

Accumulation of Vitrified Embryos Followed by Frozen Embryo Transfer in Poor Ovarian Responders According to Bologna Criteria

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Abstract

Background: The aim of current study is to compare the pregnancy outcome between natural cycle IVF-ET (NC group) and frozen ET following accumulation of vitrified embryos (ACC-E group).

Methods: The ACC-E group included 38 frozen ETs in 30 patients and the NC group included 92 natural or modified IVF-ET cycles in 91 patients. In the ACC-E group, natural cycle or modified natural cycle with minimal stimulation was used for follicular growth. Embryos were frozen by the vitrification method for several rounds and then thawed embryos were transferred.

Results: Ongoing pregnancy rate (15.8% vs. 8.7%; $p=0.24$; $\Psi=0.1$), and live birth rate (15.8% vs. 6.6%; $p=0.1$; $\Psi=0.14$) were comparable for ACC-E versus NC group, respectively. However, even though the effect size was small, the chemical pregnancy rate of the ACC-E group was significantly higher than that of the NC group (31.6% vs. 11.9%; $p=0.02$; $\Psi=0.23$).

Conclusion: Accumulation of vitrified embryos followed by frozen ET can be considered as a new strategy to improve pregnancy rate in poor ovarian responders.

Keywords: Accumulated embryos; Bologna criteria; Vitrified embryo

Introduction

The incidence of poor ovarian responders (PORs) among women who undergo treatment with assisted reproductive techniques is reported to be 9–24%, and has recently increased slightly [1].

Although the concept of POR was introduced 30 years ago, there was no common consensus for the definition of POR. In July 2011, the ESHRE working group on POR published a common standardized definition of POR. According to the Bologna criteria, the minimal criteria needed to define POR are the presence of at least two of the following three features: (i) advanced maternal age (≥ 40 years) or any other risk factor for POR; (ii) a previous POR (≤ 3 oocytes with a conventional stimulation protocol); and (iii) an abnormal ovarian reserve test showing antral follicle count (AFC) of $< 5-7$ or antimüllerian hormone (AMH) of $< 0.5-1.1$ ng/ml [2].

Various protocols have been tested in attempts to increase pregnancy rates in patients with POR. An increase in the starting dose of follicle stimulating hormone (FSH) does not result in higher pregnancy rates [3]. Conflicting results have been obtained regarding improved pregnancy rates after addition of recombinant luteinizing hormone (LH) [4,5]. Analysis of articles on randomized clinical trials (RCTs) revealed no statistically significant difference in pregnancy rates for short versus long gonadotropin-releasing hormone (GnRH) agonist protocol [6], GnRH antagonist versus long GnRH agonist protocol [7], combination clomiphene citrate (CC) with recombinant FSH versus recombinant FSH alone [8], GnRH antagonist versus short GnRH agonist protocol [9-11]. In a Cochrane Review, addition of growth hormone (GH) to a controlled ovarian stimulation protocol for patients with POR did not yield any benefit in one study [12] whereas GH adjuvant therapy significantly increased live birth rates and pregnancy rates in a meta-analysis [13].

Stringent selection of POR patients based on Bologna criteria revealed a very low ongoing pregnancy rate with the modified natural cycle (0.9% per cycle) [14] and a live birth rate of 2.6% per cycle in

natural cycle IVF [15]. Application of natural or modified natural cycle IVF to poor responders does not provide substantial benefits.

To overcome the low pregnancy rate of natural cycle IVF-ET, our institute accumulated vitrified embryos that were produced by several rounds of natural cycle or modified natural cycle IVF and transfer of embryos after thawing. The aim of this study was to compare the pregnancy outcome between natural cycle IVF-ET (NC) and frozen ET following accumulation of vitrified embryos (ACC-E).

Materials and Methods

This study was approved by the Institutional Review Board at Bundang CHA General Hospital. We reviewed the medical records of all women who underwent thawing ET following accumulation of frozen embryos (ACC-E) and those who underwent a natural or modified natural cycle IVF (NC) program at the CHA Fertility Center (CHA University, Seongnam, Korea) between January 2010 and December 2014.

Among patients sorted into ACC-E and NC groups, we included only POR patients who fulfilled two out of three Bologna criteria. Also, we excluded patients who used a controlled ovarian stimulation protocol other than natural or modified natural cycle IVF. The ACC-E

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group included 38 thawing ET cycles in 30 patients whereas the NC group included 92 fresh ET cycles in 91 patients.

