VALIDATION OF THE USE OF A LASER FOR ASSISTED HATCHING IN TIME LAPSE SPECIFIC MICROWELL DISHES

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ABSTRACT

Introduction: There has been an increase in the use of time lapse technology in recent years and more companies bringing their TL incubators to market. A shared characteristic of these systems is the need to use a specific culture dish. In order to facilitate the video capabilities of the incubator, these dishes use microwells to manage embryo movement.

Assisted hatching using a laser (LAH) is a widely used technique, either prior to embryo transfer or on day 3 prior to trophectoderm biopsy. When using a laser in small volumes of media there are concerns about media temperature changes and when using a new dish the plastic's impact on laser accuracy needs to be considered.

Methods: We used a two step method to validate the microwell dishes used in the Genea GERI TL incubator. Firstly we fired the laser on our standard dishes (Falcon 60mm) used for AH onto a water based ink and measured the hole size. We then repeated the exercise using the same dishes but prepared with 20µl media droplets under oil. This was repeated for the GERI microwell dishes: with and without media. Eight individual laser firings were performed for each group and each firing was measured in 2 dimensions using a 10µs laser shot.

Secondly 32 2-cell mouse embryos were divided into two groups. The first group were cultured in GERI microwell dishes until day 5. The second group were exposed to laser hatching on day 3, using three shots of 10µs, and then cultured to day 5. The blastulation rate was compared between the two groups.

Results: For the dishes with no media there was no significant difference between the size of the holes in 60mm dishes (7.7um SD1.56) versus GERI dishes (12.5um SD2.06) without media or between the hole size when media was added (7.8um SD0.58) vs. (7.9um SD0.72). For the blastulation rate between hatched versus unhatched mouse embryos there was no difference with 100% blastulation rate in the two groups.

Conclusions: Based on our data, LAH can be performed directly in the GERI microwell dish. This is important especially in the case of day 3 zona ablation in preparation for trophectoderm biopsy since it is desirable to limit the time embryos are removed from the time lapse environment.

OBJECTIVE

The aim of the study was to assess whether dishes designed for use in a time lapse incubation system were suitable to be used when laser assisted hatching needs to be performed on embryos.

METHODS

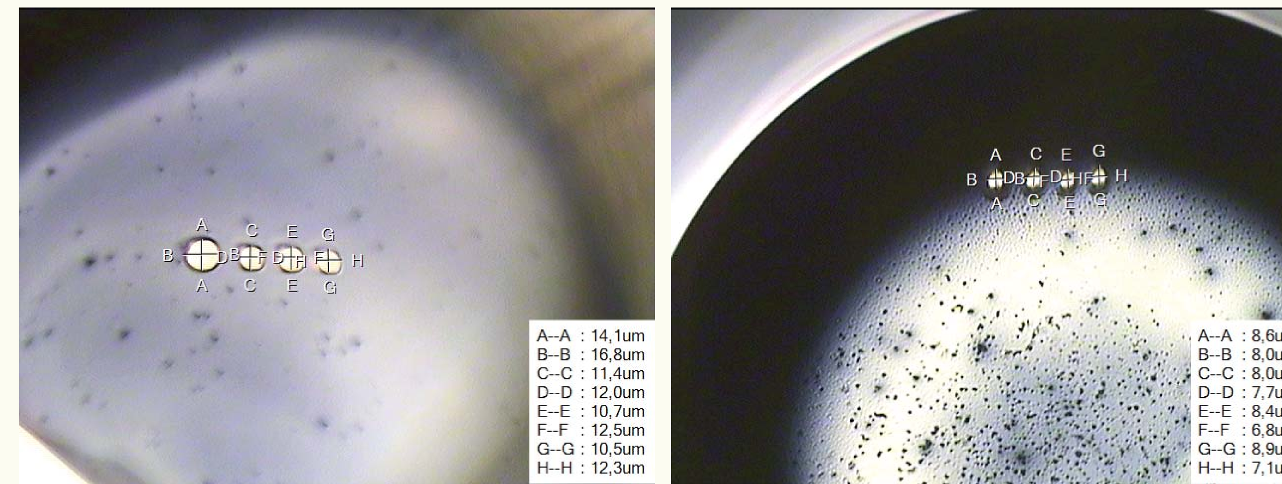
Hole size as indicated by ink ablation in our standard 60mm dish was compared to hole size in the GERI TL dish. Furthermore mouse embryo culture to blastocyst was used to assess any direct temperature effect on media and embryos.

