteria of the ESHRE consensus are not 'full-scale' and may require revision prior to implementation [8]. Recently, researchers from seven countries participated in the development of a classification for POR patients called Patient-Oriented Strategies Encompassing IndividualizeD Oocyte Number (POSEIDON) where four subgroups were suggested based on age, ovarian biomarkers, and previous ovarian response [9]. The marker of success according to the POSEIDON stratification is the number of retrieved oocytes required to obtain at least one euploid embryo for transfer in each individual patient [7].

We designed the present study to evaluate the ART outcome among various subgroups of PORs according to the POSEIDON classification and Bologna criteria with the intent to determine the most important predictive values for successful LBR in these patients.

Materials And Methods

In this cross-sectional study, we evaluated a database that contained clinical and laboratory information on infertility treatment cycles performed in the Endocrinology and Female Infertility Department of Royan Institute (Tehran, Iran) from December 2015 to December 2017. The Institutional Review Board and Ethics Committee of Royan Institute approved the study protocol. The retrospective data were collected from a registered database at the institute. The patients' data were included for analysis when all the following inclusion criteria were met: (1) history of at least one poor ovarian response (total number of retrieved oocytes ≤3) after standard COH during the in vitro fertilization/intracytoplasmic sperm injection (IVF/ICSI) cycles; (2) complete patient records on basic, clinical, ART cycle characteristics, and reproductive outcome after IVF; (3) availability of at least one measurement between anti-Mὺllerian hormone (AMH) and/or antral follicle count (AFC) in the three months before commencement of ovarian stimulation; and (4) the ovarian reserve measurements were performed at Royan Institute before entry in the ART program. The exclusion criteria consisted of: premature ovarian failure with a basal follicle stimulating hormone (FSH) level ≥25 IU/l; donor/recipient treatments; and azoospermic male partner with testicular or epididymal sperm aspiration.

Poor ovarian response was defined according to the Bologna criteria and the presence of at least two of the following criteria: 1) previous history of POR (retrieved oocytes ≤3) in a conventional stimulation protocol; 2) anamnestic risk factors of advanced maternal age (≥40 years), evidence of ovarian endometrioma at the basal ultrasound, previous ovarian surgery, previous chemotherapy, genetic abnormalities, shortening of the menstrual cycle; and 3) abnormal ovarian reserve tests (ORT) such as an AFC between <5 and <7 follicles or AMH levels between 0.5–1.1 ng/ml. There are five different phenotypes of POR that can be derived from different combinations of the Bologna criteria: I) previous poor response and presence of anamnestic risk factors; II) previous POR and abnormal markers for ovarian reserve; III) the presence of anamnestic risk factors and abnormal markers for ovarian reserve; IV) presence of a previous POR, anamnestic risk factors, and abnormal markers for ovarian reserve; and V) history of two previous POR.

The patients were also categorized into the following four groups based on the POSEIDON group classification [7]: group 1: young patients (<35 years) with normal pre-stimulation ovarian reserve parameters (AFC ≥5, AMH ≥1.2 ng/mL) and unexpected poor (<4 oocytes retrieved) or sub-optimal ovarian response (4–9 oocytes retrieved) to standard COH; group 2: older patients (≥35 years) with normal pre-stimulation ovarian reserve parameters (AFC ≥5, AMH ≥1.2 ng/mL) and unexpected poor or suboptimal ovarian response; group 3: patients <35 years with poor ovarian reserve pre-stimulation parameters (AFC <5, AMH <1.2 ng/mL); and group 4: patients ≥35 years with poor ovarian reserve pre-stimulation parameters (AFC <5, AMH <1.2 ng/mL).

We selected the ovarian stimulation protocol based on the women's age, ORT, and, when available, the results of the previous cycles. The ovarian stimulation protocols for these patients included the standard or stop gonadotropin-releasing hormone (GnRH) agonist protocol, GnRH antagonist with estradiol (E_2) priming [13], and double mild stimulation in the same cycle, which has previously been described in detail [14]. When at least one follicle that measured ≥18 mm in diameter was observed, the final stage of

oocyte maturation was induced by two pre-filled syringes of *recombinant human chorionic gonadotropin* (rhCG; Ovitrelle®, 250 µg/0.5 ml, Merck, Serono, Inc.) or by 10 000 IU of hCG (Choriomon, IBSA). If this criterion was not met after 10–12 days of stimulation, the cycle was cancelled due to an inadequate response. Transvaginal ultrasound-guided oocyte retrieval was performed 34–36 h after oocyte triggering. ICSI or IVF/ICSI was performed in all patients to prevent infrequent cases of fertilization failures by only conventional IVF. The embryos were cultured in *G1v5* Plus *media* (*Vitrolife*) until the day of the transfer. The obtained embryos were replaced by an embryo transfer catheter (Guardia™, Access ET Catheter, Cook Medical), three or five days after oocyte retrieval. Embryo quality was determined according to the number and regularity of the blastomeres and the degree of embryonic fragmentation, which was previously explained [15]. Some patients had all of their embryos frozen according to the procedure of embryo freezing (vitrification method) [16] and frozen embryo transfer (FET) cycles that have been previously described in detail [14]. All patients in the fresh ET cycles received luteal phase support in the form of a 400 mg vaginal progesterone suppository administered twice daily (Cyclogest®, Actavis, Barnstaple, UK) starting on the evening of the oocyte retrieval and continuing for 10 weeks in patients who had a positive pregnancy test. Serum ß-hCG analysis was performed 14 days after the ET, and clinical pregnancy (presence of gestational sac with a heartbeat) was determined by an ultrasound scan 14 days later.

