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Original Article

The day of embryo transfer affects delivery rate, birth weights, female-to-male ratio, and monozygotic twin rate

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ABSTRACT

Objective: To compare the reproductive outcomes between the transfer of cleavage-stage embryos and blastocysts in two different age groups of patients. The reproductive capacity of women decreases by age. This decrease in capacity is directly related to a lower ovarian reserve and errors in the meiotic spindle of the oocyte, which increase chromosomal abnormalities and the formation of aneuploidy embryos with lower chances of implantation.
Materials and Methods: A total of 1400 intracytoplasmic sperm injection cycles were analyzed. The study patients were divided into two age groups [aged < 36 years (Group I) and aged ≧ 36 years (Group II)]. The groups were subdivided according to the day of embryo transfer (ET)—Day 3 (ET3) and Day 5 (ET5).
Results: In both age groups, transfer of blastocysts resulted in a higher clinical pregnancy rate and deliveries. An increased twin birth rate was observed in patients who were younger than 36 years on both transfer days compared with those who were older than 36 years of age. There was an elevated percentage of newborn males on ET5 in both age groups. Monozygotic twinning (MZT) rate was observed only among younger patients (<36 years of age), specifically on ET5 compared with ET3. There was no significant difference in the mean birth weight of singleton and twins between the ET3 and ET5 subgroups in the younger group of patients except for the triplets who were significantly heavier in the ET5 group compared with the older group (≧36 years of age) where significant difference was found only on the mean birth weight of singleton.
Conclusion: The study suggests that if a blastocyst can be obtained in patients of advanced age (≧36 years), it improves their baby take-home rates. Younger patients (aged < 36 years) should undergo elective single blastocyst transfers to reduce multiple pregnancy rates.

Introduction

The prevalence of infertility is increasing, but its rate varies demographically and regionally [1]. This could be attributed to the fact that couples prioritize lifestyle and career over the creation of a family [2]. As the options for treatments of infertility keep increasing and the outcomes of such treatments are improving, couples delay their treatments [3]. However, there is a very strong relationship between age, decreased ovarian reserve, and errors in the meiotic spindle, which increase chromosomal abnormalities in the oocytes and the formation of aneuploid embryos with very low implantation rates [4,5]. Age has a highly negative impact on the pregnancy chances and assisted reproductive technologies cannot compensate for basic physiological preconditions of reproductive senescence [2]. Although menopause indicates the end of women's fertility, the decline actually begins many years before menopause [6]. Therefore, women older than 35 years of age should be referred for an infertility work-up after 6 months of unsuccessful attempts to conceive [3]. A number of different evaluations and treatment

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options have therefore been developed for age-dependent infertilities that improve the baby take-home rates [7].

A better understanding of the mechanisms involved in the development of human embryos has led to the production of culture media that support extended cultivation of embryos (up to the blastocyst stage) [8,9]. It is thought that the transfer of high-quality blastocysts will result in higher implantation rates [10], give a better synchronization between the blastocyst and the endometrium [11], and generate higher pregnancy and live birth rates in comparison to transfer of cleavage-stage embryos [10]. However, it seems that the cumulative clinical pregnancy rate for cleavage-stage embryos is higher than that for blastocysts [12].

Besides, there are several potential limitations to transfer of blastocysts such as higher cancellation risks [13], lower freezing rates [14], and a higher incidence of monozygotic twinning (MZT) rates [15]. There is also a risk of premature deliveries of babies with lower birth weights [16], generation of epigenetic mutations in offspring [17], and altered sex ratios [11].

The aim of this study was to compare the reproductive outcomes between cleavage-stage embryos and blastocyst transfers in two different age groups of patients. The study results should provide valuable information for making better decisions about selection of appropriate day of embryo transfer (ET; ET3 vs. ET5) in these patients (aged < 36 years vs. aged ≧ 36 years).

Materials and methods

Patient selection criteria

This retrospective study was approved by the Clinical Research and Research Ethics Committees of the General Hospital-Remedika, Skopje, Macedonia. The study encompassed the period from February 2008 to December 2013, during which 3049 cycles were performed. The exclusion criteria were donation (sperm, oocyte, or embryos) and spontaneous cycles; frozen/thawed ETs; protocols in which antagonist and microflare were used; patients with an antral follicle count lower than five follicles and basal follicle-stimulating hormone (bFSH) levels greater than 11 mIU/mL when less than five metaphase II oocytes were retrieved; and in those cycles where the ET was cancelled. After applying the aforementioned exclusion criteria, 1400 cycles were finally included in the study.

