Frozen embryo transfer can be performed in the cycle immediately following the freeze-all cycle

Kemal Ozgur¹ · Hasan Bulut¹ · Murat Berkkanoglu¹ · Peter Humaidan² · Kevin Coetzee¹

Received: 8 August 2017 / Accepted: 12 September 2017 / Published online: 22 September 2017
© Springer Science+Business Media, LLC 2017

Abstract

Purpose In this study, we investigated whether the time interval between oocyte retrieval and frozen embryo transfer (FET) affected the live birth (LB) rates of human segmented-IVF cycles.

Method A total of 1338 ICSI freeze-all cycles were performed between February 2015 and January 2016, with 1121 FET cycles being retrospectively analyzed. All vitrified-warmed blastocyst transfers were performed in artificial FET cycles, using gonadotropin-releasing hormone (GnRH) agonist downregulation and oral estrogen endometrial preparation. The primary outcome measure was LB. Cycles were investigated in oocyte retrieval-to-FET interval groups of 32–46, 47–61, 62–76, 77–91, and ≥ 92 days, with the 47–61-day group used as the reference group.

Results There were no significant differences in LB rates between the groups in the overall analysis, as well as, in sub-analyses investigating LB in terms of single blastocyst transfer (SBT), trigger type (GnRH agonist, triggers including hCG), oocyte number (≤ 5 and ≥ 15), and maternal age (> 35 years).

Conclusion The present study showed that it is feasible to perform transfers 36 days after oocyte retrieval and that delaying FET in freeze-all beyond the cycle immediately following oocyte retrieval does not increase LB rates.

Keywords Freeze-all · Frozen embryo transfer · Delay · Blastocyst · Live birth

Introduction

Live birth (LB) rates in human IVF have been seen to increase with increasing numbers of oocytes retrieved; generally, the relationship between LB rates and oocyte numbers follows a sigmoid-curve with LB rates starting to reach optimal levels when > 6 oocytes are retrieved, with the increase no longer significant when > 15 oocytes are retrieved [1, 3]. Likewise, cumulative LB (cLB) rates increase with increasing oocyte numbers; however, cLB rates continue to increase significantly in cycles with > 15 oocytes were retrieved [1]. Optimizing reproductive outcomes in IVF cycles, therefore, requires the use of high dose exogenous gonadotropin controlled ovarian stimulations (COS). Unfortunately, high dose gonadotropin COS often generates supraphysiological hormonal conditions that adversely affect endometrial development and function and, therefore, embryo-endometrium synchrony and embryo implantation [4–7]. Moreover, in autologous IVF cycles, LB rates were reported to decrease with increasing daily and total exogenous gonadotropin doses, an effect not observed in donor-oocyte cycles with increasing daily and total exogenous gonadotropin doses [8, 9].

Clinicians are presently often choose to perform elective freeze-all in autologous IVF cycles when confronted with COS-related risk factors, i.e., premature rise in progesterone levels, increased estrogen levels, and ovarian hyperstimulation syndrome symptoms [10–12]. The confidence in this strategy has grown as the result of innovations in cryopreservation technologies [13, 14] and improvements in the reproductive outcomes of frozen embryo transfers (FET) [15–19]. Moreover, the main purpose of elective freeze-all is to delay embryo transfer (ET) to a cycle with no COS-related luteal phase defect [6, 20], i.e., to a cycle with a more physiological endometrial development and, therefore, to a cycle in which embryo-endometrium synchrony can be better managed [21,

✉ Kevin Coetzee
kevincoetzee61@yahoo.co.nz

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

² The Fertility Clinic, Skive Regional Hospital and Faculty of Health,
Aarhus University, Aarhus, Denmark

22]. Exactly how long a delay is required for the endometrium to recover after COS has not been confirmed with certainty, which has important implications for the planning of FET in freeze-all cycles, as well as, for patients who have to endure the delay to FET.

The delay of ET may significantly affect the psychological state-of-mind of patients undergoing IVF with freeze-all significantly, with a delay in transfer likely to increase patient stress and anxiety and decrease reproductive success. Only two studies have thus far investigated these clinical concerns in large numbers of freeze-all cycles [23, 24]; in both studies only elective freeze-all cycles were investigated, i.e., cycles in which the risk for reduced and/or adverse outcomes was clinically increased. However, in the present study, we investigated consecutive non-elective freeze-all cycles, thereby, minimizing selection bias by including patients with and without reduced and/or adverse outcome risks. In this large retrospective study, LB outcomes were investigated according to the time interval between oocyte retrieval and FET, in a total segmented-IVF program.

