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Original Research

Which is the safer method for trophectoderm biopsy in mouse blastocyst, mechanical or laser?

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Summary

Introduction: This study was conducted to compare hatching rates after assisted hatching, re-expansion rates after trophectoderm biopsy, and survival rates after cryopreservation using different methods of assisted hatching and biopsy in mouse embryo. *Materials and Methods:* Five-week-old female mice (C57BL/CBA) were superovulated, and two-cell embryos were collected. All embryos were cultured to blastocyst stage. For assisted hatching and separating trophectoderm from blastocyst, laser device and hand-made pipette were used respectively. Hatching rates after assisted hatching, re-expansion rates after trophectoderm biopsy, and survival rates after cryopreservation were calculated. *Results:* Hatching rate was 92% in mechanically assisted hatching group and 90% in laser group, respectively. After mechanically assisted hatching, re-expansion rate was 91.3% and survival rate was 87% in biopsy by pipette and laser group, respectively. In laser hatching group, re-expansion rate was 88.9% with biopsy by pipette and survival rate was 84.4% with biopsy by laser. *Conclusion:* Throughout the study, mechanical technique and laser technique showed no differences in the safety profiles in trophectoderm biopsy procedure.

Key words: Trophectoderm; Preimplantation genetic diagnosis; IVF; Laser.

Introduction

Preimplantation genetic screening (PGS) was introduced in 1993, based on a hypothesis that pregnancy rates would be increased and abortion rates reduced by selecting normal chromosomal oocytes and embryos during in vitro fertilization [1]. However, approximately 15 years later, first-generation PGS (PGS-1.0) proved to be ineffective in increasing pregnancy rate and reducing abortion rates [2]. These disappointing results have been explained with three reasons: 1) damage to embryos during cleavage period after blastomere biopsy, 2) incomplete and limited examination of the chromosomal state by using FISH, and 3) mosaicism caused by abnormal biopsy of the embryo [3].

During the same period, enormous improvements were achieved in the assisted reproductive field, such as the use of continuous culture media combined with a low oxygen tension culture system and the introduction of vitrification for blastocyst freezing. Due to such improvement, the whole process of culture to blastocyst stage and cryopreservation of blastocyst became more efficient than before. The second-generation PGS (PGS-2.0) was then introduced, based on this high efficiency in culture and cryopreservation of blastocyst [4-6]. Combined with comprehensive chromosome screening (CCS), the blastocyst biopsy is now the most promising method to detect aneuploidy developed during meiosis and mitotic errors of embryo during pre-implantation period [7-9].

Two important steps of trophectoderm biopsy (TE) are the assisted hatching procedure at eight-cell stage embryo or blastocyst stage and the separation of five to ten trophectoderms from blastocyst. Laser device is applied in these two important steps of TE biopsy. However, concerns for negative effects of laser device, such as thermal damage to embryo development potential, have been raised after application of laser device. Regarding the use of mechanical method, there is no concern about thermal effect.

This study was conducted to compare hatching rates after assisted hatching, re-expansion rates after TE biopsy, and survival rates after cryopreservation using different methods of assisted hatching and biopsy in mouse embryo.

Material and Methods

Flow of this study was presented in Figure 1. For hatching rate comparison, 100 mouse blastocysts were used in each group. For re-expansion rate and survival rate comparison, half of mouse blastocysts in each group was used for TE biopsy in each four methods.

Five-week-old female mice (C57BL/CBA) were super-ovulated by an intraperitoneal injection of 5 IU of pregnant mare serum gonadotropin (PMSG) followed 48 hours later by 5 IU of hCG, and immediately paired with males of the same strain. On the following morning, mating was confirmed by checking for a vaginal plug. Forty-eight hours after hCG injection, two-cell embryos were collected and cultured in groups of 10 in 30 μL dr-

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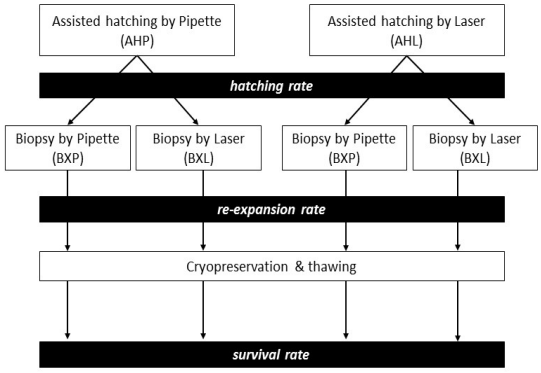

Figure 1. — Overview of study.

