Journal of Assisted Reproduction and Genetics (2019) 36:2103–2109
https://doi.org/10.1007/s10815-019-01550-6

ASSISTED REPRODUCTION TECHNOLOGIES

When next-generation sequencing-based preimplantation genetic testing for aneuploidy (PGT-A) yields an inconclusive report: diagnostic results and clinical outcomes after re biopsy

Shelby A. Neal[1,2] · L. Sun[2] · C. Jalas[2] · S. J. Morin[1,3] · T. A. Molinaro[1] · R. T. Scott Jr[1]

Received: 21 March 2019 / Accepted: 26 July 2019 / Published online: 30 August 2019

Abstract

Purpose To describe diagnostic results following re-biopsy of blastocysts with inconclusive results on preimplantation genetic screening for aneuploidy (PGT-A) and to evaluate the reproductive potential of re-biopsied blastocysts.

Methods This retrospective cohort study included all trophectoderm biopsies submitted for PGT-A by a large in vitro fertilization center to a single genetics laboratory from June 2016 to October 2018. PGT-A was performed using next-generation sequencing (NGS). No-result blastocysts that underwent re-biopsy were subsequently classified as euploid, aneuploid, mosaic/segmental, or no-result. Ongoing pregnancy and clinical loss rates were assessed following transfer of re-biopsied blastocysts. Logistic regressions were conducted to account for age and blastocyst morphology.

Results Of the trophectoderm biopsies submitted for PGT-A, 635/25,199 (2.5%) were categorized as no-result. Those that underwent re-biopsy ($n = 250$) had a 95.2% diagnostic rate with 140 (56.0%) receiving euploid diagnoses. Thirty-six re-biopsied blastocysts deemed euploid were subsequently transferred, resulting in 18 (50.0%) ongoing pregnancies and 5 (13.9%) clinical losses. After adjusting for age and blastocyst morphology, there remained a lower ongoing pregnancy rate and a trend towards higher clinical loss rate following transfer of a re-biopsied blastocyst. When compared to blastocysts that underwent the same number of vitrification-warming cycles but only one biopsy, there were no differences in outcomes.

Conclusions Failure to obtain an analytical result does not change the probability that a given blastocyst is euploid. Pregnancy outcomes following transfer of re-biopsied blastocysts are favorable, but further data must be accrued for an adequately powered comparison with outcomes after transfer of blastocysts biopsied once.

Keywords Re-biopsy · Blastocyst · Trophectoderm biopsy · Preimplantation genetic testing for aneuploidy · Next-generation sequencing · Inconclusive results

Introduction

Preimplantation genetic testing for aneuploidy (PGT-A) is a useful adjunct to IVF treatment but occasionally fails to yield a diagnostic result. The prevalence of inconclusive diagnoses reported in the literature ranges from 0.86 to 3.8% [1–4]. Factors known or hypothesized to contribute to inconclusive diagnoses include poor quality DNA secondary to poor embryo quality, day of biopsy, biopsy technique, shipping conditions, technical amplification failure, or wide scatter in the data resulting in failure to meet analytical quality control metrics [1, 2, 5]. Regardless of the etiology, inconclusive diagnoses are an inherent limitation of PGT-A and pose a challenge to both patients and clinicians.

When PGT-A yields an inconclusive diagnosis, patients typically prioritize transfer of euploid embryos if available; however, in situations where there are few or no euploid embryos, patients must choose whether to re-biopsy the no-result blastocyst, blindly transfer it or accumulate more embryos, each option posing its own potential benefits and drawbacks. Re-biopsy involves additional labor and expense for both the

✉ Shelby A. Neal
shelby.addison@gmail.com

[1] IVI/RMA New Jersey, 140 Allen Rd, Basking Ridge, NJ 07920, USA

[2] Foundation for Embryonic Competence, 140 Allen Rd, Basking Ridge, NJ 07920, USA

[3] Sidney Kimmel College of Medicine, Thomas Jefferson University, 1025 Walnut St., #100, Philadelphia, PA 19107, USA

embryology and genetics laboratories, delays time to embryo transfer, and requires an additional warming and revitrification of the embryo. Additionally, there are concerns that no-result embryos may be intrinsically less competent—less likely to yield a diagnostic result upon re-biopsy due to scant cellularity or apoptotic cells, less likely to be euploid, or less likely to result in a pregnancy.

