# Optimum Number of Oocytes Retrieved Among Patients With Polycystic Ovary Syndrome Treated Using The Follicular Phase Long-Acting Long Protocol: A Retrospective Cohort Study

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**Research**

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## Abstract

**Background:** The optimum number of oocytes retrieved by the follicular phase long-acting long protocol is still unknown. This study aimed to analyze the optimum oocyte number in patients with polycystic ovary syndrome (PCOS) undergoing this protocol.

**Methods:** A total of 1816 PCOS patients aged <35 years who were undergoing their first cycle of in vitro fertilization or intracytoplasmic sperm injection (IVF/ICSI) between January 2017 and June 2019 were identified and reviewed. All patients underwent stimulation using a follicular phase long-acting long protocol. In this retrospective cohort study, patients were categorized into seven groups according to the number of oocytes retrieved (group A, 1–5; group B, 6–10; group C, 11–15; group D, 16–20; group E, 21–25; group F, 26–30; group G, >30). The main outcome indicators were the rates of high-quality embryo, fresh cycle pregnancy, cumulative pregnancy, and “freezing all” for high ovarian response. The cumulative pregnancy and “freezing all” rates for high ovarian response were analyzed using multivariate logistic analysis.

**Results:** The high-quality embryo rate decreased with the increase in the number of oocytes retrieved (P<0.001). In the <20 oocyte group, the clinical and cumulative pregnancy rates increased with the number of oocytes retrieved, and the “freezing all” rate for high response was within 30%. In the >20 oocyte group, with an increase in the number of oocytes retrieved, no significant change was found in the clinical and cumulative pregnancy rates (P>0.05); however, the incidence of “freezing all” rate for high response was significantly increased (P<0.001). After correcting for confounding factors, the number of oocytes retrieved was an independent predictor of the “freezing all” rate for high ovarian response (odds ratio [OR], 1.085; 95% confidence interval [CI] 1.057–1.113) and cumulative pregnancy rate (OR 1.091, 95% CI 1.057–1.126). The high-quality embryo rate was significantly affected by the cumulative pregnancy rate (OR, 59.076; 95% CI: 29.591–117.938).

**Conclusion:** In PCOS patients aged <35 years treated using the follicular phase long-acting long protocol, considering clinical outcomes, laboratory indicators, and safety, appropriate ovarian stimulation should be used to control the number of oocytes retrieved at 11–20.


## Background

In vitro fertilization (IVF)/intracytoplasmic sperm injection (ICSI) is a common treatment modality for infertile women with polycystic ovary syndrome (PCOS). The goal of controlled ovarian hyperstimulation (COH) is to achieve higher clinical pregnancy rate and live birth rate while avoiding the incidence of moderate-to-severe ovarian hyperstimulation syndrome (OHSS) [1]. Studies have shown that the number of oocytes retrieved can affect the number of embryos, which in turn affects the clinical pregnancy rate [2]. When the number of oocytes retrieved is too small, the number of high-quality embryos decreases, resulting in a decline in the clinical pregnancy rate and cumulative pregnancy rate. The retrieval of too many oocytes may reduce the clinical pregnancy rate because the hyperphysiological levels of hormones affect endometrial receptivity [3], and the number of oocytes retrieved simultaneously will significantly increase the risk of OHSS [4]. Therefore, the optimum number of oocytes can be used as an important indicator of an ideal pregnancy outcome and a reduction in the complications of IVF/ICSI. Studies have shown that the optimum number of oocytes differs for different populations and according to the protocol used for ovarian stimulation. For example, in a population with normal ovarian reserve, the optimal number of oocytes is approximately 15 using the long luteal phase gonadotropin-releasing hormone (GnRH) agonist protocol [5–8], while for PCOS patients treated using the same protocol, the optimal number of oocytes is 10 [9]. The follicular phase long-acting long protocol is widely used in clinical practice because long-term GnRH agonist administration can improve endometrial receptivity and reduce the estradiol (E2) level on the days of human chorionic gonadotropin (hCG) injection, thereby improving the clinical pregnancy rate and lowering the OHSS rate. To the best of our knowledge, no relevant study has been conducted on the optimum number of oocytes among PCOS patients undergoing treatment using the follicular phase long-acting long protocol [5].

## Materials And Methods

### Aim

This retrospective cohort study aimed to comprehensively evaluate the laboratory and clinical outcomes of different oocytes retrieved from PCOS patients aged <35 years who underwent assisted pregnancy using the follicular phase long-acting long protocol and to discuss the optimum number of oocytes in this population.

### Patients

The protocol of this retrospective cohort study was approved by the Ethical Review Committee of the First Affiliated Hospital of Zhengzhou University. Written informed consent was waived due to the retrospective nature of the study. The selected research participants were patients who underwent IVF/ICSI at the Reproductive Medicine Center of the First Affiliated Hospital of Zhengzhou University between January 2017 and June 2019. The clinical data of the patients were assessed in the first cycle of IVF/ICSI-assisted pregnancy.

All patients were younger than 35 years, diagnosed according to the Rotterdam criteria for PCOS, and undergoing their first IVF/ICSI cycle with the follicular phase long-acting long protocol. For the purposes of this study, patients with cycles involving oocyte donation, rescue ICSI, uterine abnormalities, and without retrieved oocytes were excluded from the analysis. Patients who had not gotten pregnant after 2 years but still had frozen embryos remaining were also excluded from the study. Therefore, the study included 1816 patients with one complete round of treatment with a fresh transfer cycle and subsequent frozen embryo transfer (ET) cycles.

In this study, patient characteristics, including age, body mass index (BMI), duration of infertility, type of infertility, anti-Müllerian hormone (AMH) levels, and follicle-stimulating hormone (FSH) levels, were evaluated. Data regarding other parameters, including gonadotropin dose, number of days of ovarian

stimulation, number of oocytes retrieved, type of insemination, and fertilization rate, were also recorded. The primary outcome was the cumulative live birth rate.

### IVF/ICSI-ET

**Downregulation protocol:** On the second to third days of menstruation, the patients were subcutaneously injected with a long-acting GnRH agonist (Diphereline, 3.75 mg; Beaufour Ipsen Industrie, Dreux, France). Thirty days later, blood samples were obtained from the patients to determine the serum levels of FSH, luteinizing hormone (LH), E2, and progesterone. The sizes of the antral follicles were monitored via vaginal ultrasonography. The COH was initiated when the FSH level was <5 IU/L, the LH level was <3 IU/L, and the antral follicle was nearly 5 mm in diameter.

