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Original Research

Preimplantation Genetic Testing: Personal Views Regarding the Invasiveness of Trophectoderm Biopsy and Risks on Embryos Development "An Operators Survey"

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Abstract

This study aims to assess the Trophectoderm (TE) biopsy practice in Jordan in terms of the following effectiveness parameters: timing of zona breaching, risk of inner cell mass herniation if zona breaching was done on day 3, timing of TE sampling, method of biopsy (pulling or flicking), number of laser pulses, assessment of embryo survival after biopsy, and degeneration rate. An online cross-sectional survey was conducted in November 2022. The collected data presented the perception of embryologists (>10 years experience) about the difficulty of the technique and the awareness of the risks it imposes on embryonic development. Potential predictors of embryologists' awareness of the risks of trophectoderm biopsy in preimplantation genetic testing (PGT) and procedure difficulty were investigated. 125 embryologists were eligible, and 72 (57.6%) adequately filled the questionnaire, of which 51 (70.8%) perceived the procedure as moderately difficult.

However, 8 (11.1%) embryologists perceive it as very difficult. Regarding the preferred time of zona breaching, 39 (54.2%) of embryologists perform zona breaching on day 5 of embryonic life. 68% claim they primarily use flicking when performing TE biopsy. Moreover, 33 (45.8%) of the 72 surveyed embryologists claimed they use 2-3 laser pulses, and 56 (77.8%) claimed it takes 2 to 3 minutes to finish the procedure. Regarding the embryologists' awareness of the risk of Inner Cell Mass (ICM) herniation, most embryologists 46 (64%) believed there is a moderate risk if zona breaching is done on day 3. 23 (32%) acknowledge the procedure as having a low risk for embryonal development. 29 (40.3%) of embryologists assess survival by checking the re-expansion of the biopsied blastocyst after 2 hours, while 18% check blastocyst re-expansion after 15 minutes. 39 (54.2%) claimed that the incidence of degeneration rate post-TE biopsy is rare. TE biopsy strategy is one of the most promising biopsy techniques in PGT. Most embryologists in Jordan perceive the procedure as moderately difficult due to the technical considerations involved in performing the optimum TE biopsy.

Keywords
Trophectoderm biopsy (TE); embryologist; preimplantation genetic testing (PGT)

1. Introduction

One of the most important aims of assisted reproductive technologies (ART) is to select genetically normal embryos and improve clinical pregnancy rates [1]. A challenge of ART is the incidence of chromosomal abnormalities. An estimated more than 50% of the first trimester miscarriages are due to chromosomal abnormalities, with aneuploidy making the majority of them [2, 3]. Consequently, Preimplantation Genetic Testing (PGT) was developed as a clinical tool for genetic analysis and evaluation of embryos before transfer and implantation [4]. Biopsies are necessary in order to perform PGT. They can be taken in either of the following stages: the oocyte (one or two polar bodies), a cleavage-stage embryo (one blastomere cell), or the blastocyst stage embryo (5 to 10 trophectoderm cells) [5]. The blastocyst-stage biopsy, also known as Trophectoderm Biopsy (TE), consists of removing 5 to 10 trophectoderm cells on day 5 or 6 [6]. The cell sample can subsequently undergo testing for aneuploidy and single-gene disorders. This sampling procedure might have limitations, including misdiagnosis due to technical errors [7]. Despite the risks that come with trophectoderm biopsy, testing during the blastocyst stage has a lower prevalence of lethal chromosomal monosomies and other abnormalities than do cleavage-stage embryos [8, 9]. This fact makes the effort of embryo biopsy and testing more efficient in the blastocyst stage.

While this procedure is commonly employed in many in vitro fertilization (IVF) centers, achieving promising beneficial results through TE biopsy at the blastocysts stage is only possible under well-equipped IVF laboratories by highly skilled embryologists who are aware of important technical factors, including the timing of zona breaching, zona hole size, number of cells biopsied, pipette size, timing of TE sampling, pulling or flicking methods of biopsy, range and frequency of laser pulses, condition of the washing step of biopsied TE cells, and sample loading procedure [5].

Our study aims to assess the knowledge and attitudes towards TE biopsy practice (for preimplantation genetic testing of aneuploidies to select euploid blastocysts) in Jordan among senior clinical embryologists in terms of timing of zona breaching, risk of inner cell herniation, zona breaching, timing of TE sampling, method of biopsy, number of laser pulses, embryo survival after biopsy, and degeneration rate.

