Research Square

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No Additional Benefit of Eliminating Transfer Cancellations Using Cleavage-stage Transfers for Patients With Few Zygotes: A Retrospective Cohort Study

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Abstract

Purpose

This retrospective cohort study determined the relative efficacy of blastocyst and cleavage-stage transfers in patients with differing numbers of zygotes.

Methods

A total of 1116 women whose embryo transfers were planned independently of patient characteristics were included. Cleavage-stage (D3) and blastocyst-stage (D5) transfer outcomes were analyzed per number of zygotes. The D5 group included transfer cancellations as the intention-to-treat population. The effect of the embryo transfer date on the clinical outcomes (clinical pregnancy and implantation rates) was analyzed using multivariate logistic regression.

Results

Among the patients, 584 and 532 underwent D3 and D5 embryo transfers, respectively. The clinical pregnancy rates were significantly higher in D5 patients with ≥ 6 zygotes (25.7% vs 48.3%). The multivariate logistic regression analysis for clinical pregnancy did not show significant differences between the blastocyst and cleavage-stage transfers in patients with ≤ 5 zygotes (0.874). Compared to the cleavage-stage, blastocyst-stage transfers for patients with ≥ 6 zygotes resulted in a three-fold increase in clinical pregnancy rates (3.122).

Conclusion

Blastocyst transfers were not inferior to cleavage-stage embryo transfers among patients with few zygotes and were preferable for patients with several zygotes.

Introduction

Extending embryo culture to the blastocyst stage has been proposed to improve uterine and embryonic synchrony, select the most competent embryos, and allow elective single embryo transfers to avoid multiple pregnancies that create health risks for both the mother and offspring [1]. Despite concerns about the increased risk of adverse perinatal outcomes [2], blastocyst transfers are as likely to yield healthy babies as cleavage transfers [3–6], and they also have better obstetric outcomes [7].

The number of cycles needed and time to conception are significantly lower for blastocyst transfers [8]. However, most clinics offer extended culture only when abundant embryos are available because it remains unclear whether in vitro extended culture supports embryo development as effectively as in vivo.

According to the European Society for Human Reproduction and Embryology Vienna Consensus, competence and benchmark values of the blastocyst development rate are ≥ 40% and ≥ 60%, respectively [9]. Not every embryo will reach the blastocyst stage, even under optimal culture conditions. Consequently, extended culture transfers are cancelled nearly three times more often than cleavage transfers (odds ratio: 2.85; 95% confidence interval [CI]: 1.97–4.11) across all age groups [10].

Although no previous studies have elucidated whether cleavage-stage embryo transfers help patients with poor prognosis by eliminating the risk of arrested embryo development in extended culture, cleavage transfers remain the primary preference for patients with few embryos. This study was conducted to answer two questions. First, do cleavage transfers provide a benefit by avoiding transfer cancellations during extended culture? Second, what is the relative efficacy of blastocyst and cleavage transfers for patients with differing numbers of zygotes?

Materials And Methods

This retrospective cohort study included all infertile couples who attended the Reproductive Endocrinology and Assisted Reproduction Unit at Akdeniz University Hospital in Antalya, Turkey, from January 2018 to December 2019. Our clinic's digital database was screened for all fresh cycles with at least one mature egg, excluding fertility preservation, preimplantation genetic testing, and natural in vitro fertilization (IVF) cycle treatments. Only the first treatment was included for patients with multiple treatments (n = 1710). Embryo transfers were planned to avoid the weekend shift during the study period. Patients whose oocytes were collected on Monday and Tuesdays routinely received a cleavage -stage (D3), while Wednesday and Thursday pick-up patients routinely received a blastocyst-stage embryo transfer (D5). As Friday pick-up patients were eligible to be assigned to either cleavage (D3) or blastocyst (D5) transfers according to cycle characteristics, they were excluded (n = 217), and only patients whose embryo transfers were planned independently of patient and cycle characteristics were included. Total fertilization failures and cases with all embryos arrested before D3 (n = 39) were also excluded. The embryos from the remaining 1454 patients were assigned to either freeze-all (n = 338) or fresh transfer cycles (n = 1116), with the decision being made by the chief physician at the time of the oocyte pick-up based on common medical issues. Subsequently, the freeze-all cycles were excluded (n = 338) in the comparison of clinical outcomes (n = 1116) between the D3 (n = 584) and D5 (n = 532) groups (Fig. 1).

