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ORIGINAL ARTICLE

Comparison of outcomes of frozen-thawed transfer of day 5 blastocysts and day 6 blastocysts

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ABSTRACT

Objective: The aim of this study was to evaluate and compare the outcomes of frozen-thawed embryo transfer of blastocysts that were expanded at the 5th day of fertilization and blastocysts expanded at the 6th day of fertilization.

Material and Methods: Patients who underwent frozen-thawed embryo transfer cycles between 2012 and 2020 in a single infertility center were retrospectively evaluated. A total of 1814 women with frozen embryo transfers of day 5 blastocysts and 227 women with frozen embryo transfers of day 6 blastocysts were included in the study. Outcomes of embryo transfer cycles of day 5 and day 6 blastocysts were compared.

Results: Cancellation rate in day 6 blastocyst transfer group was significantly higher than day 5 transfers (5.7% vs. 0.3%; p<0.001). Post-thaw embryo quality in day 5 blastocyst group was better than day 6 blastocyst group. Clinical pregnancy rates (34% vs. 16.7%), multiple pregnancy rates (3.9% vs. 0.9%) and live birth delivery rates (31.4% vs. 15.7%) in day 5 blastocyst transfer group were significantly higher (p<0.001, p<0.001 and p<0.001). Gestational ages at birth (37.15±3.23 weeks vs. 37.97±2.52 weeks; p=0.308) and mean birthweights of infants (3153.20±681.96 g vs. 3127.72±656.75 g; p=0.994) were similar among groups. Significantly increased number of miscarriages were observed in day 5 blastocyst group (5.3% vs. 1.8%; p=0.019).

Conclusion: Clinical pregnancy and live birth delivery rates of frozen-thawed transfer of day 5 blastocysts are significantly higher in comparison to transfer of their day 6 counterparts.

Keywords: Day 5 blastocyst, day 6 blastocyst, frozen embryo transfer, *in vitro* fertilization, slow-growing embryo.

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INTRODUCTION

Embryo transfer in terms of timing and technique is a crucial step in achieving success in in-vitro fertilization. Transferring good quality embryos that have the highest capacity to survive would allow better outcomes in every initiated cycle. Among the stages of embryo development, blastocysts have the highest chance of survival with lesser implantation failures due to better embryos selection through the process of development.[1] Normally, human embryos are expected to reach to blastocyst stage at the 5th day of fertilization. However, in some occasions, no blastocyst stage embryo could be observed in the whole embryo cohort at the 5th day of fertilization. Some of these cohorts with slow-growing embryos were able to produce blastocysts at the 6th day of fertilization owing to the advances in embryo culturing techniques in recent years. Nevertheless, these slow-growing embryos are demonstrated to have higher rate of molecular abnormalities besides abnormal and reduced number of mitotic spindles.[2,3] Furthermore controlled ovarian stimulation advances endometrial receptivity for 2.5 days following oocyte retrieval.[2] Hence, fresh transfer of day 6 blastocysts could be negatively affected by asynchrony in endometrial maturation and embryo development stage.[4] Studies investigating the outcomes of day 5 and day 6 blastocyst transfers have conflicting results. Majority of the studies that evaluated these outcomes in fresh embryo transfer cycles indicate higher pregnancy rates in day 5 blastocyst transfers.[5–7] Whereas studies comparing the outcomes of frozen embryo transfers of day 5 and day 6 blastocysts present even more controversial results.[8–13]

In this study, we aimed to evaluate and compare the outcomes of frozen embryo transfer of day 5 blastocysts and day 6 blastocysts.