STATISTICS

Chi Square and Comparison of means were used to assess significance in the analysis of the two groups. P< 0.5

RESULTS

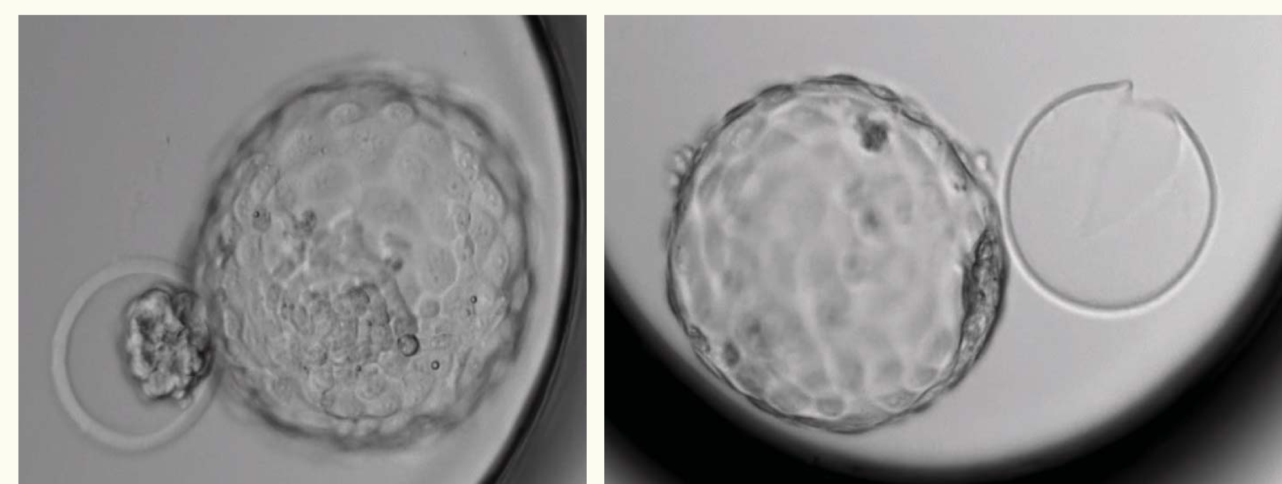
Impact of 10µm laser shot in 60mm Falcon dish: Left image without media, Right image with media



Impact of 10µm laser shot in Genea GERI TL dish: Left image without media, Right image with media

	With LAH	Without LAH	
n	16	16	
Blastocyst formation	100%	100%	
Time to blastocyst formation (h)	95	96	p<0.0001
me to hatched blastocyst (h)	100	115	p<0.0001

Development of two-cell mouse embryos in GERI TL dishes after exposure or no exposure to laser assisted hatching



CONCLUSION

Both laser assisted hatching (LAH) and time lapse technology are important tools in the embryology laboratory. One of the major benefits of TL is the ability to minimize or even eliminate manipulation of the embryos and the disturbance of their environmental stability.

In our laboratory LAH is used on day 3 of embryo culture prior to trophectoderm biopsy in cases of PGD and PGS. The use of time lapse specific dishes in the Genea GERI TL incubator meant that we would have to move the embryos temporarily on day 3 into standard culture dishes to perform LAH. This move increases the exposure time to suboptimal conditions and increases embryo manipulations. Therefore we decided to test the safety of carrying out LAH in Geri TL dishes.

Two elements can be of concern when using a laser for AH in terms of the dish that is holding the embryos. Firstly the plastic could deviate the laser beam and also absorb laser intensity and the temperature increase caused by the laser beam could heat the media if it is in a very small volume.

We compared the visual impact of a laser shot on ink in our standard Falcon 60mm culture dishes with the Genea GERI Tl dishes in order to establish if any deviation of the laser was caused by the plastic used to manufacture GERI TL dishes. Furthermore by exposing mouse embryos to LAH in the two types of dishes we were able to demonstrate no difference and therefore no apparent impact of heat increase in the small GERI culture areas.

No significant differences were seen in the hole sizes with or without culture media in the two types of culture dishes. In addition the blastocyst rate was 100% in both exposed and unexposed dishes. There was a statistically significant difference in the time to blastocyst; although not clinically significant at 1 hour difference. Unsurprisingly the group of mouse embryos exposed to LAH reached hatching faster than those without exposure. These data also support the idea that LAH can be performed directly in the Genea GERI TL culture dish without need to move embryos temporarily.

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