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ORIGINAL ARTICLE

A stepwise approach to move from a cleavage-stage to a blastocyst-stage transfer policy for all patients in the IVF clinic

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STUDY QUESTION: Is a stepwise change management approach an efficacious method to move from a Day 3 transfer policy to a Day 5 transfer policy for all patients in an IVF program?

SUMMARY ANSWER: A stepwise change from a Day 3 to a Day 5 transfer policy maintained the live birth rates per oocyte collection cycle (OCC) of the IVF program, with increased single embryo transfer (SET) and reduction of twin pregnancies.

WHAT IS KNOWN ALREADY: Evidence has shown that the probability of a live birth following IVF with a fresh embryo transfer (ET) is significantly higher after blastocyst-stage transfer than after cleavage-stage transfer. Blastocyst culture and transfer are usually performed in cases of good prognosis patients but many centers keep transferring cleavage-stage embryos for most of their patients because of the higher transfer cancelation rate in a blastocyst transfer policy.

STUDY DESIGN, SIZE, DURATION: In January 2012, a Day 5 embryo culture and blastocyst transfer policy including vitrification of supernumerary Day 5 blastocysts were implemented in a stepwise approach. The retrospective descriptive single-center analysis involving a preintervention phase consisted of Day 3 ETs and Day 3 slow freezing from 2010 until 2012. The postintervention phase involved a 6-year period from 2012 until 2017 in which three consecutive changes in the transfer policy were made, each over a 2-year period, based on the number of zygotes on Day 1. The primary outcome was live birth delivery rate per OCC during the stepwise change.

PARTICIPANTS/MATERIALS, SETTING, METHODS: All patients with at least one zygote available on Day 1 were scheduled for a fresh transfer, either on Day 3 or 5. Cycles with preimplantation genetic testing, freeze-all and oocyte donation cycles and cycles with a Day 2 transfer in the preintervention period were excluded. In the preintervention group, all cycles were scheduled for Day 3 transfer ($n = 671$ OCC) and slow freezing of the remaining Day 3 embryos. In the postintervention period, three periods were analyzed: period 1 ($n = 1510$ OCC; 1–9 zygotes: Day 3 transfer and >9 zygotes: Day 5 transfer); period 2 ($n = 1456$ OCC; 1–4 zygotes: Day 3 transfer and >4 zygotes: Day 5 transfer) and period 3 ($n = 1764$ OCC; Day 5 transfer). All remaining embryos underwent extend culture and were vitrified on Day 5, if developed to at least an early blastocyst. Data were analyzed using a mixed regression model with patient as a random factor.

MAIN RESULTS AND THE ROLE OF CHANCE: In the preintervention group, all OCC were scheduled for a Day 3 transfer. In period 1, period 2 and period 3, 20.9%, 61.5% and 100% of the OCCs were scheduled for a Day 5 transfer, respectively. More transfers per OCC were canceled in the postintervention period 2 and period 3 compared to the preintervention period (5.3% and 18.7% versus 3.4%, respectively; $P < 0.0001$). The mean number of embryos used per transfer decreased gradually after the introduction of the Day 5 transfer policy, from 1.62 ± 0.65 in the preintervention group to 1.12 ± 0.61 in period 3 ($P < 0.0001$). The percentage of SET cycles increased from 48.4% in the preintervention group to 54.6%, 73.8% and 87.8% in period 1, period 2 and period 3, respectively ($P < 0.0001$). The mean number of cryopreserved surplus embryos was significantly lower in period 3 compared to the preintervention group (1.29 ± 1.97 versus 1.78 ± 2.80; $P < 0.0001$).

Pregnancy and live birth delivery rate per fresh transfer, respectively, were significantly lower in the preintervention group (26.7% and 19.1%) as compared to period 3 (39.3% and 24.2%) ($P < 0.0001$). Twin pregnancy rate decreased gradually from 11.0% to 8.2%, 5.7% and

2.5% in the preintervention group, period 1, period 2 and period 3, respectively ($P < 0.0001$). Live birth rate and cumulative live birth delivery rates per OCC were significantly higher in group 2 compared to the preintervention period (25.6% and 35.8% versus 18.5% and 25.9%, respectively). Similar live birth and cumulative live birth delivery rates per OCC were achieved between the preintervention period and period 3 (18.5% and 25.6% versus 19.7% and 24.9%; respectively).

LIMITATIONS, REASONS FOR CAUTION: The primary limitation is the retrospective design of the study. The allocation of the cycles was done by the number of zygotes available without taking into account both embryological and clinical prognostic factors. Furthermore, the analysis was restricted to cycles where the standard transfer policy was followed. Embryos which were in the morula or compaction stage were not vitrified or cultured to Day 6, which could have contributed to the slight, not statistically significant, drop in live birth rate per OCC in group 3.

WIDER IMPLICATIONS OF THE FINDINGS: Live birth and cumulative live birth delivery rate per OCC in an unselected patient population is maintained in a Day 5 transfer policy compared to a Day 3 transfer policy. Additionally, a significantly reduction in twin pregnancy rate and a significant increase in SET were observed in a Day 5 transfer policy. For centers wanting to make the step from Day 3 to Day 5, this study provides a practical stepwise change management approach.

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TRIAL REGISTRATION NUMBER: None.

Key words: stepwise change management / Day 3 transfer / Day 5 transfer / live birth delivery rate / cumulative live birth delivery rate

WHAT DOES THIS MEAN FOR PATIENTS?

During an IVF cycle, transferring the embryo at the blastocyst stage means that there is a better selection of viable embryos available for transfer. This leads to a higher chance of pregnancy. On the other hand, IVF centers' blastocyst transfer policies also lead to a larger number of transfer cancelations and a lower number of embryos cryopreserved. For these reasons, centers are reluctant to take the step to switch from a Day 3 (cleavage-stage) transfer policy to a Day 5 (blastocyst) transfer policy for all their patients. Often only patients who have a lot of embryos are directed toward transfer of a blastocyst. This reduces the chances of not having a blastocyst to transfer on Day 5. It is indeed disappointing for patients having undergone a controlled ovarian stimulation to end up with no embryos to transfer.

This study describes the change management approach to move from a Day 3 (cleavage-stage) transfer policy for all to a Day 5 (blastocyst) transfer policy for all. We demonstrate that the blastocyst transfer policy for all showed similar live birth rates per oocyte collection cycle. We also show a higher live birth rate per fresh embryo transfer (ET) as compared to Day 3 (cleavage-stage) transfer policy. The change to a blastocyst transfer policy for all resulted in a higher number of single ETs and a lower number of twin pregnancies. For patients who have been treated in a Day 3 (cleavage-stage) ET policy and who are now being offered a Day 5 transfer (blastocyst) policy, this article may help in understanding how pregnancy rates and transfer cancelation rates change because of the difference in transfer policy.

