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ASSISTED REPRODUCTION TECHNOLOGIES

Cumulative live birth and surplus embryo incidence after frozen-thaw cycles in PCOS: how many oocytes do we need?

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Abstract
Purpose This study aimed to evaluate the cumulative live birth rate (CLBR) and surplus embryo rate of polycystic ovarian syndrome (PCOS) patients during in vitro fertilization and embryo transfer (IVF-ET) treatment.
Methods In this retrospective cohort study, we analyzed 1142 PCOS patients who underwent first IVF in our institution between January 2011 and December 2014. All patients were categorized into five groups according to the number of oocytes retrieved. Main outcomes include CLBR and surplus embryo rate.
Results A strong correlation was observed between number of oocytes retrieved and CLBR as well as surplus embryo rate in PCOS patients. CLBR was elevated with the increasing number of oocytes and plateaued when oocyte number was up to ten, whereas the surplus embryo rate steadily increased in line with the increase of oocyte number. Patients transferred with frozen embryos showed higher CLBR and LBR during first ET than patients transferred with fresh embryos.
Conclusions For PCOS patients, retrieving more than ten oocytes leads to no significant benefit to CLBR but generates surplus embryos. Thus, moderate ovarian stimulation should be reconsidered during IVF treatment.

Keywords Cumulative live birth rate · Surplus embryo rate · Polycystic ovarian syndrome

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Introduction

Since the first in vitro fertilization (IVF) baby was born in 1978, assisted reproductive technology has provided great hope for infertile couples. After that, some subsequent breakthroughs led to several innovations and improvements including controlled ovarian stimulation (COS). An individualized COS protocol is the key component that shifts clinical practice from natural mono-follicle to multi-follicle stimulated, which may ensure consequent number of oocytes retrieved, an increased number of available embryos, and ultimately a desirable cumulative live birth rate (CLBR).

The ultimate goal of IVF is a healthy baby along with the safety of mother. As an advancement of IVF, COS with exogenous gonadotropin has significantly improved the yield of oocytes in one retrieval cycle. However, it is not plausible for each oocyte to fertilize normally and develop into an available embryo, and finally to a live birth. Previous studies showed that only a small portion of embryos transferred were capable of transforming into live births [1, 2]. Another study indicated that the overall oocyte to live baby born rate was

only 4.6% [3]. While the wastage of oocytes during transfer gained more attention, rare concern was showed for the surplus embryos after birth of a live baby. In IVF, both clinicians and patients desire to obtain sufficient number of oocytes, as a result of obtaining more embryos and improving success rate. However, the number of embryos in storage around the world is steadily increasing in recent years and several reproductive centers worldwide are filled with surplus frozen embryos, which may cause considerable wastage and ethical issues [4]. Previously, researchers have advocated less aggressive protocols of ovarian stimulation for appropriate oocytes and embryos [5, 6]; and despite various innovations over the years, this value has not been appropriately implemented.

Patients with PCOS are more inclined to freeze the surplus embryos for rich ovarian reserve. PCOS is an endocrine disorder, characterized by hyperandrogenism, oligo-ovulation or anovulation, and insulin resistance. A large-scale epidemiological study from ten provinces and municipalities in China found that the prevalence of PCOS in Chinese women of reproductive age was 5.6% [7]. PCOS patients usually harbor abundant antral follicles, which would lead to retrieval of a large number of oocytes, and eventually freeze of surplus embryos. Therefore, investigating the relationship between retrieved oocyte number and clinical results including cumulative live birth and the surplus embryo percentage is essential.

The aim of this current study is to evaluate the CLBR and surplus embryo rate after one IVF cycle including all fresh and subsequent frozen-thaw embryos in PCOS women. To the best of our knowledge, this is the first study focusing on frozen surplus embryos after live baby is born and advocating less aggressive ovarian stimulation from a perspective apart from complications.

Materials and methods

This retrospective, cohort study was performed in Henan Provincial People's Hospital, China. The protocol was reviewed and approved by the Reproductive Medical Ethics Committee of Henan Provincial People's Hospital.