An intention-to-treat population was defined for the D5 group. Rather than being excluded, patients who did not have any embryos for transfer after extended culture were counted as negative. The clinical pregnancy rate was defined as the number of cycles with a viable heartbeat per number of fresh transfer cycles, with or without embryo transfer. The implantation rate was defined as the number of viable gestational sacs per number of embryos transferred; however, embryos lost in extended culture were calculated as transferred but not implanted. The primary outcome measures were clinical pregnancy and implantation rates. The secondary outcome measures were cryopreservation (CRs) and embryo utilization rates (EURs), which are defined as the sum of the transferred and cryopreserved embryos per total number of zygotes ([9].

The patients underwent IVF according to standard stimulation protocols, which involved pituitary downregulation with a gonadotropin-releasing hormone (GnRH) agonist administered in the mid-luteal phase of the prior cycle (long protocol) or a GnRH antagonist starting on the 6th day of stimulation (short protocol). Controlled ovarian stimulation was achieved with a human menopausal gonadotropin and/or recombinant follicle-stimulating hormone. In both groups, human chorionic gonadotropin was administered when the leading follicle(s) reached 17 mm.

In all cases, oocyte retrieval was performed 34–36 h after human chorionic gonadotropin injection. All inseminations were performed by intracytoplasmic sperm injection, and all embryos were cultured in a single-step culture medium (G-TL, Vitrolife, Sweden) under oil in the same benchtop incubator (Miri Multi-Room Incubator, ESCO, Singapore) under 6% CO_2 and 5% O_2 until the day of transfer. Embryo assessments were performed according to the ALPHA Istanbul Consensus guidelines [11]. For luteal support, all fresh transfer patients received 8% progesterone gel daily (Crinone, Merck, Switzerland) beginning the evening after the oocyte retrieval; this was continued until a negative pregnancy test or a viable fetus was documented by transvaginal ultrasonography.

The Chi-square test was utilized to analyze implantation rates, CRs, and EURs, and the t-test was performed to analyze clinical pregnancy rates. Multiple logistic regression analysis was performed to calculate the effects of common confounders on clinical outcomes. Data are presented as the mean ± standard deviation, and a p-value < 0.05 was considered significant. SPSS for Windows version 23.0 (IBM, USA) was used to perform the statistical analyses.

This study was conducted following ethical standards stipulated in the 1964 Declaration of Helsinki and its later amendments or comparable ethical standards and was approved by the Institutional Review Board of Akdeniz University, Faculty of Medicine (approval number: 27012021/71). The requirement of informed consent was waived owing to the retrospective nature of the study.

Results

Women in the D3 group were significantly older than those in the D5 group. The mean number of mature eggs (MIIs) was lower in the D3 group than in the D5 group. Seventy patients in the D5 group did not produce at least one viable embryo that could be transferred, and the transfer cancellation rate for the D5 group was 13.16%. The D3 group had a significantly higher number of embryos transferred per embryo transfer than that of the D5 group. Despite the lower number of mature eggs, the CRs and EURs were significantly higher in the D3 group compared with that of the D5 group (Table 1).

Table 1
Comparison of the patient and cycle characteristics of the D3 and D5 groups.

	D3 (n = 584)	D5 (n = 532)	*P*-value
Age (mean ± SD)	33.40 ± 5.11	32.40 ± 4.67	0.034
Previous IVF failure (mean ± SD)	0.52 ± 0.78	0.42 ± 0.72	NS
Baseline FSH (mean ± SD)	10.05 ± 7.22	8.76 ± 4.28	< 0.01
AFC (mean ± SD)	11.70 ± 8.12	13.82 ± 7.87	NS
COC (mean ± SD)	9.07 ± 5.84	10.27 ± 5.55	NS
MII (mean ± SD)	6.30 ± 4.39	7.25 ± 3.62	< 0.01
Fertilization rate (%)	62.67	63.77	NS
Embryo transfer rate (%)	100	86.84	< 0.01
Cryopreservation rate (%)	26.39	14.11	< 0.01
Embryo utilization rate (%)	46.02	29.14	< 0.01
Number of transferred embryos (mean ± SD)	1.31 ± 0.46	1.1 ± 0.59	< 0.01

AFC antral follicle counts, *COC* cumulus-oocyte complexes, *FSH* follicle-stimulating hormone, *IVF* in vitro fertilization, *MII* mature eggs, *SD* standard deviation, *NS* not significant

The clinical pregnancy outcomes per number of zygotes produced (1 to ≥ 9) were analyzed in total and separately. A post hoc subgroup analysis was conducted on two different numbers of zygotes (≤ 5 and ≥ 6). The clinical pregnancy rates in the D3 group were significantly lower than those in the D5 group for patients with ≥ 6 zygotes (25.7% [29/113] vs 48.3% [72/149]), respectively (Table 2). In total, the D5 group resulted in a 26.04% implantation rate, which was significantly higher than the 20.52% of the D3 group (Table 2).

