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ORIGINAL ARTICLE

No difference in cumulative live birth rates between cleavage versus blastocyst transfer in patients with four or fewer zygotes: results from a retrospective study

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STUDY QUESTION: Is the cumulative live birth rate (CLBR) per oocyte collection cycle (OCC) comparable after cleavage-stage or blastocyst-stage transfer in combination with supernumerary blastocyst vitrification on Day 5 (D5) in patients with four or fewer zygotes on Day 1?

SUMMARY ANSWER: The CLBR in a fresh blastocyst-transfer or cleavage-stage transfer policy followed by vitrification on D5 is comparable in patients with four or fewer zygotes.

WHAT IS KNOWN ALREADY: Blastocyst transfer enhances the self-selection of the embryo and shortens the time to pregnancy in patients with normal or high ovarian response. Whether these advantages are also present in patients with a low ovarian response and/or a limited number of available zygotes is a continuous debate.

STUDY DESIGN, SIZE, DURATION: This was a retrospective, observational cohort study of 2359 consecutive OCCs between January 2014 and December 2018. According to a shift in transfer policy in our center, 571 OCCs had been scheduled for a fresh transfer on Day 3 (D3) and 1788 on D5. The D5 group was matched to the D3 group by propensity score (PS) matching according to multiple maternal baseline covariates. After PS matching, there were 571 OCCs in each group.

PARTICIPANTS/MATERIALS, SETTING, METHODS: OCCs scheduled for a D3 transfer (n = 571) or for a D5 transfer (n = 1788) were matched by PS matching in a 1:1 ratio accounting for potential confounding factors associated with CLBR. The model included patient characteristics, such as maternal age and cycle rank, as well as treatment characteristics such as GnRH analog regimen and ovarian response. Embryological variables included the number of zygotes and the number of 6- to 7- and 8-cell embryos on D3. The delivery outcomes of the fresh treatment cycle and the consecutive vitrified-warmed embryo transfers were analyzed up to the first live birth. The primary endpoint of this study was CLBR per OCC. Secondary outcomes were live birth rate per fresh transfer and embryo implantation rate per transferred embryo.

MAIN RESULTS AND THE ROLE OF CHANCE: The CLBR per OCC was comparable between the D5 and D3 groups (16.8% versus 17.7%, respectively, $P = 0.600$). Live birth rates per OCC did not differ between a cleavage-stage transfer and blastocyst-stage transfer policy (15.2% versus 12.4%, respectively, $P = 0.160$). In the D5 group, 201 cycles did not result in a blastocyst to perform an embryo transfer or cryopreservation; in the D3 group, only 59 cycles did not have an embryo transfer because of poor embryo quality (35.2% versus 10.3%, respectively; $P < 0.001$). A significantly higher number of fresh double embryo transfers were performed in the D3 group compared to D5 (23.8% versus 7.0%, respectively, $P < 0.001$).

LIMITATIONS, REASONS FOR CAUTION: Although adjusted for important confounders in the PS matching, BMI and embryo quality of the transferred embryo(s) were not taken into account. This study is limited by its retrospective design and is a single-center study, which may limit the generalizability of our findings.

WIDER IMPLICATIONS OF THE FINDINGS: The CLBR in a fresh blastocyst-transfer or cleavage-stage transfer policy followed by vitrification on D5 is comparable. A fresh embryo transfer on D3 can still be considered in patients with a poor ovarian response and/or limited number of zygotes when combined with blastocyst vitrification without impacting the overall CLBR of the cycle.

STUDY FUNDING/COMPETING INTEREST(S): No external funding was obtained for this study. There are no conflicts of interest to declare.

TRIAL REGISTRATION NUMBER: This retrospective study was approved by the local ethical committee at Ghent University Hospital (B 670201731234).

Key words: propensity score matching / cleavage-stage transfer / blastocyst-stage transfer / poor responder / live birth rate / cumulative live birth rate / vitrification

WHAT DOES THIS MEAN FOR PATIENTS?

Three days after an egg is fertilized, a normally developing embryo will contain about 6–10 cells (cleavage stage). By the fifth or sixth day, the fertilized egg is known as a blastocyst and is a rapidly dividing ball of cells. There is a higher chance of obtaining a live-born baby after the transfer of a blastocyst-stage embryo (Day 5) compared to a cleavage-stage embryo (Day 3). Blastocyst transfer is, however, related to more transfer cancelations and a lower number of embryos frozen. Therefore, whether blastocyst transfer in IVF patients with a limited number of embryos is the best policy is still a matter of debate. This study describes the chance of having a live-born baby for each complete IVF cycle in a fresh blastocyst- or cleavage-stage transfer in patients with four or fewer fertilized oocytes. A complete IVF cycle takes into account all the transfers (fresh and frozen embryos) starting from one oocyte pickup. Based on the results of our study, blastocyst transfer could be an option for women with four or fewer fertilized oocytes, as we found similar results between a cleavage- and blastocyst-stage transfer policy. Whatever the transfer policy of a center is, it should be explained to patients, and counseling on expectations of outcomes and financial costs, as well as the emotional and psychological impact of the process, can help in the decision-making process.