Materials and methods

Patients

In the present study, the cycles analyzed were from a human segmented-IVF program (freeze-all) in which the majority (> 95%) of IVF treatments were performed with the intention of freeze-all. The change to segmented-IVF at the study center was implemented in 2014, based upon the evidence that the reproductive outcomes of artificial FET (art-FET) were superior to those of fresh ET [12, 18, 25]. The present 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 that included permission to use anonymised data in research.

The present study is a retrospective cohort analysis of consecutive intracytoplasmic sperm injection (ICSI) freeze-all cycles performed between February 2015 and January 2016. In total, 1338 patients underwent ICSI-freeze-all cycles, excluding cycles in which maternal age was > 42 years (Table 1). One thousand three hundred and eleven patients who had ICSI-freeze-all cycles have undergone endometrial preparation for their first FET; 1309 of whom have since undergone ET. Two patients (0.15%) failed to have an ET, as the result of post-warming blastocyst degeneration. A further 188 cycles were excluded for reasons shown in Table 1, which resulted in 1121 cycles being analyzed in the present study. At the study center, there is no policy on the duration of delay between oocyte retrieval and ET, with all ET planned to be performed with a minimum of delay or according to patient wishes. The

Table 1 Cycle tabulation

	<i>n</i>	<i>N</i>	Delay (days)
Freeze-all cycles		1338	
No FET ^a	27		
FET cycles		1311	
Cancelled ^b	2		33, 36
Termination ^c	3		33, 35, 95
Outcomes unknown ^d	3		35, 55, 69
Hysteroscopy ^e	135		66.0 (63.0–72.0) ^g
CCS ^f	47		36.0 (34.0–52.0)
FET analysis		1121	36.0 (34.0–52.0) ^g

FET frozen embryo transfer, Delay days between day of OPU and day of FET

^a No FET; patients have not completed treatment

^b Cancelled; blastocysts degenerated after warming

^c Terminations; medical terminations of pregnancy

^d Outcomes unknown; patient contact loss after FET

^e Hysteroscopy; patients had hysteroscopy between the time of oocyte retrieval and FET

^f CCS; patients had comprehensive chromosome screening

^g Mann-Whitney comparison $p < 0.001$

1121 cycles were grouped according to the time interval between oocyte retrieval and ET (i.e., 32–46, 47–61, 62–76, 77–91, and ≥ 92 days), with the chosen intervals based on the distribution of ET shown in Fig. 1. In the statistical comparisons, the 47–61-day interval group was used as a reference group, as this group represented cycles in which FET were performed two menstrual cycles from oocyte retrieval.

The reproductive outcomes were also analyzed in subgroups that included cycles with single blastocyst transfer (SBT), gonadotropin releasing hormone (GnRH) agonist triggers, human chorionic gonadotropin (hCG) triggers (including both hCG alone or in combination with GnRH agonist), maternal age > 35 years, ≤ 5 oocytes, and ≥ 15 oocytes.

Controlled ovarian stimulation, oocyte pickup, and embryo culture

Before commencing COS, all patients had an ultrasound examination on cycle day 2 or 3 for antral follicle counting (AFC; counting of follicles ≥ 3 mm). COS were performed using standard GnRH antagonist (0.25 mg, Cetrotide, Merck Serono, Istanbul, Turkey) co-treatment protocols that included the administration of recombinant follicle-stimulating hormone FSH (rFSH, 150–375 IU, Gonal-F, Merck Serono, Istanbul, Turkey) and human menopausal gonadotropin (hMG, 75–150 IU, Menopur, Ferring Pharmaceuticals, Mumbai, India) for follicular stimulation. The starting gonadotropin dose was based on maternal age, body mass index (BMI), AFC, and the outcomes of previous COS. Final oocyte

Fig. 1 Distribution of FET according to the number of days after oocyte retrieval

maturation was triggered when three or more follicles reached ≥ 17 mm, with either GnRH agonist (0.2 mg, Gonapeptyl®, Ferring Pharmaceuticals, India), hCG (250 μ g/0.05 mL, Ovidrel, Merck Serono, Istanbul, Turkey) or a combination of the two (dual trigger), at the discretion of the treating clinician. Oocyte retrieval was performed 36 h after trigger, using a transvaginal ultrasound (TVS) guided follicle aspiration (Reproline oocyte aspiration needle, 461230LF, Rheinbach, Germany) procedure. Oocyte collection and manipulation was performed using Cook Medical media (Sydney IVF, Cook Medical, Brisbane, Australia) and embryo culture was performed using SAGE 1-Step™ medium (SAGE, 67010010A, CooperSurgical Fertility Companies, Malov, Denmark), with no change of media during culture. Incubation conditions were set at 6% CO₂, 5% O₂, and 37.0 °C (G185 Long Term Flat Bed Incubators, K-Systems, Kivex Biotec Ltd, Birkerød, Denmark). All oocyte inseminations were performed using ICSI and embryo assessments performed daily.