MATERIAL AND METHODS

This study was conducted by retrospective analysis of patients who underwent frozen embryo transfer cycles between 2013 and 2020 in a university-affiliated infertility center (Memorial Atasehir Hospital affiliated with Uskudar University). Ethical approval for this study was obtained from the Ethical Committee of Uskudar University at 31 May 2021 (Approval number: 61351342/MAY 2021-47). Study protocol fits with the "Declaration of Helsinki-Ethical Principles for Medical Research Involving Human Subjects" and the ethical committee omitted the need for patients' consent owing to the retrospective design of the study. Patients with high (>30 kg/m^2) or low (<18 kg/m^2) body mass indexes (BMI), patients with endocrine disorders such as congenital adrenal hyperplasia, Addison disease, diabetes mellitus, Cushing syndrome, patients with corrected or present uterine anomalies, were excluded from the study due to possible confounding effects of these conditions.

Within the first 5 days of the menstrual cycle controlled ovarian stimulation protocols were initiated in our study population. Recombinant follicle-stimulating hormone (rFSH, Gonal-F, Merck Serono S.p.A), human menopausal gonadotropin (hMG, Merional, IBSA Institut Biochimique S.A, Menopur® Ferring Pharmaceuticals) or combination of recombinant luteinizing hormone (LH) and rFSH (Pergoveris, Merck Serono SA) is used for ovarian stimulation. Patients are monitored during stimulation for follicular growth via transvaginal ultrasound imaging and serum hormone levels. In accordance with each patient's follicular growth, adjustments in gonadotropin doses are applied. Once the leading follicle is observed to reach a diameter of 12–14 mm, Gonadotropin-releasing hormone (GnRH) antagonist (Cetrotide 0.25 mg, Pierre Fabre Medicament Production) injections commenced to suppress premature LH peak and continued to the day of oocyte maturation triggering To induce oocyte maturation, either dual-trigger method with a GnRH agonist of 0.2 mg triptorelin acetate, (Gonapeptyl, Ferring Pharmaceuticals) and 250 mcg recombinant human chorionic gonadotropin (Ovitrelle, Merck Serono) or hcg-only method with 250 mcg recombinant human chorionic gonadotropin (Ovitrelle, Merck Serono) was used when at least one follicle has reached a diameter of 18 mm. Oocytes are retrieved under transvaginal ultrasound guidance 35–36 h after oocyte maturation trigger. Fertilization was conducted by intracytoplasmic sperm injection. Obtained embryos are graded according to Istanbul consensus workshop.[14]

Media for process of vitrification and thawing was supplied from Kitazato Biopharma (Shizuoka, Japan). Steps to vitrify obtained blastocysts are as follows: Following immersion into 300 μL equilibration solution for 10 min in room temperature, embryos are placed in 300 μL vitrification solution for a maximum of 60 s. Then embryos are loaded into vitrifit straw and immersed into liquid nitrogen. For thawing of vitrified embryos, embryos are taken into 300 μL thawing medium for 1 min consequently placed into 300 μL dilution solution for 3 min. Then, embryos are consecutively put into a 300 μL washing solution for 5 min and into a second 300 μL washing solution for 1 min. Following these steps, embryos are taken into culture medium. Oral estrogen 2 mg 3 times a day (Estrofem, Novo Nordisk, Istanbul, Turkey) was administered to patients starting from the 2nd menstruation day of planned frozen embryo transfer cycle for 12 days. Patients were evaluated for endometrial thickness following 12 days of estrogen administration. Progesterone supplementation was initiated for patients that endometrial thicknesses were found to be >7 mm either with 200 mg intravaginal progesterone (Lutinus, Ferring Pharmaceuticals) twice a day or 200 mg intravaginal progesterone 3 times a day (Progestan Soft Capsules, Koçak Farma Pharmaceutical and Chemical Industry Co.) and continued through 8th–10th weeks of gestation. Embryo transfer procedure was implemented via soft embryo transfer catheters with abdominal ultrasonography guidance. A maximum of two embryos were transferred in each attempt.

