A cumulative embryo scoring system for the prediction of pregnancy outcome following intracytoplasmic sperm injection

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ABSTRACT

Introduction: The ability to select the embryos that would lead to pregnancy would help to reduce multiple pregnancy rates. The objective was to evaluate the use of a cumulative embryo scoring system (CES) based on a five- point embryo scoring system for the prediction of pregnancy outcome following intracytoplasmic sperm injection (ICSI).

Methods: A retrospective cohort study was performed on 364 triple embryo transfers from fresh ICSI cycles only. Embryo quality was assessed using a five-point scoring system. The CES was the summation of the individual scores. For the purpose of analysis, these were categorised into three groups: CES group one (score 9-10), CES group two (score 11-13) and CES group three (score 14-15). Main outcome measures were clinical pregnancy, implantation, live-births and multiple birth rates.

Results: There was a trend towards better outcome with increasing CES scores. This trend was significant with CES groups one, two and three, corresponding with increasing pregnancy rates (30.3 vs. 45.1 vs. 51.7 percent), increasing implantation rates (12.4 vs. 20.5 vs. 21.8 percent), and increasing live-birth rates (12.4 vs. 26.4 vs. 31.0 percent). Age was also a significant independent predictor of clinical pregnancy. However, only CES group score was significant in predicting live-births, while age was significant in predicting multiple births.

Conclusion: CES based on the proposed five-point scoring system is useful for the prediction of pregnancy outcome in triple embryo transfers. In younger patients, a policy of transferring fewer embryos to reduce multiple births should be adopted.

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INTRODUCTION

In order to achieve a successful outcome in assisted reproductive technology (ART) treatments, multiple embryos are often transferred, but this subsequently leads to multiple gestations, which is increasingly recognised as an undesirable complication of ART.[1-3] One solution proposed to resolve this dilemma has been blastocyst transfer. This approach reportedly generates high implantation rates, thus lowering the need for triple embryo transfers.[4] However, the effort needed for sequential blastocyst culture systems and the subsequent high cancellation rates when no embryos survive to day five, has led many centres to maintain their day two or three embryo transfer practice.[5] An effective selection of day two or three embryos therefore remains an important component of ART procedures.

Embryos are selected based on a scoring system at every *in vitro* fertilisation (IVF) centre. The main features considered in embryo scoring systems include cell number, blastomere size and shape, and degree of fragmentation.[6,7] These factors have been combined in numerous ways, often complex, to produce embryo scoring systems to identify potential embryos that would result in pregnancy.[8-11] Some systems place more emphasis on the embryo cleavage state,[12,13] while others incorporate multiple morphological criteria.[14] The decision to use one system over another is often based on the individual laboratory's familiarity and training. There is a lack of good comparative studies between different systems, as often, different criteria and outcomes are used.

Furthermore, assessment of embryo quality is a constantly evolving field. As more is learnt about the different aspects of embryo morphology in relation to pregnancy outcome, more criteria can be included in the assessment of embryos. Recently, much work has also been published regarding other screening parameters, such as assessment of the oocyte, pronuclear as well as early cleavage status. These alternative scoring systems

Table I. The five-point embryo scoring system at the IVF unit.

Features of the embryo	Yes	No
Is the embryo at a 4-cell stage at 44 hr, or a 6–8 cell stage at 68 hr, post-insemination?	1	0
Are all cells uniform in size?	1	0
Are all cells uniform in shape?	1	0
Is the cytoplasm of cells clear?	1	0
Are the anuclear fragments absent?	1	0
If present, do they exceed 25%?	-1	0

appear promising and warrant further attention as they may possibly be combined with embryo scoring to give even better prediction of pregnancy outcome. Indeed, in a study by Fisch et al,[15] such a "graduated embryo score" or GES was found to predict pregnancy outcome better than a single day three evaluation alone. The GES compromised a series of evaluations from the time of insemination, firstly at 16–18 hours for pronuclear morphology, then at 25–27 hours for early cleavage status, and finally at 64–67 hours for day-three morphology.

To implement such a system, as acknowledged by the authors, a certain degree of commitment is necessary. Whether significant time, cost and labour will have to be added is debatable. In addition, whether repeat removal of embryos from their incubators might have adverse effects on the embryos is another concern. Certainly, in implementing such a sequential system for evaluating embryos, the need for a straightforward day-two or three scoring system, such as our five-point scoring system, is even more crucial. Such a scoring system, if effective, would have the definite practical advantages of being easily performed and interpreted with little room for inter-observer variation. Although there is a lack of literature comparing the difficulty and exact amount of time required to perform different scoring methods, our embryologists have found the ease of use and objective nature of the proposed system highly advantageous.

