A freeze-all strategy does not increase live birth rates in women of advanced reproductive age

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Received: 10 March 2020 / Accepted: 25 August 2020 / Published online: 2 September 2020
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Abstract

Research question Does a freeze-all strategy improve live birth rates in women of different age groups?

Design Retrospective cohort study of 1882 first embryo transfer cycles, performed between January 2013 and December 2015. Reproductive outcomes between fresh (FRESH) or frozen (FROZEN) embryo transfers were compared in patients stratified by age: < 35, between 35 and 38, or > 38 years. Student's *t* test for independent samples and χ^2 analyses were used as needed. A multivariable logistic regression analysis was performed adjusting for age, triggering drug, number of retrieved oocytes, number of transferred embryos, and percentage of top-quality embryos.

Main results and the role of chance Live birth rates (LBR) were significantly higher for FROZEN in the < 35 years group (43.7% vs 24%; $p < 0.001$). In both the 35–38 and > 38 years groups, LBR for FROZEN vs FRESH were not statistically different (30.9% in the FROZEN group vs 29.3% in the FRESH group, $p = 0.70$, and 19.8% in the FROZEN group vs 12.7% in the FRESH group, $p = 0.07$, respectively). The multivariate analysis found a significantly positive effect of performing FROZEN on LBR in the younger group (OR 2.46, 95% CI 1.31–4.62; $p = 0.005$) but had no impact in women between 35 and 38 years (OR 1.01, 95% CI 0.55–1.83; $p = 0.98$) or older (OR 0.96, 95% CI 0.43–2.13; $p = 0.92$).

Conclusions Performing a freeze-all strategy seems to result in better reproductive outcomes when compared with a fresh ET in women under 35 years, with no significant impact on older women.

Keywords Freeze-all · IVF · Frozen embryo transfer · Embryo transfer · Endometrial receptivity

Introduction

Initially, in vitro fertilization (IVF) was developed to provide a chance to conceive to women who suffered from tubal factor infertility [1]. Since then, intracytoplasmic sperm injection (ICSI) and ovarian stimulation—and retrieval of multiple oocytes—have expanded IVF indications to most, if not all, causes of infertility and other indications such as the

prevention of chromosomal and genetic abnormalities through the use of preimplantation genetic testing (PGT). The optimization of stimulation protocols and the advent of embryo vitrification over the past decades have led to increased success rates in the field of assisted reproduction [2].

Controlled ovarian hyperstimulation (COH) is performed to improve cumulative pregnancy rates through the retrieval of multiple oocytes in one cycle. However, this increase in performance comes at a cost, namely the detrimental effect of COH on endometrial receptivity, which might be related to poorer outcomes during fresh embryo transfers secondary to an advanced receptive phase and embryo-endometrium asynchrony [3–5]. This may be in part explained by the supraphysiological estrogen levels and premature progesterone elevations during COH that affect the gene expression pattern of the normal, cyclic endometrium [6–13]. Also, COH patients carry the risk of ovarian hyperstimulation syndrome (OHSS), an iatrogenic and potentially lethal complication of COH usually associated with the use of hCG to trigger ovulation.

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The improvement in performance observed by COH is only useful in the context of cryopreservation, which proved to be a game changer in IVF and is now a successful and well-established procedure to preserve cellular viability in an arrested state [14]. Currently, embryo vitrification is associated with higher survival, metabolism and blastocyst formation rates [15], regardless of vitrification method [16]. Moreover, embryo vitrification resulted in a higher percentage of cycles resulting in transfer and higher clinical pregnancy (CPR) and live birth rates (LBR) when compared with slow freezing [17]. Moreover, embryo vitrification, at today's standards of quality, allows for similar embryo survival, development, and performance than fresh embryo culture [18], which gave rise to the “freeze-all” concept, i.e., the elective cryopreservation of the entire embryo cohort in order to preserve the best embryos to be transferred into a more favorable environment. The freeze-all strategy, together with the administration of a GnRH-agonist for final oocyte maturation, allows for a safer IVF treatment since it virtually eliminates the risk for late OHSS in what has been called “the OHSS-free clinic” [19].

