Optimum oocyte retrieved and transfer strategy in young women with normal ovarian reserve undergoing a long treatment protocol: a retrospective cohort study

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Abstract

Purpose This study aimed to investigate the relationship between the number of oocytes retrieved and clinical outcomes in young women with normal ovarian reserve who were undergoing their first in vitro fertilization and embryo transfer (IVF-ET) cycle. The transfer strategy based on yielded oocytes was also discussed in this article.

Capsule For young women with normal ovarian reserve, retrieving 10~12 oocytes might result in optimized pregnancy outcomes in consideration of both fresh and frozen transfers. With more than 16 oocytes, a “freeze-all” embryo strategy might be preferable

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Methods A total of 1567 patients who underwent first long protocol of IVF treatment in our reproductive medical center between January 2010 and June 2014 were categorized into five groups based on the retrieved oocyte number, namely, 4~6, 7~9, 10~12, 13~15, and ≥ 16 . Baseline parameters were similar among the groups. Primary outcome was defined as the cumulative live birth rate (CLBR), and secondary outcomes included the rate of patients with high risks for ovarian hyperstimulation syndrome (OHSS).

Results It was found that the CLBR increased with the number of oocytes, as well as the rate for high risks of OHSS. In fresh cycles, 10~12 oocyte group demonstrated the highest implantation rate (53.32 %), clinical pregnancy rate (CPR) (73.13 %), and live birth rate (LBR) (61.14 %), with no significant differences. Moreover, both cumulative CPR (CCPR) and CLBR became significantly higher in the 10~12 oocyte group, compared with 4~6 and 7~9 groups. However, when the retrieved oocytes increased to 13~15 or ≥ 16 , the cumulative results did not have a significant increase. Also, the high risk rate of OHSS was much lower in the 10~12 group (11.53 %) than that in the 13~15 group (29.97 %) and ≥ 16 group (77.30 %). Unconditional multivariate logistic regression analysis showed that when ≥ 10 oocytes were retrieved, the CLBR increased significantly ($P < 0.01$). When oocyte number exceeded 16, the CPR of frozen embryo transfer cycle was much higher than that of fresh cycle ($P < 0.05$).

Conclusions For young women with normal ovarian reserve, retrieving 10~12 oocytes might result in optimized pregnancy outcomes in a fresh cycle with low OHSS risk and would not compromise cumulative outcomes. When ≥ 16 oocytes were retrieved, a “freeze-all” embryo strategy might be preferable.

Keywords Oocyte number · Normal ovarian reserve · Cumulative live birth · Ovarian hyperstimulation syndrome

Introduction

The development of assisted reproductive technology (ART), in particular IVF, has provided great hope for infertile couples worldwide. More than five million IVF babies have been born since Louise Brown, the first IVF baby, in 1978 [1, 2]. Normally, only one oocyte has the chance to be matured and fertilized in a natural menstrual cycle. The efficiency and pregnancy rates of IVF are significantly improved when controlled ovarian stimulation (COS) is applied [3], which causes more mature oocytes and available embryos to be produced [4]. However, the implementation of COS is associated with concerns such as a high incidence of OHSS, financial burden, and wastage of oocytes. Although considerable efforts have focused on investigating the relationship between the optimum oocyte number and clinical outcomes, a precise conclusion has not yet been reached. The majority of researchers have found that clinical outcomes improved with the increase in the number of eggs [5–7]. However, some researchers observed no improvements in LBR if oocyte number exceeded 15 or 18 [8, 9]. A large retrospective cohort study conducted in the USA also indicated that retrieval of more than 15 oocytes resulted in a significantly increased risk of OHSS without enhancing LBR [10]. More recently, Ji et al. [11] found that in the first IVF treatment cycle of women under 34 years of age, the highest LBR obtained in fresh cycle was in the group with 6–15 oocytes, and the CLBR increased with ovarian responses. Meanwhile, another study also showed that a higher number of oocytes were associated with an increased incidence of low birth weight [12]. Besides, the quality of oocytes available for fertilization reduced when excess eggs were retrieved, resulting in a lower efficiency of oocytes [13, 14]. Therefore, it is essential to confirm the optimum number of oocytes to balance the relationship between oocyte number and the potential risks, especially for young women with a good prognosis. In this study, the role of oocyte number in CPR, LBR per transfer cycle, high risk rate of OHSS, CCPR, and CLBR per retrieval cycle in young women with normal ovarian reserve was investigated, as well as the CPR per transfer cycle between fresh and frozen-thawed embryo transfer (FET) cycle based on different amounts of yielded oocytes. To the best of our knowledge, this is the first study that divided patients into groups with much smaller class intervals and enrolled only young women with normal ovarian reserve undergoing the first IVF long protocol starting from the luteal phase. Moreover, this is a pilot study aiming to explore the difference between fresh and FET cycle when ≥ 16 oocytes are retrieved.

