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Embryo implantation in in vitro fertilization and intracytoplasmic sperm injection: impact of cleavage status, morphology grade, and number of embryos transferred

[Male Factor]

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Outline

Graphics

Abstract

Objective: The aims of this study were to compare preimplantation embryo quality in intracytoplasmic sperm injection (ICSI) with standard IVF and to examine the impact of age and number and quality of embryos transferred on implantation and pregnancy.

Design: Retrospective, controlled clinical study.

Setting: Academic tertiary center.

Patient(s): We examined 211 consecutive couples undergoing ICSI who were matched with 211 couples undergoing IVF therapy during the same time frame.

Intervention(s): In vitro embryo culture.

Main Outcome Measure(s): Day 3 embryo quality as judged by the number of blastomeres and morphology scoring.

Result(s): Patients undergoing ICSI had a significantly reduced number of embryos with good morphology and cleavage compared with IVF cases. Nevertheless, pregnancy and abortion rates were similar when adjusted by age and number of embryos transferred. Average cleavage status and age were significant predictors of implantation. Women of advanced age had significantly lower embryo cleavage and implantation rates.

Conclusion(s): [1] The cleaving status of day 3 embryos is a valuable, although limited, indicator of implantation outcome. [2] In vitro fertilization-derived embryos had better cleavage rates and morphology scores than ICSI-derived embryos; however, the implantation potential was similar for both groups. [3] The age-related decline in implantation rate was associated with impaired embryo growth rates.

Intracytoplasmic sperm injection (ICSI) has become a powerful therapeutic tool to overcome fertilization failure when dealing with various degrees and types of male infertility (1, 2). Today, ICSI is indicated after failure of other therapeutic approaches or as the initial treatment of choice, depending on the extent of sperm dysfunction(s). The versatility of ICSI is demonstrated by its success when using ejaculated (antegrade or retrograde), epididymal, or testicular sperm (3). In addition, recent evidence indicates that the outcome of ICSI is superior for implantation rate compared with IVF using a high-insemination concentration in cases with moderate and severe teratozoospermia, and with an adequate motile sperm fraction (4). This finding suggested the possibility that "toxic" sperm factors (reactive oxygen species or others) may be released by abnormal and dysfunctional sperm resulting in damage to the early embryo and leading to a lower proportion of embryos with good morphological scores compared with ICSI (4, 5).

Although the overall pregnancy rates after the transfer of multiple embryos after ICSI seem to be similar to those of standard IVF, the possibility still exists that the presence of severe sperm abnormalities and/or technical damage to the oocytes in ICSI cycles may compromise embryo developmental potential. Compared with IVF, ICSI has been reported to have either a higher fertilization rate and embryos with better morphology (6), or contrarily, fewer embryos with good morphology and lower pregnancy rates (7). Successful implantation is the major attribute of embryo quality, and morphology and cleaving status are used to evaluate embryo developmental potential before uterine transfer (8, 9). Pregnancy rates increase with the transfer of embryos of superior morphology (10) and with embryos showing a faster growth rate (11). The age of the female is another major factor determining successful implantation in ICSI (12) and IVF (13). Furthermore, embryo quality and age have been suggested as the only factors that affect pregnancy rates after IVF (10).

We sought to address these issues by taking advantage of the examination of the quality of preimplantation embryos (as revealed by cleaving stage and morphological characteristics) on being kept under culture conditions for up to 72 hours (day 3 transfers). The following questions were asked: [1] Are there differences in embryo morphology or cleaving status in ICSI versus IVF? [2] Can those embryo features be used as indicators of preimplantation embryo quality? [3] What is the combined impact of age and the quality and number of embryos transferred on pregnancy outcome? To answer these questions, we examined patients undergoing ICSI treated within a defined period of time and who were transferred two to six embryos. As controls, we examined couples undergoing standard IVF within the same period who were matched by the female's age and number of embryos transferred, and who had non-male factor infertility.