ACC-E and NC protocols

In the ACC-E group, a natural cycle or modified natural cycle with minimal stimulation was used for follicular growth. Antagonist (Cetrotide® 0.25 mg per day; Merck Serono, UK) was injected subcutaneously when a leading follicle reached 14 mm diameter in the modified natural cycle. hCG (Ovidrel®; Merck Serono, Italy) was administered when a leading follicle with a mean diameter ≥ 18 mm was observed on ultrasonography in natural or modified natural cycle. Oocytes were retrieved 35 hours after hCG injection and subsequently fertilized by IVF or intra-cytoplasmic sperm injection (ICSI). Embryos were frozen by the vitrification method described below. Embryos were pre-equilibrated in dPBS (Irvine, CA, USA) +1.5 M ethylene glycol (EG; Sigma; 102466; USA) +20% (v/v) synthetic serum substitute (SSS; Irvine, CA, USA) at 30°C for 2 min 30 sec and equilibrated in dPBS+5.5 M EG+ 1.0 M sucrose (Sigma; S-1888) + 20% (v/v) SSS (Irvine, USA) at 30°C for 20 sec before mounting on electron microscopy (EM) gold grids and immediate submersion in liquid nitrogen at -196°C. For thawing, embryos on EM gold grids were sequentially transferred to thawing solution containing 1.0, 0.5, 0.25, or 0.125 M sucrose in dPBS at 37°C at intervals of 2 min 30 sec. After four to six washes with 20% SSS in dPBS, embryos were placed into culture medium as described previously [16,17]. The thawed embryos were transferred on post-ovulation day 3 without hormonal addition in patients who were having a regular menstrual cycle or on the third day after endometrial thickness ≥ 8 mm was observed with administration of estradiol valerate (Progynova®; Schering, South Korea) in a hormonally manipulated artificial cycle. Micronized progesterone (Utrogestan® 200 mg Vaginal Capsule; Capsugel, France) was inserted into the vagina three times per day beginning on the day of ovulation for subjects in natural cycle. For those in hormonally manipulated artificial cycle, micronized progesterone was commenced when endometrial thickness was ≥ 8 mm.

In the NC group, follicular growth, oocyte retrieval, and fertilization were performed according to the natural or modified natural cycle IVF protocol as described above. Embryos were transferred on post-retrieval day 2 or 3. Micronized progesterone (Utrogestan® 200 mg Vaginal Capsule; Capsugel, France) was inserted into the vagina three times per day beginning on the day of oocyte retrieval for luteal support.

Pregnancy was determined by serum level of β human chorionic gonadotropin (hCG) 13 days after embryo transfer.

For the ACC-E group, the following factors were recorded: number of oocyte retrieval cycles, number of cycle cancelations, number of oocyte retrieval failures, total number of oocytes retrieved, number of oocytes retrieved per ET cycle (\pm SD), total number of oocytes in metaphase II, number of oocytes in metaphase II per ET cycle (\pm SD), number of injected oocytes, total number of frozen embryos, number of frozen embryos per ET cycle (\pm SD), total number of embryos transferred, number of embryos transferred per ET cycle (\pm SD), and fertilization rate (% \pm SD).

To evaluate differences in baseline characteristics of the patients, the following factors were compared between groups ACC-E and NC: age, etiology of infertility, body mass index (BMI), AMH level, antral follicle count (AFC), duration of infertility, and previous IVF-ET cycles. For comparison of pregnancy outcome in groups ACC-E and NC, the following factors were recorded: implantation rate, chemical pregnancy rate, clinical pregnancy rate, ongoing pregnancy rate, ectopic pregnancy rate, miscarriage rate, live birth rate, multiple pregnancy

rate, birth weight (g \pm SD), gestational age at delivery (weeks, \pm SD), and sex ratio. The implantation rate was defined as the number of gestational sacs divided by the number of embryos transferred. Clinical pregnancy was defined as the presence of a gestational sac with fetal heart activity. Ongoing pregnancy rates were determined as a fetus with heart beat at 12 weeks of intrauterine pregnancy.

Data are expressed as mean \pm SD or percentage, as appropriate. Student's *t*-test, χ^2 test, and Fisher's exact test were used to determine statistical significance. A *p* value <0.05 was considered statistically significant. η^2 and Ψ were calculated to measure the effect size. SPSS version 22 was used for statistical analysis.

