Which euploid single embryo transfer strategy is preferable in advanced maternal aged; Fresh versus frozen

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Abstract

Purpose: Herein, we aimed to compare two commonly used single embryo transfer (ET) strategies, fresh and frozen-thawed euploid blastocyst transfer protocols via trophectoderm biopsy after preimplantation genetic screening (PGS) in advanced maternal aged (AMA).

Study Design: This study was designed as a single-center cohort study.A total of 317 patients aged older than 37 years old who underwent single fresh or frozen-thawed day five or day 6 ET with trophectoderm biopsy examined retrospectively. PGS was performed on the embryos of 138 patients, and fresh transfers were preferred, while embryos of 179 patients were vitrified, and then frozen-thawed euploid single ET was chosen after PGS. Percentage of the euploid embryo, implantation rates, pregnancy rates, pregnancy continuity rates, clinical and biochemical abortion rates were compared.

Results: Age, body mass index, infertility duration,previous ART cycles,days of stimulation, andAnti-Mullerian hormone levels did not reveal statistically significant difference between the groups. The retrieved oocyte, MII oocytes, and fertilized ovum (2PN) numbers were higher in the Frozen-thawed group. The percentage of a euploid embryo (32% vs. 34.8%) was similar between the groups. Implantation rates (46% vs. 62%), pregnancy rates (49% vs. 66%), ongoing pregnancy rates (36.7% vs. 52.6%), and live birth percentage (35.4% vs. 51.6%) were significantly higher in the frozen-thawed embryo transfer group, while the clinical and biochemical abortion rates did not reveal significant difference.

Conclusion: Frozen-thawed single euploid ET provides sufficient time for comprehensive genetic evaluations and increases the rates of implantation and pregnancy rates compared to the fresh single euploid ET in AMA patients with PGS.

1. Introduction

Decreased implantation rates, increased miscarriage rates, or fetuses with chromosomal abnormalities are known to be related to advanced maternal age (AMA)[1]. The main reason of impaired success of assisted reproductive technology (ART) is the maternal age-dependent aneuploid embryos [2].

Preimplantation genetic screening (PGS) is a viable option that can decrease the abortion rates and the duration to receive live birth in AMA patients undergoing ART [3, 4]. Currently, trophectoderm (TE) biopsy from blastocyst may become a gold-standard technique for PGS [5, 6]. Furthermore, TE biopsy for PGS added to conventional morphologic parameters of embryo quality assessment may increase pregnancy outcomes in AMA patients and decrease recurrent implantation failures and recurrent spontaneous abortion [6].

Recently, increased cryopreservation methods provided a chance to delay the transfer and avoided non-physiological endometrium, which occurs in controlled ovarian hyperstimulation(COH). Thus the implantation rates and pregnancy outcomes improved [7]. Therefore, delayed ET, which may obtain a more receptive endometrium in the subsequent cycle, may supply to escape the negative effects of COH,

which may lead to suboptimal endometrial development, thus decreasing the possibility of embryo implantation, and leading to decreased pregnancy rates [8]. Additionally, frozen-thawed ET gives an opportunity to add a whole cohort, including the fifth and sixth-day embryos. However, embryos that grow slower may be biopsied on the fifth or sixth day in the method of fresh ET with PGS and thus may negatively affect the embryos cohort that is available for transfer [9].

PGS also provides euploid embryos with improved implantation capacity and live birth rates in AMA patients[10]. PGS is a viable option that may decrease the abortion rates and the duration to get live birth in AMA patients undergoing ART. Currently, twodifferent transfer strategies as fresh or frozen-thawed embryos are used in clinical practice [9]. The frozen embryo transfer (ET) strategy has recently been pretentious to improve pregnancy rates due to increased endometrial receptivity [8]. However, for euploid embryos, the success of the ET strategy still needs to be clarified in AMA patients. This study comparedthe transfers of single fresh and frozen-thawed euploid blastocyst after PGS with trophectoderm biopsy in AMA.