The main outcomes were the comparison of ART outcomes among patients with different POR phenotypes according to Bologna criteria and POSEIDON group classification. The predictive variables for live births in patients diagnosed with POR were evaluated as secondary outcomes.

Statistical analysis

Statistical analysis was carried out by using the Statistical Package for the Social Sciences (SPSS), version 20 (SPSS, Inc., Chicago, IL, USA. Differences between the two groups were analyzed by the independent t-test and Mann-Whitney U test for normal and non-normal continuous variables, respectively. The chi-square test was used to compare the categorical variables between groups. Descriptive data are presented as mean ± standard deviation (SD) or median (interquartile range) as indicated. Possible significant variables were entered in the multivariable logistic regression model to identify the most significant prognostic factors for live births in the studied population. The statistical significance level was set at a p-value of <0.05.

Results

There were 1100 patients who underwent IVF/ICSI cycles and had at least one poor ovarian response recorded in the database during the study period. From these, we enrolled 812 eligible patients and divided them into five groups according to the Bologna criteria; however, 41 of these patients were not included in any of the Bologna subgroups because they had only one cycle of less than four retrieved oocytes after standard ovarian stimulation and no other risk factors for POR. The number of patients in

groups III and IV was higher than the other groups. Phenotype III (58.9%, n= 478) comprised the majority of patients according to Bologna criteria.

Comparison of different poor ovarian responder (POR) patient groups based on Bolognacriteria

We compared baseline characteristics among different POR groups according to Bologna criteria (Table 1). There were significant differences among the groups in terms of age, number of previous IVF/ICSI cycles, serum AMH levels, and AFC before starting stimulation; however, this was expected because these variables are determinative factors according to Bologna criteria. Bologna group IV had the highest rate of secondary infertility compared to the other groups ($p<0.001$). Bologna group V patients had a significant difference compared with the other groups regarding the causes of infertility. Patients in this group had a lower rate of the ovulatory factor for infertility compared with the other groups ($p<0.001$). The numbers of patients with a previous history of no oocytes and no response in Bologna groups I ($p<0.001$) and II ($p=0.02$) were significantly lower than the other groups. No significant difference was found among groups regarding the other baseline characteristics (Table 1).

The outcomes of the last cycle of ovarian stimulation and embryo transfer (ET) were compared among different POR groups according to Bologna criteria (Table 2). The ovarian stimulation protocol in Bologna group IV patients differed from the other groups; the majority of protocols in this group were antagonists and double-stimulation protocols. In our institute, the COH protocol was selected based on the age of the woman, ovarian reserve, and history of previous cycles. Therefore, in patients with advanced age and low ovarian reserve or previous history of POR, the antagonist or double-stimulation protocol was used more frequently. However, the duration of ovarian stimulation, dose of gonadotropin (rFSHand/or human menopausal gonodatropin [hMG]), and number of follicles ≥15 mm on the day of oocyte triggeringin the last cycle were not different among the groups. Tukey analysis demonstrated that the number of retrieved and MIIoocytes in Bologna group III was different from the other groups; consequently, the number and quality of obtained embryos, and the number of embryos transferred was different from the other groups ($p<0.001$).However, the stage of the transferred embryo (blastocyst or cleavage) did not differ between the groups. The cancellation ($p<0.001$) and no oocyte ($p=0.007$) rates in Bologna groups IV and V were significantly higher than the other groups. Endometrial thickness on the trigger day showed a significant difference between groups and the results of the Bonferroni test showed that endometrial thickness in Bologna group IV was significantly higher compared with Bologna groups II ($p=0.03$) and III ($p=0.01$).Finally, the clinical pregnancy and LBR in Bologna groupsIIandIIIwere significantly higher than the other groups ($p=0.006$).

Comparison of different poor ovarian responder (POR) patients according toPatient-Oriented Strategies Encompassing IndividualizeD Oocyte Number (POSEIDON) group classification

Patients classified on the basis of POSEIDON group criteria were mostly in group 4 (57%, n=463). Table 3 shows the baseline characteristics of the patients in the different groups based on the POSEIDON group classification.

There were also significant differences among groups in terms of women's age, basal FSH and serum AMH levels, and AFC before starting stimulation; however, this was expected because these variables are main factors in the POSEIDON group classification. Women in POSEIDON group 4 had a significant secondary infertility type in comparison to the other groups. Also, there was a significant difference in the cause of infertility between POSEIDON group 1 and the other groups ($p<0.001$). The majority of patients in POSEIDON group 1 were with male factor diagnosis, whereas most patients were diagnosed with ovarian factor in the other groups. No significant difference was found among groups in terms of the other baseline characteristics (Table 3).

