Is there still a role for a cleavage-stage embryo transfer?

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Objective: To determine whether pregnancy outcomes are poor or futile when an intended day 5 transfer is converted to a cleavage-stage transfer because of poor embryo development or a lower number of embryos.
Design: Retrospective cohort study.
Setting: Academic medical center.
Patient(s): Women with a limited number of embryos, defined as ≤6 two pronuclear embryos, after in vitro fertilization.
Intervention(s): Patients who had a cleavage-stage transfer were age matched with patients who had a day 5 transfer.
Main Outcome Measure(s): Live birth rate.
Result(s): A total of 146 women were included in the study with 73 women in each group. Cleavage-stage transfer was associated with significantly lower implantation and clinical pregnancy rates compared with those of day 5 transfer. Although the live birth rate of the cleavage-stage transfer group was lower than that of the day 5 transfer group (25% vs. 40%, respectively), the cleavage-stage transfer still resulted in a live birth rate of 25%. A subanalysis comparing women who did and did not achieve live birth after cleavage-stage transfer demonstrated a live birth rate of 27% when at least one grade A embryo was transferred vs. 17% when a lesser quality embryo (grade B or C) was transferred.
Conclusion(s): As expected, the live birth rate after cleavage-stage transfer was lower than that after day 5 transfer. However, the live birth rate of cleavage-stage transfer still fell into acceptable practice, >5%, for patients who were otherwise at very high risk of having no day 5 embryo transfer. Extended culture may not be necessary for all patients. (Fertil Steril Rep® 2021;2:269–74. ©2021 by American Society for Reproductive Medicine.)
Key Words: Cleavage-stage transfer, blastocyst-stage transfer, embryo transfer, extended culture, IVF, live birth

Assisted reproductive technology (ART) has evolved tremendously over the past four decades as our understanding of in vitro fertilization has broadened and the available technology has improved. Emerging knowledge on embryo culture is one way that ART has progressed over the years (1, 2). In the earlier phases of ART development, transfer of cleavage-stage embryos (day 2 or 3 after fertilization) was the standard of care, because the media culture used at that time was unable to sustain the growing embryo. Although cleavage-stage embryos use pyruvate and nonessential amino acids as their main energy source, these conditions are not ideal for postcompaction embryos at the morula or blastocyst stage, which favor glucose and essential amino acids as substrates (3, 4). Recognizing this key difference was a crucial step toward practice change. As our understanding of embryo growth requirements have become more sophisticated, commercially available media that allow for extended culture to day 5 or 6 have now become routinely integrated into today's practice. However, not every embryo or clinical situation permits embryo transfer to occur on day 5 or 6. To allow a chance for implantation, earlier transfer may be preferred in some clinical scenarios.

Extended embryo culture has several advantages over an earlier cleavage-stage embryo transfer. Primarily, extended culture to day 5 allows selection of the embryos with the greatest potential for continued development and chromosomal normality (5). Because only a few embryonic genes are transcribed before the morula stage, extended culture is considered a reliable test of embryo viability and development (6–9). Additionally, extended culture results in improved temporal synchronization of the endometrium, which may

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increase embryo receptivity compared with that of cleavage-stage transfers (10–13). In addition, culturing embryos to the blastocyst stage allows the opportunity to perform preimplantation genetic testing by targeting the trophectoderm for biopsy. Several studies have demonstrated higher implantation, clinical pregnancy, and live birth rates with transfer at the blastocyst stage compared with rates with cleavage-stage embryos (14–16). Furthermore, higher implantation rates allowed for the practice of elective single-embryo transfer, which has reduced the rate of multiples without significantly compromising pregnancy outcomes (17–19).