2. Materials And Methods

This study was designed as a single-center cohort study. Patients who underwent embryo transfer in ART and Reproductive Genetics Center of Istanbul Memorial Hospital between 2011 and 2016 were examined retrospectively.The ethics committee approval for the study was obtained from Erzincan University Ethics Committee(2019-12) per the Declaration of Helsinki.

2.1 Patient Selection

A total of 317 AMA patients between the ages of 38 and 43 years were included. One hundred thirty-eight patients underwent fresh blastocyst single euploid ET (group 1), and 179 patients underwent single frozen-thawed euploid ET (group 2). All patients underwent single fresh or frozen-thawed ET on day five or day 6 with trophectoderm biopsy. Embryo implantation, abortion, and live birth rates in fresh and frozen-thawed ET with PGS were compared between the groups.

The exclusion criteria were as follows; patients with progesterone levels above 1.5 ng/mL on the trigger, fresh and frozen-thawed ET performed only with good-quality embryos, i.e., 6–10 cells with 20% fragmentation, and equal blastomere size, patients who had a preimplantation genetic diagnosis for a single-gene or chromosomal disorder, egg donor cycles, gender selection cycles, any medical or surgical intervention to fallopian tubes or uterus, and severe male factor including the need to use surgical sperm retrievement.

2.2 Study Protocol

Gonadotropin-releasing hormone (GnRH) antagonist protocols were followed, and exogenous gonadotropins including recombinant follicle-stimulating hormone (FSH) (Gonal-f; Merck Serono) and human menopausal gonadotropin (hMG) (Menogon; Merck Serono) were used for COH. The procedure

was initiated on the second or third day of the cycle. The drug doses ranged between 150 and 450 international units per day. A GnRH antagonist (cetrorelix; Cetrotide, Merck Serono) was used to suppress the pituitary gland when a leading follicle reached 14 mm in size. Intramuscular 6000 IU hCG (Ovitrelle 0,5ml; Merck Serono) was given to ensure final oocyte maturation after the follicle diameter reached 17 mm. On the trigger day, patients were tested for serum progesterone levels.Thirty-six hours later, following the trigger, ovarian follicular fluid was aspirated via transvaginal ultrasound-guided needle to accomplish oocyte retrieval. Luteal phase support started on the day of oocyte retrieval with vaginal micronized P (Crinone 8%, Merck Serono) in fresh blastocyst single euploid ET group(group 1).

2.3 Embryo culture protocol and DNA analysis

Sperm were prepared for intracellular sperm injection or insemination by the previously described technique. (10). The four-well dish, including a proximate of 400 µl single-step media (LifeGlobal® Media, USA), was used for the culturization of the two pronuclei (PN) exhibiting normally fertilized zygotes up to days. Monitorization of the embryonic development was performed with EmbryoScope (Vitrolife AB; Sweden). The blastocyst development stages were examined according to the Gardner classification [11].

TE biopsy and PGS were performed on 497 embryos in the fresh cycle group and 629 embryos in the frozen cycle group. On day three, zona pellucida perforation was performed on all the viable embryos for blastocyst biopsy.The necessary small opening on zona pellucida(ZP) was provided with Cronus 3 Laser (400 mW 0.357 mS pulse width: Cronus Research Instrument, UK). Following the procedure, the embryos were followed up until days 5 and 6. Then expanded blastocyst, which was differentiated to TE cells and inner cell mass (ICM) (better than Grade 2BB), was biopsied. TE cells herniated from the zona perforation were captured by aspiration via biopsy pipette and were withdrawn from the rest of the blastocyst gently. The tight junctions between the TE cells were separated by two direct laser pulses (400 mW 0.4mS pulse). Mechanical rubbing on the holding pipette was performed to promote separation. The biopsied cells were applied immediate rinsing in washing buffer. Following the rinsing, a PCR tube with 2ul of washing buffer was prepared, and the cells were loaded to the tube. Each tubes containing the TE biopsy was labeled with a number indicating the corresponding embryo and transferred to the reference genetic lab on dry ice for analysis. The biopsied blastocysts were vitrified with a vitrification Kit and Cryotops immediately after the biopsy (Kitazato, Japan).

