One-step vitrification of murine embryos redefines cryopreservation standards

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Introduction

Cryopreservation of embryos is amongst the most powerful tools for indefinitely preserving the genetics of laboratory animals.

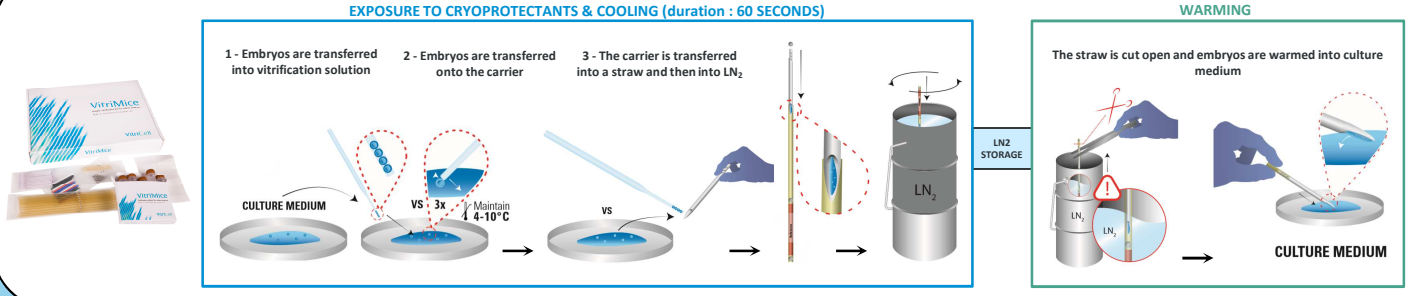
Benefits are numerous and include reduction of costs associated to strain perpetuation, limitation of mutations occurrence and spreading, ease and safety of transnational shipping, and reduction of live animal husbandry needs.

Vitrification is recognized as more efficient than slow freezing in human assisted reproduction, where it stands as the gold standard. This is equally true for murine embryos, where it better preserves chromatin integrity, induces lower intracellular ingress of cryoprotectants and ultimately yields better embryo survival and development than slow freezing.

Nevertheless, current vitrification procedures require multiple exposure steps to dedicated solutions to reach maximum effectiveness, which appears difficult to deal with when many embryos must be cryopreserved in one single session.

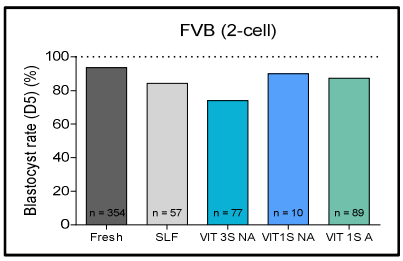
We have developed a "*one-step*" aseptic vitrification technology to address this issue.

Vitrification Protocol

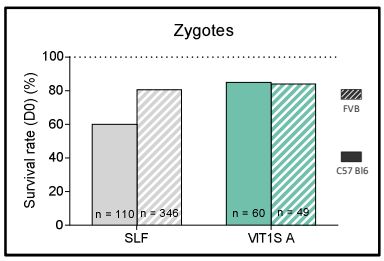


Results

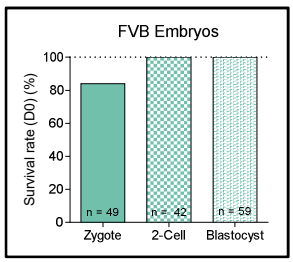
One Step Aseptic Vitrification vs. Other Cryopreservation methods



One Step Aseptic Vitrification with different mouse strains



One Step Aseptic Vitrification at different embryo stages



Newborns after transfer of One Step Aseptic vitrified zygotes

FVB/N pups

Fresh : non cryopreserved embryos / **SLF** : Slow Freezing / **Vit 1S NA** : Non-Aseptic 1-step vitrification / **Vit 1S A** : Aseptic 1-step vitrification / **Vit 3S NA** : Non-Aseptic 3-steps vitrification

1-Step Aseptic vitrification is:

- ✓ **More efficient than stepwise vitrification and slow freezing**
- ✓ **As efficient as 1-step or stepwise non aseptic vitrification**
- ✓ **Not influenced by mouse strain**
- ✓ **Suitable for cryopreservation of mouse embryos at different stages, from zygotic to blastocyst stages**
- ✓ **Giving similar birth rates as compared to fresh embryos (live birth rate = 34,6%, 18/52)**

Conclusions

We have developed and patented a unique "one-step" embryo vitrification procedure which is as efficient as the best multi-step vitrification methods. Moreover, our medium is protein-free and aseptic carriers can be used without any yield drop.
The derived kits and medium for rodents (VitriMice™, VitriCell) address the poor ergonomy issues of classical vitrification, providing scientists with efficient, biologically safe and user-friendly solutions for embryo cryopreservation.
Consequently, our technology improves efficiency and applicability of cryopreservation for laboratory rodents, thereby contributing to the reduction of live animals required to perpetuate and spread useful strains and colonies.

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