Table 2
Comparison of clinical pregnancy rates per number of zygotes and implantation rates of the subgroups for the D3 and D5 groups.

CPR	2PN	D3	D5	*P*-value
	1	13.7% (19/139)	11.9% (7/59)	NS
	2	22.9% (30/131)	18.4% (16/87)	NS
	3	31.8% (28/88)	34.8% (31/89)	NS
	4	39.1% (27/69)	26.8% (22/82)	NS
	5	36.4% (16/44)	36.4% (24/66)	NS
	6	29.2% (7/24)	42.6% (20/47)	NS
	7	25% (7/28)	62.5% (20/32)	< 0.01
	8	18.2% (4/22)	37.5% (9/24)	NS
	≥ 9	28.2% (11/39)	50% (23/46)	0.04
	≤ 5	25.5% (120/471)	26.1% (100/383)	NS
	≥ 6	25.7% (29/113)	48.3% (72/149)	< 0.01
	Total	25.5% (149/584)	32.3% (172/532)	NS
IR	< 35	29.25% (98/335)	32.87% (118/359)	NS
	≥ 35	13.72% (59/430)	18.75% (63/336)	NS
	1 ET	29.64% (83/280)	34.53% (106/307)	NS
	2 ET	15.26% (74/485)	19.33% (75/388)	NS
	Total	20.52% (157/765)	26.04% (181/695)	0.01

2PN number of fertilized zygotes, *CPR*: clinical pregnancy rate, *ET*: number of embryos transferred, *IR*: implantation rate, *NS*: not significant

A logistic regression analysis investigated the effect of the transfer date on clinical pregnancy rates. Age, previous IVF failures, follicle-stimulating hormone, antral follicle counts, cumulus-oocyte complexes, and MIIs were added into the same model, as potential confounding factors. There was no significant difference in the clinical pregnancy rates between the blastocyst and cleavage transfers (0.874 [0.635–1.204]) for patients with ≤ 5 zygotes. Conversely, for patients with ≥ 6 zygotes, blastocyst transfers resulted in a three-fold increase in clinical pregnancy rates (3.122 [1.797–5.425]) when compared to that of cleavage transfers (Table 3).

Table 3
Multivariate logistic regression analysis for clinical pregnancy.

ET day	≤5		≥6	
	aOR (95% CI)*	*P*-value	aOR (95% CI)*	*P*-value
	0.874 (0.635–1.204)	NS	3.122 (1.797–5.425)	< 0.001

*Adjusted for age, previous in vitro fertilization failures, follicle-stimulating hormone, antral follicle counts, cumulus-oocyte complexes, and mean number of mature eggs, *aOR*: adjusted odds ratio, *CI*: confidence intervals, *ET*: number of embryos transferred, *NS*: not significant

Discussion

This study, investigating whether cleavage transfers provide a benefit by avoiding transfer cancellations during extended culture and the relative efficacy of blastocyst and cleavage transfers in relation to the available number of zygotes, found that blastocyst transfers were not inferior to cleavage-stage embryo transfers among patients with few zygotes. Our findings are supported by Haas et al. [12] as comparable cumulative pregnancy rate per patient for patients with one or two cleavage-stage embryos was reported, regardless of embryo quality; however, when the pregnancy rates were analyzed per embryo transfer, blastocyst transfers resulted in higher pregnancy rates. Levi-Setti et al. [13] found that the cycle outcomes of patients (female age < 39, ≥4 zygotes) were comparable between blastocyst and cleavage transfers. Yang et al. [14], also demonstrated that the implementation of a time-lapse algorithm for cleavage transfers remains inferior to blastocyst transfers. Their findings were attributed to either embryo self-selection during extended culture [15] or better synchronization of the embryo and uterus [16]. In contrast, clinical outcomes of the blastocyst and cleavage transfers for patients with only one viable embryo on day 3 favored cleavage transfers [17], but the poorer outcomes from blastocyst transfers did not appear related to embryo loss in extended culture.