The analysis of the biopsy specimens derived from blastocysts was performed with 24 chromosomes array comparative genomic hybridization (aCGH) by Reprogenetics, LLC(Livingston, NJ, 07039),as previously described[12].

Single euploid embryotransfer was planned as fresh or frozen-thawed after obtaining the aCGH results. On the fifth day at 10 AM, the biopsy of expanded blastocysts was performed for the fresh strategy. While waiting for the results, the embryos were cultured overnight until the noon of the sixth day for a fresh ET of euploid embryos.

Frozen Thawed ET was scheduled after the patient's followingmenstruation, and endometrial preparation was performed with 6 mg/daily estradiol valerate, taken orally between the 2nd and 11th day of the menstrual cycle. Transvaginal ultrasoundwas used on the 11th day of the cycle to measure the thickness of the endometrial tissue. In the cases, where the endometrium was thicker than 7 mm, 6 mg estradiol valerate medication was continued, and on the 14th day of the cycle, vaginal progesterone (Crinone Jel 8%, Merck Serono) was added to the treatment. Frozen-Thawed ET was performed on the fifth or sixth days of the progesterone adjustment. Both estrogen and progesterone treatments were continued up to 10 weeks of pregnancy.

Ten days after embryo transferhCG concentration was tested and a value of > 10 IU/l was considered positive. The implantation rate was recorded by dividing the number of gestational sacs seen at ultrasonography to the number of transferred embryos. A clinical pregnancy is identified if an intrauterine sac and positive fetal heart activity are present. a Pregnancy proceeding beyond the 10th week of gestation was considered ongoing pregnancy.

2.4 Statistical Analysis

Statistical Package for the Social Sciences v.15.0 (SPSS Inc., Chicago, IL, USA) was used for the statistical evaluation of the collected data. Kruskal–Wallis, and Mann–Whitney U-tests were used for analyzing the non-normally distributed metric variables. T-test was used for analyzing normally distributed variables. Numbers and percentages were defined ascategorical variables, and x^2 test or its derivatives were used to compare these variables between the groups. A p-value of < 0.05 was considered statistically significant. Unless stated otherwise, the values were expressed as mean ± standard deviation.

3. Results

A total of 317 patients aged between 38–43 years old who underwent single fresh or frozen-thawed day 5 or 6 ET with trophectoderm biopsy were examined. The baseline characteristic of the study groups is given in Table 1. No statistically significant difference was present regarding age, Anti-Müllerian hormone levels, infertility duration, and previous ART cycles between the fresh and frozen-thawed ET groups. The retrieved oocytes, metaphase II oocytes, fertilized oocytes (2PN) were higher in the frozen-thawed embryo group. Euploid embryo percentage was similar between the groups and 160 out of 497 embryos (32%) in the fresh ET group, while 219 out of 629 embryos (34.8%) were euploid in the frozen ET group. (P = .32).

Table 1
Baseline parameters of the groups

	Fresh SET with PGS (n = 138)	Frozen Thawed SET with PGS (n = 179)	P value
Age (years)	39.5 ± 1.7	39.6 ± 1.8	0.24
AMH (ng/ml)	2.2 ± 2.1	2.5 ± 2.3	0.29
Infertility Duration (years)	5.9 ± 4.8	6.9 ± 5.6	0.07
Previous ART cycles	4.5 ± 3.0	4.9 ± 2.7	0.2
Mean Retrieved oocytes	9.6 ± 6.2	12.4 ± 9.4	0.002
Mean MII oocytes	8.1 ± 4.9	10.5 ± 8.2	0.001
Mean 2PNr	6.5 ± 4.0	8.6 ± 6.5	0.001
Percentage of euploid embryo (%)	160/497 (32.0)	219/629 (34.8)	0.32
D5 ET (%)	101 (70.8)	161 (89.4)	0.0001
D6 ET (%)	37 (29.2)	18 (10.6)	0.0001
AMH: Anti-Müllerian hormone SET: Single embryo transfer PGS: preimplantation genetic screening			

Patients' clinical outcomes are presented in Table 2. The incidence of positive serum β HCG per ET of frozen-thawed SET with PGS was higher than fresh SET with PGS (50 vs. 67% p = 0.003), while theimplantation rates were higher in the frozen-thawed transfer group compared with the fresh group (46.4 vs. 62.5% p = 0.006). There was no significant difference between the groups in biochemical (6.9 vs. 6.6% p = 0.77) and clinical abortion rates (16.0 vs. 14.6% p = 0.97). The rates of clinical pregnancy (42.0 vs. 58.6% p = 0.006), ongoing pregnancy (38.4 vs. 54.1% p = 0.006), and live birth (37.6 vs. 54.1% p = 0.003) were higher in the frozen-thawed group.