Several studies have sought to address these issues [1–6] but vary in terms of patient population, embryo stage at biopsy, PGT platform utilized, and fresh versus frozen embryo transfer, all factors which may be expected to influence outcomes. Only one study has reported on outcomes using a NGS platform [6]. The authors examined diagnostic outcomes following repeat trophectoderm biopsy of no-result blastocysts from 39 IVF centers and concluded that over half are euploid but did not report on subsequent pregnancy outcomes.

The objectives of this study were to describe diagnostic results of PGT-A following re-biopsy of no-result blastocysts, and to report clinical outcomes following transfer of those blastocysts found to be euploid after re-biopsy.

Materials and methods

Study population

A retrospective cohort study was performed consisting of all blastocysts that underwent trophectoderm biopsy for PGT-A at a large IVF center between June 2016 and October 2018. Blastocysts of patients undergoing PGT for indications other than aneuploidy screening (e.g., chromosomal rearrangements, single gene disorders) were excluded. Per laboratory protocol, all blastocysts were biopsied when they reached full expansion on days 5, 6, or 7, and subsequently vitrified. Biopsies were sent to a single genetics laboratory for PGT-A using a next-generation sequencing (NGS) platform. The subsequent study workflow is shown in Fig. 1.

Blastocysts were categorized as "no-result" if there was amplification failure, indicating the presence of insufficient genetic material in the biopsy for analysis. Blastocysts were also categorized as no-result if results were reported as non-concurrent, indicating wide scatter in data and failure to meet quality control standards. No-result blastocysts were compared to those that received a diagnosis on the basis of oocyte age, biopsy day, inner cell mass (ICM) grade, and trophectoderm (TE) grade to identify factors which may contribute to inconclusive PGT-A results.

Re-biopsy of no-result blastocysts

Within the cohort of no-result blastocysts, those that were warmed for re-biopsy were identified. Re-biopsy was performed according to the same laboratory procedures for the initial biopsy and followed by re-vitrification of the blastocyst. Re-biopsy specimens were submitted for PGT-A reanalysis utilizing the same NGS platform and quality control metrics for interpretation of results. Results after re-biopsy were classified as euploid, aneuploid (any result that included at least one whole chromosome aneuploidy), mosaic/segmental (any result with only mosaicism and/or segmental aneuploidies), or no-result, and were compared to the distribution of results seen after initial biopsy.

Transfer of re-biopsied blastocysts

Of the blastocysts deemed to be euploid after re-biopsy, those that were re-warmed for single embryo transfer prior to November 2018 were used to assess pregnancy outcomes. Specific pregnancy outcomes of interest included ongoing pregnancy (defined as the presence of a fetal heartbeat at 8-week gestation), clinical loss (defined as pregnancy loss