**COH protocol:** The individualized dosage of gonadotropin (GONAL-f; Merck Serono, Darmstadt, Germany) was determined based on the patient's age, AMH level, antral follicle count (AFC), BMI, and serum FSH level.

**hCG injection and corpus luteum support standard:** We administered 250 μg of Azer (Merck Serono, Italy) and 2000 IU of hCG (Zhuhai Livzon Pharmaceutical) when the diameter of one dominant follicle was ≥20 mm, the diameters of three follicles were ≥17 mm, or two-thirds of the follicles had diameters ≥16 mm. The eggs were harvested under vaginal ultrasound guidance 36–37 h after the injections were administered.

**Fresh ET:** The method of fertilization was based on semen quality. Fresh ET was performed 3–5 days after the oocyte retrieval based on embryo quality, endometrial status, and conditions of the patients. The transplant was cancelled if the patients were deemed to be at high risk for OHSS, the P level was >3 ng/mL, or a uterine effusion was demonstrated. Depending on the specific situation of each patient, different frozen-thawed ET protocols were formulated [14].

**Frozen-thawed ET:** Depending on the specific situation of each patient, different frozen-thawed ET protocols were formulated. These mainly included the following three methods: natural cycle, hormone replacement cycle, and downregulated hormone replacement cycle.

### Statistical analysis

The patients were categorized into seven groups according to the number of oocytes retrieved: group A, 1–5; group B, 6–10; group C, 11–15; group D, 16–20; group E, 21–25; group F, 26–30; and group G, >30. Continuous variables were first assessed for normality of distribution using the Kolmogorov–Smirnov test. Data with normal distributions are presented as means with their standard deviations, and skewed data are presented as medians with their interquartile ranges. One-way analysis of variance or the Kruskal–Wallis test was used to compare multiple sets of measurement data, and the chi-squared test or Fisher's exact test was used to analyze multiple sets of count data, as appropriate. The results of count data are expressed as rates (%). Between-group comparisons were performed using the chi-squared test, and comparisons among multiple groups were performed using the chi-squared segmentation method. Regarding treatment cycles, upon dividing the data into seven groups according to the number of oocytes retrieved, important aspects of the treatment outcomes were assessed, including the fresh cycle pregnancy rate, cumulative pregnancy rate, "freezing all" rate for high ovarian response, and high-quality embryos rate. Logistic regression analyses were performed to identify independent correlates of each possible confounding factor, particularly "freezing all" rate for high ovarian response and cumulative pregnancy outcomes after adjusting for other confounders identified in the univariate analysis. The number of oocytes retrieved was analyzed as a continuous variable. Linear regression analysis (stepwise regression) was used to screen independent related factors that may affect the number of oocytes retrieved, and the confounding factors identified in the single factor analysis were excluded. Regression coefficients (β values) and their 95% confidence intervals (CIs) were calculated. The size of the return effect was adjudged according to the absolute value of the standardized regression coefficient (β). Statistical tests were performed using a two-tailed alpha value of 0.05 with Bonferroni corrections ($P<0.007$ after the correction was considered to indicate a difference between groups). The statistical analyses were performed using SPSS version 24.0 (IBM Corporation, Armonk, NY, USA).

## Results

The data selection process with the number of cycles excluded (with reasons for exclusion) is shown in Figure 1. A total of 1816 cycles of young PCOS patients who underwent IVF/ICSI for the first cycle using the follicular phase long-acting long protocol were included with 16 cycles in group A, 203 cycles in group B, 480 cycles in group C, 485 cycles in group D, 309 cycles in group E, 182 cycles in group F, and 141 cycles in group G. Significant differences were found in the AMH, FSH, AFC, E2 on hCG injection day, LH, progesterone, and mean sizes of bilateral ovaries between the groups (all $P<0.05$) (Table 1 and Figure 2).

Table 1
Comparison of patients' general characteristics and ovulation induction information.

| Item | Number of oocytes retrieved (n) | | | | | | |
|---|---|---|---|---|---|---|---|
| | 1-5 | 6-10 | 11-15 | 16-20 | 21-25 | 26-30 | >3 |
| Cycle number | 16 | 203 | 480 | 485 | 309 | 182 | 14 |
| Age (year) | 29.00(27.00,32.00) | 29.00(26.00,31.00) | 28.00(26.00,30.00) | 28.00(26.00,30.00) | 28.00(26.00,30.00) | 28.00(26.00,30.00) | 28 |
| Type of infertility | | | | | | | |
| Primary infertility (%) | 75.00(12/16) | 66.50(135/203) | 71.88(345/480) | 66.39(322/485) | 66.34(205/309) | 64.84(118/182) | 64 |
| Secondary infertility (%) | 25.00(4/16) | 33.50(68/203) | 28.13(135/480) | 33.61(163/485) | 33.66(104/309) | 35.16(64/182) | 35 |
| Duration of infertility (year) | 3.50(1.25,5.75) | 3.00(2.00,5.00) | 3.00(2.00,5.00) | 3.00(2.00,5.00) | 3.00(2.00,5.00) | 3.00(2.00,5.00) | 3. |
| FSH (mIU/ml) | 6.74(6.21,7.41)$^{DEF}$ | 5.98(5.13,6.84)$^{cde}$ | 5.88(5.01,6.87)$^{\delta}$ | 5.81(4.92,6.72)$^{\square}$ | 5.58(4.79,6.36)$^{\ddagger}$ | 5.53(4.76,6.36) | 5. |
| AMH (ng/ml) | 6.21(4.90,8.52)$^{EF}$ | 6.02(4.30,9.23)$^{bcde}$ | 6.84(5.14,9.62)$^{\beta\gamma\delta}$ | 7.15(5.22,9.86)$^{\square\square}$ | 7.76(5.80,10.88)$^{\ddagger}$ | 8.92(6.76,12) | 10 |
| BMI (kg/m2) | 25.40(21.85,27.28) | 24.50(21.85,26.70) | 23.80(21.40,26.30) | 24.40(21.93,26.90) | 24.70(22.20,27.10) | 24.40(22.2,26.75) | 25 |
| AFC (n) | 24(24,24) | 24(22,24)$^{bcde}$ | 24(23,24)$^{\gamma}$ | 24(24,24) | 24(24,24) | 24(24,24) | 24 |
| Fertilization method | | | | | | | |
| IVF(%) | 93.75(15/16) | 87.68(178/203) | 89.38(429/480) | 85.57(415/485) | 89.97(278/309) | 88.46(161/182) | 88 |
| ICSI(%) | 6.25(1/16) | 12.32(25/203) | 10.63(51/480) | 14.43(70/485) | 10.03(31/309) | 11.54(21/182) | 11 |
| Starting dose of Gn(IU) | 112.50 (87.50,125.00) | 112.50 (100.00,118.75) | 112.50 (100.00,112.50) | 112.50 (100.00,112.50) | 112.50 (100.00,112.50) | 100.00 (100.00,112.50) | 11 (1 |
| Length of stimulation (d) | 15(13,19) | 14(12,16)$^{cde}$ | 14(12,16)$^{\beta\gamma\delta}$ | 14(13,16) | 15(13,17) | 15(13,17) | 15 |
| Total dosage of Gn used (IU) | 2837.50 (1687.50,4331.25) | 1937.50 (1487.50,2687.50) | 1937.50 (1468.75,2525.00) | 1925.00 (1462.50,2537.50) | 2062.50 (1500.00,2750.00) | 2118.75 (1625.00,2653.13) | 22 (1 |
| hCG injection day | | | | | | | |
| Endometrial thickness(mm) | 12.00(10.25,13.75) | 13.00(11.00,14.00) | 12.00(11.00,14.00) | 12.00(10.00,14.00) | 12.00(11.00,14.00) | 12.00(10.00,14.00) | 12 |
| E2 (pg/ml) | 1113.00 (880.65, 2423.00)$^{BCDEF}$ | 2262.00 (1644.00, 3074.00)$^{BCDEF}$ | 2906.00 (1979.50, 4170.00)$^{\alpha\beta\gamma\delta}$ | 3510.50 (2625.50, 4861.50)$^{\square\square\square}$ | 4350.00 (3286.00, 5587.00)$^{\ddagger}$ | 5191.00 (3349.50, 6895.25) | 55 (3 72 |
| P (ng/ml) | 0.27(0.09,0.54)$^{CDEF}$ | 0.48(0.29,0.71)$^{abcde}$ | 0.58(0.38,0.93)$^{\alpha\beta\gamma\delta}$ | 0.7(0.43,1.09)$^{\square\square\square}$ | 0.84(0.57,1.28)$^{\dagger\ddagger}$ | 1.01(0.65,1.53) | 1. |
| LH (mIU/ml) | 0.75(0.17,1.81) | 0.57(0.28,0.90)$^{ce}$ | 0.52(0.25,0.86)$^{\delta}$ | 0.50(0.25,0.80)$^{\square}$ | 0.41(0.19,0.70) | 0.47(0.20,0.80) | 0. |