Blastocyst assessment

Blastocysts were graded according to the 3-part grading system of Gardner and Schoolcraft [26]. The grade of a blastocyst included the assessment of blastocyst expansion (grade 1 to 6), the inner cell mass (ICM, A to C, according to the number and degree of compaction of the cells), and the trophectoderm (TE, A to C, according to the number, size, and contiguous arrangement of the trophectoderm cells). Only blastocysts with the grading of $\geq 2BB$ were cryopreserved by vitrification.

Blastocyst cryopreservation

Vitrification and warming of blastocysts were performed using ultra-rapid technologies (Cryotop® and vitrification and warming media, Kitazato BioPharma Co. Ltd., Shizouka, Japan), as described by the manufacturer. Blastocysts were warmed and transferred on the same day;

after warming, blastocysts were placed in incubation for approximately 2 h and reassessed morphologically.

Cycle programming and endometrial preparation

Standard protocols were used for endometrial preparation, with the start of endometrial preparation programmed with the administration of GnRH agonist (≈ 1.88 mg of a 3.75 mg Lucrin Depot®, Abbott Laboratories Ltd., Istanbul, Turkey) or oral contraceptive pill (OCP, Ginera®, 75 μ g Gestodene, 30 μ g ethinyloestradiol, Bayer, Istanbul, Turkey) [27, 28]. Cycles with the shortest time interval between oocyte retrieval and ET (≈ 36 days) were those in which a Lucrin Depot® was administered 7 days after oocyte retrieval and endometrial preparation was started 10 days later with oral estrogen administration [27]. Estrogen (Estrofem, Novo Nordisk, Bagsvaerd, Denmark) was taken orally in a step-up regimen: 2 mg/day from day 1 to 6, 4 mg/day from day 7 to 10, and 8 mg/day from day 11 to 14. On the 14th day of estrogen, an ultrasound examination was performed to measure endometrial thickness and a serum progesterone measurement was performed. An endometrial thickness of > 7 mm was considered optimal, however, a serum progesterone level of > 2 ng/mL resulted in the cycle being discontinued. If the endometrium was > 7 mm and serum progesterone was < 2 ng/mL, estrogen administration was continued at 6 mg/day, and daily progesterone (90 mg, twice-a-day, Crinone® 8%, Merck Serono, Turkey) administration commenced the following day (i.e., day 15). FET was performed on day 6 of progesterone administration.

Frozen embryo transfer

All transfers were performed with a glass syringe (50 μ L, Hamilton, Giarmata, Romania) attached to an embryo replacement catheter (Sure View®, Smiths Medical International Ltd., Kent, UK) and with trans-abdominal ultrasound guidance, with a maximum of two blastocysts transferred.

Outcomes and statistics

The delay to FET was calculated as the time interval in days between oocyte retrieval and FET, with the reference group (47–61 days) representing FET performed two menstruations after oocyte retrieval. The primary outcome measured in the FET cycles was LB. Patient variables recorded were maternal age (years), BMI (kg/m²), AFC (n), number of previous ET (n), infertility duration (years), and etiology. The treatment cycle variables recorded were duration of exogenous FSH administration (days), total dose of exogenous FSH (IU), trigger type (GnRHa, GnRHa plus hCG, or hCG), and the number of oocytes retrieved. LB was defined as the delivery of a live infant after ≥ 20 gestation weeks.

MedCalc version 13.0.6 (MedCalc Software, Ostend, Belgium) was used for statistical analysis in the study. All continuous data were analyzed as medians and 25th and 75th percentiles of per cycle outcomes, as data sets failed normality testing (Shapiro-Wilk), and, therefore, were analyzed with the Mann-Whitney Rank Sum Test. All categorical data are presented as percentages and were analyzed with the chi-square test to determine relative risk and 95% confidence intervals (RR 95% CI). $P < 0.05$ was considered to be significant.

