



Evaluation of the In Vitro Fertilization Success Rate in Transfer of Top-Quality Embryo Versus Poor-Quality Embryos: A Cohort Study

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Abstract

Objectives: To evaluate the in vitro fertilization success rate by transferring top- versus low-quality embryos.

Materials and Methods: This prospective cohort study was conducted on 199 infertile women. Ninety-nine patients underwent an agonist cycle (70 fresh embryo transfer [ET] and 29 frozen ET), and 100 patients received an antagonist cycle (28 fresh ET and 72 frozen ET) in the infertility department of a tertiary university-based hospital between May 2019 and March 2020. The blastocysts classified as AA, AB, and BB, as well as AC, BC, and CC were considered as top- and poor-quality embryos (TQE and PQE). The study outcomes were biochemical and clinical and determined the rate of pregnancy.

Results: The average age of the participants was 32.44 ± 5.25 years old. Women with TQE were significantly younger than those with PQE (31.35 ± 4.97 vs. 34.09 ± 5.27 , $P < 0.001$). In addition, the duration of women's infertility was significantly ($P < 0.001$) correlated with the embryo's top quality. A positive β -human chorionic gonadotropin was detected in 12.6% ($n = 25$) of women while clinical pregnancy was investigated in 8% ($n = 16$) of them. The fetal heart rate was detected in 7.5% ($n = 15$). Eventually, the clinical ($P = 0.020$) and determined ($P = 0.030$) pregnancy rates significantly differed between two study groups with a higher level in the TQE group.

Conclusions: It seems that TQP transfer should be the first recommendation for infertile women, but when the double-embryo transfer (DET) is needed according to the patient's condition, she should be informed that the quality of the second embryo may have an adverse impact on pregnancy consequences.

Keywords: IVF, Pregnancy outcome, Embryo quality, Ovulation induction protocol, Infertility

Introduction

Globally, 15% of couples are affected by infertility (1). Assistance-reproductive technology can have a considerable role in resolving this problem in such couples (2). However, many factors influence infertility treatment success, including the woman's age, medical history, and the quality of the embryos (3).

The infertility treatment cycle success tightly depends on fetal quality. In a way, clinical pregnancy and live birth rate in each embryo transfer with top quality were shown to be about twice that of poor-quality embryo transfer (4). Laboratory studies demonstrated that in an *in vitro* environment the likelihood of reaching the blastocyst stage will increase if the quality of all embryos is top. However, the presence of one or more embryo(s) with inappropriate morphology adversely influences the growth phases of other embryos. Toxic particles such as ammonia and free radicals releasing from poor quality embryos may be the related cause (5-7).

However, some studies reported that the implantation potential for each embryo (top or poor-quality embryo) is independent of each other and not affected by the other

ones (8).

Nonetheless, there is still disagreement about transferring embryos with different quality levels. For instance, Dobson et al study indicated the concurrent transfer of an inappropriate quality embryo along with the desired quality embryo reduces the rate of implantation and clinical pregnancy (9). On the contrary, Baruffi et al failed to confirm this hypothesis in their review study (10).

Although each embryo apart from its quality has logically the possibility of pregnancy, spontaneous abortions, a decrease in pregnancy outcomes, and live birth rates were mostly attributed to inappropriate quality embryos (8). Therefore, this study aimed to investigate the *in vitro* fertilization success rate in transferring top- versus low-quality embryos.

Materials and Methods

The current prospective cohort study was performed on 199 infertile women. Ninety-nine patients underwent an agonist cycle (70 fresh ET and 29 frozen ET) while the remaining 100 patients received the antagonist cycle (28 fresh ET and 72 frozen ET) in the infertility department at



Key Messages

- It is found that fetal quality is one of the most important factors of success in the infertility treatment cycle.

a tertiary university-based hospital between May 2019 and March 2020. All infertile women with each of infertility causes, who were candidates for the first or second times IVF treatment program, were participated in the study. Women having a genetic disorder in themselves or their partners and those with underlying diseases or chronic comorbidities including diabetes, hypertension, endocrine problems, and autoimmune diseases were excluded from the study. Further, patients withdrawing to participate at any time during the study were excluded from this study.