Introduction

After IVF, extending the duration of the embryo culture up to the blastocyst stage enables a better selection of embryos with a superior developmental capacity and consequently a higher implantation potential (Papanikolaou *et al.*, 2006; Wang and Sun, 2014; Glujovsky *et al.*, 2016; Martins *et al.*, 2017). Embryo transfer at the blastocyst stage increases the pregnancy rate per embryo transferred, and this is especially important in the context of single embryo transfer (SET) policies intended to reduce multiple gestations (Papanikolaou *et al.*, 2006). Although a blastocyst transfer policy does not appear to increase the cumulative live birth rate (CLBR), it optimizes live birth chances following the first embryo transfer and has the additional benefit of shortening the time to pregnancy (Cameron *et al.*, 2020). Intriguingly, these benefits have not resulted in the widespread substitution of the cleavage-stage transfer policy.

In good-prognosis patients, there is a clear consensus favoring a blastocyst- versus cleavage-stage embryo transfer (Cameron *et al.*, 2020). Opinions are less clear with respect to unselected patient cohorts given the conflicting results published (Glujovsky *et al.*, 2016; De Croo *et al.*, 2019). Moreover, little is known about the advantage of blastocyst culture in patients with a low yield of available embryos. Two retrospective studies have demonstrated that extended culture of embryos does not alter implantation potential when fewer than three embryos are available (Vlaisavljević *et al.*, 2001; Kovačič *et al.*, 2002). Conversely, other authors maintain that as *in vitro* survival of embryos does not relate to *in vivo* survival, transferring embryos at the blastocyst stage could lead to a loss of viable embryos as a result of them not surviving the prolonged culture (Maheshwari *et al.*, 2016). Xiao *et al.* (2019) confirmed this hypothesis in women with only one embryo available on D3, in whom pregnancy rates were higher when the embryo was transferred on D3 than on D5/6. However, these studies are limited by the fact that the only outcome is fresh embryo transfer. Data on subsequent cryopreservation are mostly lacking, and CLBRs are not provided. As a result, the benefits of blastocyst-stage transfer in poor-prognosis IVF patients after cryopreservation remain unknown.

In our center, the embryo transfer policy was changed stepwise from cleavage-state to blastocyst-stage in 2012. This modification was accompanied by the introduction of blastocyst vitrification for all supernumerary embryos regardless of the day of fresh transfer. With the information gathered since then, we conducted a study aimed at comparing the CLBR and other relevant IVF outcomes of the two embryo transfer policies, both combined with blastocyst vitrification on D5, in poor-prognosis patients (defined as four or fewer zygotes on D1), after controlling for confounding factors.

Materials and methods

Study design

This was a retrospective, observational, single-center, cohort study conducted at the University Hospital of Ghent (Belgium) between January

2014 and December 2018. Embryos from both fresh IVF and ICSI oocyte collection cycles (OCCs) were included. The sperm samples used were either fresh or frozen partner ejaculates, fresh or frozen surgically retrieved spermatozoa, or frozen donor ejaculates. OCCs were excluded in case of preimplantation genetic testing, oocyte donation cycles, cycles with no oocytes retrieved, no sperm available on the day of oocyte collection and no zygotes or only abnormal zygotes available on Day 1. Poor prognosis was based on our experience and defined as four or fewer zygotes on Day 1. OCCs performed between January 2014 and December 2015 were scheduled for transfer on Day D3 (n = 571), while those performed between January 2016 and December 2018 were scheduled for transfer on D5 (n = 1788; Fig. 1). Supernumerary embryos were cultured until D5 and vitrified as blastocysts. The reasons for freeze-all cycles included risk for ovarian hyperstimulation syndrome, elevated progesterone on the day of hCG trigger, or medical conditions (hydrosalpinx, suboptimal endometrium, fever). OCCs were also excluded if the transfer policy protocol in use was not followed. There was no restriction on the woman's age.

The relevant data on cycles were extracted from electronic patient records (Ideas V6, Mellowood Medical) and stored in a database. Propensity score (PS) matching was performed to reduce the bias between study groups (D3 versus D5 transfer) resulting from certain baseline demographic, clinical and embryologic confounding factors.

