

# Trophectoderm biopsy for preimplantation genetic test and technical tips: A review

Naoki Aoyama  | Keiichi Kato

Kato Ladies Clinic, Tokyo, Japan

## Correspondence

Keiichi Kato, Kato Ladies Clinic, 7-20-3 Nishishinjuku, Shinjuku-ku, Tokyo, 160-0023, Japan.

Email: k-kato@towako.net

## Abstract

**Background:** Recently, the Japan Society of Obstetrics and Gynecology initiated a clinical study of preimplantation genetic test for aneuploidy. There will be a great need for a standardized embryo biopsy technique in Japan. However, the gold standard trophoctoderm (TE) biopsy procedure has not been established, and this review outlines the clinical use of TE biopsy.

**Methods:** Based on literature, the method and associated techniques for TE biopsy, a dissection method of TE cells from blastocysts, were investigated.

**Main findings:** Two TE biopsy methods are used, namely assisted hatching (herniating) and non-assisted hatching (direct suction); however, it is not clear which of these methods is superior. It is critical to understand whether the flicking or pulling method is beneficial.

**Conclusion:** Non-assisted hatching biopsy method may cause blastocyst collapse with a higher probability, and it may extend the biopsy time. The biopsy procedure should be performed within 3 minutes, and thus direct TE suction may have greater disadvantages. It is a fact that pulling method of TE dissection with laser pulse is simple; however, excess laser shots may induce a higher frequency of mosaicism. It is important to understand that each technique of TE biopsy has benefits and disadvantages.

## KEYWORDS

assisted hatching, blastocyst, non-assisted hatching, preimplantation genetic testing for aneuploidy, trophoctoderm biopsy

## 1 | INTRODUCTION

Preimplantation genetic testing for aneuploidy (PGT-A) was developed as a clinical tool for improving implantation rates and decreasing the risk of miscarriage. However, the efficacy of this technique is still controversial. Higher implantation rates per transfer are expected compared with the control group, but the live birth rate per oocyte retrieval may be lower than in the control group.<sup>1,2</sup> Thus, misdiagnosis associated with lower diagnostic

sensitivity and specificity, and the embryonic damage caused during embryo biopsy are the major disadvantages of PGT-A. Biopsy technique can be divided into two categories: targeting of the blastomere during the cleavage stage and targeting of the trophoctoderm (TE) during the blastocyst stage. Fluorescent in situ hybridization (FISH) protocols require specimens with a wider nucleus diameter on glass slide<sup>3</sup>; this approach is appropriate for blastomere biopsy, which can be useful for obtaining larger nuclei than observed in TE cells. In contrast, more advanced platforms, such

This is an open access article under the terms of the Creative Commons Attribution License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited.

© 2020 The Authors. *Reproductive Medicine and Biology* published by John Wiley & Sons Australia, Ltd on behalf of Japan Society for Reproductive Medicine.

as next-generation sequencing (NGS) and microarray comparative genomic hybridization (aCGH), require optimum DNA from several cells for amplification are appropriate for blastocyst biopsy. TE biopsy for PGT was first reported 15 years ago,<sup>4</sup> with the development of comprehensive chromosomal screening (CCS) technique, it is becoming a major technique for PGT-A. The Japan Society of Obstetrics and Gynecology (JSOG) has prohibited PGT-A screening since 1998; however, in 2016, JSOG initiated a clinical study of PGT-A for couples with recurrent in vitro fertilization failure and pregnancy loss (ClinicalTrials.gov as UMIN000026104).<sup>5</sup> In the near future, there will be great need for a standardized embryo biopsy technique in Japan; however, the gold standard TE biopsy procedure has not been established yet. We outline the current status of blastocyst biopsy, and introduce technical tips for practitioners who are studying PGT.

### 1.1 | Biopsy method; Hatching or non-assisted hatching?

The first clinical report of preimplantation genetic diagnosis (PGD) using cleavage-stage embryo biopsy was published by Handyside in 1990.<sup>6</sup> At the same time, researchers began studying trophectoderm biopsy and demonstrated the feasibility of this method for PGD.<sup>7,8</sup> Initially, cleavage-stage embryo biopsy predominated,<sup>9-13</sup> particularly that using FISH protocols.<sup>14-23</sup> The first live birth case following blastocyst biopsy for PGD was reported in 2002.<sup>24</sup> McArthur et al<sup>25</sup> described the details of trophectoderm biopsy procedure and reproductive outcomes of PGD using this technique with FISH or real-time quantitative polymerase chain reaction (qPCR) in 2005. Notably, in their approach, zona breaching was performed on day 3 or 4, and 2-9 cells from the herniated trophectoderm were teased free from the remaining trophectoderm with laser pulse.<sup>25,26</sup> This protocol was frequently applied for clinical trials; however, researchers also proposed another approach, that is, non-assisted hatching method. Capalbo et al<sup>27</sup> reported a direct aspiration technique for biopsy of the trophectoderm without assisted hatching in 2014, demonstrating the benefits of this protocol in daily laboratory work. Because the timing of biopsy for hatching blastocyst is critical, excess herniation of trophectoderm may induce incarceration (Figure 1A). There is no evidence for the association between embryo incarceration and embryo development. However, practitioner should still avoid causing excess trophectoderm herniation. Continuous observation using time-lapse monitoring technology and direct TE aspiration with non-assisted hatching are helpful for detecting and preventing such early incarceration. Non-assisted hatching has disadvantages. For example, if blastocyst collapse occurs during TE cell aspiration, the biopsy procedure may be difficult and be associated with a higher risk of losing the inner cell mass (ICM) area. Furthermore, blastocyst collapse may extend the biopsy time, despite recommendations that the biopsy procedure should be performed within 3 minutes.<sup>28</sup> No published clinical data have shown which method is better for biopsy.