Note:Continuous data: mean±SD. Categorical data: % (n/N); BMI, body mass index; E2, estradiol; P, progesterone; FSH, follicle-stimulating hormone; AMH, Ant Antral follicular count; Gn, gonadotropin; hCG, human chorionic gonadotropin.

$^{A}$P, $^{B}$P, $^{C}$P, $^{D}$P, $^{E}$P and $^{F}$P indicate group 1 vs. groups 2, 3, 4, 5, 6 and 7 respectively;

$^{a}$P, $^{b}$P, $^{c}$P, $^{d}$P and $^{e}$P indicate group 2 vs. groups 3, 4, 5, 6 and 7 respectively;

$^{\alpha}$P,$^{\beta}$P,$^{\gamma}$P and$^{\delta}$P indicate group 3 vs. groups 4, 5, 6 and 7 respectively;

$^{\square}$P,$^{\square}$P and $^{\square}$P indicate group 4 vs. groups 5, 6 and 7 respectively;

$^{\dagger}$P and $^{\ddagger}$P indicates group 5 vs.groups 6 and 7 respectively;

$^{\#}$P indicates group 6 vs. group 7.

| Item | Number of oocytes retrieved (n) | | | | | | |
|---|---|---|---|---|---|---|---|
| | 1-5 | 6-10 | 11-15 | 16-20 | 21-25 | 26-30 | >3 |
| Mean bilateral ovarian size(mm) | 4.35(3.93,4.90)$^{CDEF}$ | 4.85(4.10,5.50)$^{bcde}$ | 5.00(4.50,5.50)$^{\alpha\beta\gamma\delta}$ | 5.30(4.85,5.90)$^{\square\square\square}$ | 5.55(5.00,6.20)$^{\ddagger}$ | 5.80(5.10,6.51) | 6. |

Note:Continuous data: mean±SD. Categorical data: % (n/N); BMI, body mass index; E2, estradiol; P, progesterone; FSH, follicle-stimulating hormone; AMH, Ant Antral follicular count; Gn, gonadotropin; hCG, human chorionic gonadotropin.

$^{A}$P, $^{B}$P, $^{C}$P, $^{D}$P, $^{E}$P and $^{F}$P indicate group 1 vs. groups 2, 3, 4, 5, 6 and 7 respectively;

$^{a}$P, $^{b}$P, $^{c}$P, $^{d}$P and $^{e}$P indicate group 2 vs. groups 3, 4, 5, 6 and 7 respectively;

$^{\alpha}$P,$^{\beta}$P,$^{\gamma}$P and$^{\delta}$P indicate group 3 vs. groups 4, 5, 6 and 7 respectively;

$^{\square}$P,$^{\square}$P and $^{\square}$P indicate group 4 vs. groups 5, 6 and 7 respectively;

$^{\dagger}$P and $^{\ddagger}$P indicates group 5 vs.groups 6 and 7 respectively;

$^{\#}$P indicates group 6 vs. group 7.

Significant differences were also noted in the fertilization rate, high-quality embryo rate, and blastocyst formation rate (all P<0.001). With an increase in the number of oocytes retrieved, the fertilization rate was maintained at a high level for 11–20 oocytes (62.82–61.42%) and then decreased, and the high-quality embryo rate was highest (67.09%) when 6–10 oocytes were retrieved and then decreased. Significant differences were found in the “freezing all” rate for high ovarian response, transplanted blastocyst rate, and cumulative pregnancy rate (P<0.001). With an increase in the number of oocytes retrieved, the “freezing all” rate for high ovarian response, transplanted blastocyst rate, and cumulative pregnancy rate increased significantly. The clinical pregnancy rate was not significantly different among the groups (P=0.768), but remained at a high level when 11–20 oocytes were retrieved (78.77–80.13%) (Table 2 and Figure 3).