Table 4 shows the outcomes of the last treatment cycle of the studied patients in the different groups based on POSEIDON group classification. In a similar way, the COH protocol was selected on the basis of age, ORT, and previous history; therefore, the antagonist and double-stimulation COH protocols were used more frequently in patients from POSEIDON group 4 ($p<0.001$).

However, the mean values of the ovarian stimulation day and dose of gonadotropins, and the number of follicles ≥15 mm on the day of oocyte triggering were not significantly different among the groups. Tukey analysis showed that the number of retrieved ($p=0.002$) and MII ($p=0.001$) oocytes in POSEIDON group 3 were greater than those of POSEIDON group 4. Meanwhile, the differences between the other groups were not statistically significant. The number of embryos obtained and the number of embryos transferred were not in line with the total retrieved oocytes; these were significantly lower in POSEIDON group 1 compared with the other three groups ($p=0.002$, $p=0.003$, and $p=0.008$). In addition, the quality of obtained embryos and the rates of fertilization, cancellation, and no oocyte cases did not significantly differ between the groups. Endometrial thickness on the trigger day in POSEIDON group 3 was significantly higher than in POSEIDON group 4 ($p=004$). Finally, the clinical pregnancy and LBR in POSEIDON group 3 were significantly higher than the other groups ($p=0.006$).

Table 5 shows the association between possible related factors and the LBR according to the univariate logistic regression test. The analysis indicated that women's age, duration of infertility, serum TSH levels, number of oocytes obtained and MII oocytes, the number of embryos obtained, the number and quality of transferred embryos, and the POSEIDON classification group showed a significant unilateral relationship with the LBR.

All of the significant possible variables related to the LBR were entered in multivariate logistic regression model. The analysis revealed that the significant independent variables that remained in the model as important predictive factors for live births in the study population were the number and quality (good and excellent) of the transferred embryos, and POSEIDON classification group 3. In other words, the likelihood of a live birth after transferring excellent embryos was approximately 5.9 times higher than the transfer of fairquality embryos.Also by increasing the number of transferred embryos, the likelihood of a live birth increased by 1.6 times.In this regression model, the likelihood of a live birth in a patient in POSEIDON group 3 was three times higher than that of a patient in POSEIDON group 4 (Table 6).

Discussion

In the present study, 812 patients diagnosed with POR were investigated; overall, 517 (63.6%) underwent ET in the last treatment cycle, resulting in a total clinical pregnancy rate of 19.3% (100 cases) and a LBR of 16.1% (86 cases). When the patients were grouped on the basis of the POSEIDON classification, the highest LBR was observed in patients in POSEIDON group III. On the other hand, according to the Bologna criteria, 41 patients were not included in any group. Analysis of the cycle outcome showed that the LBR in the Bologna group II was more than the other groups. Of note, the POSEIDON classification groups were significant predictive factors for live births in the multivariable regression analysis. According to univariate regression analysis, women age ≥35 years had a 60% *decreased likelihood of live births; therefore,* it could be interpreted that the POSEIDON group classification cut-off point for age (35 years) was a significant factor in predicting live births. It has been suggested that the POSEIDON group classification is more comprehensive and practical than Bologna criteria for diagnosing and categorizing POR patients.

Similarly, La Marca and colleagues compared the LBR in 210 POR patients from the different Bologna groups and reported the same poor prognosis for all of the groups [1]. Elsewhere, Busnelli et al., in a retrospective study, evaluated 362 patients diagnosed with POR in different groups of the Bologna criteria [4]. They reported a LBR of 6%, which was similarly poor among the different groups of Bologna criteria. In their study, positive predictive factors of live birth included previous deliveries and prior chemotherapy; however, age, serum AMH and FSH levels, and AFC were not significantly associated with live births. Also, they suggested that the Bologna criteria should be used to design future studies in this area [4]. Similarly, Bozdag and colleagues, in a retrospective study, compared LBR in 821 patients from different Bologna groups. They found no statistically significant differences in the rate of live births [11]. In contrast to the previous studies, Li and colleagues retrospectively evaluated 132 women who underwent a second IVF treatment cycle and were diagnosed with POR by the Bologna criteria [17]. In this study, women aged ≥40 years and/or a history of endometriosis or ovarian surgery were considered to be in Bologna group I; those who had three or less oocytes retrieved in the previous IVF cycle stimulated with a standard protocol were considered to be in Bologna group II, while those with AFC ≤6 were classified as Bologna group III. They concluded that the POR patients who fulfilled different combinations of the Bologna criteria did not have similar IVF outcomes. The best ovarian response and live-birth rates were observed in those classified as Bologna group (I and II) with normal AFC, and the worse were in Bologna group (I+II +III)[17].