MATERIALS AND METHODS

Patient Selection

Two hundred eleven consecutive couples who underwent IVF therapy augmented with ICSI from April 1995 to December 1997, with day 3 transfers of two to six embryos, were included in the study. In our unit, patients are selected for the ICSI program according to the following indications: [1] men with poor semen parameters predictive of partial or total fertilization failure (i.e., $<10^6$ total spermatozoa with adequate progressive motility after separation of the motile sperm function and/or a dysfunctional diagnosis as established by poor sperm-zona pellucida binding capacity under hemizona assay conditions); [2] previous failed fertilization in IVF; and [3] presence of obstructive or nonobstructive azoospermia, where ICSI is combined with sperm extraction from the testes or the epididymis (12, 14). Only couples using ejaculated sperm were examined in these studies. All ICSI couples participating in the study gave written consent under approval of the institutional review board of Eastern Virginia Medical School.

Two hundred eleven consecutive couples who underwent IVF therapy within the same time frame with non-male factor infertility were selected as controls. Female etiologic factors were tubal-peritoneal disease, endometriosis, or ovulatory dysfunction. Non-male factor infertility was defined on the basis of the presence of the following semen parameters: sperm concentration $>20 \times 10^6$/mL, >50% progressive motility, and >4% normal morphology by strict criteria. These couples were selected randomly from our computerized database during the same time frame, were matched by the female's age, and had two to six embryos transferred per attempt.

Procedures of IVF and ICSI

All women had a basal determination of cycle day 3 serum FSH, LH, and E_2 levels by a microenzymatic immunological assay (Abbott Pharmaceuticals, Chicago, IL). Controlled ovarian hyperstimulation was accomplished by a combination of a GnRH agonist (Lupron; Tap Pharmaceuticals, Abbott Park, IL) and FSH (Metrodin or Fertinex; Serono Laboratories, Randolph, MA) following protocols published elsewhere (4, 12). Transvaginal follicular aspiration was performed under local anesthesia 34-35 hours after hCG administration. The IVF and ICSI techniques conducted in our laboratory have been extensively described previously (4, 12).

Uterine transfer of embryos was performed approximately 66-72 hours after sperm injection or insemination. Embryos were transferred in transfer medium consisting of Ham's F-10 medium (GIBCO, Grand Island, NY) supplemented with 7.5% human serum albumin (Irvine Scientific, Santa Ana, CA) and loaded into a Wallace catheter (Marlow Surgical Technologies, Willoughby, OH). It is the current policy of our program to transfer three embryos to patients <=33 years of age, four embryos to women 34-38 years, and five or more embryos (if available) to women >=39 years of age. This policy reflects an attempt to minimize the risk of multiple pregnancies in the younger women and to enhance the establishment of pregnancies in the older women. The selected number of embryos to be transferred was determined immediately before transfer on the basis of those criteria and the best morphology and cleaving status. The excess embryos were cryopreserved. Results of "fresh" transfers only were considered in this study. All patients received progesterone supplementation (50 mg/d IM) of the luteal phase starting on the day of transfer.

Embryo Quality Grading

The morphological condition (grading) of cleaving embryos was assessed immediately before transfer following the criteria outlined by Veeck (15). The grading system is as follows, with grade 1 representing the best morphological condition. Grade 1: embryo with blastomeres of equal size; no cytoplasmic fragments. Grade 2: embryo with blastomeres of equal size; minor cytoplasmic fragments or blebs. Grade 3: embryo with blastomeres of distinctly unequal size; few cytoplasmic fragments or none. Grade 4: embryo with blastomeres of equal or unequal size; significant cytoplasmic fragmentation. Grade 5: embryo with few blastomeres of any size; severe or complete fragmentation. The cleaving status represented the number of blastomeres in the embryos judged immediately before transfer. The cleavage stage and morphology grade of each individual embryo was determined and entered into the database.