Ovarian stimulation and oocyte retrieval

For pituitary downregulation, short- and long-GnRH agonist and GnRH antagonist protocols were used. The short-agonist protocol was started after at least 14 days of ethinylestradiol 50/levonorgestrel 150 (M50) treatment (Microgynon 50®; Bayer Pharma AG, Berlin, Germany). After stopping M50 ('Day 0' of the IVF cycle), a GnRH agonist (GnRH-a) (Triptorelin; Decapeptyl®; Ferring, Hoofddorp, The Netherlands) was started on Day 3 until LH or hCG administration. Gonadotrophins (FSH: Gonal-F®, Serono Benelux, London, UK; or Puregon R; MSD, Oss, the Netherlands; or human menopausal gonadotropin (HMG): Menopur®; Ferring, Hoofddorp, The Netherlands) were added starting on Day 5. The long agonist protocol started using decapeptyl depot on Day 21 of the previous natural menstrual cycle. After at least 14 days of GnRH-a pretreatment, additional gonadotrophin administration was started. In both agonist protocols, controlled ovarian stimulation was achieved using daily doses of between 150 and 300 IU of gonadotrophins. For the antagonist protocol, gonadotrophins were started on Day 3 of the natural menstrual cycle, and a GnRH antagonist (Cetrorelix 0.25 mg; Cetrotide®, Merck Serono, Geneva, Switzerland) was injected s.c. as a daily dose from Day 6 of the cycle until the day before oocyte retrieval.

Follicle aspiration was performed 34–36 h after hCG (Pregnyl 5000 IU®; MSD Oss, the Netherlands) or recombinant hCG (Ovitrelle® 6500 IU, Serono Benelux, London, UK) injection. The women were treated with intravaginal progesterone (Utrogestan®, Besins Healthcare, Brussels, Belgium) starting on the day of hCG or recombinant LH injection to support the luteal phase. Biochemical pregnancy was defined as positive serum levels of hCG 16 days after oocyte retrieval. As for laboratory procedures, no changes were made to the stimulation protocols used during the study period.

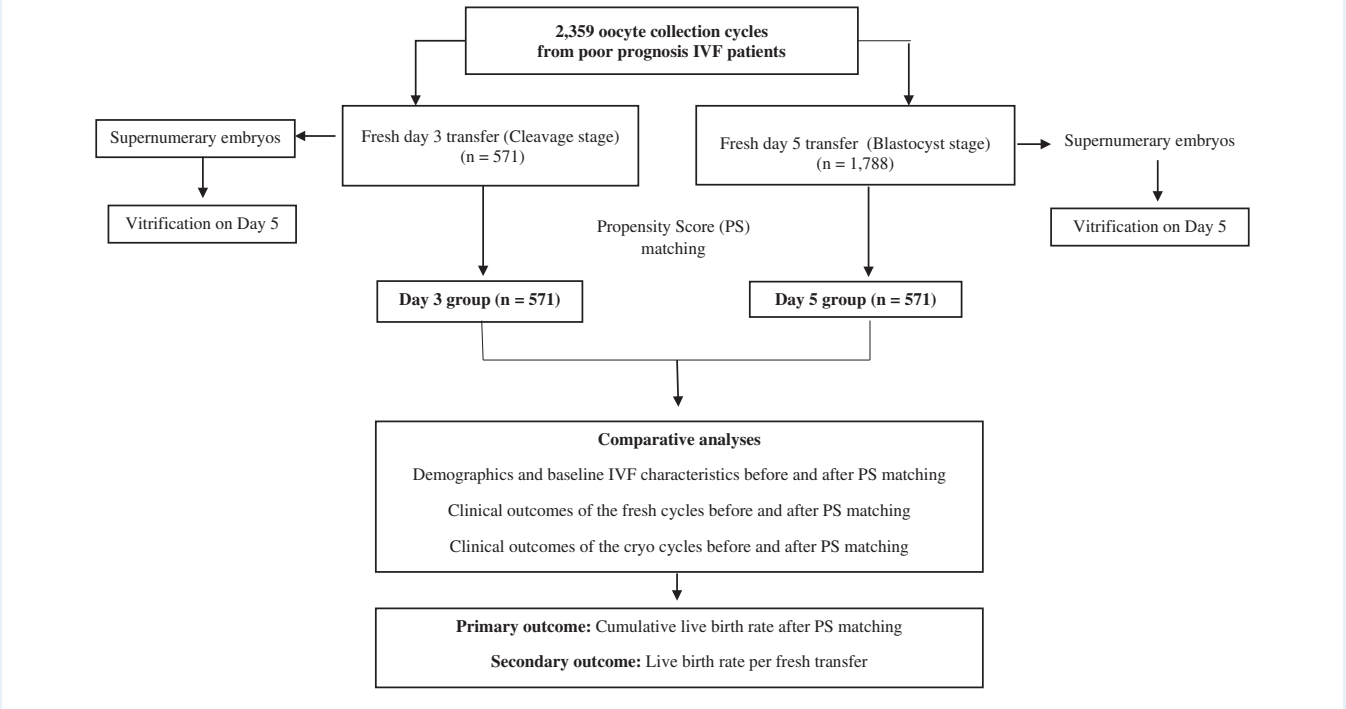

Figure 1. Flowchart of the study groups in a retrospective assessment of cumulative live birth rates after cleavage versus blastocyst transfer in patients with four or fewer zygotes. OCC, oocyte collection cycle; PS, propensity score; cryo, cryopreservation.

