



Prediction of live birth and cumulative live birth rates in freeze-all-IVF treatment of a general population

Kemal Ozgur¹ · Hasan Bulut¹ · Murat Berkkanoglu¹ · Levent Donmez² · Kevin Coetzee¹

Received: 27 September 2018 / Accepted: 12 February 2019 / Published online: 21 February 2019
© Springer Science+Business Media, LLC, part of Springer Nature 2019

Abstract

Purpose To investigate the cumulative live birth (cLB) rate of one complete freeze-all-IVF cycle in a general infertile population and to investigate patient and treatment variables that predict blastocyst development and live birth (LB).

Method In a retrospective observational study, the data of all IVF cycles performed between 1 February 2015 and 31 January 2016 at a single IVF centre was investigated. In the study, patient-couples were followed up for 18 months following oocyte retrieval. After exclusions, the patient and treatment variables of 1582 patient-couples who underwent treatment were included in the analyses.

Results The median time interval between the oocyte retrieval attempt and the frozen embryo transfer (FET) in which LB was achieved was 38.0 (35.0–67.0) days. The variables of freeze-all-IVF cycles with single blastocyst FET selected by multiple logistic regression to predict LB significantly were female age, infertility duration, FET number (i.e. 1st, 2nd, or ≥ 3rd FET), and blastocyst quality. In a regression adjusting for female age, the number of blastocysts transferred, and oocyte number group (1–3, 4–9, 10–15, and > 15), none of the oocyte number groups were selected to predict LB of 1st FET, significantly. While the per transfer LB rates decreased linearly from the 1st (56.5%) to the 3rd (36.4%) FET, the cLB rate increased from 47.3% after the 1st FET to 55.0% after a 3rd possible FET.

Conclusion The cLB rate of one complete freeze-all-IVF cycle of a general infertile population, with 18-month follow-up, was 55.0%. In freeze-all-IVF, ovarian reserve variables were not selected by regression models to predict LB, significantly.

Keywords Live birth · Cumulative live birth, freeze-all · Blastocyst · Vitrification · Frozen embryo transfer

Introduction

In an embryo-uterus model proposed for the prediction of live birth (LB) in IVF, the product of patient endometrial receptivity and embryo implantation potential was found to contribute most significantly, with both variables determined by numerous patient and treatment variables [1]. While patient variables are mostly unmodifiable, the methodologies and technologies

used in IVF have the potential to modify treatment variables. The continuous improvement in the in vitro embryo culture methodologies and technologies used in IVF has led to improved blastocyst development rates and blastocyst implantation potential. Moreover, while continuous improvement in drugs and medications of ovarian stimulation (OS) has improved the effectiveness and safety of OS (i.e. recombinant gonadotropins, gonadotropin-releasing hormone (GnRH) antagonist co-treatment stimulation, and GnRH agonist final oocyte maturation trigger [2, 3]), the iatrogenic effects of high-dose gonadotropin administration and multifollicular growth on the endometrium remain unresolved [4–6]. A high-dose-multifollicular approach, however, will continue to be used in IVF because of the strong positive association between oocyte numbers retrieved and LB [7, 8] and based on the evidence that LB only reaches maximal rates when 6–10 oocytes are retrieved [9–12]. Notwithstanding, the potential for adverse peri-implantation conditions, fresh embryo transfer (ET) has also remained the transfer strategy most often used in standard-IVF practice [4, 13–17].

Key/summary message: The cLB rate of one complete freeze-all-IVF cycle in a general infertile population was 55.0%, with oocyte numbers retrieved not independently predictive of LB.

✉ Kevin Coetzee
kevincoetzee61@yahoo.co.nz

¹ Antalya IVF, Halide Edip Cd. No:7, Kanal Mh.,
07080 Antalya, Turkey

² Medical Faculty, Public Health Department, Akdeniz University,
Antalya, Turkey

In addition to the increased implantation potential of embryos selected from in vitro embryo cultures, improvements in embryo cryopreservation methodologies and technologies have seen the reproductive rates of frozen embryo transfers (FET) also improve significantly, with rates now equal to or better than those of fresh ET [4, 18, 19]. The change in how IVF success is increasingly being viewed (i.e. LB per patient rather than LB per transfer and single LB rather than positive pregnancy test) has also seen the adjunct role of FET become increasingly important, because of its contribution to the cumulative LB (cLB) rates. Freeze-all-IVF (i.e. IVF with embryo cryopreservation and FET) has also increasingly been used in IVF for logistic and therapeutic reasons, i.e. to reduce ovarian hyperstimulation syndrome (OHSS), to avoid progesterone-induced embryo-endometrial asynchrony, to avoid polycystic ovarian syndrome (PCOS)-associated luteal phase defect, to enable preimplantation genetic testing (PGT) to be performed, and to preserve the fertility of patients who were to undergo cancer therapy. The growing confidence in FET and evidence suggesting that the physiological intrauterine conditions of FET may lead to improved implantation, placentation, and embryo-fetal growth [20–22] have led some to suggest controversially that freeze-all-IVF treatment could replace standard-IVF treatment. Moreover, there are those who oppose this suggestion of advantage, claiming that there is insufficient evidence to support such a change in IVF practice, with the evidence of advantage coming from a small number of studies with significant limitations [16, 17]. Those who oppose such a change also contend that freeze-all-IVF could decrease LB rates as the result of increased cycle cancellation and through the loss of embryo viability during the processes of vitrification and warming, and that the increase in treatment duration would unnecessarily increase the time-to-LB for patient-couples undergoing IVF.

While there is consensus that freeze-all-IVF may be the appropriate treatment strategy when > 15 oocytes are retrieved [13, 14, 16, 17], the question of whether freeze-all-IVF treatment may be appropriate for a general infertile population (i.e. poor, sub-normal, normal, and high responders) has still not adequately been answered. In a recent study, the intention was to investigate LB rates in a general infertile population; however, patient-couples with no oocytes, cycle cancellation, and no LB but with embryos in cryostorage were excluded [23]. The present study presents evidence to advance the understanding and to demonstrate the potential of freeze-all-IVF in terms of LB rates, by analysing the data of all IVF treatment cycles performed at an IVF centre during a 1-year period.

Materials and methods

Patients

In this retrospective observational study, the patient and treatment data of all IVF cycles performed at Antalya IVF between

1 February 2015 and 31 January 2016 were extracted from the centre's patient database. The treatment strategy used for all patient-couples presenting for treatment within the study period was freeze-all-IVF, with freeze-all-IVF having been implemented as the standard-IVF treatment in March 2014. At Antalya IVF, all IVF treatments are performed using autologous gametes, intracytoplasmic sperm injection (ICSI), extended in vitro embryo culture, and blastocyst vitrification. All IVF treatments in which patients did not receive a final oocyte maturation trigger were removed from the data extract, with only the first oocyte retrieval attempt performed for each patient-couple retained. Patient-couples were followed up for 18 months from the date of oocyte retrieval, with all FET performed within the follow-up period linked to the oocyte retrieval attempt in which the blastocysts transferred were cryopreserved. The study was performed in compliance with the Akdeniz University, Faculty of Medicine, Clinical Research Ethics Committee's requirements, with all patients completing a signed informed IVF consent, which included the permission to use anonymised data in research.