Currently, several observational studies report on obstetric and perinatal outcomes comparing pregnancies derived from fresh and frozen embryo transfer (FET) cycles in terms of risk of preterm birth and low birth weight [20] or risk of maternal complications, such as antenatal hemorrhage, placenta previa [21], or ectopic pregnancy [22] with results favoring FET over fresh embryo transfers. A meta-analysis by Maheshwari in 2012, and its update in 2018 both point in the same direction [23, 24], although an increase in hypertensive disorders of pregnancy can be observed in FET. Moreover, no differences between FET and fresh cycles have been reported in terms of major congenital anomalies or general physical health of children after a 3-year follow-up in two recent retrospective studies in Finland [25, 26]. Although available evidence appears to support the notion of a cryopreserving the whole embryo cohort in favor of a frozen embryo transfer strategy, the scarcity of well-designed, randomized clinical trials (RCTs) means that the universal “freeze-all” concept is not devoid of controversy and there are concerns about data and outcome reporting [27] and cycle cost-effectiveness [13, 28]. A recent Cochrane systematic review, including 4 randomized, controlled trials, shows no difference in cumulative live birth rates among freeze-all patients vs patients undergoing a fresh embryo transfer with subsequent FETs [29] although, in a supplementary analysis, the live birth rates for the first embryo transfers were statistically higher in the freeze-all group, regardless of embryo stage of development at transfer (OR 1.34; 95% CI 1.12–1.61). Since then, two RCTs have shown that in good prognosis, ovulatory patients, performing a freeze-all strategy is at least as effective as conventional fresh embryo transfer [30, 31], but safer. The mean age of the patients included in these studies was 28.5 ± 3 and 32 ± 4 years, though.

So, as of today, the answer to the central question “Who benefits from a freeze-all strategy?” is limited to very specific indications, namely OHSS prevention, polycystic ovarian syndrome (PCOS) patients [31], patients undergoing blastocyst transfers [32], endometrial abnormalities, and the need for further procedures such as PGT or endometrial receptivity tests [33], and extrapolation of these indications to a universal approach is not currently warranted. Currently, many of the published papers that demonstrate a beneficial effect of a freeze-all strategy base their analyses and conclusions on the results from young, good prognosis patients [30, 31] but evidence on women of a more advanced reproductive age is currently lacking. Preliminary reports from small studies have suggested that a freeze-all strategy could increase pregnancy rates in women of advanced reproductive age [34, 35]. This study aims to determine if a freeze-all strategy has an impact on reproductive outcomes in women of different age groups.

Materials and methods

This single-center, retrospective cohort study includes all patients who underwent a first autologous IVF cycle between January 2013 and December 2015. We analyzed the results of the first ET in patients who underwent either a fresh ET compared with those who underwent a freeze-all strategy.

Study population

Premenopausal women aged 20 to 45 years were included, and all participants had a normal karyotype. Exclusion criteria were the following: BMI over 30 kg/m^2 ; presence of endocrine pathologies or uterine abnormalities; presence of chronic, autoimmune, or metabolic diseases; need for testicular sperm extraction (TESE); evidence of meiotic chromosomal abnormalities in testicular biopsy or altered disomy or diploidy percentages in sperm by fluorescence in situ hybridization (FISH); and cycles with preimplantation genetic screening and participation, within the previous 6 months, in a clinical trial with medication.

A freeze-all approach was proposed by the physicians based on 4 indications: prevention of OHSS; unfavorable endometrium at the time of oocyte retrieval (endometrial thickness $< 7 \text{ mm}$) and/or a mid-late secretory pattern; homogeneous, hyperechoic functionalis extending from the basalis to the lumen, as described by Grunfeld [36]; serum progesterone $> 1.5 \text{ ng/ml}$ on the day of ovulation trigger; or the desire to optimize cycle outcomes. A total of 1882 consecutive cycles met the inclusion criteria; we analyzed the results of the first FET of the best available embryos between those which took

place during the fresh cycle and those performed a frozen-thaw cycle.

All patients underwent COH with exogenous gonadotropins using two possible protocols: a flexible GnRH-antagonist protocol, in which gonadotropins were started on the second day of the current menstrual cycle, introducing the antagonist when a follicle reached 14 mm in diameter, or a long GnRH-agonist protocol, in which the agonist was started in the mid-luteal phase of the preceding menstrual cycle, adding gonadotropins on the second day after menstrual bleeding.

Ovulation was triggered when follicles reached > 17 mm in diameter, using either 0.3 mg of Triptorelin (Decapeptyl®, Ipsen Pharma Biotech, France) or 250 µg of hCG (Ovitrelle®, Merck Serono, Italy) depending on the stimulation protocol.