Materials and methods

Patients

This research was a retrospective cohort study based on the medical records of patients undergoing a long protocol of IVF treatment at Reproductive Medical Center of Henan Provincial People's Hospital, China, from January 2010 to June 2014. Only the first IVF cycle of the patients was analyzed. The inclusion criteria for this study were age less than 35 years; normal ovarian reserve: antral follicle count (AFC) >6 , basal follicle stimulation hormone (bFSH) <10 IU/l, and basal estradiol (bE_2) <50 pg/ml and with normal menstrual cycle; and ≥ 4 retrieved oocytes in order to eliminate poor ovarian response. Patients with polycystic ovarian syndrome, uterine malformation, intrauterine adhesion, endometriosis, blastocyst culture, history of tuberculosis or pelvic operation, and those whose endometrial thickness was less than 7 mm on the day of human chorionic gonadotropin (hCG) test were excluded from this study. Patient characteristics, including age, duration of infertility, bFSH, and bE_2 , were evaluated. Other parameters were also recorded, such as gonadotropin (Gn) doses, endometrial thickness, serum E_2 and progesterone (P) level on hCG test day, fertilization rate, cancellation rate due to no available embryos, and number of available embryos. Additionally, pregnancy outcomes and the high risk rate of OHSS were calculated in each group. This study was reviewed and approved by the Reproductive Medical Ethics Committee of Henan Provincial People's Hospital, since this was a retrospective analysis of conventionally treated patients. The data was anonymous, and no patient-identifying information was included.

IVF and fresh embryo transfer

The standard long protocol was performed in 1567 patients. From mid-luteal phase, 0.1 mg/day Gn-releasing hormone agonist was continuously injected for 14–16 days. Gn was initiated when the down-regulation criteria were satisfied (E_2 <50 pg/ml, P <1 ng/ml, endometrial thickness <5 mm). The initial dose ranged from 112.5 to 300 IU per day. During stimulation, Gn doses were adjusted by monitoring the levels of serum luteinizing hormone, E_2 , and P and by transvaginal ultrasound. When more than three follicles reached a diameter of 18 mm or more, 4000–10,000 IU of hCG was administered. Oocytes were retrieved 36–37 h later, under ultrasonic guidance. Evidence of fertilization was assessed 16–18 h after insemination. Day 3 embryos were evaluated on a scale of one to four grades through morphology: grade 1, even sized, symmetrical blastomeres with fragmentation <10 %; grade 2, uneven sized blastomeres, 10–20 % cytoplasmic fragmentation; grade 3, obvious uneven sized blastomeres, 21–50 % cytoplasmic fragmentation; and

grade 4, >50 % cytoplasmic fragmentation [15]. Available embryos were defined as grade 1 or grade 2 ones and only available embryos were transferred or cryopreserved. Typically, transfer was performed on day 3 under ultrasound guidance, and the number of embryos transferred depended on the conditions and requests of patients; however, no more than two embryos were permitted per patient. Luteal support was supplemented from the day of oocyte retrieval with 90 mg daily of intravaginal progesterone gel (Crinone gel 8 %, Serono), and this was continued for at least 8 weeks or stopped if the hCG test was negative. For some patients, all the embryos were cryopreserved for subsequent transfer because of the presence of high risk of OHSS [16], high level of P, or uterine cavity effusion.