Procedures

Prior to commencing with IVF treatment, patient-couples underwent standard infertility work-up assessments that included a transvaginal scan (TVS) and if indicated saline-infused sonography, hysterosalpingography, and/or hysteroscopy was performed. OS were performed using flexi-start GnRH (0.25 mg, Cetrotide, Merck Serono, Istanbul, Turkey) antagonist co-treatment protocols. TVS antral follicle assessments were performed on days 2–3 of ovarian cycles, according to which gonadotropin stimulations using recombinant follicle-stimulating hormone (rFSH, 150–375 IU, Gonal-F, Merck Serono, Istanbul, Turkey) and or human menopausal gonadotropin (hMG, 75–150 IU, Menopur, Ferring Pharmaceuticals, Istanbul, Turkey) were considered and performed. Final oocyte maturation was triggered according to follicular response, using either GnRH agonist (0.2 mg, Gonapeptyl®, Ferring Pharmaceuticals, Istanbul, Turkey), human chorionic gonadotropin (hCG, 250µg/0,05 ml, Ovidrel, Merck Serono, Istanbul, Turkey), or a combination of GnRH agonist and hCG when ≥ 3 follicles reached ≥ 17 mm in diameter [24]. TVS-guided follicular aspirations were performed 36 h after the administration of triggers. Oocyte-cumulus complexes were retrieved from follicular needle aspirates received, with ICSI performed on all mature oocytes.

In vitro embryo cultures were performed using a single-step medium protocol, with incubation conditions set at 6% CO₂, 5% O₂, and 37.0 °C. Embryos from normally fertilised zygotes (2-pronuclear) were assessed daily, with blastocysts scored and selected for cryopreservation on days 4, 5, and 6 of in vitro embryo culture [25, 26]. All blastocyst vitrification-warming procedures were performed using ultra-rapid

cryopreservation technologies (Cryotop, Kitazato BioPharma Co. Ltd., Fuji City, Japan), according to the specifications of the manufacturer. PGT was performed, using blastocyst biopsies (ZILOS-tk, Hamilton Thorne Inc., Beverly, MA, USA) and comprehensive chromosome screening (Illumina, San Diego, CA, USA). All vitrified-warmed blastocyst transfers were performed in artificial FET cycles using programmed step-up oral estrogen (2 mg, Estrofem, Novo Nordisk, Istanbul, Turkey) protocols for endometrial preparation, with the start day of progesterone (90 mg, twice-a-day, Crinone® 8%, Merck Serono, Turkey) administration used to coordinate the day of the FET [27]. Blastocysts were warmed on the day of transfer according to the day of their cryopreservation, with days 5 and 6 blastocysts transferred on the 6th day and day 4 blastocysts on the 5th day of progesterone administration [25]. All blastocysts were transferred under trans-abdominal ultrasound guidance, with a maximum of two blastocysts transferred.

Outcomes

The primary outcome measure was LB, with LB defined as the delivery of a live infant at > 20 weeks of gestation. The follow-up of a patient-couple was discontinued at the successful delivery of a live infant. The delivery of multiple live infants was analysed as a single LB. The cLB rate was calculated from the ratio, total number of LB from FET performed within the 18 months follow-up period over the total number of oocyte retrieval attempts (= total number of patients), with the per transfer LB rate calculated from the ratio, total number of LB over the total number of FET. The secondary outcome measure was freeze-all (i.e. blastocyst development), with a blastocyst defined as an embryo with a blastocoel that allowed the morphological assessment of the inner cell mass (ICM) and trophoctoderm (TE) [28]. In the regression analysis that included blastocyst quality as a variable, the blastocyst morphological score was replaced with a numerical score [1–7] calculated according to the blastocyst's expansion and TE morphology (i.e. AA and BA > AB and BB > CA and CB > AC, BC, and CC) [29]. Blastocysts with a morphological score of 5AA (i.e. highest quality) were given a numerical score of 7. The blastocyst rate was calculated from the ratio, total number of blastocysts cryopreserved over the total number of 2-pronuclear (2PN) zygotes (fertilised zygotes). An antral follicle was defined as an ovarian follicle with a diameter of $> 2 \leq 10$ mm visualised on days 2–3 of a patient's ovarian cycle, with patients having an antral follicle count (AFC) of ≤ 5 diagnosed with decreased ovarian reserve (DOR). For the analysis of LB rates according to number of oocytes retrieved, the FET cycles of patient-couples were divided into four oocyte number groups, 1–3, 4–9, 10–15, and > 15 oocytes.

Statistics

SPSS 11.5 (Statistical Package for Social Science version 11.5) was used in the statistical analyses of patient and treatment variables and in the prediction of outcomes. The analyses of data were performed to produce medians and their interquartile ranges (25 and 75%) and outcome rates. In univariate analyses, Mann-Whitney Rank Sum tests, chi-square tests, or Fishers exact tests (low sample numbers) were performed, with statistical significant difference indicated by a $p < 0.05$. In multiple logistic regression analyses to predict dependent outcomes, odds ratios (OR) and 95% confidence intervals (95% CI) were reported for the variables selected in the model, with statistical significant indicated by a $p < 0.05$. The patient and treatment variables included in the multiple logistic regression to predict freeze-all (i.e. blastocyst development) are shown in Table 1. In the multiple logistic regression to predict LB, only the patient and treatment variables of single blastocyst FET cycles without PGT selection were included. In addition to the variables indicated in Table 1, blastocyst number and rate, blastocyst quality (score), FET number (1st, 2nd, or ≥ 3 rd), and endometrial thickness (mm; measured on day 14 of endometrial development) were also included. The effect of oocyte numbers retrieved on the LB of FET was investigated using one-way ANOVA using Mann-Whitney Rank Sum test and a multiple logistic regression that included only the 1st FET cycle variables of female age, number of blastocysts transferred, and oocyte number group (i.e. 1–3, 4–9, 10–15, and > 15 oocytes), with the variables selected based both on published and unpublished LB prediction analyses performed in the present study.

Results

Patients

The patient-couple population included in the study fully represents the study centre's general infertile population, with female age ≤ 42 . In Table 2, the numbers and fates of patient-couple freeze-all-IVF cycles are presented as a flow-chart. Of the 1758 patient-couples who underwent oocyte retrieval attempts, 176 were excluded for reasons shown in Table 2. The patient and treatment variables of 1582 oocyte retrieval attempts, therefore, were analysed. None of the female patients included in the analysis developed OHSS symptoms that required hospitalisation to perform paracentesis. Of the 1582 oocyte retrieval attempts included, 248 (15.7%) treatments were cancelled because no blastocysts developed. The reason for no blastocyst development was one of the following: no oocytes retrieved ($n = 20$), no mature oocytes retrieved ($n = 11$), no fertilised zygotes ($n = 33$), or embryo developmental arrest ($n = 184$). In 84.3% of patient-couple