### 1.2 | Optimal timing of zona drilling

When establishing standard approaches for trophectoderm biopsy,<sup>25,26</sup> it is essential to consider the timing of zona breaching during the cleavage or blastocyst stage (Figure 1B). Zona breaching is associated with a risk of ICM incarceration, and practitioners must be careful to avoid physical damage to the ICM during the biopsy procedure. Zona breaching during the cleavage stage could increase ICM incarceration; however, few studies have reported this phenomenon. Rubino et al<sup>29</sup> reported a double zona drilling method as a coping technique for ICM incarceration with an incidence of 3.0% (6/199) (Figure 2). Additionally, Gu et al<sup>30</sup> reported an incidence of 5.5% (590/10 730) in human blastocysts. In contrast, animal data showed incidence rates of 44.2% (42/95)<sup>31</sup> and 38.6% (49/127; N. Aoyama, Kato Ladies Clinic, unpublished data) for ICM incarceration with zona breaching during the cleavage stage, indicating large differences between human and mice. Importantly, animal experiments are performed using time-lapse monitoring, whereas clinical data do not always include such detailed information. Furthermore, 52.5% (5633/10 730) of cases were fully hatched blastocysts, which could be included many cases of ICM incarceration.<sup>30</sup> Onodera et al<sup>31</sup> suggested that the location of 8-shaped hatching influences ICM formation in mouse blastocysts, however, this concept is still controversial. Indeed, clinical data have shown that ICM incarceration does not increase monozygotic twinning delivery.<sup>30</sup> If a part of ICM is aspirated together with TE cells during biopsy, although it depends on the number of TE cells aspirated, several cells reduction from the ICM may not have a huge effect on the embryo development after biopsy. This is because it has been hypothesized that ICM splitting may generate monozygotic twinning during the repeat of collapse and expansion in the process of blastulation in a clinical study with time lapse,<sup>32</sup> or in an 8-shaped hatching animal experiment.<sup>33</sup> However, not only the number of cell compensation, but also the chorionicity and amnionicity are also very important for normal fetal development after embryo splitting.<sup>34</sup> Moreover, cell fate has already been determined at the blastocyst stage<sup>35</sup> and ICM is formed by two cell lines, which are epiblasto and primitive endoderm in early blastocyst stage.<sup>36</sup> Therefore, full attention should be paid to TE biopsy, and the double laser zona drilling may be effective to avoid impact on ICM.

Additionally, zona breaching during the cleavage stage may induce herniation in early-stage blastocyst, which are thought to have fewer cells. Although the total cell number in blastocysts have not been fully elucidated, in 1989, Hardy et al<sup>37</sup> showed that mean numbers of TE cells were 37.9, 40.3, and 80.6 at 5, 6, and 7 days after insemination, respectively. In contrast, another study by Fong et al<sup>38</sup> reported that the cell numbers ranged from 160.9 to 236.7 in morphologically high-quality blastocysts and from 43.7 to 84.0 in poor-grade blastocyst on day 6 morning. Additionally, a recent report showed that the cell number were 225.2 and 121.1 in hatching and non-hatching blastocyst.<sup>39</sup> Thus, there may be greater differences among grades of blastocysts and






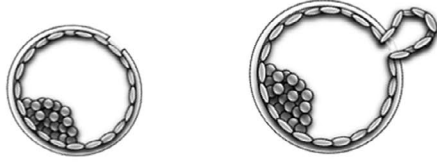




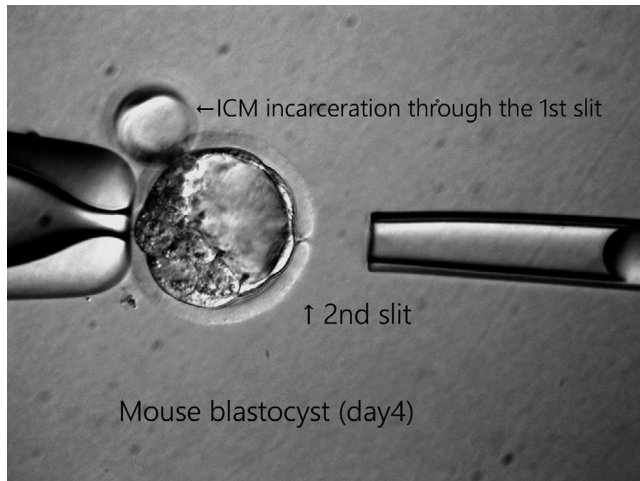
<b>(A) Biopsy method: hatching or non-assisted hatching?</b>	
<p><b>Hatching</b></p>  <ul style="list-style-type: none"> <li>•Trophectoderm herniation is extensively used for TE biopsy. (2005, McArthur, Fertil Steril.).</li> </ul>	<p><b>Non-hatching</b></p>  <ul style="list-style-type: none"> <li>•Perivitelline space dilation by expelling medium using a fine pipet makes it easier to aspirate TE cells (2014, Capalbo, Hum Reprod.).</li> </ul>
<p>Excess hatching may induce incarceration.</p>  <ul style="list-style-type: none"> <li>•There is no evidence demonstrating whether incarceration influences embryo development after biopsy. However, it may be preferable to avoid cryopreservation of segmentalized embryos through small zona pellucida hole. Practitioners may pay attention to the progression of hatching after zona breaching.</li> </ul>	<p>Is Non-hatching biopsy safe?</p>  <ul style="list-style-type: none"> <li>•Conventional embryo culture system up to the expanded blastocyst stage is conducted and no laser assisted breach is performed for the duration of culture. Practitioners can coordinate the timing of biopsy. However, the incidence of collapse during TE suction may be higher than in the assisted hatching protocol.</li> </ul>
<b>(B) Timing of zona drilling: 8 cell or blastocyst stage?</b>	
<p><b>8 cell stage</b></p>  <ul style="list-style-type: none"> <li>•Higher risk of ICM incarceration</li> <li>•Avoid ICM biopsy; Double laser drilling is recommended (2014, Rubino, Eur J Obstet Gynecol Reprod Biol.).</li> <li>•Wider perivitelline space</li> <li>•Easily handled for zona drilling</li> </ul>	<p><b>Blastocyst stage</b></p>  <ul style="list-style-type: none"> <li>•Lower risk of ICM incarceration</li> <li>•Evaluation of morphological assessment of blastocyst</li> <li>•Narrow perivitelline space</li> <li>Immerse blastocyst in hypertonic medium with 0.5 M sucrose solution to dilate perivitelline space.</li> </ul>
<b>(C) Size of hatching point (slit): small or large?</b>	
<p><b>Small hole hatching</b></p> 	<p><b>Large hole hatching</b></p>  <ul style="list-style-type: none"> <li>•Zona hole size dictates whether 8-shaped-hatching or U-shaped-hatching occurs. Small holes minimize the area of the cut plane of TE, whereas large holes lead to larger cut planes. Higher thermal energy is needed for dissection of thick TE cells and may be more invasive.</li> <li>•Suctioning pipettes (inner diameter 20-40µm) are commercially available, and smaller pipettes thin clumps of TE.</li> </ul>
<b>(D) Trophectoderm biopsy; Flicking? or pulling?</b>	
<p><b>Flicking</b></p>  <ul style="list-style-type: none"> <li>•Flicking may be recommended for TE dissection; however there is no evidence that flicking is better than pulling. Additionally, it requires time to learn this technique.</li> </ul>	<p><b>Pulling</b></p>  <ul style="list-style-type: none"> <li>•Easy and simple technique</li> <li>•It has been still unclear the toxicity of heat diffusion which must lie below 100°C, then a flick with quick movement within minimum range and number of laser pulse would be recommended.</li> </ul>

FIGURE 1 Summary of the critical point of trophectoderm biopsy



**FIGURE 2** Double zona drilling

time after insemination, and these differences may be products of the laboratory environment (incubator, culture medium), resulting in variations in embryonic developmental speeds. In either case, balancing the number of cells biopsied per total cell number may be important for applying TE biopsies, and obtaining full-size blastocyst may minimize invasion from biopsy. Moreover, continuation culture to full blastocysts may have additional benefits in evaluating morphological grading. Overall, zona breaching on days 3 to 4 after insemination may result in a higher risk of ICM incarceration with fewer benefits compared with zona breaching on day 5 to 6, thereby producing full blastocysts.