Table 2
Laboratory results and clinical outcomes.

| Item | Number of oocytes retrieved (n) | | | | | | |
|---|---|---|---|---|---|---|---|
| | A:1-5 | B:6-10 | C:11-15 | D:16-20 | E:21-25 | F:26-30 | G:> |
| Cycle number | 16 | 203 | 480 | 485 | 309 | 182 | 14 |
| Laboratory outcomes | | | | | | | |
| Tatol number of oocytes retrieved(n) | 62 | 1742 | 6297 | 8689 | 7065 | 5050 | 50 |
| Average number of oocytes retrieved(n) | 4(3.5,5)$^{BCDEF}$ | 9(8,10)$^{abcde}$ | 13(12,14)$^{\alpha\beta\gamma\delta}$ | 18(17,19)$^{\square\square\square}$ | 23(22,24)$^{\dagger\ddagger}$ | 28(27,29) | 31 |
| fertilization rate (%) | 61.29(38/62) | 60.10(1047/1742) | 62.82(3956/6297)$^{\beta\gamma\delta}$ | 61.42(5337/8689)$^{\square}$ | 60.23(4255/7065)$^{\ddagger}$ | 59.52(3006/5050) | 57 |
| high-quality embryo rate(%) | 60.53(23/38) | 67.09(693/1033)$^{cde}$ | 63.90(2503/3917)$^{\gamma\delta}$ | 63.27(3336/5273)$^{\square\square}$ | 61.61(2595/4212)$^{\ddagger}$ | 59.67(1768/2963) | 58 |
| Blastocysts rate(%) | 12.50(1/8) | 47.31(158/334)$^{acd}$ | 57.10(1130/1979) | 54.71(1783/3259)$^{\square}$ | 58.30(1718/2947)$^{\ddagger}$ | 56.20(1241/2208) | 53 |
| clinical outcomes | | | | | | | |
| “freezing all” for high ovarian response rate(%) | 0(0/16)$^{DF}$ | 8.37(17/203)$^{bcde}$ | 15.21(73/480)$^{\alpha\beta\gamma\delta}$ | 28.04(136/485)$^{\square\square\square}$ | 39.81(123/309)$^{\dagger\ddagger}$ | 63.38(119/482)$^{\#}$ | 84 |
| Blastocysts transfer rate(%) | 0(0/13)$^{CDEF}$ | 4.02(7/174)$^{abcde}$ | 19.18(75/391)$^{\alpha\beta\gamma\delta}$ | 47.02(142/302)$^{\square\square\square}$ | 70.00(112/160)$^{\dagger}$ | 85.71(42/49) | 94 |
| Clinical pregnancy rate(%) | 69.23(9/13) | 77.01(134/174) | 78.77(308/391) | 80.13(242/302) | 78.13(125/160) | 77.56(38/49) | 66 |
| Embryo implantation rate(%) | 40.00(10/25) | 57.50(184/320) | 56.24(347/617) | 56.41(176/312) | 58.95(56/95) | 57.14(8/14) | 10 |
| Blastocysts implantation rate(%) | 0(0/0) | 100.00(7/7) | 77.33(58/75) | 83.10(118/142) | 75.89(85/112) | 76.19(32/42) | 64 |
| Abortion rate(%) | 0(0/13) | 9.20(16/174) | 9.97(39/391) | 7.95(24/302) | 5.63(9/160) | 18.37(9/49) | 11 |
| Live birth rate(%) | 69.23(9/13) | 67.24(117/174) | 67.77(265/391) | 70.20(212/302) | 70.00(112/160) | 59.18(29/49) | 55 |
| Cumulative pregnancy rate(%) | 68.75(11/16)$^{CDEF}$ | 80.79(164/203)$^{abcde}$ | 88.75(426/480)$^{\beta\delta}$ | 91.34(443/485) | 95.15(294/309) | 95.05(173/182) | 96 |

Note:Continuous data: mean±SD. Categorical data: % (n/N)

$^{A}$P, $^{B}$P, $^{C}$P, $^{D}$P, $^{E}$P and $^{F}$P indicate group 1 vs. groups 2, 3, 4, 5, 6 and 7 respectively;

$^{a}$P, $^{b}$P, $^{c}$P, $^{d}$P and $^{e}$P indicate group 2 vs. groups 3, 4, 5, 6 and 7 respectively;

$^{\alpha}$P,$^{\beta}$P,$^{\gamma}$P and$^{\delta}$P indicate group 3 vs. groups 4, 5, 6 and 7 respectively;

$^{\square}$P,$^{\square}$P and $^{\square}$P indicate group 4 vs. groups 5, 6 and 7 respectively;

$^{\dagger}$P and $^{\ddagger}$P indicates group 5 vs.groups 6 and 7 respectively;

$^{\#}$P indicates group 6 vs. group 7.

The “freezing all” for high ovarian response group had 587 cycles, and the ovarian normal-response group had 1229 cycles. To reduce the interference of confounding factors, a logistic regression analysis of the following variables was performed: age, type of infertility, duration of infertility, BMI, FSH, AMH, AFC,

gonadotropin dose characteristics (starting dose, medication time, and total dose), characteristics on hCG injection day (endometrial thickness, E2, progesterone, LH, mean bilateral ovarian size), and number of oocytes retrieved. Statistically significant variables identified in the univariate analysis were included in the multivariate logistic regression analysis. The logistic regression analyses revealed that a woman's age (adjusted odds ratio [aOR]=0.932, 95% CI 0.893–0.972, P<0.001), $\log_{10}$(total gonadotropin dose) (aOR=0.225, 95% CI 0.090–0.560, P=0.001), $\log_{10}$(E2 level on hCG injection day) (aOR=5.520, 95% CI 2.784–10.946, P<0.001), mean size of the bilateral ovaries on the hCG injection day (aOR=1.585, 95% CI 1.349–1.861, P<0.001), and number of oocytes retrieved (aOR=1.132, 95% CI 1.108–1.156, P<0.001) were independent factors in the "freezing all" for high ovarian response group (Table 3).