A detailed history of all patients was recorded based on the study aim. The hormonal studies were also recorded, including follicle-stimulating hormone (FSH), anti-Mullerian hormone (AMH), and estradiol (E2) measurements.

Standard long agonist protocol therapy was applied in a normal response woman who has a history of endometriosis. Moreover, the short antagonist protocol was considered in patients with polycystic ovary, the risk for ovarian hyperstimulation syndrome (AMH >4 ng/mL), or reduced ovarian reserve (AMH <0.9 ng/mL).

Gonadotropin-releasing hormone (GnRH) agonists (1 mg/d CinnaFact, CinnaGen Company, Iran) were initiated in the mid-luteal stage (7 days before the anticipated menstruation) in the long agonist protocol. transvaginal sonography (TVS) was done on the second or third day of the next menstruation although recombinant FSH (rFSH) was initiated based on the age, antral follicle count, and AMH if ET was lower than 5 mm and the ovarian follicle or cyst size was smaller than 10 mm.

In the antagonist protocol, the GnRH antagonist (cetorelix) 250 mg/d subcutaneously, was initiated on the fifth day of the menstrual cycle and continued until the ovulation triggering day as a fixed strategy.

On the second or third day of the menstrual cycle, all patients underwent transvaginal ultrasonography. Then, the patients were checked by serial transvaginal ultrasonography, and the dose of gonadotropins was adjusted accordingly. The rFSH dosage was individually corrected according to each patient's ovarian response. Human chorionic gonadotropin (hCG, Ovitrelle, Merck Serono, Modugno, Italy) was subcutaneously prescribed 250 µg to stimulate ovulation when at least two follicles were ≥18 mm and serum E2 ≥ was 500 pg/mL.

After 36 hours, oocytes were retrieved under general or spinal anesthesia. The intracytoplasmic sperm injection method was conducted for all cycles.

If ET was ≥6 mm, oral progesterone was described as 100 mg daily. Three days after progesterone administration, embryos were transferred using the American Society for

Reproductive Medicine protocol.

According to Gardner et al (2004, the embryo grading system), the quality score of the embryos was A, B, or C. In this system, the embryos were ranked according to blastocyst growth stage from 3 to 6, and inner cell mass and trophoctoderm (TE) types were as A, B, or C and A, B, or C, respectively (11). The blastocysts classified as AA, AB, and BB, as well as AC, BC, and CC were considered as top- and poor-quality embryos (TQE and PQE), respectively (9).

The transferred embryo count was based on the woman's age and her medical conditions, which ranged from 1 to 4 embryo(s).

The primary outcome was the number of participants who became pregnant during the study. β-hCG was measured on the second week after embryo transfer. If β-hCG was positive (considered as chemical pregnancy), TVS was conducted on the sixth and the seventh week after the embryo transfer for pregnancy sac observation (i.e., clinical pregnancy) and ultrasound-proven fetal heartbeat (considered as determined pregnancy), respectively.

Statistical Analysis

All statistical analyses were performed using SPSS, version 24.0 (IBM, New York, USA), and a *P* value of less than 0.05 was determined as the level of statistical significance. An independent *t* test and non-parametric Mann-Whitney U-test were used to assess differences in the means. Finally, a chi-square test was applied to evaluate differences in proportions.

Results

One hundred and ninety-nine infertile women were assessed for eligibility. Of these, 99 patients underwent an agonist cycle (70 fresh ET and 29 frozen ET) while 100 cases received the antagonist cycle (28 fresh ET and 72 frozen ET), the data of which are illustrated in Figure 1.

The average age of the patients was 32.44 ± 5.25 years, which was 32.29 ± 5.51 and 32.58 ± 5.00 in fresh ET and frozen ET groups, respectively ($P = 0.690$). The average of infertility duration was 5.37 ± 2.62 years. All the study population's medical characteristics are provided in Table 1.