Definitions

The implantation rate was defined as the number of gestational sac(s) confirmed by ultrasound divided by the total number of embryos transferred. A clinical pregnancy was diagnosed by identification of a gestational sac(s) observed by vaginal ultrasonography. Other definitions were as follows: average morphology grade: sum of embryos' morphology grade per transfer/number of embryos transferred per transfer; average cleavage stage: sum of the number of blastomeres per transfer/number of embryos transferred per transfer; embryo of good morphology: grade 1 or 2 embryo; embryo of poor morphology: grade 4 or 5 embryo; embryo with good cleavage: embryo with eight or more blastomeres; embryo with poor cleavage: embryo with four or less blastomeres; number of embryos with good morphology (per transfer): number of embryos grade 2 or less per transfer; number of embryos with poor morphology (per transfer): number of embryos grade 4 or more per transfer; number of embryos with good cleavage (per transfer): the number of embryos with eight or more blastomeres per transfer: number of embryos with poor cleavage (per transfer): number of embryos with four or less blastomeres per transfer.

Statistical Analysis

Comparisons of age, basal serum FSH, number of embryos transferred, embryo morphology grade and cleavage, number of pregnancies, and number of abortions between ICSI and IVF patients were made with unpaired *t*-tests, $[chi]^2$, or Kruskal-Wallis one-way analysis of variance for ranks as appropriate for the data. Logistic regression was used to assess the impact of age, embryos transferred, morphology grade, cleavage, and treatment type on pregnancy. The generalized estimating equation approach with a binomial model was used to assess the impact of these variables on fertilization and implantation rates. This method treated implantation and fertilization as dichotomous variables with multiple events for each subject. Univariate and multivariable analyses were made for all variables potentially influencing pregnancy, implantation, and fertilization. Relationships between the number of embryos transferred, morphology grade, cleavage, and age were analyzed by Pearson or Spearman correlation as appropriate to the data. Data are presented as means ± standard deviation unless otherwise noted.

RESULTS

There were a total of 422 patients, 211 ICSI and 211 IVF, with 1,737 embryos transferred available for analysis. The average number of embryos transferred was 4.1 ± 1.0. There were 164 patients with gestational sacs identified by vaginal ultrasonography for a clinical pregnancy rate of 39% (164 of 422). The abortion rate was 23% (38 of 164), and the delivery rate was 30% (126 of 422).

For the overall population (combined analysis of the 422 patients), the average embryo cleavage was the most significant predictor of pregnancy ($P<.0001$). Average embryo cleavage and age were the most significant predictors of implantation ($P<.0001$). Addition of all other variables did not significantly contribute to the prediction of pregnancy or implantation. Although embryo morphology did not turn out to be significant, it was significantly correlated with cleavage ($r = -0.6$).

Table 1 presents comparisons of patients undergoing ICSI and IVF. Groups were matched for age and number of embryos transferred. The fertilization rate was significantly higher for patients undergoing IVF, but the two groups did not differ significantly in pregnancy or implantation. Compared with ICSI, IVF patients had significantly greater numbers of embryos transferred classified as good morphology and significantly fewer classified as poor morphology, and had a significantly lower average morphology grade (i.e., had embryos of superior morphology). Also, IVF patients had a significantly greater mean number of good cleavage embryos transferred, but the two groups did not differ significantly in poor cleavage embryos transferred. When analyzed as average cleavage stage, IVF patients had significantly greater mean cleavage than ICSI patients.