Introduction

Transferring the embryo at the blastocyst stage provides several theoretical advantages, including a higher rate of implantation, better selection of viable embryos for transfer and the identification of embryos that have managed to activate their embryonic genome (Wang and Sun, 2014; Glujovsky *et al.*, 2016; Martins *et al.*, 2017). On the other hand, blastocyst transfer policies are also related to an increased incidence of transfer cancelations and a lower number of embryos cryopreserved. For these and other reasons, centers are reluctant to take the step to switch from a Day 3 transfer policy to a Day 5 transfer policy for the whole of their patient population. When the decision was made in our clinic to move toward a blastocyst policy, we had the same concern. Therefore, the move from a cleavage stage transfer policy to a blastocyst transfer policy was executed in a stepwise manner, taking small steps and keeping track of our outcome parameters during this journey. During the preparation of our change management approach, some colleagues stated that it would be better for patients to hear that their embryos failed to develop to the blastocyst stage than go through with transfer of a cleavage-stage embryo having a low potential to implant. Actually, very little is known of the emotional status of couples or women presented with such choices (Zemyarska, 2019). The emotional aspect could be the reason why, until today, no randomized-controlled trial has been performed on this topic and studies only include a selected population of good prognosis patients. Indeed, most studies have analyzed the benefit of blastocyst transfer only in the good prognosis patient populations and made a selection based on age (Rienzi *et al.*, 2002; De Vos *et al.*, 2016) or on available zygotes (Fernández-Shaw *et al.*, 2015). Little is known about the advantage of blastocyst culture for poor prognosis patients; defined as patients with a low number of oocytes or embryos available and/or advanced maternal age. In a very early period of development of commercial blastocyst culture media, two studies showed that extended culture of embryos did not improve or decrease their implantation potential in cases where fewer than three developed embryos are available (Kovačič *et al.*, 2002) or in cases of unstimulated cycles (Vlaisavljević *et al.*, 2001). The study of Fernández-Shaw *et al.* (2015), selecting a patient population with at least four fertilized oocytes on Day 1, showed a significantly higher clinical pregnancy rate, ongoing pregnancy rate and cumulative pregnancy rate in women 35 years or

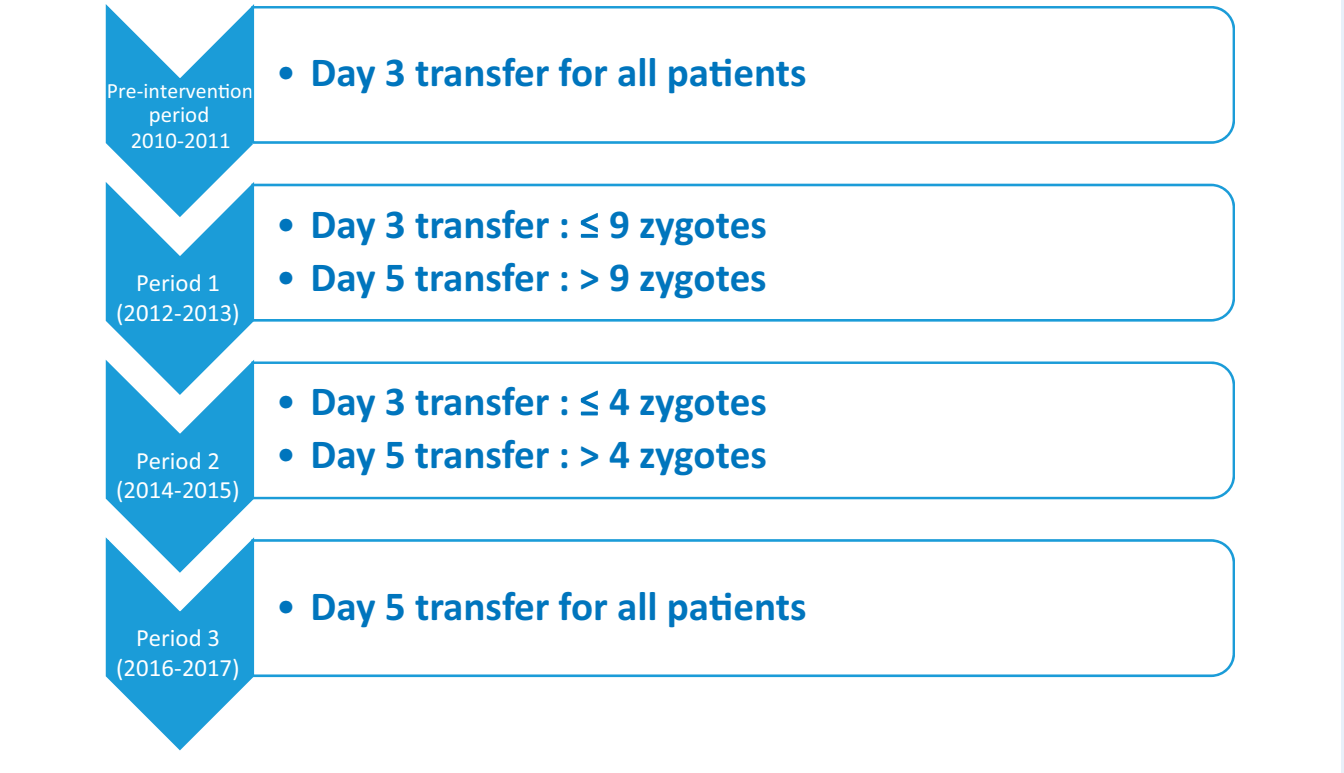

Figure 1 Flowchart of the study groups.

older after Day 5 transfer, while in women under 35 years, no significant differences were found compared to cleavage-stage transfers.

Many centers hesitate to decide between a cleavage-stage or blastocyst-stage policy for all patients and often proceed to blastocyst transfer only in good prognosis patients. It, therefore, remains questionable whether a blastocyst-stage approach is indeed beneficial for all patients seeking ART. We have taken on this challenge by using a step-wise change management approach to move from a Day 3 transfer policy for all, to a Day 5 transfer policy for all. This retrospective, observational cohort study describes the results of this approach.

Materials and methods

Study design

A retrospective, observational cohort single-center study was performed over a period of 8 years, with a preintervention period from January 2010 to December 2011 and postintervention period between January 2012 and December 2017. Both fresh IVF- and ICSI cycles were included. Sperm samples used were either fresh or frozen partner ejaculates, fresh or frozen surgically retrieved spermatozoa, or frozen donor ejaculates. Exclusion criteria were the following: cycles with preimplantation genetic testing, freeze-all and oocyte donation cycles and cycles with a Day 2 transfer in the preintervention period. Cycles with no oocytes retrieved, no sperm available on Day of oocyte collection cycle (OCC) and no zygotes or only abnormal zygotes available on Day 1 were excluded. Cycles where the transfer policy protocol, based on the number of available zygotes, was not followed were also excluded. No restriction on female age was made.

In January 2012, the intervention involving blastocyst culture and transfer was implemented in a stepwise manner. Each consecutive step to move from a Day 3 transfer policy to a Day 5 transfer policy was decided based on a selection of performance and outcome indicators. The cycles that move to a blastocyst transfer were selected on the basis of the number of zygotes available on Day 1. In order to take further steps in our change of management policy based on certain indicators, all supernumerary embryos (after a fresh transfer on Day 3) were further cultured until Day 5 and vitrified if they reached the stage of early blastocyst. Embryos in the morula or compaction stage on Day 5 were not vitrified or cultured to Day 6.

OCCs were divided into four periods according to the standard transfer strategy (Fig. 1).

Preintervention period (2010–2011).

OCC performed between July 2010 and December 2011 was scheduled for a transfer on Day 3 and supernumerary embryos were cryopreserved with a slow freezing protocol on Day 3 (n = 671).

Period 1 (2012–2013).

OCC performed between January 2012 and December 2013 and having more than nine zygotes on Day 1 was scheduled for an embryo transfer (ET) on Day 5 (n = 315), cycles with <10 zygotes were scheduled for an ET on Day 3 (n = 1195). Supernumerary blastocysts

in both groups were vitrified on Day 5. The blastocyst rate of the supernumerary embryos in combination with the pregnancy rate per transfer was monitored during the gradual implementation of the Day 5 transfer from period 1 to period 2.

Period 2 (2014–2015).

OCC performed between January 2014 and December 2015 and having more than four zygotes on Day 1 was scheduled for transfer on Day 5 (n = 896), OCC with less than five zygotes was scheduled for transfer on Day 3 (n = 560). Supernumerary blastocysts in both groups were vitrified on Day 5. Pregnancy rate per transfer was monitored during the gradually implementation of the Day 5 transfer from period 2 to period 3.

Period 3 (2016–2017).