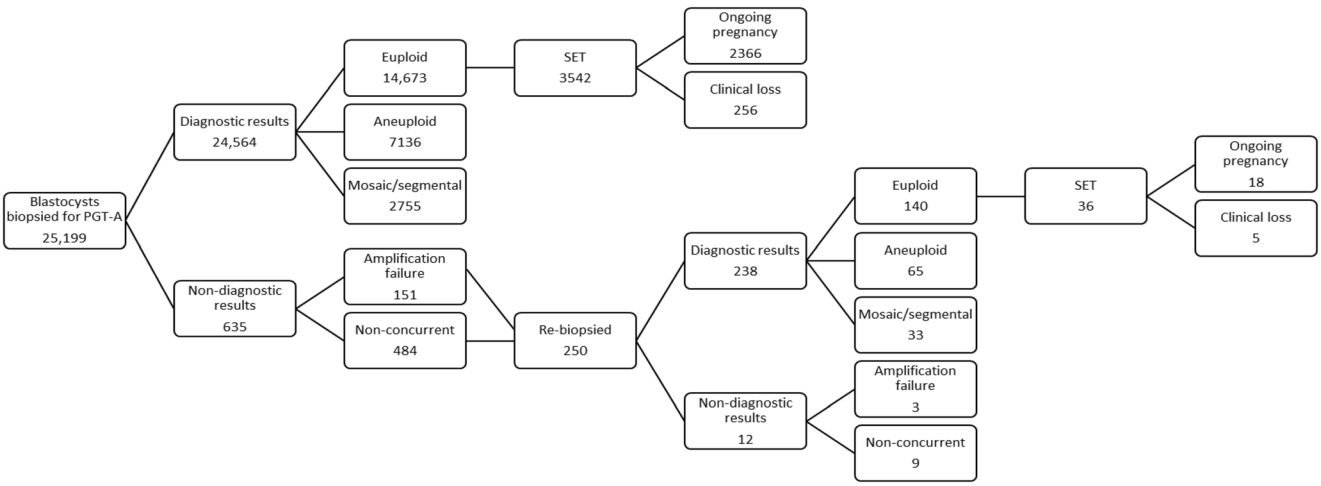

Fig. 1 Flowchart detailing distribution of PGT-A results obtained following initial and repeat (if applicable) biopsies

following observation of a gestational sac on ultrasound), and monozygotic twinning.

Pregnancy outcomes were compared to those of two control groups. The first control group consisted of patients undergoing routine care, namely transfer of euploid blastocysts biopsied once prior to a single vitrification/warming cycle. A second control group was included as an effort to isolate the impact of multiple biopsies versus multiple vitrification/warming cycles on pregnancy outcomes. This control group consisted of patients who underwent transfer of euploid blastocysts that were vitrified first and then warmed for a single biopsy prior to a second vitrification/warming cycle. Lastly, pregnancy outcomes of cycles in which no-result blastocysts were blindly transferred without re-biopsy were described.

Statistical analysis

Patient, embryo, and cycle characteristics of interest included oocyte age, age at transfer, body mass index, peak endometrial thickness, biopsy day (day 5, 6, or 7), ICM grade, and TE grade. Student's *t* test or analysis of variance (ANOVA) were used to compare continuous baseline characteristics between the groups. Pearson's chi-square test or Fisher's exact test as appropriate was used for comparison of categorical baseline characteristics and pregnancy outcomes. For the purposes of evaluating pregnancy outcome, blastocyst morphology was considered as a categorical variable according to the simplified SART embryo scoring system [7]. Logistic regression was performed to account for the impact of oocyte age and blastocyst morphology on pregnancy outcomes.

Ethical approval

This study was approved by our Institutional Review Board, and given its retrospective nature, formal consent of study participants was not required.

Results

Incidence and characteristics of no-result blastocysts

During the study timeframe, a total of 25,199 blastocysts were biopsied for PGT-A. The overall diagnostic rate was 97.5%, with 635 blastocysts receiving inconclusive reports, of which 151 (0.6%) were due to amplification failure and 484 (1.9%) were due to non-concurrent results (Table 1). There was no difference in oocyte age between blastocysts that received diagnostic results versus those that did not ($P = 0.15$). The incidence of inconclusive results did not vary with day of biopsy ($P = 0.49$) or ICM grade ($P = 0.11$); however, it was higher for blastocysts with TE grade B or C relative to TE grade A ($P < 0.01$) (Fig. 2).

Diagnostic results after re-biopsy

Of the 635 no-result blastocysts, 250 were warmed for re-biopsy and all survived the warming process. No-result blastocysts that were selected for re-biopsy were, on average, from older patients (mean age at oocyte retrieval 35.9 versus 33.4 years, $P < 0.01$). They did not differ on the basis of reason for inconclusive report (amplification failure versus non-concurrent results), biopsy day, ICM grade, or TE grade. PGT-A performed on the re-biopsy specimen yielded a diagnostic result in 95.2% of cases. The incidence of a diagnostic result upon re-biopsy did not differ by reason for initial inconclusive report (94.8% for initial non-concurrent results vs 96.6% for initial amplification failure, $P = 0.86$).

One-hundred and forty (56.0%) blastocysts were assigned a euploid diagnosis following re-biopsy. For patients < 35, no-result blastocysts undergoing re-biopsy were more likely to be categorized as no-result again upon re-biopsy (7.4% versus 2.5%, $P = 0.01$) (Fig. 3). However, the likelihood of a euploid diagnosis following re-biopsy was no different than the likelihood of a euploid diagnosis following initial assessment ($P = 0.98$); this finding was true of all age groups. Additionally, the distribution of abnormal results amongst the aneuploid and mosaic/segmental categories was not statistically different.