Variable	Patient group: ICSI	Patient group: IVF	*P* value
Age (y)	34.9 ± 4.0	35.1 ± 4.0	NS
No. of embryos transferred	4.1 ± 1.0	4.1 ± 1.0	NS
Basal FSH level (mIU/mL)	7.6 ± 2.1	7.4 ± 1.9	NS
Fertilization rate (%)	76	88	<.0001*
Pregnancy rate (%) (no. of patients who got pregnant/total no. of patients)	39 (82/211)	39 (82/211)	NS
Abortion rate (%) (no. of patients who had abortions/total no. of pregnancies)	21 (17/82)	26 (21/82)	NS
Implantation rate (%)	14	13	NS
No. of embryos with good morphology	2.3 ± 1.6	2.8 ± 1.5	<.006†
No. of embryos with poor morphology	0.9 ± 1.3	0.6 ± 1.1	<.002†
Average morphology score	2.6 ± 0.8	2.3 ± 0.8	<.0002‡
No. of embryos with good cleavage	1.8 ± 1.5	2.2 ± 1.4	<.001‡
No. of embryos with poor cleavage	0.8 ± 1.2	0.7 ± 1.1	NS
Average cleavage stage	6.3 ± 1.6	6.7 ± 1.4	<.004

Note: Values are means (±SD) unless otherwise indicated. NS = not significant.
* Estimated from the generalized estimating equation model.
† Good morphology defined as grade 1 or 2; poor morphology defined as grade 4 or 5.
‡ Good cleavage defined as 8 or more blastomeres; poor cleavage defined as 4 or less blastomeres.

TABLE 1 Comparison of ICSI and IVF patients.

Table 2 presents Spearman rank order correlations of the variables potentially influencing pregnancy and implantation for ICSI and IVF patients. A correlation of >=0.14 was statistically significantly different from zero. Overall, as age increased, so did the number of embryos transferred (a direct consequence of the policy to transfer more embryos in women of advanced age). Correlations were similar for ICSI and IVF patients except for age and number of poor cleavage embryos transferred. As age increased, the number of poor cleavage embryos transferred also increased for ICSI patients, but not for IVF patients (P = .019). As would be expected, measures of morphology and cleavage were generally highly correlated.

	Age	FSH	No. of ET	GCL	PCL	ACL	GMO	PMO	AMO
Age (y)									
FSH									
ICSI	.00								
IVF	−.07								
No. of ET									
ICSI	.46	−.04							
IVF	.55	−.09							

IVF	[illegible]	[illegible]						
GCL								
ICSI	−.04	−.13	.15					
IVF	.04	−.11	.21					
PCL								
ICSI	.31	.05	.22	−.66				
IVF	.09	.06	.20	−.62				
ACL								
ICSI	−.25	−.10	−.11	.88	−.84			
IVF	−.13	−.13	−.10	.84	−.81			
GMO								
ICSI	.09	−.09	.30	.44	−.30	.41		
IVF	.23	−.13	.44	.52	−.29	.41		
PMO								
ICSI	.17	.05	.12	−.43	.51	−.54	−.67	
IVF	.09	.06	.13	−.49	.62	−.60	−.57	
AMO								
ICSI	.08	.08	−.00	−.47	.43	−.55	−.88	.82
IVF	.08	.01	.09	−.50	.46	−.58	−.74	.75

Note: ACL = average cleavage of embryos transferred; AMO = average morphology of embryos transferred; ET = embryos transferred; GCL = good cleavage embryos transferred; GMO = good morphology embryos transferred; PCL = poor cleavage embryos transferred; PMO = poor morphology embryos transferred.

TABLE 2 Spearman's correlation coefficients for ICSI and IVF patients.

Separate regressions for ICSI and IVF patients were made for prediction of pregnancy. For ICSI patients, good and poor morphology, good and poor cleavage, average morphology, and average cleavage were all, in univariate analyses, significant predictors of pregnancy ($P<.001$ for all). Age, FSH, and number of embryos transferred were not significantly related to pregnancy. For the multivariable analysis, average cleavage and number of good morphology embryos transferred were the only significant, independent predictors of pregnancy ($P<.001$). When adjusted for these two variables, none of the others were statistically significant.

For IVF patients, good and poor cleavage, average cleavage, and poor morphology were, in univariate analyses, significant predictors of pregnancy ($P<.001$ for all). Age, FSH, number of embryos transferred, good morphology, and average morphology were not significantly related to pregnancy. For the multivariable analysis, poor cleavage was the only significant, independent predictor of pregnancy ($P<.001$). When adjusted for poor cleavage, none of the others were statistically significant.

