ORIGINAL ARTICLE: ASSISTED REPRODUCTION

Preimplantation genetic testing for aneuploidy versus morphology as selection criteria for single frozen-thawed embryo transfer in good-prognosis patients: a multicenter randomized clinical trial

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Objective: To evaluate the benefit of next-generation sequencing (NGS)–based preimplantation genetic testing for aneuploidy (PGT-A) for embryo selection in frozen-thawed embryo transfer.
Design: Randomized controlled trial.
Setting: Not applicable.
Patient(s): Women aged 25–40 years undergoing IVF with at least two blastocysts that could be biopsied.
Intervention(s): Randomization for single frozen-thawed embryo transfer with embryo selection based on PGT-A euploid status versus morphology.
Main Outcome Measure(s): Ongoing pregnancy rate (OPR) at 20 weeks' gestation per embryo transfer.
Result(s): A total of 661 women (average age 33.7 ± 3.6 years) were randomized to PGT-A (n = 330) or morphology alone (n = 331). The OPR was equivalent between the two arms, with no significant difference per embryo transfer (50% [137/274] vs. 46% [143/313]) or per intention to treat (ITT) at randomization (41.8% [138/330] vs. 43.5% [144/331]). Post hoc analysis of women aged 35–40 years showed a significant increase in OPR per embryo transfer (51% [62/122] vs. 37% [54/145]) but not per ITT.
Conclusion(s): PGT-A did not improve overall pregnancy outcomes in all women, as analyzed per embryo transfer or per ITT. There was a significant increase in OPR per embryo transfer with the use of PGT-A in the subgroup of women aged 35–40 years who had two or more embryos that could be biopsied, but this was not significant when analyzed by ITT.

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The high incidence of chromosome aneuploidy in human gametes and embryos is a major cause of in vitro fertilization (IVF) failure and miscarriage (1). Most aneuploidies arise in maternal meiosis, and they increase exponentially in women over the age of 35 years, coinciding with rapidly declining IVF success and live birth rates in patients of advanced maternal age. For example, the Society for Assisted Reproductive Technology (SART) compilation of U.S. IVF cycle data for 2016 shows that final cumulative live birth rate per egg-retrieval cycle decreased from 54.5% in young patients to 13.4% in women aged 41–42 years (2). This is mirrored by the increased incidence of aneuploidy from 30% to 50% in patients under 35 years of age to 80% in women 42 years of age or older (3, 4). In contrast, the transfer of a euploid embryo results in similar implantation rates regardless of maternal age (5). Indeed, the 2016 SART data showed no age-related decrease in implantation rates after frozen-thawed euploid embryo transfer following preimplantation genetic testing for chromosome aneuploidy (PGT-A) (2).

Morphologic assessment has always been the primary method of prioritizing IVF embryos for transfer, but the chromosome status of cultured embryos cannot be accurately ascertained through either static or dynamic morphologic evaluation (6, 7). PGT-A, formerly known as preimplantation genetic screening (PGS), was proposed as a method to select IVF embryos with the highest potential of ongoing implantation (8). Initial studies with the use of cleavage-stage biopsy on day 3 after insemination and fluorescence in situ hybridization (FISH) showed an improvement in pregnancy outcomes, but this was not confirmed in randomized control trials (RCTs) (9). With improvements in blastocyst culture, embryo vitrification, and molecular techniques that can test copy number of all chromosomes, several, mainly single-center, RCTs performed in the past decade have all shown significant improvement in ongoing pregnancy rates (OPRs) per embryo transfer procedure (10–13). Furthermore, the recent introduction of next-generation sequencing (NGS)–based methods have increased the sensitivity and resolution of copy number variation genome wide (14–16).

Here, we present the results of a multinational multicenter RCT (Single Embryo Transfer of Euploid Embryo (STAR) study; ClinicalTrials.gov registration number NCT02268786), in women aged 25–40 years undergoing IVF with at least two blastocysts for transfer. OPRs and live birth rates after single vitrified-warmed blastocyst transfer were compared with selection of euploid embryos with the use of NGS-based PGT-A or selection by morphology alone. This RCT replicated real-life conditions in which each clinic could follow their own clinical and laboratory protocols and genetic testing laboratories determined their own criteria for identifying aneuploid embryos.

MATERIALS AND METHODS

Study Design

We conducted a randomized controlled trial (RCT) with patients recruited from 34 clinics and testing in nine laboratoriess across the United States, Canada, the United Kingdom, and Australia. Each clinic followed their own standard of care regarding ovarian stimulation, endometrial preparation, luteal-phase support, and IVF laboratory procedures. Genetics laboratories were required to meet predetermined sequencing quality metrics and have established processes for running Veriseq PGS (Illumina); each laboratory followed its own internally validated testing and reporting. Site-specific appropriate Institutional Review Board or Ethics Committee approvals were obtained. Written informed consents were obtained from patients before any study-specific procedures were performed.

Inclusion criteria were female age 25–40 years undergoing IVF with autologous oocytes with at least two blastocysts of sufficient quality for biopsy and vitrification by day 6. Exclusion criteria are detailed in Supplemental Appendix 1 (available online at www.fertstert.org), but the primary exclusion criteria included diminished ovarian reserve, more than two previous failed IVF-ETs, more than one miscarriage, azoospermia, or severe oligospermia. All patients meeting specified inclusion criteria were able to participate in the study, with no requirements for a minimum E_2 level, follicle number, or expected oocyte recovery number.

Enrollment was completed before oocyte retrieval to allow patients adequate time for informed consent. Patients received no financial incentives for patient participation other than free access to embryo biopsy, vitrification, and PGT-A for surplus embryos. Eligibility for randomization occurred 110–150 hours after fertilization, when the IVF laboratory confirmed the development of at least two blastocysts of sufficient quality for biopsy and vitrification. As per the clinical study protocol, the intention-to-treat (ITT) population was defined as patients undergoing randomization. The patient, physicians, and IVF clinical staff (not the embryologists) were all blinded to the patient's randomization status. An electronic data capture system with a randomization module was used to randomize subjects 1:1 into the control and PGT-A arms. Randomization was stratified by three female age groups: <35 years, 35–37 years, and 38–40 years. This ensured a well-matched population across the age spectrum by assigning equivalent randomization to each arm within each age group; there was no requirement for equivalent total

numbers among the three age groups. There was no minimum number of enrolled patients per clinic, and clinics were not capped at a maximum enrollment number. Individual patients were randomized to PGT-A or control group as they entered the study, but they were not randomized by clinic of origin.