OCC performed between January 2016 and December 2017 was all scheduled for transfer on Day 5 (n = 1764). Supernumerary blastocysts were vitrified on Day 5.

No major changes in the clinical and laboratory practices were introduced during the study period. The preintervention analysis started in July 2010: this time point was chosen as we moved the IVF laboratory into a cleanroom facility from that date onwards. The environmental conditions of the IVF laboratory have thus been tightly controlled ever since and we have been working in a background Good Manufacturing Practice class C, in contrast to the D environment issued by the European Union Tissue and Cells Directives. Today we are still using the same sequential media, oil and type of incubators. Although ongoing optimization of laboratory protocols are part of our daily quality management, in the specific time window of the paper, the biggest change for the laboratory was the introduction of blastocyst culture and transfer together with blastocyst vitrification.

Ovarian stimulation and oocyte retrieval

For pituitary downregulation, three protocols (two agonists, one antagonist) were used. The short agonist protocol started following at least 14 days ethinylestradiol 50/levonorgestrel 150 (M50) (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 HMG: Menopur®; Ferring, Hoofddorp, The Netherlands) were added starting on Day 5. The longer 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 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 cycle Day 6 until the day prior to oocyte retrieval.

Between 34 and 36 h after the HCG (Pregnyl 5.000IU ®; MSD Oss, the Netherlands) or recombinant HCG (Ovitrelle® 6500IU, Serono Benelux, London, UK) injection, follicle aspiration was performed. To support the luteal phase, all women were treated with intravaginal progesterone (Utrogestan®, Besins Healthcare, Brussels, Belgium) starting on the day of HCG or recombinant LH injection. Biochemical pregnancy was considered as positive serum levels of HCG at 16 days after oocyte retrieval.

IVF/ICSI treatment, embryo culture and fresh ET

Oocytes were fertilized either by insemination (IVF) or by ICSI. The embryos were cultured individually in sequential media (Cleavage and Blastocyst medium, Cook, 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 insemination or ICSI. Embryo development was evaluated daily until the day of transfer.

Briefly, the embryo quality on Days 2 and 3 was assessed on the basis of the number of blastomeres, the rate of fragmentation and the presence of multinucleation. ET on Day 3 was carried out using embryos having at least 6 blastomeres, <50% fragmentation and showing no multinucleation. On Day 4, the evaluation included assessment of the compaction stage. Assessment on Day 5 was based on the classification system of Gardner and Schoolcraft (1999), where the embryo ideally develops to the blastocyst stage. Embryos in compaction stage were not transferred or vitrified. All transfers were performed using a Cook embryo replacement catheter (Sydney IVF, Cook, USA).

Slow freezing, vitrification and frozen ET

In the preintervention group, after fresh transfer on Day 3, supernumerary Day 3 embryos with at least six blastomeres and <30% fragmentation were cryopreserved following a slow-freezing protocol with 1,2-propanediol-sucrose as cryoprotectant (Sydney IVF cryopreservation kit, Cook, USA) using CBS High-Security straws (CryoBiosSystem, L'aigle, France).

In postintervention groups, supernumerary blastocysts with at least expansion stage 1, inner cell mass score A, B or C and trophectoderm score 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 dimethylsulphoxide-ethylene glycol-sucrose as the cryoprotectant (Irvine Scientific Vit Kit-Freeze, Ireland).

The patients having regular ovulatory cycles underwent natural cycle frozen embryo transfer (FET) (natural cycles). During natural cycles, patients were monitored with transvaginal ultrasound and serum estradiol (E2) and LH concentrations. In case patients did not have a regular ovulatory cycle, endometrial preparation was initiated by oral administration of 6–12 mg estradiol valerate dd (Progynova®, Bayer, Belgium) until the endometrial thickness was >6 mm on transvaginal ultrasound (artificial cycles). At that moment, 3 × 200 mg dd micronized progesterone vaginally (Utrogestan®, Besins, Belgium) was added to the daily oral estradiol intake. The first day of start of progesterone application was set as Day 0 for calculating the day of thawing.

The FET was performed on the fourth or sixth day after ovulation depending on the day of cryopreservation (pre- or postintervention periods, respectively). Embryos were thawed 1 day before the day of transfer and a maximum of two embryos were transferred. All

Table I Period 1 (2012–2013) compared with preintervention (2010–2011): patient characteristics and pregnancy outcome.

	Pre-intervention period		Period 1		*P*-value
	1–9 zygotes Day 3	>9 zygotes Day 3	*1–9 zygotes Day 3*	>9 zygotes Day 5	
Number of OCC	*529*	142	*1195*	315	
Number of transfers (%)	*507 (95.8%)*	141 (99.3%)	*1156 (96.7%)*	306 (97.1%)	NS
Number of cycles with no transfer because of poor embryo quality (%)	*22 (4.2%)*	1 (0.7%)	*39 (3.3%)*	9(2.9%)	NS
Number of SET	*237 (44.8%)*	77 (54.6%)	*629 (54.4%)*	171 (55.9%)	NS
Mean female age (mean ± SD) (years)	*32.87 ± 4.3*	32.43 ± 4.4	*35.27 ± 5.09*	32.69 ± 4.78	NS
Number of previous cycles (mean ± SD)	*3.03 ± 2.44*	1.98 ± 2.24	*2.89 ± 2.07*	1.73 ± 2.01	NS
Antagonist protocol (%)	*22.3%*	28.9%	*21.1%*	24.4%	NS
Agonist protocol (%)	*77.7%*	70.4%	*78.9%*	75.5%	NS
Number of oocytes retrieved (mean ± SD)	*8.87 ± 4.92*	20.45 ± 7.05	*8.60 ± 4.80*	20.50 ± 6.17	NS
Number of zygotes (mean ± SD)	*4.41 ± 2.48*	13.61 ± 4.09	*4.34 ± 2.37*	13.52 ± 3.81	NS
Number of embryos transferred (mean ± SD)	*1.57 ± 0.63*	1.52 ± 0.66	*1.50 ± 0.61*	1.45 ± 0.64	NS
Number of embryos cryopreserved (mean ± SD)	*0.97 ± 2.10*	4.83 ± 4.14	*0.66 ± 1.22*	3.06 ± 2.97	<0.0001
Number of pregnancies	*122*	51	*317*	157	
Number of live birth deliveries	*88*	36	*205*	105	
Number of twin pregnancies (%)	*15 (12.3%)*	4 (7.8%)	*25 (7.9%)*	14 (8.9%)	NS
% pregnancies/transfer	*24.1%*	36.2%	*27.4%*	51.3%	0.0030
% live birth/ transfer	*17.3%*	25.5%	*17.7%*	34.3%	NS
%live birth/OCC	*16.6%*	25.3%	*17.2%*	33.3%	NS

Data are presented as mean (± SD) or number (%); OCC, oocyte collection cycles; SET, single embryo transfer, pregnancy, positive serum levels of hCG performed 16 days after OCC; *P*-value of <0.05 was considered statistically significant. Statistical analysis was performed on the data in the gray columns and data in italics were added for the completeness. Dichotomous outcome variables were tested with a mixed logistic regression. For the continuous variable, a mixed linear regression was applied. For the analysis of counts, a mixed negative binominal regression was used.

transfers were performed using a Cook embryo replacement catheter (Sydney IVF, Cook, USA).

Outcomes and statistical analysis

The primary outcome of this study was live birth delivery rate after fresh transfer per OCC. Secondary outcomes were fresh transfer cancelation rate, live birth delivery rate per fresh and FET, and cumulative live birth delivery rate per OCC. Live birth delivery rate was defined as the number of deliveries that resulted in at least one live birth, expressed per 100 cycle attempts (Zegers-Hochschild *et al.*, 2017). Cumulative live birth delivery rate per OCC included fresh and frozen ETs over a 2-year period to account for the first live birth ((Maheshwari *et al.*, 2015). Relevant data for all cycles were extracted from electronic patient records and stored in a database. To compare the different conditions and periods, descriptive statistics were expressed as mean (±SD) for continuous data. Frequencies and percentages within the study groups were used to present categorical data.

