

Optimizing Embryo Selection: Developmental Competence and Reproductive Outcomes Following Single versus Combined Transfer of Top- and Poor-Quality Embryos

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Abstract

Embryo selection based on developmental competence is a key determinant of successful assisted reproductive technology outcomes. This study aimed to evaluate whether transferring poor-quality embryos (PQEs) alongside a top-quality embryo (TQE) influences pregnancy and live birth outcomes in fresh intracytoplasmic sperm injection (ICSI) cycles. In this retrospective cohort study, data from 400 women who underwent fresh ICSI cycles at Mehr Outpatient Surgical and Infertility Center, Rasht, Iran, between May 2022 and December 2023 were analyzed. Participants were classified into nine groups according to the number of embryos transferred (single, double, or triple embryo transfer) and embryo quality (different combinations of TQEs and PQEs). Biochemical pregnancy, clinical pregnancy, ongoing pregnancy, and live birth rates were compared among the groups using multivariate logistic regression adjusted for maternal age, endometrial thickness, and day of embryo transfer. Significant differences were observed in biochemical pregnancy ($P = 0.001$), clinical pregnancy ($P = 0.004$), ongoing pregnancy ($P = 0.006$), and live birth rates ($P = 0.003$) among the study groups. However, after adjustment for potential confounders, embryo transfer group was not independently associated with reproductive outcomes. Increasing maternal age was negatively associated with live birth, whereas greater endometrial thickness was positively associated with treatment success. The addition of one or more PQEs to a TQE did not improve live birth outcomes or increase multiple pregnancy rates in fresh ICSI cycles. These findings support elective single top-quality embryo transfer as an effective embryo selection strategy for optimizing reproductive outcomes while minimizing the risk of multiple gestations.

Keywords: Assisted Reproductive Technology, Embryo Transfer, Embryo Viability, Intracytoplasmic Sperm Injection, Live Birth, Pregnancy Outcome

Introduction

Despite remarkable advances in assisted reproductive technologies (ART), achieving a successful live birth remains a major challenge. Successful embryo implantation depends on the

coordinated interaction between a developmentally competent embryo and a receptive endometrium. During the preimplantation stage, embryo developmental competence is reflected by its ability to sustain normal development,

establish effective embryo–maternal communication, and ultimately result in a viable pregnancy. Although morphological assessment remains the most widely used method for embryo selection in clinical practice, the developmental potential of morphologically different embryos and their influence on reproductive outcomes continue to be subjects of considerable interest. Accumulating evidence suggests that the human endometrium functions as a biological sensor capable of recognizing embryo quality and preferentially supporting embryos with greater developmental competence while limiting the progression of developmentally compromised embryos (1).

In *in vitro* fertilization (IVF) and intracytoplasmic sperm injection (ICSI) cycles, embryo transfer (ET) is the final and one of the most critical steps determining treatment success. Both embryo quality and the number of embryos transferred influence implantation, clinical pregnancy, and live birth outcomes. When the number of transferred embryos is controlled, top-quality embryos (TQEs) consistently demonstrate superior implantation competence and higher live birth rates than poor-quality embryos (PQEs) (2). Consequently, current international guidelines, including those of the European Society of Human Reproduction and Embryology (ESHRE), recommend elective single embryo transfer (SET) in appropriately selected participants to maximize reproductive success while reducing the risks associated with multiple gestations (3,4)

Nevertheless, double embryo transfer (DET) and triple embryo transfer (TET) continue to be practiced in many ART centers, particularly for participants with advanced maternal age, previous implantation failure, or limited embryo availability. A common clinical challenge arises when only one TQE is available while the remaining embryos exhibit poor morphological quality. Under these circumstances, clinicians must decide whether transferring one or more PQEs together with a TQE could improve reproductive outcomes or whether the additional embryos provide no clinical benefit (5-7)

The biological and clinical implications of co-transferring PQEs with a TQE remain controversial. While several clinical studies suggest that implantation potential is largely determined by the intrinsic developmental competence of each embryo, experimental evidence indicates that embryos may interact with the maternal endometrium through paracrine signaling pathways capable of modulating endometrial receptivity and implantation dynamics (8). Differences in embryo quality have also been associated with distinct molecular and inflammatory responses at the maternal–embryo interface, raising the possibility that developmentally compromised embryos could influence the implantation environment. Conversely, several clinical investigations have shown that the addition of PQEs does not adversely affect pregnancy or live birth outcomes, although findings remain inconsistent and may vary according to embryo developmental stage, transfer strategy, and participant characteristics (5-8).