We sought to determine if a simplified five-point scoring system developed in our centre would suffice in predicting pregnancy outcome. There are definite practical advantages of a simplified embryo scoring system, which is easily performed and interpreted with little room for inter-observer variation. The objective of our study was to evaluate the use of a cumulative embryo score (CES) based on this five-point scoring system for the prediction of pregnancy outcome in intracytoplasmic sperm injection (ICSI) pregnancies. Most studies have shown that embryo morphology is similar in patients undergoing conventional IVF vs. ICSI, apart from the timing of pronuclear development as the first cleavage generally takes place four hours earlier in ICSI embryos.[16,17] However, after prolonged culture, the ICSI procedure results in a reduced capacity for blastocyst formation.[18-20] The need for an effective day-two or three embryo scoring system is therefore of particular importance in ICSI cycles. If shown to be effective for the prediction of pregnancy outcome, a policy of transferring fewer embryos based on good CES scores may be adopted to reduce multiple pregnancies. We have also included age and infertility diagnosis in our analysis as these parameters have previously been shown to also influence outcome.[21,22]

METHODS

364 consecutive cycles of triple embryo transfers performed in our institution from January 2002 to January 2004 were analysed in this retrospective cohort study. Only fresh ICSI cycles were included. In our country, patients older than 45 years of age are not allowed to undergo IVF, unless prior approval from the Ministry of Health has been obtained. In addition, a standard number of three embryos are allowed for transfer. Therefore, in order to maximise their chances of pregnancy, most of our patients with a sufficient number or embryos deemed suitable for transfer would have three embryos transferred. Institutional Review Board approval was not obtained as the study involved a retrospective review of laboratory data and did not affect patient management.

All patients received the long protocol for down regulation with leuprolide acetate (Lupron, TPA Pharmaceuticals, IL, USA) beginning on day 21 of the previous cycle for 10–14 days. Once pituitary suppression was achieved as evidenced by ultrasonography showing all follicles < 10 mm, endometrial thickness < 8 mm and oestradiol levels < 183 pmol/ml, ovarian stimulation with recombinant follicle stimulating hormone (rFSH; Puregon, Organon, France) was initiated. A standard ovulating dose of human chorionic gonadotrophin (hCG; Profasi, Italy) was given when ultrasonography showed three follicles of 17–18mm in diameter. Oocytes were retrieved 36–38 hours after hCG administration and fertilised via ICSI with sperm collected from fresh semen samples obtained on the day of oocyte retrieval. ICSI was performed according to Payne,[23] with some modifications. Oocytes were assessed for fertilisation at 18–24 hours post-ICSI. Embryo quality was assessed at 44 hours post-ICSI for day-two, or 68 hours post-ICSI for day-three transfers.

The proposed embryo score is based on the number of blastomeres or cells observed in relation to number of hours post-ICSI, the uniformity of cells in terms of size and shape, the clarity of the cytoplasm in terms of presence or absence of granulation, as well as the degree of anuclear fragmentation (Table I). Embryos resulting from

Table II. Infertility diagnoses and age versus clinical pregnancy.

	No. of cases (%)	% clinical pregnancy	p-value*
Infertility diagnoses			
Male factor only	215 (59)	45	0.317
Female factor only	108 (30)	35	0.317
Mixed	6(2)	33	0.317
Idiopathic	35 (9)	49	0.317
Age (years)			
< 35	177 (49)	49	0.001
35–37	110 (30)	42	0.001
38–40	52 (14)	33	0.001
> 40	25 (7)	12	0.001

*p-values for significance in differences between groups by chi-square and Fisher's exact tests

abnormal fertilisation or embryos with mutinucleation were excluded from scoring and transfer. The best embryos obtained a score of five, while the minimum cut-off score for embryos deemed suitable for transfer or cryopreservation was three. The CES was calculated by the summation of the individual embryo scores of the three embryos transferred. For this study, the minimum CES for transfer was nine and the maximum was 15.