Morphologic assessment was performed in all patients before vitrification. In the control group, the embryo with the most favorable morphologic assessment (17) was vitrified; the remaining nonselected good-quality blastocysts underwent biopsy and vitrification. In the PGT-A group, all good-quality blastocysts underwent biopsy then vitrification. A single frozen-thawed embryo transfer was performed in a subsequent cycle. The embryo transferred in the control arm was the intact embryo previously identified as having the most favorable morphologic assessment. The embryo transferred in the PGT-A arm was selected based on a euploid result according to PGT-A as well as the most favorable morphologic assessment. The IVF laboratory staff thawed the embryo for transfer based on study protocol. The providers performing the transfer were blinded to the randomization status and PGT-A results at least until after the embryo transfer; the study subjects were blinded to the randomization status until pregnancy outcome. The genetic laboratory investigators and personnel performing PGS were blinded to the aggregate pregnancy outcomes until study completion.

Preimplantation Genetic Testing for Aneuploidy

Preimplantation genetic testing for aneuploidy of all samples was performed with the use of an NGS-based assay (Veriseq PGS) following standard protocols and manufacturer recommendations (see Supplemental Appendix 1 for details). Results for all samples were reported as "no chromosome abnormality detected," indicating a euploid embryo eligible for transfer, or "abnormal," indicating that chromosomal copy number changes consistent with aneuploidy were detected. Mosaic chromosome copy number changes, segmental copy number variants, or polyploidy was identified by the laboratory director and considered to be "abnormal" for the purposes of the study and the embryo was not transferred. A report of "no result" indicated that the sample did not yield informative results.

Outcomes

Pregnancy outcomes per ITT at randomization and per transfer are reported. Study participants consented to the collection of clinical follow-up information. This included the results of a biochemical pregnancy test, ultrasound confirmation of a gestational sac at 4–5 weeks after transfer, pregnancy viability at 10 and 20 weeks of gestation, and birth outcome. The primary study outcome was the ongoing pregnancy rate at 20 weeks' gestation. Biochemical pregnancy was defined as a positive serum β-hCG level (>5 MIU/mL). Clinical pregnancy was defined as the presence of a gestational sac according to ultrasound. Pregnancy viability was confirmed by presence of a fetal heartbeat at or beyond 10 and 20 weeks' gestation according to ultrasound.

Statistical Analysis

Sample-size calculations incorporated assumptions made from the published literature, clinic-estimated 20-week OPRs per transfer without PGT-A, and predicted rates of improvement with PGT-A. It was determined that 600 embryo transfers, 300 patients in each arm, would provide 85% power at an alpha value of 0.045 (adjusted for a single interim analysis).

The efficacy of PGT-A for euploid embryo selection was determined by comparing the 20-week OPRs between the control and PGT-A arms by means of the Cochran-Mantel-Haenszel test, stratified for maternal age at randomization. The confidence interval for the difference in 20-week OPRs between the two arms was estimated with the use of the Miettinen-Nurminen method, stratified for maternal age at randomization.

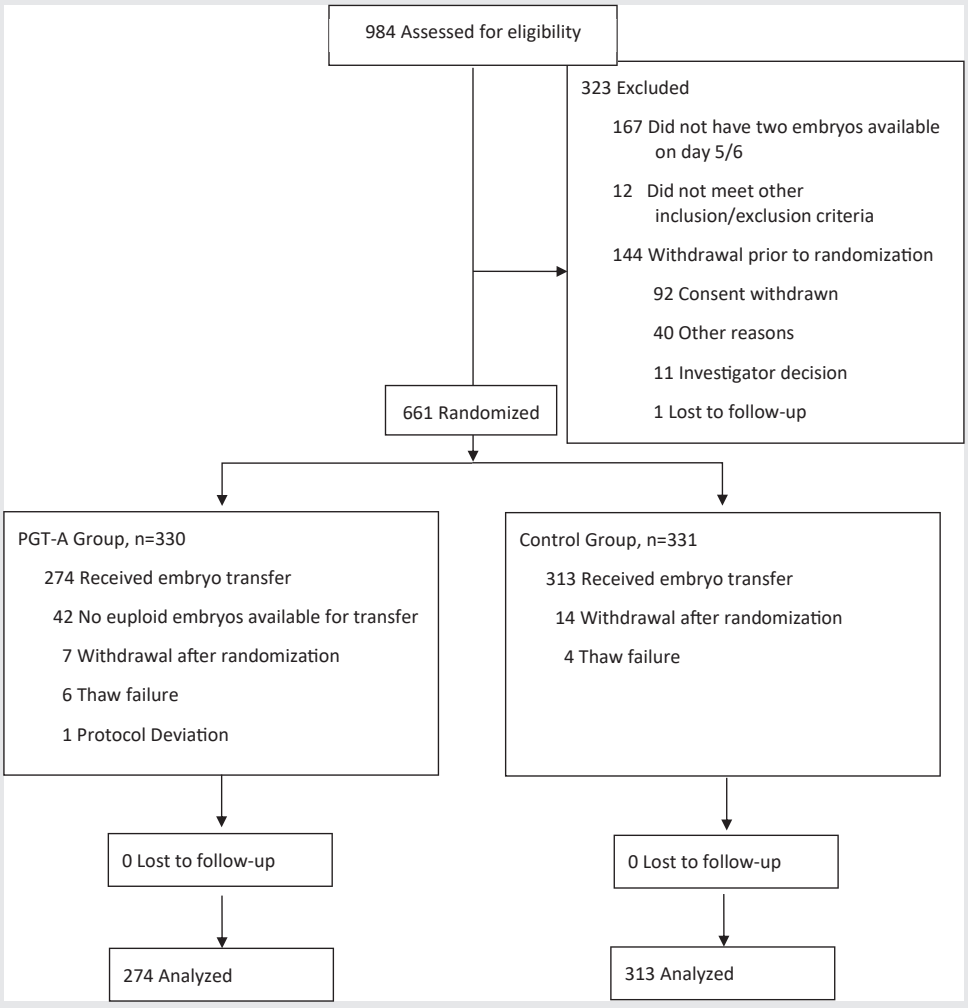
A post hoc analysis using the Fisher exact test was performed to evaluate clinical outcomes in two age populations: female ages 25–34 years and 35–40 years. Two age groups, 35–37 and 38–40 years, used during randomization were pooled for the post hoc analysis because of the small numbers in these groups.