The primary outcome of this study was live birth delivery rates. Secondary outcomes are implantation rates (gestational sacs observed/transferred embryos), clinical pregnancy rates, and miscarriage rates. Outcome parameters were defined in accordance with The International Glossary on Infertility and Fertility Care, 2017.[15]

Statistical analysis was conducted by IBM SPSS 23 (Evaluation version). Descriptive statistics were expressed as mean±standard deviations for normally distributed data. Categorical variables were expressed as numbers and percentages (%). Significance of differences between means were assessed with Student's t-test. Categorical variables were assessed with Pearson's Chi-Squared test or Fisher's exact test. P<0.05 are considered as significant.

Table 1: Demographic characteristics of day 5 blastocyst transfer group and day 6 blastocyst transfer group

	Day 5 blastocyst transfer n=1814	Day 6 blastocyst transfer n= 227	p
Age (years)	32.92±4.84	33.51±4.93	0.080
BMI (kg/m^2)	25.09±3.10	25.17±3.09	0.718
Etiology of ınfertility			0.891
Anovulation	500 (27.6%)	61 (26.9%)	
Combined	112 (6.2%)	17 (7.5%)	
Diminished ovarian reserve	97 (5.3%)	14 (6.2%)	
Endometriosis	124 (6.8%)	16 (7.0%)	
Male factor	442 (24.4%)	59 (26%)	
Tubal factor	306 (16.9%)	37 (16.3%)	
Unexplained	233 (12.8%)	23 (10.1%)	
Estradiol levels (pg/ml)	211.55±82.61	219.74±85.19	0.181
Progesterone levels (ng/ml)	0.56±0.31	0.55±0.34	0.250
Endometrial thickness (mm)	10.18±1.79	10.44±1.88	0.051

BMI: Body mass index.

RESULTS

A total of 1814 frozen-thawed embryo transfers of day 5 blastocysts and 227 frozen-thawed transfers of day 6 blastocysts that are eligible for this study were included that were applied between the selected period of time. Mean age, BMI, estradiol and progesterone levels as well as endometrial thicknesses at the time of transfer were found similar within day 5 blastocyst transfer group and day 6 blastocyst transfer group. Distribution of prevalence of infertility etiologies was not significantly different among groups. Demographic data and comparison of characteristics of day 5 blastocyst transfers and day 6 blastocyst transfers were given in Table 1.

A total of 1809 embryo transfer procedures were performed in the day 5 blastocyst group and 213 in the day 6 blastocyst transfer group. Cancellation rate in day 6 blastocyst transfer group was significantly higher than day 5 transfers (5.7% vs. 0.3%; p<0.001). The number of transferred embryos were similar among groups whereas post-thaw quality of embryos in the day 5 blastocyst transfer group has a significant tendency to be superior than their counterparts in the day 6 blastocyst transfer group. Live birth delivery rates (31.4% vs. 15.7%), clinical pregnancy rates (34% vs. 16.7%) and multiple pregnancy rates (3.9% vs. 0.9%) in day 5 blastocyst transfer group were significantly higher in comparison to day 6 transfers (p<0.001, p<0.001 and p<0.001). Although gestational ages at birth (37.15±3.23 weeks vs. 37.97±2.52 weeks; p=0.308) and mean birthweights of infants (3153.20±681.96 g vs. 3127.72±656.75 g; p=0.994) were similar among groups significantly higher number of patients delivered via cesarean section in day 6 blastocyst transfer group. A significantly increased number of miscarriages were observed in day 5 blastocyst group in comparison to day 6 transfers (5.3% vs. 1.8%; p=0.019). Comparison of outcomes of frozen-thawed embryo transfers in day 5 blastocyst transfer group and day 6 blastocyst transfer groups were summarized in Table 2.