Patients

This study included a total of 1142 PCOS patients who underwent first IVF/intracytoplasmic sperm injection (ICSI) treatment at a single institution, between January 2011 and December 2014. PCOS was diagnosed according to the Rotterdam criteria, i.e., the presence of ≥2 criteria after excluding other diseases such as congenital adrenal hyperplasia, Cushing syndrome, and androgen-secreting tumors: (1) oligo-ovulation or anovulation, (2) clinical manifestations of androgen excess and/or hyperandrogenism, and (3) ultrasonography showing polycystic ovary (unilateral ovary or bilateral ovaries have >12 ovarian follicles, 2–9 mm in diameter, and/or ovarian volume >10 mL) [8].

Patients were excluded from this study if they did not perform the oocyte pickup for some certain reasons. Patients with unclear data were also excluded. No patients with oocyte donation, oocyte freezing, preimplantation genetic diagnosis, or preimplantation genetic screening were included. In addition, women who met the above mentioned criteria but still had frozen embryos remaining without delivery a live baby by collection date (November 2015) were excluded in order to minimize the risk of misclassification bias (Fig. 1). We included only women who either delivered a baby or used all their embryos after their first stimulated cycle. To evaluate the impact of oocytes retrieved, all the included patients were categorized into five groups according to oocyte number: 1–3, 4–9, 10–15, 16–20, and ≥21. The specific categorization was based on previous consensus studies and recent evidences [9–11].

COS protocol

All patients were subjected to a standard GnRH agonist protocol, wherein daily injection of triptorelin (0.1 mg) was administered from the mid-luteal phase. The desensitization was verified 14–18 days after initiation of GnRH agonists. Daily injection of recombinant FSH (rFSH) was started only if the estradiol levels were <50 pg/mL, progesterone <1 ng/mL, endometrial thickness < 5 mm, and in the absence of a functional ovarian cyst. The initial dose varied between 75 and 225 IU/day according to age, body mass index (BMI), and ovarian reserve. During stimulation, the gonadotropin doses were adjusted based on monitoring levels of serum luteinizing hormone (LH), estradiol, and progesterone, and transvaginal ultrasound examinations. Ovarian triggering was performed with the administration of 4000–10,000 IU human chorionic gonadotropin (hCG) based on clinicians' discretion as soon as three leading follicles reached a diameter of ≥18 mm. The oocyte retrieval was performed by transvaginal ultrasound-guided needle aspiration, 36–37 h after hCG injection.

IVF and embryo culture

IVF or ICSI was carried out 39–40 h after hCG injection, and different fertilization methods were performed depending on sperm parameters. The evidence of fertilization was assessed 16–18 h after insemination, and appearance of two pronuclei indicated normal fertilization.

Embryo morphology was observed 48 (day 2) and 72 h (day 3) after oocyte retrieval. The grading criteria for the cleavage embryos were as follows: 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%