Despite increasing evidence regarding embryo quality and reproductive outcomes, few studies have comprehensively evaluated different combinations of embryo number and quality while accounting for important clinical confounders. Therefore, this retrospective cohort study aimed to investigate whether transferring one or more PQEs alongside a TQE influences reproductive outcomes in fresh ICSI cycles. Biochemical pregnancy, clinical pregnancy, ongoing pregnancy, and live birth rates were compared across nine embryo transfer groups defined by embryo number and morphological quality. The findings may contribute to optimizing embryo selection and transfer strategies by providing further evidence regarding the clinical significance of embryo quality in relation to developmental competence and successful implantation.

Materials and Methods

This retrospective cohort study was conducted using data from women who underwent fresh ICSI cycles at Mehr Outpatient Surgical and Infertility Center, Rasht, Iran, between May

2022 and December 2023. Clinical and embryological data were retrieved from the institute's computerized database, which is routinely maintained for participant management. The study protocol was approved by the Research Ethics Committees of Guilan University of Medical Sciences (IR.GUMS.REC.1401.081).

Women aged ≤ 43 years who underwent fresh embryo transfer following ICSI and received at least one embryo were eligible for inclusion. Participants were excluded if they had undergone testicular sperm extraction (TESE), experienced more than two previous failed ART cycles, or had incomplete clinical or laboratory records. After applying the eligibility criteria, 400 women were included in the final analysis.

Embryo morphology was assessed as a surrogate marker of developmental competence using standardized grading systems. Cleavage-stage embryos were evaluated on day 3 according to the criteria described by Stensen et al. [9], based on blastomere number, blastomere symmetry, and the degree of cytoplasmic fragmentation. Blastocysts were evaluated on day 5 using Gardner's grading system [10], which considers the degree of blastocyst expansion together with the quality of the inner cell mass and trophectoderm. Based on these morphological characteristics, embryos were classified as TQEs or PQEs according to the routine clinical criteria of the study center.

Participants were categorized into nine groups according to both the number and morphological quality of embryos transferred during the fresh embryo transfer cycle (Table 1).

This classification enabled a comprehensive comparison of reproductive outcomes across different combinations of embryo number and morphological quality. Controlled ovarian stimulation was performed using either a long gonadotropin-releasing hormone (GnRH) agonist protocol or a GnRH antagonist protocol according to ovarian reserve assessment and clinical judgment. In the long protocol, a half-dose depot GnRH agonist (Variopeptyle 1.875 mg; Varian Pharmed, Iran) was administered on day 21 of the preceding menstrual cycle. In antagonist cycles, ovarian stimulation commenced on day 2 of the menstrual cycle, and a GnRH antagonist

Table 1. Table 1. Participant Grouping According to Embryo Transfer Strategy

Group Number	Morphological quality of embryos transferred
Group 1	SET with one TQE
Group 2	SET with one PQE
Group 3	DET with two TQEs
Group 4	DET with two PQEs
Group 5	DET with one TQE and one PQE
Group 6	TET with three TQEs
Group 7	TET with two TQEs and one PQE
Group 8	TET with one TQE and two PQEs
Group 9	TET with three PQEs

(Cetrotide®, 0.25 mg/day; EMD Serono) was initiated when two to three follicles reached a mean diameter of 18–20 mm.

Ovarian stimulation was initiated with 150 IU recombinant follicle-stimulating hormone (rFSH; Karma-FSH, Homa Pharmed, Iran), followed by individualized dose adjustment using recombinant FSH and human menopausal gonadotropin (hMG; KarmahMG, Homa Pharmed, Iran) according to ovarian response. Follicular development was monitored by transvaginal ultrasonography together with serum estradiol and progesterone measurements. Final oocyte maturation was induced using human chorionic gonadotropin (hCG; PD PREG, Pooyesh Darou, Iran) when at least two follicles reached a diameter of 17–18 mm.