The parameters selected for evaluation in our five-point embryo scoring system were based on the laboratory's historical experience, and current knowledge of the prognostic value of the embryo cleavage stage and morphology on pregnancy outcome.[6,12-14] Multinucleated blastomeres have previously been found to be associated with markedly impaired implantation rates and have therefore been excluded from our embryo transfers.[7] Our scoring system therefore assesses embryo cleavage stage in terms of cell number in relation to the number of hours post-ICSI, and morphological features in terms of blastomere size, shape, cytoplasmic clarity or granularity and degree of fragmentation. Compared with the four-point scoring system used by Terriou et al 2001,[21] which includes cell number, symmetry and fragmentation, our system also considers cell cytoplasmic granularity, which has been shown to be important in recent studies.[14] In addition, the score places particular emphasis on the degree of fragmentation, which has been found to be associated with an increased risk of aneuploidy,[6] thus the '–1' if fragmentation exceeds 25%.

Embryos were transferred on day two or three (if day two fell on a Sunday) after oocyte retrieval, using Wallace 1816N soft catheter (SIMS Portex Ltd, Kent, UK). Luteal support was provided with either hCG or IM progesterone, depending on the estimated risk of ovarian hyperstimulation syndrome. When ≤ 15 eggs or embryos were obtained or if the patient was older than 40 years of age, hCG was given. However, when > 15 eggs or embryos were obtained or if the patient had ovarian hyperstimulation syndrome, intramuscular progesterone was given up to day 19. Biochemical pregnancy was established on day 19 with serum level of βhCG, and clinical pregnancy was determined by identifying the presence of a gestational sac at six weeks gestation on transvaginal ultrasonography. Both intra- and extrauterine pregnancies were included. Biochemical pregnancies were excluded from the analysis. Clinical pregnancy rate was defined as the fraction of embryo transfers resulting in a gestational sac. Implantation rate was defined as the fraction of transferred embryos resulting in a gestational sac. Live-birth rates as well as mutiple birth rates were also assessed.

The variables analysed were type of infertility (male, female, mixed, unexplained), female age, and CES based on the five-point embryo score as shown in Table I. Statistical analysis was performed using the Statistical Package for Social Sciences version 11.0 (SPSS Inc, Chicago, IL, USA). Chi-square and Fisher's exact tests were used to investigate correlation between qualitative variables, with $p < 0.05$ considered as significant. Multivariate binary logistic regression was used to define the most significant predictor for pregnancy outcome.

RESULTS

In this series of 364 triple embryo transfers, 1,092 embryos were transferred, of which ten had an embryo score of two. However, these embryos belonged to patients with too few embryos of "transferable quality" for transfer and as the CES of three embryos attained a minimum CES of nine, they were also included in the analysis. This culminated in 151 clinical pregnancies, including 104 singleton and 45 multiple gestations (39 twins, five triplets and one quadruplet), and two ectopic pregnancies. The overall clinical pregnancy rate was 42% (151/364) and the overall implantation rate was 19% (203/1,092). The live-birth rate per cycle, when multiple births from one pregnancy were counted as one live-birth, was 23% (85/364). Of the live-births, the multiple pregnancy rate was 29% (25/85) with 23 pairs of twins and two sets of triplets.

The clinical pregnancy rate involving only female infertility was lower than in cases involving only male

Table III. Percentage of CES in each age group.

Age group (years)	Total	CES group 1, no. (%)	CES group 2, no. (%)	CES group 3, no. (%)
< 35	177	41 (23.2)	121 (68.4)	15 (8.5)
35–37	110	29 (26.4)	73 (66.4)	8 (7.3)
38–40	52	14 (26.9)	33 (63.5)	5 (9.6)
> 40	25	5 (20.0)	19 (76.0)	1 (4.0)

p = 0.951 for difference in CES group distribution between different age groups by chi- square and Fisher's exact tests

Table IV. Clinical pregnancy, implantation and live-birth rates by CES group.

	Grand total	CES group 1 Cases/total (%)	CES group 2 Cases/total (%)	CES group 3 Cases/total (%)
Clinical pregnancy rate[a]	364	27/89 (30.3)	111/246 (45.1)	15/29 (51.7)
Implantation rate[b]	1,092	33/267 (12.4)	151/738 (20.5)	19/87 (21.8)
Live-birth rate[c]	364	11/89 (12.4)	65/246 (26.4)	9/29 (31.0)

[a] p = 0.02 for difference between CES groups by Fisher's exact test
[b] p = 0.01 for difference between CES groups by chi-square test
[c] p = 0.01 for difference between CES groups by Fisher's exact test

infertility (Table II). This, however, did not reach statistical significance. The age of our patients ranged from 22.9 to 44.95 years, with a mean and median of 35 years. The clinical pregnancy rate decreased significantly with increasing female age, with a sharp decline seen after 40 years (Table II). For the purpose of analysis, CES scores were categorised into three groups: CES group one (score 9–10), CES group two (score 11–13) and CES group three (score 14–15). There were, in total, 89 embryo transfers in CES group one, 246 in CES group two, and 29 in CES group three. The distribution of CES group scores did not differ significantly in the various age populations (Table III), with the majority of scores distributed around the mean. Thus, there was no bias towards better embryo scores in the younger age groups.