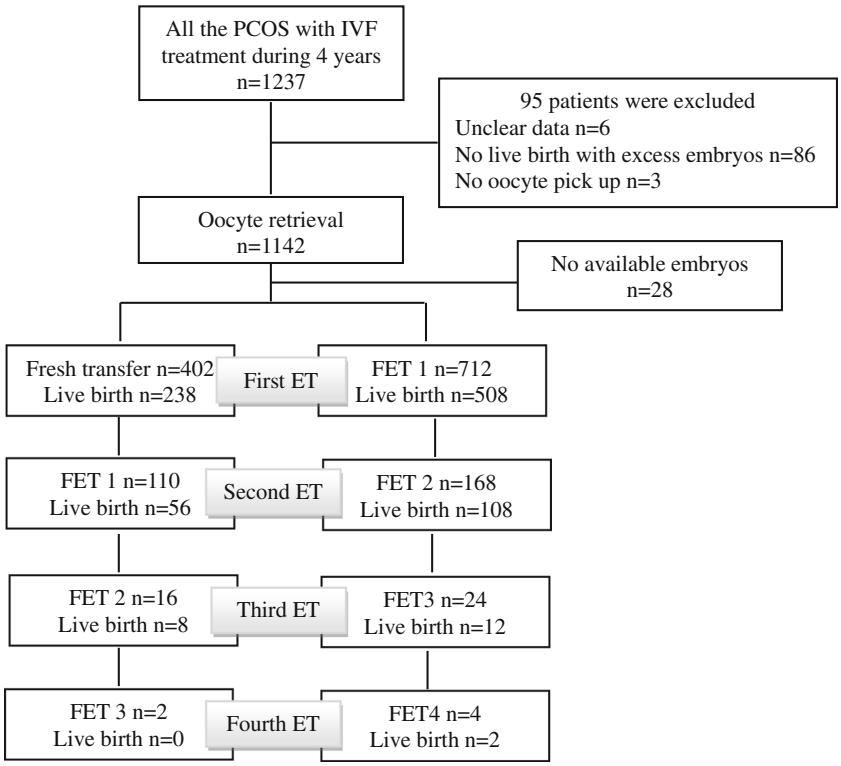

Fig. 1 Trial flow chart. An overview of the patient selection and the overall reproductive comes of all included oocyte retrieval cycle

cytoplasmic fragmentation; and grade 4, >50% cytoplasmic fragmentation [12]. Available embryos were defined as with more than four cells and grade 1 or 2 3 days after oocyte retrieval. Both transferred and frozen embryos met the criteria of available embryos.

In the case of at least six cleavage embryos, blastocyst culture was performed according to the patients' opinion. The blastocysts were observed on the fifth and sixth day after oocyte retrieval, and each blastocyst was evaluated by the Gardner grading system, based on the degree of cyst expansion [13]. The blastocysts with grade ≥3BB were transferred or frozen. Furthermore, a "freeze-all" policy was performed in case of risk for ovarian hyperstimulation syndrome (OHSS) (definition and management for high risk of OHSS were the same as previously published article) [14].

Embryo transfer and freezing

In fresh cycles, one or two available cleavage embryos were transferred on the 3rd day after oocyte retrieval and 1–2 blastocysts of grade ≥3BB were transferred on the 5th day after oocyte retrieval. Luteal support, with 90 mg daily of intravaginal progesterone gel (Crinone gel 8, Serono, Geneva, Switzerland), was supplemented from the day of oocyte retrieval and was continued for at least 8 weeks. The administration was ceased if the hCG test was negative.

Vitrification of human embryo freezing was performed using a Cryotop device. The endometrial preparation for frozen-thawed cycles included natural cycle, exogenous steroid replacement cycle, and mild stimulation cycle. In the replacement cycle, estradiol was administered at a dosage of 6–8 mg/day, beginning from day 2 or 3 of menstruation, and the dose was modified based on endometrial thickness and morphology. Micronized natural progesterone of 600 mg/day (Utrogestan, Laboratoires Besins International SA, France) or intravaginal progesterone gel of 90 mg/day was administered when the endometrial thickness reached 8 mm, approximately on the 12th day of the cycle. The cleavage embryos were transferred on the fourth day after progesterone was administered, and blastocysts were transferred 2 days later. In all types of frozen-thawed embryo transfer cycles, the luteal phase support was administered vaginally with 600-mg/day micronized natural progesterone or 90-mg/day progesterone gel. This regimen was continued for a minimum of 8 weeks or stopped if the hCG test was negative.

Definition of outcomes

Clinical pregnancy was defined by ultrasound scan of at least one gestational sac exhibiting fetal heart activity after 4–6 weeks of embryo transfer. Live birth was defined as one or more live babies delivered beyond 28 weeks of gestation. The cumulative live birth was calculated with one whole protocol treatment round of a fresh transfer cycle and subsequent frozen transfer cycles. The surplus embryo rate was defined as

the ratio between number of surplus embryos after delivery and number of total available embryos.

Statistical analysis

The statistical analysis was performed using the Statistical Package for Social Sciences version 17.0 (SPSS Inc., Chicago, IL, USA). Continuous variables were presented as mean ± SD and compared by analysis of variance. On the other hand, categorical variables, such as live birth, were presented as frequencies and percentages and compared using one-way ANOVA test. *P* values of <0.05 were considered as statistically significant.