RESULTS

From October 2014 to January 2016, a total of 984 patients were consented for the study and assessed for eligibility (Fig. 1). A randomization cutoff of June 2016 and embryo transfer cutoff of August 2016 were put in place for study inclusion. Clinical outcome follow-up ended on December 31, 2016. Of the consented patients, 661 were determined to be eligible and were randomized to a study group, with 331 allocated to the control arm and 330 to the PGT-A arm. The primary reasons for exclusion from the ITT population were a failure to achieve at least two blastocysts by day 5 or 6 of embryo culture (n = 167) and withdrawal before randomization (n = 144). As a proportion of the consented patients for each age group, the frequency of patients without two blastocysts by day 5 or 6 of culture increased with maternal age: 13.7% (70/510) in women 25–34 years of age versus 20.5% (97/474) in women aged 35–40 years (Supplemental Table 1, available online at www.fertstert.org). Common reasons cited for withdrawal of patient consent were discomfort with some aspect of the study design (e.g., requirement for a frozen-thawed embryo transfer cycle, requirement for a single-embryo transfer) and unwillingness to accept treatment blinding.

Within the control arm, 313 (94.6%) received the allocated treatment. Within the PGT-A arm, 274 (83.0%) received the allocated treatment. The reasons for randomized patients not receiving their allocated treatment are detailed in Figure 1. Of note, 42 patients in the PGT-A arm (12.7%) did not receive an embryo transfer because no euploid embryos were available for transfer. For these 42 patients, 25 (59.5%) had at least one embryo classified as a chromosomally abnormal because one to five chromosomes were classified as a mosaic aneuploidy: 15 patients had one mosaic embryo, seven patients had two mosaic embryos, 1-patient had three mosaic embryos, and two patients had four mosaic embryos.

FIGURE 1



Enrollment, assignment, treatment, and analysis of patients. PGT-A = preimplantation genetic testing for aneuploidy.

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When evaluated as a proportion of the ITT population, the frequency of no euploid embryos increased with maternal age: 8.9% (16/179) in women 25–34 years of age versus 17.2% (26/151) in women aged 35–40 years.

The demographics for the ITT population are presented in Table 1. Characteristics were similar for the two arms. Embryo characteristics are presented in Table 2. An average of 7.4 day-5/6 blastocysts were obtained per patient in both arms. Of the 2,178 blastocysts analyzed by means of PGT-A in the PGT-A arm, 939 (43.1%) were reported as euploid and 1,181 (54.2%) as aneuploid. When analyzed within maternal age ranges, the percentage of euploid embryos decreased with increasing maternal age, from 48.0% for women <35 years of age to 35.5% in women aged 35–40 years (Supplemental Table 2, available online at www.fertstert.org). The distribution of chromosome abnormalities observed is shown in Supplemental Figure 1 (available online at www.fertstert.org). Of note, of the 1,181 chromosomally abnormal embryos, 366 (31.0%; 16.8% of all embryos) were reported to have a whole or partial chromosome mosaic aneuploidy for one or more chromosomes (Table 2). A detailed breakdown of the chromosome abnormalities observed in embryos classified as mosaic is shown in Supplemental Figure 2 (available online at www.fertstert.org); the majority (82%) had one to three chromosomes classified as mosaic aneuploid, with no other chromosome abnormalities.

Clinical outcomes for transferred embryos are presented in Table 3. All randomized patients with an embryo transfer had clinical outcome information. All patients with an ongoing pregnancy at 20 weeks' gestation continued to a live birth. Thus, the OPR at 20 weeks also reflects the live birth rate in this study cohort. The overall OPRs per transfer at 20 weeks' gestation was not significantly different between the PGT-A and control arms for either the embryo transfer population (50.0% [137/274] vs. 45.7% [143/313]; $P=.32$) or the ITT population (41.8% [138/330] vs. 43.5% [144/331]; $P=.65$). Similarly, the rates of negative β-hCG ($P=.09$), biochemical pregnancy ($P=.33$), miscarriage

TABLE 1

Characteristics of the intention-to-treat study population.

Characteristic	PGT-A (n = 330)	Control (n = 331)
Age, y		
Mean ± SD	33.7 ± 3.59	33.8 ± 3.58
Median	34.0	34.0
Range	25–40	25–40
<35 y	179 (54.2%)	177 (53.5%)
35–37 y	95 (28.8%)	96 (28.9%)
38–40 y	56 (17.0%)	58 (17.5%)
BMI,[a] kg/m^2	25.24 ± 5.285	25.10 ± 5.204
Nulliparous, n (%)	219 (66.4%)	211 (63.7%)
Clinical infertility diagnosis[b]		
Low ovarian reserve	8 (2.4%)	5 (1.5%)
Ovulatory dysfunction	77 (23.3%)	80 (24.2%)
Tubal factor	29 (8.8%)	29 (8.8%)
Endometriosis	17 (5.2%)	17 (5.1%)
Uterine abnormality	7 (2.1%)	11 (3.3%)
Other female factor	68 (20.6%)	72 (21.8%)
Combination factor	19 (5.8%)	21 (6.3%)
Male factor	117 (35.5%)	121 (36.6%)

[a] Body mass index was calculated from patient height and weight information collected at the time of enrollment.
[b] Some patients had more than one infertility diagnosis.

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(*P*=.90), and elective termination (*P*=.66) per embryo transfer were not significantly different between study arms (Table 3). There were two elective terminations in the control arm and one in the PGT-A arm. All were attributed to medical reasons: One control-arm case had multiple serious abnormalities noted at the 18-week ultrasound; one control-arm case was terminated at 14 weeks after observation of fetal anomalies during a first-trimester ultrasound; and one PGT-A–arm twin pregnancy was terminated at 20 weeks because multiple fetal anomalies were apparent on ultrasound. Karyotype information was not available for these three cases. Two control-arm cases with spontaneous pregnancy losses had products of conception karyotype results: one 46,XY and one triploid, sex chromosomes unconfirmed.