### 1.3 | The ideal procedure for trophectoderm biopsy

#### 1.3.1 | An appropriate number of biopsied TE cells is required to maintain implantation potential

The number of biopsied cells is one of the most critical factors affecting TE biopsy,<sup>40</sup> the general consensus among researchers is that this number may be 5 cells.<sup>26,41</sup> However, it is difficult to apply this in the clinical setting, and attempts have been shown to result in poor-grade TE and fragmentation. Fewer cell biopsies may be less invasive but result in a higher risk of amplification failure, whereas increased cell biopsies may lead to a lower risk of amplification failure but increase blastocyst disruption. Several clinical reports have described the incidence of DNA amplification and inconclusive results using a CCS device, at rates of 12.5% and 4.5-8.2% by polar body biopsy,<sup>42,43</sup> 2.9%-12.3% and 0%-18.6% by single blastomere isolation,<sup>44-48</sup> and 0%-4.0% and 0%-4.6% by TE,<sup>28,49-55</sup> respectively (Table 1). Blastocyst biopsy results in lower amplification failure rates because of the superior cell numbers obtained from biopsy comparing with that of polar body or blastomere biopsy. Cimadomo et al showed that 8.0 trophectoderm cells were required for successful DNA amplification and conclusive diagnostic results; thus, the most suitable cell number for biopsy may be 5-10 cells rather than

1-5 cells. Overall, the use of these invasive techniques for TE biopsy may reduce the rate of live births by 5% live births.<sup>56</sup>

#### 1.3.2 | When should we perform TE biopsy?

No conclusion has yet been reached regarding the best timing of TE biopsy, and we interpret that a point of contention may be the number of biopsied cell per the total cell number. The day 3 zona opening method may prompt hatching in early-stage blastocysts, which have 40-60 cells. On the other hand, zona opening in developing or developed blastocyst stage, which have 60-100 cell can avoid hatching in blastocysts with a fewer cell number. Goossens et al<sup>57</sup> reported that 16 the removal of one or two cells of blastomere in cleavage stage had a significant influence on embryo development on Day 5, that is; removing a smaller number of cells by embryo biopsy is less invasive for embryo development. Namely, removing 5 to 10 TE cells by biopsy may be less invasive for expanded blastocyst, which have a larger number of total cells than early-stage blastocyst, which have fewer cells. Actually, we performed TE biopsy when the blastocysts reached full size (>160  $\mu\text{m}$ ) with the hatching method in the pilot study of PGT-A,<sup>5</sup> because there is no way to assess the total cell number.

On the other hand, from the results of our clinical study, blastocysts, which have earlier developmental speed, showed better reproductive outcomes such as clinical pregnancy and live birth rate<sup>58</sup>; meanwhile, smaller size blastocysts (<160  $\mu\text{m}$ ) until day 7 had little chance to achieve clinical pregnancy. We hypothesize that biologically high-potential embryos reaching full-size blastocyst until 130 hours after insemination have a higher chance to be euploidy, similar to the report that day 7 blastocysts showed a lower euploidy rate.<sup>59,60</sup> Finally, the timing of biopsy may be better in the developed or developing blastocyst, which have larger cell numbers. Additionally, if an embryo has an earlier developmental speed, such as developed blastocyst in day 5, favorable result may be obtained.

#### 1.3.3 | Size of hatching point and time length to perform the biopsy

In general, single TE biopsy should be performed within 3 minutes.<sup>28</sup> This can be achieved using a simple biopsy. However, thick TE samples may be difficult to dissect from blastocysts. Thinning the TE area of the cut plane makes biopsy less difficult; thus, we suggest that smaller zona holes may produce less constriction of the hatching site for micro-cutting (Figure 1C). However, this process may be more complicated. Indeed, if a practitioner ignores the optimal timing of biopsy, a large 8-shaped hatching site may be generated, resulting in incarceration (Figure 1A). To prevent this, a time-lapse system may be useful. Furthermore, the size of the suction pipette for TE may be altered to minimize the area of the cut plane; several suction pipettes with inner diameters of 20-40  $\mu\text{m}$  have been developed and are available from commercial sources.

TABLE 1 The incidence of amplification and diagnostic failure using biopsied cell and reproductive outcome with PGT-A

Outcomes (WGA and diagnostic)		Reproductive outcomes									
		PGT-A group					Control group				
No. of cells	Amplification method	Amplification failure	CCS device	No available result	Age	Result	Age	Result	P-value	Reference	
Biopsy sample: PB1/PB2											
1-2	SurePlex	N/A	aCGH (24sure V3)	4.5% (24/530)	39.5	LB/transfer 26.4% (29/110)	38.4	LB/transfer 14.9% (60/403)	.015	2015, Feichtinger, PLoS One.	
1-2	SurePlex	12.5% (128/1023)	aCGH (24Sure)	8.2% (84/1023)	38.6	Pregnancy/1st transfer 38% (57/149) Cumulative LB rate/Pt. 24% (50/205)	38.6	Pregnancy/1st transfer 32% (54/171) Cumulative LB rate/Pt. 24% (45/191)	.71	2018, Verpoest, Hum Reprod.	
Biopsy sample: Blastomere											
1	DOP-PCR	12.3% (9/73)	metaphase-CGH	0.0% (0/64)	N/A	a validation report using surplus embryos	N/A	2000, Wells, Mol Hum Reprod.			
1	① GenomePlex ② SurePlex	11.2% (18/161) 2.9% (19/654)	aCGH (BlueGnome) aCGH	3.5% (5/143) 2.5% (16/634)	37.5	Pregnancy rate 57.9%/BT (44/76) 42.3%/OR (44/104)	N/A	N/A		2011, Gutierrez-Mateo, Fertil Steril.	
1	MDA	5.5% (5/91)	aCGH (8K)	18.6% (16/86)	N/A	a validation report using surplus embryos	N/A	2013, Mertzaniidou, Hum Reprod.			
1	PicoPLEX WGA	N/A	Ion PGM Sequencing	8.3% (21/252)	34.0	Implantaion rate 61.5% (40/65)	34.4	Implantaion rate 34.8% (31/89)	.001	2015, Lukaszuk, Fertil Steril.	
1	Sureplex	N/A	aCGH (24sure arrays V2)	2.8% (15/538)	38-41	Implantaion rate 52.8% (47/89)	38-41	Implantaion rate 27.6% (48/174)	<.0001	2017, Rubio, Fertil Steril.	
Biopsy sample: Trophectoderm											
5	qPCR	0% (0/71)	N/A	1.4% (1/71)	N/A	a validation report using cell lines	N/A	2012, Treff, Fertil Steril.			
5	qPCR	N/A	N/A	1.4% (7/483)	32.2	Implantaion rate 79.8% (107/134)	32.4	Implantaion rate 63.2% (103/163)	.02	2013, Scott, Fertil Steril.	
6-10	SurePlex SurePlex	1.5% (3/195)	① aCGH (24sure V3) ② NGS (VeriSeq)	6.3%* (12/191)	39.9	Implantaion rate 64.0% (32/50)	N/A	N/A		2014, Fiorentino, Hum Reprod.	
3-5	SurePlex SurePlex qPCR	1.4% (6/418) 2.1% (9/427) 1.2% (30/2586)	① aCGH (24sure) ② NGS (HiSeq 2000) N/A	0.0% (0/418) 0.0% (0/427) 4.6% (119/2586)	35.5 35.2 39.4	Implantaion rate 66.2% (88/133) Implantaion rate 70.2% (92/131) Implantaion rate 53.4% (264/494)	P = .56	N/A N/A N/A		2015, Yang Z, BMC Med Genomics. 2015, Capalbo, Hum Reprod.	