Results

ACC-E group

In the ACC-E group 38 frozen ET procedures were performed in 30 patients. A total of 154 follicular growth cycles were attempted and 22 cycles (14.3%) were cancelled because of no follicular growth or early ovulation. No oocytes were retrieved in 23 cycles out of 132 oocyte retrieval trials (17.4%). An average of 3.6 ± 2 oocyte aspiration cycles were needed to perform one frozen ET cycle and the mean fertilization rate was 73.6 ± 19.1 . Six oocytes could not be used for fertilization: two oocytes were not arrested at metaphase I stage, one oocyte was arrested at the germinal vesicle (GV) stage, and three oocytes were abnormal. 4.5 ± 2.1 embryos were frozen per frozen ET cycle and 3.6 ± 1.0 embryos were transferred per frozen ET cycle (Table 1).

Comparison between ACC-E group and NC group

In the NC group, 92 natural or modified IVF-ET cycles were performed in 91 patients. The ACC-E and NC groups were not significantly different with respect to etiology of infertility, BMI, AMH, AFC, or previous IVF-ET cycles. However, there was a significant difference in age ($p=0.02$; $\eta^2=0.04$): 37.8 ± 4.7 years in ACC-E group compared with 39.9 ± 4.2 years in NC group but the effect size was extremely small. Duration of infertility was significantly higher in the NC group than in the ACC-E group: 5.9 ± 4.0 in NC group compared with 4.2 ± 3.6 in ACC-E group ($p=0.02$; $\eta^2=0.04$) but the effect size was extremely small. Implantation rate [12/136 (8.8%) vs. 10/101 (9.9%); $p=0.77$; $\Psi=0.02$], clinical pregnancy rate [8/38 (21.1%) vs. 10/92 (10.9%); $p=0.13$; $\Psi=0.14$], ongoing pregnancy rate [6/38 (15.8%) vs. 8/92 (8.7%); $p=0.24$; $\Psi=0.01$], ectopic pregnancy rate [0% vs. 0%], miscarriage rate [2/12 (16.7%) vs. 2/11 (18.2%); $p=1$; $\Psi=0.02$], live birth

No. of patients	30
No. of frozen ET cycles	38
No. of oocyte retrieval(cycles)	132
No. of oocyte retrieval cycles per ET cycle (\pm SD)	3.6 ± 2.1
No. of cycle cancellation	22/154(14.3%)
Cycles without oocytes	23/132(17.4%)
Total no. of oocyte retrieved	237
No. of oocyte retrieved per ET cycle(\pm SD)	6.2 ± 2.5
Total no. of oocytes in metaphase II	156
No. of oocytes in metaphase II per ET cycle(\pm SD)	4.1 ± 2.4
No. of oocytes used for fertilization	231
Total no. of frozen embryos	172
No. of frozen embryos per ET cycle(\pm SD)	4.5 ± 2.1
Total no. of embryos transferred	136
No. of embryos transferred per ET cycle(\pm SD)	3.6 ± 1.0
Fertilization rate(%)(\pm SD)	73.6 ± 19.1

Table 1: Oocytes and embryologic outcomes of patients with accumulation of embryos.

rate [6/38 (15.8%) vs. 6/91(6.6%); $p=0.1$; $\Psi=0.14$], multiple pregnancy [3/6 (50%) vs. 0], gestational age at delivery [36.8 ± 3.2 weeks vs. 37.9 ± 1.2 weeks; $p=0.36$], and sex ratio [7 males:5 females vs. 5 males:1 female; $p=0.6$] were comparable between the ACC-E and NC groups. However, even though the effect size was small, the chemical pregnancy rate of the ACC-E group was significantly higher than that of the NC group [12/38 (31.6%) vs. 11/92 (11.9%); $p=0.02$]. Birth weight of the ACC-E group was significantly lower than that of the NC group [2608.9 ± 562.6 g vs. 3130.8 ± 644.1 g; $p=0.049$] (Table 2).

Discussion

Even though it has been difficult to achieve new breakthroughs to overcome the very low pregnancy rate and live birth rate of natural or modified natural cycle IVF in POR patients defined according to Bologna criteria, the current study shows that accumulation of vitrified embryos followed by thawing ET should be considered as a new strategy to improve the pregnancy rate in POR patients.