Study group

The patients were divided into two groups according to their age: younger than 36 years of age (Group I) and 36 years of age or older (Group II). Each group was further divided in two subgroups according to the day of ET: Day 3 (ET3) and Day 5 (ET5). If the patient had more than four excellent or good embryos according to selected criteria on Day 3 or had a previous history of implantation failure, the embryos were selected for blastocyst transfer.

Both groups were comparable in terms of average female age, bFSH and E2 levels on Day 3, dose of exogenous gonadotropins, yield of metaphase II oocytes, fertilization rates, and grade of embryos transferred in both age groups (Tables 1 and 2).

Male infertility factor (oligozoospermia, asthenozoospermia, teratozoospermia, or combined) contributed to 70% of infertility in Group I and 73% in Group II. Consequently, intracytoplasmic sperm injection (ICSI) was chosen as the fertilization method for all patients.

Ovarian stimulation

A long stimulation protocol was used in all patients. Down-regulation was initiated with buserelin acetate (Suprefact, Sanofi Aventis, Kalithea, Athens, Greece) during the mid-luteal phase.

Table 1
Demographics and cycle characteristics of female patients younger than 36 years of age ($N = 958$).

Parameter	ET3	ET5
Age (y)	30.0 ± 3.3	29.4 ± 3.1
Basal FSH (IU/L)	7.2 ± 2.2	7.0 ± 2.3
Estradiol on the day of hCG injection (pg/mL)	1509 ± 666	1620 ± 918*
Retrieved number of oocytes/cycle	13.1 ± 6.1	13.9 ± 7.3
Number of mature oocytes/cycle	9.4 ± 5.5	10.8 ± 6.4
Fertilization rate (%)	70 ± 0.19	69 ± 0.2
No. of ICSI cycles embryo transfer	654	304
No. of embryos transferred/cycle	2.65 ± 0.7	2.6 ± 0.6
Embryos transferred	1777	825

Data are presented as *n* (%) or mean ± standard deviation.
* $p < 0.05$ was considered statistically significant.
ET = embryo transfer; FSH = follicle-stimulating hormone; hCG = human chorionic gonadotropin; ICSI = intracytoplasmic sperm injection.

Injections of recombinant follitropin beta (Puregon, N.V. Oregon, Os, The Netherlands) were initiated on the 3rd day of menstrual cycle. The doses were adjusted to the patient's age and the number of preantral follicles in the ovary. When at least two follicles measuring over 18 mm were visible in the ovaries, human chorionic gonadotropin (hCG, 10,000 IU; Pregnyl, N.V. Organon, Os, The Netherlands) was injected for triggering maturation of oocytes.

Egg collection routines and culture technique

Transvaginal ultrasound-guided oocyte retrieval was performed under short intravenous anesthesia (Propofol-Lipuro 1%, Braun Melsungen AG, Melsungen, Germany). Thirty-six to thirty-seven hours after the hCG injection, the cumulus–oophorous complexes (COCs) were aspirated using a single-lumen aspiration needle (17GA/30 cm, Cook Medical, Eight Mile Plains, Brisbane, Australia) at an aspiration pressure of 80–100 mmHg. All retrieved COCs were washed free from the follicular fluid in Quinn's Advantage medium containing HEPES (SAGE, CooperSurgical, Trumbull, CT, USA). All oocytes and embryos were cultured in preincubated Quinn's Advantage sequential media under mineral oil (SAGE, CooperSurgical, Trumbull, CT, USA).

Denudation of oocytes and ICSI techniques

Four hours after egg collection, the COCs were transferred into a 100-μL drop of hyaluronidase solution (80 IU/mL hyaluronidase, SAGE, CooperSurgical, Trumbull, CT, USA) and repeatedly aspirated through a Pasteur pipette for 20–30 seconds. Mechanical

Table 2
Demographics and cycle characteristics of female patients aged 36 years or older ($N = 442$).