Figure 1 plots predicted pregnancy rates from the regression models for ICSI and IVF patients. For ICSI patients (Fig. 1A), number of good morphology embryos transferred is plotted on the horizontal axis. The predicted probabilities of pregnancy for an ICSI patient with average cleavage at the mean level (6.3

blastomeres) is plotted for each number of good morphology embryos transferred along with 1 SD below and above the mean average cleavage. This plot illustrates the differential effects of average cleavage along with number of good morphology embryos transferred. The predicted probability of pregnancy for an ICSI patient with average cleavage of 6.3 ranged from approximately 24% with zero good morphology embryos transferred to 61% with six good morphology embryos transferred. Figure 1B plots the number of poor cleavage embryos transferred for IVF patients. The probability of pregnancy ranged from 46% if no poor cleavage embryos were transferred to <5% if six were transferred.

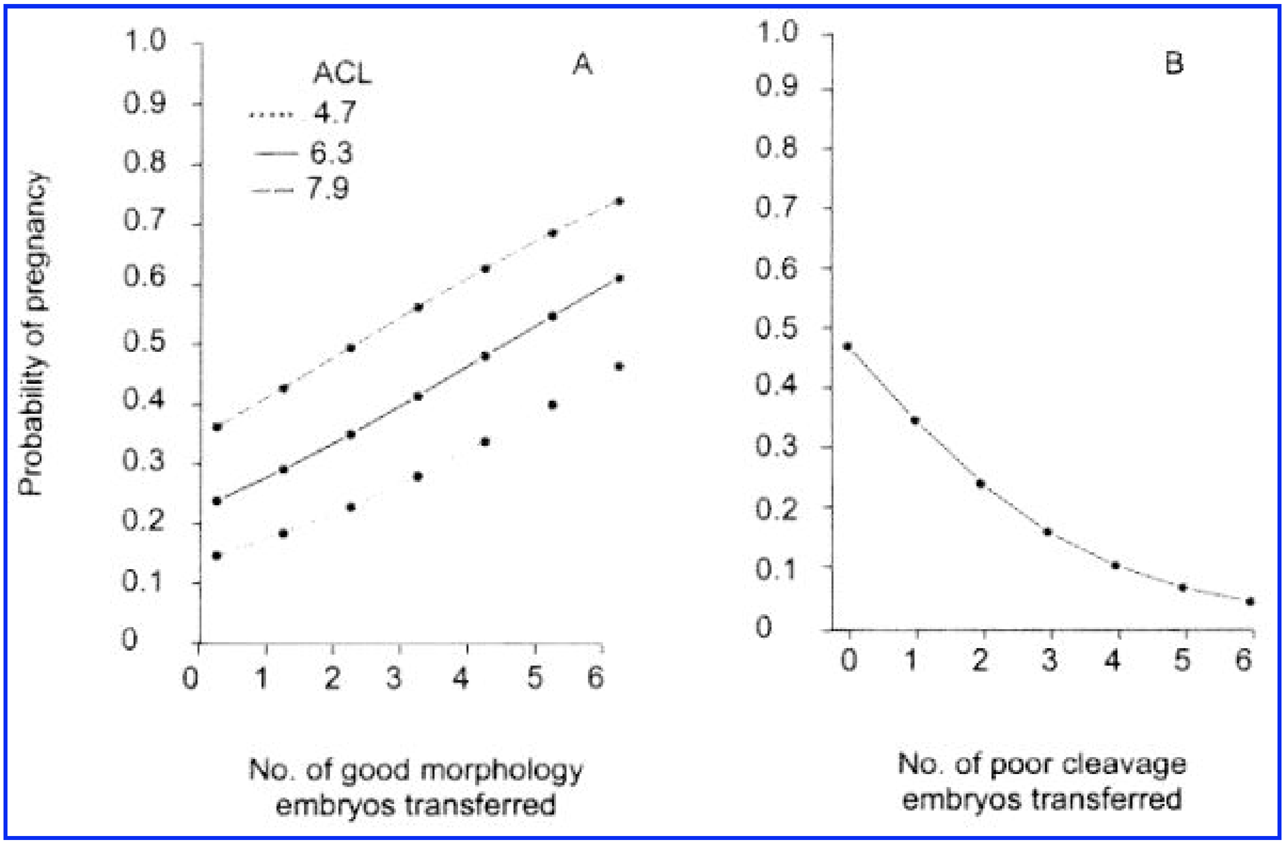

FIGURE 1 Predicted clinical pregnancy rates for ICSI (**A**) and IVF (**B**) patients from logistic regression models.

Separate regressions for ICSI and IVF patients were made for prediction of implantation. For ICSI patients, age, number of embryos transferred, good and poor morphology, good and poor cleavage, average morphology, and average cleavage were all, in univariate analyses, significant predictors of implantation ($P<.05$ for all). Follicle-stimulating hormone was not significantly related to implantation. For the multivariable analysis, age and number of poor cleavage embryos transferred were the only significant, independent predictors of implantation ($P<.001$). When adjusted for these two variables, none of the others were statistically significant.

For IVF patients, age, number of embryos transferred, good and poor cleavage, and average cleavage were, in univariate analyses, significant predictors of implantation ($P<.02$ for all). FSH, good and poor morphology, and average morphology were not significantly related to implantation. For the multivariable analysis, age and poor cleavage were the only significant, independent predictors of implantation ($P<.001$). When adjusted for these two variables, none of the others were statistically significant.

Figure 2 plots predicted implantation rates from the generalized estimating equation models for ICSI and IVF patients. The number of poor cleavage embryos transferred is plotted on the horizontal axis. The predicted probabilities of implantation for a patient age 35 (mean age) is plotted for each number of poor cleavage embryos transferred (solid line). The dashed line represents a patient age 31 (1 SD below the mean age of 35), and the dotted line represents a patient age 39 (1 SD above the mean). The predicted probability of implantation for an ICSI patient age 35 ranged from approximately 19% if no poor cleavage embryos were transferred to <1% if six were transferred (Fig. 2A). The range was similar for a 35-year-old IVF patient, approximately 16% if no poor cleavage embryos were transferred to <1% if six were transferred (Fig. 2B).