Table 3
Logistic regression analysis of "freezing all" for high ovarian response

| | No "freezing all" (n=1229) | "freezing all" for high ovarian response (n=587) | OR(95%CI) | P value | [a]OR(95%CI) | [a]P value |
|---|---|---|---|---|---|---|
| Age (year) | 28.41±3.13 | 27.83±3.11 | 0.942(0.913,0.972) | <0.001 | 0.932(0.894,0.972) | 0.001 |
| Type of infertility | | | 0.829(0.67,1.026) | 0.085 | | |
| Primary infertility (%) | 66.31(815/1229) | 70.36(413/587) | | | | |
| Secondary infertility (%) | 33.69(414/1229) | 29.64(174/587) | | | | |
| Duration of infertility (year) | 3.81±2.42 | 3.54±2.12 | 0.948(0.906,0.991) | 0.020 | 0.989(0.935,1.046) | 0.700 |
| BMI (kg/m2) | 24.50±3.32 | 23.87±3.35 | 0.945(0.917,0.973) | <0.001 | 0.949(0.895,1.007) | 0.083 |
| FSH (mIU/ml) | 5.90±1.59 | 5.62±1.54 | 0.893(0.836,0.953) | 0.001 | 0.968(0.889,1.054) | 0.458 |
| AMH (ng/ml) | 7.77±3.90 | 9.69±4.60 | 1.111(1.085,1.137) | <0.001 | 1.020(0.986,1.054) | 0.249 |
| AFC (n) | 22.19±4.78 | 22.81±4.33 | 1.032(1.008,1.057) | 0.009 | 1.000(0.970,1.030) | 0.993 |
| Gn | | | | | | |
| Starting dose of Gn(IU) | 109.05±19.42 | 103.80±15.33 | 0.982(0.976,0.988) | <0.001 | 1.002(0.990,1.013) | 0.770 |
| Length of stimulation (d) | 14.59±2.65 | 14.6±2.62 | 1.001(0.965,1.039) | 0.948 | | |
| Log10(Total dosage of Gn used) (IU) | 3.31±0.17 | 3.27±0.17 | 0.227(0.126,0.41) | <0.001 | 0.225(0.090,0.560) | 0.001 |
| hCG injection day | | | | | | |
| Endometrial thickness(mm) | 12.38±2.36 | 12.17±2.43 | 0.964(0.925,1.005) | 0.085 | | |
| Log10(E2 )(pg/ml) | 3.48±0.23 | 3.66±0.21 | 41.707(24.793,70.16) | <0.001 | 5.520(2.784,10.946) | <0.001 |
| P (ng/ml) | 0.77±0.51 | 0.97±0.58 | 1.005(0.902,1.119) | 0.927 | | |
| LH (mIU/ml) | 0.71±0.91 | 0.71±0.93 | 1.962(1.636,2.353) | <0.001 | 0.982(0.847,1.140) | 0.815 |
| Mean bilateral ovarian size(mm) | 5.14±0.86 | 5.90±1.01 | 2.477(2.188,2.804) | <0.001 | 1.585(1.349,1.861) | <0.001 |
| Number of oocytes retrieved(n) | 16.20±5.95 | 23.90±8.35 | 1.168(1.148,1.189) | <0.001 | 1.132(1.108,1.156) | <0.001 |

Note:Continuous data: mean±SD. Categorical data: % (n/N); [a]OR, [a]P, adjusted by the variables which were statistically significant in the univariate logistic analysis.

The cumulative pregnancy group included 1648 cycles, and 168 cycles did not reach pregnancy. The factors and methods included in the analysis were the same as those used in the logistic regression analysis of "freezing all" for high ovarian response. The results showed that $\log_{10}$ (E2 level on hCG injection day), number of oocytes retrieved, and high-quality embryo rate were factors that influenced the cumulative pregnancy rate (P<0.05). The multivariate logistic regression analysis showed that the number of oocytes retrieved (aOR=1.091, 95% CI 1.057–1.126, P<0.001) and high-quality embryo rate (aOR=59.076, 95% CI 29.591–117.938, P<0.001) were independent predictors of cumulative pregnancy (Tables 4 and 5).

Table 4
Single factor logistic regression analysis of cumulative pregnancy

| | No cumulative pregnancy (n=168) | Cumulative pregnancy (n=1648) | OR(95%CI) | P value |
|---|---|---|---|---|
| Age (year) | 28.26±3.23 | 28.22±3.13 | 0.996(0.947,1.048) | 0.875 |
| Type of infertility | | | 1.181(0.834,1.674) | 0.348 |
| Primary infertility (%) | 119/168 | 1109/1648 | | |
| Secondary infertility (%) | 49/168 | 539/1648 | | |
| Duration of infertility (year) | 4.01±2.48 | 3.69±2.31 | 0.948(0.890,1.010) | 0.098 |
| BMI (kg/m2) | 24.30±3.38 | 24.30±3.34 | 1.000(0.954,1.049) | 1.000 |
| FSH (mIU/ml) | 5.93±1.78 | 5.80±1.56 | 0.95(0.860,1.049) | 0.309 |
| AMH (ng/ml) | 8.19±4.15 | 8.41±4.25 | 1.013(0.974,1.052) | 0.527 |
| AFC (n) | 22.34±4.25 | 22.40±4.69 | 1.003(0.969,1.037) | 0.880 |
| Gn | | | | |
| Starting dose of Gn(IU) | 108.41±22.04 | 107.25±17.95 | 0.997(0.989,1.005) | 0.435 |
| Length of stimulation (d) | 14.74±2.52 | 14.57±2.66 | 0.976(0.92,1.036) | 0.429 |
| Log10(Total dosage of Gn used) (IU) | 3.31±0.16 | 3.29±0.17 | 0.49(0.191,1.255) | 0.137 |
| hCG injection day | | | | |
| Endometrial thickness(mm) | 12.49±2.62 | 12.30±2.36 | 0.966(0.905,1.033) | 0.312 |
| Log10(E2 )(pg/ml) | 3.49±0.26 | 3.55±0.23 | 2.629(1.352,5.111) | 0.004 |
| P (ng/ml) | 0.80±0.53 | 0.84±0.54 | 1.121(0.828,1.518) | 0.459 |
| LH (mIU/ml) | 0.67±0.80 | 0.72±0.92 | 1.077(0.884,1.313) | 0.460 |
| Mean bilateral ovarian size(mm) | 5.33±0.98 | 5.39±0.98 | 1.065(0.904,1.255) | 0.452 |
| Number of oocytes retrieved(n) | 15.20±6.91 | 19.05±7.70 | 1.085(1.057,1.113) | <0.001 |
| high-quality embryo rate(%) | 0.37±0.31 | 0.65±0.25 | 57.494(28.797,114.786) | <0.001 |
| Note:Continuous data: mean±SD. Categorical data: % (n/N); | | | | |

Table 5
Multivariate logistic regression analysis of cumulative pregnancy

| | Single factor logistic regression analysis | | Multivariate logistic regression analysis | |
|---|---|---|---|---|
| | OR(95%CI) | P value | aOR(95%CI) | aP value |
| $Log_{10}$(hCG injection day E2) (pg/ml) | 2.629(1.352,5.111) | 0.004 | 1.048(0.459,2.395) | 0.911 |
| Number of oocytes retrieved(n) | 1.085(1.057,1.113) | <0.001 | 1.091(1.057,1.126) | <0.001 |
| high-quality embryo rate(%) | 57.494(28.797,114.786) | <0.001 | 59.076(29.591,117.938) | <0.001 |

The patient's age, duration of infertility, AMH level, FSH level, AFC, gonadotropin characteristics (starting dose, medication time, and total dose), and other variables as alternative independent variables and the number of oocytes retrieved as a dependent variable were entered into the multiple regression equation. The independent variables identified in the regression and order of the size of the influence on the number of eggs obtained were FSH (β=-0.731, P<0.001), duration of gonadotropin administration (β = 0.374, P<0.001), AMH level (β = 0.322, P<0.001), and AFC (β = 0.085, P=0.035) (Table 6).