IVF/ICSI treatment, embryo culture and fresh embryo transfer

Oocytes were fertilized by IVF or ICSI. The embryos were cultured individually in sequential media (Cleavage and Blastocyst medium, Cook, Bloomington, IN, USA) in 25 µl microdroplets under oil (Irvine Scientific, Ireland) in a 6% CO_2, 5% O_2 and 89% N_2 incubator at 37°C (Binder 210, VWR, Belgium). Fertilization was checked 16–19 h after IVF or ICSI. Embryo development was evaluated daily until the transfer day. The quality of embryos on Days 2 and 3 was assessed on the basis of the number of blastomeres, the rate of fragmentation and the presence of multinucleation. On Day 4, the evaluation included an assessment of the compaction stage. Assessment on Day 5 was based on the classification system proposed by Gardner and Schoolcraft (1999), where the embryo ideally develops to the blastocyst stage.

Criteria for embryo transfer on Day 3 was having at least six cells, <30% fragmentation and no signs of multinucleation. Criteria for embryo transfer on Day 5 were having blastocysts with at least an expansion stage 1. From expansion stage 3 onwards, an inner cell mass score of A, B or C and trophectoderm score of A, B or C was required. Embryos in the compaction stage were not transferred or vitrified.

All transfers were performed using a Cook embryo replacement catheter (Sydney IVF, Cook, USA). The maximum number of embryos transferred was calculated and performed according to the Belgian legislation (De Neubourg *et al.*, 2013; Peeraer *et al.*, 2014). This calculation takes into account the women's age, the embryo quality and the rank of the cycle. Women aged <36 years in their first treatment cycle receive one embryo, regardless of the quality of the embryo. For the same age group, in a second treatment cycle, one embryo is transferred unless it is of insufficient quality (in which case double embryo transfer (DET) is possible). In older women or in subsequent cycles, the number of embryos never exceeds two except in women aged >39 years, in whom there is no maximum quantity of embryos to be transferred.

Vitrification and cryopreserved embryo transfer

Supernumerary blastocysts with at least an expansion stage 1, inner cell mass score of A, B or C, and trophectoderm score of A, B or C were cryopreserved on Day 5. The vitrification procedure was performed using CBS-VIT High-Security straws (CryoBiosSystem, L'aigle, France) with dimethyl sulfoxide-ethylene glycol-sucrose as the cryoprotectants (Irvine Scientific Vit Kit-Freeze, Ireland).

For patients with regular ovulatory cycles, embryos were transferred in a natural cycle. During natural cycles, patients were monitored with transvaginal ultrasound and serum estradiol (E2) and LH concentrations. For patients with irregular ovulatory cycles, endometrial preparation was initiated by oral administration of 6–12 mg estradiol valerate (Progynova®, Bayer, Belgium) once a day until the endometrial thickness was >6 mm on transvaginal ultrasound (artificial cycles). At that moment, once daily 3 × 200 mg micronized progesterone vaginally (Utrogestan®, Besins, Belgium) was added to the daily oral estradiol intake. The first day of progesterone application was set as Day 0 for calculating the day of thawing.

The transfer of warmed embryos was performed on the sixth day after ovulation. Embryos were warmed 1 day before the transfer day. In accordance with Belgian legislation, a maximum of two embryos were transferred per woman.

Outcomes

The primary outcome was CLBR per OCC, which included fresh and vitrified-warmed transfers to account for the first live birth (Maheshwari *et al.*, 2015). CLBR was assessed for transfers occurring up to 18 months following OCC. The secondary outcome was live birth rate (LBR) per fresh transfer, defined as the number of deliveries that resulted in at least one live birth, expressed per 100 cycle attempts (Zegers-Hochschild *et al.*, 2017).