To describe the outcomes per period, crude unadjusted descriptive statistics were provided together with the *P*-values resulting from unadjusted mixed models with patient as a random factor, taking the clustered nature of the data into account (a patient may have undertaken more than one cycle).

For the dichotomous outcome variables ('live birth deliveries', 'down regulation protocol', 'No transfer', 'pregnancy', 'cumulative live birth deliveries'), a mixed logistic regression was applied. For the continuous variable, mean female age, a mixed linear regression was applied. For the analysis of counts ('number of oocytes retrieved', 'number of zygotes', 'number of embryos transferred', 'number of embryos cryopreserved', 'number of previous cycles'), a mixed negative binominal regression was used. Statistical analysis was performed using R version 3.5.3 (R Studio, Boston, MA, USA) A value of $P < 0.05$ was considered statistically significant.

Ethical approval

This retrospective study was approved by the local ethics committee of the UZ Ghent (B 670201731234).

Results

Evaluation of cycle outcomes between period 1 (Day 5 ET) and the preintervention period (Day 3 ET) in patients with >9 zygotes

Period 1 included 1510 fresh IVF and ICSI OCCs, as summarized in Table I. In this period, a Day 3 transfer was scheduled in 1195 OCCs and a Day 5 transfer in 315 OCCs. The proportion of OCCs scheduled for Day 5 transfer was 20.9% (315/1510) in period 1. Of interest

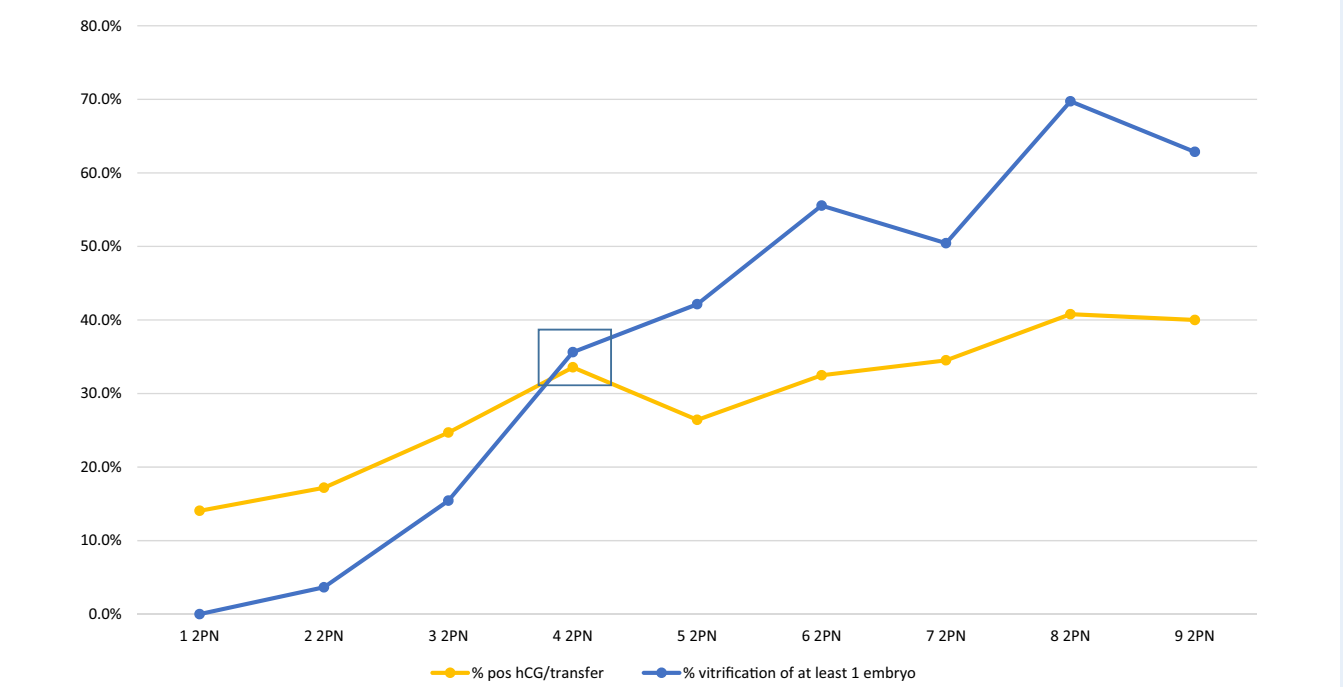

Figure 2 Pregnancy rate after fresh transfer on Day 3 and vitrification rate of supernumerary blastocysts on Day 5 in the 1–9 zygote group (period 1). 2PN, two pronuclei.

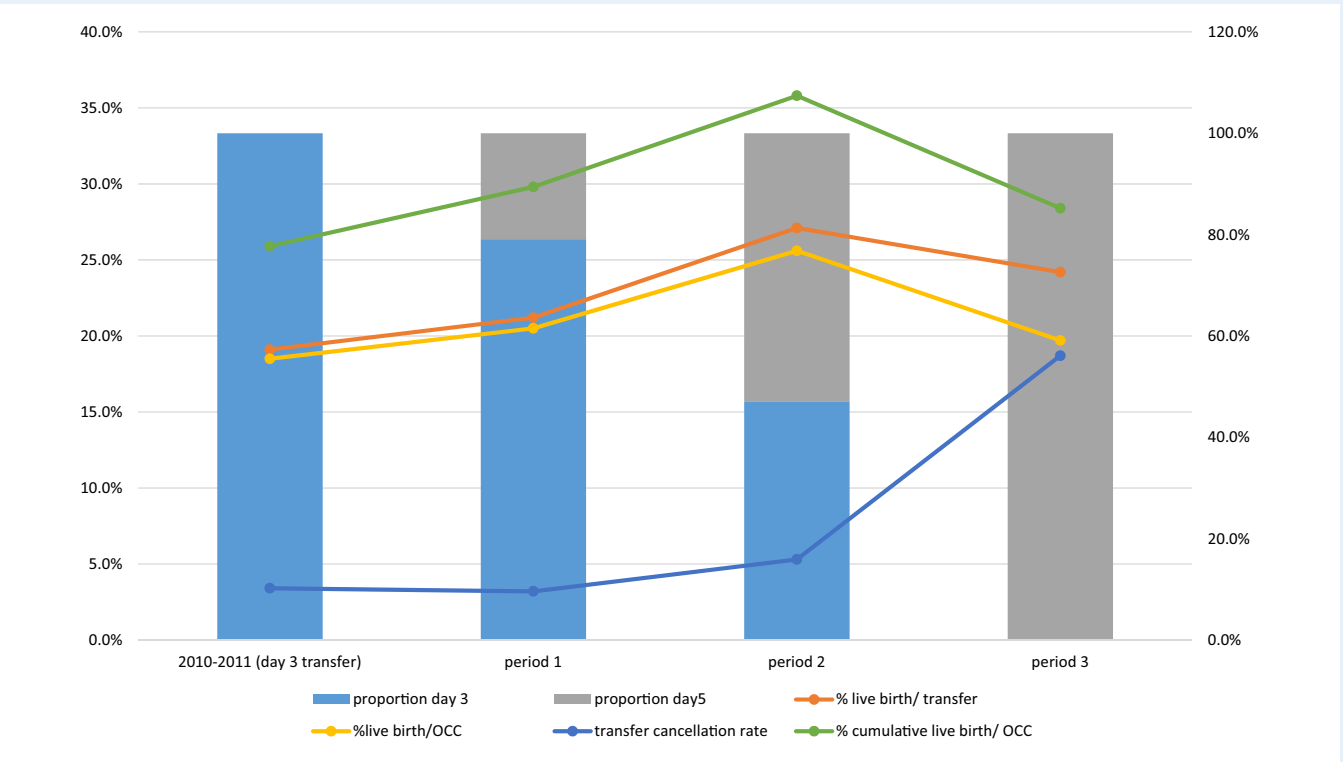

Figure 3 Live birth rate and transfer cancelation rate per OCC between preintervention period and the postintervention periods: period 1, period 2 and period 3. OCC, oocyte collection cycle.