Table 2
Clinical outcomes of the patients

	Fresh SET with PGS	Frozen Thawed SET with PGS	P
% positive serum β HCG per ET	69/138 (50%)	120/179 (67%)	0.003
Implantation rates	64/138 (46.4%)	112/179 (62.5%)	0.006
Biochemical Abortion	5/72(6.9%)	8/120 (6.6%)	0.77
Clinical Abortion	11/64 (16.0 17.1%)	15/112 (14.6 13,3%)	0.97
Clinical Pregnancy	58/138 (42.0%)	105/179 (58.6%)	0.006
Ongoing Pregnancy	53/138 (38.4%)	97/179 (54.1%)	0.006
Live Birth	52/138 (37.6%)	97/179 (54.1%)	0.003
ET: Embriyo transfer SET: Single embryo transfer PGS: preimplantation genetic screening			

4. Discussion

PGS is the most pivotal strategy promotingthe clinical success rates of ART cycles in AMA patients. Additionally, another option to increase pregnancy rates is ET in the presence of a euploid embryo. In this study,clinical pregnancy, ongoing pregnancy, and live birthrates were statistically higher in the frozen ET group in AMA patients.

Currently, the frozen-thawed ET strategy has been trending due to improved implantation and pregnancy rates. Optimal endometrial preparation has a critical role in accomplishing successful results in frozen ET, and the impaired results after fresh ET strategy point out the importance of the endometrial receptivity and implantation window [13]. [In review study of Evans J, it was stated that the endometrial receptivity was reduced during COH cycles, and FET had higher beneficial outcomes regarding the mother and baby(8). Roque M analyzed 530 cycles, compared the fresh transfer and frozen transfer outcomes, and reported a 1,33 RR of implantation rate and 1,28 RR ofongoing pregnancy rate for the frozen group. Additionally, this study suggests that the implantation failure could be resulting from COH(14). Both the CPR (RR 1.31; 95% CI 1.10– 1.56) and ongoing pregnancy rates (RR 1.32;95% CI 1.10 – 1.59) were higher in the eFET group than fresh embryo transfer group according to a systematic review subjecting three randomized controlled trials and a total of 633 women. In this review, the miscarriage rates (RR 0.83; 95% CI 0.43 – 1.60) did not reveal a significant difference(16). Freezing all embryos ensures that all blastocysts are included in the embryo cohort transferability, which results in a higher proportion of successful ET [9]. Recently, Coates et al. study revealed that frozen-thawed euploid embryo transfer after PGS had higher pregnancy outcomes. (9) Our study also demonstrated that AMA patients had higher implantation and pregnancy rates via frozen single euploid embryo transfer.