Definition and management for high risk of OHSS

The criteria for the high risk of OHSS were in line with one of the following items: (1) number of oocytes retrieved ≥ 25 ; (2) E₂ level ≥ 6000 pg/ml on the day of hCG administration; (3) ovary diameter ≥ 10 cm on one or both sides on day 3 after oocyte retrieval; and (4) puncture follicle number ≥ 30 (follicular diameter ≥ 14 mm on oocyte retrieval day). Excepting for freezing all the embryos, patients with high risk of OHSS were also given hydroxyethyl starch and albumin for plasma expander, as well as 7.5 mg aromatase inhibitor for 5 days to prevent early-onset OHSS starting from oocyte retrieval.

Frozen-thawed embryo transfer

Endometrial preparation for frozen-thawed cycles included natural cycle, exogenous steroid replacement cycle, and mild stimulation cycle. In the replacement cycle, E₂ was administered at a dose of 6–8 mg per day from day 2 or 3 of menstruation, and the dose was modified according to endometrial thickness and morphology. Micronized natural progesterone (Utrogestan, Laboratoires Besins International SA, France) was administered at a dose of 600 mg per day when the endometrial thickness reached 8 mm, approximately on cycle day 15. Embryos were transferred on the fourth day after Utrogestan was administered. In all types of FET cycles, the luteal phase support was given by vagina with 90 mg daily of progesterone gel or 200 mg micronized natural progesterone three times a day, and this was continued for at least 8 weeks or stopped if the hCG test was negative. Clinical pregnancy was defined by ultrasound scan of an intrauterine gestational sac after 4–6 weeks of embryo transfer. Live birth was defined as one or more live babies delivered beyond 28 weeks of gestation. Cumulative outcomes were calculated with one complete long protocol treatment round of a fresh transfer cycle and subsequent frozen transfer cycles.

Statistical analysis

Frequencies and proportions were reported for categorical outcomes, and means and standard deviations were reported for continuous outcomes. Continuous data with abnormal distribution were presented as median (min-max) plus to means and standard deviations. Normal distribution of continuous variables was examined using one-sample Kolmogorov-Smirnov test. One-way ANOVA, Kruskal-Wallis tests, or χ^2 tests were used to ascertain the significant differences among groups, as appropriate. Unconditional multivariate logistic regression analysis was conducted to identify independent correlations between oocyte number and CLBR after controlling for other confounders. $P < 0.05$ was considered as statistically significant. Analyses were performed using the Statistical Package for Social Sciences version 19.0 (SPSS Inc, Chicago, IL, USA).

Results

Data regarding the selected process including the number of cycles and the reasons for their inclusion or exclusion is provided in Fig. 1. A total of 1567 patients with 8246 initial cycles were eligible for analysis. The distribution of retrieved oocyte number is presented in Fig. 2. Sixteen or more oocytes were retrieved for the majority of patients, accounting for 38.2 % of the cohort. The major characteristics of eligible patients for this cohort analysis are shown in Table 1. Women who retrieved more oocytes had lower serum bFSH level and amount of total Gn dosage, more AFC, and available embryos ($P < 0.05$). There were no statistically significant differences in age, duration of infertility, body mass index (BMI), bE₂, endometrial thickness on hCG test day, fertilization rate, cleavage rate, cancellation rate due to no available embryos, and number of fresh embryos transferred among the five groups. However, the serum level of E₂ and P on hCG test day increased in accordance with the increase in oocyte number because of the development of multiple follicles.

Relationship between number of oocytes retrieved and clinical outcomes in the five groups

Of 1567 eligible cycles, 902 were transferred in a fresh cycle, 26 were discarded because there are no available embryos. And 639 were frozen all the embryos, of which 598 accounting for high risk of OHSS. There were a total of 1782 FET cycles. In line with the increase in oocyte number, the CCPR and CLBR per retrieval cycle steadily increased (Fig. 3). When up to 10–12 oocytes were retrieved, both CCPR and CLBR significantly increased compared to the case when 4–6 oocytes were retrieved. Meanwhile, the CLBR as well as CCPR in the group of women in which more than 16 oocytes

Fig. 1 Data regarding the selected process including the numbers of cycles and the reasons for their inclusion or exclusion

were retrieved were the highest ($P<0.001$, Fig. 3); however, when the oocyte number exceeded 16, the cryopreservation rate for all embryos owing to high risk of OHSS was also the highest ($P<0.001$, Fig. 4). Cases of OHSS were clarified according to previously published criteria from Golan et al. [17].