Table 6
Factors that affect the number of oocytes retrieved

| Model | Regression coefficients b | Standard error Sx | Standardized regression coefficient B | T value | Sig. | 95.0% CI for b |
|---|---|---|---|---|---|---|
| constant | 12.828 | 1.401 | | 9.159 | <0.001 | 12.828-1.401 |
| AMH (ng/ml) | 0.322 | 0.044 | 0.177 | 7.340 | <0.001 | 0.322-0.044 |
| FSH (mIU/ml) | -0.731 | 0.116 | -0.151 | -6.279 | <0.001 | -0.731-0.116 |
| Length of stimulation (d) | 0.374 | 0.071 | 0.128 | 5.297 | <0.001 | 0.374-0.071 |
| AFC (n) | 0.085 | 0.040 | 0.050 | 2.108 | 0.035 | 0.085-0.040 |
| Stepwise regression:Incorporate possible influencing factors into the analysis, including Age, Type of infertility, Duration of infertility, BMI, FSH, AMH, AFC, Starting dose of Gn, Length of stimulation, Log10(Total dosage of Gn used),hCG injection day: Endometrial thickness, Log10(E2),P, LH. | | | | | | |

## Discussion

In this study, we determined the optimum number of oocytes for PCOS patients aged < 35 years. Considering the clinical outcomes, laboratory indicators, and safety, the optimum number of oocytes was 11–20. Studies have shown differences in the optimum number of oocytes between different ovarian response groups and patients treated using different ovarian stimulation protocols [16–17]. In the population with a normal ovarian response, the GnRH antagonist protocol has the best effect in obtaining 10–11 oocytes, while the GnRH agonist protocol can achieve the highest clinical pregnancy rate when 8–11 oocytes are obtained [18]. After comprehensively evaluating the cumulative live birth rate and remaining embryo rate of PCOS patients who underwent assisted pregnancy using the luteal phase long GnRH agonist protocol, a retrospective study suggested that the optimum number of oocytes was 10 [9]. Because PCOS patients with high ovarian responsiveness have a large number of antral follicles, their AMH levels are higher than those of normal people [19]. During the COH process, the number of oocytes retrieved is often large; however, the quality is poor, resulting in a low fertilization rate and a higher risk of OHSS [20]. Therefore, the optimum number of oocytes for women with normal ovarian response may not apply to PCOS patients. Since the follicular phase long-acting long protocol uses a lower dose than the luteal phase long GnRH agonist protocol, the E2 level on the day of hCG administration is lower and the long-acting GnRH agonist improves endometrial receptivity. This can increase the fresh cycle transplantation rate and clinical pregnancy rate, and reduce the risk of OHSS ($P<0.01$) [12, 21]. In China, the follicular phase long-acting long protocol has become one of the predominant COH protocols used in the field of assisted reproduction. To reduce the risk of OHSS, GnRH antagonist protocols are often used to assist pregnancy among PCOS patients. Current research shows no significant difference in the OHSS incidence between patients treated using the follicular phase long-acting long protocol and those treated using the GnRH antagonist protocol [10–11]. Therefore, it is of great clinical significance to study the optimum number of oocytes among PCOS patients treated using the follicular phase long-acting long protocol.

As the strategy of transplanting fresh embryos is adopted in the follicular phase long-acting long protocol, it is particularly important to obtain an appropriate number of oocytes. In this study, laboratory indicators were combined with clinical results to determine the optimum number of oocytes. The results showed that the high-quality embryo rate decreased gradually with an increase in the number of oocytes retrieved. When <20 oocytes were retrieved, the fertilization rate was higher, and this rate decreased with an increase in oocytes retrieved when >20 were retrieved ($P<0.007$). No significant difference was found in the clinical pregnancy rate among the groups; however, the clinical pregnancy rate was the highest (80.13%) when 16–20 oocytes were retrieved. Additionally, the excessive number of oocytes retrieved may be related to adverse pregnancy outcomes. A study involving 65,868 single live births showed that >20 oocytes were retrieved in assisted reproductive technology, which may be related to the increased risk of premature birth and low birth weight among infants [22]. The cumulative pregnancy rate/live birth rate has become a more meaningful index for evaluating IVF-ET [23]. A systematic retrospective analysis showed that the retrieval of 12–18 oocytes can yield the maximum fresh cycle live birth rate and a continuous positive correlation exists between the number of oocytes retrieved and the cumulative live birth rate [24]. A study involving 39,387 individuals treated using the GnRH agonist or GnRH antagonist protocol, including 77,956 fresh cycles and 36,270 frozen cycles, suggested that the retrieval of 18–20 oocytes can achieve the desired cumulative live birth rate and maintain the incidence of OHSS at a reasonable level [25]. Another study including 221,221 treatment cycles showed that women aged 18–34 years achieved the highest cumulative live birth rate when 25 oocytes were retrieved and that with the continuous increase in the number of oocytes retrieved, the cumulative live birth rate increased a little.

The multiple logistic regression analysis showed that the number of oocytes retrieved and the high-quality embryo rate were independent risk factors for cumulative pregnancy. When <20 oocytes were retrieved, the cumulative pregnancy rate increased significantly with an increase in the number of oocytes retrieved ($P<0.007$). When >20 oocytes were retrieved, the cumulative pregnancy rate did not increase. According to the comprehensive clinical and laboratory results, when 11–20 oocytes were retrieved, the high-quality embryo rate was high, the “freezing all” for high ovarian response rate was controlled within an acceptable range, and fresh cycle pregnancy and cumulative pregnancy rates both reached peak values.