Parameter	ET3	ET5
Age (y)	38.5 ± 2.11	38.8 ± 2.55
Basal FSH (IU/L)	7.4 ± 2.2	7.6 ± 2.2
Estradiol on the day of hCG injection (pg/mL)	1636 ± 851	1508 ± 652*
Retrieved number of oocytes/cycle	12.0 ± 6.3	12.3 ± 6.2
Number of mature oocytes/cycle	8.8 ± 5.1	9.8 ± 5.3
Fertilization rate (%)	70 ± 0.19	69 ± 0.2
No. of ICSI cycles embryo transfer	275	167
No. of embryos transferred/cycle	2.6 ± 0.65	2.5 ± 0.63
Embryos transferred	718	393

Data are presented as *n* (%) or mean ± standard deviation.
* $p < 0.05$ was considered statistically significant.
ET = embryo transfer; FSH = follicle-stimulating hormone; hCG = human chorionic gonadotropin; ICSI = intracytoplasmic sperm injection.

denudation was carried out in enzyme-free HEPES-buffered medium (50 μL droplets) by repeated aspiration into stripper tips (Flexipet, Cook Medical, Bloomington, IN, USA).

All metaphase II oocytes were injected using a 35-degree angled ICSI pipette (Cook Medical, Bloomington, IN, USA). The oocytes were considered normally fertilized when they had two pronuclei and two polar bodies 16–18 hours after ICSI. Embryos were classified according to the scoring system of Hardarson et al [18].

Sperm preparation techniques

The semen samples were obtained by masturbation after 2–5 days of abstinence. Semen analysis was performed 30 minutes following liquefaction [19] followed by semen sample preparation by density gradient centrifugation using 90% and 50% PureCeption (SAGE, Trumbull, CT, USA).

Embryo transfer technique

On Day 3, all embryos were transferred into Quinn's blastocyst medium and the embryos were selected either for transfer on the same day or for culture and transfer on Day 5. Evaluation of embryo quality on Day 3 was based on blastomeres number, fragmentation rate, multinucleation, and early compaction. The selection criteria of Day 3 embryos were early cleavage on Day 1, four cells on Day 2, and eight cells or early compaction on Day 3, with minimal fragmentation and no multinucleation. After applying the selection criteria, transfer was only performed using Grade I (excellent quality) embryos (with at least eight blastomeres on Day 3, of equal size, with <10% of fragmentation, and no multinucleation) and Grade II (good quality) embryos (6–10 blastomeres, equal or moderate in size, with <15–20% of fragmentation, and no multinucleation). Grade III (fair quality) and Grade IV (bad quality) embryos were not transferred.

All transfers were performed under abdominal ultrasonography guidance using a soft embryo catheter (K-SOFT 5000, Cook Medical, Eight Mile Plains, Brisbane, Australia). The number of embryos for transfer varied from one to three, considering the age and previous history of the patient. The remaining surplus high-quality embryos were cryopreserved with vitrification (SAGE Vitrification kit, SAGE Trumbulll, IN, USA). Intravaginally administrated progesterone (Crinone 8%, Merck Serono, Darmstadt, Germany) was used as the luteal phase support.

Statistical analysis and definition

A database for 1400 samples was generated. Statistical analysis included descriptive statistics and Chi-square test using SPSS software 13.0 (SPSS, Inc., Chicago, IL, USA). Differences were considered significant when p values were less than 0.05.

Pregnancy test was considered positive when positive serum hCG levels (>5.3 mIU/ml) were detected 14 days after ET. A clinical pregnancy was considered established when at least one visible sac with heart beating was detected by transvaginal ultrasonography 5 weeks after ET. A biochemical pregnancy was defined as a pregnancy without an intrauterine sac. Spontaneous abortion until 12 gestation weeks was registered as having empty gestation sac (blighted ovum) or fetal demise detected by transvaginal ultrasound. Second-trimester abortion was defined as the case in which the fetus was lost between gestation weeks 13 and 26. Preterm deliveries were defined as babies born alive before 37 weeks of pregnancy.

Results

Outcomes of ICSI in Group I

The outcome of the ICSI cycles in the younger age group (patient age < 36 years; $N = 958$) is shown in Table 3.

There was neither significant difference in the fertilization rates nor in the mean numbers of embryos transferred on the 3rd day (2.65 ± 0.7) or 5th day (2.6 ± 0.6; Table 1).

The quality of the transferred embryos and the pregnancy rates [positive beta-hCG/ET, 63% (415/654) and 62.5% (190/304) for ET3 and ET5, respectively] were not significantly different between the two subgroups.

