



## Expansion potential of vitrified and warmed embryos might be affected by extent of post-warmed laser-assisted hatching

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### Purpose

The purpose of this study was to evaluate the re-expansion potential of vitrified-warmed embryos that were conventionally hatched pre-vitrified, or post-warmed compared to those that had been extensively hatched post-warming.

### Materials and Methods

Data for this study was collected from donated vitrified embryos at a private fertility clinic between 2017 and 2020. Following warming, we randomly assigned embryos to one of two treatment groups (Table 1). To determine the expansion percent of an embryo, we obtained measurements of embryo diameter at two different time points after warming; 0h and 5h. Embryo volume was calculated from the diameters, and expansion percent was calculated by dividing 5h volume by the 0h volume. We also included the zona pellicuda (ZP) thickness, which was determined as mean thickness from four measurements taken at 0h.

### Results

Embryos in the extensive hatching group had an average of 31% of the surface area of the zona removed (range of 24-42%). Meanwhile, control embryos had a single laser width of 5uM removed representing <1% of the zona surface area. Embryos had an initial (0h) post-warm volume of 46% and 60% of the pre-freeze volume (control and extensive hatching respectively) and a 5-hour post-warm volume of 115% and 113% of the pre-freeze volume (control and extensive hatching respectively). There were no significant differences observed at either time point ( $P>0.05$ ), and embryos in both groups showed recovery to a volume greater than the pre-freeze volume within the 5-hour time allowance, indicating that embryos had survived treatment and were continuing growth. No significant differences found between hatching methods or PGT status ( $P>0.05$ )

### Conclusion

In conclusion, we showed that both laser assisted hatching techniques are not detrimental, and additional laser assisted hatching is safe to use for biopsied embryos. However, a randomized controlled study with a large sample size should also include IVF outcomes in future investigations.

### Table

Table 1 Experimental setting

N=161	PGT (n=70)	Non-PGT (n=91)
Control (n=79)	D3 single pulse width hatch only (5 uM) (n=23)	Simple hatching post-warm (n=56)
Extensive Hatching (n=83)	D3 hatching, plus hatch 1/4 of the ZP (n=47)	Hath 1/4 of the ZP (n=35)