Statistical analysis

The R packages 'MatchIt' and 'optmatch' were used to apply optimal matching in a 1:1 ratio (Ho *et al.*, 2011). The matchit() function of the R package 'MatchIt' was applied to estimate the PS using logistic regression (logit) based on the following variables: women's age at start cycle, cycle rank, type of GnRH analog, number of oocytes retrieved, number of zygotes on Day 1, number of 6-cell embryos on D3, number of 7-cell embryos on D3 and number of 8-cell embryos on D3. The Box-Tidwell method was used to test the linearity of the continuous predictors versus the log(odds) and to apply the appropriate transformation. PSs were compared using density plots. The model converged after four iterations, matching was applied without replacement, and no caliper was used. The baseline characteristics, ovarian stimulation outcomes and clinical outcomes were evaluated before and after matching. The matched dataset was used to compare primary and secondary outcomes. Generalized estimating equation (GEE) with the patient as subject was applied to adjust the clustering of cycles within women. Descriptive statistics were expressed as mean ± SD for continuous data with a normal distribution, whereas those with skewed distributions were expressed as median with interquartile ranges. Frequencies and percentages were used to present categorical data. Differences were analyzed with GEE linear regression (women's age, gonadotrophins dose), GEE Poisson regression (cycle rank, number of 6- to 7- and 8-cell embryos on D3, number of oocytes retrieved, number of zygotes) and GEE logistic regression (cause of infertility, type of GnRH analog, fresh transfer, freeze-all cycles, OCC with no transfer owing to poor embryo quality, clinical outcomes per fresh transfer, clinical outcomes per cryo transfer and cumulative outcomes per OCC). A *P*-value of <0.05 was considered statistically significant. Statistical analysis was performed using R version 3.5.1. (R Foundation for Statistical Computing, Vienna, Austria).

Ethical approval

This retrospective study was approved by the local ethics committee of the University Hospital of Ghent, Belgium (B 670201731234).

Results

Histogram was used to compare PS visually before and after matching. On the matched dataset, the PS for D3 and D5 showed a very similar distribution.

Based on the Box-Tidwell method, the variables 'cycle rank' and 'number of oocytes retrieved' were transformed to obtain linearity in the PS matching logistic regression model. Density plots of the distance after matching were almost identical in both groups. After PS matching with the D3 group (see Materials and methods section), the D5 group consisted of 571 OCCs.

Demographic and baseline IVF characteristics after PS matching

The demographic and baseline cycle characteristics and ovarian stimulation data of the study groups after PS matching are shown in Table I. The women's age, cycle rank and type of GnRH analog were comparable between the D3 group and D5 group. The laboratory parameters, including the number of oocytes retrieved, number of zygotes, number of 6- to 7- and 8-cell embryos on D3, were similar between both groups.

The percentage of OCCs leading to fresh transfers was significantly higher in the D3 group compared to the D5 group (89.7 versus 59.2%; $P < 0.001$). The percentage of freeze-all cycles was significantly higher in the D5 group than in the D3 group (5.6% versus 0.0%; $P < 0.001$). A similar percentage of OCCs with or without cryopreservation was observed in both groups after PS matching ($P = 0.299$). A small proportion of unsuccessful cycles still had cryopreserved blastocysts in cryostorage (8/571 in the D3 group and 10/571 in the D5 group; Table I).

Clinical outcomes of fresh transfers

The percentage of DET was significantly higher in the D3 group (23.8% versus 7.0%; $P < 0.001$). Positive hCG rates (26.3% versus 32.8%, $P = 0.296$) and twin pregnancy positive hCG (2.6% versus 4.5%, $P = 0.736$) rates did not differ between the D3 and D5 groups, respectively. After PS matching, the ongoing pregnancy rate ($P = 0.603$) and LBR per fresh transfer ($P = 0.171$) were similar in the two groups (Table II).

Clinical outcomes of vitrified-warmed transfers

No differences were observed between the two groups in the percentages of SET ($P = 1.000$). Positive hCG rates ($P = 0.862$) and twin pregnancies positive hCG rates per vitrified-warmed transfer ($P = 0.488$) were similar in the two groups. Ongoing pregnancy and LBR per vitrified-warmed transfer were similar in the two groups (both $P = 0.118$; Table III).

Cumulative outcomes

No significant difference was observed for the CLBR (17.7% in the D3 group versus 16.8% in the PS-matched D5 group; $P = 0.600$). The LBR from fresh transfers was also similar (15.2% in the D3 group versus 12.4% in the PS-matched D5 group; $P = 0.160$). No differences were observed in the cumulative twin LBRs per OCC retrieved ($P = 0.999$; Table IV).

Discussion

In this study, comparing a cleavage-stage versus a blastocyst-stage transfer policy in populations matched according to relevant demographic, clinical and embryologic characteristics, no differences were observed in the CLBR per OCC, both being close to 17% when combined with blastocyst vitrification on Day 5. The LBR per fresh transfer was also comparable between both groups.