It is known that ART success declines relatively in women at the age of forty and older. It has been shown that the most important reason for the decrease in fertility in this age group is related to the increased risk of age-related aneuploidy. [1] Lee HL et al. analyzed AMA and ART results, in which the mean participant age was 41.2 and 41.3 for study groups, retrospectively. Biopsies were taken from 451 blastocysts in the PGS group (n:170), and as a result of the ploidy analysis, 20.4% of the blastocysts were found to be euploid, while aneuploidy was observed in 74.3% of the blastocysts, 3% of the remaining blastocysts could not be diagnosed and 2.2% had chaotic profiles after amplification. Implantation rates and live delivery per transferred embryo incidence were significantly higher for the PGS group (10). Ma GC et al. analyzed rapid array comparative genomic hybridization (aCGH) in the selection of blastocysts for fresh SET and compared the results with the methodsusing vitrified embryo transfer cycle as a pilot study. Despite the small sample size (8 for fresh and 13 for the frozen group) and non-randomized character of the study, the revealed clinical pregnancy rate (87.5% in the fresh transfer group and 76.9% in the frozen embryo transfer group) was successful (15). Capalbo A demonstrated that aneuploidy rates were significantly higher at advancing age females (odds ratio (OR) ¼ 1.12) and pregnancy rates were 57.3% and 75.6% in euploid ET when mean age was 38.8 and 36.1 respectively (6). For euploid embryos, implantation potential was higher in AMA patients [10]. In fresh or frozen-thawed cycles, only one selective euploid ET increased the pregnancy rates [6, 15]. Moreover, single euploid ET reduced the chance of multiple pregnancies, which was associated with increased abortion rates and premature deliveries with newborn complications. In our study, a single ET strategy was chosen in both groups, and the euploid embryo rates of the groups were similar. The main difference between the groups was the transfer strategy, and endometrial receptivity was vital for implantation. Our study revealed high implantation rates in the frozen ET group.

In literature, too many studies compared the outcomes of fresh and frozen ET [16], but the number and sample size of studies comparing two transfer strategies with euploid embryos are limited. Although this study is retrospective, it has the largest number of participants comparing fresh versus frozen ET results for euploid embryos. Only one prospective randomized controlled study investigated fresh vs. frozen ET for euploid embryos. Coates A et al. analyzed the fresh vs. frozen transfers after PGS with next-generation sequencing(NGS) in their prospective randomized controlled study in a total of 179 patients. The mean transferred embryo numbers were 1,4 for the fresh ET group and 1.5 for the frozen ET group ($p = 0,27$). Ongoing PRs (62.2% vs. 40.9%) and live birth rates (61.5% vs. 39.8) were significantly higher in the freeze-all group than the fresh group ($p < 0,01$ and $p < 0,01$ respectively). The results were significantly higher in the frozen ET group than the fresh group according to the subgroup analysis of day-5 biopsied euploid blastocyst; however, the difference was not significant. The authors explained the result with the small sample size.We included 317 participants and found that ongoing pregnancy rates and live birth rates were significantly higher in the frozen group. Although our study is the first to choose the single ET strategy that compares fresh or frozen, the results were comparable with Coates A et al.'s study. The most significant effects on the success rates of ART are the embryo and endometrium. It is also known that a higher estrogenic environment may cause reduced endometrial receptivity and decreased pregnancy outcomes [16]. Our study considers all of the factors and may solely examine the effect of the

endometrial factor on pregnancy outcomes. Frozen-thawed single euploid ET strategy results in higher implantation rates and pregnancy rates compared to fresh single euploid ET in our study. Additionally, this transfer strategy reduces the risk of ovarian hyperstimulation syndrome and multiple pregnancies, which is a critical condition and has high morbidity and mortality rates in ART.

5. Conclusion

In conclusion, this present study indicates that frozen-thawed single euploid ET provides sufficient time for comprehensive genetic evaluations, increased implantation, and pregnancy rates compared with the fresh single euploid ET in AMA patients with PGS. This study is the most extensive study examining the two treatment protocols and the first study that used a single euploid to decrease the embryo factor on the success of ART. Our findings suggest a trend toward frozen-thawed single euploid ET strategy, and further prospective randomized controlled studies with large groups may be needed to clarify the outcomes of ART. Finally, frozen-thawed single euploid ET provides sufficient time for comprehensive genetic evaluations in addition to increased implantation and pregnancy rates than the fresh single euploid ET in AMA patients with PGS.

Declarations

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Competing interests

The authors have no relevant financial or non-financial interests to disclose.

Author contribitions

F Ozdemir: Manuscript writing/editing, Data analysis

G Oner: Data collection or management, Data analysis

S Kahraman: Protocol/project development

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H Yelke: Data collection or management

Ethics approval

This study was performed in line with the principles of the Declaration of Helsinki. Approval was granted by the Ethics Committee of University Erzincan University Ethics Committee(2019-12).

Consent to participate

Informed consent was obtained from all individual participants included in the study.

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