Table 1 Patient and treatment variable comparisons

	Patient cycles			¹ <i>p</i> value (A vs B)	Freeze-all cycles		¹ <i>p</i> value (A1 vs A2)
	Total group	Freeze-all (A)	Cancelled (B)		Live birth (A1)	No live birth (A2)	
<i>N</i>	1582	1334	248		871	453	
x Female age (years)	32.6 (28.2–36.3)	32.0 (27.8–35.7)	35.1 (31.1–38.7)	< 0.001	31.2 (27.4–34.6)	34.2 (29.0–38.4)	< 0.001
≤ 25 (%)	11.6 (184)	12.7 (169)	6.0 (15)	0.009	14.1 (123)	9.7 (44)	
26–30 (%)	25.7 (407)	27.5 (367)	16.1 (40)	0.004	30.0 (261)	22.7 (103)	0.039
31–35 (%)	32.8 (519)	33.5 (447)	29.0 (72)		37.2 (324)	26.9 (122)	0.009
36–40 (%)	23.4 (370)	21.4 (286)	33.9 (84)	0.002	16.3 (142)	30.9 (140)	< 0.001
40–42 (%)	6.4 (102)	4.9 (65)	14.9 (37)	< 0.001	2.4 (21)	9.7 (44)	< 0.001
x Infertility duration (years)	4 (2–8)	4 (2–7)	4.8 (2–9)		4 (2–7)	5 (3–8)	< 0.001
x Primary infertility	80.3 (1270)	80.1 (1069)	81.0 (201)		81.2 (707)	77.9 (353)	
x Secondary infertility	19.7 (312)	19.9 (265)	19.0 (47)		18.8 (164)	22.1 (100)	
x Number previous ET (<i>n</i>)	0 (0–1)	0 (0–1)	1 (0–2)	< 0.001	0 (0–1)	0 (0–1)	0.005
x AFC (<i>n</i>)	13 (7–19)	14 (9–20)	6 (3.8–11.0)	< 0.001	15 (10–23)	11 (7–17)	< 0.001
x BMI (kg/m ²)	25 (23–29)	25 (23–29)	25 (22–29)		25 (22–28)	26 (23–30)	0.026
x Aetiology: male (%)	27.7 (438)	28.1 (375)	25.4 (63)		29.9 (260)	25.2 (114)	
x Aetiology: unexplained (%)	28.8 (455)	29.3 (391)	25.8 (64)		29.5 (257)	28.5 (129)	
x Aetiology: DOR (%)	13.9 (220)	9.5 (127)	37.5 (93)	< 0.001	6.4 (56)	15.4 (70)	< 0.001
x OS duration (days)	9 (8–10)	9 (8–10)	8 (7–10)	< 0.001	9 (8–10)	9 (8–10)	0.016
x Total FSH dose (IU)	3300 (2400–4050)	3375 (2400–4050)	2862 (2100–3713)	< 0.001	3375 (2400–4050)	3325 (2400–4050)	
x Trigger: GnRH agonist (%)	43.2 (684)	47.6 (635)	19.8 (49)	< 0.001	48.7 (424)	46.1 (209)	
x Trigger: hCG (%)	17.8 (281)	16.1 (215)	26.6 (66)	0.002	34.9 (304)	38.6 (1759)	
x Trigger: dual (%)	39.0 (617)	36.3 (484)	53.6 (133)	0.001	16.4 (143)	15.2 (69)	
x Oocyte number (<i>n</i>)	12 (7–21)	14 (8–22)	4 (2–9.8)	< 0.001	16 (10–23)	11 (6–18)	< 0.001
Oocyte number: 0	1.3 (20)	0 (0)	8.1 (20)	< 0.001	0 (0)	0 (0)	
Oocyte number: 1–3	11.2 (177)	6.1 (81)	38.7 (96)	< 0.001	3.9 (34)	10.2 (46)	< 0.001
Oocyte number: 4–9	25.5 (404)	25.0 (334)	28.2 (70)		20.6 (179)	33.6 (152)	< 0.001
Oocyte number: 10–15	22.8 (360)	24.4 (326)	13.7 (34)	0.003	23.8 (207)	25.2 (114)	
Oocyte number: > 15	39.3 (621)	44.5 (593)	11.3 (28)	< 0.001	51.8 (451)	31.1 (141)	< 0.001
x Mature oocyte number (<i>n</i>)	9 (5–16)	11 (6–17)	3 (1–6)	< 0.001	12 (7–8)	8 (5–13)	< 0.001
x Mature oocyte rate (%)	80.0 (66.7–91.4)	80.0 (66.7–90.9)	70.7 (50.0–100.0)	< 0.001	80.0 (68.0–90.0)	80.0 (66.7–93.0)	
x Fertilised zygote number (<i>n</i>)	7 (3–12)	8 (5–13)	2 (0–3)	< 0.001	9 (6–15)	6 (4–10)	< 0.001
x Fertilisation rate (%)	81.8 (66.7–93.3)	82.4 (70.0–92.3)	70.0 (40.0–100)	< 0.001	82.4 (70.8–90.9)	83.3 (70.0–100.0)	
Blastocyst number (<i>n</i>)		3 (2–6)			4 (2–6)	2 (1–4)	< 0.001
Blastocyst rate (%)		46.2 (33.3–63.6)			50.0 (33.3–63.6)	41.7 (28.6–65.2)	0.008
Endometrial thickness (mm)					9.5 (8.3–11.0)	9.5 (8.0–11.0)	

Items with “x” indicate patient and treatment variables included in the multiple logistic regression to predict freeze-all (i.e. blastocyst development). Statistical analysis: all data presented and analysed as medians and interquartile ranges (25 and 75%) or as rates, with statistical analysis generating a ¹*p* < 0.05 indicating significance

ET embryo transfer, AFC antral follicle count, BMI body mass index, DOR decreased ovarian reserve (AFC of ≤ 5), OS ovarian stimulation, FSH follicle-stimulating hormone, GnRH agonist gonadotropin-releasing hormone agonist, hCG human chorionic gonadotropin, dual GnRH-a plus hCG

Table 2 Flowchart of patient-couple cycles

Oocyte retrievals	1758
Excluded cycles	176 (10.0%)
Natural cycle IVF	3
Fresh blastocyst transfers	67
Maternal age > 42 years	99
No sperm	7
Patient-couple cycles	1582
Cancelled cycles ¹	248 (15.7%)
Freeze-all cycles	1334
Discontinued cycles ²	10 (0.75%)
1st FET cycles ³	1324
2nd FET cycles	231
3rd FET cycles	22
4th FET cycles	1
Cumulative FET cycles ⁴	1578
Incomplete cycles ⁵	156

¹ Cancelled cycles—oocyte retrieval cycles that failed to produce blastocysts

² Discontinued cycles—in the 18-month follow-up, 8 patients never returned for FET, and for two FET cycles, the live birth outcomes were unknown

³ FET cycles—patients who underwent FET following freeze-all, with known final outcomes

⁴ Cumulative FET cycles—the total number of FET performed in the 18-month follow-up from the freeze-all cycles

⁵ Incomplete cycles—the number of patients with no live birth, but still with blastocysts in storage at the end of the 18-month follow-up period (includes discontinued cycles)

cycles ($n = 1334$), the in vitro culture of 2PN zygotes produced blastocysts suitable for cryopreservation (i.e. freeze-all). Of the 1334 freeze-all-IVF cycles included, 261 patient-couples underwent PGT. Patient-couples predicted to have no euploid (i.e. normal) blastocysts for transfer were recorded to have had one failed FET cycle. The freeze-all-IVF cycles of 10 (0.75%) patient-couples were recorded as discontinued; eight patient-couples did not return for FET within the 18-month follow-up period and the final treatment outcomes of two patient-couples were not available at the end of the 18-month follow-up period. The FET cycles of three (3/1578, 0.19%) patient-couples were cancelled because all vitrified-warmed blastocysts degenerated, with no blastocysts available for transfer (two 1st FET cycles and one 2nd FET cycle). These patient-couples were also recorded to have had one failed FET cycle.

Blastocyst development prediction

The patient and treatment variable outcomes of the 1582 patient-couples included in the analysis are presented in Table 1. In the total group, the median female age was 32.6 years, with 70.2% of female partners ≤ 35 years old.