The decrease in ovarian reserve (29.1%) and endometriosis (16.5%) were the two most prevalent infertility causes in PQE while malefactor was the most prevalent factor in TQE, and this difference was significant in infertility causes ($P < 0.001$).

Approximately 17% of women had a history of abortion. The mean of endometrium thickness was 9.08 ± 1.27 with a range of 7 to 13 millimeters. There was no significant difference in endometrium thickness according to the treatment protocol ($P = 0.786$). The estradiol level, ovule, and the embryo counts were not significantly varied between the groups according to the treatment cycle type (Table 2).

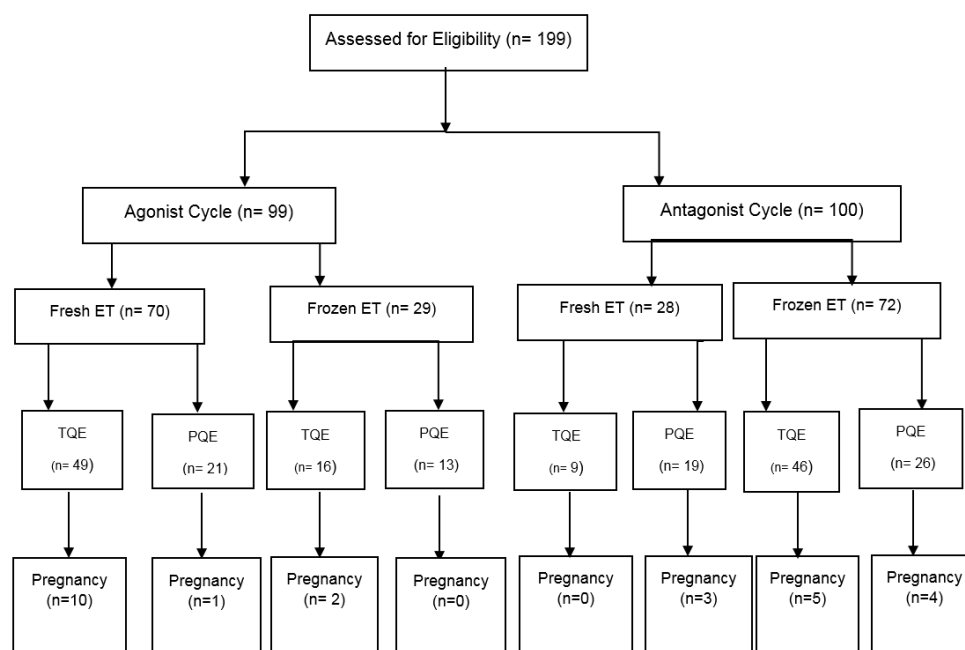


Figure 1. The Participants' Cycle Type, Type of Embryo Transfer, Embryo Quality, and the Number of Pregnancy. Note. ET: Embryo transfer; TQE: Top-quality embryo; PQE: Poor-quality embryo.

The mean number of transferred embryos was 2.71 ± 0.73 . TQEs and PQEs were transferred in 60.3% ($n=120$) and 39.7% ($n=79$) of the participants, respectively.

Women with TQE were significantly younger than those with PQE (31.35 ± 4.97 vs. 34.09 ± 5.27 , $P < 0.001$). Although the body mass index was higher in the PQE group, this diversity was not significant ($P = 0.137$). Additionally, the duration of women's infertility was significantly ($P < 0.001$) correlated with the quality of the embryos. The evaluated FSH and AMH levels on the 3rd day of menstruation were significantly in more acceptable ranges compared to the TEQ group ($P \leq 0.001$ for both), the details of which are presented in Table 3.