In a recent retrospective study, Eftekhar et al. evaluated LBR in 245 POR patients from different POSEIDON group classifications and concluded that LBR in groups 1 and 2 were higher than those from groups 3 and 4. In their study, the ovarian stimulation protocol (microdose agonist flare-up) was used in all of the study groups. The authors mentioned that in contrast to previous studies, which reported women's age as the most important predictor of ART success, their study results indicated that ovarian reserve factor (AMH and AFC) were the main predictive factors[9]. In the present study, we found no relationship between LBR and different ovarian stimulation protocols in our study population according

to univariate regression analysis. This issue is challenging for the management of POR patients. Youssef et al., in a multicenter randomized trial, concluded that a mild ovarian stimulation strategy in women with poor ovarian reserve who underwent IVF led to similar ongoing pregnancy rates as a conventional ovarian stimulation strategy [18]. In similar way, Pilehvari and colleagues have suggested that a minimal stimulation protocol that used lower gonadotropin could be considered as a patient-friendly and cost-effective substitute for PORs [19]. In the present study, the number and quality of transferred embryos were important related variables to the probability of live births in POR patients. Therefore, we suggest the pooling method for collection of additional oocytes and embryos by consecutive minimal stimulation protocols in order to improve the success rate in POR patients.

In line with our results, Haung and colleagues evaluated 1957 patients with diminished ovarian reserve. In their study, the patient's age, and the number and quality of the transfer embryos were the most predictive factors for live birth in this population[20]. Xu et al. conducted a 15-year survey of final in vitro fertilization outcomes by evaluating the cumulative live birth rates in more than 3000 patients with poor ovarian response. They reported the following age-related decreases in CLBR - from 22% for women ≤30 years, 18.3% for women aged 31–34 years, 17.2% for 35–37 years, 13.5% for 38–40 years, 10.5% for 41–43 years, and 4.4% among women >43 years in their conservative analysis. Therefore, an optimistic estimate in these patients is a challenging subject for clinicians[21].

The strengths of the current study are the large numbers of POR patients, in addition to the cycle outcomes according to two main diagnostic criteria in this field. As a limitation of present study, we could not apply the same protocol COH for all POR patients according to our institute policy and the individualized COH protocol considering the age and ovarian reserve and the previous patient's treatment cycles was used. Therefore, we used multivariable logistic regression analysis to determine the main predictive factors for LBR in POR patients.

Conclusion

According to the current study results, a high prevalence of POR patients according to Bologna criteria and POSEIDON group classification were in groups III and IV. Therefore, most clinical trials should be designed to improve cycle outcomes in these patients. The study findings suggested that the POSEIDON group classification could be more comprehensive and practical than Bologna criteria for diagnosing and categorizing POR patients. In addition, we noted that the number and quality of transferred embryos were the most important prognostic factors for live births in POR patients; therefore, we suggest that clinicians consider COH protocols that have a freezing embryos strategy and the collection of more good quality embryos in order to improve the probability of live births in these patients.

Abbreviations

AFC: Antral follicle count; AMH: Anti-Mὺllerian hormone; COH: Controlled ovarian hyperstimulation; ET: Embryo transfer; E_2:Estradiol; FSH: Follicle stimulating hormone; GnRH: Gonadotropin-releasing hormone;

hMG: Human menopausal gonadotropins; hCG: Human chorionic gonadotropin; IVF/ICSI: In vitro fertilization/intra-cytoplasmic sperm injection; ORT: Ovarian reserve test; POSEIDON: Patient-Oriented Strategies Encompassing IndividualizeD Oocyte Number; POR: Poor ovarian responder; SD: Standard deviation.

Declarations

Ethics approval and consent to participate

The Institutional Review Boards and the Ethics Committees of Royan Institute, Tehran, Iran approved this study (ethics code: IR.ACECR.ROYAN.REC.1395.136).

Consent for publication

Not applicable.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding authors upon reasonable request.

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Competing interests

All authors have no competing interests.

Authors' contributions

MM, AA: Designed the research. FB, MM, and AA: Evaluated patients' charts for study eligibility, performed data collection, data interpretation, and manuscript writing/editing. MM and AA: Wrote the manuscript. ZZ: Assisted with data analysis. All authors read and approved the final manuscript.

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Tables

Table I : Baseline characteristics different POR groups according to Bologna criteria

Data are mean +SD or number (%)