Results

In the present study, the reproductive outcomes of 1121 art-FET cycles, following blastocyst freeze-all, were analyzed in relation to the time interval between oocyte retrieval and ET (Table 1). In all the segmented-IVF cycles, the intention was to minimize patient stress and anxiety that might be related to treatment delay and, therefore, ET were performed with minimal delay. The decision to delay transfer for > 62 days was made by patients, with no procedures such as hysteroscopy performed between oocyte retrieval and ET. The protocols used to perform FET resulted in the first ET being performed approximately 36 days after oocyte retrieval, with 67.4% performed within 47 days and 87.6% within 62 days of oocyte retrieval (Table 2). The 137 (12.2%) transfers performed after 62 days were distributed across the longer time interval groups without any group containing a particularly large number of FET (Fig. 1). Overall, the maternal age of the study population was 31.6 years (± 5.32) and the overall LB rate for the treatment population was 57.8%.

The analysis of patient demographics of cycles in the interval groups are presented in Table 2. The only significant difference observed was between the infertility duration of the 62–76-day interval group and the reference interval group. In the analysis of the cycle characteristics, presented in Table 3, the only significant difference found was between the total FSH dose of the 77–91-day interval group and the reference interval group. In the two interval groups with significant differences, low numbers of FET (33 and 35) were performed.

In the analysis of LB rates, presented in Table 4, there were no significant differences between the LB rates of any of the time interval groups and that of the reference group (47–61 days). The LB rate of FET performed after only one menstruation (57.8%) was similar to the LB rate for FET performed after four menstruations (54.9%). In the sub-analyses of cycles with SBT, GnRH agonist triggers, hCG triggers, maternal age > 35 years, ≤ 5 oocytes, and ≥ 15 oocytes there were also no significant differences between the LB rates of any of the time interval groups and that of the reference group.

Table 2 Characteristics of patients within the time interval groups

Intervals	Days	47–61	32–46	62–76	77–91	≥ 92	<i>p</i> value	<i>p</i> value
Cycles	<i>n</i>	226	756	33	35	71		
Maternal age	Years	31.9 (27.2–35.3)	31.5 (27.6–35.2)	31.3 (26.4–35.8)	31.3 (27.8–37.9)	32.2 (28.2–34.8)	0.839	0.732
Body mass index	kg/m ²	26.0 (22.0–29.0)	25.0 (23.0–29.0)	25.0 (23.0–28.3)	25.0 (22.5–29.5)	24.5 (23.0–29.0)	0.653	0.770
Infertility duration	Years	5.0 (2.8–7.3)	4.0 (2.0–7.0)	3.0 (1.5–5.5)	5.0 (3.0–8.0)	5.0 (3.0–8.0)	0.06	0.339
Previous ET	<i>n</i>	0 (0–1)	0 (0–1)	1 (0–1.5)	1 (0–2)	1.0 (0–1)	0.362	0.050
Antral follicle count	<i>n</i>	14.0 (8.3–20.8)	14.0 (8.0–20.0)	15.0 (8.0–23.0)	14.0 (7.0–19.0)	14.0 (8.0–17.5)	0.738	0.623

Statistical analysis; all data presented and analyzed as medians and percentiles (25 and 75%). The outcomes in each of the time interval groups were compared with the 47–61-day reference group, with the time interval representing the time in days between oocyte retrieval and frozen embryo transfer. $p < 0.05$ indicating statistical significance

Table 3 Cycle characteristics of patients within the time interval groups

Intervals	Days	47–61	32–46	<i>p</i> value	62–76	<i>p</i> value	77–91	<i>p</i> value	≥ 92	<i>p</i> value
Cycles	<i>n</i>	226	756		33		35		71	
COS duration	Days	9.0 (8–10)	9.0 (8–10)	0.849	8.5 (8.0–9.8)	0.100	10.0 (8–10)	0.141	9.0 (8–10)	0.847
Total FSH	IU	3293 (2400–4050)	3150 (2400–4050)	0.168	3300 (2138–4050)	0.362	4050 (2925–4500)	0.036	3600 (2475–4050)	0.903
Maturation trigger										
Agonist		46.9 (106)	47.1 (356)	0.970	48.5 (16)	0.950	42.9 (15)	0.912	38.0 (27)	0.484
Dual		33.6 (76)	35.6 (269)		30.3 (10)		40.0 (14)		38.0 (27)	
hCG		19.5 (44)	17.3 (131)		21.2 (7)		17.1 (6)		23.9 (17)	
Oocytes	<i>n</i>	13 (8–22)	14 (9–22)	0.068	18 (8–23)	0.373	13 (8–19)	0.851	13 (8–19)	0.710