Table II Period 2 (2014–2015) compared with preintervention (2010–2011): patient characteristics and pregnancy outcome.

	Preintervention period		Period 2		*P*-value
	1–4 zygotes Day 3	>4 zygotes Day 3	*1–4 zygotes Day 3*	>4 zygotes Day 5	
Number of OCC	*272*	399	*560*	896	
Number of transfers (%)	*255 (93.7%)*	393 (98.4%)	*495 (88.4%)*	883 (98.5%)	NS
Number of cycles with no transfer because of poor embryo quality (%)	*17 (6.3%)*	6 (1.5%)	*65 (11.6%)*	13 (1.5%)	NS
Number of SET (%)	*128 (50.2%)*	186 (47.3%)	*382 (77.2%)*	635 (71.9%)	<0.0001
Mean female age (mean ± SD) (years)	*35.37 ± 5.02*	33.52 ± 4.78	*35.15 ± 4.97*	33.37 ± 4.78	0.0389
Number of previous cycles (mean ± SD)	*3.15 ± 2.69*	1.93 ± 2.12	*2.56 ± 1.89*	1.62 ± 1.99	0.0041
Antagonist protocol (%)	*21.0%*	21.8%	*21.5%*	31.2%	NS
Agonist protocol (%)	*79.0%*	78.2%	*78.5%*	68.8%	NS
Number of oocytes retrieved (mean ± SD)	*6.20 ± 3.16*	14.83 ± 7.01	*6.36 ± 3.58*	13.34 ± 5.33	<0.0001
Number of zygotes (mean ± SD)	*2.35 ± 1.09*	9.15 ± 4.27	*2.47 ± 1.12*	8.08 ± 3.20	<0.0001
Number of embryos transferred (mean ± SD)	*1.48 ± 0.73*	1.63 ± 0.69	*1.23 ± 0.62*	1.28 ± 0.46	<0.0001
Number of embryos cryopreserved (mean ± SD)	*0.42 ± 0,69*	2.85 ± 3.18	*0.34 ± 0.64*	2.28 ± 2.36	0.0098
Number of pregnancies	*47*	126	*148*	411	
Number of live birth deliveries	*35*	89	*90*	283	
Number of twin pregnancies (%)	*8 (17.0%)*	11 (8.7%)	*3 (2.0%)*	32 (7.8 %)	NS
% pregnancies/transfer	*18.4%*	32.1%	*29.9%*	46.5%	<0.0001
% live birth/ transfer	*13.7%*	22.6%	*18.2%*	32.0%	<0.0001
% live birth/OCC	*12.9%*	22.3%	*16.1%*	31.6%	<0.0001

Data are presented as mean (± SD) or number (%); *P*-value of <0.05 was considered statistically significant. Statistical analysis was performed on the data in the gray columns and data in italics were added for the completeness. Dichotomous outcome variables were tested with a mixed logistic regression. For the continuous variable, a mixed linear regression was applied. For the analysis of counts, a mixed negative binominal regression was used.

in this first intervention period is the analysis of the outcome data of the Day 5 group (>9 zygotes) compared to data from a subgroup in the preintervention group (>9 zygotes Day 3). The characteristics of the OCC with 1–9 zygotes (Day 3 ET) in period 1 and the preintervention period (Day 3 ET) were also included in Table I in order to give a complete overview of the data.

Female age, number of previous cycles, number of oocytes retrieved, number of zygotes and number of embryos transferred were similar in the pre- and postintervention group (Table I). The number of embryos cryopreserved per cycle was significantly higher in the preintervention group compared to the number of blastocysts vitrified in the Day 5 group (4.83 ± 4.14 versus 3.06 ± 2.97, respectively; $P < 0.0001$). In the preintervention group, 99.3% of the cycles resulted in a fresh transfer, which was similar compared to the Day 5 group (97.1%). In the Day 5 group, in nine cycles none of the embryos reached the blastocyst stage to perform the ET; in the Day 3 group, one cycle did not result in an ET due to impaired embryo quality (2.9% versus 0.7%, respectively).

The percentage of OCCs having single embryo transfer (SET) was comparable in both group (55.9% versus 54.6%), which was reflected in a similar twin pregnancy rate for both groups (8.9% versus 7.8%).

Owing to improved embryo selection in the Day 5 group, a significantly increased pregnancy rate per fresh transfer was observed: 51.3% versus 36.2% ($P = 0.0030$). The difference in live birth rate delivery per fresh transfer and OCC did not reach statistical significance (34.3% and 33.3% versus 25.5% and 25.3% for Day 5 compared to Day 3, respectively).

After these first 2 years of performing blastocyst transfer for a subgroup of cycles, the data were analyzed in order to find out which OCCs with fresh transfer on Day 3 had at least one supernumerary blastocyst vitrified. These OCC could have been eligible for blastocyst transfer. This group of patients would be selected for the next step of the intervention study in the stepwise move toward a general blastocyst transfer policy. The subanalysis of the pregnancy rate per fresh transfer and the vitrification rate in relation to the amount of zygotes showed an intersection around four zygotes (Fig. 2). In patients with four zygotes on Day 1, a pregnancy rate per fresh transfer of 33.6% was observed and in 35.6% of these cycles at least one supernumerary blastocyst was vitrified after transfer on Day 3 (Fig. 2). Based on these results, the group of patients scheduled for blastocyst transfer in period 2 (January 2014 to December 2015) of the stepwise change approach was those having more than four zygotes. Patients with less than five zygotes were scheduled for a transfer on Day 3.

Evaluation of cycle outcomes between period 2 (Day 5 ET) and preintervention period (Day 3 ET) in patients with >4 zygotes

Period 2 included 1456 fresh IVF and ICSI OCCs, as summarized in Table II. In this period, a Day 3 transfer was scheduled in 560 OCCs and a Day 5 transfer in 896 OCCs. The proportion of OCCs scheduled for Day 5 transfer was 61.5% (896/1456) in period 2. Results of

Table III Period 1 (2012–2013), period 2 (2014–2015) and period 3 (2016–2017) compared with preintervention (2010–2011): patient characteristics and pregnancy outcome.