Variables	POR categories					
	Group I (Anamnestic risk factors + One previous POR)	**Group II** (One previous POR + abnormal ORT)	**Group III** (Anamnestic risk factors + abnormal ORT)	**Group IV** (Anamnestic risk factors + One previous POR + abnormal ORT)	**Group V** (Two previous POR after standard COH protocol)	**P**
No. of patients	30 (3.7)	57 (7)	478 (58.9)	154 (19)	52 (6.4)	
Age (yr)	39.4 ± 4.3	38.8 ± 4.8	34.5 ± 4.1	42.6 ± 1.4	34.8 ± 5.7	**<0.001**
Menarche age (yrs)	13.3 ± 1.6	13.1 ± 1.5	12.7 ± 1.5	12.8 ± 1.6	12.9 ± 1.2	0.21
Duration of marriage	8.8 ± 7.1	8.9 ± 6.5	8.2 ± 4.9	9.0 ± 7.7	8.0 ± 4.4	0.53
Day 2 or 3 serum FSH (IU/ml)	9.0 ± 3.9	8.0 ± 3.172	8.6 ± 4.1	9.1 ± 4.3	7.3 ± 2.8	0.51
Day 2 or 3 serum LH (IU/ml)	5.7 ± 2.3	4.9 ± 2.7	5.0 ± 3.1	5.1 ± 3.2	4.8 ± 3.3	0.77
AMH (ng/ml)	1.5 ± 0.4	0.6 ± 0.3	0.5 ± 0.375	0.463 ± 0.3	1.8 ± 0.8	**<0.001**
Total AFC	7.8 ± 2.902	6.0 ± 2.4	6.1 ± 3.1	5.1± 2.8	6.7 ± 3.0	**<0.001**
Left ovary size (mm^2)	813.3 ± 523.9	569.7 ± 220.4	703.4 ± 498.8	662.2 ± 376.4	613.3 ± 239.9	0.32
Right ovary size (mm^2)	602.5 ± 189.1	749.6 ± 364.9	698.5 ± 506.8	548.8 ± 171.3	607.3 ± 268.4	0.16
Duration of infertility (yr)	5.8± 6.2	6.7 ± 5.6	6.0 ± 4.5	6.7 ± 7.0	5.9 ± 4.5	0.64
Type of infertility						**0.001**
Primary	22(73.3)	44(77.1)	366(76.5)	92(59.7)	42(80.7)	

Secondary	8(26.6)	13(22.8)	112(23.4)	62(40.2)	10(19.2)	
Cause of infertility						**<0.001**
Ovulatory F	13(43.3)	22(38.5)	221(46.2)	85(55.1)	14(26.9)	
Male factor	2(6.66)	9(15.7)	85(17.7)	4(2.59)	13(25)	
Mix	15(50)	26(45.6)	172(35.9)	65(42.2)	25(48.0)	
No. of previous IUI cycles	1.1 ± 2.2	0.6 ± 0.9	0.8 ± 1.3	0.5 ± 0.9	0.6 ± 1.1	0.32
No. of previous IVF/ICSI cycles	2.3 ± 1.3	2.1 ± 1.3	2.1 ± 1.4	2.2 ± 1.4	3.1 ± 2.0	**0.005**
No. of previous cycle with no oocyte	0 (0)	2 (3.57)	78 (16.3)	34 (22.0)	11 (21.1)	**<0.001**
No. of previous cycle with no response	2 (6.66)	1 (1.81)	70 (14.7)	17 (11.0)	9 (17.3)	**0.02**

POR: Poor ovarian response; ORT: ovarian reserve tests; COH: controlled ovarian hyperstimulation; SD: standard deviation

Table II: The outcome of the last treatment cycle in different groups according to Bologna criteria.

Data are mean +SD or number (%)

Variables	POr categories					
	Group I (Anamnestic risk factors for POR + One previous POR)	**Group II** (One previous POR + abnormal ORT)	**Group III** (Anamnestic risk factors for POR + abnormal ORT)	**Group IV** (Anamnestic risk factors for POR + One previous POR + abnormal ORT)	**Group V** (Two previous POR after standard COH protocol)	**P value**
No. of patients	30(3.7%)	57(7%)	478(58.9%)	154(19%)	52(6.4%)	
Type of COH protocol						**<0.001**
Long	12(40 %)	19(34.5 %)	142(29.8 %)	13(8.4 %)	19(36.5 %)	
Antagonist	14(46.7 %)	31(56.4 %)	219(45.9 %)	85(55.2 %)	19(36.5 %)	
Double-stimulation	4(13.3 %)	5(8.7 %)	100(20.9 %)	50(32.4 %)	12(23.4 %)	
Mini flare	0(0 %)	0(0 %)	16(3.4 %)	6(3.9 %)	2(3.9 %)	
Duration of stimulation (days)	10.2 ± 1.39	9.5 ± 0.68	9.327 ± 0.41	8.77 ± 0.58	8.22 ± 1.06	0.71
Total dosage of rFSH used (IU)	23 ± 27.6	44 ± 30.9	326 ± 27.5	83 ± 27.0	30 ± 24.7	0.22
Total dosage of HMG used (IU)	19 ± 10.6	28 ± 12.3	281 ± 11.3	88 ± 12.6	31 ± 10.7	0.56
Number of follicles (≥ 15 mm) on hCG day	2.6 ± 0.4	2.8 ± 0.55	1.89 ± 0.15	1.84 ± 0.21	1.44 ± 0.24	0.081
No. of retrieved oocytes	3.13 ± 0.34	5.90 ± 0.28	2.34 ± 0.07	1.54 ± 0.11	1.65 ± 0.20	**<0.001**
No. of metaphase II oocytes	2.5 ± 0.27	5.02 ± 0.25	1.90 ± 0.07	1.25 ± 0.10	1.42 ± 0.18	**<0.001**
No. of	2.333 ±	3.204 ±	2.107 ±	1.709 ± 0.865	1.571 ±	**<0.001**