The superiority of the cumulative outcomes after blastocyst embryo transfer with respect to cleavage-stage embryo transfer in poor-prognosis IVF patients is currently a matter of debate. Our observations support the findings of the retrospective study conducted by Yin *et al.* (2017) in matched patients who had only one or two embryos on Day 3, in whom embryos were transferred either on Day 2/3 or 5/6 (n = 217 for each group). In the Yin *et al.* (2017) study, the CLBR was comparable between the two groups (53.0% for Day 5/6 versus 49.8% for Day 2/3; P = NS). The cumulative pregnancy rate was also similar (57.1% versus 53.5% for Day 5/6 versus Day 2/3, respectively; P = NS). However, the time to live birth was significantly lower in the Day 5/6 group. The authors concluded that a Day 5/6 embryo transfer was a more cost- and time-efficient policy. In another retrospective study in patients with one or two cleavage-stage embryos, Haas *et al.* (2019) also reported a similar cumulative pregnancy rate per retrieval (22% versus 24.6%; P = NS) and ongoing pregnancy rate per retrieval (20% versus 20.2%; P = NS) in patients with 1–2 cleavage-stage embryos that were transferred either on Day 3 (n = 102) or Day 5 (n = 429), respectively. On Day 3, the number of mature oocytes and cleavage-stage embryos was similar in the two groups. Conversely, in another retrospective study in patients with only one viable embryo on Day 3, Xiao *et al.* (2019) showed that embryo transfer on this day (cleavage stage) resulted in a higher clinical pregnancy rate (14.7% versus 6.8%) and LBR (9.7% versus. 4.4%) than culturing until the blastocyst stage (Days 4–6), respectively, with these differences persisting after adjusting for significant confounders. The superiority of the cleavage-stage transfer policy in patients with few oocytes was also reported by Freeman *et al.* (2000) despite both policies achieving similar success rates (clinical and ongoing pregnancy rates) in unselected populations. The advantages of blastocyst transfer in patients with low ovarian response have also been gradually recognized. There have been few studies targeting the best embryo transfer strategy for patients with different ovarian responses. Two studies (Papanikolaou *et al.* 2008; Alfaraj *et al.*, 2017) indicated that in patients with low ovarian response, the LBR was higher in a blastocyst transfer than in a cleavage-stage transfer. The study of Freeman *et al.* (2000) showed similar success rates in cleavage- and blastocyst-stage transfer strategies, with the exception of patients with few oocytes. Our definition of poor-prognosis patients is based on less than five zygotes on Day 1, which includes a very heterogeneous population associated with differences in the underlying etiology. The low yield of zygotes may be related to poor ovarian response to stimulation yielding few mature oocytes or intrinsic abnormalities in either the oocyte or the sperm, impacting fertilization. However, since we matched on female age, number of oocytes retrieved and number of zygotes on Day 1, subpopulations of women with low ovarian reserve and/or low fertilization rate were not analyzed. These discrepancies are to be elucidated in the ongoing multicenter, non-inferiority, randomized controlled PRECISE trial (ClinicalTrials.gov Identifier: NCT03764865) aimed at comparing blastocyst to cleavage-stage

Table I Demographic and baseline IVF characteristics of the study groups after propensity score matching.

	OCCs Day 3 group (n = 571)	OCCs Day 5 group (n = 571)	*P*-value
Demographic characteristics			
Women's age (years), mean ± SD*	35.3 ± 4.9	35.2 ± 5.2	0.378
Number of patients	467	513	
Cycle characteristics			
Cause of infertility, n (%):			
Endometriosis	46 (8.0)	31 (5.4)	0.112
Ovulation disorder/PCOS	39 (6.8)	51 (8.9)	0.088
Tubal factor	41 (7.2)	43 (7.5)	0.669
Men's factor	249 (43.6)	240 (42.0)	0.446
Unexplained	196 (34.3)	206 (36.1)	0.332
Cycle rank, median (IQR)*	2.0 (1.0–4.0)	2.0 (1.0–3.0)	0.957
Ovarian stimulation data			
Type of GnRH analog, n (%)*:			0.674
Agonist	469 (82.1)	473 (82.8)	
Antagonist	102 (17.9)	98 (17.2)	
Gonadotrophins dose (IU), mean ± SD	3064 ± 1.347	3071 ± 1224	0.667
Oocytes retrieved, median (IQR)*	6.0 (4.0–8.0)	6.0 (4.0–9.0)	0.542
Embryologic outcomes			
Zygotes, median (IQR)*	2.0 (1.0–3.0)	2.0 (1.0–3.0)	0.947
Six-cell embryos on Day 3, n (%)*	1.7 ± 1.2	1.8 ± 1.1	0.286
Seven-cell embryos on Day 3, n (%)*	1.5 ± 1.1	1.6 ± 1.1	0.494
Eight-cell embryos on Day 3, n (%)*	1.2 ± 1.0	1.3 ± 1.0	0.678
Cycle outcomes			
Fresh transfers, n (%)	512 (89.7)	338 (59.2)	**<0.001**
Freeze-all cycles, n (%)	0 (0.0)	32 (5.6)	**<0.001**
Reasons, n:			
OHSS risk	0	1	
Progesterone elevation on day of trigger	0	7	
Medical condition	0	24	
OCCs with no transfer owing to poor embryo quality, n (%)	59 (10.3)	201 (35.2)	**<0.001**
OCCs with cryopreservation, n (%):			
0 embryos			0.299
1 embryo	429 (75.1)	428 (75.0)	
2 embryos	96 (16.8)	80 (14.0)	
3 embryos	42 (7.4)	50 (8.7)	
4 embryos	4 (0.7)	13 (2.3)	
OCCs with no live birth and cryopreserved embryos left	0 (0.0)	0 (0.0)	
	8	10	