Statistical analysis; all data presented and analyzed as medians and percentiles (25 and 75%). The outcomes in each of the time interval groups were compared with the 47–61-day reference group, with the time interval representing the time in days between oocyte retrieval and frozen embryo transfer. *p* < 0.05 indicating statistical significance

COS controlled ovarian stimulation, *FSH* follicle stimulating hormone, *Agonist* gonadotropin releasing hormone agonist, *hCG* human chorionic gonadotropin, dual a combination of agonist and hCG

Discussion

In the present study, the LB rates of non-elective blastocyst freeze-all cycles were analyzed; the patients fully represent the infertile population that attended our ART center and included both patients at high and low risk of adverse COS-related outcomes. The cycles were analyzed in 14-day interval groups based on the distribution of FET performed following oocyte retrieval (Fig. 1), to confirm whether COS performed in the oocyte retrieval cycle affected the endometrial receptivity of subsequent cycles. The analysis of FET cycles confirmed that delaying FET of freeze-all cycles beyond the cycle immediately following oocyte retrieval does not increase LB rates, irrespective of patient, and cycle characteristics (i.e., final oocyte maturation trigger, oocyte number, and maternal age).

For the majority of IVF patients, the delay between oocyte retrieval and FET is a major concern. Considering the tremendous psychological and physical burden of infertility and the treatment of infertility, it is important that patients are provided with not only the most effective treatment, but also the most efficient. In the present study, the majority (87.6%) of treatments were completed within 62 days or within three menstrual cycles of oocyte retrieval, with an overall LB rate of 57.8%. Importantly, the LB rates were similar for FET performed < 62 and > 92 days after oocyte retrieval.

There are many factors playing a role in the success of an IVF treatment; the most important of which are factors related to embryo quality and endometrial receptivity. Therefore, any procedure that has an effect on endometrial development has the potential to impair embryo implantation, placentation, intrauterine fetal growth, and ultimately the chances of LB [12]. The use of high gonadotropin dose COS is acknowledged to be pivotal to the success of IVF, in terms of oocyte numbers [1–3, 8]. However, the use of high gonadotropin dose COS significantly affects the early luteal phase [10, 12, 17] of a treatment cycle, with the supraphysiological levels of estrogen and progesterone that often arise affecting the ability of the endometrium to proliferate, differentiate, and mature [6, 29–31]. Moreover, the capacity of the endometrium to regenerate is undeniable, with studies reporting that localized injury to the endometrium in the cycle preceding ET even improving the chances of pregnancy [32]. However, the endometrium has been assumed to need a longer time to fully recover after COS, because of its systemic affects. In the past, IVF patients have been advised to delay continuing with treatment for 2–3 months after a failed cycle, with the length of the recommended delay a concern for many patients. Currently, because of the increasing use of segmented-IVF as a novel treatment strategy, the need for such a delay is being questioned by clinicians.

The primary purpose of segmented-IVF is to optimize LB rates by performing embryo transfers under more physiologic luteal phase conditions. The question of when these