Results

Overall, a total of 1142 PCOS patients with first IVF/ICSI treatment were eligible for analysis.

Patients' characteristics

Baseline characteristics were comparable with respect to age, duration of infertility, BMI, basal FSH, basal LH, and antral follicle number of the five groups (Table 1). No significant difference was observed for duration of ovarian stimulation, total doses of gonadotropin, insemination method, fertilization rate, moderate-severe OHSS rate, and the average transfer times (Table 1). The estradiol level and progesterone level on hCG day, cancelation rate with no available embryos, freeze-all embryo rate for high risk of OHSS, and blastocyst percentage showed a significant difference between five groups (Table 1).

CLBR including live births of fresh and frozen cycles and surplus embryo rate

Overall, a total of 1439 transfers were performed, 932 (81.61%) patients achieved a final live birth after one stimulation cycle and 210 (18.39%) patients did not.

As presented in Fig. 2, both of CLBR and surplus embryo rate rose with increasing number of oocytes, while CLBR plateaued at ten oocytes and the surplus embryo rate steadily increased with the number of oocytes retrieved. Besides, CLBR was found to be significantly lower in groups 1–3 and 4–9 than the other three groups ($P < 0.001$). On the other hand, the difference did not reach statistical significance among groups 11–15, 16–20, and ≥21. The surplus embryo rate increased significantly from 0 in 1–3 oocyte group to 69.64% in ≥21 oocyte group ($P < 0.001$). Furthermore, the surplus embryo rate of blastocyst was higher than that of cleavage embryo in different oocyte groups with surplus embryos (Fig. 2).

Overall, 18 out of 1142 patients (1.58%) presented moderate-severe OHSS (Table 1). The classification of OHSS was based on Golan criteria [15]. No significant differences of moderate-severe OHSS rate were found between the five groups ($P = 0.607$).

CLBR and LBR of first transfer of fresh transfer group and freeze-all embryo group

Overall, a total of 740 patients did not have a fresh embryo transfer either because of no available embryos (28 patients) or because of freeze-all embryos (712 patients), of which 660 patients were for high risk of OHSS.

For patients with freeze-all embryos, both CLBR (88.48 vs. 75.12%, $P < 0.001$) and LBR of first ET (71.35 vs.71.35%, $P < 0.001$) were much higher than patients with fresh embryo transfer (Fig. 3).

Discussion

Our study demonstrated a strong relationship between number of oocytes retrieved and CLBR as well as surplus embryo rate in PCOS patients. Concurrently, with the increase of oocyte number, CLBR plateaued at ten oocytes and surplus embryo rate steadily increased to as high as 69.64% when oocyte number exceeded 20. Too many oocytes do not indicate a higher CLBR but rather a higher surplus embryo rate, higher doses of gonadotropin, and longer days of ovarian stimulation. Altogether, only less than half embryos were transferred, and the remaining ones were either frozen for an indefinite period or abandoned, which is definitely a large wastage. Thus, a protocol of less aggressive stimulation for appropriate oocyte number should be considered.

IVF is a major treatment for infertility, with more than five million IVF babies born worldwide [10]. Live birth is the principal clinical outcome and ultimate goal of IVF treatment, while CLBR is a critical parameter of evaluating the efficiency of IVF. Upon the initiation of an IVF cycle, a live birth result without complications is desired. The best approach is to maximize the overall interest, a healthy baby, safe mother, less time, cost-effective, and sufficient embryos. Our findings showed that CLBR could reach as high as 81.91% in group 10–15, and no higher CLBR was observed in groups 16–20 and ≥21. More oocytes showed no benefits for ultimate goal of live baby in PCOS patients.