A post hoc subgroup analysis revealed a higher OPR per transfer in women aged 35–40 years after PGT-A (50.8% vs. 37.2%; *P*=.035), but not in women aged 25–34 years (49.3% vs. 53.0%; *P*=.58). The OPRs were not significantly different between arms for women aged 25–34 years (42.5% vs. 50.3%; *P*=.17) or 35–40 years (41.1% vs. 35.7%; *P*=.35) when analyzed per ITT. Miscarriage rates per transfer were ~10% regardless of maternal age in both the PGT-A arm (11.2% at <35 years, 8.2% at ≥35 years) and the control arm (8.3% at <35 years, 11.0% at ≥35 years).

For this multicenter and multilaboratory RCT, each clinical site and genetic testing laboratory followed its own internal protocols and procedures for IVF and PGT-A assessment and reporting. To evaluate whether individual practices may have contributed to the lack of significance in the primary outcome, we evaluated variability relating to morphology classification as well as variability at the level of the clinical sites and genetic testing laboratories. Supplemental Figure 3 (available online at www.fertstert.org) shows a comparison of the Gardner scores for the transferred embryos in each arm. Embryos with a broad range of Gardner scores were transferred, but the overall pattern was similar between the two arms. When the Gardner scores were mapped to a good, fair, or poor quality score (see Supplemental Table 3 [available online at www.fertstert.org] for mapping of Gardner scores to quality scores), it appeared that more poor-quality embryos and fewer good quality embryos were transferred in the PGT-A arm than in the control arm (Supplemental Fig. 3).

Sample size at the clinical site level was variable: 26.5% (9/34) of clinics had fewer than 10 randomized patients, 58.8% (20/34) of clinics had 10–30 randomized patients, and 14.7% (5/34) of clinics had more than 30 randomized patients. Supplemental Figure 4 (available online at www.fertstert.org) shows some key metrics at the clinical site level. Of note, the percentage of euploid embryos in the PGT-A arm was variable between sites, but this did not directly correlate with the 20-week OPR, which was also highly variable.

The number of samples in the PGT-A arm analyzed at each genetics laboratory varied: three labs analyzed fewer than 100 samples each, five labs analyzed 100–500 samples each, and one laboratory analyzed more than 500 samples (Supplemental Table 4, available online at www.fertstert.org). The euploid and mosaic rates for each genetics testing laboratory, with subgroup classification by the way that mosaic samples were handled, are presented in Supplemental Table 4. Within the five genetic testing laboratories reporting mosaicism, the rate of mosaic embryo calls varied from 10.5% to 26.4%. There appeared to be a trend toward a higher percentage of euploid embryos and higher OPRs in the genetic testing laboratories that did not report mosaics.

DISCUSSION

The use of trophectoderm biopsy at the blastocyst stage and NGS-based PGT-A to select euploid embryos for single vitrified-warmed blastocyst transfer did not significantly improve OPRs and live birth rates per transfer in women aged 25–40 years undergoing IVF, compared with embryo selection by morphology alone, analyzed either per embryo transfer (50% vs. 46%; *P*=.32) or per ITT (42% vs. 44%; *P*=.65). The failure to replicate numerous previous RCTs, in women of a broad range of ages, was unexpected. However, this was a large-scale, multinational, multicenter RCT which allowed reputable clinics to follow their own clinical and embryology protocols and qualified genetic testing laboratories to use their own internally validated criteria for identifying aneuploid embryos. Thus, among the top enrolling clinics, outcomes were highly variable. For example, the percentage of euploid embryos after PGT-A ranged from 38% to 100% in younger patients and from 17% to 75% in older patients, and the combined OPRs per transfer ranged from 30% to 60%. The generally small number of patients enrolled per clinic prevented any meaningful comparisons between clinics.

To qualify for the trial, genetic testing laboratories had to pass strict criteria in preliminary tests to ensure that the NGS-based assay was being performed optimally. However, in practice, there were variations in protocol, and laboratories set their own criteria for identifying aneuploid samples.

TABLE 2

In vitro fertilization laboratory and genetic testing results for the intention-to-treat population.

Characteristic	PGT-A (n = 330)	Control (n = 331)
Oocytes retrieved, mean ± SD per retrieval	18.6 ± 9.2	18.9 ± 10.0
Mature oocytes, mean ± SD per retrieval	14.6 ± 7.6	15.0 ± 8.1
Fertilization method, n (%)		
Conventional insemination	37 (11.2%)	42 (12.7%)
Intracytoplasmic sperm injection	293 (88.8%)	288 (87.0%)
Fertilized oocytes, mean ± SD per retrieval	11.8 ± 6.5	12.1 ± 6.9
Day 5/6 blastocysts, mean ± SD per retrieval	7.4 ± 4.5	7.4 ± 5.4
Biopsied embryos, mean ± SD per retrieval	2,178 (6.6 ± 4.1)[a]	1,758 (5.5 ± 4.1)
Embryo classification by preimplantation genetic screening, n (% of embryos biopsied)		
Undetermined[b]	61 (2.8)	59 (3.4)
Euploid	939 (43.1)	719 (40.9)
Aneuploid	1,181 (54.2)	987 (56.1)
Monosomy	196 (9.0)	181 (10.3)
Trisomy	163 (7.5)	137 (7.8)
Mosaic[c]	366 (16.8)	285 (16.2)
Subsegmental	105 (4.8)	86 (4.9)
Other[d]	10 (0.5)	11 (0.6)
Complex[e]	341 (15.7)	287 (16.3)

[a] An additional five embryos had failed biopsies: one embryo was discarded and four were cryopreserved.
[b] Potential reasons for an undetermined preimplantation genetic testing for aneuploidy (PGT-A) result included failed biopsy, no DNA amplification, a chaotic profile suggesting degraded DNA, and no diagnosis.
[c] Mosaics reflect data from the five laboratories that reported mosaicism. The remaining four laboratories designated samples as "no aneuploidy detected" or "abnormal" depending on whether the perceived level of mosaicism was below or above, respectively, set chromosome-specific thresholds.
[d] Triploidy or tetrasomy aneuploidy call.
[e] Combination of >1 trisomy, monosomy, and/or subsegmental aneuploidy call.