There was a trend towards better clinical pregnancy, implantation and live-birth rates with increasing CES scores, which did not reach statistical significance. When CES was analysed according to the defined groups, this trend was significant by chi-square test and Fisher's exact test (Table IV). The correlation between CES and clinical pregnancy rates within each age group is not as obvious in the older age groups, probably because of the smaller numbers of transfers in the older age groups, the majority of patients (79%) being < 38 years of age (Fig. 1).

Logistic regression was performed to determine the significant predictors of pregnancy outcome in terms of clinical pregnancy and live-birth (Table V). Age and CES group score were included in the analyses. Infertility diagnosis was excluded, as this was not found to be statistically significant in the initial analyses, as shown in Table II. It was found that both age and CES group score were independent predictors of clinical pregnancy. However, for the prediction of live-births, logistic regression showed that only CES group score was significant. With regard to multiple pregnancies, it was found that the risk was higher in the younger patients compared to older patients (Table VI). The numbers were however too small for meaningful analysis of the influence of CES on multiple births.

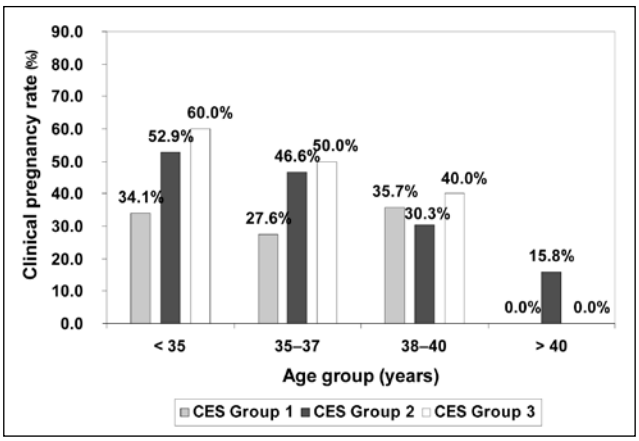

Fig. 1 Bar chart shows the clinical pregnancy rate for each CES group by age group.

DISCUSSION

The objective of this study was to assess the effectiveness of using a simple five-point embryo scoring system in predicting pregnancy outcome. Apart from the traditional

Table V. Variables selected as predictive of pregnancy outcome.

Factor	Odds-ratio (95% CI)
Variables predictive of clinical pregnancy	
Female age <35 years compared to >40 years[a]	7.32 (2.11–25.47)[b]
Female age 35–38 years compared to >40 years[a]	5.54 (1.56–19.72)[c]
CES group 1 compared to group 3[d]	1.95 (1.15–3.30)[e]
CES group 2 compared to group 3[d]	2.44 (1.02–5.83)[f]
Variables predictive of live birth	
CES group 1 compared to group 3[g]	2.58 (1.29–5.18)[h]
CES group 2 compared to group 3[g]	3.13 (1.13–8.67)[i]

[a] p = 0.005 for overall significance of female age in prediction of clinical pregnancy
[b] p = 0.002
[c] p = 0.008
[d] p = 0.028 for overall significance of CES group score in prediction of clinical pregnancy
[e] p = 0.013
[f] p = 0.045
[g] p = 0.020 for overall significance of CES group score in prediction of live birth
[h] p = 0.008
[i] p = 0.028

Table VI. Age and multiple births.

	Total	Mean age (years)	SD	SEM
Singleton births	60	34.5	3.5	0.45
Multiple births	25	31.9	3.1	0.62

SD: standard deviation; SEM: standard error of mean
Two-tailed significance 0.001 if equal variances assumed

outcome measures of clinical pregnancy and implantation rates, we also analysed live-birth and multiple pregnancy rates. Such a scoring system if effective, would have definite practical advantages being easily performed and interpreted with little room for inter-observer variation. Although there is a lack of literature comparing the difficulty and exact amount of time required to perform different scoring methods, our embryologists have found the ease of use and objective nature of the proposed system highly advantageous.