Furthermore, our study showed that surplus embryo rate steadily increased to as high as 69.64% in line with oocyte number and the surplus embryo rate of blastocyst was higher than that of cleavage embryo. Though with large scale of surplus embryos in storage, there are still no legal regulations or deadlines for destruction of cryopreserved embryos in China at present time, while legislation has stipulated a

Table 1 Baseline characteristics and IVF outcomes of the cohort

	1–3 oocytes $n = 26$	4–9 oocytes $n = 206$	10–15 oocytes $n = 376$	16–20 oocytes $n = 232$	≥21 oocytes $n = 302$	*P* value
Age (years)	27.46 ± 1.90	28.52 ± 4.52	28.18 ± 3.45	27.27 ± 4.03	27.86 ± 3.59	0.132
Duration of infertility (years)	3.67 ± 2.09	4.48 ± 3.06	4.02 ± 2.65	3.68 ± 2.35	3.61 ± 2.04	0.078
BMI (kg/m^2)	25.42 ± 4.01	25.34 ± 3.80	24.77 ± 3.81	23.46 ± 3.54	23.26 ± 3.54	0.059
bFSH (IU/L)	6.51 ± 2.04	5.90 ± 1.37	5.95 ± 1.55	5.85 ± 1.55	5.80 ± 1.34	0.121
bLH (IU/L)	10.21 ± 3.99	9.30 ± 5.03	9.72 ± 5.31	9.74 ± 4.86	10.02 ± 5.57	0.868
AFC	20.0 ± 4.6	20.8 ± 5.1	21.9 ± 6.0	21.2 ± 4.5	21.7 ± 5.2	0.056
Duration of stimulation	14.3 ± 3.1	13.3 ± 2.7	13.7 ± 3.1	13.1 ± 3.1	13.5 ± 3.4	0.441
Total dose of Gn (IU)	2071.2 ± 1049.9	1937.9 ± 772.2	1967.2 ± 828.6	1762.7 ± 926.7	1812.4 ± 889.3	0.193
E_2 on HCG trigger day (pg/mL)	1183.8 ± 523.7	2274.7 ± 1158.9	3370.5 ± 1234.6	4900.5 ± 2031.9	5589.8 ± 2026.7	0.001
P on HCG trigger day (ng/mL)	0.71 ± 0.27	0.75 ± 0.33	0.87 ± 0.32	0.99 ± 0.33	1.17 ± 0.43	0.000
Insemination methods						
IVF	24 (92.31%)	170 (82.52%)	316 (84.04%)	196 (84.48%)	236 (78.15%)	0.139
ICSI	2 (7.69%)	36 (14.56%)	60 (15.96%)	36 (15.52%)	66 (21.85%)	
Fertilization rate (%)	73.08 (38/52)	72.91 (872/1196)	71.73(2888/4026)	71.30 (2524/3540)	73.06 (4810/6584)	0.144
Cancelation rate with no available embryos (%)	7.69(2/26)	6.80(14/206)	1.60(6/376)	2.59(6/236)	0(0/302)	<0.001
Freeze-all embryo rate for high risk of OHSS (%)	0(0/26)	5.83(12/206)	41.49(156/376)	81.36(192/236)	99.34(300/302)	<0.001
Moderate-severe OHSS	0(0/26)	0.97(2/206)	1.06(4/376)	2.12(5/236)	2.32(7/302)	0.607[a]
No. of available embryos						
Blastocyst	0 (0)	24 (3.30%)	400 (20.06%)	652 (44.55%)	1768 (72.34)	<0.001
Cleavage embryo	38 (100%)	704 (96.70%)	1594 (79.94%)	812 (55.46%)	676 (27.66)	
Transfer times	0.92 ± 0.28	1.19 ± 0.60	1.32 ± 0.62	1.31 ± 0.61	1.24 ± 0.55	0.080

[a] Fisher exact test.

storage limit of human embryos in some countries such as Australia [16]. Besides, embryo donation to other couples is prohibited in China; nonetheless, few couples are willing to donate their surplus embryos for scientific research. A previous study of attitudes towards surplus frozen embryos of Chinese people showed that 58.8% of the couples preferred to dispose of surplus embryos rather than donate them for research [4]. Taken together, currently maintaining large number of frozen embryos is quite a burden for IVF centers in China. On the other hand, the high surplus embryo rate might be attributed to the policy of "one family one child" in China. In cases that the couple already had one baby, they could not transfer the remaining embryos. However, the changed "two-child" policy has been implemented from November 2015, and several couples have the chance to transfer the surplus embryos, which may increase the usage of remaining embryos.