DISCUSSION

Results of studies involving pregnancy outcomes of day 5 and day 6 blastocyst transfers are controversial in literature.[2,8] Besides the higher rate of intrinsic flaws in slow-growing day 6 blastocysts, endometrial asynchrony and a subsequent reduction in endometrial receptivity was suggested as a concern in fresh transfers of these blastocysts.[16] Frozen embryo transfers allow transfer of obtained embryos in the consecutive menstrual cycle hence eliminating the possible effects of a proposed endometrial asynchrony and reduced endometrial receptivity that emerges as a result of controlled ovarian stimulation cycle. Therefore, we conducted this study to evaluate the outcomes of frozen-thawed embryo transfer cycles of day 5 and day 6 blastocysts. Some of the previous studies about this topic showed similar cycle outcomes in frozen embryo transfers of day 5 and day 6 blastocysts.[5,9,17] In a review conducted by Sunkara et al.[8] frozen embryo transfers of day 5 blastocysts were shown to have better clinical outcomes comparing to frozen transfer of day 6 blastocysts. However when the findings were adjusted in accordance with morphologic qualities of day 5 and day 6 blastocysts, they found comparable rates of clinical pregnancies among day 5 and day 6 frozen embryo transfers. Higher rate of morphologic abnormalities in slow-growing day 6 blastocysts such as cellular cytoskeletal abnormalities were identified in previous studies.[2,3] Moreover, both Piccolomini et al.[18] and Taylor et al.[13], demonstrated increased rate of aneuploidies in day 6 slow growing blastocysts in comparison to day 5 blastocysts and suggested that clinical pregnancy rates in euploid day 5 and day 6 blastocysts could be similar.In our study we found significantly higher rates of clinical pregnancy and live birth delivery rates in the day 5 blastocyst group in comparison to day 6 blastocyst transfers. Although the quality of embryos was similar among these two groups, none of the obtained embryos in our study population underwent preimplantation

Table 2: Comparison of outcomes of frozen-thawed embryo transfers in day 5 blastocyst transfer group and day 6 blastocyst transfer group

	Day 5 blastocyst transfer n=1814	Day 6 blastocyst transfer n=227	p
Number of embryo transfer procedure	1809	213	
Cancellation rate	5 (0.3%)	14 (5.7%)	<0.001
Number of embryo transfers			0.294
1 embryo transfer	1192 (65.9%)	148 (69.5%)	
2 embryo transfer	617 (34.1%)	65 (30.5%)	
Quality of transferred embryos			<0.001
Grade 1	1691 (93.5%)	181 (84.6%)	
Grade 2	118 (6.5%)	32 (15.4%)	
Number of previous ıvf attempts	2.32.±1.79	2.48±2.58	0.323
Live birth delivery rate	591 (31.4%)	36 (15.7%)	<0.001
Clinical pregnancies	617 (34%)	38 (16.7%)	<0.001
Miscarriages	97 (5.3%)	4 (1.8%)	0.019
Multiple pregnancies	71 (3.9%)	2 (0.9%)	<0.001
Gestational weeks at birth (weeks)	37.15±3.23	37.97±2.52	0.308
Mean birthweights of infants (gr)	3153.20±681.96	3127.72±656.75	0.994
Route of delivery			<0.001
Vaginal delivery	153 (29.37%)	3 (0.09%)	
Cesarean section	368 (70.63%)	31 (91.17%)	

genetic testing. Since we theoretically by-passed the proposed effects of reduced endometrial receptivity at the 6th day after oocyte retrieval by transferring frozen-thawed embryos, lower rates of pregnancies achieved in the day 6 blastocyst group could largely be attributed to higher rates of un-diagnosed genetic abnormalities in this group.

Findings of some previous studies comparing the outcomes of frozen embryo transfer of day 5 and day 6 blastocysts indicated increased number of multiple gestations in day 5 blastocyst transfers.[6,11] Parallel with these previous findings, in our study we found significantly higher rates of multiple pregnancies in day 5 blastocyst transfer group.

In a study conducted by Ferreux et al.[11] frozen day 6, blastocyst transfers resulted with higher birth weights. They suggested that the day of blastocyst expansion might affect placentation and resultant infant birth weight. In our study, we found similar mean birth weights in day 5 and day 6 blastocyst groups. Discrepancy in our findings could be a consequence of different compositions of culture media, cryopreservation techniques, or endometrial preparation methods.