(Continues)

TABLE 1 (Continued)

Outcomes (WGA and diagnostic)		Reproductive outcomes								
		PGT-A group			Control group					
No. of cells	Amplification method	Amplification failure	CCS device	No available result	Age	Result	Age	Result	P-value	Reference
5-10	PicoPLEX	N/A	aCGH (SurePrint G3)	1.3% (3/224)	28-44	Implantaion rate 42.6% (26/61)		N/A		2016, Liu M, Reprod Biol Endocrinol.
2.1-7.5	qPCR	2.0% (176/8990)	N/A	0.5% (52/8990)	38.5	LB/transfer 38.8% (19/49)		N/A		2018, Cimadomo, Hum Reprod.
TE:5-10 ICM:1-10	SurePlex	4.0% (4/99)	②NGS (VeriSeq)	0.0% (0/95)	N/A, a validation report using surplus embryos					2018, Chuang TH, Mol Hum Reprod.

Abbreviations: CCS, comprehensive chromosomal screening; LB, live birth; MDA, multiple displacement amplification; N/A, not applicable; Pt, patient; WGA, whole genome amplification.

\*It is a non-concordant rate in the 2 methods.

### 1.3.4 | Trophectoderm biopsy technique using flicking or pulling

The first solid-state laser to be used for ART, which was applied to trap spermatozoa, was neodymium:yttrium–aluminum–garnet (Nd:YAG) at a wavelength of 1064 nm<sup>61,62</sup>; however, this method can have the adverse effect of potential risk of DNA damage from ultraviolet wavelength. Thereafter, erbium:YAG laser, which operates at a wavelength of 2900 nm was introduced<sup>63,64</sup>; however, it required direct contact using laser fiber, raising concerns related to damage and contamination. Furthermore, holmium:yttrium–scandium–gallium–garnet (Ho:YSGG)<sup>65,66</sup> laser using at 2100 nm wavelength exhibited different absorption behavior in water than earlier lasers; moreover, it required quartz slides. Several studies have applied ArF,<sup>67</sup> KrF,<sup>68</sup> XeCl<sup>69</sup>; however, they were impracticable. Finally, a non-contact 1480-nm diode laser-induced micro-drilling procedure has been introduced<sup>70</sup>; six types of lasers are now available for assisted reproductive technology based on infrared-emitting diodes currently in the market.<sup>71</sup> The use of this approach is helpful for shortening the duration and reducing the complexity of zona breaching and TE biopsy. However, the potential disadvantages of this approach have not been clarified. For example, the toxicity of heat diffusion, which must remain below 100°C, has not been determined.

Does the laser-assisted biopsy introduce mosaic or chaotic changes to biopsied cells? This discussion has just started, and there is also a controversy. At American Society for Reproductive Medicine meeting in 2017, Kelka et al<sup>72</sup> concluded that laser-assisted trophectoderm biopsy (TEB) did not have an impact on DNA profiles, however, at the European Society of Human Reproduction and Embryology meeting in 2019, Herrero et al<sup>73</sup> concluded that laser assisted TEB may increase the risk of mosaicism. This mechanism may not be complicated; the number of laser shots and power of laser pulse may influence the induction of mosaicism. However, sample quality and biopsy technique may influence the results. Further investigation is needed to assess this mechanism, and it is necessary to conclude whether flicking method is superior to pulling method (Figure 1D).

### 1.3.5 | Tubing procedure

Blastocoel fluid and blastocyst culture medium contain cell-free DNA, and PGT-A protocols have already been established using liquid biopsy technique<sup>74,75</sup> and culture media.<sup>76-79</sup> The efficacy of these minimally invasive PGT approaches is controversial,<sup>80-83</sup> and regardless of diagnostic accuracy of PGT. These data showed biopsied TE samples are contaminated with higher concentrations of cell-free DNA. Therefore, practitioners should be careful to avoid this contamination and may apply a washing step to improve purity. Furthermore, retrieval of fragmented TE cells may increase inconclusive results. Accordingly, practitioners should avoid suction of fragmented TE during the biopsy procedure. Unamplified samples mainly result from the absence of TE cells, and recent data have shown that the rate is <1.5% when analyzing 5 TE cells using a whole-genome amplification kit. To decrease the

**TABLE 2** Critical points for trophectoderm biopsy

Timing of zona breaching: day3-4 or day5-6
Zona hole size: small or large
How many cells biopsied?
Pipette size: 20-40 mm (commercially available)
Timing of TE sampling: herniation or direct suction
Biopsy method: pulling or flicking
Size of laser pulses, and times of the laser shot
Condition of the washing step of biopsied TE cells
Tubing procedures (sample loading)

rate of “No result” is very important because it is associated with a higher probability of error during the tubing procedure. We may avoid this error to a certain degree with a technique to confirm that a mass of TE cells is not brought out in the pipette with back current after sample loading in the PCR tube. By expelling the content of the capillary pipette into an empty dish, we can determine the remaining TE cells in the solution. If TE cells are retrieved in the dish, it is recommended to try sample loading again. Actually we experienced 2 cases of re-loading in 149 TE biopsies and these 2 blastocysts were completely analyzed, and we consequently achieved a 100% (149/149) of diagnostic rate.<sup>5</sup>