In addition, the clinical pregnancy rate/ET was not significantly higher on Day 5 (57%; 172/304) compared with Day 3 (55%; 360/654); however, there was a difference in the implantation rates between ET on Day 3 (32.6%) and Day 5 (38.9%).

There was also a significantly higher biochemical pregnancy rate, missed abortion rate (until the 12th gestation week), and second-trimester abortion rate (12th–26th gestation week) after ET on Day 3 in this age group of patients.

For patients in the ET3 subgroup, 447 babies were born from 305 deliveries, consisting of 250 (56%) females and 197 (44%) males, with a multiple delivery frequency of 43% (132/305), 40% (122/305) twins, and 3.3% (10/305) triplets (Table 3). In the ET5 subgroup, 234 babies were born with a statistical significance between males (57%, 133/234) and females (43%, 101/234; Table 3).

There was no significant difference in the mean birth weight of singleton and twins between the ET3 and ET5 subgroups, except for the triplets who were significantly heavier in the ET5 subgroup (Table 5). A statistical difference in only height was found in twins between the two subgroups.

In this group, there was a significant difference in the percentage of monozygotic twins [0.5% (2/360) for ET3 vs. 2.9% (5/172) for ET5].

Outcomes of ICSI in Group II

The outcome of the ICSI cycles for the patients (aged 36 years or older) in Group II ($N = 442$) is presented in Table 4. The fertilization rates were 70% and 69% for the ET3 and ET5 subgroups, respectively (Table 2). A beta-hCG test was positive in 50% of patients (12% biochemical) in the ET3 subgroup. Thirty-eight percentages (106/275) of these patients had a viable fetal heartbeat and 6.9% (19/275) had a miscarriage or had their pregnancy aborted therapeutically. The final ongoing pregnancy rate was 31% (84/275) with 100 newborn babies.

In this group, 55% (92/167) of patients in the ET5 subgroup were positive for beta-hCG. There was no significant difference in the implantation rates between ET3 and ET5, but the clinical pregnancy rate (48%, 80/167) was significantly higher in the ET5 subgroup than that in the ET3 subgroup. There was a significant difference in the percentage of deliveries between ET3 and ET5 (31% and 43%, respectively).

There was a significant difference in multiple birth rates (singleton and twins) between transfer of cleavage-stage embryos and blastocysts. The sex ratio of the delivered babies was 53% versus 44% for females and 47% versus 56% male in the ET3 and ET5 subgroups, respectively.

There was a significant difference in the mean birth weight of singletons, but not in twins between the ET3 and ET5 subgroups (Table 6). Statistical difference was found only in the height of twins ($p < 0.05$) between these two subgroups. However, statistical analysis for these two parameters (height and weight) could not be

Table 3
Pregnancy outcomes in intracytoplasmic sperm injection cycles for female patients younger than 36 years ($N = 958$).

Parameter	ET3	ET5
Positive beta-hCG rate (%)	63 (415/654)	62.5 (190/304)
İmplantation rate (%)	32.6 (581/1777)	38.9 (321/825) *
Biochemical pregnancy (%)	8.0 (55/654)	6.3 (18/304) *
Clinical pregnancy rate (%)	55 (360/654)	57 (172/304)
Missed abortion (until 12 gestation weeks, %)	4.1 (27/654)	2.0 (6/304) *
Second-trimester abortion (12–26 gestation weeks, %)	4.2 (28/654)	3.0 (9/304) *
Deliveries (%)	46.6 (305/654)	51.6 (157/304) *
Singleton (%)	56.7 (173/305)	56.0 (88/157)
Twins (%)	40 (122/305)	39 (61/157)
Triplets (%)	3.3 (10/305)	5.0 (8/157) *
Monozygotic twins (%)	0.5 (2/360)	2.9 (5/172) *
Total preterm deliveries (%)	23 (71/305)	25 (40/157)
Preterm deliveries singleton (%)	19.7 (14/71)	10.0 (4/40) *
Newborn females (%)	56 (250/447)	43 (101/234) *
Newborn males (%)	44 (197/447)	57 (133/234) *

Data are presented as n (%) or mean ± standard deviation.
* $p < 0.05$ was considered statistically significant.
ET = embryo transfer; hCG = human chorionic gonadotropin.

