



# Effects of Temporary Storage of Cryopreserved C57BL/6 Mouse Sperm on Dry Ice



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## INTRODUCTION

- Cryopreservation of sperm, eggs, and embryos provides a valuable means of maintaining transgenic mouse strains used in biomedical research.<sup>1,2</sup>
- Sperm is easy and inexpensive to collect, store, and transport between research institutes.<sup>3</sup>
- Liquid nitrogen (LN<sub>2</sub>; -196 °C) is a reliable media to maintain cryopreserved sperm at low temperatures, but is considered a hazardous material incurring additional shipment cost.<sup>4</sup>
- Dry ice may maintain adequately low (-79 °C) temperatures to maintain cryopreserved sperm and does not incur additional shipment costs like LN<sub>2</sub>.<sup>4</sup>
- The European Mutant Mouse Archive has recently described the potential for dry ice to be used as effective shipment media for cryopreserved mouse sperm, but has yet to be confirmed by other repositories.<sup>4</sup>

## HYPOTHESIS

The use of dry ice as a temporary transport media will not significantly impact sperm cells compared to standard LN<sub>2</sub> storage.

If true, we predict cryopreserved sperm stored on dry ice will:

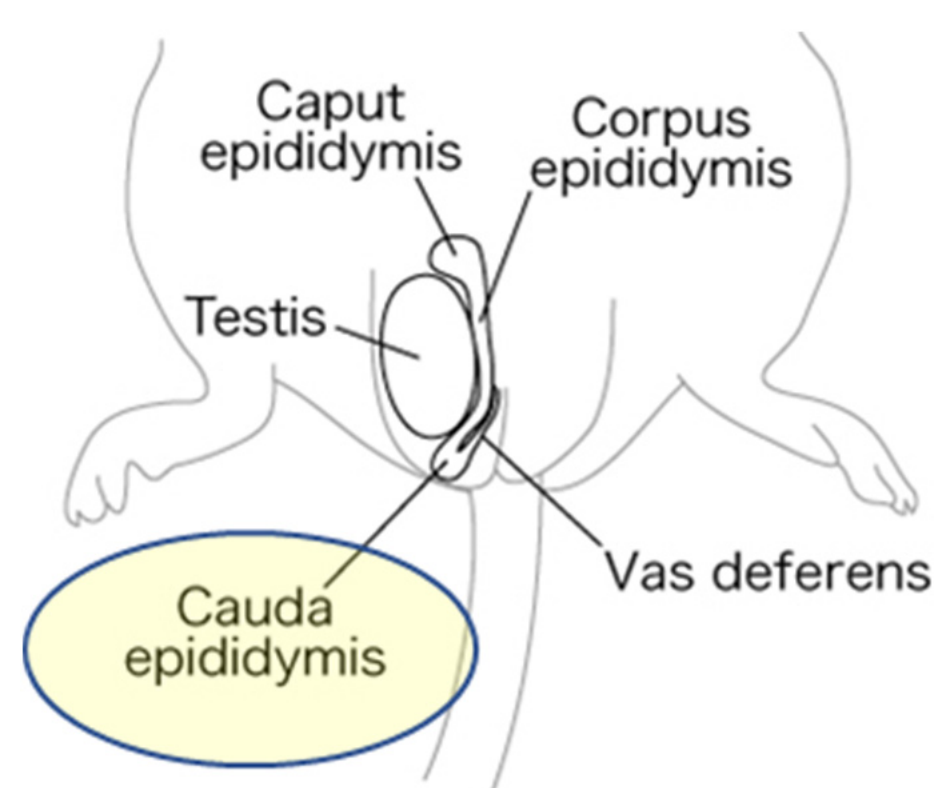
- Exhibit minimal changes in motility compared to initial cryopreservation with LN<sub>2</sub>
- Produce viable embryos when used for in vitro fertilization