Oocyte collection was performed 36 h after triggering by ultrasound-guided transvaginal follicle aspiration. Oocytes were cultured for 4 h post-harvest before being inseminated for IVF or decumulated for intracytoplasmic sperm injection (ICSI). Sperm was capacitated using density gradients (PureSperm®, Nidacon Internacional AB, Sweden).

Embryo culture was performed in single droplets, using a Lifeglobal® protein supplement media. Embryos were scored following the system of morphological assessment of oocytes, early embryos, and human blastocysts of the Spanish Association of Reproductive Biology (ASEBIR, III Journal of Clinical Embryology, Third Edition, 2015). This classification divides cleavage stage embryos into four categories (A, B, C, and D) according to the number and symmetry of the blastomeres and grade of fragmentation, with top-quality embryos being those graded A and B.

In fresh ET, 1, 2, or 3 embryos were transferred at day 2 or 3 of development and surplus embryos were vitrified. In freeze-all cycles, all embryos were vitrified at day 2 or 3 of development in a closed device system (CryoTip®, Irvine Scientific, USA); FET of 1, 2, or 3 embryos was performed 1 day after thawing (day 3/4) to assess embryo viability. Cleavage stage embryos were considered suitable for transfer if they presented at least 50% of blastomeres intact post-thawing and showed signs of development, in accordance with Alpha consensus guidelines [37]. Regardless of embryo stage of development, single-embryo transfer (SET) was recommended to patients in order to decrease multiple pregnancy rate.

Endometrial preparation for FET was achieved by an artificial cycle in which transdermal exogenous estradiol hemihydrate (150 mg every other day; Estradot® Novartis Pharma, Germany) or oral exogenous estradiol valerate (6 mg/day; Meriestra® Novartis Farmacéutica, Spain) was administered.

In both fresh and frozen-thawed embryo transfer cycles, micronized progesterone (600 mg/day) was administered vaginally for luteal phase support (Utrogestan®, SEID, Spain).

Luteal phase support was started on the day of egg retrieval in fresh embryo transfers, 3 days before embryo transfer for day 2 embryos, and 4 days before embryo transfer for day 3 embryos, as the embryos were transferred 1 day post-thaw. The luteal phase was supported during the whole first trimester prior to April 2015. From then on, the duration was restricted to 9–10 weeks of gestational age [38].

Statistical analysis

The primary outcome of this study was the live birth rate (LBR) during the first embryo transfer of the IVF cycle. Secondary endpoints were pregnancy rate (PR), clinical pregnancy rate (CPR), implantation rate (IR), and pregnancy loss (PL). All calculations were made on a “per embryo transfer” basis.

Pregnancy rate is defined as a positive serum pregnancy test performed 15 days after oocyte retrieval in fresh cycles or on day 15 of embryo development in FET. Clinical pregnancy is defined as the presence of an intrauterine gestational sac with embryonic cardiac activity, evaluated by ultrasound at 7 weeks of gestation. Implantation rate is defined as the number of gestational sacs observed, divided by the number of embryos transferred. Pregnancy loss is defined as the loss of a pregnancy before 20 completed weeks of gestation, calculated using the total number of positive pregnancy tests as the denominator. The live birth rate is the number of live birth deliveries per FET.

Data were analyzed after stratifying patients into three age categories: women under 35 years of age, women between 35 and 38 years of age, and women over 38 years of age, in order to account for the impact of age on results.

Accepting an alpha risk of 0.05 and a beta risk of 0.2 in a two-sided test, 137 subjects are necessary for each study group (within each age category) to find a statistically significant a proportion difference, assuming an increase of 15 percentage points in live birth rate, starting from a LBR of 20%.

Data analysis was performed using the IBM SPSS Statistics 20 (SPSS Inc., Chicago, IL) software. A comparison of quantitative variables was performed using Student's *t*, while categorical variables were compared using a χ^2 analysis. Differences were considered significant if two-tailed *p* values were < 0.05.

A multivariable logistic regression analysis was performed to determine the variables that could be independently associated with pregnancy, live birth, or pregnancy loss and could affect outcomes across woman's age categories. The age of the woman (continuous), drug used for ovulatory trigger (agonists vs hCG), number of retrieved oocytes, number of transferred embryos (2–3 vs 1), percentage of top-quality embryos, and type of cycle (FROZEN vs FRESH) were included in the analysis.