	Preintervention	Postintervention			*P*-value
	Total	Period 1 total	Period 2 total	Period 3 total	
Number of OCC	671	1510	1456	1764	
Number of transfers	648	1462	1378	1434	
% Day 5 OCC	0% (0/671)	20.9% (315/1510)	61.5% (896/1456)	100% (1764/1764)	
Number of cycles with no transfer because of poor embryo quality	23 (3.4%)[a,b]	48 (3.2%)	78 (5.3%)[a]	330 (18.7%)[b]	<0.0001
Number of SET	314 (48.4%)[c,d,e]	800 (54.6%)[c]	1017 (73.8%)[d]	1259 (87.8%)[e]	<0.0001
Mean female age (mean ± SD) (years)	34.27 ± 4.90[f,g,h]	34.73± 5.14[f]	34.05 ± 4.92[g]	34.65 ± 4.92[h]	<0.0001
Number of previous cycles (mean ± SD)	2.02 ± 2.18i[i,j]	1.78 ± 2.03[i]	1.60 ± 1.98[j]	1.80 ± 1.88	<0.0001
Antagonist protocol (%)	22.5 %	21.6%	27.2%	20.1 %	0.0125
Agonist protocol (%)	75.8%	77.5%	71.8%	78.8%	0.0043
Number of oocytes retrieved (mean ± SD)	11.32 ± 7.24[k,l]	11.09 ± 7.04	10.66 ± 5.83[k]	10.20 ± 5.36[l]	<0.0001
Number of zygotes (mean ± SD)	6.52 ± 4.64[m,n]	6.26 ± 4.63	5.93 ± 3.77[m]	5.45 ± 3.55[n]	<0.0001
Number of embryos transferred (mean ± SD)	1.62 ± 0.65[o,p,q]	1.50 ± 0.61[o]	1.26 ± 0.62[p]	1.12 ± 0.61[q]	<0.0001
Number of embryos cryopreserved (mean ± SD)	1.78 ± 2.80[r,s]	1.16 ± 1.99[r]	1.54 ± 2.12	1.29 ± 1.97[s]	<0.0001
Number of pregnancies	173	474	559	564	
Number of live birth deliveries	124	310	373	347	
Number of twin pregnancies (%)	19 (11.0%)	41 (8.2%)	36 (5.7%)	15 (2.5%)	<0.0001
% pregnancies/transfer	26.7%[t,u,v]	32.4%[t]	40.6%[u]	39.3%[v]	<0.0001
% live birth/ transfer	19.1%[w,x]	21.2%	27.1%[w]	24.2%[x]	<0.0001
%live birth/OCC	18.5%[a2]	20.5%	25.6%[a2]	19.7%	<0.0001

Data are presented as mean (± SD) or number (%);*P*-value of <0.05 was considered statistically significant. Differences between groups are indicated with a character: [a]$P = 0.0403$; [b]< 0.0001; [c–e]$P < 0.0001$; [f]$P = 0.0239$; [g,h]$P < 0.0001$; [i,j]$P < 0.0001$; [k]$P = 0.0045$; [l]$P = 0.0015$; [m]$P = 0.0012$; [n]$P < 0.0001$; [o]$P = 0.0187$; [p,q]$P < 0.0001$; [r]$P < 0.0001$; [s]$P = 0.01584$; [t]$P = 0.0078$; [u,v]$P = 0.0001$; [w]$P < 0.0001$; [x]$P = 0.0086$; [y]$P < 0.0001$; [z]$P < 0.0001$; [a1]$P = 0.0001$; [a2]$P < 0.0001$. Dichotomous outcome variables were tested with a mixed logistic regression. For the continuous variable, a mixed linear regression was applied. For the analysis of counts, a mixed negative binominal regression was used.

the OCCs in the preintervention period and period 2 with 1–4 zygotes will not be discussed in this Results section as they are not part of the intervention group but are added in Table II for the completeness of the data. The data of Day 5 transfer (>4 zygotes) were compared with data from a subgroup of the preintervention group (>4 zygotes Day 3 ET).

Number of oocytes retrieved, number of zygotes and number of embryos transferred were significantly higher in the preintervention group as compared with the postintervention group (14.83 ± 7.01, 9.15 ± 4.27 and 1.63 ± 0.69 versus 13.34 ± 5.33, 8.08 ± 3.20 and 1.28 ± 0.46, respectively; $P < 0.0001$). The ET cancelation rate was similar in the pre- and postintervention group. Women were significantly younger in the postintervention group compared to the preintervention group (33.52 ± 4.78 versus 33.37 ± 4.78; $P = 0.0389$).

A significantly higher percentage of SET had been performed in the Day 5 group versus the preintervention group (71.9% versus 47.3%, respectively; $P < 0.0001$). Twin pregnancy rates were similar for both groups (7.8% versus 8.7%).

Improved embryo selection in the Day 5 group resulted in a significantly increased pregnancy rate and live birth delivery rate per fresh transfer: 46.5% and 32.0% versus 32.1% and 22.6%, respectively ($P < 0.0001$). Live birth delivery rate per OCC was significantly higher in the Day 5 group compared to the pre-intervention group (45.9% and 31.6% versus 31.6% and 22.3%, respectively, $P < 0.0001$).

After this 2-year period, based on a pregnancy rate of 46.5% per fresh transfer and a transfer cancelation rate of 1.5% in the group with >4 zygotes, we were confident that the blastocyst culture and selection process worked in our hands. We decided to take the leap and perform a Day 5 transfer policy for all patients from 2016 onwards.

Evaluation of cycle outcomes between the pre- and postintervention periods

Period 3 is characterized by the Day 5 transfer policy for all patients.

In Table III and Figure 3, the patient characteristics and cycle outcomes of the pre- and postintervention groups were compared in their totality. All outcome data were calculated for the whole of each patient population. In the preintervention group, none of the OCCs was scheduled for a Day 5 transfer. In period 1, period 2 and period 3, 20.9%, 61.5% and 100% of the OCCs were scheduled for Day 5 transfer, respectively.

Patients in the preintervention group were significantly younger than patients in period 3 (34.27 ± 4.90 versus 34.65 ± 4.92 years; $P < 0.0001$). The mean number of embryos used for transfer decreased gradually after the introduction of the Day 5 transfer policy, from 1.62 ± 0.65 in the preintervention group to 1.12 ± 0.61 in period 3 ($P < 0.0001$). The percentage of cycles having SET increased from 48.4% in the preintervention group to 54.6%, 73.8% and 87.8% in

Table IV Period 1 (2012–2013), period 2 (2014–2015) and period 3 (2016–2017) compared with preintervention (2010–2011): cumulative live birth rate.

	Preintervention	Postintervention		
	Total	Period 1 total	Period 2 total	Period 3 total
Number of OCC	671	1510	1456	1764
Number of live birth deliveries after fresh transfer	124	310	373	347
Number of cryo transfers	211	704	707	791
Number of live births deliveries after cryo transfer	50	140	149	154
Number of embryos thawed/warmed	725	980	995	995
% live birth/cryo transfers	23.7%	19.9%	21.1 %	19.5%
Number of cumulative live birth deliveries	174	450	522	493
% cumulative live birth/OCC	25.9%[a]	29.8%	35.8%[a]	28.4%

Data are presented as number (%). *P*-value of <0.05 was considered a statistically significant. Differences between groups are indicated: [a]$P = 0.00$. Dichotomous outcome variables were tested with a mixed logistic regression.

period 1, period 2 and period 3, respectively ($P < 0.0001$). The mean number of cryopreserved surplus embryos was significantly lower in period 3 versus the preintervention group (1.29 ± 1.97 versus 1.78 ± 2.80; $P < 0.0001$).

Relatively more transfers per OCC were canceled in the postintervention period 3 and period 2 compared to the preintervention period (18.7% and 5.3% versus 3.4%, respectively; $P < 0.0001$).

Five hundred and sixty-four cycles led to a positive HCG value which resulted in a pregnancy rate of 39.3% per fresh transfer and 32.0% per OCC for period 3. Three hundred and forty-seven pregnancies resulted in a live birth with a live birth delivery rate of 24.2% per fresh transfer and 19.7% per OCC.

Pregnancy rate per fresh transfer was significantly lower in the preintervention groups as compared to period 1 (32.4%), period 2 (40.6%) and period 3 (39.3%) ($P < 0.0001$). Live birth delivery rate per fresh transfer was significantly lower in the preintervention group as compared to period 2 and period 3 (19.1% versus 27.1% and 24.2%, respectively; $P < 0.0001$). Live birth delivery rate per OCC was significantly lower in the preintervention group (18.5%) as compared to period 2 (25.6%). Similar live birth delivery rates per OCC were achieved between preintervention and period 3 (18.5% (124/671) versus 19.7% (347/1764)). Twin rate per pregnancy decreased gradually from 11.0%, 8.2%, 5.7% to 2.5% in the preintervention period, period 1, period 2 and period 3, respectively ($P < 0.0001$).