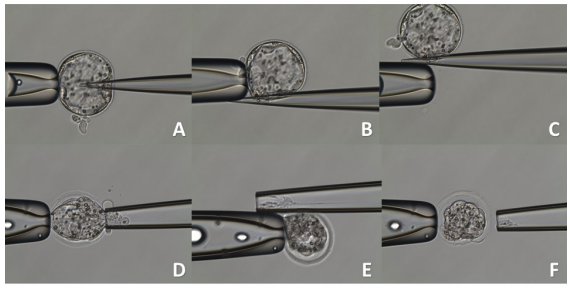

Figure 2. — Assisted hatching and trophectoderm biopsy by mechanical method. A) Hatching pipette insertion in the perivitelline space. B) Penetration of opposite zona pellucida. C) Rubbing zona pellucida with assisted hatching pipette and holding pipette. D) Cells are drawn into the biopsy pipette. E) Splitting of the trophectoderm with holding and biopsy pipette. F) Biopsied blastocyst and biopsy sample in biopsy pipette.

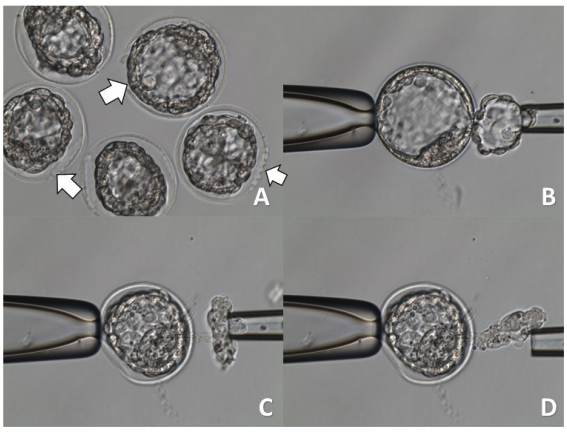

Figure 3. — Assisted hatching and trophectoderm biopsy by laser device. A) Assisted hatched mouse blastocysts by laser (arrow; thinning area by laser). B) Expanding blastocyst with trophectoderm herniating through an opening of the zona pellucida made by laser. C) and D) A cluster of trophectoderm cells split by laser.

ops of medium under mineral oil. All embryos were cultured to the eight-cell stage in G1.1 culture medium and to the blastocyst stage in G2.2 culture medium.

At the early blastocyst stage, assisted hatching was carried out by either hand-made hatching pipette or laser. After fixing the embryo using a holding pipette, a hand-made hatching pipette was inserted in the perivitelline space and penetrated opposite the zona pellucida. The zona was split with holding and assisted hatching pipettes (partial zona dissection) (Figures 2, A-C).

For laser, each embryo was immobilized by holding pipette, and partial zona thinning was made by pulses of 10-12 ms with a laser system. Zona pellucida thinning was made along the periphery of zona to leave a thin rim (~10%) of the original thickness, covering an area of 1/4 of the zona pellucida circumference (Figures 3, A).

After one hour of assisted hatching, the blastocysts were positioned using the holding pipette to locate the herniating TE at 3 o'clock position. A piece of TE away from the inner cell mass was aspirated with a biopsy pipette and dissected with laser or with using a mechanical method of rubbing the holding pipette and the biopsy pipette (Figures 2, D-F and Figures 3, B-D).

Equilibration solutions consisted of EBS1 (10% glycerol) and EBS2 (10% glycerol + 20% ethylene glycol). EBS1 contained 1 mL glycerol, 2 mL serum substitute supplement, and 7 mL phosphaste buffer 1. EBS2 contained 1 mL glycerol, 2 mL ethylene glycol, 2 mL SSS, and 5 mL PB1. For vitrification, two different solutions were prepared. The vitrification solution was composed of 25% glycerol and 25% ethylene glycol and contained 2.5 mL glycerol and 2.5 mL ethylene glycol in 3 mL PB1 plus 2 mL SSS. The thawing solution was composed of sucrose solution (0.5 M, 0.25 M, and 0.125 M), PB1, with 20% SSS.

The re-expanding blastocysts that were undergone TE biopsy by two different methods were fixed with a holding pipette after turning the inner cell mass (ICM) to 6 or 12 o'clock. Then, an intracytoplasmic sperm injection pipette was inserted into the blastocoelic cavity and about 70% to 80% of the blastocoelic fluid was aspirated.

After artificial shrinkage, the shrunken blastocyst was equilibrated in 10% glycerol and 10% glycerol+20% ethylene glycol solution for three minutes at room temperature, in sequence, and transferred to the vitrification solution. After ten seconds, the blastocysts were re-equilibrated and loaded in a capped-pulled straw and frozen. After seven days of cryopreservation, for the thawing process, blastocysts were rehydrated with 0.5 M, 0.25 M, and 0.125 M sucrose, 20% SSS-PBS solution for three minutes at room temperature, in sequence and rinsed with PB1 three times. The expansion rate was evaluated six hours after thawing.