Ethical committee approval was obtained from the Hashemite University, Zarqa, Jordan.

Ref. Number: IRB 4/9/2022/2023. As it is a survey, patients' consent was waived by the ethical committee.

Informed consent was obtained from all participants (the embryologists) before participating in the study by answering an "I agree/disagree" question at the beginning of the survey. No private or identifying information was collected through the form.

2. Material and Methods

2.1 Study Design

An online cross-sectional survey was conducted among clinical embryologists in Jordan in November 2022. The online questionnaire was conducted using Google Forms and distributed to embryologists through social media platforms (WhatsApp and Facebook). The study population included currently employed Senior embryologists (with more than 10 years of experience) working in hospitals and laboratories in Jordan who performed trophectoderm biopsies. Embryologists with no practical expertise in trophectoderm biopsy in PGT were excluded. Participants of the study consented prior to filling out the questionnaire form. Five to ten minutes were required to complete the online survey.

2.2 Questionnaire Development

The questionnaire items were developed by the research team in English and validated by experts in the field. It consists of two parts (Table S1); the first has two main sections covering two main aspects, and it tackles embryologists' perception of the technique's difficulty, while the second aspect assesses their awareness of the risks that trophectoderm biopsy imposes on embryo development. The second part tackled specific questions to assess embryologists' awareness of TE biopsy effectiveness parameters, including timing of zona breaching, risk of ICM herniation on day 3, timing of TE sampling, method of biopsy, number of laser pulses, assessment of embryo survival after biopsy, and degeneration rate.

2.3 Data Analysis

After data collection, all completed questionnaire forms were included and analyzed using SPSS (IBM Corp. Released 2017. IBM SPSS Statistics for Windows, Version 25.0. Armonk, NY: IBM Corp.). Descriptive categorical (counts and percentages) statistics were calculated and used to disclose findings.

3. Results

3.1 Awareness of Invasiveness of TE Biopsy Technique

3.1.1 Procedure Difficulty

125 embryologists were eligible, and 72 (57.6%) completed and submitted the questionnaire. In response to the question about their evaluation of the difficulty of trophectoderm biopsy as a procedure (Table S2), 51 (70.8%) perceive the procedure as moderately difficult, 8 (11.1%) perceive it as very difficult, and 13 (18.1%) believe it is an easy procedure.

3.1.2 Time of Zona Breaching

72 embryologists completed the second part of the questionnaire. Regarding zona breaching timing, 54.2% perform zona breaching on day 5, 40.2% perform this step on day 3, and the remaining 5.6% perform zona breaching on day 4 of embryonic development.

3.1.3 Method of TE Biopsy

The method used for TE biopsy, according to the embryologists' practice of the 72 surveyed embryologists, 68% claim they primarily use flicking when performing TE biopsy, 28% use the pulling technique, and 4% use both techniques in their practice.

3.1.4 The Number of Laser Pulses

The number of laser pulses used in their approach was also assessed. Pulling approach, 45.5% of the 72 surveyed embryologists claim they use 2-3 laser pulses, while 40.9% use a higher range of 3-5 laser pulses. A small percentage (9.1%) use more than 5 laser pulses using the pulling approach. However, 4.5% claim to utilize 2-3 pulses with the flicking approach.

3.1.5 Duration of TE Biopsy

Regarding the time embryologists take to complete the TE biopsy (including the preparation time), 77.8% claim it takes 2 to 3 minutes to finish the procedure, and the remaining 22.2% need approximately 3 to 5 minutes to complete it.

3.1.6 Risk of Inner Cell Mass (ICM) Herniation

In the second part of the survey, we also assessed the embryologists' awareness (their perception based on their experience) of the risk of ICM herniation if zona breaching is done on Day 3. 32% of the sample claimed that the risk of ICM herniation is low, 64% state that there is a moderate risk, and 4% consider ICM herniation risk is high and significant.

3.2 Awareness of TE Biopsy Technique Risks on Embryonic Development

Questions about the awareness of the risks trophectoderm biopsy imposes on embryo development (Table S3): 57 (79.2%) acknowledge it is of low risk, 11 (15.3%) believe it is highly risky, and 4 (5.5%) believe it imposes zero risks on the developing embryo.

3.2.1 Assessment of Blastocyst Survival After TE Biopsy

Assessing blastocyst survival is one of the indicators of TE biopsy effectiveness. We have inquired about the methods used by embryologists to assess blastocyst survival after the procedure. Most embryologists 40.3% assess survival by checking the re-expansion of the biopsied blastocyst after 2 hours, while 18% check blastocyst re-expansion after 15 minutes. On the other hand, 36.1% of embryologists use immediate freezing regardless of re-expansion to assess blastocyst survival, while 5.6% freeze the biopsied blastocyst one hour after the procedure.

