Laser-assisted zona opening and trophectoderm biopsy at the blastocyst stage: a video guide

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Summary

There are mainly two protocols to perform trophectoderm biopsy. The mostly used approach shows some intrinsic putative drawbacks due to the need for a zona pellucida drilling step at the cleavage stage. On the contrary, a more recent approach does not entail this step, since the opening of the zona pellucida and the retrieval of the biopsy fragment are conducted simultaneously. Indeed, the embryo may be left undisturbed up to the fully-expanded blastocyst stage without any potential perturbation to its development. This paper is a video guide to this latter biopsy protocol.

KEY WORDS: blastocyst biopsy, trophectoderm, PGD, PGS.

Introduction

There are mainly two protocols to perform trophectoderm (TE) biopsy.

The first method (1, 2) entails the opening of the Zona Pellucida (ZP) in day 3 of embryo development through 2-3 laser shots and embryo culture up to the blastocyst stage. Potentially, some TE cells will hatch from that hole and their laser-assisted removal will be an easy procedure. Even if this is the protocol mainly used worldwide to conduct TE biopsy, it shows some limitations: embryo culture should be interrupted in day 3 of embryo development to drill the ZP, an unnecessary source of stress; further embryo development could be impacted in the processes of ZP thinning, blastocyst expansion, increase of the number of TE cells; there is a moderate risk for the herniation of cells of the Inner Cell Mass (ICM), which would make the procedure delicate.

The second method has been described in 2014 (3) and does not entail any micromanipulation in day 3, thus embryo development is preserved from a sub-optimal environment up to the blastocyst stage. This protocol entails the selection of the TE cells to retrieve, since ZP opening and biopsy are sequentially conducted whenever the blastocyst reaches full expansion (day 5, 6 or 7). The ICM, in particular, will be clearly visible, and it is thus prevented from being accidentally involved in the procedure. Specifically, ZP opening is targeted to an area of the blastocyst on the other side with respect to the ICM, then some media is blown through the biopsy pipette to make the TE gently collapse and retrieve 5-10 cells. The cells are moved out from the ZP to expose the junctions between them and the body of the blastocyst. Three-five laser shots are targeted to these junctions to remove the TE fragment. This method is compatible with the use of a continuous culture media and time-lapse incubators. Theoretically then, the embryo can be left undisturbed to develop as a fully-expanded blastocyst.
and cultured in an ideal environment from ICSI up to biopsy.

Importantly, any embryo that reaches the fully-expanded blastocyst stage is candidate for TE biopsy, regardless its morphology and developmental rate assessed following either a static or dynamic approach (3, 4).

No studies have been performed up to date to compare the two different protocols. It is though reasonable to think that the less stress is introduced during embryo development, the lower the putative impact on embryo reproductive competence.

A multicenter study by Capalbo et al. (5) has recently investigated the reproducibility of the TE biopsy method without ZP opening in day3 among 7 different operators who performed more than 2500 blastocyst biopsies. No differences were reported for both technical (qPCR-based analysis was performed for aneuploidy testing) and clinical outcomes among them. Importantly, the no amplification rate was just ≈1% and the mean estimated number of cells to retrieve to obtain a good quality of the chromosomal analysis was reported as ≈7.

It is advisable to implement an efficient blastocyst culture and vitrification system before introducing TE biopsy in the clinical practice of an IVF centre (6), as well as to perform a failure modes and effects analysis to guarantee the safety and the traceability of the whole procedure (7).

Hereafter a brief description of the “ZP opening and TE biopsy at the blastocyst stage” procedure shown in the video.

Biopsy protocol

The procedure should be performed in presence of a witness and with DNA-free personal protective equipment and devices. A mouth-controlled biopsy pipette should be used:

1) Orient the blastocyst to have the TE cells to be retrieved on the opposite side with respect to the ICM. They should be small and accessible. Fasten the blastocyst with the holding pipette [0’5’’].
2) Move to the 40X laser objective. Start firing to drill the ZP. The hole should be large enough to allow the biopsy pipette to enter the ZP [0’13’’].
3) Start blowing some media with the biopsy pipette through the hole to make the TE gently collapse [0’18’’].
4) Suck 5-10 cells with the biopsy pipette and slowly start moving backwards [0’33’’].
5) Once out of the ZP, start firing with the laser to the junctions between the cells and the body of the biopsy. Keep moving backwards with the biopsy pipette while firing to expose and loosen the junctions. Three-five shots are sufficient to remove the biopsy fragment [0’44’’].
6) Release the TE fragment in an area of the dish sufficiently far from the blastocyst [0’50’’].
7) The blastocyst takes 1.5-2 hours to re-expand after the TE biopsy [1’14’’]. In case the blastocyst is to be cryopreserved, it is suggested to prevent it from re-expanding by vitrifying it within 30-45 minutes after the procedure.

References