Given the clear benefits to extended culture, many ART clinics have implemented protocols to only transfer embryos that make it to the blastocyst stage or only euploid blastocyst embryos. However, when the embryo numbers are low and the extended culture for embryo selection is no longer necessary, the disadvantages of extended culture must be considered. Embryo culture attempts to replicate the ideal environment of the reproductive tract but may increase exposure to oxidative stress or reactive oxygen species (20, 21). The primary disadvantage from a clinical perspective is the potential cancellation of an embryo transfer that would have resulted in a live birth (22). In unselected patients randomized to cleavage vs. blastocyst transfers, a significantly higher number of transfers were cancelled after extended embryo culture (15). Although one may argue that an embryo that does not survive in the embryology laboratory up to day 5 may be of poor quality, this may in addition be interpreted as a missed opportunity when considering the financial and emotional commitment that embryo creation requires. In situations in which there is concern about transfer cancellation because of limited quantity or poor embryo development, our clinic implements the option for a cleavage-stage transfer. The rationale behind offering a cleavage-stage transfer is to provide a more natural embryo environment for the developing embryo in a situation in which the embryo may otherwise fail to reach the blastocyst stage in an artificial culture.

Although cleavage-stage embryo transfers are offered as an option to our patients, the success of this effort was not clear. If this practice is ineffective in achieving a live birth, then it may be futile to continue with such practice. The American Society for Reproductive Medicine (ASRM, formerly The American Fertility Society) Ethics Committee recommended specific counseling and special considerations when care is thought to be of very poor prognosis (<5% success rate) or futile (<1% success rate) (23). The objective of this study was to assess the live birth rates after a cleavage-stage transfer in women with limited pronuclear embryos to ensure that this practice is consistent with effective care and to help counsel patients who require cleavage-stage transfer when extended culture is planned.

MATERIALS AND METHODS

This retrospective cohort study was conducted at the Mayo Clinic in Rochester, Minnesota. Institutional review board approval was obtained before the initiation of the study. The Mayo Clinic Assisted Reproductive Technology database and the electronic medical records were used to query pertinent controlled ovarian stimulation cycles from the years 2014 to 2017. We included women aged 18– 45 years with ≤6 two pronuclear embryos created after controlled ovarian stimulation with conventional or intracytoplasmic sperm injection (ICSI) fertilization. This was selected because we found on internal review in our clinic that patients creating ≤6 two pronuclear embryos were at risk of transfer cancellation and therefore we captured the population at greatest risk of needing an earlier cleavage-stage transfer. Only women planning a fresh day 5 embryo transfer were included in the study. Women were excluded if the controlled ovarian stimulation resulted in cancellation before oocyte retrieval, they were converted to a freeze all, or they elected to have limited oocyte insemination. Patients with a trigger progesterone serum level of ≥1.5 ng/mL were converted to a freeze all because of concern about embryo-endometrial dyssynchrony. In addition, frozen embryo transfers were not included. For patients with multiple stimulation cycles, only the first cycle was included. Women who underwent a cleavage-stage transfer were age matched with women who had a day 5 transfer.

The baseline patient characteristics collected included age at stimulation, race, body mass index, parity, antimüllerian hormone serum level, follicle-stimulating hormone serum level, antral follicle count, thyroid-stimulating hormone serum level, and etiology of infertility. The stimulation protocols were chosen on the basis of ovarian reserve and patient history. The protocols included gonadotropin-releasing hormone (GnRH) agonist long, microdose GnRH agonist flare, or GnRH antagonist stimulation. All patients received progesterone supplementation starting the day of oocyte retrieval. The cycle characteristics collected included total stimulation days, total gonadotropin dose, maximum estradiol level, trigger day progesterone serum level, endometrial stripe thickness, number of mature oocytes retrieved, maturity rate, use of ICSI and assisted hatching, fertilization rate, number of embryos transferred and their respective morphologic grade, and any additional embryos frozen at the blastocyst stage. The embryos were cultured using a time-lapse imaging system. The embryos were graded on the basis of morphologic characteristics assessed by the embryologist. At the cleavage stage, the embryo was defined as grade A if there was appropriate timing of cell division, <10% fragmentation, and perfect cell symmetry; grade B if there was inappropriate timing of cell division by the day of transfer, 11%–25% fragmentation, and moderate cell symmetry; or grade C if there was significant deviation in the timing of cell division, >25% fragmentation, and absence of symmetry. The pregnancy outcomes measured included the rates of implantation, clinical pregnancy, live birth, miscarriage, and twin gestation. Clinical pregnancy was defined as ultrasound evidence of an intrauterine pregnancy with cardiac activity at approximately 7 weeks gestation, and live birth was defined as birth of a viable fetus. Both the miscarriage and twin gestation rates were calculated from patients that had a positive human chorionic gonadotropin quantitative serum level.

