ORIGINAL ARTICLE

Comparison of Two Embryo Scoring Systems for Prediction of Outcome in Assisted Reproductive Techniques Cycles

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Abstract- Cumulative embryo score (CES) is one of the many embryo scoring methods which have been developed to help clinicians to transfer high quality embryos and predict pregnancy rate in assisted reproductive techniques (ART) cycles. Regarding the existing difference in CES calculation this study was done to compare two methods in order to determine the more practical and preferable one. In a retrospective, cross sectional descriptive analytical study, a total of 508 ART cycles in infertile patients treated from November 2002 until March 2004, were evaluated using two methods of CES calculation in embryonic scoring to predict ART outcome. According to one method, CES was obtained by adding the individual scores of all transferred embryos. Whereas in the other reference method, CES was calculated by the sum of each embryo score multiplied by its number of blastomeres on the day of transfer. The mean score of transferred embryos (MSTE) was referred to CES divided by the total number of embryos transferred in either method. A total of 109 clinical pregnancies (pregnancy rate 21.5%) including 96 singletons, 10 twins and triplets occurred in the 508 ART cycles. The pregnancy rate was strongly correlated to CES & MSTE. According to one method, CES was 12.6±6.4 in pregnant versus 9.2±5.8 in non-pregnant group ($P<0.0001$). According to the other one, in the pregnant group CES was 86.7±48 versus 68.7±55 in the non-pregnant group ($P<0.002$). Both methods showed a significant difference. Regarding MSTE, using the first method, in the pregnant group it was 3±0.6 versus 2.8±0.7 in the non-pregnant group ($P<0.011$) whereas with the other approach it was 21.3±8.6 in the pregnant group versus 19.9±9.07 in non-pregnant ($P<0.152$) showing that the first method can also predict pregnancy outcome with MSTE. Considering that both MSTE and CES in the first method can significantly predict outcome in ART cycles, it seems this method is preferable and more useful in practice. Moreover, sometimes due to continuous division, on the third post oocyte retrieval day the blastomere number cannot be counted precisely which can be misleading if taken into account according to the method introduced by Steer.

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Keywords: Cumulative embryo score; Mean Score of Transferred Embryos; Assisted Reproductive techniques

Introduction

Since the introduction of assisted reproductive techniques (ART) in 1978, transferring several embryos in these cycles has been a common trend for many years in order to overcome the consistently low conception rates (1). Multiple pregnancies as a major consequence of ART have been of great concern in recent years with increased perinatal mortality and morbidity (2-4). Different modalities have been implemented to decrease these complications. It seems that the best way to avoid multiple order pregnancies is to transfer the least number of embryos without affecting ART outcome (5). It is obvious that many factors with especially endometrial receptivity and embryo quality being the most important ones, contribute to successful ART. While endometrial receptivity has been assessed to be effective from 31% to 64%, embryo quality has a contribution rate of 21% to 32% in mathematically devised formulas. Embryo quality is practically an easier

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variable to be assessed and applied (4). In this regard, many different embryo scoring methods have been developed starting from 1986 to help clinicians to transfer high quality embryo and /or embryos with the best results (6-13).

The first reports of embryo scoring (14) have concentrated on embryo growth rates with attention to its morphology. In 1987 Puissant *et al.* (15) suggested that consideration of an embryo scoring system including cell number, blastomere size and shape, fragmentation degree is essential to identify high quality embryos that would lead to pregnancy. This idea was followed by Steer *et al.* (4) who proposed a mathematical scoring which is cumulative embryo score (CES), created by the summation of the score of all embryos transferred. The score for each embryo in this method is the result of multiplication of the morphological grade of the embryo by the number of blastomeres to produce a quality score for each embryo. This scoring method has been used to predict ART outcome in several studies (16,17)

In 2001 Terriou *et al.* also proposed the efficacy of CES in predicting ART outcome using the sum of scores of the embryos evaluated by a 4 points scoring method. They also suggested using mean score of transferred embryos (MSTE) which was referred to CES divided by the total number of embryos transferred as a parameter for assessing ART outcome (18).

Finally in 2006 Loi *et al.* suggested the 5 point embryo score to be used as a reference for CES calculation (6).

Regarding the difference observed in various studies in expressing CES values this study was done in order to assess embryo quality in a more simple and coordinated way in practice.