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Therefore, there was wide variation between laboratories (and associated clinics) in the proportion of embryos identified as euploid. Most importantly, the significance of intermediate copy number changes detected by means of NGS-based methods, indicating possible chromosome mosaicism among the biopsied trophectoderm cells, was unknown at the outset of the trial. Therefore, mosaic embryos, which constituted 16.8% of embryos tested, were excluded from transfer, and if all other embryos were aneuploid in that cycle the patient was removed from the trial. It is now clear that a significant proportion of embryos with only mosaic aneuploidies, particularly of few chromosomes, are viable and result in live births after transfer (14, 15, 18). However, the criteria for identifying mosaic copy number changes by means of NGS-based and other methods has been evolving as more is known about clinical outcomes, and during the trial they varied among the testing laboratories. This resulted in the exclusion of 25 patients with no euploid embryos and one or more mosaic embryos, which in retrospect could potentially have resulted in live births if they had been transferred.

Post hoc analysis of pregnancy outcomes in women aged 35–40 years demonstrated a significant improvement in OPR and live birth rate (14%) with the use of PGT-A per embryo transfer (51% [62/122[vs. 37% [54/145]; P=.035). This is similar to the trend observed with PGT-A cycles in the U.S. SART 2016 registry data, which shows an improvement

TABLE 3

Outcomes in patients undergoing an embryo transfer with embryo selection by means of preimplantation genetic testing for aneuploidy (PGT-A) versus morphology (Control), n (%).

	< 35 y		35–40 y		All patients		
Outcome	PGT-A (n = 152)	Control (n = 168)	PGT-A (n = 122)	Control (n = 145)	PGT-A (n = 274)	Control (n = 313)	*P* value[a]
Negative β-hCG	46 (30.3)	53 (31.5)	34 (27.9)	59 (40.7)	80 (29.2)	112 (35.8)	.0934
Positive β-hCG	106 (69.7)	115 (68.5)	88 (72.1)	86 (59.3)	194 (70.8)	201 (64.2)	ND
Biochemical pregnancy	14 (9.2)	10 (6.0)	15 (12.3)	16 (11.0)	29 (10.6)	26 (8.3)	.3315
Miscarriage	17 (11.2)	14 (8.3)	10 (8.2)	16 (11.0)	27 (9.9)	30 (9.6)	.8979
Elective termination	0	2 (1.2)	1 (0.8)	0	1 (0.4)	2 (0.6)	.6603
Ongoing pregnancy at 20 weeks' gestation	75 (49.3)	89 (53.0)	62 (50.8)	54 (37.2)	137 (50.0)	143 (45.7)	.3177
P value for age subgroups	*P*=.5757		*P*=.0349				

Note: ND = not determined.
[a] *P* value determined by means of Cochran-Mantel-Haenszel test.

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from the age of 35 years onward per euploid embryo transfer (2).

The targeted sample size of 300 transfers in each arm was not achieved, reflecting the patients in the PGT-A arm lacking a euploid embryo to transfer. There was no control of the demographics of patients recruited to the trial, and the average maternal age (33.7 ± 3.6 years), with more than half of the patients aged <35 years, is much lower than the experience of most clinics. One reason for the failure to reach significance per ITT in older women may be the lack of standardization as previously discussed, but also the limitations of the trial itself, particularly the eligibility criteria, which were biased toward good-prognosis patients. In addition, in contrast to routine practice, patients over 40 years of age or with a history of multiple miscarriages or multiple IVF failures were considered to be ineligible.

It was surprising that despite finding a relatively high aneuploidy rate in both the PGT and control arms, PGT-A did not improve implantation or OPR per embryo transfer in the younger patients. One limitation of the study is that IVF laboratory staff could not be blinded to patient participation in the study or study group assignment. A possible explanation for failure to achieve a more significant beneficial effect of PGT-A is that more embryos of poor quality were biopsied and vitrified because of study participation that otherwise may have been discarded in standard clinic practice. It is possible that there is a detrimental effect of the biopsy previtrification on the embryo viability that is outweighed by the benefit of PGT-A. A recent publication demonstrated a negative impact on the implantation rate if a biopsied embryo was not expanded and hatching at the time of biopsy; for unbiopsied embryos, expansion at the time of vitrification had no effect on pregnancy rates (19). In the present study, the priority for embryo selection for transfer was based on PGT-A results rather than morphology, which may have been devalued.

In conclusion, the STAR study showed no overall improvement in OPR and live birth rate in women aged 25–40 years, but does support the use of PGT-A for women aged 35–40 years to improve outcomes per frozen-thawed embryo transfer. The trial also demonstrated the pitfalls and difficulties of large-scale multicenter RCTs in the context of a complex multifactorial medical treatment such as IVF and the importance of standardizing new diagnostic tests and laboratory procedures before introducing them into clinical practice.

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On January 25, 2018, during the preparation of this manuscript, Dr. Gysler sadly passed away. He will be greatly missed by colleagues and friends worldwide.

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SUPPLEMENTARY METHODS

STUDY DESIGN

From September 1, 2014, we screened 985 eligible women who were medically cleared, and without any documented contraindications to pregnancy (as determined by their participating clinical provider), to undergo an IVF cycle with the intent to receive a single embryo transfer (SET) after vitrification and subsequent warming. Women were considered eligible for the trial if: they were 25 to 40 years of age at the time of oocyte retrieval; they were using their own oocytes; they were not using a gestational carrier; they had a history of two or fewer prior implantation failures following IVF (with implantation failure defined as an embryo transfer that did not result in a gestational sac); they had at most one prior miscarriage (with a miscarriage defined as the loss of a viable intrauterine pregnancy before 20 weeks of gestation); neither they nor their partner were a known genetic carrier of an autosomal recessive mutation; neither they nor their partner were a known genetic carrier of an autosomal dominant mutation; neither they nor their partner were a known carrier of a chromosomal abnormality predisposing them to aneuploid embryos; their partner did not have severe oligospermia (<1,000,000 sperm/ml) and/or a surgical requirement for microsurgical sperm retrieval; they did not have a diminished ovarian reserve (follicle stimulating hormone >10 IU/L on day 2-4 of their menstrual cycle and/or anti-mullerian hormone[AMH] <1.0 or 2.0 ng/ml [depending on date of enrolment]; 99 patients were enrolled under the initial, more conservative [<2 ng/ml] AMH exclusion criteria, of which 1 patient had an AMH level between 1 and 2 ng/ml); they were not undergoing any other (non-study related) PGT-A or preimplantation genetic diagnosis in the current cycle; and they were not doing a gender selection cycle.