The majority (80.3%) of patient-couples had primary infertility, with 27.7% diagnosed with male factor and 13.9% with as DOR. The median number of oocytes retrieved was 12, with no oocytes retrieved in 1.3% and ≤ 3 oocytes in 11.2% of oocyte retrievals. In comparison to the freeze-all group (A), the median female age was significantly higher ($p < 0.001$), the median AFC was significantly lower ($p < 0.001$), the DOR rate was significantly increased ($p < 0.001$), the median number of oocytes retrieved was significantly lower ($p < 0.001$), the median number of mature oocytes was significantly lower ($p < 0.001$), and the fertilisation rate was significantly lower ($p < 0.001$) in the cancelled group (B). In the cancelled group’s OS, the duration was significantly shorter ($p < 0.001$) and the total FSH dose significantly lower ($p < 0.001$). The main reason for cycle cancellation (80.7%; 184/228) in cycles with oocytes retrieved was embryo development arrest. The patient and treatment variables marked in Table 1 were included in a multiple logistic regression to predict freeze-all. The variables selected to predict freeze-all in the final model are presented in Table 3, with the prediction of the following variables reaching a $p < 0.001$ significance: DOR, oocyte fertilisation rate, and oocyte maturation rate. Although total FSH dose was selected in the model, the OR of 1.0 may suggest limited clinical significance.

Live birth prediction

In Table 1, the freeze-all group was divided into two groups: (A1) patient-couples with an LB and (A2) patient-couples with no LB. In the no LB group (A2) as compared to the LB group (A1), the median female age was significantly higher ($p < 0.001$), infertility duration significantly longer ($p < 0.001$), number of previous ET significantly higher ($p = 0.005$), AFC significantly lower ($p < 0.001$), BMI

Table 3 Multiple logistic regression analysis for the prediction of freeze-all in all cycles ($n = 1582$)

Independent variables	OR (95% CI) (rCoef ± se)	<i>p</i> value
Number of previous ET	0.88 (0.782–0.999) (− 0.124 ± 0.063)	0.049
DOR (aetiology)	0.27 (0.152–0.464) (− 1.325 ± 0.285)	< 0.001
OS duration (days)	0.87 (0.760–0.984) (− 0.145 ± 0.066)	0.028
Total FSH dose (IU)	1.00 (1.000–1.001) (0.000 ± 0.000)	0.019
Mature oocyte rate	5.88 (2.595–13.317) (1.771 ± 0.417)	< 0.001
Fertilisation rate	9.40 (3.116–28.325) (2.240 ± 0.563)	< 0.001
Constant	0.11 (− 2.224 ± 0.655)	0.001

The variables selected in the final model for the prediction of freeze-all (i.e. blastocyst development), reported as adjusted Odds Ratios (OR) and 95% confidence intervals (95% CI), with a $p < 0.05$ indicating significance

rCoef regression coefficient, *se* standard error, *ET* embryo transfer, *DOR* decreased ovarian reserve, *OS* ovarian stimulation, *FSH* follicle-stimulating hormone

significantly higher ($p = 0.026$), and chance of DOR significantly higher ($p < 0.001$). In addition, the no LB group had significantly fewer oocytes retrieved ($p < 0.001$), fewer mature oocytes ($p < 0.001$), fewer blastocysts ($p < 0.001$), and a lower blastocyst rate ($p = 0.008$). The patient and treatment variables of only single blastocyst FET cycles without PGT were included in a multiple logistic regression to predict LB. The variables selected in the final model to predict LB are presented in Table 4, with female age, infertility duration, and blastocyst quality (i.e. score 1–7) predicting LB with a significance of $p < 0.001$ and performing a 2nd FET predicting LB with a significance of $p = 0.029$. The odds of a patient-couple having an LB increased with the transfer of a blastocyst with an increasing blastocyst score.

Live birth according to oocyte numbers retrieved

In Table 5, the 1st FET cycles of patient-couples were grouped according to the number of oocytes retrieved (1–3, 4–9, 10–15, and > 15 oocytes). The 20 cycles with no oocytes retrieved and 10 discontinued cycles were excluded from this analysis. The > 15 oocyte number group was not sub-divided further for analysis, as the LB rates showed no tendency of either increasing or decreasing in subsequent subgroups. In the trend analysis, the per transfer LB rates increased linearly and significantly ($p < 0.001$) from 42.5% in the 1–3 oocyte number group to 62.3% in the > 15 oocyte number group. The linearity of LB rates was lost in the analysis of per patient LB rates, because of the high cycle cancellation rate (54.5%) in the 1–3 oocyte number group. In the 1–3 oocyte number group, the

cycle cancellation rate declined according to the number of oocytes retrieved: 65.4% for one (34/52), 58.2% for two (32/55), and 43.4% for three oocytes (30/69) retrieved, and the per transfer LB rate increased from 33.3, 43.5, to 46.1%, respectively. In the analysis of female age, female age was found to decrease with increasing oocyte numbers retrieved. A multiple logistic regression analysis for the prediction of LB, therefore, was performed, adjusting for the variables of female age, blastocyst number transferred, and oocyte number group. In this regression, female age (OR = 0.93, 95% CI (0.912–0.954), $p < 0.001$) and the transfer of two blastocysts (OR = 1.67, 95% CI (1.312–2.127), $p < 0.001$) were selected as significant predictors of LB. The retrieval of > 15 oocytes retrieved predicted LB with a significance of $p = 0.056$. In the > 15 oocyte number group, 75.5% of patient-couples still had blastocysts remaining in cryostorage as compared to the only 5.1% in the 1–3 oocyte number group.

Cumulative live birth

The cLB analysis of consecutively performed FET (1st, 2nd, 3rd, 4th), within the 18-month follow-up period of the study, is presented in Table 6. The median time interval between oocyte retrieval and the 1st FET was 37.0 (34.0–54.0) days, between the 1st and the 2nd FET 188.0 (117.0–290.0), and between the 2nd and 3rd FET 198.0 (126.8–280.0). The median time interval between the oocyte retrieval attempt and the FET in which LB was achieved was 38.0 (35.0–67.0) days. The per transfer LB rates decreased linearly from the 1st (56.5%) to the 3rd (36.4%) FET, despite an increase in the

Table 4 Multiple logistic regression analysis for the prediction of live birth in single blastocyst FET cycles ($n = 843$)

Independent variables	OR (95% CI) (rCoef ± se)	p value
Female age (years)	0.94 (0.913–0.973) (− 0.059 ± 0.016)	< 0.001
Infertility duration (years)	0.91 (0.872–0.951) (− 0.094 ± 0.022)	< 0.001
1st FET		0.093
2nd FET	0.54 (0.305–0.939) (− 0.625 ± 0.287)	0.029
3rd FET	0.0 (0.0) (− 20.823 ± 16,866.33)	0.999
Blastocyst quality		< 0.001
Blastocyst quality (1)	0.22 (0.061–0.787) (− 1.521 ± 0.654)	0.020
Blastocyst quality (2)	0.13 (0.033–0.539) (− 2.019 ± 0.714)	0.005
Blastocyst quality (3)	0.10 (0.040–0.257) (− 2.288 ± 0.475)	< 0.001
Blastocyst quality (4)	0.39 (0.165–0.942) (− 0.931 ± 0.445)	0.036
Blastocyst quality (5)	0.66 (0.361–1.205) (− 0.415 ± 0.307)	0.176
Blastocyst quality (6)	0.81 (0.455–1.431) (− 0.214 ± 0.292)	0.463
Constant	17.51 () (2.863 ± 0.552)	< 0.001

The variables selected in the final model for the prediction of live birth, reported as adjusted odds ratios (OR) and 95% confidence intervals (95% CI), with a $p < 0.05$ indicating significance. Cycles excluded: 81 SET cycles in which PGT-A was used to select blastocysts

rCoef regression coefficient, se standard error, FET frozen embryo transfer, Blastocysts quality a numerical score (i.e. 1–7), based on blastocyst expansion grade (i.e. 1–5) and TE morphology (A, B, or C)

Table 5 Live birth outcomes of the 1st FET according to the number of oocytes retrieved