obtained embryo	1.340	1.607	0.997		0.634	
Fertilization rate	0.76 ± 0.35	0.58 ± 0.31	0.575 ± 0.40	0.51 ± 0.44	0.57 ± 0.40	0.07
Cycle cancellation; n (%)	0(0 %)	0(0 %)	22(4.60 %)	13(8.44 %)	6(11.5 %)	**<0.001**
No oocyte rate; n (%)	0(0 %)	0(0 %)	47(9.83 %)	22(14.2 %)	8(15.3 %)	**0.006**
Fertilization failure; n (%)	5(16.6 %)	5(8.77 %)	101(21.1 %)	30(19.4 %)	6(11.5 %)	0.11
All freeze for poor endometrium; n (%)	1(3.33 %)	3(5.26 %)	11(2.30 %)	3(1.94 %)	4(7.69 %)	0.1
Type of embryo transfer Fresh	19 (79.1%)	38 (77.5%)	238 (80.1%)	59 (68.6%)	19 (67.8%)	0.3
Frozen	5 (20.9%)	11 (22.5%)	59 (19.9%)	27 (31.4%)	9 (32.2%)	
No. of embryos transferred	1.95 ± 0.75	2.34± 0.75	1.90 ± 0.77	1.65 ± 0.664	1.48 ± 0.57	**<0.001**
Stage of embryo transferred Cleavage stage	20(83.3 %)	42(85.7 %)	264(88.5 %)	77(89.5 %)	25(86.2 %)	0.47
Blastocyst Stage	4(16.6 %)	7(14.2 %)	34(11.4 %)	9(10.4 %)	4(13.7 %)	
Quality of embryos	22(91.6 %)	27(55.1 %)	139(46.6 %)	39(45.3 %)	12(42.9 %)	**0.007**
Excellent Good	1(4.16 %)	16(32.6 %)	104(34.8 %)	30(34.8 %)	10(35.7 %)	
Fair	1(4.16 %)	6(12.2 %)	55(18.4 %)	17(19.7 %)	6 (21.4 %)	
Endometrium thickness on trigger day	9.0 ± 1.7	9.0 ± 1.5	8.8± 1.7	8.4 ± 1.5	8.5 ± 1.4	**0.013**
Implantation rate	0.5 ± 0.1	0.545 ± 0.1	0.61 ± 0.3	0.52 ± 0.2	0.62 ± 0.2	0.93

Clinical pregnancy rate /ET	3/24 (12.5%)	10/49 (20.4 %)	69/297 (23.2 %)	5/ 86(5.81 %)	3/ 28(10.7 %)	**0.006**
Blighted ovum rate/ET	0(0 %)	1(2.04 %)	4(1.34 %)	3(3.48 %)	1(3.57 %)	**0.045**
Abortion rate /ET	2(8.33 %)	1(2.04 %)	10(3.36 %)	1(1.16 %)	0(0 %)	0.17
Live birth rate /ET	1/24 (4.2 %)	9/49 (18.3 %)	59/297 (19.8 %)	5/86 (5.81 %)	3/28 (10.7 %)	**0.006**

Table III: Baseline characteristics different POR groups according to POSEIDON group classification.

Data are mean +SD or number (%)

Variable	POR categories				
	Group I (Age<35 + normal ORT+ oocyte retrieved <9)	**Group II** (Age≥35 + normal ORT+ oocyte retrieved <9)	**Group III** (Age <35 + abnormal ORT+oocyte retrieved <5)	**Group IV** (Age≥35 + abnormal ORT+oocyte retrieved <5)	**P value**
No. of patients	50 (6.2%)	75(%9. 2)	224 (27.6%)	463 (57%)	
Age (yr)	30.5 ± 2.9	39.8 ± 2.9	30.5± 2.9	39.4 ± 3.0	**<0.001**
Menarche age (yrs)	12.9 ± 1.32	13.0 ± 1.3	12.8 ± 1.5	12.8 ± 1.6	0.73
Duration of marriage	7.7 ± 3.6	8.6 ± 6.4	7.0 ± 3.3	9.163 ± 6.5	0.11
Basal FSH serum level	7.58 ± 3.0	7.9 ± 3.4	8.3± 4.0	8.9 ± 4.1	**0.03**
Basal LH serum level	5.1 ± 3.346	5.2 ± 3.1	4.7 ± 2.8	5.1 ± 3.1	0.60
Basal AMH serum level	1.9 ± 1.2	1.84 ± 1.2	0.5 ± 0.04	0.5 ± 0.01	**<0.001**
Antral follicle count	7.4 ± 2.8	7.3 ± 2.8	6.3 ± 3.1	5.7 ± 3.0	**<0.001**
Left ovary size (mm^2)	728.1 ± 460.3	679.7 ± 353.4	680.4 ± 477.3	686.4 ± 447.6	0.70
Right ovary size (mm^2)	631.5 ± 301.4	634.3 ± 344.0	674.3 ± 410.5	669.2 ± 455.8	0.74
Duration of infertility (yr)	6.1 ± 3.8	5.9 ± 5.7	5.3 ± 3.4	6.7± 5.9	0.30
Type of infertility	42 (84 %)	55 (73.3 %)	191 (85.3 %)	314 (67.8 %)	**<0.001**
	8 (16 %)	20 (26.7 %)	33 (14.7 %)	149 (32.2 %)	