### 1.3.6 | Timing of cryopreservation after TE biopsy

There are two transfer strategies for euploidy embryos currently in clinical practice: vitrified-warmed or fresh embryo transfer. Biopsy of blastomeres on day 3 permits analysis of diagnostic outcomes before day 5 using advanced molecular biology techniques. Several reports have described PGT-A using the fresh transfer strategy.<sup>47,84,85</sup> However, the use of TE biopsy and vitrified-thawed blastocyst transfer protocols is increasing worldwide.<sup>86-90</sup> On the other hand, the optimal timing of vitrification after TE biopsy has not been thoroughly discussed. In one study, the optimal time was reported to be 10-15 minutes,<sup>91</sup> whereas another study reported that 30 minutes or less was the optimal.<sup>92</sup> In contrast, another report showed that the optimal time was >3 hours to enable blastocysts to reach re-expansion.<sup>93</sup> From our experience in a pilot study, most blastocysts are vitrified within 30 minutes after TE biopsy, enabling high rates of clinical pregnancy per transfer. However, additional clinical studies are needed to clarify the optimal protocol. In the step of euploidy blastocyst transfer after thawing, basically, we did not assume a special procedure for embryo transfer, and we performed a zona-free transfer, similar to the routine protocol in the pilot study.<sup>5</sup>

## 2 | CONCLUSION

The efficacy of PGT-A is still controversial, and various factors, including diagnostic accuracy (sensitivity and specificity) and biopsy and tubing procedures, affect reproductive outcomes. In particular, Paulson showed that PGT-A may cause 20% embryo loss.<sup>1</sup> Two

TE biopsy methods were used; however, the gold standard trophectoderm (TE) biopsy procedure has not established yet. Based on our experience, zona breaching on days 5-6 after insemination has greater benefits than that on days 3-4. Moreover, biopsy of herniated TE samples simplifies and shortens the procedure, and a non-assisted hatching protocol may make the approach more complex if blastocysts collapse during TE suction. Recent studies have indicated that excess laser pulses may induce a higher frequency of mosaicism; thus, a rapid flick within the minimum range and fewer laser pulses are recommended. Smaller zona holes produce thinner TE cells at the hatching point, which may facilitate biopsy. Five to 10 TE cells are recommended for biopsy to decrease amplification failure. Achieving proficiency in these various techniques (Table 2) is expected to lead to optimal results. Since, biopsy techniques have not been discussed extensively in the literature, we hope that this review will facilitate further advancements in the study of this procedure.

### ACKNOWLEDGEMENTS

None.

### CONFLICT OF INTEREST

The authors declare no conflict of interest.

### HUMAN AND ANIMAL RIGHTS

This article does not contain any studies with human and animal subjects performed by the any of the authors. This review focused on the literatures previously published and it did not contain ethical issues with human subjects; thus, there was no need for the study to be evaluated by an ethical committee.

### ORCID

Naoki Aoyama  <https://orcid.org/0000-0003-4944-908X>

### REFERENCES

1. Paulson RJ. Preimplantation genetic screening: what is the clinical efficiency? *Fertil Steril.* 2017;108(2):228-230. Epub 2017/07/13.
2. Murphy LA, Seidler EA, Vaughan DA, et al. To test or not to test? A framework for counselling patients on preimplantation genetic testing for aneuploidy (PGT-A). *Hum Reprod.* 2019;34(2):268-275. <https://doi.org/10.1093/humrep/dey346>
3. Aoyama NKM, Takehara Y, Kawachiya S, et al. A simple efficient method of blastomere fixation for preimplantation genetic diagnosis of aneuploidy with interphase FISH. *Fertil Steril.* 2006;86(Issue 3):S482-S483.
4. de Boer KA, Catt JW, Jansen RP, et al. Moving to blastocyst biopsy for preimplantation genetic diagnosis and single embryo transfer at Sydney IVF. *Fertil Steril.* 2004;82(2):295-298. Epub 2004/08/11.
5. Sato T, Sugiura-Ogasawara M, Ozawa F, et al. Preimplantation genetic testing for aneuploidy: a comparison of live birth rates in patients with recurrent pregnancy loss due to embryonic aneuploidy or recurrent implantation failure. *Hum Reprod.* 2019;34(12):2340-2348. Epub 2019/12/08.
6. Handyside AH, Kontogianni EH, Hardy K, et al. Pregnancies from biopsied human preimplantation embryos sexed by Y-specific DNA amplification. *Nature.* 1990;344(6268):768-770. Epub 1990/04/19.

