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Research Article

A Cohort Study to examine the Result of Transferring a Poor Quality Embryo not Suitable for Freezing along with a Good Quality Embryo?

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Abstract

Objective: To improve the live birth rate and multiple birth rates, we created a strategy to balance the benefits/risks of embryo transfer approaches, in which a combination of a high-score embryo and a transferable lower-score embryo were transferred.

Design: Retrospective analysis.

Setting: A single university medical center.

Population or Sample: We performed a cohort study of 3,830 patients receiving first cycle of IVF /ICSI in the Reproduction center of Nanjing Medical University.

Methods: To compare the outcomes of three approaches embryonic transfer strategies: (1) elective single embryo transfer (eSET), (2) double embryo transfer 1 (DET1) with a high-score embryo and a low-score transferable embryo, and (3) double embryo transfer 2 (DET2) with two high-score embryos.

Results: For the three groups, the clinical pregnancy rate per cycle was 32.1%, 49.4% and 63.2% respectively. Pregnancy resulting in at least one live birth occurred in 659 of 1523 women (43.3%) in the (DET1) group as compared with 152 of 468 women (28.8%) in the eSET group and 1017 of 1839 women (55.3%) in the (DET2) group. The increase of live birth rate in DET1 group vs.. eSET is accompanied with a decrease in the multiple birth rates (24.7%) vs.. the DET2 group (34.1%). No significant difference was found in miscarriage rates among the three groups. The newborn characteristics were also analyzed among the three groups, no significant different was found in the newborn characteristics of singleton and twins among three groups.

Conclusion: The data indicate that the combined transferring strategy of a high-score embryo with a transferable low-score embryo yields an optimized rate of live birth rates and multiple birth rates compared with eSET and the traditional DET. It could be a beneficial alternative for lower-score embryo in some ethnicity and institutes. It seemed that there might be no adverse effect of lower-score embryo on the higher-score embryo.

Keywords: Poor quality embryo; Double embryos transfer; Multiple pregnancy rates; Embryos interaction

Introduction

The overall proportion of deliveries with twins and triplets from in vitro fertilization (IVF) and intracytoplasmic sperm injection (ICSI) was 25.1 and 1.8%, respectively, but varied widely by country and region. Most countries have standardized the double embryo transfer strategy (DET) [1]. This has allowed the rate of twins resulting from ART has remained stable: 43.4%% in 2010 in USA [2], 21.00% in 2005 in Europe [3] and 22.10% in 2001 in Australia [4]. Accumulated studies in perinatal medicine have revealed that multiple gestation lead to higher maternal/fetal complications, adverse perinatal outcomes, and economical burden for the health care system [5].

The most effective strategy to decrease the risk of multiple gestations from IVF/ICSI is to limit the number of embryos transferred. Elective single embryo transfer (eSET), defined as the transfer of one embryo when more than one high-score embryos are available, has been considered as the best way of avoiding multiple gestations and has been able to reduce the incidence of twin pregnancy in IVF. However, eSET hasn't been as widely implemented due to the limited pregnancy rate per transfer cycle, which discouraged the intentions of both doctors and couples with long standing infertility [5].

Thus DET strategy is still more widely accepted than eSET. Embryo quality appeared to be predictors of pregnancy in cleavage-stage embryos transfers. High-score embryos are an important factor in achieving high pregnancy rate, as a result of these facts many IVF' programs have adopted the policy of transferring good-quality embryos.

Whereas poor-quality embryos are more likely to be damaged during the cryopreservation process and associated with lower rate to grow to the blastocyst stage, which lead most of the poor-quality embryo to be discarded [6,7]. In the past few years, much effort has been focused on embryo quality and selection. On the other hand, no effective noninvasive clinical tools are available to evaluate which embryo is the really good-quality, a certain degree of bias is unavoidable because of the developmental potential of an individual embryo in vitro is not 100%.

To achieve an optimized single birth rate in women receiving the first cycle of IVF /ICSI by compromising the limited pregnancy rate of eSET and the multiple gestation risk of traditional DET, we introduced an alternative approach of embryo transfer in our Reproductive Center, in which a combination of a high-score embryo with a transferable lower-score embryos were co-transferred. To our knowledge, no published studies have used this method to select embryos for transferring. Therefore we performed this study to take

new insights on low-score embryo serves as a backup candidate for further maturation to win the priority of successful pregnancy comparable to eSET.

Materials and Methods

Participants

It was a retrospective study of couples who had undergone their first IVF/ICSI cycle at the Centre for Reproductive Medicine, the First Affiliated Hospital of Nanjing Medical University from 2007 to 2012. The first inclusion criteria included: (1) women younger than 35 years, (2) The Lupron down-regulation Ovarian Stimulation Protocol was used for all patients.