Materials and Methods

A total of 508 ART cycles with embryo transfer which were performed between November 2002 and March 2004, were included in this cross sectional, descriptive-analytical study. The efficacy of two embryo scoring systems in selecting the embryos best suited for transfer was compared retrospectively. All patients underwent the IVF/ICSI procedure at the Reproductive Health Research Center, Tehran University of Medical Sciences, Iran. The data used for the study from the patients were kept private and the sources were not revealed elsewhere.

All patients were stimulated with 150 IU recombinant follicle stimulating hormone (FSH) (Gonal F; Serono, Switzerland) 12-15 days after pituitary function was down-regulated with daily 0.5 ml *s.c.* doses of gonadotropin releasing hormone (GnRH-agonist; Superfact®, Hoechst, Germany) starting on the 21st day of the cycle preceding ART treatment. Follicular development was monitored using serial vaginal ultrasound and serum estradiol levels. Human chorionic gonadotropin (HCG) was administered when two or more follicles reached 18 mm in mean diameter. Oocytes were transvaginally retrieved under ultrasound guidance 34-36 h after triggering ovulation. Of all patients, the following information was collected for analysis: Maternal age, duration and type of infertility, morphological embryo scores (CES & MSTE) and pregnancy outcome. Embryo development was evaluated shortly before embryo transfer, and the best embryos were selected. On day 2 or 3 after ovum pick up, between 1-5 (4-8 celled) embryos were transferred according to the patient's age and their quality. Each embryo was graded as follows: Grade 4, equal sized symmetrical blastomeres without fragmentation; Grade 3, uneven blastomeres with 10%-20% fragmentation; Grade 2, uneven blastomeres with 20-50% fragmentation; Grade 1, uneven blastomeres with >50% fragmentation.

Chemical pregnancy was confirmed by serum β-HCG measurement 14 d after embryo transfer and clinical pregnancy was defined as the presence of a gestational sac on ultrasound scan performed 2 weeks thereafter. The data available from the scoring was retrospectively used to calculate CES by the methods described by Terriou (18) and Steer (4).

Terriou *et al.* calculated CES by adding the individual scores of all transferred embryos (18). Whereas in the method introduced by *Steer et al.* (4), CES was calculated by the sum of each embryonic score multiplied by its number of blastomeres on the day of transfer. MSTE was calculated by CES divided by the total number of embryos transferred in each method.

Statistical analysis

The SPSS 15 statistical package was used for data analysis. All P-values were two-sided, and $P<0.05$ was considered statistically significant. Multivariate analysis was used to evaluate the relationship between CES and MSTE score and pregnancy outcome.

Results

The study group included 508 cycles who underwent IVF/ICSI and had at least one embryo transferred,

Table 1. Age and fertility background of the two groups

	Pregnant	Non- pregnant	*P* value
Age (yrs) (mean ± SD)	29.5 ± 5.20	30.3 ± 5.00	0.14
Infertility type***N (%)*			
Primary	82 (21%)	314 (79%)	
Secondary	27 (24%)	85 (76%)	0.43
Infertility duration* yrs (mean ± SD)	8.04 ± 5.92	8.84 ± 5.34	0.48
Infertility cause**N (%)			
Male	48 (24%)	218 (55%)	
Female	42 (38%)	108 (27%)	
Both	6 (5%)	32 (8%)	
Unexplained	13 (13%)	41 (10%)	0.08
Female Infertility** N (%)			
Tubal	26 (54%)	72 (51%)	
Ovulatory & PCOS	13 (27%)	36 (26%)	
Endometriosis	4 (8%)	18 (13%)	
Immunologic	4 (8%)	9 (6%)	
Others	1 (3%)	5 (4%)	0.54

t*-test;Chi-square test

Table 2. Embryo score and ART outcome

Scores / mean±SD	Pregnant	Non-Pregnant	*P* value*
CES (1st method)**	12.6 ± 6.4	9.2 ± 5.8	<0.0001
CES (2nd method)***	86.7 ± 48	68.7 ± 55	<0.002
MSTE (1st method)**	3 ± 0.6	2.8 ± 0.7	<0.011
MSTE (2nd method)***	21.3 ± 8.6	19.9 ± 9.07	0.152

* Multivariate analysis, ** Method introduced by Terriou, *** Method introduced by Steer *et al.*

resulting in 109 clinical pregnancies (pregnancy rate 21.5%) including 96 singletons, 13 twins and triplets. The mean age of the patients was 30.2±4.6 years. Mean number of embryos transferred was 3.4±1.8. The general characteristics of the pregnant and non-pregnant groups are shown in table 1.