Table 4 Live birth rates of patients within the time interval groups

	Days	47–61	32–46	RR (95% CI) <i>p</i> = value	62–76	RR (95% CI) <i>p</i> = value	77–91	RR (95% CI) <i>p</i> = value	≥ 92	RR (95% CI) <i>p</i> = value
Total cycles	% (n)	59.7 (135/226)	57.8 (437/756)	0.92 (0.682–1.250) <i>p</i> = 0.606	63.6 (21/33)	1.18 (0.553–2.516) <i>p</i> = 0.669	45.7 (16/35)	0.57 (0.277–1.162) <i>p</i> = 0.121	54.9 (39/71)	0.82 (0.480–1.407) <i>p</i> = 0.474
Single blastocyst transfer cycles	% (n)	56.3 (80/142)	54.7 (260/475)	0.94 (0.642–1.367) <i>p</i> = 0.736	72.2 (13/18)	2.02 (0.682–5.954) <i>p</i> = 0.205	52.3 (9/17)	0.87 (0.318–2.390) <i>p</i> = 0.790	43.2 (16/37)	0.59 (0.285–1.225) <i>p</i> = 0.157
Agonist trigger cycles	% (n)	57.5 (61/106)	57.6 (205/356)	1.00 (0.646–1.553) <i>p</i> = 0.995	56.3 (9/16)	0.95 (0.329–2.738) <i>p</i> = 0.922	40.0 (6/15)	0.49 (0.163–1.481) <i>p</i> = 0.207	59.3 (16/27)	1.07 (0.455–2.533) <i>p</i> = 0.872
hCG included trigger cycles	% (n)	61.7 (74/120)	58.0 (232/400)	0.86 (0.565–1.304) <i>p</i> = 0.474	70.6 (12/17)	1.49 (0.494–4.510) <i>p</i> = 0.478	50.0 (10/20)	0.62 (0.240–1.608) <i>p</i> = 0.327	52.3 (23/44)	0.68 (0.339–1.366) <i>p</i> = 0.279
≤ 5 oocyte cycles	% (n)	46.2 (18/39)	43.8 (39/89)	0.91 (0.427–1.938) <i>p</i> = 0.807	40.0 (2/5)	0.78 (0.117–5.183) <i>p</i> = 0.795	33.3 (2/6)	0.23 (0.025–2.186) <i>p</i> = 0.202	50.0 (5/10)	1.75 (0.426–7.190) <i>p</i> = 0.438
≥ 15 oocyte cycles	% (n)	63.1 (65/103)	63.9 (241/377)	1.04 (0.659–1.628) <i>p</i> = 0.878	78.9 (15/19)	2.19 (0.678–7.087) <i>p</i> = 0.190	46.2 (6/13)	0.50 (0.157–1.601) <i>p</i> = 0.244	58.6 (17/29)	0.83 (0.357–1.919) <i>p</i> = 0.660
Maternal age > 35 years	% (n)	45.5 (25/55)	40.7 (72/177)	0.82 (0.447–1.514) <i>p</i> = 0.531	33.3 (3/9)	0.60 (0.136–2.646) <i>p</i> = 0.500	30.8 (4/13)	0.53 (0.147–1.941) <i>p</i> = 0.340	43.8 (7/16)	0.93 (0.304–2.864) <i>p</i> = 0.904

Statistical analysis; all data presented and analyzed as percentages (%). The outcomes in each of the time interval groups were compared with the 47–61-day reference group, with the time interval representing the time in days between oocyte retrieval and frozen embryo transfer. *p* < 0.05 indicating statistical significance

Agonist trigger GnRH agonist trigger, hCG included trigger cycles all triggers that included the use of hCG, ≤ 5 oocytes cycles all cycles with < 6 oocytes recovered at oocyte retrieval, ≥ 15 oocyte cycles all cycles with > 14 oocytes recovered at oocyte retrieval

conditions are present after COS was the subject of recent investigations [23, 24]. While the results of the two studies did not confirm a minimum time delay, both studies showed that the reproductive outcomes from FET performed in the cycle immediately following oocyte retrieval were in no way compromised. Santos-Ribeiro et al. [23], in their univariate analysis, found the clinical pregnancy rate to be significantly higher in the immediate FET group compared to the delayed FET group (52.9 vs 41.6%, respectively), while Lattes et al. [24], in their univariate analysis, found LB rates to be significantly higher (37.6 vs 27.3%) in FET performed in the cycle immediately following oocyte retrieval compared to FET performed after two cycles. The generalizability of the evidence from these studies, however, is questionable, as both only investigated elective freeze-all cycles of patients at risk of adverse outcomes. In contrast, we investigated non-elective freeze-all cycles in the present study, with similar LB rates spanning the five intervals (i.e., 32–46, 47–61, 62–76, 77–91, and ≥ 92 days). In terms of the number of menstrual cycles, the LB rates of FET performed within one (32–46 days) and two menstrual cycles (47–61 days) of oocyte retrieval were 57.8 and 59.7%, respectively. In the present study, the same FET programming protocols were used as previously described for freeze-all cycles [26], with the earliest endometrial preparations starting 14 days after oocyte retrieval and the earliest FET performed 36 days after oocyte retrieval. While the methods may not result in the shortest time delay between oocyte retrieval and FET, the use of GnRH agonist programming does limit the chances of cycle cancellation ($\approx 0\%$) and, therefore, any further unwanted delay. The certainty of having a FET on the planned date avoids increasing patient anxiety and stress (i.e., state anxiety), which has been found to be a significant confounding factor in both pregnancy and LB outcomes [33].