7. Dokras A, Sargent IL, Ross C, et al. Trophoctoderm biopsy in human blastocysts. *Hum Reprod.* 1990;5(7):821-825. Epub 1990/10/01.
8. Dokras A, Sargent IL, Gardner RL, et al. Human trophoctoderm biopsy and secretion of chorionic gonadotropin. *Hum Reprod.* 1991;6(10):1453-1459. Epub 1991/11/01.
9. Hardy K, Martin KL, Leese HJ, et al. Human preimplantation development in vitro is not adversely affected by biopsy at the 8-cell stage. *Hum Reprod.* 1990;5(6):708-714. Epub 1990/08/01.
10. Jones AE, Wright G, Kort HI, et al. Comparison of laser-assisted hatching and acidified Tyrode's hatching by evaluation of blastocyst development rates in sibling embryos: a prospective randomized trial. *Fertil Steril.* 2006;85(2):487-491. Epub 2006/04/06.
11. De Vos A, Staessen C, De Rycke M, et al. Impact of cleavage-stage embryo biopsy in view of PGD on human blastocyst implantation: a prospective cohort of single embryo transfers. *Hum Reprod.* 2009;24(12):2988-2996. Epub 2009/09/24.
12. Tarin JJ, Conaghan J, Winston RM, et al. Human embryo biopsy on the 2nd day after insemination for preimplantation diagnosis: removal of a quarter of embryo retards cleavage. *Fertil Steril.* 1992;58(5):970-976. Epub 1992/11/01.
13. Dumoulin JC, Bras M, Coonen E, et al. Effect of Ca<sup>2+</sup>/Mg<sup>2+</sup>-free medium on the biopsy procedure for preimplantation genetic diagnosis and further development of human embryos. *Hum Reprod.* 1998;13(10):2880-2883. Epub 1998/11/06.
14. Munne S, Lee A, Rosenwaks Z, et al. Diagnosis of major chromosome aneuploidies in human preimplantation embryos. *Hum Reprod.* 1993;8(12):2185-2191. Epub 1993/12/01.
15. Verlinsky Y, Cieslak J, Ivakhnenko V, et al. Prepregnancy genetic testing for age-related aneuploidies by polar body analysis. *Genetic testing.* 1997;1(4):231-235. Epub 1997/01/01.
16. Munne S, Chen S, Fischer J, et al. Preimplantation genetic diagnosis reduces pregnancy loss in women aged 35 years and older with a history of recurrent miscarriages. *Fertil Steril.* 2005;84(2):331-335. Epub 2005/08/09.
17. Schoolcraft WB, Katz-Jaffe MG, Stevens J, et al. Preimplantation aneuploidy testing for infertile patients of advanced maternal age: a randomized prospective trial. *Fertil Steril.* 2009;92(1):157-162. Epub 2008/08/12.
18. Meyer LR, Klipstein S, Hazlett WD, et al. A prospective randomized controlled trial of preimplantation genetic screening in the "good prognosis" patient. *Fertil Steril.* 2009;91(5):1731-1738. Epub 2008/09/23.
19. Blockeel C, Schutyser V, De Vos A, et al. Prospectively randomized controlled trial of PGS in IVF/ICSI patients with poor implantation. *Reprod Biomed Online.* 2008;17(6):848-854. Epub 2008/12/17.
20. Hardarson T, Hanson C, Lundin K, et al. Preimplantation genetic screening in women of advanced maternal age caused a decrease in clinical pregnancy rate: a randomized controlled trial. *Hum Reprod.* 2008;23(12):2806-2812. Epub 2008/06/28.
21. Staessen C, Verpoest W, Donoso P, et al. Preimplantation genetic screening does not improve delivery rate in women under the age of 36 following single-embryo transfer. *Hum Reprod.* 2008;23(12):2818-2825. Epub 2008/10/22.
22. Staessen C, Platteau P, Van Assche E, et al. Comparison of blastocyst transfer with or without preimplantation genetic diagnosis for aneuploidy screening in couples with advanced maternal age: a prospective randomized controlled trial. *Hum Reprod.* 2004;19(12):2849-2858. Epub 2004/10/09.
23. Twisk M, Mastenbroek S, Hoek A, et al. No beneficial effect of preimplantation genetic screening in women of advanced maternal age with a high risk for embryonic aneuploidy. *Hum Reprod.* 2008;23(12):2813-2817. Epub 2008/06/24.
24. De Boer K, McArthur S, Murray C. First live birth following blastocyst biopsy and PGD analysis. *Reprod Biomed online.* 2002;4(Supplement 2):35. Epub 2002.
25. McArthur SJ, Leigh D, Marshall JT, et al. Pregnancies and live births after trophoctoderm biopsy and preimplantation genetic testing of human blastocysts. *Fertil Steril.* 2005;84(6):1628-1636. Epub 2005/12/20.
26. Schoolcraft WB, Fragouli E, Stevens J, et al. Clinical application of comprehensive chromosomal screening at the blastocyst stage. *Fertil Steril.* 2010;94(5):1700-1706. Epub 2009/11/27.
27. Capalbo A, Rienzi L, Cimadomo D, et al. Correlation between standard blastocyst morphology, euploidy and implantation: an observational study in two centers involving 956 screened blastocysts. *Hum Reprod.* 2014;29(6):1173-1181. Epub 2014/03/01.
28. Capalbo A, Ubaldi FM, Cimadomo D, et al. Consistent and reproducible outcomes of blastocyst biopsy and aneuploidy screening across different biopsy practitioners: a multicentre study involving 2586 embryo biopsies. *Hum Reprod.* 2016;31(1):199-208. Epub 2015/12/08.
29. Rubino P, Cotarello RP, Alteri A, et al. Trophoctoderm biopsy performed by a double laser zona drilling: first safety evidence. *Eur J Obstet Gynecol Reprod Biol.* 2014;181:344-345. Epub 2014/09/10.
30. Gu YF, Zhou QW, Zhang SP, et al. Inner cell mass incarceration in 8-shaped blastocysts does not increase monozygotic twinning in preimplantation genetic diagnosis and screening patients. *PLoS ONE.* 2018;13(1):e0190776. Epub 2018/01/10.
31. Onodera Y, Takahashi K, Goto M, et al. The location of "8"-shaped hatching influences inner cell mass formation in mouse blastocysts. *PLoS ONE.* 2017;12(4):e0175150. Epub 2017/04/07.
32. Mio Y, Maeda K. Time-lapse cinematography of dynamic changes occurring during in vitro development of human embryos. *Am J Obstet Gynecol.* 2008;199(6):660.e1-660.e5. Epub 2008/10/01.
33. Yan Z, Liang H, Deng L, et al. Eight-shaped hatching increases the risk of inner cell mass splitting in extended mouse embryo culture. *PLoS ONE.* 2015;10(12):e0145172. Epub 2015/12/19.
34. McNamara HC, Kane SC, Craig JM, et al. A review of the mechanisms and evidence for typical and atypical twinning. *Am J Obstet Gynecol.* 2016;214(2):172-191. Epub 2015/11/10.
35. Marikawa Y, Alarcon VB. Establishment of trophoctoderm and inner cell mass lineages in the mouse embryo. *Mol Reprod Dev.* 2009;76(11):1019-1032. Epub 2009/05/30.
36. Morris SA, Teo RT, Li H, et al. Origin and formation of the first two distinct cell types of the inner cell mass in the mouse embryo. *Proc Natl Acad Sci USA.* 2010;107(14):6364-6369. Epub 2010/03/24.
37. Hardy K, Handyside AH, Winston RM. The human blastocyst: cell number, death and allocation during late preimplantation development in vitro. *Development.* 1989;107(3):597-604. Epub 1989/11/01.
38. Fong CY, Bongso A. Comparison of human blastulation rates and total cell number in sequential culture media with and without co-culture. *Hum Reprod.* 1999;14(3):774-781. Epub 1999/04/30.
39. Iwasawa T, Takahashi K, Goto M, et al. Human frozen-thawed blastocyst morphokinetics observed using time-lapse cinematography reflects the number of trophoctoderm cells. *PLoS ONE.* 2019;14(1):e0210992. Epub 2019/01/17.
40. Neal SA, Fransiak JM, Forman EJ, et al. High relative deoxyribonucleic acid content of trophoctoderm biopsy adversely affects pregnancy outcomes. *Fertil Steril.* 2017;107(3):731-736 e1. Epub 2016/12/13.
41. Zhang S, Luo K, Cheng D, et al. Number of biopsied trophoctoderm cells is likely to affect the implantation potential of blastocysts with poor trophoctoderm quality. *Fertil Steril.* 2016;105(5):1222-1227 e4. Epub 2016/01/29.
42. Feichtinger M, Stopp T, Gobl C, et al. Increasing live birth rate by preimplantation genetic screening of pooled polar bodies using array comparative genomic hybridization. *PLoS ONE.* 2015;10(5):e0128317. Epub 2015/05/30.
43. Verpoest W, Staessen C, Bossuyt PM, et al. Preimplantation genetic testing for aneuploidy by microarray analysis of polar bodies