The patient and cycle characteristics were summarized and compared by Wilcoxon's rank sum test for continuous

TABLE 1

Patient characteristics.

	Day 5 transfer (N = 73)	Cleavage-stage transfer (N = 73)	*P* value
Age, years	34.4 (33.1–38.2)	35.8 (32.7–38.8)	.07
Body mass index, kg/m^2	25.0 (21.7–31.8)	25.6 (22.6–31.9)	.6
Race, No. (%)			.6
White	61 (83.6)	54 (74.0)	
Black	0	1 (1.4)	
Asian	6 (8.2)	8 (11.0)	
Nulliparous, No. (%)	39 (53.4)	41 (56.2)	.1
Infertility diagnosis			
Unexplained, No. (%)	12 (16.4)	9 (12.3)	.5
Diminished ovarian reserve, No. (%)	20 (27.3)	32 (43.8)	.04
Male factor, No. (%)	26 (35.6)	21 (28.8)	.4
Polycystic ovarian syndrome, No. (%)	6 (8.2)	8 (11.0)	.6
Uterine factor, No. (%)	1 (1.4)	3 (4.1)	.6
Endometriosis, No. (%)	5 (6.8)	8 (11.0)	.6
Tubal factor, No. (%)	11 (15.1)	8 (11.0)	.4
Preimplantation genetic testing, No. (%)	5 (6.8)	8 (11.0)	.6
Follicle-stimulating hormone level, IU/L	6.9 (5.7–8.9)	7.6 (6.3–9.4)	.08
Antimüllerian hormone, ng/mL	1.7 (0.8–2.7)	1.2 (0.8–2.0)	.1
Antral follicle count, No.	11.0 (8.0–16.0)	10.0 (7.0–14.5)	.07
Thyroid-stimulating hormone level, mIU/L	1.6 (1.1–2.1)	1.7 (1.2–2.2)	.6

Note: Values are median (1st and 3rd interquartile ranges) unless stated otherwise.

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variables, whereas Chi-square and Fisher's exact tests were used for categorical variables. The outcome measures assessed using Chi-square and Fisher's exact tests were based on the data distribution. All statistics were assessed using JMP Pro 10. A P value of $<.05$ was used to determine statistical significance. Our primary outcome was the live birth rate. A multivariate logistic regression was additionally performed to control for the effects of both the year of transfer and the number of embryos transferred on live birth rate. The secondary outcomes evaluated were rates of implantation, clinical pregnancy, miscarriage, and twin gestation. Finally, subanalyses were performed with age group comparisons of live birth rates and outcomes of women who did and did not achieve a live birth after a cleavage-stage transfer.

RESULTS

A total of 146 women with creation of $\leq$6 two pronuclear embryos were included in the study. Of these women, 73 had a cleavage-stage transfer and were age matched with 73 women who had a day 5 transfer. The patients' characteristics are shown in Table 1. There were no differences in age, body mass index, race, and percent of nulliparous women between the two groups. Women who had a cleavage-stage transfer were more likely to have a previous diagnosis of diminished ovarian reserve as the etiology for infertility compared with those who had a day 5 transfer (43.8% vs. 27.3%, respectively, P=.04). However, ovarian reserve measurements including follicle-stimulating hormone level, antimüllerian hormone level, and antral follicle count were not statistically different between the two groups.