Table 4
Pregnancy outcomes in intracytoplasmic sperm injection cycles for female patients 36 years or older ($N = 442$).

Parameter	ET3	ET5
Positive beta-hCG rate (%)	50 (138/275)	55 (92/167) *
İmplantation rate (%)	30 (201/673)	32 (125/393)
Biochemical pregnancy (%)	12 (32/275)	7 (12/167) *
Clinical pregnancy rate (%)	38 (106/275)	48 (80/167) *
Missed abortion (until 12 gestation weeks, %)	5.4 (15/275)	4.0 (7/167) *
Second-trimester abortion (12–26 gestation weeks, %)	1.4 (4/275)	1.0 (2/167)
Deliveries (%)	31 (84/275)	43 (71/167) *
- Singleton (%)	80 (67/83)	72 (51/71) *
- Twins (%)	19 (16/83)	25 (18/71) *
- Triplets (%)	0	1.5 (1/71) *
- Perinatal mortality (%)	1 (1/84)	1.5 (1/71) *
Monozygotic twins (%)	0	0
Total preterm deliveries (%)	18 (15/84)	18 (13/71)
- Preterm deliveries singleton (%)	47 (7/15)	0
Newborn females (%)	53 (53/100)	44 (40/91) *
Newborn males (%)	47 (47/100)	56 (51/91) *

Data are presented as n (%) or mean ± standard deviation.
* $p < 0.05$ was considered statistically significant.
ET = embryo transfer; hCG = human chorionic gonadotropin.

performed for triplets because there were no triplets in the ET3 subgroup. Group II had no monozygotic twins.

Discussion

One of the most important factors affecting *in vitro* fertilization outcomes is maternal age, as increasing age is associated with reduced oocyte quality and high chromosomal instability [20]. It is still unclear whether advanced female age has an opposing effect on blastocyst formation.

In this study, the highest clinical pregnancy rates were found in women undergoing ET on Day 5 (57% in Group I and 48% in Group II). However, in the higher age group (age of patients ≧36 years; Group II), there was a significant decrease in the clinical pregnancy and delivery rates in comparison to those of the younger age group (age of patients < 36; Group I). This suggests that women of advanced age, despite having low basal FSH level (<10 mIU/mL) have a lower chance of establishing a pregnancy via assisted reproductive technology. This decrease in fertility might primarily be due to oocyte senescence rather than due to poor endometrial receptivity, because older women produce less oocytes and have lower implantation rates, which suggests the lower response of the follicles to exogenous hormones and the retrieval of a lower number of high-quality oocytes [21]. Older women may have

Table 5
Mean birthweight and height of singleton, twins, and triplet newborns resulting from ET3 and ET5 in female patients younger than 36 years.

Deliveries	Weight (ET3/ET5)	Height (ET3/ET5)
Singleton	3189 ± 457/3211 ± 417	49.9 ± 1.6/50.6 ± 0.5
Twins	2263 ± 408/2474 ± 376	46.6 ± 1.9/49.6 ± 1.0 *
Triplets	1863 ± 164.7/2252 ± 474 *	43.7 ± 0.6/43.7 ± 3.3

Data are presented as weight (g) or height (cm) with mean ± standard deviation.
* $p < 0.05$ was considered statistically significant.

Table 6
Mean birthweight and height of singleton, twins, and triplet newborns resulting from ET3 and ET5 in female patients aged 36 years or older.

Deliveries	Weight (ET3/ET5)	Height (ET3/ET5)
Singleton	2872 ± 751/3467 ± 373 *	49.2 ± 3.3/50 ± 1.4
Twins	2555 ± 348/2697 ± 312	48.1 ± 2.4/48.5 ± 0.7 *
Triplets	0/1893 ± 347	0/42.6 ± 5.0

Data are presented as weight (g) or height (cm) with mean ± standard deviation.
* $p < 0.05$ was considered statistically significant.

decreased level of stored maternally transcribed messenger RNA responsible for trophectoderm function and maintenance of the blastocoel [22].

Despite the adverse effects of multiple pregnancies, many clinics still transfer more than one embryo to increase the chances of establishing a pregnancy [23]. In this study, we found very high multiple pregnancy rates in patients aged less than 36 years (Group I) with a twinning rate of 40% in the ET3 subgroup and 39% in the ET5 subgroup. This is in contrast to the much lower twinning rate found in the older age group (patients aged ≧ 36 years), where twinning rates of 19% and 25% were reported in the ET3 and ET5 subgroups.