Oocyte number group	Patients (N)	Cycle cancellation rate	Embryo development arrest rate	Female age (years)	LB/FET (%)	LB/patient (%)	Blastocysts in cryostorage
1–3	176	54.5 (96)	36.9 (65)	37.0 (33.9–39.5)	42.5 (34)	19.3 (34)	5.1 (9)
4–9	401	17.5 (70)	16.0 (64)	35.1 (31.5–38.6)	50.8 (168)	41.9 (168)	31.4 (126)
10–15	355	9.6 (34)	8.5 (30)	32.1 (28.2–35.1)	55.1 (177)	49.9 (177)	54.9 (195)
> 15	620	4.5 (28)	4.0 (25)	29.7 (26.4–33.6)	62.3 (369)	59.5 (369)	75.5 (468)

Cycles excluded: the 20 cycles in which no oocytes retrieved and 10 cycles of patients who had discontinued cycles

Cycle cancellation, as described in Table 2; embryo development arrest, cancelled cycles with greater than or equal to one 2PN zygote that produced no blastocysts; blastocysts in cryostorage, patients with blastocysts still remaining in cryostorage at the end of the 18-month follow-up period

number of blastocysts transferred. The cLB rate of patient-couples within the 18-month follow-up duration which allowed for a possible 3rd FET to be performed was 55.0%, increasing from 47.3% after the 1st FET.

Discussion

In the present study, the patient and treatment variables of a general infertile population (with female age ≤ 42) who underwent freeze-all-IVF treatments during a 1-year period were investigated to identify which variables predicted in freeze-all and which predicted LB. In addition, the association between oocyte numbers retrieved and LB and the cLB rate of freeze-all-IVF were investigated. Analysing the variables and outcomes of the 1582 patient-couples included in the present study revealed that female age, infertility duration, FET number (1st, 2nd, or 3rd), and blastocyst quality were variables that predict LB significantly, with oocyte number either as a continuous or categorical variable not included in the multiple logistic regression models to predict LB. The cLB rate after patient-couples having a possible 3rd FET within the 18-month follow-up period of the study was 55.0%, with an LB rate of 47.3% after the 1st FET and a median time interval of 38.0 (35.0–67.0) days between the oocyte retrieval attempt and the FET of LB. The suggested implementation of freeze-all-IVF as standard-IVF practice has long been a subject of controversy, mostly because of the lack of quality and balanced evidence, with scientific studies investigating select patient populations (i.e. normal and high ovarian responders) and epidemiology (i.e. registry) studies analysing data from the use of out-dated methodologies, technologies, and treatment strategies (<https://www.hfea.gov.uk/media/2563/hfea-fertility-trends-and-figures-2017-v2.pdf>) [30]. While a substantial amount of evidence has been accumulated over the past 40 years of IVF on which variables predict LB in standard-IVF, the evidence in freeze-all-IVF treatment is limited. In a recent study, specifically designed to investigate the treatment of a general infertile population using freeze-all-IVF [23], shortcomings in methodology, unfortunately, may also

have limited its quality of evidence. Many of the questions salient to freeze-all-IVF, therefore, remain inadequately answered, i.e. do the same patient and treatment variables predict LB, do oocyte numbers retrieved predict the LB rates of FET, will poor ovarian reserve patients have reduced LB rates, and will performing the 1st embryo transfer of standard-IVF in a FET cycle affect cLB rates.

The rationale for performing extended in vitro embryo culture for blastocyst development is based on the understanding that the intrauterine transfer of a blastocyst is analogous to that occurring in a spontaneous cycle, and on the experience that blastocyst development is a method of embryo self-selection. An embryo that has developed into a blastocyst is believed to have an increased chance of being developmentally competent and of implanting [7, 31, 32]. The mean or median blastocyst development rate is a Key Performance Indicator (KPI) incorporated into the quality assurance schemes of many IVF centres, because it is also believed to reflect the quality of an in vitro embryo culture system. The “desirable range” for a day 5 blastocyst development rate has been reported to be 40–60% [33]; however, blastocyst rates may vary (0–100%) significantly according to patient and treatment variables [34]. In the present study, the median blastocyst rate in the freeze-all group was 46.2 (33.3–63.6), with 24.0% (5527 blastocysts/23014 oocytes) of oocytes developing into viable blastocysts suitable for cryopreservation. In two previous studies, investigating the prediction of blastocyst development and or cycle cancellation in select patient-couples (i.e. specific numbers of 2PN zygotes), regressions selected female age, AFC, fertilisation rate, and tubal factor infertility [35], and female age, fertilisation method, number of day 3 embryos, and quality ratio of day 3 embryos [36] as significant predictors. In a study in which all 2PN zygotes were cultured for a minimum of 4 days, regression selected oocyte number retrieved and grade of day 3 cleavage-stage embryos as significant predictors, with increasing oocyte numbers positively associated with increasing numbers and quality of blastocysts [7]. In the present study, multiple logistic regression to predict blastocyst development (i.e. freeze-all) selected three variables with a significance of $p < 0.001$: DOR (i.e. poor ovarian

Table 6 Cumulative live birth rate from consecutive FET

Patient cycles	Cancelled cycles	Discontinued cycles	1st FET	ET number	2nd FET	ET number	3rd FET	ET number	4th FET
1582	248	10	56.5 (748/1324) cLB/patient	1.37 ± 0.493	49.4 (114/231) 54.5 (862/1582)	1.66 ± 0.489	36.4 (8/22) 55.0 (870/1582)	1.64 ± 0.569	100.0 (1/1)

Cancelled and discontinued cycles, as described in Table 2

FET frozen embryo transfer, ET number number of blastocysts transferred, LB live birth, cLB cumulative live birth

reserve), mature oocyte rate, and fertilisation rate. Overall, the variables selected were associated with ovarian function, and with variables determining the number of 2PN zygotes.

The rationale for performing freeze-all-IVF rather than standard-IVF is based on the assumptions that the physiological and predictable intrauterine conditions of FET in freeze-all-IVF potentiates optimal endometrial receptivity and embryo-endometrial synchrony [21] and, therefore, embryo implantation, embryo placentation, and fetal development. In standard-IVF, the intrauterine conditions at transfer are often unpredictable and considered unphysiological, with the variables determining ovarian response (i.e. gonadotropin dosages) and the outcomes of ovarian response (i.e. estrogen and progesterone levels and multifollicular growth) contributing to adverse risks (i.e. OHSS, ovarian torsion, and bleeding) and outcomes (i.e. reduced embryo implantation, [15]). LB is generally considered to be the primary KPI of IVF and, therefore, an appropriate outcome measure to investigate the effectiveness of alternate treatment strategies, such as freeze-all-IVF. Of the variables selected to be predictive of LB, female age has often been selected as the most significant, with the physiological dynamics of female ageing known to affect both the endometrial and embryonic components of reproduction [7, 30, 37, 38]. In a study analysing LB according to the embryo-uterus model, regression selected female age, infertility diagnosis, previous IVF treatment, infertility duration, embryo quality, and controversially cryopreservation as significant predictors [38]. Investigating the prediction of LB in SET (using day 2 embryos), regression selected seven significant variables, i.e. embryo score, treatment history, ovarian sensitivity index (OSI; number of oocytes/total dose of FSH), female age, infertility cause, endometrial thickness, and female height [39]. In the present study, multiple logistic regression to predict LB from single blastocyst FET selected variables that were very similar to those selected for standard-IVF with fresh ET, with variables associated with embryo quality and endometrial receptivity represented. The variables selected to predict LB significantly were female age, infertility duration, FET number (1st, 2nd, or 3rd), and blastocyst quality (score of 1–7). Interestingly, none of the variables directly associated with ovarian function (i.e. ovarian reserve and ovarian response) were selected, including that of oocyte numbers retrieved.