Results

We analyzed the results of the first embryo transfer of 1882 cycles, stratified by age, comparing between those which took place during a fresh cycle (FRESH; 1412 cycles) and those who underwent a freeze-all cycle (FROZEN; 470). The overall mean age of the study population was 36.8 ± 4.1 years: 27.8% (524/1882) patients were younger than 35 years (range 20–34), 33.5% (631/1882) were between 35 and 38 years (range 35–38), and 38.6% (727/1882) were over 40 years of age (range 39–44). The mean embryo survival rate on the day of thawing for the study period was 95%. The overall pregnancy loss and live birth rates were 16.4% and 23.9%, respectively. Cycle characteristics, overall and stratified by age, are presented in Table 1.

Univariate analysis

We found a significantly higher live birth rate (43.7% vs 24%; $p < 0.001$) when comparing FROZEN vs FRESH, respectively, in women under 35 years of age (Table 2). In this age group, differences were also significant for pregnancy rate (PR; 57.9% vs 39.9%; $p < 0.001$), clinical pregnancy (CPR; 50.8% vs 31.4%; $p < 0.001$), and pregnancy loss (24.5% vs 38.8%; $p = 0.026$).

In the intermediate age group (women between 35 and 38 years of age), PR and CPR were significantly higher in the fresh group, as was the pregnancy loss rate (Table 2). However, the LBR was not statistically different for both groups (30.9% in the FROZEN group vs 29.3% in the FRESH group, $p = 0.701$).

In women over 38 years of age, both PR and CPR were significantly higher in the FROZEN group, as well as implantation rate (IR; 75.7% vs 66.6%, $p = 0.048$), but the pregnancy loss rate and the LBR were similar among groups (19.8% in the FROZEN group vs 12.7% in the FRESH group, $p = 0.066$).

Multivariable analysis

Women under 35 years of age

In this group age, for the secondary outcomes of PR and CPR, we observed no significant impact of age, drug used for ovulation trigger, total number of oocytes retrieved, and day of embryonic development at transfer. The only variables that had an impact on outcomes were the type of cycle performed (frozen vs fresh, with an OR of 2.33, 95% CI 1.25–4.32, $p = 0.007$ for PR and OR of 2.31, 95% CI 1.25–4.29, $p = 0.008$ for CPR), the number of transferred embryos (1 vs 2+ embryos, with an OR of 0.49, 95% CI 0.33–0.72, $p < 0.001$ for PR and OR of 0.52, 95% CI 0.35–0.78, $p = 0.002$ for CPR), and the

transfer of top-quality embryos (OR of 2.19, 95% CI 1.32–3.63, $p = 0.002$ for PR and OR of 2.24, 95% CI 1.32–3.81, $p = 0.003$ for CPR).

For the main outcome, LBR, age did not have a significant impact and the same was observed for ovulation trigger, number of retrieved oocytes, and day of embryo transfer. The only variables that had a significant impact on LBR were the cycle type, number of transferred embryos, and transfer of top-quality embryos (Table 3).

Women between 35 and 38 years of age

In the intermediate age group, when assessing the odds of achieving a pregnancy or clinical pregnancy, we observed no significant impact of the number of retrieved oocytes or day of embryonic development at transfer. The variables that had an impact on PR were age (OR 1.21, 95% CI 1.04–1.4, $p = 0.015$), the type of cycle performed (frozen vs fresh, with an OR of 0.33, 95% CI 0.19–0.58, $p < 0.001$), the drug used for trigger (agonist vs hCG, OR of 2.09, 95% CI 1.09–4.0, $p = 0.026$), the number of transferred embryos (OR 0.44, 95% CI 0.3–0.65, $p < 0.001$), and the use of top-quality embryos (OR of 2.26, 95% CI 1.46–3.48, $p < 0.001$). For CPR, the variables that impacted results were the type of cycle performed (frozen vs fresh, with an OR of 0.39, 95% CI 0.22–0.68, $p = 0.001$), the number of transferred embryos (OR 0.51, 95% CI 0.35–0.75, $p = 0.001$), and the use of top-quality embryos (OR of 1.76, 95% CI 1.15–2.69, $p = 0.009$) as we observed no impact of age, trigger, or number of retrieved oocytes on results.

For LBR, there was no significant impact of the type of cycle (frozen vs fresh) on outcomes, and the only variable that did exhibit an impact was the number of transferred embryos (Table 3).