## METHODS

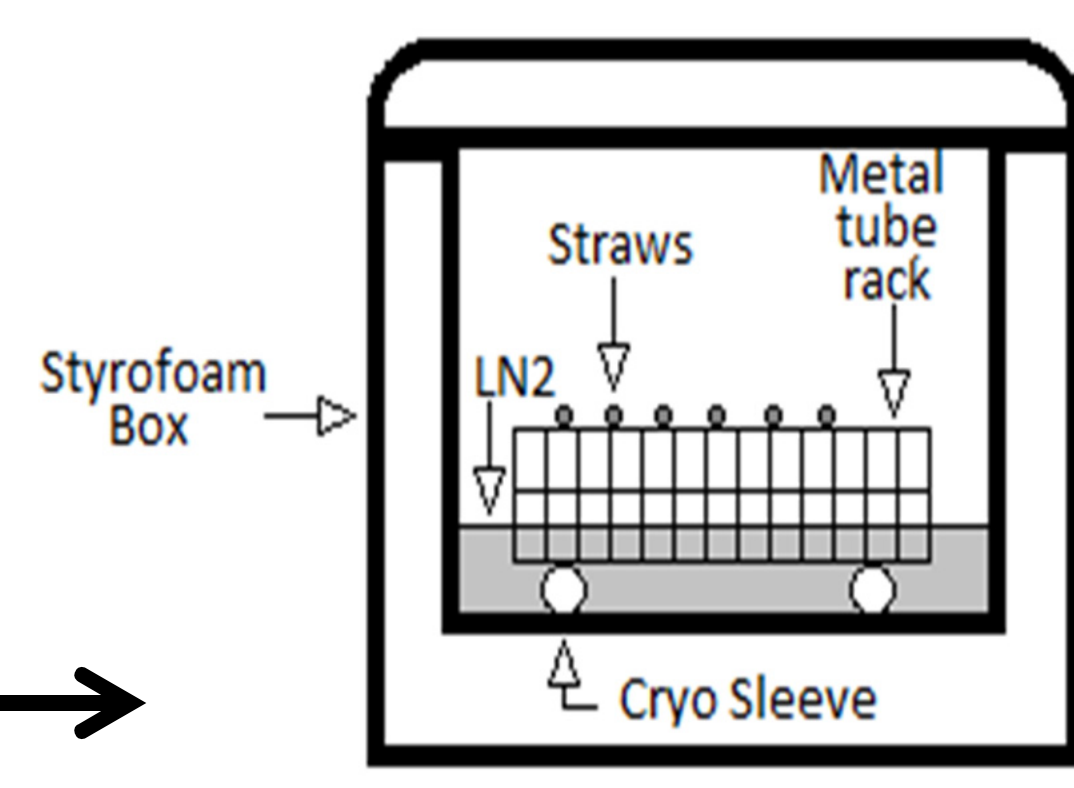


C57BL/6

- Widely used strain for gene targeting studies
- Moderate fresh sperm quality
- Very poor post-thaw sperm viability



Fresh sperm collection via dissection of cauda epididymis



Sperm mixed with freezing solution and frozen via suspension in LN<sub>2</sub> vapour followed by plunging directly into LN<sub>2</sub>

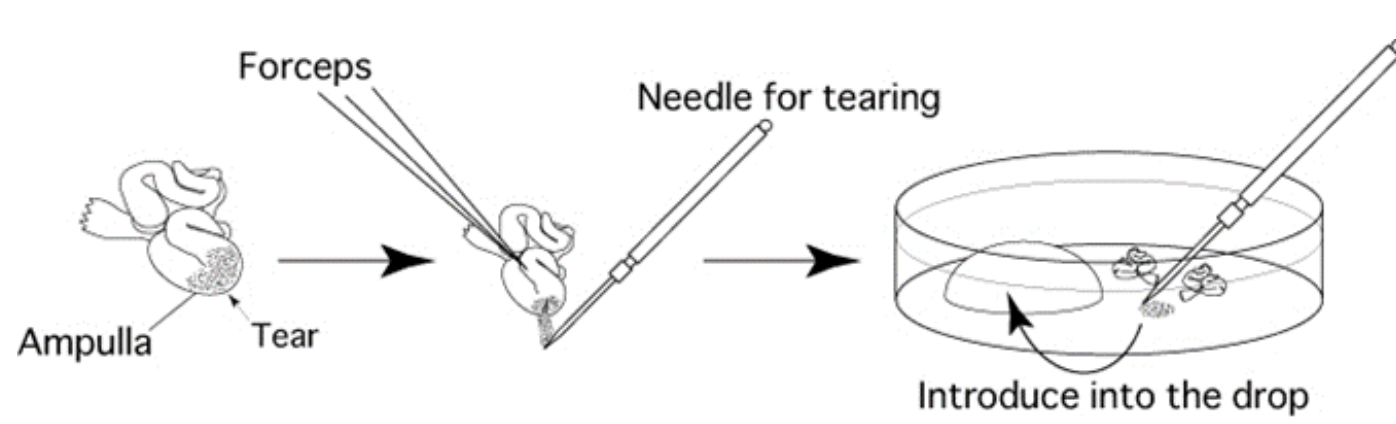


Frozen sperm stored on dry ice in a Styrofoam container for 1 week mimicking shipment