Total 4562 couples were initially included in the study. There are 108 couples had freeze all embryos because of risk of hyper stimulation or high progesterone on the day of hCG administration, 301 couples who have no high score embryo, 265 couples who have no surpluses embryo for freezing, 58 couples who have no low score embryo. The above mentioned 723 cycles were excluded. The final 3830 couples were met the inclusion criteria.

The study was approved by the ethics committees of the First Affiliated Hospital of Nanjing Medical University.

IVF process

The Lupron down-regulation Ovarian Stimulation Protocol was used for all patients. Desogestrel and Ethinylestra (OC) pre-treatment with 1 table daily starting from day 3 of the preceding cycle. Start gonadotropin releasing hormone agonist (Triptorelin, Ferring, Netherlands) by 0.05-0.1 mg/day after 21 days of OC until the day of "trigger". If down-regulation achieved after 2 weeks, 112.5-225U of gonadotropins (Gonal-F, Merck Serono S.p.A. and Puregon, Merck Sharp and Dohme) was injected daily for follicular stimulation. When at least three leading follicles ≥17mm in diameter were produced, 250ug of recombinant human chorionic gonadotropin (hCG, Merck Serono S.p.A.) was administered. 36 hours after the administration of hCG, Oocytes were aspiration was performed under the guidance of trans-vaginal ultrasound. Luteal support was provided both oral and intra-vaginal progesterone until 2 weeks following embryo transfer. Embryos transfers were performed at day 3. Pregnancy was tested by quantitative definition of serum β-hCG. Clinical pregnancies were confirmed ultrasonically by the presence of a gestational sac with fetal heart beat 4 weeks after the transfer. All pregnancies were followed up until the delivery.

Embryo qualities Assessment

The cleavage-stage embryos are scored based on their cell number, morphology and degree of fragmentation under the 400x inverted microscope [8,9]. The embryo quality can be scored as 1-4 grades:

Grade 1: Embryos growth with normal development speed, equal size of blastomeres, cytoplasm is not granular, no vacuoles, fragmentation <5 %

Grade 2: Embryos growth with normal development speed, equal size or almost equal size of blastomeres, cytoplasm is not granular, no vacuoles, fragmentation 5% -10%

Grade 3: Embryo's development speed is close to normal, equal or unequal size of blastomeres, small amount of vacuoles, fragmentation 10% --15%

Grade 4: Embryos growth with abnormal development speed, unequal size of blastomeres, granular cytoplasm, a lot of vacuoles, fragmentation 15%--50%

Grade 1 and 2 were considered to be high-score embryos, and Grade 3 and 4 were considered lower-score. Specifically, embryos of grade 3 were defined transferable.

Embryo transfer

All embryo transfers were performed on the day-3 post fertilization. The patients were informed about the possible procedures involving the transfer of only one high-score embryo would be expected to result mainly in singleton pregnancies but might also lead to a considerable decrease in the pregnancy rate, the transfer of two high-score embryo would result in higher pregnancy rate but also lead to increase in multiple pregnancy rate, and the combined one high-score and one lower-score embryos transfer strategy would be expected to obtain a compromise outcomes. Patients could voluntary choose which strategy they prefer. Based on the number and score of the embryos transferred, the patients were divided into three groups: eSET group (Elective high-score single-embryo transfer, n=468), DET1 group (combination of a high-score embryo with one transferable lower-score embryo, n=1,523), and DET2 group (two high-score embryos, n=1,839). The patients of three groups all have more than one high score embryos.

Statistical analysis

Statistical analysis was performed using the SPSS 16.0 software. One way ANOVA was used to compare the differences among groups. Differences in categorical data were analyzed by Chi-square (χ^2) test. P value <0.05 was considered statistically significant.

Results

Patient Characteristics

A total of 3,830 patients were included in the study. The demographic and clinical characteristics of the patients are shown in Table 1.

Characteristic (mean ± SD)	eSET (n=468)	DET1 (n=1523)	DET2 (n=1839)
Mean age(years)	29.1 ± 3.2	28.8 ± 3.2	28.9 ± 3.1
Body Mass Index(kg/m^2)	21.5 ± 2.5	21.7 ± 2.5	21.7 ± 2.6
Basis FSH(U/L)	7.4 ± 2.0	7.4 ± 2.1	7.5 ± 2.0
Antral follicle number	12.6 ± 5.9	12.0 ± 5.7	12.1 ± 7.1
oocytes/retrieval	12.2 ± 2.6	12.0 ± 2.8	11.9 ± 2.5
embryos2PN	9.1 ± 2.0	9.5 ± 2.5	9.2 ± 2.5
high score embryos	4.9 ± 1.6	5.1 ± 2.2	5.4 ± 2.0
low score embryos	4.2 ± 1.5	4.4 ± 1.2*	3.8 ± 1.5*

freezing embryos	6.1 ± 1.3	5.7 ± 2.1	5.7 ± 2.0

Table 1: Characteristics of the patients *P<0.05 vs. SET

Fresh embryo transfer was performed with one or two embryo for all patients, who have more than one embryo for freezing. All the patients have both low-score and high-score embryos.