LB rates are generally believed to increase with increasing oocyte numbers retrieved, with studies reporting that LB rates were optimal when 6–10 oocytes were retrieved, plateaued when 10–15 oocytes were retrieved [8–12], and declined when > 15 oocytes were retrieved [9, 11]. Similarly, cLB rates were also found to be positively associated with increasing numbers of oocytes retrieved [11, 12]. In the present study, the importance of oocyte numbers retrieved to IVF success was evident from the univariate analyses, with significantly more oocytes retrieved in the freeze-all than in the cancelled

group (14 vs 4) and in the LB than in the no LB group (16 vs 11). In the trend analysis, per transfer LB rates increased significantly from 42.5% in the 1–3 oocyte number group to 62.3% in the > 15 oocyte number group, with no apparent decline when > 15 oocytes were retrieved (16–25 oocytes; 61.7% (235/381) and > 26 oocytes; 63.5% (134/211)). In the study of Zhu et al. [23], the cLB rate increased from 20.60% in the 1–5 oocyte number group to 90.09% in the > 25 oocyte number group, with also no apparent decline in LB rates when > 15 oocytes were retrieved. The increasing LB rates associated with increasing oocyte numbers retrieved have been suggested to be the result of increasing oocyte euploidy, embryo implantation potential, and increasing endometrial receptivity [7, 38, 40–42]. Moreover, in the present study, it was observed that female age, which contributes to endometrial receptivity and embryo competence significantly, decreased with increasing oocyte numbers retrieved. In a multiple logistic regression adjusting for female age and number of blastocysts transferred in 1st FET, none of the oocyte number groups were selected as predictors of LB. This limited variable regression confirming the hierarchical importance of ovarian response variables that was observed in the multiple regression performed to predict LB of SET. In standard-IVF, high numbers of oocytes (> 15 or ≥ 16) have also been found to be associated with increases in adverse treatment complications, such as OHSS [9–12]. OHSS is a serious and potentially life-threatening complication of OS, with the pathogenesis of this iatrogenic complication often observed to be unpredictable. In the present study, no patients developed moderate to severe OHSS, including the 620 patients from whom > 15 oocytes were retrieved. In the Cochrane review by Wong et al., freeze-all-IVF was also found to be associated with lower prevalence of OHSS and, therefore, suggested to be an appropriate treatment strategy for patient-couples with > 15 oocytes retrieved [43].

While freeze-all-IVF has been investigated in normal and high ovarian response patients, the present study is the first to investigate this treatment strategy in poor ovarian reserve (i.e. 1–3 oocyte number group) patient-couples of a general infertile population. The lowest oocyte number group in the recently published study of Zhu et al. [23] was 1–5 oocytes. In standard-IVF treatments, poor ovarian response patients generally have lower LB rates than normal ovarian response patients. This lower reproductive potential was confirmed in studies showing that selected DOR patients had higher embryo aneuploidy rates and an increased chance of having no euploid embryos for transfer [44, 45]. In the present study, the per transfer LB rate in the 1–3 oocyte number group was 42.5%, with a multiple logistic regression suggesting that embryo implantation potential and/or endometrial receptivity was not adversely affected in this group, at least in 1st FET following oocyte retrieval. These assumptions are supported by studies showing that implantation rates were similar for decreased and normal ovarian reserve patients (61.0 vs 59.0%)

when euploid blastocysts were transferred [45], and that ovarian stimulation and response were not associated with adverse oocyte and embryo aneuploidy outcomes [42, 46]. In the present study, the per transfer LB rate in the 1–3 oocyte number subgroup with female age ≤ 35 years was 64.3%, which was similar to that of the > 15 oocyte number group (62.3%). In the > 15 oocyte number group, the median female age was 29.7 (26.4–33.6) years. This evidence serves to highlight the clinical importance of female age in reproduction and, therefore, the reason for including it in IVF treatment planning and patient counselling. Female age may be especially important in patients who may potentially have a poor ovarian response, according to the present study. Moreover, the per patient LB rate of the 1–3 oocyte number group was significantly reduced (19.3 vs > 41.9%), because of the high cancellation rate (54.5%) in this group. The reasons for cycle cancellation suggests that further research is required in the development of patient-specific OS (i.e. dose-correct and drug-correct) protocols that ensure the developmentally competent oocytes [5, 40, 42, 47, 48], and in the development of tests that identify patient ovarian cycles with optimal or suboptimal ovarian response potential [49, 50]. In addition, multiple inter- or intra-cycle OS in conjunction with blastocyst freeze-all has also been suggested to be a feasible treatment strategy to increase the number of viable blastocysts for poor ovarian response patient-couples [48, 50, 51].

Now that FET results in LB rates that are at least statistically equal to those of fresh ET [18], the potential of complete IVF cycles is changing the way IVF treatment is being perceived by both clinicians and patient-couples (i.e. in regard to cost and effort). In a single-centre study, in which both single cleavage and blastocysts stage embryos were transferred to patient-couples undergoing their first standard-IVF cycles, the LB rate after fresh ET was 30.5% (335/1099) and the cLB rate was of 45.9% (504/1099) [12]. In another study in which a blastocyst transfer strategy was used to investigate cLB in a select patient population (i.e. female age < 36 years), the LB rate after fresh ET was 36.8% (229/623) and the cLB rate was of 48.0% (299/623) [31]. In a study investigating the cLB rate of a general infertile population in freeze-all-IVF, a cLB rate of 50.74% was reported for first complete cycles [23]. In the present study, the per transfer LB rates were found to decrease in consecutively performed FET cycles: 56.5% in 1st, 49.4% in 2nd, and 36.4% in 3rd FET cycles. Moreover, the cLB rate increased from 47.3% after the 1st FET to 55.0% after the 3rd FET. This decrease in LB rate was also observed in the consecutive transfers of standard-IVF [52]. The lower LB rates reported for the fresh ET of standard-IVF than for the 1st FET of freeze-all-IVF observed in the present study might be clinically revealing, especially as a general infertile population was investigated. In the Cochrane review of Wong et al. [43], the cLB rates of freeze-all-IVF and standard-IVF were found not to be significantly different; however, the quality of

evidence included in the review was low. In multiple logistic regressions to select variables that predict cLB significantly, oocyte number or oocyte response category (and BMI [11]; and fertilisation [12]; and female age, secondary infertility, and ICSI [23]; and female age, embryo cryopreservation, and stage of embryos transferred [53]) and embryo quality (two or ≥ 3 top embryos [54]) were selected. While a multiple logistic regression with cLB as dependent outcome was not performed in the present study, the evidence certainly suggests that oocyte number retrieved may affect cLB rates, as the percentage of patient-couples with blastocysts still in cryostorage at the end of the present study increased with increasing number of oocytes retrieved.