To achieve a breakthrough in the low pregnancy rate of NC IVF in POR patients we need to increase the number of embryos transferred, and therefore in the current study we accumulated vitrified embryos. In one cycle of embryo transfer, an average of 4.5 ± 2.1 embryos were frozen and 3.6 ± 1.0 embryos were transferred. The cancellation rate of 14.3% among 154 attempted cycles in the ACC-E group is comparable to the reported rate of 18.6% of 1,504 cycles for other modified NC IVF procedures [16]. Similarly, the rate of 17.4% cycles without oocytes is

not greatly different from the rate of 21.9% in another study of 500 NC IVF cycles [17]. Modified NC IVF was used in addition to NC IVF to try to decrease the rate of cycle cancellation and oocyte retrieval failure in the ACC-E group; however, the cycle cancellation rate and the oocyte retrieval failure rate were not decreased in the current study.

When baseline characteristics were compared between the ACC-E and NC groups, age and duration of infertility were statistically significantly higher in the NC group compared to the ACC-E group. Most of the patients in both the ACC-E and NC group underwent no or only one follicular growth despite conventional controlled ovarian stimulation. Also, the number of previous IVF cycles of the ACC-E group was comparable to that of the NC group. Past history did not differ greatly between the ACC-E group and NC group. The time point at which we calculated age, duration of infertility, and previous IVF cycles in the ACC-E group was the time when the first IVF cycle was initiated. Between two and nine oocyte retrieval cycles (average 3.6 ± 2.1) were needed to complete one frozen ET cycle. With increasing time during the accumulation of vitrified embryos patient age and the duration of infertility increased, although this increment was not reflected in our analyses. As a result, age and duration of infertility appear to be lower in the ACC-E group. However, the effect size for age and duration of infertility was extremely low. There was no difference between the groups in BMI, etiology of infertility, number of previous IVF cycles, and ovarian reserve indicators (AFC, AMH).

After the establishment of a common consensus for the definition of POR—the Bologna criteria—in 2011, [15] analyzed the effect of natural cycle IVF in POR patients fulfilling these criteria. In a comparison between POR patients according to Bologna criteria and normal responders, the chemical pregnancy rate per treatment cycle in normal responders was significantly higher than that in POR patients (15.2% vs. 4.6%). The live birth rate per treatment cycle was significantly lower for POR patients compared with normal responders (2.6% vs. 8.9%). Thus, the authors suggested that natural cycle IVF in POR patients as described by Bologna criteria has a very poor prognosis and cannot provide a substantial benefit [15]. Because modified natural cycle IVF has a lower cancellation rate compared with NC IVF, one study group assumed that modified NC IVF in POR patients selected by Bologna criteria would improve pregnancy outcome. However, the clinical pregnancy rate per aspiration cycle was 0.9%, and this modified NC IVF approach falls in the range of futile treatment according to the Ethics Committee of the ASRM [13]. There therefore seems to be no alternatives available to POR patients other than egg donation or adoption.

However, the bioethics laws are very strict in some nations and it is difficult to perform IVF-ET by oocyte donation. Also, as the number of identified poor responders increases with time, it will become less feasible to recommend oocyte donation and adoption to so many POR patients. As an alternative approach, a previous study used fertilized oocytes that were thawed after accumulation of vitrified oocytes over several rounds together with fresh oocytes and then transferred embryos. Although this method resulted in a high LBR, further verification is needed because the study population included POR patients within a broader spectrum than the Bologna criteria [18].

Even though the effect size was small, the chemical pregnancy rate per transfer in the current study was significantly higher in the ACC-E group, demonstrating the efficacy of this method in treating POR patients defined according to Bologna criteria. When we consider the chemical pregnancy rate of 4.6% in another report of natural cycle IVF for POR patients selected stringently according to Bologna criteria [15] our chemical pregnancy rate of 31.6% in the ACC-E