Controlled ovarian stimulation cycle characteristics such as total stimulation days, total gonadotropin dose, maximum estradiol level, trigger day progesterone serum level, endometrial stripe thickness, total mature oocytes retrieved, maturity rate, and use of ICSI and assisted hatching were similar between the two groups (Table 2). An equal number of patients were placed on the various stimulation protocols in each group, with most (89%) receiving the GnRH antagonist protocol. The fertilization rate was lower in the cleavage-stage transfer group compared with that in the day 5 transfer group (58% vs. 72%, respectively, $P<.001$). More embryos were transferred in the cleavage-stage transfer group (mean $\pm$ SD, 1.6 $\pm$ 0.6 day 5 transfer vs. 2.1 $\pm$ 0.7 cleavage-stage transfer, $P<.001$) with a significantly higher number of grade B and C embryos transferred compared with the results in the day 5 transfer group (P=.03). After embryo transfer, a significantly higher number of additional blastocysts were cryopreserved in the day 5 transfer group (1.4 $\pm$ 1.3 day 5 transfer vs. 0.07 $\pm$ 0.3 cleavage-stage transfer, $P<.001$).

Several outcome measures were found to be lower with a cleavage-stage transfer. The rates of implantation and clinical pregnancy were significantly lower after a cleavage-stage transfer (Fig. 1). Live birth rate in the cleavage-stage transfer group was lower than that of the day 5 transfer group and trended toward significance (25% vs. 40%, respectively, P=.05); however, a cleavage-stage transfer still resulted in a live birth rate of 25%. When controlling for both the year of the transfer and the number of embryos transferred, the live birth rate conclusion remained consistent after a day 5 transfer vs. a cleavage-stage transfer (odds ratio = 1.89, 95% confidence interval 0.85 to 4.21, P=.12). Another subanalysis revealed no significant difference in live birth rates between age group comparisons, especially with increasing age >35 years, which trended toward decreased significance (Table 3). No statistical difference was observed in rates of miscarriage (28% day 5 transfer vs. 15% cleavage-stage transfer, P=.28) or twin gestation (8% day 5 transfer vs.

TABLE 2

Cycle characteristics.

	Day 5 transfer (N = 73)	Cleavage-stage transfer (N = 73)	*P* value
Total stimulation days, No.	10.0 (9.0–12.0)	10.0 (8.5–11.0)	.2
Total gonadotropin dose, units	3,900 (3,150–4,500)	4,050 (2,925–4,687)	.5
Maximum estradiol serum level, pg/mL	1,424 (1,055–1,923)	1,451 (1,091–1,896)	.7
Trigger day progesterone serum level, ng/mL	0.9 (0.7–1.1)	0.9 (0.6–1.1)	.6
Endometrial stripe thickness, mm	10.0 (8.0–12.0)	10.0 (9.0–12.0)	.2
Oocytes retrieved, No.	8.0 (6.0–10.0)	7.0 (5.0–12.0)	.5
Mature oocytes, No.	6.0 (5.0–8.0)	5.0 (4.0–8.0)	.1
Maturity rate, %[a]	79 ± 15.8	75 ± 19.8	.1
Intracytoplasmic sperm injection, No. (%)	48 (65.8)	49 (67.1)	.9
Fertilization rate, %[a]	72 ± 20.2	58 ± 25.9	< .001
Assisted hatching, No. (%)	21 (28.8)	23 (31.5)	.8
Embryos transferred, No.[a]	1.6 ± 0.6	2.1 ± 0.7	< .001
Embryo grade, No. (%)			.03
Grade A	56 (76.7)	45 (61.6)	
Grade B	16 (21.9)	20 (27.4)	
Grade C	1 (1.4)	8 (11.0)	
Embryos frozen, No.[a]	1.4 ± 1.3	0.07 ± 0.3	< .001

Note: Values are medians (1st and 3rd interquartile ranges) unless stated otherwise.
[a] Mean value (standard deviation).