The providers performing the transfer and the study subjects were blinded to the randomization status and PGT-A results until after the embryo transfer or pregnancy outcome was known. The genetic laboratory investigators and personnel were blinded to the aggregate pregnancy outcomes until study completion.

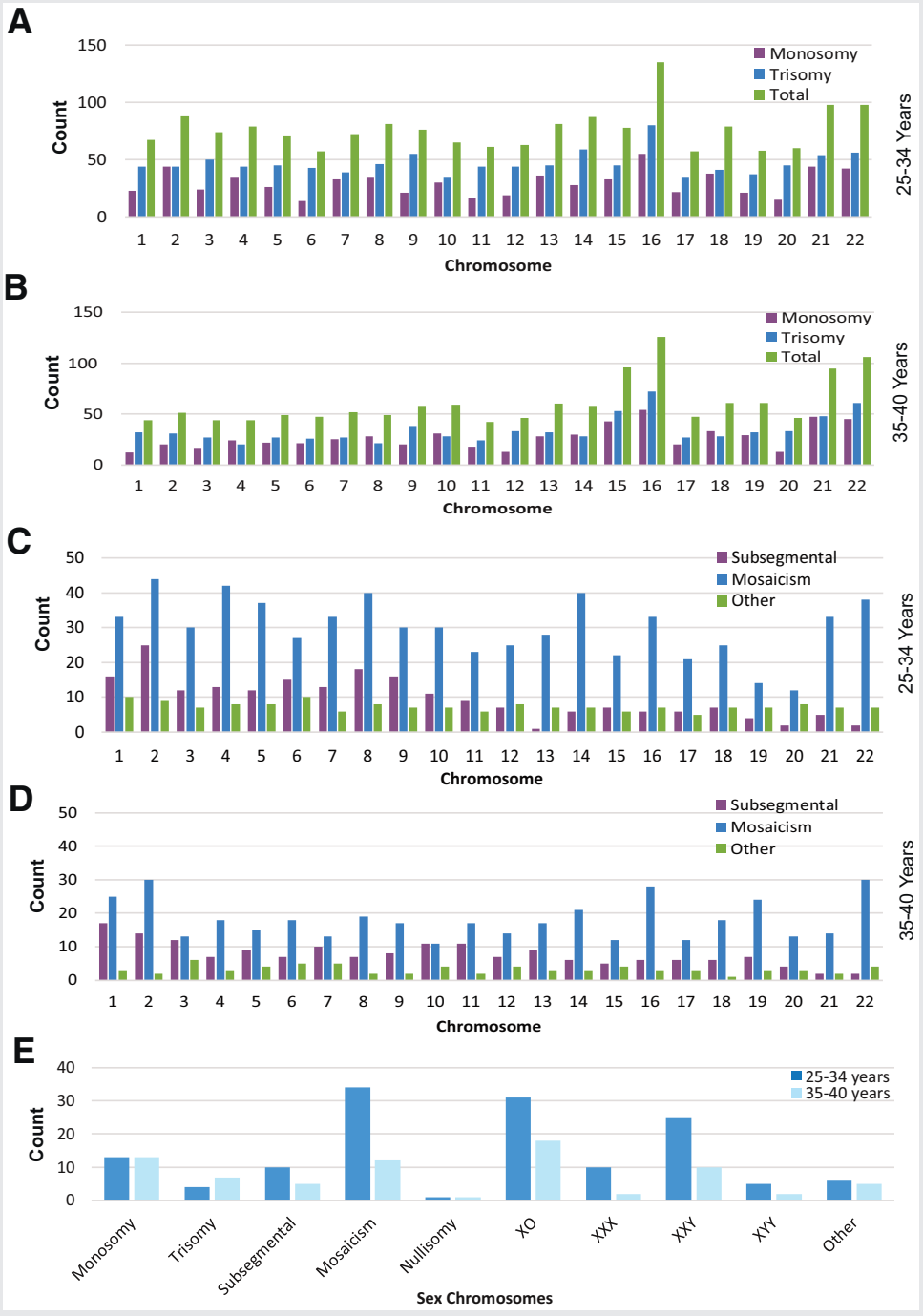
PREIMPLANTATION GENETIC SCREENING

Preimplantation genetic screening of all samples was performed using the next-generation sequencing (NGS)-based assay VeriSeq PGS following standard protocols and manufacturer recommendations (Illumina Inc., San Diego, USA; SurePlex Summary Protocol, Part #15053626; VeriSeq PGS Library Prep Reference Guide, Part #15052877). Biopsy samples were prepared and delivered to genetic testing laboratories as per standard operating procedures and included the randomization status. The genetic testing laboratory maintained a log to ensure the subject randomization was appropriately maintained during and after the study.

Biopsies were lysed and genomic DNA randomly fragmented and amplified using the SurePlex DNA Amplification System prior to preparation of libraries and indexing using the VeriSeq Library Preparation Kit-PGS and VeriSeq Index Kit-PGS. After sequencing on a MiSeq™ (Illumina Inc.), copy-number calls were automatically generated by the BlueFuse™ Multi Software (BFM version 4.1, Illumina Inc.) followed by manual assessment by qualified laboratory staff, as per standard protocols for each genetic testing laboratory.

All laboratories participating in the study evaluated samples for mosaicism, but the standard protocol for reporting mosaicism differed between the clinical laboratories: five laboratories reported mosaicism when observed; four laboratories designated samples as "no aneuploidy detected" or "abnormal" depending on whether the perceived level of mosaicism was below or above, respectively, set chromosome-specific thresholds. A low-level (10-20% abnormal cells) mosaic sample would likely be reported as euploid by all labs, a 20-50% mosaic may be reported as mosaic by the former labs and as euploid by the latter labs, a 50-80% mosaic may be reported as mosaic by the former labs and as aneuploid by the latter labs, and a high-level (80-90%abnormal cells) mosaic would likely be reported as aneuploid by all labs.