Fig. 2 Distribution of the number of oocytes retrieved

The results showed that moderate-severe OHSS incidence rates of the five groups were very low [0(0/101), 0(0/239), 1.25(4/321), 0.65(2/307), 1.67(10/599), respectively], and the difference did not reach statistical significance. In fresh cycles, the group of women in which 10~12 oocytes were retrieved had the highest implantation rate, CPR, and LBR; however, no significant differences were found among the groups ($P=0.241$, $P=0.114$, $P=0.300$, respectively) (Fig. 4). The same patterns were also observed in the rates of first-trimester miscarriages (3.51, 7.97, 8.67, 4.93, and 6.33 % ($P=0.595$), respectively), and multiple pregnancies (19.95, 26.17, 29.85, 29.90, and 27.87 % ($P=0.308$), respectively).

The results of CLBR per retrieval cycle were subjected to unconditional multivariate logistic regression analysis to address the confounding factors (Table 2). The group in which 4~6 oocytes were retrieved was used as a reference. When controlling for age, duration of infertility, BMI, bFSH, bE₂, and AFC, we found that the odds ratio (OR) for CLBR increased steadily. Compared with the reference and the group in which 7~9

Table 1 Characteristics of the cohort ($N=1567$)

Group	4~6 ($n=101$)	7~9 ($n=239$)	10~12 ($n=321$)	13~15 ($n=307$)	≥ 16 ($n=599$)	<i>P</i> value
Age (years)	29.73 $\pm$ 3.51 30 (21–35)	29.47 $\pm$ 3.58 29 (21–35)	29.37 $\pm$ 3.48 30 (21–35)	29.32 $\pm$ 3.64 30 (20–35)	28.93 $\pm$ 3.56 29 (20–35)	NS
Duration of infertility (years)	4.05 $\pm$ 3.03 3 (1–13)	3.81 $\pm$ 2.61 3 (1–14)	3.84 $\pm$ 2.76 3 (1–13)	3.84 $\pm$ 2.60 3 (1–13)	3.74 $\pm$ 2.48 3 (1–14)	NS
BMI (kg/m^2)	23.81 $\pm$ 3.49 23.73 (18.03–33.33)	22.99 $\pm$ 3.07 22.85	22.77 $\pm$ 2.94 22.66	22.35 $\pm$ 2.81 22.59	22.73 $\pm$ 2.98 22.04	NS
bFSH (IU/l)	7.25 $\pm$ 1.51	(17.68–34.29) 7.14 $\pm$ 1.35	(17.29–32.03) 7.00 $\pm$ 1.42	(16.80–34.48) 6.90 $\pm$ 1.33	(16.98–33.91) 6.73 $\pm$ 1.36	<0.05
bE ₂ (pg/ml)	31.69 $\pm$ 11.09	31.65 $\pm$ 10.37	31.75 $\pm$ 10.25	31.23 $\pm$ 9.56	32.09 $\pm$ 9.83	NS
AFC	10.89 $\pm$ 3.51 10 (7–20)	11.51 $\pm$ 4.10 10 (7–32)	11.68 $\pm$ 3.92 11 (7–24)	12.31 $\pm$ 4.23 11 (7–24)	13.47 $\pm$ 4.57 12 (7–28)	<0.05
Total dose of Gn (IU)	2364.05 $\pm$ 699.35 2200 (1050–5775)	2100.71 $\pm$ 780.66 1950 (850–4900)	2031.97 $\pm$ 754.84 1950 (525–4200)	1942.91 $\pm$ 696.42 1800 (850–4950)	1841.58 $\pm$ 727.07 1650 (750–5137.50)	<0.05
Endometrial thickness on hCG day (mm)	11.14 $\pm$ 2.81 11.00 (6.00–18.00)	11.34 $\pm$ 2.76 11.00	11.19 $\pm$ 2.71 11.00	11.35 $\pm$ 2.89 11.00	11.15 $\pm$ 2.75 11.00	NS
E ₂ on hCG day (pg/ml)	2309.45 $\pm$ 3249.96 1862.00 (606.00–4300.00)	(6.20–22.000) 2366.54 $\pm$ 1038.77 2308.00	(3.00–21.00) 3402.77 $\pm$ 2842.96 3121.00	(6.00–20.00) 3945.18 $\pm$ 1576.65 3823.00	(5.80–21.00) 5454.54 $\pm$ 3383.84 4500.00	<0.05
P on hCG day (ng/ml)	0.89 $\pm$ 0.53 0.76 (0.20–4.87)	(570.10–6204.00) 0.89 $\pm$ 0.31 0.84 (0.13–1.80)	(643.10–8600.00) 0.97 $\pm$ 0.36 0.93 (0.20–2.32)	(738.60–9248.00) 1.04 $\pm$ 0.38 1.00 (0.12–2.54)	(754.00–9138.00) 1.16 $\pm$ 0.47 1.10 (0.17–6.13)	<0.05
Fertilization rate (%)	62.80 (341/543)	59.68 (1150/1927)	59.50 (2095/3531)	59.33 (2543/4286)	58.60 (7384/1260)	NS
Cleavage rate (%)	95.60 (326/341)	95.48 (1098/1150)	96.13 (2014/2095)	96.30 (2449/2543)	95.53 (7054/7384)	NS
No. of embryos available	2.53 $\pm$ 1.21 2 (0–6)	3.63 $\pm$ 1.63 4 (0–8)	4.77 $\pm$ 2.17 5 (0–11)	5.74 $\pm$ 2.53 6 (0–13)	7.77 $\pm$ 3.77 7 (0–24)	<0.05
No. of fresh embryos transferred	1.86 $\pm$ 0.38 2 (1–2)	1.93 $\pm$ 0.27 2 (1–2)	1.97 $\pm$ 0.27 2 (1–2)	1.96 $\pm$ 0.20 2 (1–2)	1.94 $\pm$ 0.24 2 (1–2)	NS