Table 1. The Basic Characteristics of Participants

Characteristics	
Age (y)	32.44±5.25
Partner's age (y)	36.58±5.79
Duration of infertility (y)	5.37±2.62
BMI (kg/m ²)	25.92±3.06
AMH (day 3)	2.97±2.47
FSH (day 3)	6.07±1.50
Cause of infertility, n (%)	
Tubal factor	24 (12.1)
Decreased ovarian reserve	28 (14.1)
PCO	16 (8)
Endometriosis	25 (12.6)
Male factor	36 (18.1)
Unexplained	36 (18.1)
Multiple factors	34 (17.1)

Note. BMI: body mass index; AMH: anti-Mullerian hormone; FSH: follicle-stimulating hormone; PCO: polycystic ovary.

Totally, positive β -hCG, clinical pregnancy, the fetal heart rate was observed in 25 (12.6%), 16 (8%), and 15 (7.5%) women, respectively. Based on the results (Table 4), pregnancy measures were higher in the TQE group, and there were significantly higher in clinical pregnancy ($P = 0.020$) and determined pregnancy ($P = 0.030$). As shown in Figure 2, women with C embryo quality had a lower chance of pregnancy and progress of pregnancy to a live birth.

The implantation rate (clinical pregnancy/transferred fetus numbers) was 28%. Additionally, miscarriage and

Table 2. Treatment Outcomes According to Participants' Cycle Type

Characteristics	Cycle Type		P Value
	Agonist	Antagonist	
Endometrial thickness (mm)	9.10±0.13	9.06±0.12	0.786
Estradiol (pg/mL)	1835.01±107.99	1908.17±150.23	0.693
Ovule number	9.68±0.33	9.09±0.49	0.323
Embryo number	2.66±0.07	2.77±0.07	0.277

Table 3. The Basic Characteristics According to Participants' Embryo Quality

Variables	Embryos Quality		P Value
	Top Quality	Antagonist	
Female age	n = 120	Poor Quality	<0.001
BMI (kg/m ²)	n = 79	26.32±2.81	0.137
Infertility duration (y)	4.83±2.42	6.18±2.72	<0.001
FSH day 3 (IU/mL)	5.77±1.25	6.54±1.72	0.001
AMH day 3 (IU/mL)	3.51±2.64	2.16±1.95	<0.001

Note. BMI: body mass index; AMH: anti-Mullerian hormone; FSH: follicle-stimulating hormone.

Table 4. The Comparison of Different Types of Pregnancy According to Participants' Embryo Quality

Variables	Embryos Quality		P Value
	Top Quality (n = 120)	Antagonist (n = 79)	
Chemical pregnancy	17 (14.2)	8 (10.1)	0.400
Clinical pregnancy	14 (11.7)	2 (2.5)	0.020
Determined pregnancy	13 (10.8)	2 (2.5)	0.030

live/dead birth rate were assessed, and based on the results, miscarriages, ectopic pregnancy, and live births were reported in 3 cases, 1 case, and 8 cases, respectively, and 3 participants were still pregnant.

Discussion

The findings demonstrated that embryo quality was affected by many different factors in infertile women, including age, infertility duration, and infertility cause that may have a highly important impact on the result of IVF and pregnancy and pregnancy maintenance in infertile women.