Motility analysis performed

Motility analysis performed

Motility analysis performed

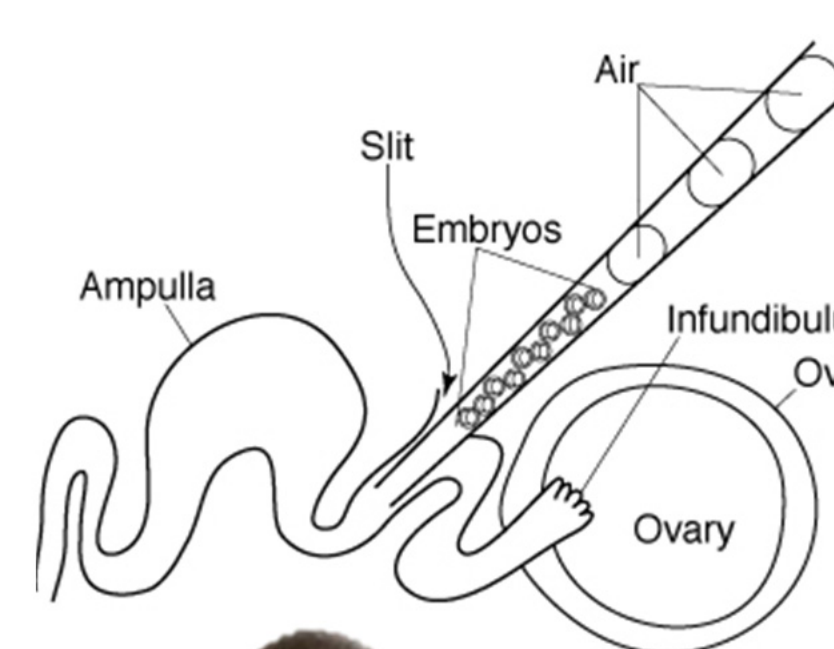


Female C57BL/6 superovulation and cumulus oocyte collection



In vitro fertilization with sperm from each condition and embryo culture in KSOM media

## FUTURE DIRECTIONS



Embryo transfer into pseudopregnant surrogates and production of viable pups

## ACKNOWLEDGMENTS

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Mice photo credits: The Jackson Laboratory  
Diagram credits: The Manual for Lab Mouse  
IVC method photo credits: Embryologist Media