Notwithstanding the general homogeneity presented in the present study, there are many more variables that may independently and significantly affect reproductive outcomes. A series of sub-analyses were performed in which specific variables were controlled for that are known to be associated with the outcomes of COS and the reproductive outcomes of IVF [7, 8, 34]. In the sub-analyses, transfer number (SBT), trigger type (GnRH agonist and hCG trigger), oocyte number (≤ 5 and ≥ 15 oocytes), and maternal age (> 35 years) were controlled for in the comparison of LB rates. In none of the sub-analyses were significant differences found in comparison to the LB rate of the reference group. Not only were the LB rates similar across the different interval groups, but they also followed conventional norms, with reduced LB rates in older patients, patients who had low oocyte numbers and patients who had SBT. In addition, the LB rates of cycles that were potentially at increased risk of adverse outcomes (i.e., high oocyte number cycles) were also similar across the different time intervals.

The LB evidence from the analyses of the present study suggests that FET from freeze-all cycles can be planned for the cycle immediately following the oocyte retrieval cycle, with patients who, however, wish to delay FET able to do so without fear of compromising their LB chances. The results of the present study confirm that FET in freeze-all cycles can be successfully performed 36 days after oocyte retrieval and that delaying transfer does not benefit reproductive outcomes. Moreover, while segmented-IVF may be perceived to increase patient stress and anxiety as the result of the delay in treatment completion, its separation from COS and oocyte retrieval may in fact result in patients having less stress and anxiety at the time of transfer.

Even though the study size was large, fully representative of the infertile population of our ART center, and included cycles performed in a 1-year period only, its retrospective design means that the evidence presented may not be free of bias and confounding. In addition, as all FET were performed in art-FET, it is uncertain whether the evidence can be extrapolated to the use of natural cycle FET. In conclusion, the LB rates of the present study show that there is no benefit in delaying FET in segmented-IVF beyond the cycle immediately following the oocyte retrieval cycle.

Compliance with ethical standards The present 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 that included permission to use anonymised data in research.

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

References

1. Ji J, Liu Y, Hong Tong X, Lihua Luo L, Jinlong Ma J, Zijiang CZ. The optimum number of oocytes in IVF treatment: an analysis of 2455 cycles in China. *Hum Reprod*. 2013;28:2728–34.
2. Steward RG, Lan L, Shah A, Yeh J, 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.
3. 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.
4. Papanikolaou EG, Bourgain C, Kolibianakis E, Tournaye H, Devroey P. Steroid receptor expression in late follicular phase endometrium in GnRH antagonist IVF cycles is already altered, indicating initiation of early luteal phase transformation in the absence of secretory changes. *Hum Reprod*. 2005;20:1541–7.
5. Van Vaerenbergh I, Van Lommel L, Ghislain V, In't Veld P, Schuit F, Fatemi HM, et al. In GnRH antagonist/rec-FSH stimulated cycles, advanced endometrial maturation on the day of oocyte retrieval correlates with altered gene expression. *Hum Reprod*. 2009;24:1085–91.