Oocyte retrieval was performed 34–36 hours after hCG administration under transvaginal ultrasound guidance. Following denudation, metaphase II oocytes were fertilized by ICSI using morphologically normal spermatozoa. Fertilization was confirmed 16–18 hours later by the presence of two pronuclei and two polar bodies.

Embryo transfer was performed on day 3 or day 5 under ultrasound guidance. Luteal phase support consisted of 400 mg vagi-

Table 2. Baseline and Stimulation characteristics of women undergoing intracytoplasmic sperm injection compared in 9 groups based on

Groups Variables	G1: SET-TQ	G2: SET-PQ	G3: DET-2TQ	G4: DET-2PQ	G5: DET-TQ+PQ	G6: TET-3TQ	G7: TET-2TQ+PQ	G8: TET-TQ+2PQ	G9: TET-3PQ	P-value
Number (n)	55	37	67	23	34	84	40	34	26	
Age (yr)*	35.74±5.16	36.78±5.24	34.27±5.92	36.40±6.26	33.26±6.38	35.23±6.16	34.52±5.79	33.41±6.35	32.34±6.13	0.58--
Basal AMH (ng/ml) *	1.50±1.18	1.63±1.64	1.99±1.93	2.05±2.26	1.71±1.21	1.78±1.30	2.82±2.46	2.66±2.53	2.35±2.61	0.01
Male factor	14	24	17 (25.4)	16 (69.6)	20 (58.8)	28	26 (65)	25 (73.5)	17 (65.4)	0.001
Number of collected oocytes*	6.21±4.58	4.29±4.24	6.89±3.92	6.43±5.38	5.35±3.61	9.41±4.15	7.82±4.27	7.85±3.79	7.31±3.35	0.001
Day of embryo transfer*	3.45±0.91	3.62±1.01	3.25±1.02	4.00±1.04	3.61±0.89	3.05±0.81	3.71±0.88	3.82±0.94	3.92±1.05	0.001

* Data presented as mean ± standard deviation with ANOVA test followed by Tukey's post-hoc test. ** Data presented as n (%), chi-square test or Fisher's exact test when appropriate. G: group; SET: single embryo transfer; TQ: top quality; DET: double embryo transfer; PQ: poor quality; TET: triple embryo transfer, AMH: Anti-müllerian hormone.

nal progesterone (Cyclogest®, Alpha, UK) together with 50 mg intramuscular progesterone administered daily from the day of oocyte retrieval until the 12th week of gestation in women with confirmed pregnancy.

Clinical and laboratory variables extracted from the medical records included maternal age, body mass index (BMI), infertility duration and etiology, basal anti-Müllerian hormone (AMH) concentration, ovarian stimulation protocol, total gonadotropin dose, stimulation duration, serum hormone levels on the day of hCG administration, endometrial thickness, number of retrieved oocytes, embryo quality, day of embryo transfer, and the number and quality of transferred embryos.

The primary outcomes were biochemical pregnancy, defined by a positive serum β -human chorionic gonadotropin (β -hCG) test, clinical pregnancy confirmed by visualization of a gestational sac with fetal cardiac activity on transvaginal ultrasonography, and ongoing pregnancy. Secondary outcomes included live birth rate, number of gestational sacs, and multiple pregnancy rate.

Continuous variables were tested for normality using the Kolmogorov–Smirnov test. Normally distributed variables were compared using one-way analysis of variance (ANOVA) followed by Tukey's post hoc test. Categorical variables were analyzed using the chi-square or Fisher's exact test, as appropriate. Univariate and multivariate logistic regression analyses were performed to estimate crude and adjusted odds ratios for reproductive outcomes while controlling for maternal age, endometrial thickness, and day of embryo transfer. Statistical analyses were conducted using SPSS version 26 (SPSS Inc., Chicago, IL, USA), and a two-sided P value < 0.05 was considered statistically significant.