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13% cleavage-stage transfer, $P=.70$) between the two groups. When comparing women who did and did not achieve a live birth after a cleavage-stage transfer, there were no significant differences in patient characteristics or cycle characteristics except for embryo grade. When at least one grade A embryo was transferred during a cleavage-stage transfer, the live birth rate was 27%. However, if a lesser quality grade B or C embryo was transferred, the live birth rate was 17%.

DISCUSSION

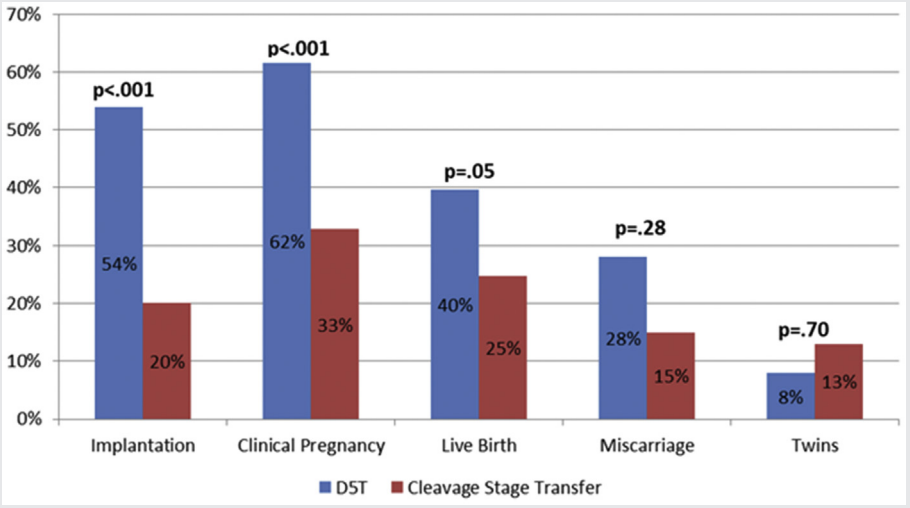
In women with a limited number of fertilized embryos after IVF or ICSI, proceeding with a cleavage-stage transfer, not surprisingly, resulted in lower implantation, clinical pregnancy, and live birth rates compared with those of women who had day 5 transfers. Despite this, the absolute live birth rate after a cleavage-stage transfer remained encouraging at 25% in comparison with no transfer. Therefore, an earlier cleavage-stage transfer is a justifiable alternative in a proportion of women who may otherwise be at risk of transfer cancellation.

Extended embryo culture with transfer on day 5 has several advantages over earlier cleavage-stage transfers. One of the main advantages of extended culture is the self-selection process of competent embryos, which allows the transfer of good quality embryos with the highest potential for pregnancy (6, 8, 9). However, for poor responders with a limited number of embryos to select from, continued in vitro culture could theoretically lead to developmental arrest (15). With several possible confounding environmental factors playing a role in embryo arrest, it has been postulated that perhaps the uterus is the ideal incubator for the embryo (24, 25). Although fertility clinics in the United States have generally transitioned to preferentially transferring blastocyst over cleavage-stage embryos, primary cleavage-stage transfers are still performed in various parts of the world. One reason for performing transfers of cleavage-stage embryos over blastocyst embryos is the lack of clear results and mixed findings demonstrated in some previous studies. A Cochran review concluded that although the live birth rates were higher after a fresh blastocyst transfer compared with those after a fresh cleavage-stage transfer, the evidence for this was of low quality (16). A previous study demonstrated that patients aged ≥35 years had significantly higher ongoing pregnancy rates per transfer and cumulative ongoing pregnancy rates with embryo transfer on day 5 compared results with transfer on day 3. However, such differences were not observed in women aged <35 years (26). Another randomized controlled study comparing outcomes with fresh blastocyst vs. cleavage-stage embryo transfers in women <39 years found no differences in the implantation rate, pregnancy rate, or delivery rate per cycle between the two groups (27). A subanalyses of live birth rates in our study with age group comparisons demonstrated acceptable rates (>5% success rates) of cleavage-stage transfers with all ages and in particular similar live birth rates when comparing cleavage vs. blastocyst stage transfer for patients >35 years.