3.2.2 Degeneration Rate Post TE Biopsy

54.2% claim that the incidence of degeneration post TE biopsy (before vitrification) is rare, 9.7% often report incidents of degeneration, and 36.1% claim that they have never encountered this complication during their practice.

4. Discussion

Of most embryologists, 70.8% perceive the procedure as moderately difficult. 8 (11.1%) embryologists perceive it as difficult, and 13 embryologists, 18.1%, believe it is easy. This discrepancy could be attributed to the variation in the embryologists' expertise. Nevertheless, a retrospective study has been conducted on 2586 consecutive blastocyst biopsies, showing a highly consistent and reproducible approach to blastocyst biopsy among embryologists from different IVF centers when the practitioners have similar training and use similar laboratory settings [10]. These results were confirmed by the consistency of genetic and clinical outcomes in terms of the quality of genetic analysis and the rate of biochemical pregnancy, implantation, and miscarriage after frozen euploid embryo transfer cycles. Another retrospective study showed no significant difference in euploidy rate, low mosaicism, or no results after experienced embryologists and newly trained ones had operated TE biopsy. However, it was compelling to find that experienced embryologists had a significantly higher level of mosaicism than less experienced practitioners, which could be related to difficult cases usually being performed by more experienced operators [11].

The consideration of many technical factors in achieving a successful TE biopsy includes timing of zona breaching, biopsy method, number of laser pulses, TE biopsy duration, and risk of inner cell mass (ICM) herniation. In this study, more than half of the embryologists 54.2% in Jordan perform zona drilling on day 5 at the blastocyst stage, while the rest 40.2% prefer to do zona breaching on day 3, and a tiny percentage 5.6% may do it on day 4. Even though zona drilling could be easier on day 3 due to the larger perivitelline space, this procedure has more advantages when done on day 5 due to the lower risk of ICM herniation and better assessment of blastocyst morphology [12].

Another study has supported the approach of zona drilling on day 5 and reported a significantly higher rate of mosaic blastocysts combined with zona opening on day 3 than on day 5. However, no statistically significant difference was found in the rate of a euploid and aneuploid blastocyst or the clinical pregnancy outcome in the blastocysts following the two different approaches of zona opening time [13].

In Jordan, the flicking method of TE biopsy without using laser pulses is the dominant technique over the pulling method and laser shots to cut the pulled cells out of the trophectoderm. TE biopsy presents challenges, necessitating the assurance of the safety of both the biopsied blastocyst and the extracted trophectoderm (TE) cells. A recent study was conducted to assess the effect of the two methods (flicking and pulling) on the integrity of the pulled TE cells and to study the impact of each method on the euploidy rate of the blastocysts. The results demonstrated that the integrity of the excised cells was higher in the pulling method than in the flicking method. However, if both procedures were implemented correctly, no effect was reported on the euploidy rate in either procedure [14].

Also, laser pulses during the TE pulling mechanism do not impact the DNA profiles of the extracted cells [15]. On the other hand, using laser pulses in the pulling technique may induce mosaicism in the biopsied cells as the genetic materials from these damaged cells (by the heat produced by laser) might affect the sequencing results, leading to an overdiagnosis of normal embryo mosaics [12, 16].

In our study, 50% of the embryologists used 2-3 laser pulses during TE biopsy, 40.9% used 3-5 laser pulses, and a small percentage used more than 5. This approach is consistent with some published reports that indicated the use of 3-5 laser pulses during TE biopsy [13, 17]. Even with higher intensity, repeated application of laser pulses may not affect the genetic results of the PGT-A. However, it may adversely affect the embryo and reduce its implantation rate [18]. This survey showed that most embryologists 77.3% need 2-3 minutes to complete the TE biopsy procedure, consistent with the literature, while 22.7% need 3-5 minutes to finish this procedure. The risk of inner cell mass (ICM) herniation after zona breaching on day 3 is a valid possibility, and 63.6% of embryologists believe there is a moderate risk. However, 31.8% think the risk is low, and only 4.6% find the risk significantly high [12]. Few studies have investigated the association between ICM herniation following laser-assisted hatching of the zona pellucida on day 3 before TE biopsy on day 5 and the incidence of monozygotic twinning (MZT). Gu et al., 2018 reported a higher grade of ICM and a lower grade of TE in 8-shaped blastocysts with ICM herniation compared to partially and fully hatched blastocysts. Moreover, they have not detected any effect on the rate of MZT or a negative impact on the neonatal outcome of the PGT patients [19]. On the other hand, Sellers *et al.,* 2021 studied the rate of MZT following PGT cycles at the blastocyst stage with zona drilling on day 3, compared to non-PGT. Their results showed that the MZT rate in PGT cycles was 3.5% versus 0.8% in ICSI cycles. They concluded that embryo biopsy on day 5 for PGT increases the MZT rate. This discrepancy in the published data should be considered, and further work is still needed to validate the timing of zona drilling to avoid any damage to the ICM during blastocyst biopsy [20].