Women over 38 years of age

In this group, the variables that had a significant impact on pregnancy rate were age (OR 0.9, 95% CI 0.81–0.99, $p = 0.04$), the number of transferred embryos (OR 0.46, 95% CI 0.31–0.66, $p < 0.001$), and the use of top-quality embryos (OR 1.54, 95% CI 1.01–2.35, $p = 0.047$).

For CPR and LBR, the impacting variables were age (with an OR of 0.83, 95% CI 0.74–0.93, $p = 0.002$ for CPR and OR of 0.8, 95% CI 0.69–0.94, $p = 0.005$ for LBR), the number of transferred embryos (OR of 0.54, 95% CI 0.36–0.81, $p = 0.003$ for CPR and OR of 0.5, 95% CI 0.3–0.85, $p = 0.01$ for LBR), and the use of top-quality embryos (OR of 1.77, 95% CI 1.11–2.81, $p = 0.016$ for CPR and OR of 2.12, 95% CI 1.14–3.96, $p = 0.018$ for LBR, respectively) (Table 3).

Table 1 Demographic characteristics overall and by study group for all patients and for each age category

All patients	Overall (n = 1882)	FRESH (n = 1412)	FROZEN (n = 470)	p - value*
Age, mean (SD)	36.82 (4.09)	37.31 (4.02)	35.32 (3.92)	< 0.001
Latency, mean (SD)	50.48 (36.47)		50.48 (36.47)	
Retrieved oocytes, mean (SD)	9.95 (6.68)	7.85 (4.43)	16.26 (8.16)	< 0.001
Total number of embryos, mean (SD)	6.32 (4.65)	4.97 (3.28)	10.36 (5.71)	< 0.001
Ovulation trigger, n (%)				< 0.001
hCG	1608 (85.4%)	1412 (100%)	196 (41.7%)	
GnRH agonist	274 (14.6%)	0 (0.0%)	274 (58.3%)	
Origin of sperm, n (%)				0.72
Partner	1694 (90.0%)	1273 (90.2%)	421 (89.6%)	
Donor	188 (10.0%)	139 (9.8%)	49 (10.4%)	
Top quality embryos, n (%)	2033/3176 (64%)	1500/2440 (61.5%)	533/736 (72.4%)	< 0.001
Transferred embryos, mean (SD)	1.69 (0.54)	1.73 (0.54)	1.57 (0.51)	< 0.001
1, n (%)	660 (35.1%)	207 (17.8%)	453 (63.2%)	
2, n (%)	1150 (61.1%)	890 (76.3%)	260 (36.4%)	
3, n (%)	72 (3.8%)	69 (5.9%)	3 (0.4%)	
Day of embryo transfer, mean (SD)	2.96 (0.76)	2.65 (0.55)	3.90 (0.46)	< 0.001
Age < 35	Overall (n = 524)	FRESH (n = 341)	FROZEN (n = 183)	p - value
Age, mean (SD)	31.72 (2.45)	31.90 (2.42)	31.39 (2.49)	0.022
Latency, mean (SD)	44.50 (30.99)		44.50 (30.99)	
Retrieved oocytes, mean (SD)	12.22 (7.29)	9.40 (4.88)	17.46 (8.09)	< 0.001
Total number of embryos, mean (SD)	7.61 (4.97)	5.89 (3.69)	10.80 (5.46)	< 0.001
Ovulation trigger, n (%)				< 0.001
hCG	394 (75.2%)	341 (100%)	53 (29.0%)	
GnRH agonist	130 (24.8%)	0 (0.0%)	130 (71.0%)	
Origin of sperm, n (%)				0.58
Partner	491 (93.7%)	321 (94.1%)	170 (92.9%)	
Donor	33 (6.3%)	20 (5.9%)	13 (7.1%)	
Top quality embryos, n (%)	602/863 (69.8%)	384/574 (66.9%)	218/289 (75.4%)	0.01
Transferred embryos, mean (SD)	1.65 (0.51)	1.68 (0.51)	1.58 (0.51)	0.027
1, n (%)	194 (37.0%)	116 (34.0)	78 (42.6%)	
2, n (%)	321 (61.3%)	217 (63.6%)	104 (56.8%)	
3, n (%)	9 (1.7%)	8 (2.3%)	1 (0.5%)	
Day of embryo transfer, mean (SD)	3.13 (0.75)	2.72 (0.55)	3.90 (0.45)	< 0.001
Age (35–38)	Overall (n = 631)	FRESH (n = 450)	FROZEN (n = 181)	p - value
Age, mean (SD)	36.43 (1.12)	36.46 (1.11)	36.38 (1.6)	0.45
Latency, mean (SD)	54.65 (41.14)		54.65 (41.14)	
Retrieved oocytes, mean (SD)	10.34 (6.86)	8.04 (4.14)	16.07 (8.70)	< 0.001
Total number of embryos, mean (SD)	7.61 (4.97)	5.24 (3.14)	10.61 (6.10)	< 0.001
Ovulation trigger, n (%)				< 0.001
hCG	529 (83.8%)	450 (100%)	79 (43.6%)	
GnRH agonist	102 (16.2%)	0 (0.0%)	102 (56.4%)	
Origin of sperm, n (%)				0.28
Partner	564 (89.4%)	406 (90.2%)	158 (87.3%)	
Donor	67 (10.6%)	44 (9.8%)	23 (12.7%)	
Top quality embryos, n (%)	709/1093 (64.9%)	505/810 (62.3%)	204/283 (72.1%)	0.003
Transferred embryos, mean (SD)	1.73 (0.51)	1.80 (0.50)	1.56 (0.52)	< 0.001
1, n (%)	190 (30.1%)	109 (24.2%)	81 (44.8%)	
2, n (%)	420 (66.6%)	322 (71.6%)	98 (54.1%)	
3, n (%)	21 (3.3%)	19 (4.2%)	2 (1.1%)	