Few studies about the comparison of frozen day 5 and day 6 blastocyst transfers reported miscarriage rates and most of them indicated similar miscarriage rates in these two groups.[8] However, contrary to expectations Levens et al.[19] found increased rates of spontaneous abortions in frozen day 5 blastocyst transfers in comparison to day 6 transfers. Furthermore, they reported much lower rates of miscarriages in day 6 frozen blastocyst transfer group than expected rates of spontaneous abortions in cryopreserved embryo transfers. They speculated that the difference in miscarriage rates could be a result of higher implantation capacity of day 5 blastocysts. Conflicting with the rest of the literature and aligning with Levens et al.[19] we found significantly increased rate of miscarriage in the frozen-thawed transfer of day 5 blastocysts. Hashimoto et al.[3] showed that the rate of cytoskeletal abnormalities is higher in day 6 blastocysts. These abnormalities could either be results of point mutations or post-translational protein alterations and known to reduce implantation capacity of embryos regardless of the ploidy status.[3] Although aneuploidy rates among day 6 blastocysts have shown to be higher in comparison to day 5 blastocysts in previous studies, a day 5 blastocyst with chromosomal anomaly could expected to have higher chance of implantation that might explain significantly increased rate of miscarriages in day 5 blastocyst transfer group in our study.

The rate of cesarean delivery in the day 6 blastocyst transfer group in our study was 91.17%, far above the general rates in assisted reproduction and significantly higher than the day 5 blastocyst transfer group. This increased rates of cesarean might be a result of maternal anxiety as well as physicians' uneasiness in the management of patients with a whole cohort of slow-growing embryos.

CONCLUSION

Clinical pregnancy and live birth delivery rates of frozen-thawed transfer of day 5 blastocysts are significantly higher in comparison to transfer of their day 6 counterparts probably due to higher rate of aneuploidies in slow-growing day 6 blastocysts.

Statement

Ethics Committee Approval: The Üsküdar University Non-Interventional Research Ethics Committee granted approval for this study (date: 31.05.2021, number: 61351342/MAY 2021-47).

Informed Consent: Written informed consent was obtained from patients who participated in this study.

Peer-review: Externally peer-reviewed.

Author Contributions: Concept – FT, AK; Design – FT, AK; Supervision – FT; Data Collection and/or Processing – FT, AK; Analysis and/or Interpretation – FT, AK; Literature Search – FT, AK; Writing – AK; Critical Reviews – FT, AK.

