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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. Downregulation was initiated with buserelin acetate (Suprefact, Sanofi Aventis, Kalithea, Athens, Greece) during the mid-luteal phase. 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.

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.

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.