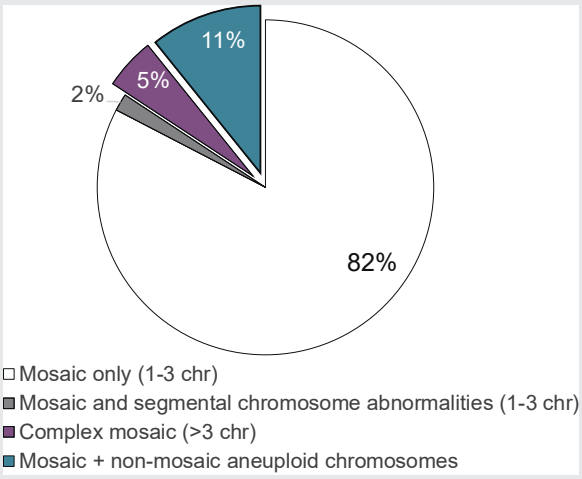
SUPPLEMENTAL FIGURE 1



Screening results by chromosome in the preimplantation genetic testing for aneuploidy (PGT-A) arm. (**A**) Autosomal monosomies and trisomies in patients 25–34 years of age. (**B**) Autosomal monosomies and trisomies in patients 35–40 years of age. (**C**) Autosomal mosaic aneuploidies and segmental copy number changes in patients 25–34 years of age. (**D**) Autosomal mosaic aneuploidies and segmental copy number changes in patients 35–40 years of age. (**E**) Sex chromosomes for patients of all ages.

Munné. RCT evaluating NGS-based PGT-A. Fertil Steril 2019.

SUPPLEMENTAL FIGURE 2



Mosaic embryo classifications.

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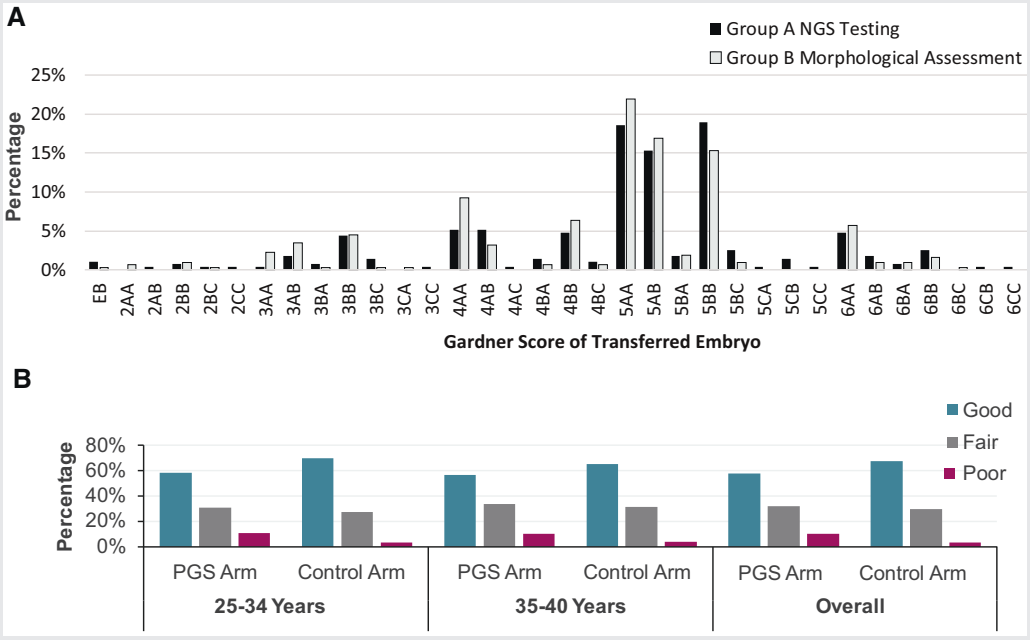
SUPPLEMENTAL FIGURE 3



(**A**) Frequency distribution of Gardner scores for transferred embryos. (**B**) Comparison of morphologic quality ratings for transferred embryos. NGS = next-generation sequencing; PGS = preimplantation genetic screening.

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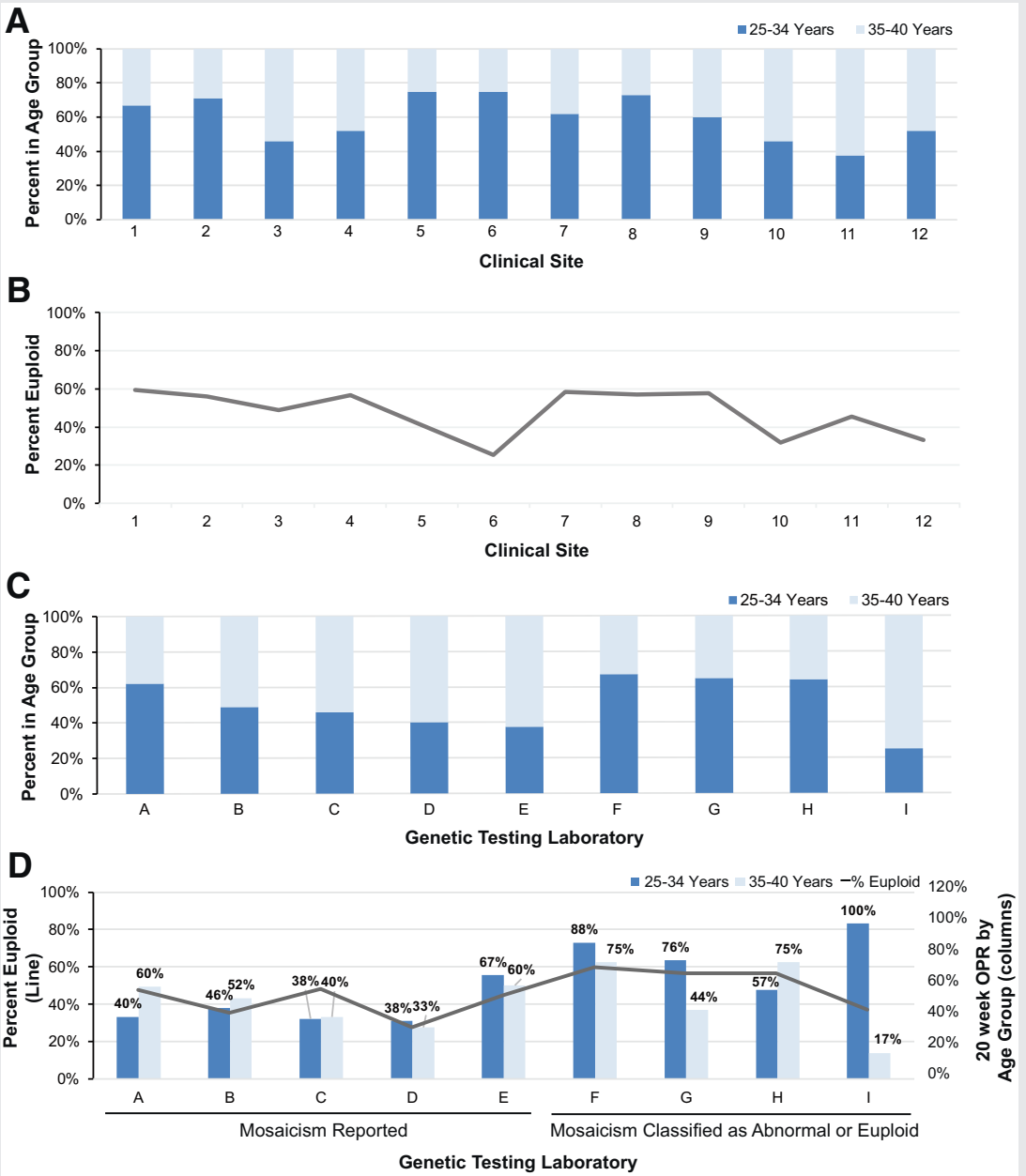
SUPPLEMENTAL FIGURE 4