	ACC-E group	NC group	p value	Effect size(η^2 or Ψ)
Patients(n)	30	91		
Cycles of embryo transfer(n)	38	92		
Age(years)(\pm SD)	37.8 ± 4.7	39.9 ± 4.2	0.02	0.04
Etiology			0.41	
Tubal factor	2	2		
Endometriosis	0	4		
POR	36	86		
BMI(Kg/m ²)(\pm SD)	21.5 ± 2.3	21.9 ± 3.2	0.31	0.01
AMH(ng/ml)(\pm SD)	1.0 ± 1.3	0.8 ± 0.8	0.37	0.01
AFC(\pm SD)	9.7 ± 3.2	10.5 ± 4.0	0.24	0.01
Duration of infertility(yrs.)(\pm SD)	4.2 ± 3.6	5.9 ± 4.0	0.02	0.04
Previous IVF-ET cycles	2.9 ± 2.1	3.2 ± 3.3	0.66	0.001
Implantation rates	12/136(8.8%)	10/101(9.9%)	0.77	0.02
Chemical pregnancy rates	12/38(31.6%)	11/92(11.9%)	0.02	0.23
Clinical pregnancy rate	8/38(21.1%)	10/92(10.9%)	0.13	0.14
Ongoing pregnancy rates	6/38(15.8%)	8/92(8.7%)	0.24	0.1
Ectopic pregnancy	0	0		
Miscarriage rate	2/12(16.7%)	2/11(18.2%)	1	0.02
Live birth rate	6/38(15.8%)	6/91(6.6%)	0.1	0.14
Multiple pregnancy	3/6(50%)	0		0.58
Birth weight(gram)(\pm SD)	2608.9 ± 562.6	3130.8 ± 644.1	0.049	
Gestational age at delivery(weeks)(\pm SD)	36.8 ± 3.2	37.9 ± 1.2	0.36	
Sex ratio			0.6	
Male	7	5		
Female	5	1		

POR=poor ovarian response, AFC=antral follicle count
 η^2 for Student's *t*-test and Ψ for χ^2 test

Table 2: Baseline characteristics and pregnancy outcome in POR patients according to Bologna criteria.

group suggests that accumulation of embryos is a revolutionary approach to improving the pregnancy rate for POR patients. A total of 136 out of 172 frozen embryos were transferred and 32 embryos were discarded during the freeze-thawing process. This selection process during the freeze-thawing and the increase in the number of embryos transferred (an average of 3.6 ± 1.0 in this study) results in an enormous improvement in pregnancy rate. Even though there was no statistical significance, clinical pregnancy rate, ongoing pregnancy rate, and LBR were higher in the ACC-E group compared to the NC group (21.1% vs. 10.9%, 15.8% vs. 8.7%, and 15.8% vs. 6.6% respectively). The ongoing pregnancy rate and LBR for the ACC-E group in the current study are notable improvements over the 2.6% ongoing pregnancy rate and LBR for natural cycle IVF in a previous report [15]. The current study included only poor responders who were stringently selected by the Bologna criteria. Therefore, the promising results of our ACC-E method can be considered promising for genuine POR patients [19,20].

Multiple pregnancy rates were 50% in the ACC-E group compared with 0% in the NC group. As a result, birth weight at delivery was statistically significantly lower in the ACC-E group compared to the NC group (2608.9 ± 562.6 vs. 3130.8 ± 644.1 g). Generally, single ET should be recommended for normal responders in order to avoid multiple pregnancies. However, since single ET cannot sufficiently help POR patients because of the low pregnancy rate, the ACC-E method is the optimal strategy for poor responders in spite of the risk of multiple pregnancies.

It should be noted that the current study has several limitations, such as the small sample size of the ACC-E group and the retrospective nature of the study. Although the ACC-E strategy has been applied to poor responders in our institute since January 2010 many patients did not participate in ACC-E treatment because of concerns about the efficacy of the new method. Despite these limitations, the current study has important significance because the ACC-E method resulted in a higher pregnancy rate in a study population selected by the stringent Bologna criteria.

The frequency of chromosomal abnormality increases over time and can be a barrier to successful pregnancy because most of the poor responders are older (average age 37.8 ± 4.7 in the current study). To accomplish a higher pregnancy rate using the ACC-E method, a chromosomal selection process called pre-implantation genetic screening (PGS) should be added before vitrification of embryos in future applications of this method.

Conclusion

Accumulation of vitrified embryos followed by frozen embryo transfer notably improved the pregnancy rate compared with natural cycle IVF in poor responders who fulfilled the Bologna criteria. At the present time, considering the very low pregnancy rate and low live birth rate for natural or modified natural cycle IVF in poor ovarian responders, accumulation of vitrified embryos followed by frozen embryo transfer represents a promising alternative treatment for such patients.

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