Table 1 (continued)

Day of embryo transfer, mean (SD)	3.05 (0.75)	2.71 (0.55)	3.91 (0.44)	< 0.001
Age > 38	Overall (n = 727)	FRESH (n = 621)	FROZEN (n = 106)	p - value
Age, mean (SD)	40.82 (1.72)	40.91 (1.77)	40.30 (1.33)	0.001
Latency, mean (SD)	53.68 (35.67)		53.68 (35.67)	
Retrieved oocytes, mean (SD)	7.97 (5.36)	6.86 (4.11)	14.53 (6.96)	< 0.001
Total number of embryos, mean (SD)	4.99 (3.83)	4.28 (2.97)	9.18 (5.34)	< 0.001
Ovulation trigger, n (%)				< 0.001
hCG	685 (94.2%)	621 (100%)	64 (60.4%)	
GnRH agonist	42 (5.8%)	0 (0.0%)	42 (39.6%)	
Origin of sperm, n (%)				0.96
Partner	639 (87.9%)	546 (87.9%)	93 (87.7%)	
Donor	88 (12.1%)	75 (12.1%)	13 (12.3%)	
Top quality embryos, mean (SD)	722/1220 (59.2%)	611/1056 (57.9%)	111/164 (67.7%)	0.02
Transferred embryos, mean (SD)	1.68 (0.58)	1.70 (0.59)	1.55 (0.50)	0.012
1, n (%)	276 (38.0%)	228 (36.7%)	48 (45.3%)	
2, n (%)	409 (56.3%)	351 (56.5%)	58 (54.7%)	
3, n (%)	42 (5.8%)	42 (6.8%)	0 (0.0%)	
Day of embryo transfer, mean (SD)	2.75 (0.71)	2.56 (0.56)	3.83 (0.53)	< 0.001

*Student's *t* test or Pearson's χ^2 test

Discussion

According to our data, LBR appears to be improved by performing a freeze-all strategy in women under 35 years of age. For older women, we observed no difference in live birth rate between fresh or FET.

We observed higher pregnancy and clinical pregnancy in the freeze-all group in older women (> 38 years) although the LBR—before adjustment by potentially confounding factors—was significantly higher only in the younger women

group (43.7% vs 24.5%). After controlling for potential confounders at multivariable analysis, this difference remained significant (OR 2.43; 95% CI 1.32–4.51; *p* = 0.003). In women between 35 and 38 years of age, while the pregnancy rate was significantly higher in the fresh group, this was accompanied by a significantly higher pregnancy loss rate in said group, which explains why we observe no statistically significant differences between performing a fresh transfer or a freeze-all cycle. These results are in concordance with previously published data [39].