Most recently, De Croo et al. [18] retrospectively analyzed the live birth rates of four different embryo transfer strategies: cleavage transfers for all, blastocyst transfer for patients with > 9 zygotes, for patients with > 4 zygotes, and blastocyst transfer for all per oocyte collection cycle. They found that blastocyst transfers resulted in comparable live birth rates per retrieval among all the groups; however, the clinical outcomes regarding the number of zygotes available were not reported. In the present study, the effect of the zygote cohort size on the efficacy of embryo self-selection in extended culture was investigated. The clinical pregnancy rates for the patients with ≥ 6 zygotes after extended culture to the blastocyst stage were higher than those at the cleavage stage. One possible explanation for the poor cleavage transfer outcomes is that the conventional morphological criteria for cleavage embryos might be insufficient for choosing the most competent embryos [19]. In addition to the conventional morphological evaluation, the time-lapse selection of cleavage embryos resulted in lower implantation rates than in blastocyst transfers for patients with > 10 mature oocytes [14]. This result is understandable, given that more potentially competent embryos will be produced when the ovarian reserve and response are increased, thereby increasing the effectiveness of embryo self-selection. Recent studies support this conclusion, as clinical

pregnancy and live birth rates have been shown to increase according to the number of eggs retrieved [20] and oocytes fertilized [21]. Consequently, the probability of finding at least one euploid embryo increases with the number of oocytes retrieved [22] and embryos biopsied [23]. The blastocyst cohort size is also associated with at least one euploid embryo being found [24]. Here, the improved implantation and pregnancy rates of blastocyst transfers for patients with a larger zygote cohort could be attributed to the production of more embryos with implantation potential and improved embryo selection properties resulting from extended culture.

The live birth [25] and clinical pregnancy [26] rates of first fresh embryo transfers are higher for blastocyst transfers, consistent with our study's findings. However, for patients with a poor prognosis who produce a limited number of embryos, the current literature is insufficient to assist practitioners in developing embryo transfer date strategies. Despite the benefits of blastocyst transfers, it is speculated that an in vitro environment is inferior to an in vivo environment. Higher transfer cancellation rates of blastocyst transfers are mainly attributed to suboptimal extended in vitro culture conditions [27]. Per the UK National Institute of Health guidelines (NICE Guidelines), cleavage transfers are recommended to avoid transfer cancellations when few embryos are available [28]. The American Society for Reproductive Medicine also recommends avoiding transfer cancellations [10]. Performing a cleavage transfer eliminates the risk of transfer cancellation for those patients. To date, it remains unclear whether these patients benefit from cleavage transfers.

A major challenge of assisted reproduction is the treatment's cost-effectiveness. Reducing the number of failed embryo transfers, thereby decreasing the time to conception, is critical [29]. To date, clinical pregnancy and live birth rates for blastocyst transfers are higher than those of cleavage transfers for first fresh embryo transfers [30]. Further studies have shown that blastocyst transfers reduce the mean number of cycles and days needed per live birth compared to those of cleavage transfers [8]. According to current knowledge, most aneuploid embryos show arrested development during extended culture [15, 16]. Aneuploidy rates of slow-developing embryos in extended culture are significantly elevated, regardless of patient age [31]. The clinical pregnancy and live birth rates of embryos with delayed blastulation and poor expansion patterns are also lower than those of fully expanded blastocysts [32]. Therefore, performing an extended culture for all patients may decrease the transfer of incompetent embryos, which will reduce costs and shorten the time to conception [33].

To our knowledge, the following methods used here were a novel approach to the dilemma of cleavage or blastocyst transfers for patients with few embryos. First, transfer date decisions were made independent of patient characteristics, and patients with a low zygote number were also allowed to extend the culture to D5. Second, transfer cancellations in the D5 group were not excluded from the analysis, they were counted as transferred but not pregnant to compare pregnancy rates per patient rather than per embryo transfer.

Blastocyst transfers are associated with a sex imbalance, favoring men and monozygotic twinning [34–36]. They are also associated with increased birth weights [37]. However, they are as safe as cleavage

transfers in terms of pregnancy complications, obstetric outcomes, and congenital abnormalities [25, 27, 36, 38]. Consequently, patients should be thoroughly counseled about the potential benefits and risks of blastocyst transfers.

This study has several limitations. Although the embryo transfers were planned independently of patient characteristics, the study's retrospective nature introduces potential bias, as the patient characteristics were not completely matched between groups. The patients receiving blastocyst transfers were younger with more mature oocytes. Furthermore, the ongoing pregnancy and live birth rates were not available for analysis. Further prospective studies are needed to verify these results.

In conclusion, blastocyst transfers provide significantly better results for patients with many zygotes, suggesting that it is preferable to perform extended culture and delay embryo transfer to find or select competent embryos. Moreover, extended culture seems to have no or negligible influence on embryo viability. Patients with few zygotes undergoing either D3 or D5 transfer have similar clinical outcomes. This can guide practitioners and patients in avoiding cleavage transfers that will not be successful.