Results

A total of 400 women who underwent fresh ICSI cycles between 2022 and 2023 were included in this retrospective cohort study. The mean maternal age of the study population was 34.76 ± 5.98 years. Baseline demographic and clinical characteristics of the nine study groups are presented in Table 2.

No significant differences were observed among the groups regarding maternal age, body mass index (BMI), or the distribution of primary and secondary infertility. However, significant differences were identified in basal anti-Müllerian hormone (AMH) levels, the prevalence of male-factor infertility, the number of retrieved oocytes, and the day of embryo transfer. In contrast, the total gonadotropin dose ($P = 0.18$) and endometrial thickness on the day of hCG administration ($P = 0.29$) were comparable across all groups.

Overall, biochemical pregnancy, clinical pregnancy, ongoing pregnancy, and live birth occurred in 108 (27.0%), 94 (23.5%), 74 (18.5%), and 70 (17.5%) participants, respectively. As summarized in Table 3 and 4, significant differences were observed among the embryo transfer groups for biochemical pregnancy ($P = 0.001$), clinical pregnancy ($P = 0.004$), ongoing pregnancy ($P = 0.006$), and live birth rates ($P = 0.003$). Among the nine groups, the DET-2TQ group demonstrated rates ($P = 0.003$). Among the nine

groups, the DET-2TQ group demonstrated the highest live birth rate (32.8%), whereas no pregnancies were observed in the DET-2PQ group.

Morphological embryo assessment remains the most widely applied method for embryo selection in clinical practice because it reflects, albeit imperfectly, embryo developmental competence and implantation potential. Experimental studies have suggested that preimplantation embryos may communicate through paracrine signaling and secrete bioactive molecules capable of influencing the surrounding microenvironment and endometrial receptivity (11,12). Nevertheless, accumulating clinical evidence indicates that the implantation potential of individual embryos is largely independent, and the presence of a PQE does not substantially alter the developmental capacity of a co-transferred TQE (13–15). Our findings are consistent with this concept and further support the clinical value of morphology-based embryo selection.

Previous clinical studies evaluating combined transfer of top- and poor-quality embryos have reported inconsistent find-

ings. Several investigations demonstrated that SET and DET achieved comparable live birth rates, whereas DET was associated with a substantially higher risk of multiple pregnancies (13,14). Similarly, another study reported that adding a PQE did not significantly improve live birth rates but increased the likelihood of multiple gestations, particularly after cleavage-stage embryo transfer (15). In blastocyst-stage transfers, some authors observed that DET involving one good-quality and one poor-quality blastocyst increased both live birth and multiple birth rates, potentially resulting in less favorable neonatal outcomes (16). Conversely, others reported that co-transfer of a PQE with a high-quality blastocyst did not compromise reproductive outcomes and may provide a modest increase in live birth rate (17). These discrepancies may reflect differences in embryo developmental stage, participant characteristics, embryo grading criteria, and transfer policies across ART centers.

Multivariate analysis identified maternal age and endometrial thickness as the principal independent predictors of reproductive success. Increasing maternal age was associated with a progressive decline in live birth probability, whereas greater endometrial thickness was positively associated with ongoing pregnancy. These observations are biologically plausible and are consistent with previous reports emphasizing the importance of both embryo developmental competence and endometrial receptivity for successful implantation (1,7).

Taken together, our findings support current evidence favoring elective single transfer of a TQE when available. Although transferring additional PQEs does not appear to compromise reproductive outcomes, it also does not provide an independent improvement in live birth after adjustment for relevant To evaluate the independent association between embryo transfer strategy and reproductive outcomes, univariate and clinical factors. Therefore, routine addition of PQEs to a TQE cannot be justified solely to increase the probability of treatment success.

Table 3. Pregnancy outcomes (Biochemical Pregnancy and Clinical Pregnancy) across study groups and the association with clinical predictors: from univariate to multivariate adjusted analysis.