Table 2 Univariable analysis (Pearson's χ^2 test, Student's *t* test) of reproductive results overall and by study group, for all patients and for each age category

All patients	Overall (n = 1882)	FROZEN (n = 470)	FRESH (n = 1412)	p - value	OR (95% CI)
Biochemical pregnancy, n (%)	833 (44.3%)	229 (48.7%)	604 (42.8%)	0.025	1.27 (1.03, 1.56)
Clinical pregnancy, n (%)	698 (37.1%)	196 (41.7%)	502 (35.6%)	0.017	1.30 (1.05, 1.61)
Live birth, n (%)	450 (23.9%)	157 (33.4%)	293 (20.8%)	< 0.001	1.92 (1.52, 2.41)
Miscarriage, n (%)	309 (16.4%)	72 (15.3%)	237 (16.8%)	0.45	0.90 (0.67, 1.20)
Implantation rate, mean (SD)	80.0% (25.9%)	76.1% (26.6%)	69.0% (25.3%)	0.001	-
Age < 35	Overall (n = 524)	FROZEN (n = 183)	FRESH (n = 341)	p - value	OR (95% CI)
Biochemical pregnancy, n (%)	240 (45.8%)	106 (57.9%)	134 (39.9%)	< 0.001	2.13 (1.48, 3.06)
Clinical pregnancy, n (%)	200 (38.2%)	93 (50.8%)	107 (31.4%)	< 0.001	2.26 (1.56, 3.27)
Live birth, n (%)	162 (30.9%)	80 (43.7%)	82 (24.0%)	< 0.001	2.45 (1.67, 3.60)
Miscarriage, n (%)	78 (14.9%)	26 (14.2%)	52 (15.2%)	0.75	0.92 (0.55, 1.53)
Implantation rate, mean (SD)	76.5% (27.0%)	77.7% (28.0%)	75.6% (26.2%)	0.58	-
Age (35–38)	Overall (n = 631)	FROZEN (n = 181)	FRESH (n = 450)	p - value	OR (95% CI)
Biochemical pregnancy, n (%)	355 (56.3%)	77 (42.5%)	278 (61.8%)	< 0.001	0.46 (0.32, 0.65)
Clinical pregnancy, n (%)	307 (48.7%)	66 (36.5%)	241 (53.6%)	< 0.001	0.50 (0.35, 0.71)
Live birth, n (%)	188 (29.8%)	56 (30.9%)	132 (29.3%)	0.70	1.08 (0.74, 1.57)
Miscarriage, n (%)	167 (26.5%)	21 (11.6%)	146 (32.4%)	< 0.001	0.27 (0.17, 0.45)
Implantation rate, mean (SD)	69.0% (25.0%)	74.0% (25.5%)	67.6% (24.8%)	0.07	-
Age > 38	Overall (n = 727)	FROZEN (n = 106)	FRESH (n = 621)	p - value	OR (95% CI)
Biochemical pregnancy, n (%)	238 (32.7%)	46 (43.4%)	192 (30.9%)	0.014	1.71 (1.13, 2.61)
Clinical pregnancy, n (%)	191 (26.3%)	37 (34.9%)	154 (24.8%)	0.025	1.62 (1.05, 2.52)
Live birth, n (%)	100 (13.8%)	21 (19.8%)	79 (12.7%)	0.050	1.70 (1.00, 2.89)
Miscarriage, n (%)	138 (19%)	25 (23.6%)	113 (18.2%)	0.191	1.39 (0.85, 2.27)
Implantation rate, mean (SD)	68.3% (25.2%)	75.7% (25.3%)	66.6% (24.9%)	0.048	-

Table 3 Multivariable analysis results (logistic regression). Association of frozen embryo vs fresh transfer to live birth stratified by woman's age

		95% CI			
		OR	Lower	Upper	<i>p</i> - value
< 35	Frozen vs Fresh* ET	2.46	1.31	4.62	0.005
	Age (years)	1.05	0.96	1.13	0.29
	GnRh agonist vs hCGr* for trigger	0.94	0.47	1.90	0.87
	Number of oocytes (ln of oocytes number)	1.10	0.74	1.62	0.64
	1 vs 2+* transferred embryos	0.48	0.31	0.74	0.001
	Top-quality embryos (% of transferred embryos)	1.98	1.13	3.49	0.017
(35–38)	Frozen vs Fresh* ET	1.01	0.55	1.83	0.98
	Age (years)	1.07	0.92	1.26	0.38
	GnRh agonist vs hCGr* for trigger	1.56	0.79	3.06	0.20
	Number of oocytes (ln of oocytes number)	1.00	0.72	1.38	0.98
	1 vs 2+* transferred embryos	0.46	0.29	0.71	0.001
	Top-quality embryos (% of transferred embryos)	1.45	0.91	2.32	0.12
> 38	Frozen vs Fresh* ET	0.96	0.43	2.13	0.92
	Age (years)	0.80	0.69	0.94	0.005
	GnRh agonist vs hCGr* for trigger	1.61	0.55	4.68	0.35
	Number of oocytes (ln of oocytes number)	1.19	0.79	1.78	0.41
	1 vs 2+* transferred embryos	0.50	0.30	0.85	0.011
	Top-quality embryos (% of transferred embryos)	2.12	1.14	3.96	0.018