It is important to remember what the ultimate goal of IVF is when choosing between using standard-IVF or freeze-all-IVF, i.e. IVF needs to lead to the safe and term delivery of a healthy infant. While many patient-couples have achieved an LB after standard-IVF with fresh ET, there have been concerns on the long-term health of infants and on the lost-opportunity costs of patient-couples who fail to achieve an LB as result of compromised peri-implantation conditions. It has been suggested that having a clinical tool able to predict endometrial receptivity in the fresh cycle may help to identify patients that would be negatively affected by fresh ET; however, biological and technical limitations may significantly hamper the accuracy of such predictions and the cost of such a tool at present may preclude its general use. Whether FET replaces fresh ET as the embryo transfer strategy of standard-IVF will depend on whether the benefits of having physiological and predictable intrauterine conditions outweigh the burden of increased treatment costs and duration. Moreover, the implications of failing to achieve a LB from the 1st embryo transfer of a IVF treatment are significant, as LB rates decline in consecutive transfers, time increases between a failed transfer and the next transfer with every failure, and approximately 20% of patient-couples drop-out of treatment following a failed transfer. Notwithstanding, the patient-couple inclusivity of the present study, its retrospective design and having no control group may also limit the quality of evidence reported. In addition, the list of patient and treatment variables included in the analyses was not exhaustive, with variables of ethnicity, smoking, alcohol consumption, and male-partner not investigated. Also, while the patient-couples included in the study fully represent the general infertile patient population of the study centre, the patient and treatment variables may be specific to the ethnic and socio-cultural demographics of the national population, which may limit the *generalizability* of evidence. In conclusion, the present study shows that cLB rates of $> 55.0\%$ can be achieved in the treatment of a general infertile population with freeze-all-IVF. Moreover, whether performing the 1st embryo transfer of IVF treatment in the uncompromised peri-implantation conditions of FET improves the LB rates per transfer and consequently, the per

patient LB rates of a general infertile population require further direct-comparison studies. The evidence that ovarian reserve associated variables were not overtly predictive of LB in the present study shows the importance of knowing which and understanding how patient and treatment variables affect the reproductive outcomes of IVF.

Acknowledgements The authors wish to thank Dr. Peter Humaidan (MD, DMSc) for his significant contribution to the conceptualisation and design of the study, to the scientific and semantic content of the manuscript, and the critical revision of the reviewed manuscript.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflicts of interest.

Publisher's note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

References

1. Rogers PA, Milne BJ, Trounson AO. A model to show human uterine receptivity and embryo viability following ovarian stimulation for in vitro fertilization. *J In Vitro Fert Embryo Transf.* 1986;3: 93–8.
2. Practice Committee of American Society for Reproductive Medicine. Gonadotropin preparations: past, present, and future perspectives. *Fertil Steril.* 2008;90:S13–20.
3. Devroey P, Polyzos NP, Blockeel C. An OHSS-free clinic by segmentation of IVF treatment. *Hum Reprod.* 2011;26:2593–7.
4. Evans J, Hannan NJ, Edgell TA, Vollenhoven BJ, Lutjen PJ, Osianlis T, et al. Fresh versus frozen embryo transfer: backing clinical decisions with scientific and clinical evidence. *Hum Reprod Update.* 2014;20:808–21.
5. Baker VL, Brown MB, Luke B, Smith GW, Ireland JJ. Gonadotropin dose is negatively correlated with live birth rate: analysis of more than 650,000 assisted reproductive technology cycles. *Fertil Steril.* 2015;104:1145–52.
6. Haahr T, Roque M, Esteves SC, Humaidan P. GnRH agonist trigger and LH activity luteal phase support versus hCG trigger and conventional luteal phase support in fresh embryo transfer IVF/ICSI cycles—a systematic PRISMA review and meta-analysis. *Front Endocrinol (Lausanne).* 2017;8:116.
7. Stone BA, March CM, Ringler GE, Baek KJ, Marrs RP. Casting for determinants of blastocyst yield and of rates of implantation and of pregnancy after blastocyst transfers. *Fertil Steril.* 2014;102:1055–64.
8. Baker VL, Brown MB, Luke B, Conrad KP. Association of number of retrieved oocytes with live birth rate and birth weight: an analysis of 231,815 cycles of in vitro fertilization. *Fertil Steril.* 2015;103: 931–8.
9. Ji J, Liu Y, Tong XH, Luo L, Ma J, Chen Z. The optimum number of oocytes in IVF treatment: an analysis of 2455 cycles in China. *Hum Reprod.* 2013;28:2728–34.
10. Steward RG, Lan L, Shah AA, Yeh JS, Price TM, Goldfarb JM, et al. Oocyte number as a predictor for ovarian hyperstimulation syndrome and live birth: an analysis of 256,381 in vitro fertilization cycles. *Fertil Steril.* 2014;101:967–73.
11. Chen YH, Xu XH, Wang Q, Zhang SD, Jiang LL, Zhang CL, et al. Optimum oocyte retrieved and transfer strategy in young women with normal ovarian reserve undergoing a long treatment protocol: a