Recently, Xiao et al. (25) compared the clinical outcomes in a cohort of women with even more limited embryo development than that in our cohort. In their study, they compared day 3 embryo transfers with day 4–6 embryo transfers in a cohort of women with just a single embryo, and they found that an earlier transfer was associated with a higher live birth rate (9.7% vs. 4.4%, $P=.002$). These results confirmed our hypothesis that not only is cleavage-stage transfer acceptable, but it may be beneficial when you are considering a single embryo's reproductive potential rather than that of a cohort of embryos.

Our finding of improved clinical outcomes in the day 5 transfer group compared with those of the cleavage-stage

FIGURE 1



Overall pregnancy outcome rates. D5T = day 5 transfer.

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TABLE 3

Live birth rates of the transfer types based on maternal age.

Age, years	Live birth rates		
	Day 5 transfer (%)	Cleavage-stage transfer (%)	*P* value
<35	21/41 (51%)	9/30 (30 %)	.07
35–40	5/20 (25%)	6/30 (20%)	.67
>40	3/12 (25%)	3/13 (23%)	.91

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transfer group is not surprising. This finding is consistent with previous studies that demonstrated improved implantation, clinical pregnancy, and live birth rates with blastocyst embryo transfers compared with the results of cleavage-stage embryo transfers (14–16, 26). Compared with the day 5 transfer group, women in the cleavage-stage group had a significantly lower rate of fertilization and a higher rate of transferring a lower quality embryo. Additionally, after embryo transfer, the cleavage-stage group had a significantly lower number of extra embryos that progressed to the blastocyst stage for subsequent cryopreservation (1.4 ± 1.3 day 5 transfer vs. 0.07 ± 0.3 cleavage-stage transfer, $P<.001$). Taken together, this suggests that women who had a cleavage-stage transfer had poorer embryo quality compared with that of those who had a day 5 transfer, resulting in substantial selection bias. Despite all of this, it was reassuring to find that the live birth rate after a cleavage-stage transfer was not consistent with very poor prognosis care (<5% anticipated success).

In a subanalysis of women who had a live birth after a cleavage-stage transfer, 27% had a live birth after transfer of at least one grade A embryo. The live birth rate was 17% after transfer of a lesser quality embryo. This finding suggests that performing a cleavage-stage transfer with at least one grade A embryo may still result in an acceptable live birth rate in a proportion of women who otherwise would be at risk of not having a transfer at all. Additionally, performing a cleavage-stage transfer with a grade B or C embryo was found to be consistent with acceptable live birth rates.

A limitation to this study was the nature of the retrospective data collection, which relied on accurate and complete data from the medical records. In addition, we only reported per transfer outcomes rather than cumulative outcomes, which may be more clinically applicable for patient counseling. Additionally, this study was conducted at a tertiary referral center and therefore the results may not be generalizable given our subset of patient population. Furthermore, as patient care was transferred to a primary obstetrician at approximately 6–8 weeks gestation, not all data were available to evaluate both obstetric and neonatal outcomes. The strengths of our study included analysis of current laboratory practice, age-matched cohorts to minimize age-related differences in pregnancy outcomes, and the primary outcome of live birth.

CONCLUSION

In spite of plans for a day 5 transfer, alternatives should be considered for women with a limited number of embryos. The financial burden as well as the emotional and psychological toll of patients must be factored into the overall treatment decision. For patients at risk of cycle cancellation, alternatives such as an earlier cleavage-stage transfer should be considered in the overall decision-making process. A cleavage-stage transfer resulted in modest, yet acceptable, live birth rates of >5%. When offering cleavage-stage transfer as an option to patients, thorough counseling on expectations,

financial costs, and live birth rates should be included in an individualized decision-making process.

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