Pregnancy outcomes

Thirty-six of the 140 re-biopsied blastocysts found to be euploid were re-warmed for transfer with a 100% survival rate. These blastocysts were subsequently used for single embryo transfers, resulting in 18 (50.0%) ongoing pregnancies and 5 (13.9%) clinical losses. When compared to the control groups (1 biopsy + 1 vitrification/warming cycle and 1 biopsy + 2 vitrification/warming cycles), re-biopsied blastocysts that were transferred were more likely to be from older patients and less likely to be classified as good quality (Table 2). Logistic regression analyses which adjusted for these variables identified a lower ongoing pregnancy rate (OR 0.49, 95% CI 0.25–0.96) for re-biopsied blastocysts when compared to those that underwent 1 biopsy + 1 vitrification/warming cycle. There was a trend towards a higher clinical loss rate (OR 2.03, 95% CI 0.69–4.83) as well. However, when compared to blastocysts that underwent 1 biopsy + 2 vitrification/warming cycles, differences in ongoing pregnancy rate ($P = 0.91$) and clinical loss rate ($P = 0.30$) were no longer apparent.

Only three cases were identified in which no-result blastocysts were blindly transferred without undergoing re-biopsy for repeat analysis. All three blastocysts received non-concurrent results on initial assessment and were not re-biopsied. One transfer (for which the patient was 28 years of age) resulted in an ongoing pregnancy, and the other two

Table 1 Characteristics of blastocysts that received a diagnosis after initial biopsy for PGT-A versus those that did not (no-result blastocysts)

Characteristic	Diagnosed blastocysts ($n = 24,564$)	No-result blastocysts		P value
		Amplification failure ($n = 151$)	Non-concurrent results ($n = 484$)	
Oocyte age (years)	34.4 ± 4.8	35.0 ± 4.3	34.2 ± 4.4	0.15
Biopsy day				0.52
5	8096 (33.0%)	46 (30.5%)	163 (33.7%)	
6	15,704 (63.9%)	101 (66.9%)	300 (62.0%)	
7	764 (3.1%)	4 (2.6%)	21 (4.3%)	
ICM grade				0.19
A	4696 (19.1%)	28 (18.5%)	78 (16.1%)	
B	15,792 (64.3%)	99 (65.6%)	308 (63.6%)	
C	4076 (16.6%)	24 (15.9%)	98 (20.2%)	
TE grade				< 0.01
A	5618 (22.9%)	27 (17.9%)	75 (15.5%)	
B	12,224 (49.8%)	95 (62.9%)	254 (52.5%)	
C	6722 (27.3%)	29 (19.2%)	155 (32.0%)	

transfers (for which both patients were 38 years of age) resulted in no pregnancy.

Discussion

To date, there are limited data available regarding diagnostic and clinical outcomes following re-biopsy of no-result blastocysts. The available publications vary with respect to study population (aneuploidy screening only or single gene disorder/chromosomal rearrangement; single or multiple IVF centers), biopsy stage (cleavage stage versus blastocyst), PGT-A platform, fresh versus frozen transfer, and number of embryos transferred. Our study is the first one to our knowledge to evaluate distribution of diagnostic outcomes following re-biopsy of blastocysts when utilizing a NGS-based PGT-A platform. In addition, we present pregnancy outcomes following subsequent transfer of blastocysts deemed to be euploid upon re-biopsy. Our results affirm findings of previous studies, suggesting that no-result blastocysts are often deemed to be euploid upon repeat PGT-A and such blastocysts have reproductive potential.

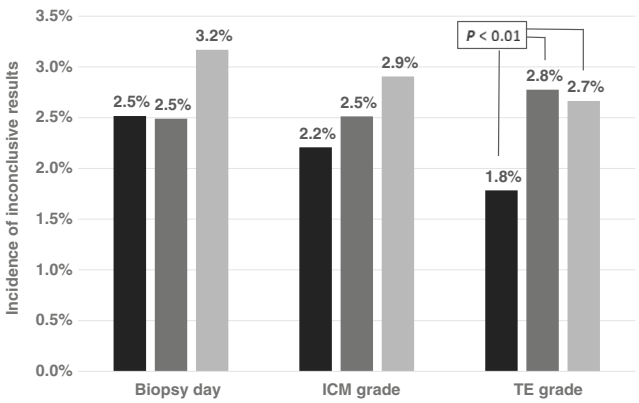

Fig. 2 Incidence of inconclusive results by day of biopsy, inner cell mass grade, and trophectoderm grade

In this study, blastocyst biopsy for NGS-based PGT-A yielded inconclusive results in a small minority (2.5%) of cases. This finding is consistent with those of previous studies which employed quantitative polymerase chain reaction [1] and array comparative genomic hybridization [2–4]-based methods of PGT-A, demonstrating that the risk of inconclusive results with NGS is not increased over other platforms.