- in advanced maternal age: a randomized clinical trial. *Hum Reprod.* 2018;33(9):1767-1776. Epub 2018/08/08.
44. Wells D, Delhanty JD. Comprehensive chromosomal analysis of human preimplantation embryos using whole genome amplification and single cell comparative genomic hybridization. *Mol Hum Reprod.* 2000;6(11):1055-1062. Epub 2000/10/25.
  45. Gutierrez-Mateo C, Colls P, Sanchez-Garcia J, et al. Validation of microarray comparative genomic hybridization for comprehensive chromosome analysis of embryos. *Fertil Steril.* 2011;95(3):953-958. Epub 2010/10/26.
  46. Mertzaniidou A, Wilton L, Cheng J, et al. Microarray analysis reveals abnormal chromosomal complements in over 70% of 14 normally developing human embryos. *Hum Reprod.* 2013;28(1):256-264. Epub 2012/10/12.
  47. Lukaszuk K, Pukszta S, Wells D, et al. Routine use of next-generation sequencing for preimplantation genetic diagnosis of blastomeres obtained from embryos on day 3 in fresh in vitro fertilization cycles. *Fertil Steril.* 2015;103(4):1031-1036. Epub 2015/01/28.
  48. Rubio C, Bellver J, Rodrigo L, et al. In vitro fertilization with preimplantation genetic diagnosis for aneuploidies in advanced maternal age: a randomized, controlled study. *Fertil Steril.* 2017;107(5):1122-1129. Epub 2017/04/24.
  49. Treff NR, Tao X, Ferry KM, et al. Development and validation of an accurate quantitative real-time polymerase chain reaction-based assay for human blastocyst comprehensive chromosomal aneuploidy screening. *Fertil Steril.* 2012;97(4):819-824. Epub 2012/02/22.
  50. Scott RT Jr, Upham KM, Forman EJ, et al. Blastocyst biopsy with comprehensive chromosome screening and fresh embryo transfer significantly increases in vitro fertilization implantation and delivery rates: a randomized controlled trial. *Fertil Steril.* 2013;100(3):697-703. Epub 2013/06/05.
  51. Fiorentino F, Bono S, Biricik A, et al. Application of next-generation sequencing technology for comprehensive aneuploidy screening of blastocysts in clinical preimplantation genetic screening cycles. *Hum Reprod.* 2014;29(12):2802-2813. Epub 2014/10/23.
  52. Yang Z, Lin J, Zhang J, et al. Randomized comparison of next-generation sequencing and array comparative genomic hybridization for preimplantation genetic screening: a pilot study. *BMC Med Genomics.* 2015;8:30. Epub 2015/06/24.
  53. Liu M, Su Y, Wang WH. Assessment of clinical application of preimplantation genetic screening on cryopreserved human blastocysts. *Reprod Biol Endocrinol.* 2016;14:16. Epub 2016/04/10.
  54. Cimadomo D, Rienzi L, Romanelli V, et al. Inconclusive chromosomal assessment after blastocyst biopsy: prevalence, causative factors and outcomes after re-biopsy and re-vitrification. A multicenter experience. *Hum Reprod.* 2018;33(10):1839-1846. Epub 2018/09/22.
  55. Chuang TH, Hsieh JY, Lee MJ, et al. Concordance between different trophoctoderm biopsy sites and the inner cell mass of chromosomal composition measured with a next-generation sequencing platform. *Mol Hum Reprod.* 2018;24(12):593-601. Epub 2018/10/30.
  56. Scott RT Jr, Upham KM, Forman EJ, et al. Cleavage-stage biopsy significantly impairs human embryonic implantation potential while blastocyst biopsy does not: a randomized and paired clinical trial. *Fertil Steril.* 2013;100(3):624-630. Epub 2013/06/19.
  57. Goossens V, De Rycke M, De Vos A, et al. Diagnostic efficiency, embryonic development and clinical outcome after the biopsy of one or two blastomeres for preimplantation genetic diagnosis. *Hum Reprod.* 2008;23(3):481-492. Epub 2007/12/25.
  58. Kato K, Ueno S, Yabuuchi A, et al. Women's age and embryo developmental speed accurately predict clinical pregnancy after single vitrified-warmed blastocyst transfer. *Reprod Biomed online.* 2014;29(4):411-416. Epub 2014/08/19.
  59. Hernandez-Nieto C, Lee JA, Slifkin R, et al. What is the reproductive potential of day 7 euploid embryos? *Hum Reprod.* 2019;34(9):1697-1706. Epub 2019/08/10.
  60. Tiegs AW, Sun L, Patounakis G, et al. Worth the wait? Day 7 blastocysts have lower euploidy rates but similar sustained implantation rates as Day 5 and Day 6 blastocysts. *Hum Reprod.* 2019;34(9):1632-1639. Epub 2019/08/14.
  61. Tadir Y, Wright WH, Vafa O, et al. Micromanipulation of sperm by a laser generated optical trap. *Fertil Steril.* 1989;52(5):870-873. Epub 1989/11/01.
  62. Tadir Y, Wright WH, Vafa O, et al. Force generated by human sperm correlated to velocity and determined using a laser generated optical trap. *Fertil Steril.* 1990;53(5):944-947. Epub 1990/05/01.
  63. Feichtinger W, Strohmmer H, Radner KM. Erbium YAG laser for micromanipulation of oocytes and spermatozoa. *Lancet.* 1992;340(8811):115-116. Epub 1992/07/11.
  64. Strohmmer H, Feichtinger W. Successful clinical application of laser for micromanipulation in an in vitro fertilization program. *Fertil Steril.* 1992;58(1):212-214. Epub 1992/07/01.
  65. Neev J, Schiewe MC, Sung VW, et al. Assisted hatching in mouse embryos using a noncontact Ho:YSGG laser system. *J Assist Reprod Genet.* 1995;12(4):288-293. Epub 1995/04/01.
  66. Schiewe MC, Neev J, Hazeleger NL, et al. Developmental competence of mouse embryos following zona drilling using a non-contact holmium:yttrium scandium gallium garnet (Ho:YSGG) laser system. *Hum Reprod.* 1995;10(7):1821-1824. Epub 1995/07/01.
  67. Palanker D, Ohad S, Lewis A, et al. Technique for cellular microsurgery using the 193-nm excimer laser. *Lasers Surg Med.* 1991;11(6):580-586. Epub 1991/01/01.
  68. Blanchet GB, Russell JB, Fincher CR Jr, et al. Laser micromanipulation in the mouse embryo: a novel approach to zona drilling. *Fertil Steril.* 1992;57(6):1337-1341. Epub 1992/06/11.
  69. El-Danasouri I, Westphal LM, Neev Y, et al. Zona opening with 308 nm XeCl excimer laser improves fertilization by spermatozoa from long-term vasectomized mice. *Hum Reprod.* 1993;8(3):464-466. Epub 1993/03/01.
  70. Rink K, Delacretaz G, Salathe RP, et al. Non-contact microdrilling of mouse zona pellucida with an objective-delivered 1.48-microns diode laser. *Lasers Surg Med.* 1996;18(1):52-62. Epub 1996/01/01.
  71. Davidson LM, Liu Y, Griffiths T, et al. Laser technology in the ART laboratory: a narrative review. *Reprod Biomed online.* 2019;38(5):725-739. Epub 2019/03/30.
  72. Kelka DA, Sawarkarb SS, Liua Y, et al. Does laser assisted biopsy introduce mosaic or chaotic changes to biopsied cells? *Fertil Steril.* 2017;108(3, Supplement):O-215, e88.
  73. Herrero Grassa L, Cascales Romero L, Ortiz Salcedo JA, et al. Does the trophoctoderm biopsy technique affect the result of the genetic analysis in PGT-A cycles? *Hum Reprod Abstract book.* 2019;34(Supplement 1):O-248 i111.
  74. Tobler KJ, Zhao Y, Ross R, et al. Blastocoel fluid from differentiated blastocysts harbors embryonic genomic material capable of a whole-genome deoxyribonucleic acid amplification and comprehensive chromosome microarray analysis. *Fertil Steril.* 2015;104(2):418-425. Epub 2015/05/27.
  75. Capalbo A, Romanelli V, Patassini C, et al. Diagnostic efficacy of blastocoel fluid and spent media as sources of DNA for preimplantation genetic testing in standard clinical conditions. *Fertil Steril.* 2018;110(5):870-879.e5. Epub 2018/10/15.
  76. Wu H, Ding C, Shen X, et al. Medium-based noninvasive preimplantation genetic diagnosis for human alpha-thalassemias-SEA. *Medicine.* 2015;94(12):e669. Epub 2015/03/31.
  77. Shamonki MI, Jin H, Haimowitz Z, et al. Proof of concept: preimplantation genetic screening without embryo biopsy through analysis of cell-free DNA in spent embryo culture media. *Fertil Steril.* 2016;106(6):1312-1318. Epub 2016/08/28.
  78. Xu J, Fang R, Chen L, et al. Noninvasive chromosome screening of human embryos by genome sequencing of embryo culture medium for in vitro fertilization. *Proc Natl Acad Sci USA.* 2016;113(42):11907-11912. Epub 2016/10/30.