	Biochemical Pregnancy				Clinical Pregnancy			
	Univariate OR (95% CI)	P- value	Multivariate OR (95% CI)	P- value	Univariate OR (95% CI)	P- value	Multivariate OR (95% CI)	P- value
Maternal age	0.93 (0.90 to 0.97)	0.001	0.94 (0.90 to 0.98)	0.005	0.94 (0.90 to 0.98)	0.003	0.95 (0.90 to 0.98)	0.01
Endometrial thickness	1.22 (1.04 to 1.44)	0.01	1.17 (0.98 to 1.41)	0.07	1.23 (1.03 to 1.26)	0.02	1.17 (0.97 to 1.41)	0.09
Day of embryo transfer	0.72 (0.56 to 0.92)	0.008	0.74 (0.55 to 0.99)	0.04	0.71 (0.55 to 0.92)	0.01	0.68 (0.46 to 0.87)	0.12
Group 1 SET-TQ, (n=55)	Reference		Reference		Reference		Reference	
Group 2 SET-PQ, (n=37)	0.24 (0.06 to 0.88)	0.03	0.26 (0.07 to 0.97)	0.05	0.28 (0.07 to 1.08)	0.06	0.32 (0.08 to 1.27)	0.11
Group 3 DET-2TQ, (n=67)	1.91 (0.89 to 4.12)	0.09	1.48 (0.62 to 3.51)	0.37	2.05 (0.93 to 4.53)	0.07	1.70 (0.70 to 4.12)	0.24
Group 4 DET-2PQ, (n=23)	0	-	0	-	0	-	0	-
Group 5 DET-TQ+PQ, (n=34)	1.11 (0.43 to 2.86)	0.83	0.89 (0.29 to 2.31)	0.71	0.99 (0.36 to 2.72)	0.99	0.72 (0.24 to 2.17)	0.56
Group 6 TET-3TQ, (n=84)	1.19 (0.56 to 2.53)	0.64	0.93 (0.41 to 2.18)	0.87	1.22 (0.55 to 2.67)	0.62	0.97 (0.41 to 2.28)	0.95
Group 7 TET-2TQ+1PQ, (n=40)	1.01 (0.41 to 2.52)	0.98	1.03 (0.38 to 2.69)	0.95	0.81 (0.29 to 2.18)	0.67	0.81 (0.28 to 2.30)	0.69
Group 8 TET-1TQ+2PQ, (n=34)	1.27 (0.51 to 3.23)	0.61	1.11 (0.41 to 3.02)	0.85	1.16 (0.44 to 3.11)	0.76	0.98(0.34 to 2.85)	0.98
Group 9 TET-3PQ, (n=26)	0.48 (0.14 to 1.64)	0.24	0.41 (0.11 to 1.46)	0.17	0.58 (0.17 to 2.01)	0.39	0.51 (0.14 to 1.85)	0.30

Table 4. Pregnancy outcomes (Ongoing Pregnancy and Live Birth) across study groups and the association with clinical predictors: from univariate to multivariate adjusted analysis.