- retrospective cohort study. *J Assist Reprod Genet.* 2015;32:1459–67.
12. Drakopoulos P, Blockeel C, Stoop D, Camus M, de Vos M, Tournaye H, et al. Conventional ovarian stimulation and single embryo transfer for IVF/ICSI. How many oocytes do we need to maximize cumulative live birth rates after utilization of all fresh and frozen embryos? *Hum Reprod.* 2016;31:370–6.
 13. Shapiro BS, Daneshmand ST, Garner FC, Aguirre M, Hudson C. Clinical rationale for cryopreservation of entire embryo cohorts in lieu of fresh transfer. *Fertil Steril.* 2014;102:3–9.
 14. Blockeel C, Drakopoulos P, Santos-Ribeiro S, Polyzos NP, Tournaye H. A fresh look at the freeze-all protocol: a SWOT analysis. *Hum Reprod.* 2016;31:491–7.
 15. Ozgur K, Humaidan P, Coetzee K. Segmented ART—the new era in ART? *Reprod Biol.* 2016;16:91–103.
 16. Ata B, Seli E. A universal freeze all strategy: why it is not warranted. *Curr Opin Obstet Gynecol.* 2017;29(3):136–45.
 17. Dieamant FC, Petersen CG, Mauri AL, Comar V, Mattila M, Vagnini LD, et al. Fresh embryos versus freeze-all embryos—transfer strategies: nuances of a meta-analysis. *JBRA Assist Reprod.* 2017;21:260–72.
 18. Coates A, Kung A, Mounts E, Hesla J, Bankowski B, Barbieri E, et al. Optimal euploid embryo transfer strategy, fresh vs frozen, following PGS with NGS. A randomized controlled trial. *Fertil Steril.* 2017;107:723–30.
 19. Roque M, Valle M, Kostolias A, Sampaio M, Geber S. Freeze-all cycle in reproductive medicine: current perspectives. *JBRA Assist Reprod.* 2017;21:49–53.
 20. Maheshwari A, Raja EA, Bhattacharya S. Obstetric and perinatal outcomes after either fresh or thawed frozen embryo transfer: an analysis of 112,432 singleton pregnancies recorded in the Human Fertilisation and Embryology Authority anonymized dataset. *Fertil Steril.* 2016;106:1703–8.
 21. Teh WT, McBain J, Rogers P. What is the contribution of embryo-endometrial asynchrony to implantation failure? *J Assist Reprod Genet.* 2016;33:1419–30.
 22. Zhao J, Xu B, Zhang Q, Li YP. Which one has a better obstetric and perinatal outcome in singleton pregnancy, IVF/ICSI or FET?: a systematic review and meta-analysis. *Reprod Biol Endocrinol.* 2016;14:51.
 23. Zhu Q, Chen Q, Wang L, Lu X, Lyu Q, Wang Y, et al. Live birth rates in the first complete IVF cycle among 20 687 women using a freeze-all strategy. *Hum Reprod.* 2018;33(5):924–9.
 24. Ozgur K, Bulut H, Berkkanoglu M, Humaidan P, Coetzee K. Increased body mass index associated with increased preterm delivery in frozen embryo transfers. *J Obstet Gynaecol.* 2018. accepted for publication.
 25. Ozgur K, Bulut H, Berkkanoglu M, Humaidan P, Coetzee K. Artificial frozen embryo transfer cycle success depends on blastocyst developmental rate and progesterone timing. *Reprod BioMed Online.* 2018;36:269–76.
 26. Gardner DK, Schoolcraft WB. In vitro culture of human blastocysts. In: Jansen R, Mortimer D, editors. *Toward reproductive certainty: fertility and genetics beyond 1999.* London: Parthenon Publishing; 1999. p. 378–88.
 27. Ozgur K, Berkkanoglu M, Bulut H, Humaidan P, Coetzee K. Agonist depot versus OCP programming of frozen embryo transfer: a retrospective analysis of freeze-all cycles. *J Assist Reprod Genet.* 2016;33:207–14.
 28. ALPHA Scientists in Reproductive Medicine and ESHRE Special Interest Group of Embryology. The Istanbul consensus workshop on embryo assessment: proceedings of an expert meeting. *Hum Reprod.* 2011;26:1270–83.
 29. Ahlström A, Westin C, Reismer E, Wikland M, Hardarson T. Trophectoderm morphology: an important parameter for predicting live birth after single blastocyst transfer. *Hum Reprod.* 2011;26:3289–96.
 30. McLernon DJ, Steyerberg EW, Te Velde ER, Lee AJ, Bhattacharya S. Predicting the chances of a live birth after one or more complete cycles of in vitro fertilisation: population based study of linked cycle data from 113 873 women. *BMJ.* 2016;355:i5735.
 31. De Vos A, Van Landuyt L, Santos-Ribeiro S, Camus M, Van de Velde H, Tournaye H, et al. Cumulative live birth rates after fresh and vitrified cleavage-stage versus blastocyst-stage embryo transfer in the first treatment cycle. *Hum Reprod.* 2016;31:2442–9.
 32. Glujovsky D, Farquhar C, Quinteiro Retamar AM, Alvarez Sedo CR, Blake D. Cleavage stage versus blastocyst stage embryo transfer in assisted reproductive technology. *Cochrane Database Syst Rev.* 2016;6:CD002118.
 33. ESHRE Special Interest Group of Embryology and Alpha Scientists in Reproductive Medicine. The Vienna consensus: report of an expert meeting on the development of ART laboratory performance indicators. *Reprod BioMed Online.* 2017;35:494–510.
 34. Swain JE, Carrell D, Cobo A, Meseguer M, Rubio C, Smith GD. Optimizing the culture environment and embryo manipulation to help maintain embryo developmental potential. *Fertil Steril.* 2016;105:571–87.
 35. Thomas MR, Sparks AE, Ryan GL, Van Voorhis BJ. Clinical predictors of human blastocyst formation and pregnancy after extended embryo culture and transfer. *Fertil Steril.* 2010;94:543–8.
 36. Dessolle L, Fréour T, Barrière P, Darai E, Ravel C, Jean M, et al. A cycle-based model to predict blastocyst transfer cancellation. *Hum Reprod.* 2010;25:598–604.
 37. ACOG Committee on Gynecologic Practice; Practice Committee of the ASRM. Female age-related fertility decline. Committee Opinion No. 589. *Obstet Gynecol.* 2014;123:719–21.
 38. Roberts SA, Hann M, Brison DR. Factors affecting embryo viability and uterine receptivity: insights from an analysis of the UK registry data. *Reprod BioMed Online.* 2016;32:197–206.
 39. Vaegter KK, Latic TG, Olovsson M, Berglund L, Brodin T, Holte J. Which factors are most predictive for live birth after in vitro fertilization and intracytoplasmic sperm injection (IVF/ICSI) treatments? Analysis of 100 prospectively recorded variables in 8,400 IVF/ICSI single-embryo transfers. *Fertil Steril.* 2017;107:641–8.
 40. Gianaroli L, Magli MC, Cavallini G, Crippa A, Capoti A, Resta S, et al. Predicting aneuploidy in human oocytes: key factors which affect the meiotic process. *Hum Reprod.* 2010;25:2374–86.
 41. Ata B, Kaplan B, Danzer H, Glassner M, Opsahl M, Tan SL, et al. Array CGH analysis shows that aneuploidy is not related to the number of embryos generated. *Reprod BioMed Online.* 2012;24:614–20.
 42. Labarta E, Bosch E, Mercader A, Alamá P, Mateu E, Pellicer A. A higher ovarian response after stimulation for IVF is related to a higher number of euploid embryos. *Biomed Res Int.* 2017;2017:5637923.
 43. Wong KM, van Wely M, Mol F, Repping S, Mastenbroek S. Fresh versus frozen embryo transfers in assisted reproduction. *Cochrane Database Syst Rev.* 2017;3:CD011184.
 44. Katz-Jaffe MG, Surrey ES, Minjarez DA, Gustofson RL, Stevens JM, Schoolcraft WB. Association of abnormal ovarian reserve parameters with a higher incidence of aneuploid blastocysts. *Obstet Gynecol.* 2013;121:71–7.
 45. Shahine LK, Marshall L, Lamb JD, Hickok LR. Higher rates of aneuploidy in blastocysts and higher risk of no embryo transfer in recurrent pregnancy loss patients with diminished ovarian reserve undergoing in vitro fertilization. *Fertil Steril.* 2016;106:1124–8.
 46. Sekhon L, Shaia K, Santistevan A, Cohn KH, Lee JA, Beim PY, et al. The cumulative dose of gonadotropins used for controlled ovarian stimulation does not influence the odds of embryonic aneuploidy in patients with normal ovarian response. *J Assist Reprod Genet.* 2017;34:749–58.

47. Lainas TG, Sfontouris IA, Venetis CA, Lainas GT, Zorzovilis IZ, Tarlatzis BC, et al. Live birth rates after modified natural cycle compared with high-dose FSH stimulation using GnRH antagonists in poor responders. *Hum Reprod*. 2015;30:2321–30.
48. Reed BG, Babayev SN, Bukulmez O. Shifting paradigms in diminished ovarian reserve and advanced reproductive age in assisted reproduction: customization instead of conformity. *Semin Reprod Med*. 2015;33:169–78.
49. Munne S, Held KR, Magli CM, Ata B, Wells D, Fragouli E, et al. Intra-age, intercenter, and intercycle differences in chromosome abnormalities in oocytes. *Fertil Steril*. 2012;97:935–42.
50. Ubaldi FM, Capalbo A, Vaiarelli A, Cimadomo D, Colamaria S, Alviggi C, et al. Follicular versus luteal phase ovarian stimulation during the same menstrual cycle (DuoStim) in a reduced ovarian reserve population results in a similar euploid blastocyst formation rate: new insight in ovarian reserve exploitation. *Fertil Steril*. 2016;105:1488–95.
51. Vaiarelli A, Cimadomo D, Ubaldi N, Rienzi L, Ubaldi FM. What is new in the management of poor ovarian response in IVF? *Curr Opin Obstet Gynecol*. 2018;30:155–62.
52. Chambers GM, Paul RC, Harris K, Fitzgerald O, Boothroyd CV, Rombauts L, et al. Assisted reproductive technology in Australia and New Zealand: cumulative live birth rates as measures of success. *Med J Aust*. 2017;207:114–8.
53. McLernon DJ, Maheshwari A, Lee AJ, Bhattacharya S. Cumulative live birth rates after one or more complete cycles of IVF: a population-based study of linked cycle data from 178,898 women. *Hum Reprod*. 2016b;31:572–81.
54. Niinimäki M, Veleva Z, Martikainen H. Embryo quality is the main factor affecting cumulative live birth rate after elective single embryo transfer in fresh stimulation cycles. *Eur J Obstet Gynecol Reprod Biol*. 2015;194:131–5.