There were no differences between no-result blastocysts and those blastocysts that received a diagnostic result on the basis of oocyte age, biopsy day, or ICM grade. TE grade B or C, however, was associated with an increased incidence of inconclusive results, relative to TE grade A. These findings make intuitive sense, as a biopsy from a blastocyst with a lesser TE grade may be less cellular than one from a blastocyst with TE grade A, thereby increasing the risk of an inconclusive result. We did not quantify trophectoderm biopsy cellularity in the present study; however, a recent study by Cimadomo et al. looked at this parameter and found that the mean number of trophectoderm cells was significantly lower in biopsies that yielded inconclusive results [1]. This finding supports the notion that inconclusive results may, in some cases, be attributed to an underlying biologic etiology.

It is conceivable that poor quality blastocysts may be less likely to be subjected to the stress of warming, re-biopsy, and repeat vitrification. In this study cohort, there was no difference in ICM or TE grade between no-result blastocysts selected for re-biopsy and those that were not re-biopsied. There was, however, a difference in mean age, with re-biopsied blastocysts tending to be from older subjects. As a potential explanation for this finding, older patients may be more likely to pursue re-biopsy of a no-result blastocyst due to their

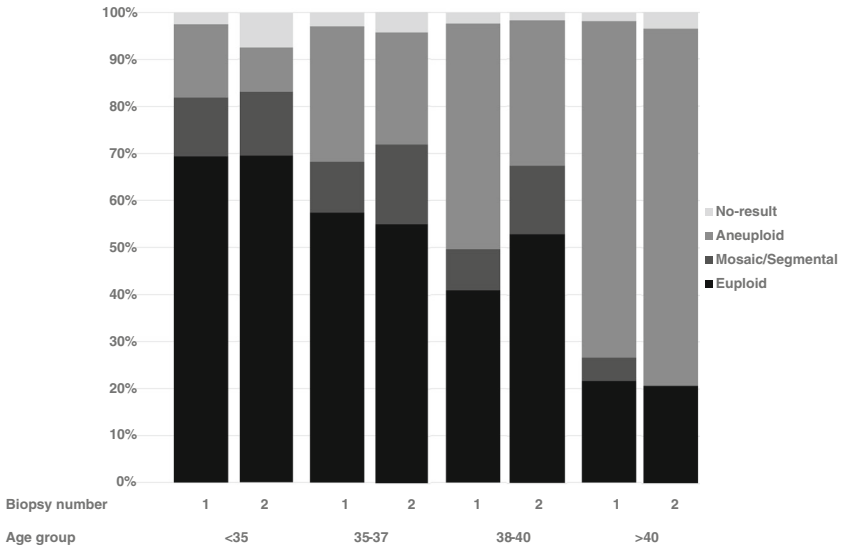

Fig. 3 Distribution of PGT-A diagnoses by number of biopsies and oocyte age

increased risk of aneuploidy or their lower likelihood of having another blastocyst suitable for transfer.

With respect to diagnostic outcomes following re-biopsy of no-result blastocysts, the overall euploidy rate was 56.0% which did not differ from that of blastocysts receiving a diagnosis after the first trophectoderm biopsy. This is consistent with previous reports which suggest euploidy rates of 44–70% upon re-biopsy [1–3, 5, 6, 8]. When stratifying by age, the distribution of PGT-A results following initial biopsy was no different than that following re-biopsy of no-result embryos, with the exception of the < 35 age group. In this group, the incidence of inconclusive results was notably higher after re-biopsy than after initial biopsy (7.4% versus 2.5%, $P = 0.01$), suggesting that no-result blastocysts in this age group are more likely to receive an inconclusive result again. However, the clinically relevant outcome which is the proportion of euploid blastocysts did not differ after re-biopsy when compared to that seen after initial biopsy.