We found that transfer of Day 3 embryos resulted in higher miscarriage rate in comparison to transfer of Day 5 embryos between the two age groups (Groups I and II). These observations are in line with a previous report [24], which indicated that the transfer of blastocysts generates higher pregnancy rates. These observations suggest that blastocyst selection is more reassuring in terms of pregnancy achievement, because the selection is made through a cohort of cleavage-stage embryos that have already survived the process of initial selection. These results also demonstrate the important role of uterine receptivity in successful implantation.

In both age groups, the transfer of blastocysts resulted in a higher percentage of male babies (57% and 56% for Group I and Group II, respectively), which might depend on the selection of fast developing expanded blastocysts for ET [25]. In addition, it is thought that *in vitro* culture induces a precocious inactivation of the X-chromosome and that ICSI decreases the number of trophectoderm cells in female blastocysts, thereby increasing the risk of early postimplantation failure [26]. The genes controlling glucose uptake and metabolism (glucose-6-phosphate dehydrogenase) and antioxidants (hypoxanthine phosphoribosyl transferase) are located on the X chromosome. The activities of these genes and potentially certain enzymes, might delay the development of female embryos [27]. Orvos et al [28] found a maternal and paternal age effect on the sex ratio in ICSI cycles [28]; however, no such correlation was found in our study. In addition, it has been suggested that the source of protein in the selected culture media might alter the sex ratio after ICSI [29].

Blastocyst transfers also seem to increase the MZT rate. The exact pathogenesis for embryo splitting is still unknown, but multiple factors seem to affect the outcome such as maternal age, micromanipulation of the zona pellucida (ICSI and assisted hatching), prolonged embryo culture, and altered *in vitro* culture condition [30,31]. In our study, ICSI was performed in all patients, but assisted hatching was not done. Despite this, the incidence of MZT was much higher on Day 5 (2.9%) compared with Day 3 (0.5%). It has also been suggested that high MZT rates relate to longer culture periods [32,33] and components within the culture media, such as high levels of glucose [34] and low levels of calcium, which increase the division in the inner cell mass and destabilize the intracellular bonds between the cells [35]. In addition, the influence of growth factors such as insulin receptors (insulin-like growth factors 1 and 2) may induce changes in signaling, cytoplasmic shifting, and polarity changes in the embryo, which may increase MZT [36,37]. Previous reports have also suggested that advanced maternal age increases the MZT rate as a result of a gradual decrease of the average thickness of the zona pellucida altering the normal hatching process [35]; however, Knopman et al [38] only found a relationship between the age of female patient (<35 years) and MZT.

There were also no significant differences in the mean birth weights if embryos were transferred on Day 3 (3189 ± 457 g) or Day 5 (3211 ± 417 g) in younger patients (age < 36 years), which was also found in singleton births after blastocyst transfers [39]. However the age of the patients was not documented in the latter study [39]. The mean birth weight in the older age group in our study was significantly higher for ETs on Day 5 (3467 ± 373 g) in comparison to that for ETs on Day 3 (2872 ± 751 g).

Interestingly, our results also show a statistical difference in the height of twins in both age groups, with infants born after blastocyst transfer being taller (Tables 5 and 6).

We did not find statistical significance in the total preterm deliveries between ET3 and ET5 in both age groups; however, a statistical difference was found in the preterm deliveries of the singletons, with the highest rate achieved for ET3 in both age groups. In our study the number of infants born after blastocyst transfer is too small to evaluate rare outcomes, when compared with data presented in meta analysis and systematic reviews [40]. We will continue to follow up the outcomes of neonates for future consideration of the background risk of neonatal and obstetrics complications.

In summary, it seems that if a blastocyst can be obtained in patients of advanced age (≧36 years), it improves their baby take-home rates (Table 4). In younger patients, we suggest that an elective single blastocyst transfer should be performed to reduce the high multiple pregnancy rates.

Finally, the retrospective nature of the study may pose certain limitations to our study. The possibility of bias was reduced such that we isolated the effect of fertilization rate on implantation rate. Furthermore, this study did not investigate whether the sperm quality significantly influences the outcomes although only ICSI cases were included.

Conflicts of interest

The authors have no conflicts of interest relevant to this article.

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