Maternal outcome

The clinical pregnancy rate and live birth rate of DET1 and DET2 group were higher than eSET (49.4% (753/1523) and 63.2% (1163/1839) vs. 32.1% (150/468) p<0.01, 43.3% (659/1523) and 55.3% (1017/1839) vs. 28.8% (135/468) p<0.05). On the other hand, the DET1 approach resulted in a much less multiple birth rate than the DET2 approach (24.7% (186/753) vs. 34.1% (396/1163), p<0.01). The implantation rates of eSET and DET2 group were 32.5% (152/468) and 42% (1544/3678) respectively, which were higher than DET1 (30.3%, 923/3046). The difference was statistically significant (p<0.05). However, the miscarriage rates showed no statistical differences between eSET, DET1 and DET2 groups (11.3% (17/150), 15.5% (117/753), 13.2% (153/1163), p>0.05) (Table 2).

Neonatal outcome

The outcome of live newborn is present in Table 2. We made analysis of both gestational weeks and birth weight between three groups who all had single live newborn. The gestational weeks in eSET, DET1 and DET2 groups was 38.6 ± 1.2, 38.4 ± 1.6 and 38.5 ± 1.8 respectively, no statistical difference was found (p>0.05). The birth weight was 3448.3 ± 420.1 (g), 3419.8 ± 456.9 (g) and 3324.4 ± 546.6 (g) respectively, showed no statistical differences (p>0.05). In twin newborn, DET1 and DET2 showed gestational weeks was 37.8 ± 1.8 and 37.5 ± 1.9 respectively. The birth weight was 3147.7 ± 594.4 (g) and 3028 ± 612.3 (g) in DET1 and DET2. Both gestational weeks and birth weight in twin newborn showed no statistical difference (Table 3).

Results	eSET (n=468)	DET1 (n=1523)	DET2 (n=1839)
Pregnancy rates	32.1** (150/468)	49.4 (753/1523)	63.2** (1163/1839)
Implantation rates	32.5** (152/468)	30.3 (923/3046)	42.0** (1544/3678)
Live birth rates	28.8** (135/468)	43.3 (659/1523)	55.3** (1017/1839)
Multiple birth rates	1.3** (2/150)	24.7 (186/753)	34.1** (396/1163)
Miscarriage rates	11.3 (17/150)	15.5 (117/753)	13.2 (153/1163)
OHSS rates	1.3 (6/468)	0.9 (14/1523)	1.5 (27/1839)

Table 2: Clinical outcome **P<0.01 vs. DET1

	Single live newborn			Twin live newborn	
mean ± SD	eSET (n=133)	DET1 (n=287)	DET2 (n=225)	DET1 (n=81)	DET2 (n=219)
Gestational weeks (weeks)	38.6±1.2	38.4 ± 1.6	38.5 ± 1.8	37.8 ± 1.8	37.5 ± 1.9
Birth weight (g)	3448.3 ± 420.1	3419.8 ± 456.9	3324.4 ± 546.6	3147.7 ± 594.4	3028.8 ± 612.3

Table 3: Characteristics of live newborn

Discussion

The clinical most important finding of the present study was the strategy of transferring a high-score with a transferable lower-score embryo, leading to an optimized rate of live birth rates (43.3%) and multiple birth rates (24.7%) compared with eSET and the double high-score embryo transfer cycles. This strategy leads to maximize the use of transferable lower-score embryos not suitable for freezing.

Clinical reproductive centers produce large amounts of surplus poor-quality embryos annually. To optimize the benefit of those surplus embryos has become a problem. Those poor-quality embryos which have lower development potential and failure recovery in the subsequent frozen cycle might be given up [7].

Zygote transfers at day 1 have been performed in 1985 [10]. Some authors reported no difference in the implantation and pregnancy rates between day-1 and day-2/3 embryo transfers in 1998 and 2002 [11,12]. Alternatively, according to others reports in 2003 and 2004, pregnancy rates are significantly lower after zygote transfer compared with embryo transfers at cleavage stage [13,14]. It was reported that poor-quality embryo that transfer at zygote stage with clinical pregnancy rate per transfer of 26.4% [15]. At some clinics, poor-quality cleavage stage embryos are cultured to blastocyst stage. It was reported that the treatments program by using blastocysts developing from poor-quality, in which 19.7% of poor quality embryos can reach the blastocyst stage [7] Similar to the reports before which estimate about 21% of poor quality embryos can reach the blastocyst stage [16]. Reports showed

that 16% and 24.8% respectively poor quality embryos were cultured to blastocyst stage [17,18]. However, they have not paid attention to the disadvantages of this strategy, like cost increasing and laboratory burden, especially in china.