The safety of re-biopsy and multiple vitrification/warming cycles has been debated. We did not find evidence of an inferior blastocyst survival rate. In fact, no-result blastocysts which were warmed for re-biopsy had a 100% survival rate in this study, as did those blastocysts found to be euploid upon re-biopsy and subsequently warmed for transfer. These findings are in accordance with those from most prior studies [1–3, 8, 9] with the exception of a study by Bradley et al. in which the thaw survival rate was lower amongst blastocysts that were twice biopsied and vitrified/warmed [10]. However, the thaw survival rate was not significantly different when compared to a group of blastocysts that were biopsied only

Table 2 Baseline characteristics and pregnancy outcomes resulting from transfer of euploid blastocysts with varying numbers of trophectoderm biopsies and vitrification/warming cycles

Characteristic or outcome	1 Bx + 1 V/W ($n = 3542$)	1 Bx + 2 V/W ($n = 155$)	2 Bx + 2 V/W ($n = 36$)	*P* value
Oocyte age (years)	34.8 ± 4.4	32.0 ± 4.4	35.5 ± 4.4	*< 0.01*
Age at transfer (years)	36.0 ± 4.4	35.8 ± 5.6	36.2 ± 4.4	0.87
Body mass index (kg/m^2)	26.1 ± 5.6	26.5 ± 5.6	26.0 ± 5.8	0.71
Peak endometrial thickness (mm)	10.5 ± 2.5	10.6 ± 2.8	11.0 ± 2.4	0.48
Biopsy day				
5 6	1765 (49.8%) 1723 (48.7%)	63 (40.6%) 91 (58.7%)	13 (36.1%) 22 (61.1%)	0.06
7	54 (1.5%)	1 (0.6%)	1 (2.8%)	
Embryo quality				
Good Fair	1348 (38.1%) 1985 (56.0%)	44 (28.4%) 99 (63.9%)	10 (27.8%) 26 (72.2%)	*0.03*
Poor	209 (5.9%)	12 (7.7%)	0 (0%)	
Ongoing pregnancy	2366/3542 (66.8%)	98/155 (63.2%)	18/36 (50.0%)	0.07
Clinical loss	256/2622 (9.8%)	18/116 (15.5%)	5/23 (21.7%)	0.08
Monozygotic twins	44/2622 (1.7%)	0/116 (0%)	1/23 (4.3%)	0.23

Bx biopsy, *V/W* vitrification/warming cycle

once, but vitrified/warmed twice, suggesting that the diminution in survival seen in this study was primarily related to the additional vitrification/warming cycle and not the second biopsy.

With respect to pregnancy outcomes, two control groups were utilized (blastocysts that underwent 1 biopsy + 1 vitrification/warming cycle and blastocysts that underwent 1 biopsy + 2 vitrification/warming cycles) in an effort to identify whether re-biopsy or multiple vitrification/warming cycles impacted outcomes. The mean oocyte age for the latter control group was notably younger, and this is likely due to the fact that young patients are less likely to pursue blastocyst biopsy for PGT-A initially but may elect to do so later, after a failed transfer or clinical loss. Conversely, it is not surprising that the mean oocyte age for re-biopsied blastocysts was older, as older patients are less likely to have other euploid blastocysts to transfer and therefore more likely to pursue re-biopsy of a no-result blastocyst. Re-biopsied blastocysts that were transferred in this study were less likely to be classified as good quality; this finding is likely due to the fact that patients transferring a re-biopsied blastocyst are unlikely to have a cohort of euploid blastocysts from which to choose the best quality embryo for transfer.

When utilizing logistic regression to account for the differences in oocyte age and blastocyst morphology, there was a statistically significant lower ongoing pregnancy rate and a trend towards higher clinical loss following transfer of re-biopsied blastocysts when compared to blastocysts that underwent 1 biopsy + 1 vitrification/warming cycle. Potential explanations for these findings include an adverse impact from multiple vitrification/warming cycles, an adverse impact from multiple biopsies, or the possibility of a lower reproductive potential inherent to no-result blastocysts. In order to control for the multiple vitrification/warming cycles, pregnancy outcomes were compared to blastocysts that underwent 1 biopsy + 2 vitrification/warming cycles. In this setting, the comparison of ongoing pregnancy rates was no longer statistically significant, suggesting that an additional vitrification/warming cycle may contribute to the lower ongoing pregnancy rate seen following transfer of re-biopsied blastocysts; however, it is unlikely to be the sole explanation. The remaining two possibilities—an adverse impact of multiple biopsies and lower reproductive potential inherent to no-result blastocysts—must, therefore, be entertained.