Data are presented for oocyte collection cycles (OCCs); IQR, interquartile range; OHSS, ovarian hyperstimulation syndrome; GEE, generalized estimating equation.
*Selected for matching in the propensity score model. Women's age, gonadotrophin dose: *P*-value based on GEE linear regression; cycle rank, number of 6- to 7- and 8-cells embryos on Day 3, number of oocytes retrieved, number of zygotes: *P*-value based on GEE Poisson regression; Cause of infertility, type of GnRH analog, fresh transfer, freeze-all cycles and OCC with no transfer owing to poor embryo quality: *P*-value based on GEE logistic regression.
Statistically significant values are indicated in bold.

embryo transfer in poor-prognosis patients (defined as having ≤5 zygotes on Day 1 after fertilization), whose protocol has recently been published (Neuhausser *et al.*, 2020). The primary outcome is LBR per retrieval (time frame: 9 months). The study will enroll 658 eligible women at six IVF centers over the course of 22 months. The estimated completion date is 28 February 2024.

Table II Clinical outcomes per fresh embryo transfer after propensity score matching.

	Day 3 (n = 512)	Day 5 (n = 338)	*P*-value
Embryos transferred, n	636	363	
Double embryo transfers, n (%)	122 (23.8)	25 (7.0)	**<0.001**
Positive hCG, n (%)	150 (26.3)	111 (32.8)	0.296
Twin pregnancies†, n (%)	4 (2.7)	5 (4.5)	0.763
Biochemical pregnancy loss (%)	20 (13.3)	14 (12.6)	1.000
Miscarriages, n (%)	35 (23.3)	24 (21.6)	0.767
Ectopic pregnancy, n (%)	2 (1.3)	0 (0.0)	0.509
Ongoing pregnancy rate, n (%)	93 (18.1)	73 (21.5)	0.603
Live births, n (%)	87 (17.0)	71 (20.4)	0.171

Data are presented for fresh transfers unless indicated. Statistically significant values are indicated in bold, *P*-value based on generalized estimating equation logistic regression; †Per positive hCG.

Table III Clinical outcomes per cryotransfer after propensity score matching.

	Day 3 (n = 92)	Day 5 (n = 85)	*P*-value
Single embryo transfer, n (%)	91 (98.9)	85 (100.0)	1.000
Positive hCG, n (%)	22 (23.9)	22 (25.9)	0.862
Twin pregnancies*, n (%)	2 (9.1)	0 (0.0)	0.488
Ongoing pregnancy rate, n (%)	14 (15.2)	20 (23.5)	0.118
Live birth rate, n (%)	14 (15.2)	20 (23.5)	0.118

P-value based on generalized estimating equation logistic regression; *Per positive hCG.

As in other studies comparing both transfer policies in unselected populations (Papanikolaou *et al.*, 2008; Glujovsky *et al.*, 2016; Alfaraj *et al.*, 2017), the stepwise change from a cleavage-stage to a blastocyst-stage transfer policy in our center resulted in a similar CLBR per OCC (25.9% for Day 3 versus 28.4% for Day 5, *P* = NS; De Croo *et al.*, 2020). However, we observed a dramatic increase in transfer cancelation rates, especially in poor-prognosis women (1–4 zygotes available on Day 1). This change in transfer policy was accompanied by the introduction of blastocyst vitrification for all supernumerary embryos, regardless of the day of fresh transfer. This policy gave us the opportunity to analyze the data retrospectively and see if a hybrid transfer and cryopreservation policy might benefit poor-prognosis patients, i.e. fresh embryo transfer on Day 3 and cryopreservation policy on Day 5.