To our knowledge, this study is the first to analyze the optimal number of oocytes among PCOS patients undergoing their first IVF/ICSI cycle with the follicular phase long-acting long protocol by considering clinical outcomes, laboratory indicators, and adverse outcomes, such as “freezing all” for high ovarian response. This study provides a basis for assisted conception management of PCOS patients in clinical practice via IVF/ICSI-ET. Simultaneously, we realize that our study had some limitations. First this study utilized a retrospective design without considering all confounding factors. Second, the study only included PCOS patients aged <35 years. Third, the conclusions derived herein cannot be extended to all age groups or patients with low or normal ovarian reserves. Meanwhile, the cut-off point of the observation time was 2 years, and some patients had frozen embryos that had not been transplanted. These

members of the population were excluded from this study because the number of patients was small (69 cycles in total). The results of the present study need to be further confirmed in large, multicenter, and prospective studies.

## Conclusion

This study comprehensively analyzed the clinical pregnancy rate, cumulative pregnancy rate, high-quality embryo rate, and “freezing all” rate for high ovarian response. Among young PCOS patients undergoing assisted pregnancy using the follicular phase long-acting long protocol, an optimum number of 11–20 oocytes is required to retrieved better clinical pregnancy outcomes and a low risk of OHSS.

## Abbreviations

AMH = anti-Müllerian hormone

AFC = antral follicle count

BMI = body mass index

CI = confidence interval

COH = controlled ovarian hyperstimulation

ET = embryo transfer

FSH = follicle-stimulating hormone

GnRH = gonadotropin-releasing hormone

hCG = human chorionic gonadotropin

IVF/ICSI = in vitro fertilization or intracytoplasmic sperm injection

OR = odds ratio

OHSS = ovarian hyperstimulation syndrome

PCOS = polycystic ovary syndrome

## Declarations

**Ethics approval and consent to participate:** The protocol of this retrospective cohort study was approved by the Ethical Review Committee of the First Affiliated Hospital of Zhengzhou University. Written informed consent was waived due to the retrospective nature of the study.

**Consent for publication:**Not applicable.

**Availability of data and materials :**The datasets that were used and/or analysed during the current study are available from the corresponding author upon reasonable request.

**Competing interests:** Not applicable

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**Authors' contributions:** JZ contributed to the conception of this study. TY was responsible for the study design, statistical analyses, and manuscript writing. DW contributed by revising the manuscript. All the authors contributed to the manuscript and approved the submitted version.

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## Figures

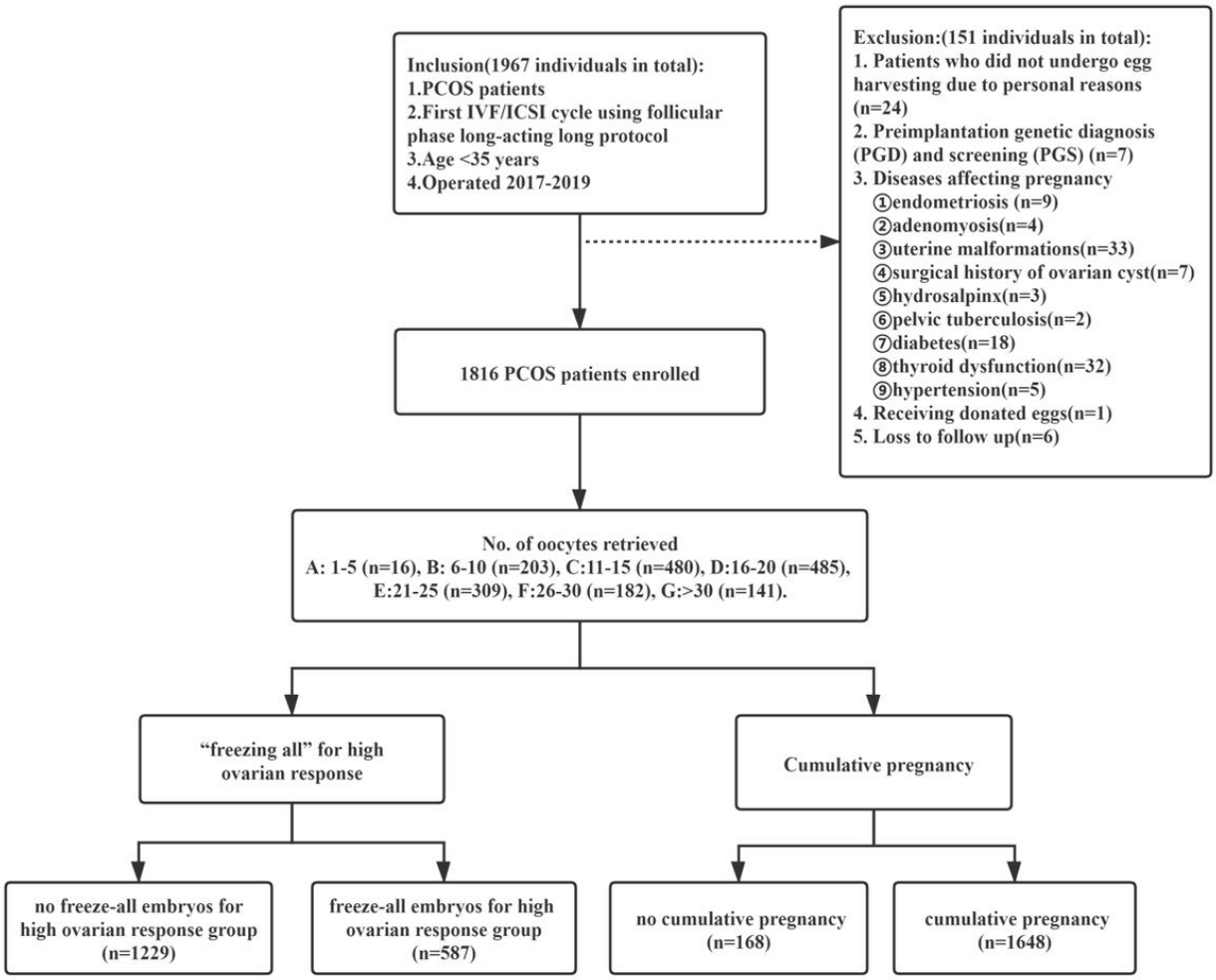


**Figure 1**

Flow chart depicting the patient selection.