Declarations

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Conflicts of interest

The authors report no conflict of interest.

Availability of data and material

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Code availability

Not applicable

Ethics approval

Approved by the Institutional Review Board of Akdeniz University, Faculty of Medicine (approval number: 27012021/71).

Consent to participate

Approved

Consent for publication

Approved

Bibliographical notes

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Conceptualization, formal analysis, methodology, writing.

Mehmet Sakıncı:

Formal analysis, supervision, writing

Mete Çağlar:

Methodology, supervision, visualization

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Figures

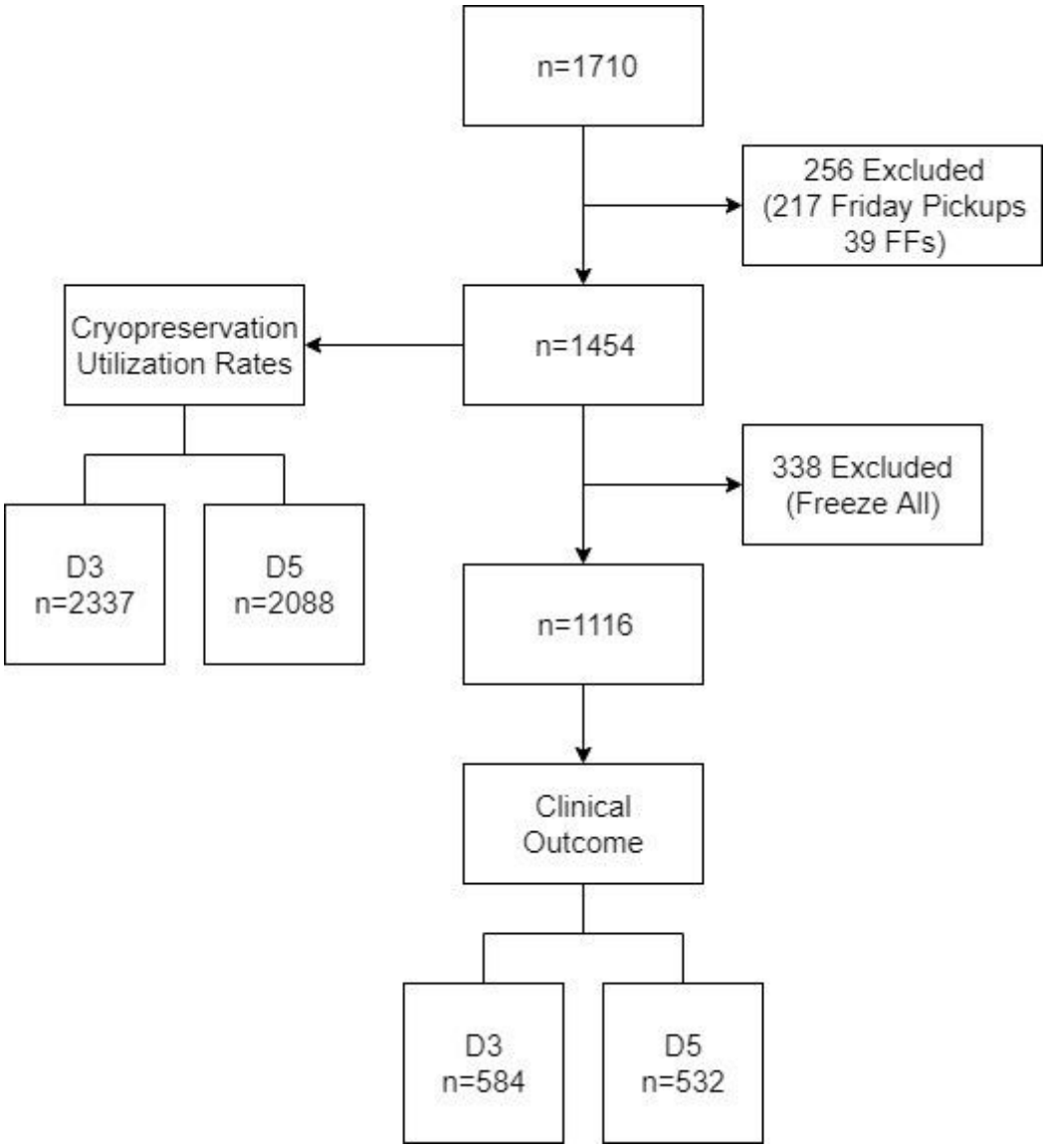

Figure 1

Patient flow chart. Cryopreservation and utilization rates are shown per zygote. Clinical outcomes are shown per patient