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REFERENCES

1. Kovacic B, Vlaisavljevic V, Reljic M, Cizek-Sajko M. Developmental capacity of different morphological types of day 5 human morulae and blastocysts. Reprod Biomed Online 2004;8(6):687–94.
2. Park DS, Kim JW, Chang EM, Lee WS, Yoon TK, Lyu SW. Obstetric, neonatal, and clinical outcomes of day 6 vs. day 5 vitrified-warmed blastocyst transfers: Retrospective cohort study with propensity score matching. Front Endocrinol (Lausanne) 2020;11:499.
3. Hashimoto S, Amo A, Hama S, Ito K, Nakaoka Y, Morimoto Y. Growth retardation in human blastocysts increases the incidence of abnormal spindles and decreases implantation potential after vitrification. Hum Reprod 2013;28(6):1528–35.
4. Mirkin S, Nikas G, Hsiu JG, Díaz J, Oehninger S. Gene expression profiles and structural/functional features of the peri-implantation endometrium in natural and gonadotropin-stimulated cycles. J Clin Endocrinol Metab 2004;89(11):5742–52.
5. Shapiro BS, Daneshmand ST, Garner FC, Aguirre M, Ross R. Contrasting patterns in *in vitro* fertilization pregnancy rates among fresh autologous, fresh oocyte donor, and cryopreserved cycles with the use of day 5 or day 6 blastocysts may reflect differences in embryo-endometrium synchrony. Fertil Steril 2008;89(1):20–6.
6. Barrenetxea G, Delarruzea A, Ganzabal T, Jimenez R, Carbonero K, Mandiola M. Blastocyst culture after repeated failure of cleavage-stage embryo transfers: A comparison of day 5 and day 6 transfers. Fertil Steril 2005;83(1):49–53.
7. Shapiro BS, Richter KS, Harris DC, Daneshmand ST. A comparison of day 5 and day 6 blastocyst transfers. Fertil Steril 2001;75(6):1126–30.
8. Sunkara SK, Siozos A, Bolton VN, Khalaf Y, Braude PR, El-Toukhy T. The influence of delayed blastocyst formation on the outcome of frozen-thawed blastocyst transfer: A systematic review and meta-analysis. Hum Reprod 2010;25(8):1906–15.
9. Yang H, Yang Q, Dai S, Li G, Jin H, Yao G, et al. Comparison of differences in development potentials between frozen-thawed D5 and D6 blastocysts and their relationship with pregnancy outcomes. J Assist Reprod Genet 2016;33(7):865–72.
10. Haas J, Meriano J, Laskin C, Bentov Y, Barzilay E, Casper RF, et al. Clinical pregnancy rate following frozen embryo transfer is higher with blastocysts vitrified on day 5 than on day 6. J Assist Reprod Genet 2016;33(12):1553–7.
11. Ferreux L, Bourdon M, Sallem A, Santulli P, Barraud-Lange V, Le Foll N, et al. Live birth rate following frozen–thawed blastocyst transfer is higher with blastocysts expanded on day 5 than on day 6. Hum Reprod 2018;33(3):390–8.
12. Desai N, Ploskonka S, Goodman L, Attaran M, Goldberg JM, Austin C, et al. Delayed blastulation, multinucleation, and expansion grade are independently associated with live-birth rates in frozen blastocyst transfer cycles. Fertil Steril 2016;106(6):1370–8.
13. Taylor TH, Patrick JL, Gitlin SA, Wilson JM, Crain JL, Griffin DK. Comparison of aneuploidy, pregnancy and live birth rates between day 5 and day 6 blastocysts. Reprod Biomed Online 2014;29(3):305–10.
14. Alpha Scientists in Reproductive Medicine and ESHRE Special Interest Group of Embryology. The Istanbul consensus workshop on embryo assessment: Proceedings of an expert meeting. Hum Reprod 2011;26(6):1270–83.
15. Zegers-Hochschild F, Adamson GD, Dyer S, Racowsky C, de Mouzon J, Sokol R, et al. The international glossary on infertility and fertility care, 2017. Fertil Steril 2017;108(3):393–406.
16. Tannus S, Cohen Y, Henderson S, Al Ma'mari N, Shavit T, Son WY, Dahan MH. Fresh transfer of day 5 slow-growing embryos versus deferred transfer of vitrified, fully expanded day 6 blastocysts: Which is the optimal approach? Hum Reprod 2019;34(1):44–51.
17. Kaye L, Will EA, Bartolucci A, Nulsen J, Benadiva C, Engmann L. Pregnancy rates for single embryo transfer (SET) of day 5 and day 6 blastocysts after cryopreservation by vitrification and slow freeze. J Assist Reprod Genet 2017;34(7):913–9.
18. Piccolomini MM, Nicolielo M, Bonetti TC, Motta EL, Serafini PC, Alegretti JR. Does slow embryo development predict a high aneuploidy rate on trophectoderm biopsy? Reprod Biomed Online 2016;33(3):398–403.
19. Levens ED, Whitcomb BW, Hennessy S, James AN, Yauger BJ, Larsen FW. Blastocyst development rate impacts outcome in cryopreserved blastocyst transfer cycles. Fertil Steril 2008;90(6):2138–43.