Blastocyst recovery after TE biopsy, represented by re-expansion of blastocoel, is one of the most critical indicators that affect the future blastocyst implantation and pregnancy outcome.

Cryopreservation of biopsied blastocysts for future embryo transfer is the worldwide gold standard. In the current survey, 40.9% of the embryologists assess the recovery of blastocoel

expansion after two hours, and the others vary between 15 minutes (18.2%) and one hour (4.6%). Nevertheless, 36.4% of them freeze blastocysts immediately after blastocyst biopsy, regardless of the re-expansion status. The literature shows a discrepancy between the proper timing of blastocyst vitrification post TE excision. One study has reported a 96.9% survival rate of cryopreserved-thawed biopsied blastocysts that were vitrified after 10-15 minutes using a large-volume vitrification method [21]. Another study revealed that the vitrification of the blastocysts was after 30 min from TE biopsy while they were still collapsed. The overall re-expansion rate of the cryopreserved blastocysts was 97.5% after warming [22]. The finding above aligns with the data from a pilot study, which suggested a high clinical pregnancy rate when blastocyst vitrification was conducted within 30 minutes [12]. Conversely, another study conducted by Chen *et al.* in 2017 showed that the optimal timing of biopsied blastocyst is more than 3 hours with a 100% survival rate [23]. The incidence of degenerated embryos post-biopsy was reported as "rare" by 54.2% of the embryologists, and 9.7% answered "often" in this survey. However, 36.1% affirmed that they had not encountered any incidental embryo loss in their experience. The safety of trophectoderm biopsy tends to be higher than blastomere biopsy due to different reasons indicated in one study, including the percentage of one biopsied blastomere out of 8-cell stage embryo representing 13% of the total cell content. In contrast, 5 cells biopsied from around 200 trophectoderm cells may represent around 2-3% of the total cell content [24]. Another reason is that the trophectoderm cells are already committed to an extra-embryonic cell, but the fate of each biopsied blastomere is still uncertain. In addition, the tolerance of the blastocyst to micromanipulation is higher than that of the cleaved embryo since the blastocyst has already started the genomic activation, as embryo loss may reach 20% during the blastocyst biopsy procedure [25].

5. Strength and Limitations

This research is the first study in the field to assess the level of embryologist expertise and awareness around the technicalities of TE biopsy and its risks to embryonic development in Jordan. The survey was distributed through online social media platforms to recruit participants. However, the sample size was relatively small due to limited access to the survey, as only embryologists with social media profiles had access to it. Moreover, the subjectivity of each answer is dependent on the operator's perceptions rather than a measured value. A possible selection bias could be present due to the voluntary, non-randomized recruitment of participants.

6. Conclusion

TE biopsy strategy is one of the most promising biopsy techniques in preimplantation genetic testing. Most embryologists in Jordan perceive the procedure as moderately difficult, recognizing the technical considerations involved in performing the optimal TE biopsy. Further large-scale statistical data and intensive discussion should be carried out to reach a consensus on the best practice of blastocyst biopsy to optimize embryologist proficiency skills and provide the best clinical service to patients for healthy baby outcomes. We suggest that more training and practice will broaden the technique's use in the country.

Author Contributions

F.R: Research question, data collection, manuscript editing. L.A: Manuscript writing, final Editing. N.Y: Data analysis, manuscript writing. R.A: Data analysis, manuscript writing. A.F: Questionnaire development, data collection. All authors read and approved the final manuscript. Acknowledgements: None.

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Competing Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

Data Availability Statement

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Additional Materials

The following additional materials are uploaded at the page of this paper.

1. Table S1: Questions that the embryologists answered.
2. Table S2: Awareness of invasiveness of TE Biopsy technique.
3. Table S3: Awareness of TE biopsy risks on embryonal development.

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