Evaluation of cumulative cycle outcomes between pre- and postintervention periods

In Table IV, the cumulative cycle outcomes of the pre- and postintervention groups in their totality were compared. Live birth delivery rate per FET was 23.7% in the preintervention group which was similar to the live birth delivery rate/FET of the postintervention period 1, period 2 and period 3 (19.9%, 21.1% and 19.5%, respectively). Although the live birth delivery rate per FET was similar for all groups, a significantly higher number of embryos were thawed in the preintervention group as compared to the postintervention periods to reach this similar live birth rate per FET. In the preintervention group, 725 embryos needed to be thawed to achieve 50 live births as compared to 995 in period 3 to achieve 154 live births. Cumulative live birth delivery rate per OCC in the preintervention group (25.9%) was significantly lower as compared to period 2 (35.8%) ($P = 0.002$). Similar cumulative live birth delivery rates per OCC were achieved between the preintervention and period 3 (25.9% versus 28.4%).

Evaluation of cycle outcomes between period 2 and period 3 in patients with 1–4 zygotes

A subanalysis of the poor prognosis patients was performed to further investigate the impact of the blastocyst policy in this specific patient group. As blastocyst transfer is often not pursued in patients with low amount of zygotes, therefore a comparison was made between period 2 and period 3 where specifically cycles with 1–4 zygotes with Day 3 or Day 5 ET were selected (Table V).

In period 2, OCCs with 1–4 zygotes were scheduled for a Day 3 transfer while in period 3 all OCCs were scheduled for a Day 5 transfer.

In period 2, a significantly higher percentage of OCCs resulted in a fresh transfer compared to period 3 (88.4% versus 68.4%; $P < 0.0001$). In period 2, 77.2% SETs were performed, which was significantly lower than in period 3 (87.4%; $P < 0.0001$). Transfer cancelation rate was significantly lower in period 2 as compared to period 3 (11.6% versus 32.0%; $P < 0.0001$). Interestingly, similar numbers of blastocysts were vitrified in both groups. Reassuring and similar pregnancy and live birth rates per fresh transfer were observed between both periods (30.4% and 18.1% versus 29.9% and 18.2%, respectively). The live birth delivery rate per OCC did not reach a statistical difference between the Day 5 transfer and the Day 3 transfer for patients with 1–4 zygotes (12.3% versus 16.1%, respectively).

Discussion

This retrospective, observational cohort study describes the stepwise approach that we applied to change from a cleavage-stage transfer

Table V Comparison between period 2 and period 3: patient characteristics and outcome in patients with 1–4 zygotes.

	Period 2 1–4 zygotes Day 3	Period 3 1–4 zygotes Day 5	*P*-value
Number of OCC	560	831	
Number of transfers	495 (88.4%)	565 (68.0%)	<0.0001
Number of cycles with no transfer because of poor embryo quality	65 (11.6%)	266 (32.0%)	<0.0001
Number of SET (%)	382 (77.2%)	494 (87.4%)	<0.0001
Mean female age (mean ± SD) (years)	35.15 ± 4.97	35.5 ± 4.89	<0.0001
Number of previous cycles (mean ± SD)	1.52 ± 1.94	1.88 ± 1.99	<0.0001
Number of oocytes retrieved (mean ± SD)	6.36 ± 3.58	6.96 ± 3.70	0.0042
Number of zygotes (mean ± SD)	2.47 ± 1.12	2.58 ± 1.05	NS
Number of embryos transferred (mean ± SD)	1.23 ± 0.62	1.13 ± 0.61	NS
Number of embryos cryopreserved (mean ± SD)	0.34 ± 0.64	0.32 ± 0.95	NS
Number of pregnancies	148	172	
Number of live birth	90	102	
Number of twin pregnancies	4 (2.7%)	7 (4.1%)	NS
% pregnancies/transfer	29.9%	30.4%	NS
% live birth/ transfer	18.2%	18.1%	NS
%live birth/OCC	16.1%	12.3%	NS

Data are presented as mean (± SD) or number (%); *P*-value of <0.05 was considered statistically significant. Dichotomous outcome variables were tested with a mixed logistic regression. For the continuous variable, a mixed linear regression was applied. For the analysis of counts, a mixed negative binominal regression was used.

policy to a blastocyst-stage transfer policy for all patients. Our study indicates that it is possible to successfully move to a blastocyst transfer policy for all patients. Until December 2011, a Day 3 transfer policy with a satisfactory live birth delivery rate of 17% per OCC was applied for all patients in our clinic (De Croo *et al.*, 2019). An ET policy at the blastocyst stage challenges the extended *in vitro* development of certain embryos resulting in an IVF cycle without ET. For this exact reason, many centers are reluctant to decide between a cleavage-stage or blastocyst-stage policy for all patients and often opt for a mixed transfer policy in which blastocyst transfer is only performed in good prognosis patients.

The efficacy of an extended embryo culture strategy has been evaluated in a recent meta-analysis (Glujovsky *et al.*, 2016) comparing the outcomes of transfers on Day 2 and Day 3 versus Day 5 and Day 6. In case of fresh blastocyst transfer, live birth rate was significantly higher compared to a fresh cleavage stage ET. Most studies included in this meta-analysis were performed in a good prognosis population, either based on age (Rienzi *et al.*, 2002) or on available zygotes (Fernández-Shaw *et al.*, 2015). None of the studies included a blastocyst ET policy for all patients.

Our approach to choose for a stepwise change from a cleavage-stage transfer policy to blastocyst-stage transfer policy has allowed us to evaluate pregnancy and vitrification rates per transfer after a first 2-year period and to make a decision for the next period depending on these results. The change in transfer policy started very prudently with a small selection of good prognosis patients. The decision to start the change in transfer policy so carefully was to minimize the risk for the patient experiencing a transfer cancelation, as many of these patients had already undergone IVF with a Day 3 ET. These good prognosis patients for blastocyst transfer were defined as patients with at least 10 zygotes on Day 1 and constituted of only 20.9% (315/1510) of our patient population. After a first 2-year period, an overall pregnancy rate of 31.4% per OCC was observed in the whole of the patient group in our clinic. More specifically in the group of patients experiencing the blastocyst intervention policy (with >9 zygotes), a pregnancy rate of 49.8% per OCC was achieved. Before the patient population was expanded for Day 5 transfer, a subanalysis was performed in the Day 3 group (1–9 zygotes) to evaluate the pregnancy and vitrification rate per fresh transfer. In the patient group with four zygotes, a pregnancy rate of 33.6% per fresh transfer and a vitrification rate (defined as having at least one supernumerary blastocyst vitrified) of 35.6% was accomplished. With these results, we were confident to move to a blastocyst transfer for patients with more than four zygotes. Also in literature, a minimum of four zygotes is a common decision parameter to go for blastocyst transfer (Fernández-Shaw *et al.*, 2015) because of the limited risk of transfer cancelations.

From 2014 (period 2), we decided on blastocyst transfer for patients with more than four zygotes (61.5%; 896/1456), while patients with less zygotes were scheduled for a Day 3 transfer. Transfer cancelation rates were similar between the preintervention group and the subgroup of period 2 in patients with >4 zygotes. The pregnancy rate per fresh transfer significantly increased from 32.1% in the preintervention period to 46.5% in the subgroup of period 2 (patients with >4 zygotes) ($P < 0.0001$). Fernández-Shaw *et al.* (2015) reported an ongoing pregnancy rate per OCC of 43.1%, which is comparable with our results in the group of patients with >4 zygotes on Day 5. Based on these reassuring results in terms of pregnancy rates per transfer and transfer cancelation rate for the blastocyst group, the decision was made to move to a blastocyst transfer policy for all patients from 2016 onwards.