Most studies comparing CLBR with different transfer policies use selected patient cohorts based on age (Rienzi *et al.*, 2002; De Vos *et al.*, 2016), available zygotes (Rienzi *et al.*, 2002; De Vos *et al.*, 2016; Levi-Setti *et al.*, 2018), number of available embryos on Day 3 (De Vos *et al.*, 2016; Yin *et al.*, 2017) or a combination of these factors (Milki *et al.*, 2000; De Vos *et al.*, 2016; Levi-Setti *et al.*, 2018). Deciding the transfer policy based on the number of oocytes, on the one hand, or the number of embryos on Day 3, on the other hand, has disadvantages, as: the effect of low fertilization is not taken into account when the number of retrieved oocytes is chosen to decide the day of transfer; decision-making on Day 3 based on the number of embryos will have the consequence of additional communications with the patients, who will have to be able to come to the clinic on that same day, and patients also need to be available for a transfer on both Day 3 and Day 5; and allocation of patients for the day of transfer based on the number of available embryos on Day 3 is often a clinical decision (De Vos *et al.*, 2016) influenced by unknown variables. The possibility of deciding the day of transfer on Day 1, based on the number of zygotes, has the benefit of being able to communicate the day of transfer to patients when the fertilization results are reported. The decision between a cleavage-stage transfer policy and a blastocyst-stage policy, in our study, is the result of a change in transfer policy during the two time periods and is thus independent of any individual clinical decision, which adds to an objective allocation of patients to the transfer policy.

The use of a high-quality vitrification blastocyst cryopreservation policy on Day 5 could be an interesting combination with a fresh embryo transfer on Day 3 in patients with few zygotes. The proportion of cryopreserved embryos per OCC was similar for cleavage-stage and blastocyst-stage transfer ($P = 0.299$). Two publications evaluated the added value of the vitrified embryos in a Day 3 transfer policy (Fernández-Shaw *et al.*, 2015; De Vos *et al.*, 2016). Although survival after warming of cleavage-stage embryos with vitrification is higher than with slow-freezing, these studies failed to confirm a higher cumulative success rate despite a higher number of embryos vitrified on Day 3. These reports show that it is not the higher number of vitrified embryos that make a difference in CLBR. A more selective cryopreservation program on Day 5 can equilibrate the CLBR between fresh transfers on Day 3 and Day 5, as shown in our study.

Transfer cancelation rates in blastocyst transfer policies range between 0% and 28% (Papanikolaou *et al.*, 2008). We observed a cancelation rate of 33.3% in the Day 5 group, which is slightly higher than the 30% OCC cancelation rate with 1–2 oocytes, 14% with 3 oocytes and 10% with 4 oocytes reported in the literature (Connell *et al.*, 2019). This may be explained by our strict transfer policy, as we only transfer on Day 5, and no compaction or earlier stages are considered.

Our data show similar LBRs with a significantly higher percentage of SET in the Day 5 group (93.0% versus 76.2% SET in the Day 3 group; $P < 0001$). Single blastocyst-stage transfer not only reduces multiple gestations but also results in a higher pregnancy rate per transfer compared to a single cleavage-stage transfer (Papanikolaou *et al.*, 2006). It has been reported that the LBR after transfer of a single fresh blastocyst is similar to that of transfer of two fresh cleavage-stage embryos (Thurin *et al.*, 2004).

The main limitation of our study stems from its retrospective nature. Despite being nonrandomized, the PS matching allowed comparability of the study populations with respect to confounding factors in a 'context-based medicine' approach (Macklon and Fauser, 2020). PS matching is gaining interest in studies evaluating infertility treatments (Yin *et al.*, 2017; Gu *et al.*, 2019; Qu *et al.*, 2019; Haviland *et al.*, 2020).

Table IV Cumulative outcomes after propensity score matching.

	Day 3 (n = 571)	Day 5 (n = 571)	*P*-value
Live birth fresh, n (%)	87 (15.2)	71 (12.4)	0.160
Cumulative live birth, n (%)	101 (17.7)	96 (16.8)	0.600
Cumulative twin live birth, n (%)	3 (0.5)	3 (0.5)	0.999

Data are presented for oocyte collection cycles. *P*-value based on generalized estimating equation logistic regression.

No major changes were made to the laboratory practices during the shift from a cleavage-stage to blastocyst-stage transfer policy.

In conclusion, our retrospective PS matching study in poor-prognosis patients (four or fewer zygotes) shows a similar CLBR per OCC, and LBR per fresh transfer, regardless of the day of fresh transfer, when combined with blastocyst vitrification on Day 5. The blastocyst-stage transfer policy was accompanied by a higher percentage of SET, and the cumulative outcomes were achieved regardless of a higher transfer cancelation rate in the blastocyst group. Our results may benefit clinics not willing to shift to a blastocyst transfer policy in all patients, as similar cumulative outcomes may be achieved in patients with four or fewer zygotes on Day 1 when transfer on Day 3 is combined with blastocyst vitrification on Day 5.

Data availability

The data underlying this article will be shared on reasonable request to the corresponding author.

Authors' roles

Study design: I.D.C. and K.T. Acquisition of data: I.D.C. and K.T. Statistical analysis: R.C. Writing of the manuscript: I.D.C. and K.T. Interpretation of the data, critical review of the manuscript: I.D.C., R.C., K.T. D.S. and P.D.S.

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Conflict of interest

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