79. Jiao J, Shi B, Sagnelli M, et al. Minimally invasive preimplantation genetic testing using blastocyst culture medium. *Hum Reprod.* 2019;34(7):1369-1379. Epub 2019/06/30.
80. Magli MC, Pomante A, Cafueri G, et al. Preimplantation genetic testing: polar bodies, blastomeres, trophoctoderm cells, or blastocoelic fluid? *Fertil Steril.* 2016;105(3):676-683.e5. Epub 2015/12/15.
81. Hammond ER, Shelling AN, Cree LM. Nuclear and mitochondrial DNA in blastocoelic fluid and embryo culture medium: evidence and potential clinical use. *Hum Reprod.* 2016;31(8):1653-1661. Epub 2016/06/09.
82. Zhigalina DI, Skryabin NA, Artyukhova VG, et al. Preimplantation genetic diagnosis by blastocentesis: problems and perspectives. *Genetika.* 2016;52(1):5-13. Epub 2016/05/18.
83. Hammond ER, McGillivray BC, Wicker SM, et al. Characterizing nuclear and mitochondrial DNA in spent embryo culture media: genetic contamination identified. *Fertil Steril.* 2017;107(1):220-228 e5. Epub 2016/11/21.
84. Coates A, Kung A, Mounts E, et al. Optimal euploid embryo transfer strategy, fresh versus frozen, after preimplantation genetic screening with next generation sequencing: a randomized controlled trial. *Fertil Steril.* 2017;107(3):723-730.e3. Epub 2017/02/01.
85. Coll L, Parriego M, Boada M, et al. Transition from blastomere to trophoctoderm biopsy: comparing two preimplantation genetic testing for aneuploidies strategies. *Zygote.* 2018;26(3):191-198. Epub 2018/05/26.
86. Huang J, Yan L, Lu S, et al. Validation of a next-generation sequencing-based protocol for 24-chromosome aneuploidy screening of blastocysts. *Fertil Steril.* 2016;105(6):1532-1536. Epub 2016/02/24.
87. Rodriguez-Purata J, Lee J, Whitehouse M, et al. Reproductive outcome is optimized by genomic embryo screening, vitrification, and subsequent transfer into a prepared synchronous endometrium. *J Assist Reprod Genet.* 2016;33(3):401-412. Epub 2016/01/16.
88. Parriego M, Coll L, Vidal F, et al. Inconclusive results in preimplantation genetic testing: go for a second biopsy? *Gynecol Endocrinol.* 2019;35(1):90-92. Epub 2018/09/06.
89. Nazem TG, Sekhon L, Lee JA, et al. The correlation between morphology and implantation of euploid human blastocysts. *Reprod Biomed online.* 2019;38(2):169-176. Epub 2018/12/24.
90. Cimadomo D, Soscia D, Vaiarelli A, et al. Looking past the appearance: a comprehensive description of the clinical contribution of poor-quality blastocysts to increase live birth rates during cycles with aneuploidy testing. *Hum Reprod.* 2019;34(7):1206-1214. Epub 2019/06/28.
91. Reed ML, Said AH, Thompson DJ, et al. Large-volume vitrification of human biopsied and non-biopsied blastocysts: a simple, robust technique for cryopreservation. *J Assist Reprod Genet.* 2015;32(2):207-214. Epub 2014/12/04.
92. Cimadomo D, Capalbo A, Levi-Setti PE, et al. Associations of blastocyst features, trophoctoderm biopsy and other laboratory practice with post-warming behavior and implantation. *Hum Reprod.* 2018;33(11):1992-2001. Epub 2018/09/29.
93. Chen HH, Huang CC, Cheng EH, et al. Optimal timing of blastocyst vitrification after trophoctoderm biopsy for preimplantation genetic screening. *PLoS ONE.* 2017;12(10):e0185747. Epub 2017/10/06.

**How to cite this article:** Aoyama N, Kato K. Trophoctoderm biopsy for preimplantation genetic test and technical tips: A review. *Reprod Med Biol.* 2020;19:222-231. <https://doi.org/10.1002/rmb2.12318>