*Reference group

We also found that, in women younger than 35 years of age, and after adjusting for potential confounders, there was no impact on LBR of age or the drug used for ovulation trigger. The only other factors that had a significant influence in LBR were—as expected—the number of transferred embryos and the use of top-quality embryos.

In the univariable analysis, we observe significantly higher pregnancy rates for FRESH vs FROZEN in the 35–38 years group; however, the miscarriage rate was also significantly higher in this group, which in the end renders a live birth rate that is comparable among groups. We have no biologically plausible explanation for these findings other than this could be attributed to the effect of imbalanced confounding factors, which have been taken into account for the multivariable analysis.

Previous studies have attempted to address the impact of maternal age on IVF outcomes. In a retrospective, matched cohort study of good prognosis patients, comparing the performance of freeze-all cycles vs fresh embryo transfers, stratified by maternal age and progesterone levels on the day of ovulation trigger, the authors only observed differences in ongoing pregnancy rates in favor of frozen embryo transfers when progesterone levels were high (> 1 ng/ml). When progesterone levels were low, there was no difference in OPR between fresh and frozen embryo transfers, regardless of maternal age. Hence, the beneficial effect of frozen embryo transfer appears to be related to progesterone levels and not to maternal age per se [40].

It has been proposed until now that the potential advantage of performing a freeze-all policy decreases with a reduction in ovarian response, and different stratification methods have been proposed to use the ovarian response as a better predictor of IVF outcomes [41, 42]. In our population, patients undergoing a freeze-all strategy yielded a higher number of retrieved oocytes in all age groups (Table 1) and could be considered a group of patients with better prognosis; however, in women older than 35 years, the LBR did not increase despite the higher number of oocytes obtained. Furthermore, in the multivariate analysis, after adjusting for these differences, the number of retrieved oocytes did not have a significant impact on results in any of the age groups.

In our opinion, our results show that although there is a potential detrimental effect of ovarian hyperstimulation on endometrial receptivity, it appears that, in aging patients, correcting the potentially deleterious endometrial modifications induced by COH and its alleged negative impact of embryo-endometrium synchrony is not enough to compensate for the physiological decrease in embryo quality associated with the increasing aneuploidy rate related to oocyte aging.

In the past years, we have witnessed an important number of studies being published that intend to shed some light on the question of who are the patients that benefit from a freeze-all strategy. In light of the evidence published so far, however, and excluding the cases in which special interventions are needed—such as preimplantation genetic testing or

endometrial receptivity studies—a freeze-all strategy appears to be effective in improving cumulative live birth rates in hyper-responders or women affected by polycystic ovarian syndrome, with outcomes being comparable with fresh embryo transfers in other groups of patients [29, 39, 43, 44].

The primary limitation of our study is the uncontrolled potential for biases of the retrospective design. This implies that relevant variables, such as endometrial thickness or ovarian reserve markers, were not available for analysis in all patients. Also, in some of the cases, the decision to forgo a fresh embryo transfer in favor of a freeze-all strategy was solely based on physician preference, which constitutes bias in itself.

On the other hand, its main strength is being, to the best of our knowledge, the first study to assess reproductive results of a freeze-all strategy during the first embryo transfer of the first IVF cycle of patients of different age groups. Prospective, randomized, controlled trials are needed to confirm these results.

Acknowledgments We would like to thank Rosa Borràs, Maite Castro, Enrique Fabián, Manuel Gómez, Sara López, and Alicia Maqueda for their collaboration in recruiting, providing, and caring for study patients. We would also like to thank Francesc Figueras for statistical advice.

Authors' roles KL initiated and designed the study, interpreted the data, and wrote the manuscript. SL participated in data collection and interpretation and manuscript revision. MAC participated in data interpretation, provided expert knowledge, and was involved in manuscript revision. MB provided expert knowledge. DG performed the statistical analysis. RV was involved in study supervision, provided expert knowledge, and participated in data analysis and manuscript preparation.

Compliance with ethical standards

This study was approved by the local Research Ethics Committee.

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

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