Clinical site and laboratory variables. (**A**) Maternal age distribution by clinical site for sites with 20 or more transfers. (**B**) Percentage of euploid embryos in the preimplantation genetic testing for aneuploidy (PGT-A) arm for sites with 20 or more transfers. (**C**) Maternal age distribution by genetic testing laboratory. (**D**) Percentage of euploid embryos in the PGT-A arm per genetic testing laboratory by age group. Ongoing pregnancy rate in each arm per genetic testing laboratory.

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SUPPLEMENTARY TABLE 1

Age breakdown for consented patients excluded from the intent to treat population.

Reason for exclusion from ITT Population	25-34 years (N = 510)	35–37 years (N = 269)	38–40 years (N = 205)
Did not have 2 Blastocysts on Day 5/6	70 (13.7)	40 (14.9)	57 (27.8)
Did not meet other inclusion/exclusion criteria	5 (1.0)	1 (0.4)	6 (2.9)
Withdrawal (prior to randomization)	79 (15.5)	37 (13.8)	28 (13.7)

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SUPPLEMENTARY TABLE 2

PGT-A results for embryos in the PGT-A arm by maternal age.

PGT-A results, n (%)	Maternal age < 35 yr (N=1325)	Maternal age ≥35 yr (N=853[a])
No result	36 (2.7)	25 (2.9)
Euploid	636 (48.0)	303 (35.5)
Aneuploid	653 (49.3)	528 (61.9)
Monosomy	93 (7.0)	103 (12.1)
Trisomy	76 (5.7)	87 (10.2)
Mosaicism	231 (17.4)	135 (15.8)
Subsegmental	64 (4.8)	41 (4.8)
Other[b]	7 (0.5)	3 (0.4)
Complex[c]	182 (13.7)	159 (18.6)

[a] The difference in the total number of PGT-A results from the number of patients was because of 3 repeat biopsies.
[b] Triploidy or tetrasomy aneuploidy call.
[c] Combination of >1 trisomy, monosomy, and/or subsegmental aneuploidy call.

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SUPPLEMENTARY TABLE 3

Gardner score map to quality.

Gardner Score	Quality Grade	Gardner Score	Quality Grade	Gardner Score	Quality Grade
EB	Poor	4AA	Good	6AA	Good
2	Poor	4AB	Good	6AB	Good
2AA	Fair	4AC	Fair	6AC	Fair
2AB	Fair	4BA	Good	6BA	Good
2AC	Poor	4BB	Fair	6BB	Fair
2BA	Fair	4BC	Poor	6BC	Poor
2BB	Fair	4CB	Poor	6CB	Poor
2BC	Poor	4CC	Poor	6CC	Poor
2CA	Poor	4C	Poor	6C	Poor
2CB	Poor	5AA	Good		
2CC	Poor	5AB	Good		
3AA	Good	5AC	Fair		
3AB	Good	5BA	Good		
3AC	Fair	5BB	Fair		
3BA	Good	5BC	Poor		
3BB	Fair	5CA	Poor		
3BC	Poor	5CB	Poor		
3CA	Poor	5CC	Poor		
3CB	Poor	5B	Fair		
3CC	Poor	5C	Poor		
3C	Poor				

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SUPPLEMENTARY TABLE 4

Outcomes in patients with embryo selection by PGT-A or morphology for women aged 25 to 34 years, 35 to 37 years, and 38 to 40 years.

	25-34 years		35–37 years		38–40 years	
Outcome	**PGT-A Arm N = 152**	**Control Arm N = 168**	**PGT-A Arm N = 80**	**Control Arm N = 89**	**PGT-A Arm N = 42**	**Control Arm N = 56**
Negative β-HCG, n (%)	46 (30.3)	53 (31.5)	22 (27.5)	37 (41.6)	12 (28.6)	22 (39.3)
Positive β-HCG, n (%)	106 (69.7)	115 (68.5)	58 (72.5)	52 (58.4)	30 (71.4)	34 (60.7)
Biochemical pregnancy, n (%)	14 (9.2)	10 (6.0)	11 (13.8)	9 (10.1)	4 (9.5)	7 (12.5)
Miscarriage, n (%)	17 (11.2)	14 (8.3)	5 (6.3)	10 (11.2)	5 (11.9)	6 (10.7)
Elective termination, n (%)	0	2 (1.2)	0	0	1 (2.4)	0
Ongoing pregnancy at 20 weeks' gestation, n (%)	75 (49.3)	89 (53.0)	42 (52.5)	33 (37.1)	20 (47.6)	21 (37.5)

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