Primary Secondary					
Cause of infertility	11(22 %)	30(40 %)	90(40.1 %)	237(51.2 %)	**<0.001**
Ovarian factor	21(42 %)	10(13.3 %)	54(24.1 %)	46(9.95 %)	
Male factor	18(36 %)	35(46.6 %)	80(35.7 %)	179(38.7 %)	
Mixed					
No. of previous IUI cycles	0.8 ± 1.2	0.6 ± 1.5	0.8± 1.2	0.6 ± 1.1	0.13
No. of previous IVF.ICSI cycles	2.4 ± 1.7	2.4 ± 1.7	2.1 ± 1.2	2.2 ± 1.5	0.89
No. of no oocyte history	6(12 %)	6(8 %)	30(13.5 %)	83(18 %)	0.85
No. of no response history	8(16 %)	9(12 %)	35(15.7 %)	54(11.8 %)	0.48

POR: Poor ovarian response; ORT: ovarian reserve tests; COH: controlled ovarian hyperstimulation; SD: standard deviation

Table IV: The outcome of the last treatment cycle in different groups according to POSEIDON group classification. Data are mean +SD or number (%).

Variable	POR categories				
	Group I (Age<35 + normal ORT+ oocyte retrieved <9)	**Group II** (Age≥35 + normal ORT+ oocyte retrieved <9)	**Group III** (Age<35 + abnormal ORT+ oocyte retrieved <5)	**Group IV** (Age≥35 + abnormal ORT+ oocyte retrieved <5)	**P value**
No. of patients	50 (6.2%)	75(%9. 2)	224 (27.6%)	463 (57%)	
Type of ovarian stimulation protocol Long	23(46 %)	33(44 %)	77(34.4 %)	92(20 %)	**<0.001**
Antagonist	22(44 %)	29(38.7 %)	102(45.5 %)	235(51.1 %)	
Double-stimulation protocol	5(10 %)	11(14.6 %)	36(16.1 %)	120(26 %)	
Mini-flare	0(0 %)	2(2.7 %)	9(4 %)	13(2.9 %)	
Duration of stimulation (days)	7.6 ± 3.6	9.5 ± 2.9	10.3 ± 3.6	8.8 ± 2.7	0.13
Total dosage of rFSH used (IU)	24.8 ± 7.7	26.5 ± 10.9	26.7 ± 10.9	28.5± 12.0	0.14
Total dosage of hMG used (IU)	8.3 ± 4.6	12.51 ± 7.0	11.14 ± 6.5	11.9 ± 7.9	0.07
Number of follicles (≥ 15 mm) on hCG day	7.6 ± 3.6	9.5 ± 2.9	10.3 ± 3.6	8.8 ± 2.7	0.35
No. of retrieved oocytes	1.6 ± 0.5	1.9 ± 1.1	2.4 ± 1.3	1.9 ± 1.2	**0.005**
No. of metaphase II oocytes	2.6 ± 1.7	2.5 ± 1.9	2.9 ± 2.1	2.3± 1.9	**0.002**
Number of MI	1.8 ± 1.2	2.2 ± 1.7	2.4 ± 1.9	1.9 ± 1.7	0.15
Number of GV	0.3 ± 0.7	0.1 ± 0.3	0.2 ± 0.5	0.2 ± 0.5	0.44
No. of	1.5 ± 0.5	2.5 ± 1.4	2.3 ± 1.1	2.1 ± 1.1	**0.001**

obtained embryo					
Cycle cancellation; n (%)	0 (0%)	6 (8%)	13 (5.8%)	22 (4.75%)	0.22
No oocyte rate; n (%)	3(6 %)	5(6.66 %)	19(8.48 %)	50(10.7 %)	0.46
Fertilization failure; n (%)	6(12 %)	17(22.6 %)	38(16.9 %)	93(20.0 %)	0.36
All freeze for poor endometrium; n (%)	2(4 %)	4(5.33 %)	6(2.67 %)	9 (2.0 %)	0.52
Type of embryos transfer Fresh	35 (89.7%)	33 (76.7%)	126 (85.1%)	210 (72.6%)	0.08
Frozen	4 (10.3%)	10 (23.3%)	22 (14.9%)	79 (27.4%)	
No. of embryos transferred	1.4 ± 0.5	2.0 ± 0.8	1.9 ± 0.743	1.9 ± 0.7	**0.003**
Stage of embryo transferred	35(89.7 %)	33 (76.7 %)	131(88.6 %)	253 (87.5 %)	0.16
Cleavage stage	4 (10.3 %)	10(23.3 %)	17(11.4 %)	36 (12.5 %)	
Blastocyst Stage					
Quality of embryos	18(46.1 %)	29 (67.5 %)	68(45.6 %)	139(48.1 %)	0.28
Excellent	14(35.8 %)	9(20.9 %)	54(36.2 %)	102(35.3 %)	
Good	7 (17.9 %)	5(11.6 %)	26(18.1 %)	48(16.6 %)	
Fair					
Endometrium thickness on trigger day	9.0 ± 1.4	8.9± 1.6	9.080 ± 1.9	8.6 ± 1.5	**0.019**
Fertilization rate	0.5 ± 0.3	0.5 ± 0.4	0.5 ± 0.3	0.56 ± 0.4	0.93
Implantation rate	0.5 ± 0.2	0.5 ± 0.2	0.6 ± 0.2	0.56 ± 0.2	0.58