The pregnancy rate was strongly correlated to CES & MSTE. Table 2 shows the mean difference of these scores between pregnant and non-pregnant women.

Discussion

In recent years with the widespread use of ART and better standards of IVF labs, in order to avoid unacceptably high multiple pregnancy rates, most centers in the world have shifted to the trend of transferring one or a maximum of two embryos. Although the effect of woman's age and number of embryos transferred on pregnancy rate has been shown in various studies, the importance of embryo quality in ART outcome has gained more attention in recent years especially when single embryo transfer strategy has been considered in IVF centers with high standard labs (11). Also in centers without freezing systems when no limitation in number of transferred embryos is practiced, cumulative embryo scoring (CES) methods are being increasingly used to predict ART outcomes. Various studies have shown the efficiency of CES in determining the rate of pregnancy (4,18), multiple (6,17,19) and ongoing pregnancy (6,18). In this study, the two different methods used to define CES were compared in 508 ART cycles. With the method shown by Terriou *et* *al.* (18), CES was 12.6±6.4 in the pregnant versus 9.2±5.8 in the non-pregnant group ($P<0.0001$). CES according to Steer method was 86.7±48 in the pregnant versus 68.7±55 in the non-pregnant group ($P<0.002$).

CES scoring was introduced for the first time by Steer et al as criteria to predict ART outcome, optimal selection of embryos and also determine the number of transferred embryos in order to attain the highest outcome with lowest risk of multiple pregnancy. In this method the morphological score of the embryo using a 4-point grading was then multiplied by the number of blastomeres. In his study on 390 IVF transfer cycles he showed that the pregnancy rate rose progressively to a maximum of 33% per embryo transfer as the CES increased to a value of 42 (4).

In a retrospective study by Terriou *et al.* which was performed on 10,000 embryo transfer cycles to evaluate the predictive value of several parameters on IVF outcome, he introduced a new CES method which was obtained by adding the individual scores of all transferred embryos (based on a 4 point score). He believed CES, according to this method is the best predictor of pregnancy outcome. His study showed that pregnancy rate was strongly correlated with CES with a linear increase in pregnancy rates as scores increased from 1 to 11 with a plateau at 12 points. According to his findings each CES point corresponds to a theoretical pregnancy rate of 4%.The multivariate analysis in this study showed that CES is a better variable to predict pregnancy compared to female age and number of retrieved oocytes (18).

In another study by Loi *et al.* (6) they suggested CES to be used for prediction of ART outcome with a trend towards better results with increasing CES. He calculated CES scores using a method similar to that of Terriou *et al.* (18) except that he used a five-point scoring system, also taking into account the clarity of the cytoplasm in terms of presence or absence of granulation. He categorized CES scores into three groups with group1 (score 9-10), group2 (score 11-13) and group 3 (score 14-15). He found higher pregnancy rates with increasing CES scores (30.3 % vs. 45.1% vs. 51.7%) in the three level CES groups respectively which did not reach statistical significance. Nevertheless in the logistic regression analysis it was shown that CES group score was significant in prediction of live births (6).

MSTE is another parameter which has been mentioned to predict ART outcome in centers where multiple embryo transfer is practiced. The only one study in which MSTE was analyzed showed that MSTE was significantly correlated with pregnancy in all age groups. This correlation was particularly strong in oldest age group. The pregnancy rate was 3 times higher in older women with higher MSTE than in women with lower MSTE (22% vs7% respectively) (18).

In our study we used this variable and calculated MSTE using the CES in both methods divided by the number of embryos transferred. According to Terriou, MSTE was 3±0.6 and 2.8±0.7 in pregnant versus non-pregnant group ($P<0.011$) respectively. Using the CES calculation by Steer, MSTE was not statistically significant with 21.3±8.6 in pregnant versus19.9±9.07 in non-pregnant group ($P<0.152$), showing that the first method can predict pregnancy outcome with MSTE.

Considering that both MSTE and CES in Terriou *et al.* method can significantly predict outcome in ART cycles, it seems this method is preferable and more useful in practice (18). Moreover, sometimes due to continuous division, on the third post oocyte retrieval day the blastomere number cannot be counted precisely which can be misleading if taken into account according to the method introduced by Steer *et al.* (4) In conclusion, the CES and MSTE based on a 4-point embryo grading method according to Terriou *et al.* (6) is the best predictor of pregnancy and should be used in IVF-ET programs to choose the best embryos for transfer especially where more than one or two embryos are transferred.

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