As the transfers involved only fresh embryos derived from IVF/ ICSI cycles, and were performed over a period of two years by a single team of embryologists and reproductive specialists, any bias due to treatment changes was avoided. There is a host of other parameters for which there has been conflicting data regarding their influence on IVF outcome. These include basal follicle-stimulating hormone,[24-26] day-one stimulation levels of luteinising hormone, progesterone, oestradiol,[21,22, 27,28] as well as duration of infertility, length and type of stimulation and rank of attempt.[21,28-30] However, these parameters were not included in our analysis, as our pregnancy outcome was calculated from the time of embryo transfer.

Our overall pregnancy, implantation and live-birth rates are comparable with those reported by other centres.[31] With regard to infertility diagnoses, we found that the clinical pregnancy rate was lower in cases involving female infertility than in cases involving male infertility. Although this finding may not have reached statistical significance, it is in keeping with previous studies,[21,22] and emphasises the importance of the female genital tract in pregnancy and implantation. The increasing proficiency in ICSI is likely to have a mitigating effect on male infertility. It is thought that male factors may decrease fertilisation and transfer rates, but have a diminished effect on clinical pregnancy and implantation rate.[18,22] Female age, on the other hand, clearly had a profound effect on clinical pregnancy rate, which was independent of the CES score. The negative effects of age on reproductive, capacity are widely accepted,[31,32] and again probably reflect the importance of the female genital tract in pregnancy and implantation. Increasing age may have a detrimental effect on uterine receptivity, or more likely, result in increased genetic defects in the oocyte, which have an adverse impact on implantation.[33,34]

This study confirms that our simplified five-point embryo scoring system is effective in predicting pregnancy outcome in triple embryo transfers. While some authors have preferred to make an assessment of the likelihood of a successful pregnancy based on the best embryo transferred, others have incorporated all transferred embryos either as a cumulative score or an average score.[21,22] In most Asian countries, where the practice is to transfer more than two embryos, the "cumulative" score may provide a simple and effective means of prognosticating outcome. Nevertheless, it is recognised that the mean score may be useful or equitable when comparing outcomes of cycles where a different number of embryos is transferred.

While there was a trend towards better pregnancy

outcomes with increasing CES, this only became significant when the scores were analysed in three groups. We therefore propose that for triple embryo transfers scored by the five-point scoring system, embryo transfers may be categorised into three groups: (1) "Fair" (CES group one with scores 9–10); (2) "Good" (CES group two with scores 11–13); (3) "Excellent" (CES group three with scores 14–15). This categorisation would, in turn, help to prognosticate outcome in terms of clinical pregnancy, implantation and live-birth. Although it was difficult to find a good comparative study because different inclusion criteria and outcome measures tend to be used in different papers, our results appear comparable with those of Ziebe et al.[12] This comparison is not perfect, as Ziebe's study included single and dual transfers, in addition to triple transfers of embryos of identical cleavage stage and identical quality score. Our study of triple embryo transfers, on the other hand, may be a more realistic model for IVF, where most patients have several embryos available for transfer but of different cleavage stage and quality. Nevertheless, it indicates that the predictive value of our simplified embryo scoring system is not compromised.

Furthermore, our findings show that there is a strong correlation between age and risk of multiple births. The numbers were, however, too small for the analysis of the correlation between CES and multiple births. Although earlier publications indicated that good embryo quality might be associated with increased multiple pregnancy rates,[8-10] a more recent observational study, which takes into consideration the independent effect of age and number of embryos transferred, has reported that while embryo quality is correlated with live-birth rate, it may not be correlated with multiple-birth rate.[35] Certainly, the risk of multiple pregnancies is strongly associated with the number of embryos transferred and the patient's age, being higher in the younger, as opposed to the older, maternal age group.[36,37]

Our ultimate aim would be to utilise this scoring system for the elective transfer of two or even single embryos, particularly in the younger age group, where the risk of multiple pregnancies is especially high. We are currently in the process of accumulating data on elective double embryo transfers. Recently, much work has also been published regarding other screening parameters, such as oocyte scoring,[38] pronuclear zygote scoring[39-42] and early cleavage status.[15,43,44] These alternative scoring systems appear promising, and warrant further attention as they may possibly be combined with our five-point embryo scoring system to give a even better prediction of pregnancy outcome.

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