Clinical pregnancy rate .ET	5/39 (12.8 %)	5/43 (11.6 %)	43/148 (29.0 %)	47/289 (16.3 %)	**0.001**
Blighted ovum rate/ET	1/39 (2.5 %)	0 (0 %)	3/148 (2.0 %)	5/289 (1.7 %)	0.81
Abortion rate /ET	0/39 (0 %)	1/43 (2.3 %)	3/148 (2.0 %)	10/289 (3.4 %)	0.57
Live birth rate/ET	5/39 (12.8%)	4/ 43 (9.3%)	40/148 (27%)	37/289 (12.8%)	**0.001**

POR: Poor ovarian response; ORT: ovarian reserve tests; COH: controlled ovarian hyperstimulation; SD: standard deviation

Table V: Interaction between main variables and live birth in POR population.			
Variables	**Odds Ratio**	**Confidence interval**	**P-value**
Body mass index (BMI) kg/m^2	0.95	(0.90 -1.01)	0.13
Total dose of used gonadotropins	1.008	(0.98-1.02)	0.41
History of previous ART cycle	0.94	(0.80-1.09)	0.43
Women age	0.91	(0.87-0.95)	**0.01**
Women age groups			**0.001**
< 35 years	Reference group		
≤ 35 years	0.44	(0.28-0.71)	
Reason of infertility (Mixed factors)	Reference group		
Ovulatory Factor	0.68	(0.40-1.17)	0.17
Male factor	1.34	(0.73-2.45)	0.3
Type of infertility (secondary)	1.00	(0.58-1.71)	0.99
Duration of infertility	0.95	(0.90-1.01)	0.12
Total number of retrieved oocytes	1.25	(1.12-1.40)	**<0.001**
Number of MII oocytes	1.28	(1.12-1.45)	**<0.001**
Number of 2PN	1.41	(1.21-1.65)	**<0.001**
Number of obtained embryos	1.38	(1.15-1.65)	**<0.001**
Quality of embryos (Fair)	Reference group		
Quality of embryos (Excellent)	7.46	(2.26-24.53)	**0.0009**
Quality of embryos (Good)	4.82	(1.42-16.40)	**0.01**
Day of ET (cleavage stage)	0.96	(0.48-1.92)	0.91
Number of ET	1.6	(1.19-2.19)	**0.001**
Endometrial thickness on ET day	1.06	(0.92-1.22)	0.37
AMH (ng/ml)	0.85	(0.55-1.31)	0.48
AMH group AMH > 0.5(ng/ml)	Reference group		0.7
AMH ≤ 0.5 (ng/ml)	0.93	(0.58-1.48)	
Basal serum LH	1.04	(0.96-1.11)	0.26

Basal serum FSH	1.02	(0.96-1.08)	0.37
Basal serum TSH	0.74	(0.58-0.96)	**0.02**
POSEIDON groups POSEIDON group (4)	Reference group		
POSEIDON group (1)	0.9	(0.36-2.70)	0.99
POSEIDON group (2)	0.7	(0.23-2.05)	0.57
POSEIDON group (3)	2.5	(1.51-4.12)	**0.0003**
Type embryo transfer (FET)	1.267	(0.74-2.16)	0.38
Type of COH protocol			
Long agonist	Reference group		
Antagonist	0.71	(0.42-1.17)	0.18
Double-stimulation	0.69	(0.33-1.41)	0.31
Mini-flare	0.6	(0.07-5.68)	0.70
Bologna groups (Phenotype V)	Reference group		
Phenotype I	0.18	(0.01-1.96)	0.161
Phenotype II	1.10	(0.25-4.84)	0.893
Phenotype III	1.59	(0.44-5.69)	0.471
Phenotype IV	0.42	(0.09-1.97)	0.277

Table VI: The multivariate logistic regression (by backwards manner) analysis to determine the predictive variables of the live birth in the studied population (n=812)			
Variables	**Odds Ratio**	**Confidence interval**	**P-value**
Quality of transferred embryos Fair	Reference group		-
Excellent	5.96	(1.7-20.47)	**0.005**
Good	4.56	(1.2-16.13)	**0.018**
Number of transferred embryos	1.608	(1.09-2.35)	**0.015**
POSEIDON group 4	Reference group		-
POSEIDON group 1	1.73	(087-5.139)	0.317
POSEIDON group 2	0.63	(0.1-2.327)	0.496
POSEIDON group 3	3.04	(1.6-5.634)	**<0.0001**