The statistical software R version 3.3.2 was used for data analysis. Statistical analysis was performed with Chi-square test. $P < 0.05$ was considered statistically significant.

Results

Table 1 shows the comparison of hatching rate, re-expansion rate, and survival rate of mouse blastocysts after TE sampling by mechanical and laser method. The hatching rate was 92% and 90% in assisted hatching by pipette,

Table 1. — *Comparison of hatching rates, re-expansion rates, and survival rates of mouse blastocysts after assisted hatching and trophectoderm biopsy with hand-made pipette and laser respectively.*

	Hatching rate (%)		Re-expansion rate (%)	Survival rate (%)
AHP (n=100)	92 (92)	BXP (n=46)	42 (91.3)	40 (87.0)
		BXL (n=46)	40 (87.0)	40 (87.0)
AHL (n=100)	90 (90)	BXP (n=45)	40 (88.9)	39 (86.7)
		BXL (n=45)	41 (91.1)	38 (84.4)
p-value	0.806		0.947	0.955

AHP: assisted hatching by pipette; AHL: assisted hatching by laser; BXP: biopsy by pipette; BXL: biopsy by laser.

and assisted hatching by laser group, respectively. The re-expansion rate was the highest in assisted hatching by pipette-biopsy by pipette (AHP-BXP) group, but there was no statistical difference among the four groups. The survival rate also showed no statistical difference.

Discussion

In the beginning of the application of embryo biopsy, embryos of eight-cell stage were used. The effects of biopsy to development potential of biopsied embryos were inconsistent. An early study reported that removal of one or two cells at the eight-cell stage did not adversely affect the further development of biopsied embryos in vitro [10], but some studies have shown that even the one-cell biopsy of cleavage-stage embryos significantly impairs embryonic implantation potential [11, 12].

After widely adopting blastocyst culture and cryopreservation of blastocyst, now TE biopsy is widely conducted as a method for PGS. This approach seems to preserve embryo viability and to provide the most reliable results for PGS associated with an improved clinical outcome [13-15]. A major advantage of blastocyst biopsy is that multiple cells can be retrieved from each embryo without touching the embryonic mass. Therefore, the invasiveness of the procedure, if not null, is certainly reduced compared with blastomere biopsy, with the additional benefit of a lower degree of mosaicism [16, 17].

Generally, laser device is used for TE biopsy, and it intervenes two points of the TE biopsy procedure. First, it is applied for zona breaching for promoting hatching process of blastocyst. In this study, the authors used laser device or hand-made pipette for zona pellucida breaching and could not find any differences in hatching rate according to assisted hatching method. This indicates the safety of laser device on assisted hatching. Zakharchenko *et al.* reported that zona pellucida perforation by laser device at the blastocyst stage had a negative effect and that hatching did not occur after perforation of zona pellucida [18]. Therefore, we could assume that the safety or detrimental effect of laser device on blastocyst is dependent on the method of laser application. Appropriate method of laser application is not the perforation but zona thinning.

Another application point of laser is detaching process of TE from embryonic mass after hatching. TE biopsy also was conducted using laser device and hand-made pipette by rubbing with holding pipette. In case of laser application, damages to adjacent TE are unavoidable. However, in case of application of mechanical way, damage to adjacent TE could be minimized. To compare these two methods, the authors checked the re-expansion rate after TE biopsy and survival rate after freezing-thawing process. The results showed no difference between the two groups, suggesting the safety of laser method.

This study has indicated that the safety of laser device on TE biopsy. For TE biopsy, assisted hatching has to be accomplished by zona thinning, not by perforation. Also, TE detaching from embryonic mass does not make detrimental effect on embryos. On the other hand, this study shows the safety and efficacy of TE biopsy by using hand-made pipette. In spite of increasing demands for PGS, not all infertility centers have laser device. Therefore, hand-made pipette method could be an alternative way for TE biopsy in some facilities.

This study has several weak points. First, this study was conducted with mouse blastocyst, and consequent result might be different from human data. However, due to ethical problems, this kinds of study hardly processed in human blastocyst. Second, the authors followed general guide in controlling intensity and duration of laser application. Thus, the results could be different in case of different setting of laser intensity and duration. However, in this study, the whole procedure using laser device was conducted without problems associated with laser intensity. Third, the hand-made pipette method might require professional skills and consequently could not be applied immediately in every infertility center. Despite these limitations, this study contains valuable interpretations. There are scarce studies to compare the safety of laser device in TE biopsy procedure; most of the TE biopsy cases are carried out with laser device, and it would be difficult to compare laser method with another method. Finally, this study suggests the alternative way of TE biopsy other than using laser device. Further studies are needed to evaluate the safety and efficacy of TE biopsy method.

Conclusion

In conclusion, there was no advantage to laser over mechanical method in survival rate of mouse blastocysts TE biopsy.

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