	Ongoing Pregnancy				Live Birth			
	Univariate OR (95% CI)	P- value	Multivariate OR (95% CI)	P- value	Univariate OR (95% CI)	P- value	Multivariate OR (95% CI)	P- value
Maternal age	0.94 (0.90 to 0.98)	0.007	0.94 (0.89 to 0.99)	0.02	0.96 (0.92 to 1.001)	0.06	0.96 (0.91 to 1.01)	0.13
Endometrial thickness	1.26 (1.05 to 1.53)	0.01	1.24 (1.01 to 1.52)	0.04	1.24 (1.02 to 1.51)	0.03	1.22 (0.99 to 1.50)	0.06
Day of embryo transfer	0.67 (0.50 to 0.89)	0.007	0.75 (0.54 to 1.05)	0.09	0.68 (0.51 to 0.92)	0.01	0.78 (0.56 to 1.10)	0.15
Group 1 SET-TQ, (n=55)	Reference		Reference		Reference		Reference	
Group 2 SET-PQ, (n=37)	0.32 (0.08 to 1.21)	0.09	0.37 (0.09 to 1.49)	0.16	0.28 (0.07 to 1.08)	0.06	0.33 (0.08 to 1.27)	0.11
Group 3 DET-2TQ, (n=67)	1.87 (0.83 to 4.23)	0.13	1.58 (0.63 to 3.94)	0.33	1.58 (0.71 to 3.53)	0.26	1.31 (0.53 to 3.21)	0.56
Group 4 DET-2PQ, (n=23)	-	-	-	0.99	-	-	-	0.99
Group 5 DET-TQ+PQ, (n=34)	0.77 (0.26 to 2.28)	0.63	0.51 (0.15 to 1.72)	0.28	0.69 (0.23 to 2.04)	0.50	0.48 (0.15 to 1.60)	0.23
Group 6 TET-3TQ, (n=84)	0.84 (0.36 to 1.95)	0.69	0.60 (0.24 to 1.52)	0.28	0.65 (0.28 to 1.51)	0.31	0.45 (0.17 to 1.15)	0.09
Group 7 TET-2TQ+1PQ, (n=40)	0.40 (0.12 to 1.34)	0.14	0.34 (0.11 to 1.40)	0.15	0.26 (0.07 to 0.99)	0.05	0.26 (0.07 to 1.02)	0.05
Group 8 TET-1TQ+2PQ, (n=34)	0.65 (0.19 to 2.26)	0.50	0.77 (0.24 to 2.42)	0.65	0.69 (0.23 to 2.04)	0.50	0.71 (0.23 to 2.19)	0.55
Group 9 TET-3PQ, (n=26)	0.65 (0.18 to 2.26)	0.50	0.57 (0.15 to 2.12)	0.41	0.42 (0.11 to 1.63)	0.21	0.39 (0.09 to 1.58)	0.18

multivariate logistic regression analyses were performed.

Maternal age was significantly associated with reproductive outcomes in the univariate analysis, including biochemical pregnancy ($P = 0.001$). After adjustment for potential confounding factors, maternal age remained an independent predictor of live birth (adjusted odds ratio [aOR]: 0.94, 95% confidence interval [CI]: 0.89–0.99), indicating a progressive decline in the likelihood of live birth with increasing maternal age.

Endometrial thickness showed a significant positive association with live birth in the univariate analysis ($P = 0.03$). Following multivariable adjustment, greater endometrial thickness remained independently associated with ongoing pregnancy (aOR: 1.24, $P = 0.04$).

Although the day of embryo transfer was significantly associated with live birth in the univariate analysis ($P = 0.01$), this association was no longer significant after multivariate adjustment ($P > 0.05$). Likewise, after adjustment for maternal age, endometrial thickness, and day of embryo transfer, the embryo transfer group was not independently associated with biochemical pregnancy, clinical pregnancy, ongoing pregnancy, or live birth.

Discussion

The present study investigated whether the transfer of one or more PQEs alongside a TQE influences reproductive outcomes in fresh ICSI cycles. Although significant differences in pregnancy and live birth rates were observed among the embryo transfer groups in the unadjusted analysis, these differences were no longer significant after adjustment for maternal age, endometrial thickness, and day of embryo transfer. These findings suggest that the addition of PQEs to a TQE does not independently improve reproductive outcomes; supporting the concept that embryo developmental competence is primarily determined by the intrinsic biological quality of each embryo rather than by the presence of additional morphologically inferior embryos.

The strengths of this study include the simultaneous evaluation of nine embryo transfer strategies representing different combinations of embryo number and morphological quality, together with adjustment for important clinical confounders. However, several limitations should be acknowledged. The retrospective design may have introduced selection bias, and the moderate sample size may have limited statistical power for some subgroup analyses. Furthermore, embryo quality was assessed exclusively by morphological criteria without adjunctive technologies such as time-lapse morphokinetic assessment or preimplantation genetic testing. Prospective multicenter studies with larger populations are warranted to validate these findings and to further elucidate the biological mechanisms underlying embryo developmental competence and embryo–endometrium interactions during implantation.

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Conflict of interest

The authors declare that there is no conflict of interest regarding the publication of this manuscript.

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