6. Humaidan P, Van Vaerenbergh I, Bourgain C, Alsbjerg B, Blockeel C, Schuit F, et al. Endometrial gene expression in the early luteal phase is impacted by mode of triggering final oocyte maturation in recFSH stimulated and GnRH antagonist co-treated IVF cycles. *Hum Reprod*. 2012;27:3259–72.
7. Fatemi HM, Polyzos NP, van Vaerenbergh I, Bourgain C, Blockeel C, Alsbjerg B, et al. Early luteal phase endocrine profile is affected by the mode of triggering final oocyte maturation and the luteal phase support used in recombinant FSH–GnRH antagonist IVF cycles. *Fertil Steril*. 2013;100:742–7.
8. 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.
9. 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.
10. 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.
11. 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.
12. Ozgur K, Humaidan P, Coetzee K. Segmented ART—the new era in ART? *Reprod Biol*. 2016;16:91–103.
13. Edgar DH, Gook DA. A critical appraisal of cryopreservation (slow cooling versus vitrification) of human oocytes and embryos. *Hum Reprod Update*. 2012;18:536–54.
14. Li Z, Wang YA, Ledger W, Edgar DH, Sullivan EA. Clinical outcomes following cryopreservation of blastocysts by vitrification or slow freezing: a population-based cohort study. *Hum Reprod*. 2014;29:2794–801.
15. Feng G, Zhang B, Zhou H, Shu J, Gan X, Wu F, et al. Comparable clinical outcomes and live births after single vitrified–warmed and fresh blastocyst transfer. *Reprod BioMed Online*. 2012;25:466–73.
16. Roy TK, Bradley CK, Bowman MC, McArthur SJ. Single-embryo transfer of vitrified–warmed blastocysts yields equivalent live-birth rates and improved neonatal outcomes compared with fresh transfers. *Fertil Steril*. 2014;101:1294–301.
17. 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.
18. Ozgur K, Berkkanoglu M, Bulut H, Isikli A, Coetzee K. Higher clinical pregnancy rates from frozen–thawed blastocyst transfers compared to fresh blastocyst transfers: a retrospective matched-cohort study. *J Assist Reprod Genet*. 2015;32:1483–90.
19. Coates A, Kung A, Mounts E, Hesla J, Bankowski B, Barbieri E, et al. Optimal euploid embryo transfer strategy, fresh versus frozen, after preimplantation genetic screening with next generation sequencing: a randomized controlled trial. *Fertil Steril*. 2017;107:723–30.
20. Humaidan P, Ejdrup Bredkjaer H, Westergaard LG, Yding AC. 1, 500 IU human chorionic gonadotropin administered at oocyte retrieval rescues the luteal phase when gonadotropin-releasing hormone agonist is used for ovulation induction: a prospective, randomized, controlled study. *Fertil Steril*. 2010;93:847–54.
21. Shapiro BS, Daneshmand ST, Garner FC, Aguirre M, Hudson C, Thomas S. Evidence of impaired endometrial receptivity after ovarian stimulation for in vitro fertilization: a prospective randomized trial comparing fresh and frozen–thawed embryo transfer in normal responders. *Fertil Steril*. 2011;96:344–8.
22. Mainigi MA, Olalere D, Burd I, Sapienza C, Bartolomei M, Coutifaris C. Peri-implantation hormonal milieu: elucidating mechanisms of abnormal placentation and fetal growth. *Biol Reprod*. 2014;90:1–9.
23. Santos-Ribeiro S, Polyzos NP, Thi Ngoc Lan V, Siffain J, Mackens S, Van Landuyt L, et al. The effect of an immediate frozen embryo transfer following a freeze-all protocol: a retrospective analysis from two centres. *Hum Reprod*. 2016;31:2541–8.
24. Lattes K, Checa MA, Vassena R, Brassocco M, Vernaev V. There is no evidence that the time from egg retrieval to embryo transfer affects live birth rates in a freeze-all strategy. *Hum Reprod*. 2017;32:368–74.
25. Ozgur K, Berkkanoglu M, Bulut H, Humaidan P, Coetzee K. Perinatal outcomes after fresh versus vitrified–warmed blastocyst transfer—a retrospective analysis. *Fertil Steril*. 2015;104:899–907.
26. Gardner DK, Lane M, Stevens J, Schenkler T, Schoolcraft WB. Blastocyst score affects implantation and pregnancy outcome: towards a single blastocyst transfer. *Fertil Steril*. 2000;76:1155–8.
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. İşkoğlu M, Özdem S, Berkkanoglu M, Jamal H, Şentürk Z, Özgür K. Single dose 1.88 mg depot leuprolide is as efficient as daily short acting leuprolide in ICSI cycles. *Hum Reprod*. 2007;22:1657–61.
29. Fauser BC, Devroey P. Reproductive biology and IVF: ovarian stimulation and luteal phase consequences. *Trends Endocrinol Metab*. 2003;14:236–42.
30. Venetis CA, Kolibianakis EM, Bosdou JK, Tarlatzis BC. Progesterone elevation and probability of pregnancy after IVF: a systematic review and meta-analysis of over 60 000 cycles. *Hum Reprod Update*. 2013;19:433–57.
31. Imudia AN, Goldman RH, Awonuga AO, Wright DL, Styer AK, Toth TL. The impact of supraphysiologic serum estradiol levels on peri-implantation embryo development and early pregnancy outcome following in vitro fertilization cycles. *J Assist Reprod Genet*. 2014;31:65–71.
32. Nastri CO, Lensen SF, Gibreel A, Raine-Fenning N, Ferriani RA, Bhattacharya S, et al. Endometrial injury in women undergoing assisted reproductive techniques. *Cochrane Database Syst Rev*. 2015;22(3):CD009517.
33. An Y, Sun Z, Li L, Yajuan Zhang Y, Ji H. Relationship between psychological stress and reproductive outcome in women undergoing in vitro fertilization treatment: psychological and neurohormonal assessment. *J Assist Reprod Genet*. 2013;30:35–41.
34. Kyrou D, Al-Azemi M, Papanikolaou EG, Donoso P, Tziomalos K, Devroey P, et al. The relationship of premature progesterone rise with serum estradiol levels and number of follicles in GnRH antagonist/recombinant FSH-stimulated cycles. *Eur J Obstet Gynecol Reprod Biol*. 2012;162:165–8.