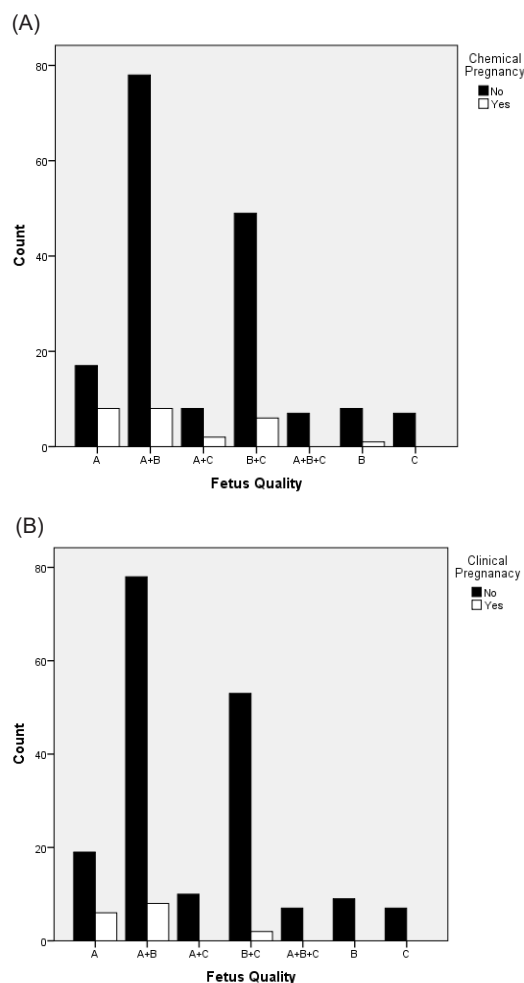
Two previous relevant studies reported that further pregnancy rates and embryo quality were affected by women's age, type of infertility, indications for IVF, and seasonal variations (12,13), which is in accordance with the results of the present study.

As mentioned earlier, the older age of an infertile woman was strongly correlated with PQE. This finding is in line with the literature and can be one of the reasons women younger than 33 years had a higher chance of live birth compared to women older than ≥ 33 years (9,14).

In this study, the rate of pregnancy was higher in pregnant women with TQP, and it was found that PQE might negatively influence the TQE during double-embryo transfer (DET). These results are in conformity with those of some recent studies, reporting that transferring a bad quality embryo, along with a good quality embryo meaningfully lowered both pregnancy and implantation rates as compared with transferring a good quality embryo lonely (15). Nonetheless, this finding is not confirmed by another study (8).

On the other hand, transferring more than one embryo, regardless of the adverse impact of PQE, results in more multiple pregnancies. This situation is related to a higher risk of low birth weight, premature birth, and the need for care in a neonatal intensive care unit in comparison with single pregnancies (16).

Although it seems that single-embryo transfer (SET) is a better choice, DET is mostly preferred in several infertile women. The causes consist of a higher pregnancy rate, the expense of paying for two cycles in comparison with one cycle, and anxiety. Therefore, it is worthy of taking the risk of multiple pregnancies in selected women when balanced versus the little increment in pregnancy rates reported with DET (8,15,17,18). In such cases, well recommendation

**Figure 2.** The Comparison of Chemical (A) and Clinical Pregnancy (B) According to Participants' Embryo Quality.

and giving useful medical data are of necessity since these women must be capable of equating these factors against the hazards of multiple pregnancies to themselves and any unborn infant. However, one may consider that without a certain reform in the live birth rate, patients may be better treated by having SET when a TQE is available.

Study Limitations

Despite the strong point of our study, the small sample size was one of our study limitations. Another limitation was the lack of evaluating late pregnancy sequels, containing preterm labor, and low birth weight, and it was a single-center study.

Thus, it is suggested that future studies use the matching method to eliminate the possible confounders. However, they should not compare pregnancy outcomes with respect to different fetal grading systems.

Conclusions

It seems that TQP transfer should be the first recommendation for infertile women. However, when DET is needed according to the patient's condition,

women can be aware that the quality of the second embryo may have an adverse effect on pregnancy outcomes, and the risk of pregnancy complications may increase due to multiple pregnancies.

Authors' Contribution

FAA and ME: concept and design. FDT and ZR: performing of the study. TH and MT: data collection and interpretation of the data. AA and EF: analysis of the data and writing of the manuscript. All authors read and approved the study.

Conflict of Interests

Authors declare that they have no conflict of interests.

Ethical Issues

This study was conducted in compliance with the Helsinki Declaration and approved by the Ethics Committee of Tehran University of Medical Sciences (IR.TUMS.MEDICINE.REC.1398.629). All the participants signed an informed agreement form.

Financial Support

The study was supported by the Vice-chancellor for the Research and Technology of Tehran University of Medical Sciences, Tehran, Iran and was extracted from the infertility fellowship of Tayebe Hemmati.

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