To our knowledge, no published studies have used the strategy that transfer a high-score with a lower-score but transferable embryo. Therefore, we performed this transitional strategy in order to maximize the availability of transferable embryos both good and poor quality.

At present the average number of embryos transferred was 2.35 and double embryo transfers accounted for 73.2% of cycles [1]. In European IVF cycles, the average number of embryos transferred varies between countries, but is often more than one [19,20]. Embryo quality is one of the most important factors determining the clinical pregnancy. If two good-quality embryos are transferred, there is no doubt that it will lead to higher multiple pregnancy rates.

About one-third of all twin pregnancies are the result of in vitro fertilization (IVF) or intracytoplasmic sperm injection (ICSI) [21]. eSET may result in a lower pregnancy rate per cycle [22]. Nevertheless, the implementation of eSET in daily practice is not yet overwhelming [5]. The purpose of the current study was to design a transitional strategy of double embryo transfers which can reduce the rate of multiple gestations versus the traditional high quality DET outcome, with a substantially higher rate of successful pregnancy and live birth than the eSET.

In this prospective clinical study, our combined transfer strategy results in a marked reduction in the rate of multiple gestations versus the traditional high quality DET outcome, with a substantially higher rate of successful pregnancy and live birth than the eSET.

Compared with good-quality cleavage-stage embryos, poor-quality embryos are more often related to chromosomal abnormalities, resulting in lower implantation [23]. Notably, our study showed that DET1 yielded a lower implantation rate than the other two groups. Because of embryo plasticity, the proportion of chromosomally abnormal cells varies during the culture, and the corrupted cells can be eliminated resulting in a good-quality embryo developing from a poor-quality cleavage stage embryo [24].

Kalstrom has argued although the high score embryos seem to have a good predictive value for implantation, the slow-developing "transferable embryos" might still have relative potential for implantation [25]. Although embryo morphology is considered a major predictor for the development potential of embryos leading to successful pregnancy, the quality of embryos are determined by multiple factors including genetics, culture environment, techniques, etc., the day-3 morphology is not considered an absolute predictor for subsequent blastocyst formation [26]. Despite many improvements, even the best culture media imperfectly reproduces the natural environment for embryo developing in vivo. Therefore, a "transferable embryo" still has the potential to evolve if shorted the culture time in-vitro.

It has been an ongoing debate whether the poor quality embryo would influence the developmental potential of the good quality embryo. Some factors derived from poor quality embryos may have negative influences on the surrounding embryo development [26,27]. The study of Zhu in 2014 indicated that the transfer of poor-quality embryos resulted in higher miscarriage but no increase the risk of adverse outcome to pregnancy complications [28]. However, it has been reported that group culture may promote embryo development vie secretion of embryo trophic factors, and embryos cultured in group developed better than those cultured individually [29]. Grouping of embryos after Day 3 based on embryo quality may benefit blastocyst formation, no impacts on pregnancy or implantation outcomes were observed. In vitro studies have shown no difference in the embryo morphologic features or cleavage speed of the embryos cultured together or cultured separately, this may be due to secretion of beneficial factors by good embryos, or removal of detrimental factors from poor embryos [29]. In our study, as compared with the eSET group, the DET1 group held a higher pregnancy rate despite an equivalent miscarriage rate, the gestational age and birth weight in single and twin live newborn is no difference between three groups both in single and twin live newborn, indicating that the lower-score embryo have no adverse effect on the high-score embryo. The interaction between lower-score and high-score embryo have not been well explained. The embryos maybe can modify their surrounding environment by creating localized zones of secreted and depleted factors

In the present study, all the children born from the poor-quality cleavage stage embryos cultured to blastocyst stage were healthy and no chromosomal abnormalities or malformations were reported. Therefore we can consider this practice of poor-quality transferring is safe [17].

In the practice of our reproductive center, eSET is a feasible option for the perspective of newborns. However, most couples favor the overall successful pregnancy per ET cycle indifferent to the risk of multiple pregnancies in spite of the knowledge about multiple pregnancy complications [5]. Our transfer program might be a more transitional and favorable protocol for doctors and patients to balance the benefit of successful pregnancy and the risk of multi-gestation.

In conclusion, achieving a healthy singleton pregnancy is the goal of IVF treatment. Our combined embryo strategy shows a significant reduction in multiple gestation rate despite an improvement in pregnancy rate and live birth rate per ET cycle. These finding provide an insight into the potential interaction between the co-transferred embryos, especially the potential role of the low score embryos. More prospective randomized studies are required to confirm the efficacy of this new strategy to provide evidence for a better selection of patients whom can benefit the most from this strategy.

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