In view of both CLBR and surplus embryo rate, mild ovarian stimulation should be taken into consideration for PCOS patients in China. Mild ovarian stimulation in IVF refers to use of low doses of gonadotropin to produce a maximum of ten oocytes [17]. Besides controlling oocyte number, mild

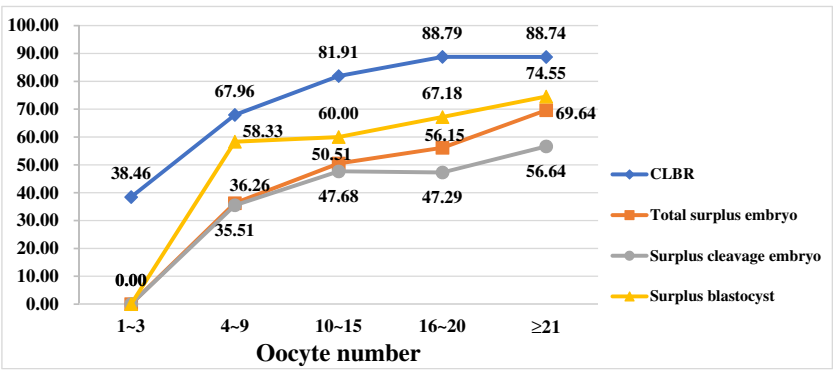

Fig. 2 CLBR and surplus embryo rate with respect to the number of oocytes retrieved

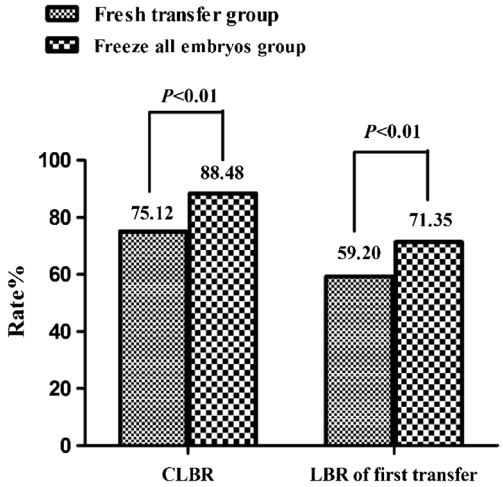

Fig. 3 CLBR and LBR of first transfer between fresh transfer group and freeze-all embryo group

stimulation protocols also could offer patients with the advantages of reducing total cost and shortening time lost from work. Furthermore, it would also lessen number of clinical visits and blood withdrawals [18], which is more "patient-friendly."

However, there are still concerns about CLBR of mild stimulation for a lower number of oocytes and embryos. A randomized study showed that the proportions of cumulative pregnancies resulting in live birth after 1 year were 43.4% with mild treatment and 44.7% with standard treatment, without a significant difference [19]. Mild stimulation seems not to be the optimal treatment protocol for all patients; thus, we speculate it as an option for some certain patients and should not be dismissed due to misconceptions about low success rates.

The results of our study clearly illustrate a significant higher surplus embryo rate, while the CLBR do not increase when oocyte number is ≥10 for PCOS patients. The chance that IVF can produce a healthy baby (or babies) needs to be weighed considering the risk of complications and costs associated with treatment, though the endpoint is always a live baby. The success rates of IVF have increased over the last 30 years; however, the efficacy and safety necessitate further focus. Thus, it is essential to re-investigate the usage of mild stimulation, especially for patients with well-predicted prognosis. Nevertheless, all patients in our study originated from only one reproductive medical center, and the data might not be adequate for a reliable conclusion. Therefore, further prospective analyses and multi-center studies with larger sample sizes are warranted.

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Author contributions All authors made substantial contributions to the conception and design of this research study. The first author analyzed the data and wrote the manuscript. The second author collected the data. The other authors critically revised the manuscript. The corresponding author finally approved the manuscript. All authors read and approved the final manuscript.

Compliance with ethical standards

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

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