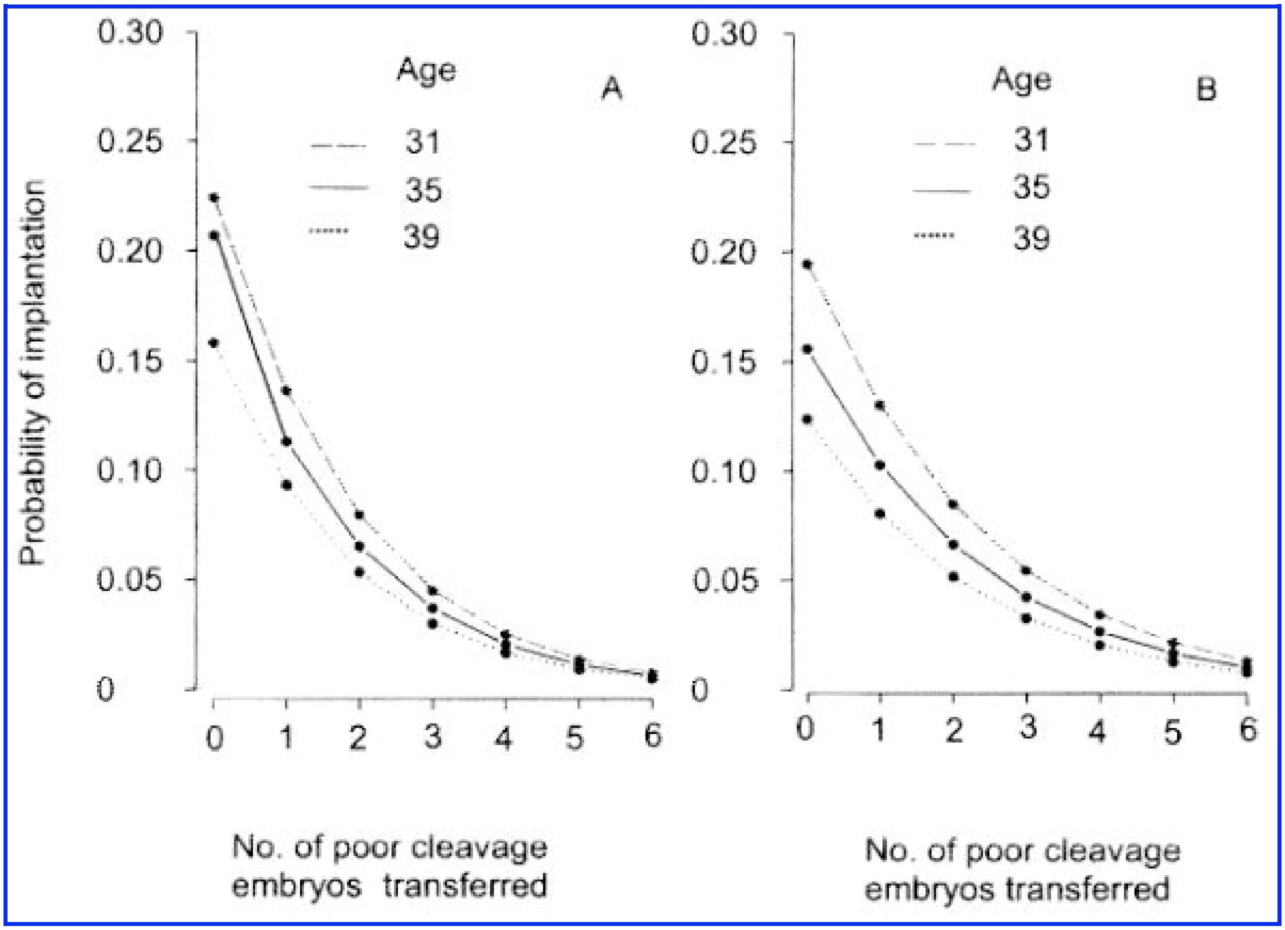

FIGURE 2 Predicted implantation rates for ICSI (**A**) and IVF (**B**) patients from generalized estimating equation models.

DISCUSSION

The accurate evaluation of embryo implantation potential becomes crucial when trying to enhance pregnancy outcome and to reduce the complication of multiple pregnancies. The morphological appearance and growth rate (cleavage stage) of embryos, which can be observed with noninvasive techniques, are commonly used as parameters to judge embryo quality. Most published studies have analyzed implantation rates based on individual embryo parameters (8, 9, 11) by assessing single ETs (9) or by the examination of single, dual, or triple transfers of embryos of identical cleavage or morphological conditions (16). We analyzed the combined impact of the various embryo numbers and different embryo qualities transferred on pregnancy outcome, a daily dilemma in the clinical IVF and ICSI setting.

From the multiple comparisons performed in the study for the overall population, it can be established that embryo cleavage is a superior indicator of implantation outcome compared with embryo morphology. Although embryo cleavage and morphology are strongly correlated with each other, morphology grading adds little to the overall logistic regression analysis. McKiernan and Bavister (17) suggested that the morulae and blastocysts developing in vitro from faster or slower cleaving embryos may be qualitatively as well as quantitatively different (17). These findings concur with our analysis of in vitro cultured day 3 embryos. However, the overall predictive power of these indicators is weak; even when the transfer of good-quality embryos alone was considered, the correlation with pregnancy was relatively low. Therefore, although the analysis of cleavage and morphology is a relatively valuable, noninvasive technique for the evaluation of embryo quality the limitation of their application is obvious. Other indicators or biomarkers of implantation potential need to be sought.