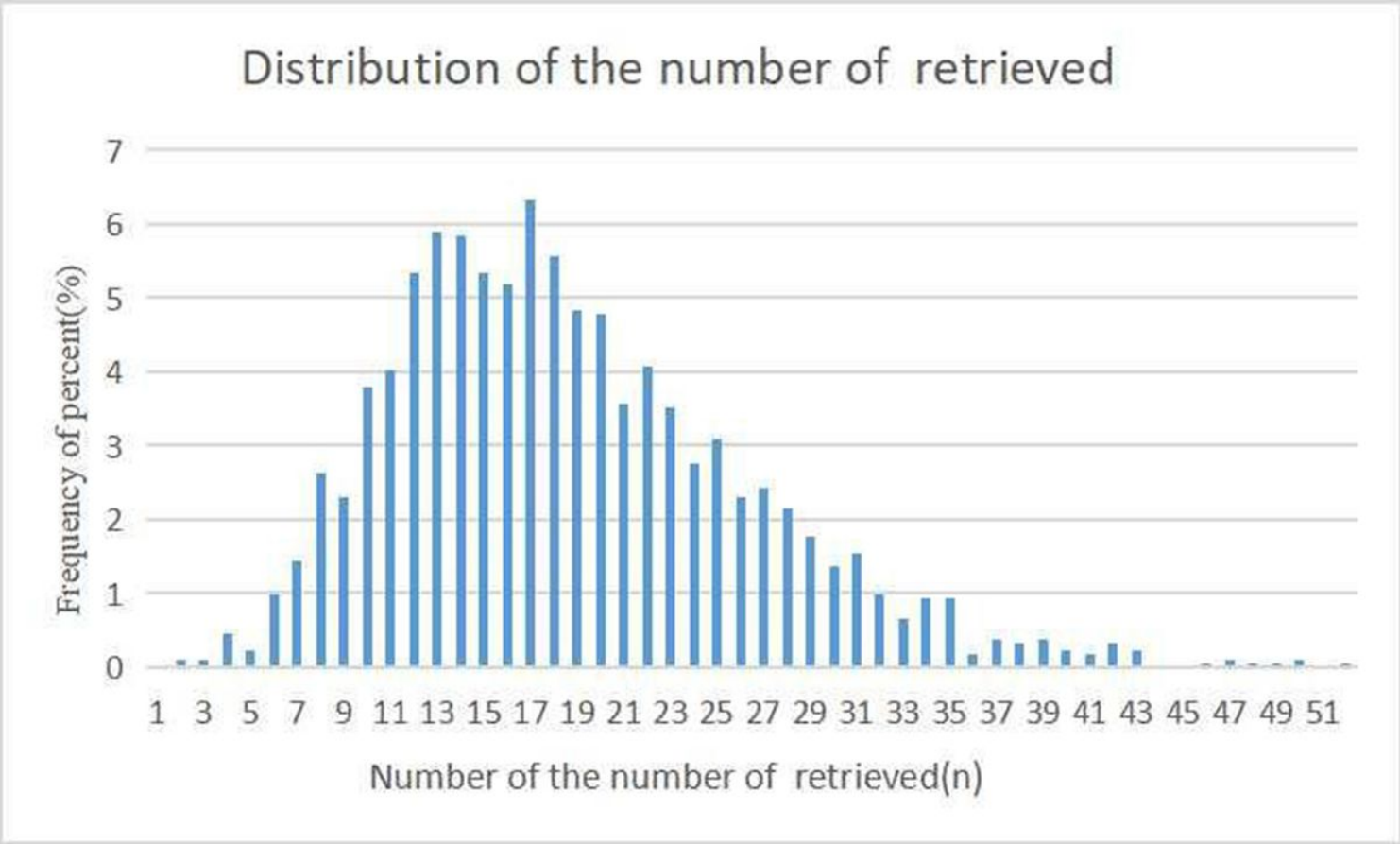


Figure 2

Distribution of the number of oocytes retrieved

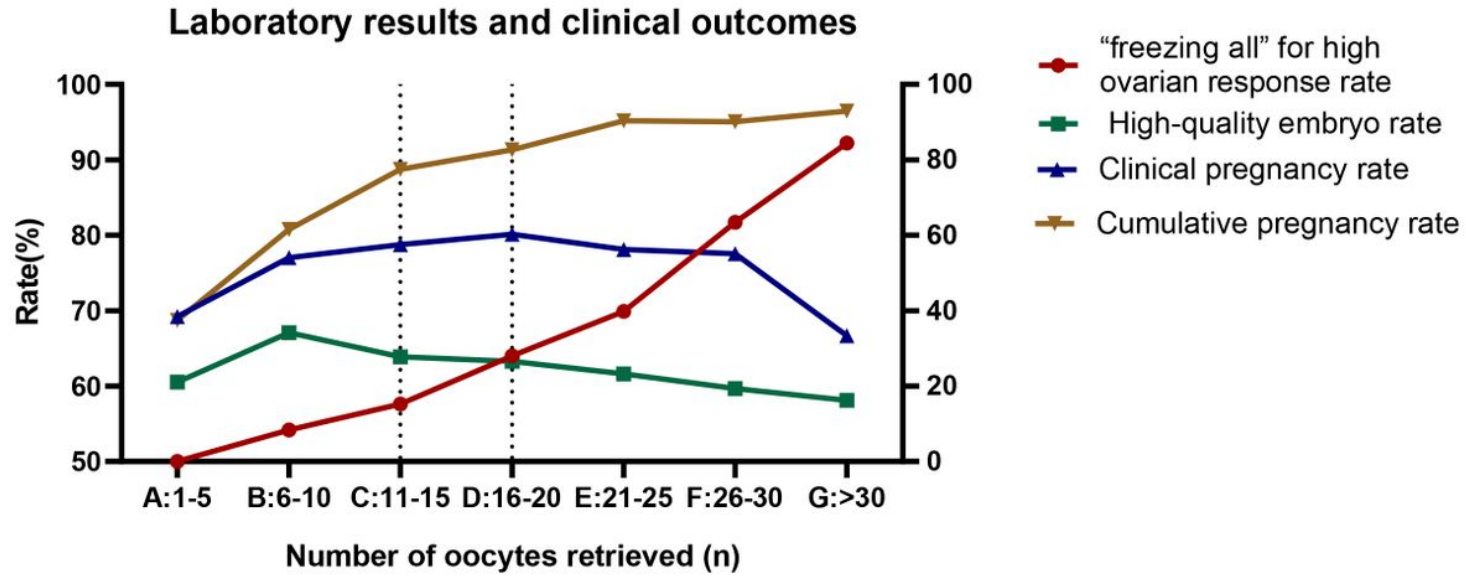


Figure 3

Laboratory results and clinical outcomes Note: High-quality embryo, clinical pregnancy rate, and cumulative pregnancy rate are referring to the ordinate axis on the left; "freezing all" for high ovarian response are referring to the ordinate axis on the right