One alternative to re-biopsy of a no-result blastocyst is to blindly transfer it without a result. At our center, there were only three such cases during the study timeframe, one of which resulted in an ongoing pregnancy. Insufficient sample size precludes comparison of pregnancy outcomes following blind transfer versus re-biopsy. However, of the 250 no-result blastocysts that underwent re-biopsy, 98 (39.2%) were subsequently assigned an aneuploid diagnosis, at least three of which are known to be compatible with life (45,X; 47,XX,+18; and 47,XY,+22). Blindly transferring no-result blastocysts without re-biopsy, therefore, confers a low but present risk of an ongoing aneuploid gestation.

Due to the low number of live births ($n = 13$) resulting from transfer of re-biopsied blastocysts at our IVF center since 2016 when utilization of the NGS-based platform began, perinatal outcomes were not assessed in this study. Two published studies thus far have identified no differences in birth weight or gestational age at delivery [1, 10]; however, small numbers preclude definitive conclusions regarding perinatal outcomes at this time. Further data must be accrued regarding this important issue.

In summary, our data demonstrate that re-biopsy of no-result blastocysts yields a euploid result over half of the time, suggesting that this strategy warrants consideration for all patients who wish to understand the ploidy status of their entire embryo cohort, and particularly for those patients who do not have other embryos available for transfer. Re-biopsy, as well as the additional vitrification/warming cycle that it entails, does not appear to impact blastocyst survival. Pregnancy outcomes may be adversely impacted when transferring a re-biopsied blastocyst; however, the current analysis is underpowered to detect a difference. Further data are needed in order to make a definitive conclusion regarding the impact of re-biopsy on pregnancy outcomes. Regardless, it is clear that re-biopsied blastocysts have reproductive potential and re-biopsy for repeat PGT-A should be offered to patients who have blastocysts with inconclusive results following initial biopsy.

References

1. Cimadomo D, Rienzi L, Romanelli V, Alviggi E, Levi-Setti PE, Albani E, et al. Inconclusive chromosomal assessment after blastocyst biopsy: prevalence, causative factors and outcomes after re-biopsy and re-vitrification. A multicenter experience. Hum Reprod Oxf Engl. 2018;33(10):1839–46.
2. Brower M, Hill D, Danzer H, Surrey M, Ghadir S, Chang W, et al. "No diagnosis" embryos after PGS should not be discarded: rebiopsy and reanalysis demonstrate the majority are euploid. Fertil Steril. 2014;102(3):e31.
3. Kaing A, Kroener L, Brower M, Hill D, Danzer H, Barritt J. Rebiopsy and preimplanation genetic screening (PGS) reanalysis demonstrate the majority of originally "no diagnosis" embryos are euploid with comparable pregnancy rates. Fertil Steril. 2015;104(3):e277.
4. Lee H, McCulloh DH, Olivares R, Goldstein-Tufaro A, McCaffrey C, Grifo J. Live births after transfer of rebiopsy and revitrification of blastocyst that had "no diagnosis" following trophectoderm biopsy. Fertil Steril. 2016;106(3):e164.
5. Neitzel D, Alouf CA, Robinson K, Zhu M, Faulkner N. Re-biopsied pgs embryos yield actionable results. Fertil Steril. 2017;108(3):e290.
6. Priner S, Altarescu G, Schonberger O, Holzer H, Rubinstein E, Dekel N, et al. The effect of repeated biopsy on pre-implantation

genetic testing for monogenic diseases (PGT-M) treatment outcome. J Assist Reprod Genet. 2018.
7. Heitmann RJ, Hill MJ, Richter KS, DeCherney AH, Widra EA. The simplified SART embryo scoring system is highly correlated to implantation and live birth in single blastocyst transfers. J Assist Reprod Genet. 2013;30(4):563–7.
8. Zhang S, Tan K, Gong F, Gu Y, Tan Y, Lu C, et al. Blastocysts can be rebiopsied for preimplantation genetic diagnosis and screening. Fertil Steril. 2014;102(6):1641–5.
9. Swain JE, Schoolcraft WB, Katz-Jaffe M. Dual trophectoderm biopsy on the same blastocyst does not impair clinical outcomes. Fertil Steril. 2015;104(3):e186.
10. Bradley CK, Livingstone M, Traversa MV, McArthur SJ. Impact of multiple blastocyst biopsy and vitrification-warming procedures on pregnancy outcomes. Fertil Steril. 2017;108(6):999–1006.