The major characteristics of eligible patients for this cohort analysis

BMI body mass index, *FSH* follicle stimulation hormone, *E2* estradiol, *AFC* antral follicle count, *Gn* gonadotropin, *hCG* human chorionic gonadotropin, *P* progesterone

Fig. 3 **a** CCPR in five groups with different retrieved oocytes. Patients who did not become pregnant but still had frozen embryos remaining were included in our study but excluded when calculating the CCPR. CCPR cumulative clinical pregnancy rate; α indicates $P < 0.05$, compared with 4–6 and 7–9 oocyte groups. **b** CLBR in five groups

with different retrieved oocytes. Patients who did not give live birth but still had frozen embryos or had already been pregnant but not delivered by the data collection date were also included in our study, but were excluded when calculating the CLBR. CLBR cumulative live birth rate; α indicates $P < 0.05$, compared with the 4–6 and 7–9 oocyte groups

oocytes were retrieved, groups with 10–12, 13–15, and ≥ 16 oocytes retrieved demonstrated a higher chance of CLBR (OR 2.203 [95 % CI 1.255–3.869], 2.526 [95 % CI 1.428–4.470], 2.842 [95 % CI 1.665–4.851], respectively; both P values < 0.001). The BMI also had a significant influence on CLBR (OR 0.928, 95 % CI 0.885–0.972). However, for age, duration of infertility, bFSH, bE₂, and AFC, the effect was not significant.

Comparison between CPR of fresh and first FET cycle after embryo cryopreservation

In order to compare the influence of the supraphysiological concentrations of estrogen and progesterone on the

endometrium resulting from the development of multiple follicles, CPR per transfer cycle of fresh and the first FET cycle after the cryopreservation of all embryos was compared. One or two of the available embryos were transferred from both the groups. It was observed that the CPR was much higher when oocyte number exceeded 16 (Fig. 5), which may be attributed to the better endometrial receptivity.