Similar live birth rates per OCC were observed between the Day 5 for all policy (19.7%) compared to the preintervention period where Day 3 for all was the ET policy (18.5%). These results were achieved regardless of the dramatic increase in transfer cancelation rate from 3.4% in the preintervention group (Day 3 ET for all) to 18.7% in period 3 (Day 5 ET for all) ($P < 0.0001$). This increase was largely attributed to the change in transfer policy from period 2 to period 3 (from 5.3% to 18.7%, respectively) and could only be due to incorporation of the patient population with 1–4 zygotes available on Day 1. Connell *et al.* (2019) showed in a recent study that cancelation of transfer due to lack of blastocysts on Day 5 occurred in 10% of OCC with less than five oocytes. The subanalysis for patients with 1–4 zygotes scheduled for Day 3 versus Day 5 in period 2 and period 3 confirmed this. Although the pregnancy and live birth rate per fresh transfer were similar for both groups (29.9% and 18.2% versus 30.4% and 18.1% in period 2 and period 3, respectively), transfer cancelation rates for the blastocyst group were significantly higher as compared to the Day 3 group (32.0% versus 11.6%; $P < 0.0001$). While not significant, the latter was responsible for the lower live birth rate per OCC in the Day 5 group as compared to the Day 3 group (12.3% versus 16.1%, respectively). Our data are in accordance with the recent publication of the group of Haas *et al.* (2019) comparing Day 3 versus Day 5 in patients with one or two cleavage-stage embryos and found similar ongoing pregnancy rates per OCC in both groups (20.0% versus 20.2%).

The success of a blastocyst transfer policy largely depends on the embryo selection criteria. Embryos arrested at the cleavage stage or morulas with delayed and/or incomplete compaction on Day 5 were not selected for transfer in our study. Additionally, a rigorous transfer and vitrification policy were established only at Day 5 and extended culture to Day 6 or 7 for slow-growing embryos was not performed during this study. It has, however, been shown that embryos, even under the same conditions, can display heterogeneity in their development rate and can reach the blastocyst stage on Days 5, 6 and 7 (Ivec *et al.*, 2011). Studies showed that up to 30% of embryos can be slow growing (Shapiro *et al.*, 2008; Capalbo *et al.*, 2014) and that advanced age and aneuploidy are associated with delayed embryo development (Shapiro *et al.*, 2008; Minasi *et al.*, 2016; Cimadomo *et al.*, 2019). In a recent study by Tannus *et al.* (2017), the authors reported that delayed blastulation (morula ET) and fresh Day 5 transfer resulted in very low live birth rates. The authors suggested that in these cases the fresh ET could be postponed and culture extended until fully expanded blastocysts are achieved. For patients with 1–4 zygotes available, extended culture until Day 6/7 could potentially have produced a blastocyst in our current study. However, it is known that the success rates for Day 6 ET are lower than Day 5 ET (32.3% versus 59.3% (Shapiro *et al.*, 2008) and 11.1% versus 38.3% (Barrenetxea *et al.*, 2005)). A recent study of Tiegs *et al.* (2019) suggested that routine culture extended to Day 7 could successfully increase the pool of transferrable embryos for all patients. This would particularly be true for older patients (i.e. older than 35 years) whose embryos take longer to blastulate (Forman *et al.*, 2013; Shapiro *et al.*, 2016).

Since our laboratory policy during this intervention study did not extend embryo culture for fresh transfer or vitrification to Day 6 or 7, there is a possibility that slow-growing embryos were discarded before reaching their full developmental potential. The study of Cimadomo *et al.* (2019) provided evidence that euploid poor-quality embryos result in an absolute increase in live birth of 2.6%. According to this study, our outcome results for the Day 5 blastocyst policy for all patients could thus potentially and theoretically be increased.

On the other hand, embryo development may not be solely based on inherent survival potential and embryonic activation. Some believe that *in vitro* survival does not equate to *in vivo* survival and by committing to ET at the blastocyst stage there is a risk of losing some embryos, which might not survive the challenge of extended culture but might have, if transferred to the uterus, survived *in vivo*, implanted and resulted in a pregnancy (Maheshwari *et al.*, 2016). This is supported by the findings from the study of Xiao *et al.* (2019), which demonstrated that in women with only one embryo available on Day 3, transferring the embryo into the uterine environment achieved higher pregnancy rates than culturing the embryo longer with the goal to transfer on Day 5/6.

Limitations of this study include its retrospective design and although no major changes in the clinical and laboratory practices were introduced during the study period, the periods were all in different (sequential) years. A more appropriate design, a prospective randomized trial, should entail patient's randomization and cumulative live birth rate per intention to treat as a primary outcome. Cumulative live birth per OCC was, however, not our primary outcome as the decision to take the next step in our gradual implementation of a Day 3 transfer policy to a Day 5 transfer policy was made on the pregnancy rate per fresh transfer and the blastocyst rate on Day 5 of the supernumerary embryos. The analysis of the cumulative data was performed after the completion of the implementation of the Day 5 transfer policy for all patients. Reassuring similar cumulative live birth delivery rates per OCC were observed between the Day 5 for all policy (28.4%) compared to the preintervention period where Day 3 for all was the transfer policy (25.9%).

Live birth rate per FET was 23.7% in the preintervention group which was similar to the live birth rate per FET in the postintervention period 1, period 2 and period 3 (19.9%, 21.9% and 20.1%, respectively). Although the live birth rate per FET was similar for all groups, a significantly higher number of embryos were thawed in the preintervention group as compared to the postintervention periods to reach this similar live birth rate per transfer. In the preintervention group, 725 embryos needed to be thawed to achieve 50 live births as compared to 995 in period 3 to achieve 154 live births. This indicated that smaller numbers of cryo cycles were required to obtain a first live birth after blastocyst-stage transfer compared to cleavage-stage transfer. A significantly higher number of embryos was thus needed to achieve the same live birth rate/OCC in a Day 3 transfer policy than in a Day 5 transfer policy.

In this study, blastocyst transfer policy for all patients was implemented in a single IVF clinic. The pregnancy results and live birth rates were carefully analyzed during the stepwise change management method. This slow but prudent approach to change from a cleavage-stage transfer policy to a blastocyst-transfer policy can be used by other clinics as it shows a method with minimal and calculated risks for the patient in terms of live birth and transfer cancelation rates. This stepwise approach additionally showed a significantly lower twin pregnancy rate and a significant increase in SETs in a Day 5 transfer policy, without a loss in outcome success. Although randomized trials are able to truly compare a Day 3 transfer policy with a Day 5 transfer policy, we felt that our approach was more patient-friendly. Also, the staff involved were given the time to adjust their counseling and get

acquainted with the pros and cons of this change in transfer policy. We have been implementing the Day 5 transfer policy for all patients and all types of IVF cycles with own or donated gametes from 2016 onwards. We have shown that the live birth and cumulative live birth delivery rate per OCC was no different than in our Day 3 policy, and this was achieved in combination with a higher percentage of SET and a lower twin pregnancy rate. We do believe that our current strategy can further be optimized. Especially in the group of patients with low numbers of zygotes (1–4 zygotes) available on Day 1, we do experience lower (not statistically different) live birth rates per OCC due to significantly higher transfer cancelation rates in a Day 5 transfer policy. In this specific patient population, deferring fresh ET in favor of Day 6/7 until the full potential of a blastocysts is reached, might even further improve the outcome of our current blastocyst transfer policy for all.

Authors' roles

Study design, acquisition of data and writing of the manuscript: I.D.C and K.T. Interpretation of the data and critical review of the manuscript: I.D.C., K.T. and P.D.S.

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