When pregnancy was analyzed as the main outcome, average embryo cleavage and number of embryos with good morphology (ICSI), and number of embryos with poor morphology (IVF) were demonstrated as best predictors. Superior average cleavage rates, higher numbers of good morphology embryos, and lower number of poor morphology embryos transferred were predictive of a clinical pregnancy. When implantation was analyzed as the main outcome, age and number of embryos with poor cleavage were the most significant predictors in both ICSI and IVF settings. Increasing maternal age and larger number of poor cleavage embryos transferred were inversely related to implantation.

Patients from the IVF group appeared to have embryos of more advanced cleaving status and better morphological appearance than the ICSI patient group. However, the implantation and postimplantation developmental capacity of embryos transferred after IVF and ICSI was similar. It remains to be established whether the observed differences in embryo quality are the result of damage secondary to the oocyte micromanipulation or are due to more intrinsic gamete abnormalities. We acknowledge that these results may vary among different IVF programs as the micromanipulation technique may vary significantly. Our data confirmed the relationship between embryo cleaving status and IVF pregnancy outcome as demonstrated by other investigators (7-10, 16); however, both embryo cleaving status and morphology showed a significant relationship with ICSI outcome in terms of pregnancy. Zhu et al. (18) also reported that the stage of embryo development at transfer appeared to exert a powerful influence on the successful establishment of pregnancy after ICSI when analyzing day 2 embryos (18).

The presence of embryos with poor cleavage and morphology had a significant negative impact on pregnancy outcome. However, this finding may be biased by the fact that more embryos are replaced with increasing age or in those cases with poor embryo quality. Therefore, these poor prognosis cases are more likely to have additional embryos of lower quality transferred. The ideal way to determine the impact of poor-quality embryos on pregnancy outcome is to perform a prospective analysis controlled for age. If corroborated, those results might suggest that the transfer of poor quality embryos should be reconsidered if enough embryos of good quality are present. Tasdemir et al. (19) reached a different conclusion (i.e., the transfer of poor quality embryos may enhance pregnancy rates) using a different embryo quality definition. Culturing human embryos in groups may enhance the cleavage rates and embryo scores of embryos in vitro (20), but the implantation rate of individual embryos was not affected by the number of embryos transferred (21).

Age is one of the major factors related to pregnancy outcome (10, 12, 13). The cleaving status was more dramatically impacted by age than embryo morphological appearance, i.e., the major problem in the embryos of older women was a decreased growth rate rather than an impaired morphological appearance. Compared with the embryos of younger women, the embryos of older women had lower implantation rates even within the same morphological grade, thus, confirming previous reports (9, 13).

The differing implantation ability of embryos within the same morphological or cleaving status category and its variation with age demonstrate the limitation of morphological or cleaving status to represent total embryo quality. Minaretzis and colleagues (22) studied day 2 transfers with use of a multivariate analysis approach and concluded that only maternal age negatively influenced live birth rates, whereas good-quality embryos positively influenced this rate. As was suggested earlier (23), they demonstrated that each good-quality embryo has a different potential for live and multiple births (22). The present study corroborates and extends these findings as related to day 3 embryo transfers.

We conclude the following: [1] embryo cleavage and age are the most significant independent predictors of implantation in IVF and ICSI; however, overall, average embryo quality parameters (growth rate and morphology) have only limited power to predict pregnancy or implantation when multiple embryos are transferred; [2] ICSI is associated with a decrease in morphological and cleaving parameters compared with IVF, but the implantation potential of a given cohort of embryos transferred after IVF or ICSI is similar; and [3] the embryos from women of advanced age demonstrate a significant impairment in cleaving status. Whether these variables or other biomarkers have more predictive value for implantation when assessing blastocysts immediately before transfer has not been established.

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Key Words: Implantation; IVF; ICSI; average cleavage status; morphology score

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