Discussion

Live birth is the principal clinical outcome and ultimate goal of IVF treatment, while CLBR is an important parameter to evaluate the efficiency of IVF. The quality and quantity of transferred embryos are the two most important factors to predict the cumulative outcomes in IVF cycles [18]. However, normally, only one oocyte has the chance to be mature and ovulated in a natural cycle. COS, which can recruit adequate follicles, is utilized in order to obtain sufficient number of high-quality oocytes and embryos in ART. However, this treatment is expensive and also increases the risk of associated complications such as OHSS. Up to now, there is still no consensus on optimum oocyte number of long protocol, the most commonly used protocol. Fewer oocytes result in fewer available embryos, and ultimately poor pregnancy outcomes. Saldeen et al. found that CPR was only 14 % and cancellation rate due to no available embryos was as high as 40 % of young women with no more than 5 oocytes [19]. However, too many oocytes are always accompanied with complications, financial burden, and wastage of oocytes. Therefore, retrieving appropriate number of oocytes is central to ovarian stimulation. This retrospective study was designed to explore the relationship between CLBR and the number of oocytes retrieved, in order to

Table 2 Multivariable analysis for cumulative live birth

Variables	OR (95 % CI)	P value
Age (years)	0.989 (0.949–1.031)	0.601 ^a
Duration of infertility (years)	0.984 (0.933–1.038)	0.554 ^a
BMI (kg/m ²)	0.928 (0.885–0.972)	0.002 ^a
bFSH	1.089 (0.983–1.205)	0.102 ^a
bE ₂	0.990 (0.977–1.004)	0.166 ^a
AFC	1.007 (0.974–1.040)	0.702 ^a
Oocytes retrieved		< 0.001 ^a
4–6	1	
7–9	1.335 (0.752–2.370)	0.323 ^b
10–12	2.203 (1.255–3.869)	0.006 ^b
13–15	2.526 (1.428–4.470)	0.001 ^b
≥ 16	2.842 (1.665–4.851)	< 0.001 ^b

BMI body mass index, FSH follicle stimulation hormone, E₂ estradiol, AFC antral follicle count

^a P value of each variable's overall effects after adjusting for the other variables

^b P value between each variable's subgroups and reference group

Fig. 4 Percentage of implantation rate, CPR, LBR, and high risk of OHSS in the five groups with different retrieved oocytes. All rates are for fresh cycles. *CPR* clinical pregnancy rate; *LBR* live birth rate; α indicates $P < 0.05$, compared with the 4~6 and 7~9 oocyte groups; $\alpha\beta$ indicates $P < 0.05$, compared with the 4~6, 7~9, and 10~12 oocyte groups; $\alpha\beta\gamma$ indicates $P < 0.05$, compared with the 4~6, 7~9, 10~12, and 13~15 oocyte groups

determine the optimum oocyte number for achieving the maximum clinical outcomes.

Our study suggests that in a fresh IVF cycle, the optimal number of retrieved oocytes is between 10 and 12 in order to obtain maximum CPR and LBR, and to balance the high risk of OHSS. As the clinical outcomes in our study indicated, in fresh cycles, 10~12 oocytes group demonstrated the highest implantation rate (53.32 %), CPR (73.13 %), and LBR (61.14 %), with no significant differences (Fig. 4). Moreover, both CCPR and CLBR became significantly higher in 10~12 oocytes group, compared with 4~6 and 7~9 groups

(Fig. 3). However, when the retrieved oocytes were increased to 13~15 or ≥ 16 , the cumulative results did not have a significant increase (Fig. 3). At the same time, the high risk rate of OHSS was 11.53 % with 10~12 oocytes retrieved, which was dramatically lower than that of 13~15 oocyte category (29.97 %) and ≥ 16 oocyte category (77.30 %). With the influence of confounders such as age, duration of infertility, BMI, bFSH, bE₂, and AFC controlled in multivariable logistic regression analysis, a strong relationship between oocyte number and CLBR was found in an IVF cycle including both the fresh and frozen-thawed transfers. The results indicated that CLBR was significantly higher when oocyte number was up to 10~12 (OR 2.203 [95 % CI 1.255–3.869]). The results were generally consistent with the previous study of Stanger et al., which from a global view presented the interaction between oocyte number and outcomes [4].

OHSS is a serious and potentially life-threatening iatrogenic complication caused by COS, characterized by an excessive discharge of body fluid from the blood vessels which may lead to pleural fluid, ascites, and even mortality. At present time, the pathogenesis remains unknown and there are no effective treatments for moderate-severe OHSS, so prevention might be a primary strategy [20]. The strong relationship between the high risk of OHSS and the number of oocytes retrieved has already been validated [10]. Papanikolaou et al. reported that the number of follicles ≥ 11 mm in diameter was an effective predictor; the sensitivity was 85.5 % and specificity for OHSS risk was 69 % when more than 13 follicles were measured before oocyte retrieval [21]. It was observed in our study that the high risk rate for OHSS and the accompanied cancellation rate could reach as high as 77.30 % in

Fig. 5 CPR of fresh and first FET cycle after “all embryo” cryopreservation in five groups with different retrieved oocytes. *CPR* clinical pregnancy rate, *FET* frozen-thawed embryo transfer; ϵ indicates $P < 0.05$ when compared to CPR of fresh cycle and first FET cycle in the ≥ 16 oocyte group

the ≥ 16 oocyte category. Meanwhile, a decrease of CPR and LBR in fresh cycle was observed in patients in whom more than 13 oocytes were retrieved in our study, though with no significant differences.

Moreover, some previous studies suggested that too many oocytes accompanied with supraphysiological concentrations of steroid hormone might compromise pregnancy outcomes [22–24]. Our research showed that in the group of women in which 16 or more oocytes were retrieved, the CPR of first FET cycles after freezing all embryos was significantly higher than that of fresh cycles, suggesting endometrial damage. The results indicated that when ≥ 16 oocytes were retrieved, a “freeze-all” embryo strategy might be preferable with low OHSS risk and higher CPR. Also, the comparable rates of first-trimester miscarriage between the groups suggested that too many oocytes deleteriously influenced endometrial receptivity, but the quality of the transferred embryos was not affected. The reasons for failure of a successful pregnancy with ≥ 16 retrieved oocytes are still unclear; however, some possible theories are as follows. First, a greater proportion of immature oocytes from high responders may reduce the fertilization rate without impairing ongoing implantation rates [25]. Second, the supraphysiological concentrations of serum E_2 and P level impose deleterious effects on the endometrial receptivity, consequently affecting embryo implantation [23–25]. Finally, the recruited oocytes are generated from the oocytes that have been selected for atresia in a natural situation.

In summary, this study showed a strong relationship between oocyte number and the CLBR in young women with good ovarian reserve who were undergoing IVF treatment. Regulation of the number of oocytes retrieved is the key step to balance the clinical outcomes and high risks of OHSS. The optimal oocyte number to achieve the best chance of live birth in both fresh and cumulative cycles is between 10 and 12. Too many eggs could not increase clinical outcomes. With more than 16 oocytes, in consideration of the influence on endometrial receptivity of fresh cycles, a freeze-all embryo strategy might be preferable. Overstimulation of ovaries resulting in high oocyte numbers may give rise to increased costs and ethical dilemmas about the residual embryos. Less aggressive stimulation protocols need to be reconsidered, especially in high responders. This study aims to advocate more effective and gentler ovarian stimulation protocols for IVF treatment to obtain optimal number of oocytes and pregnancy outcomes. However, all patients in our study were from only one reproductive medical center, and the sample size was not big enough to reach a reliable conclusion. Further prospective analyses and multicenter studies with larger sample sizes are warranted.

Abbreviations

IVF-ET	In vitro fertilization and embryo transfer
LBR	Live birth rate
CLBR	Cumulative live birth rate
OHSS	Ovarian hyperstimulation syndrome
CPR	Clinical pregnancy rate
CCPR	Cumulative clinical pregnancy rate
FET	Frozen-thawed embryo transfer
COS	Controlled ovarian stimulation
Gn	Gonadotropin
AFC	Antral follicle count
BMI	Body mass index
E_2	Estradiol
hCG	Human chorionic gonadotropin
P	Progesterone
OR	Odds ratio

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Conflict of interest The authors declare that they have no competing interests.

Author contributions All authors made substantial contributions to the conception and design of this research study. C-yH and X-xH acquired and analyzed the data and wrote the manuscript. QW collected the data. Z-sD, J-lL, and G-zJ critically revised the manuscript. Z-cL finally approved the manuscript. All authors read and approved the final manuscript.

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