## SPERM STORAGE ON DRY ICE DECREASES PROGRESSIVE MOTILITY, BUT ALLOWS FERTILIZATION AND EMBRYO DEVELOPMENT

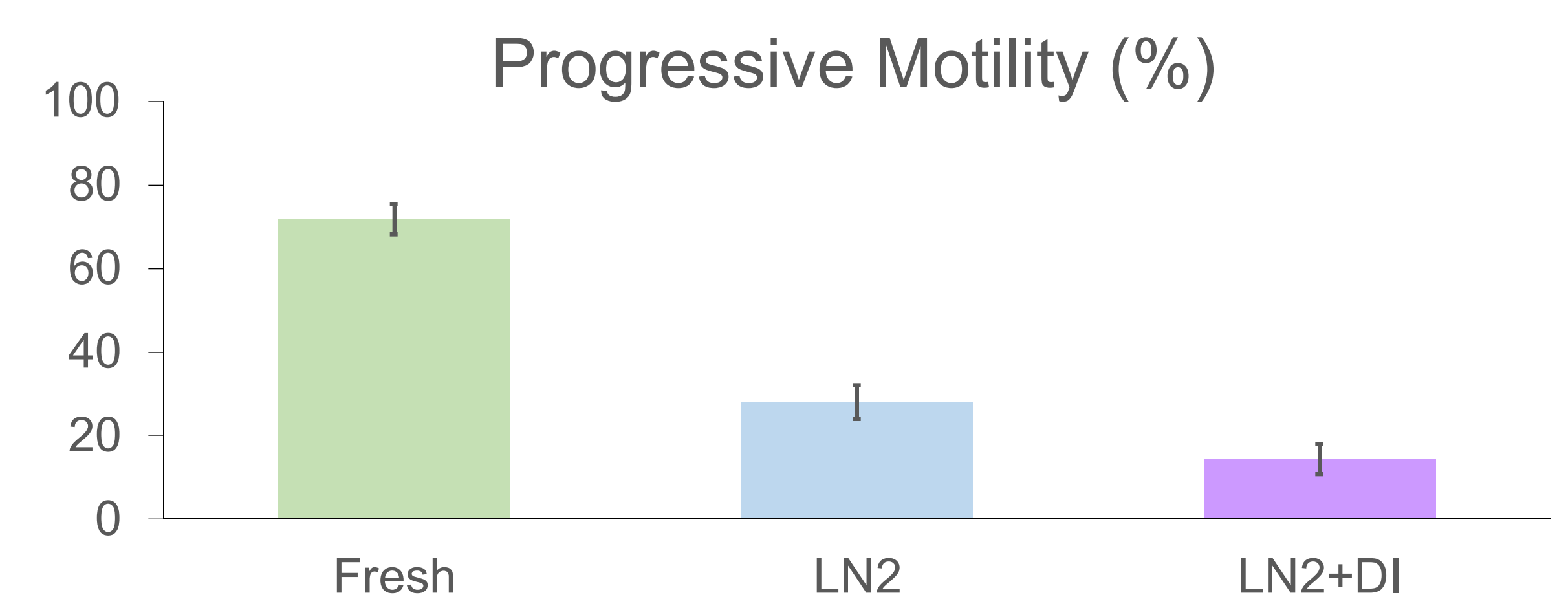


Figure 1: Percent progressive motility of fresh sperm, frozen sperm cryopreserved in LN<sub>2</sub>, and frozen sperm stored on dry ice for 1 week.

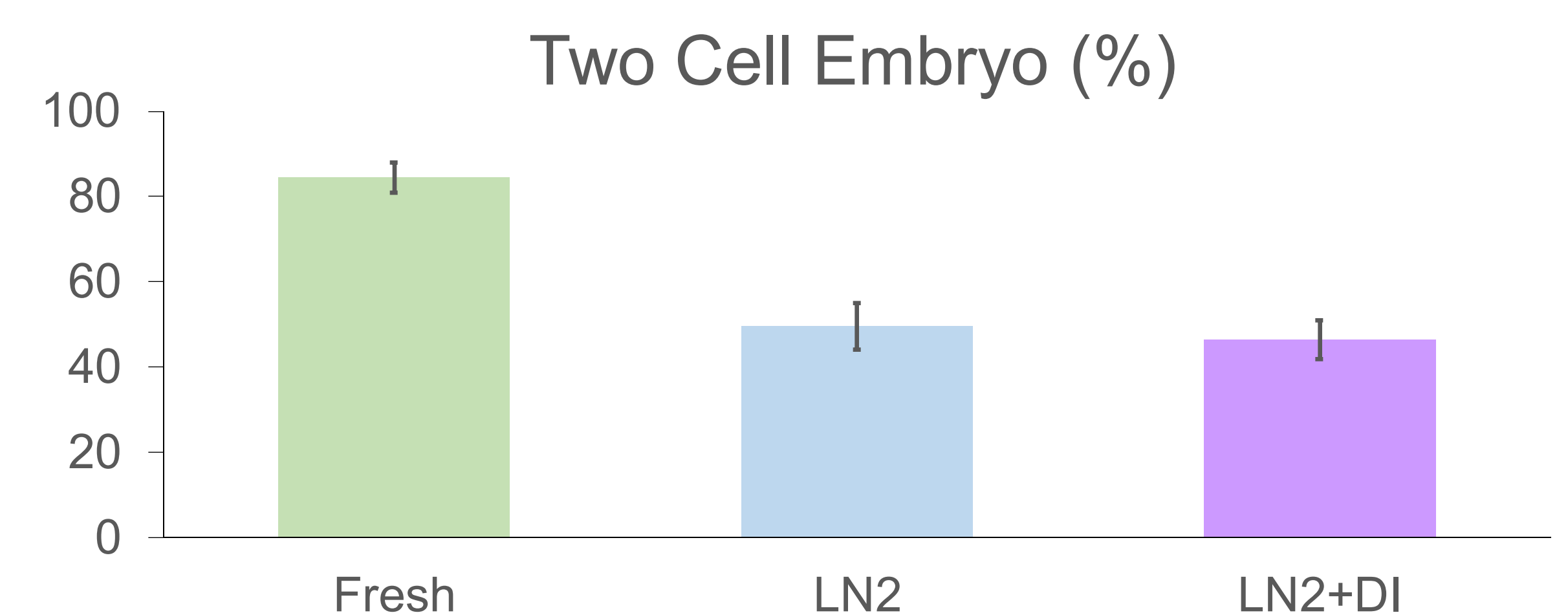


Figure 2: Percent two cell embryo development of oocytes fertilized in vitro with fresh sperm, frozen sperm cryopreserved in LN<sub>2</sub>, and frozen sperm stored on dry ice for 1 week.

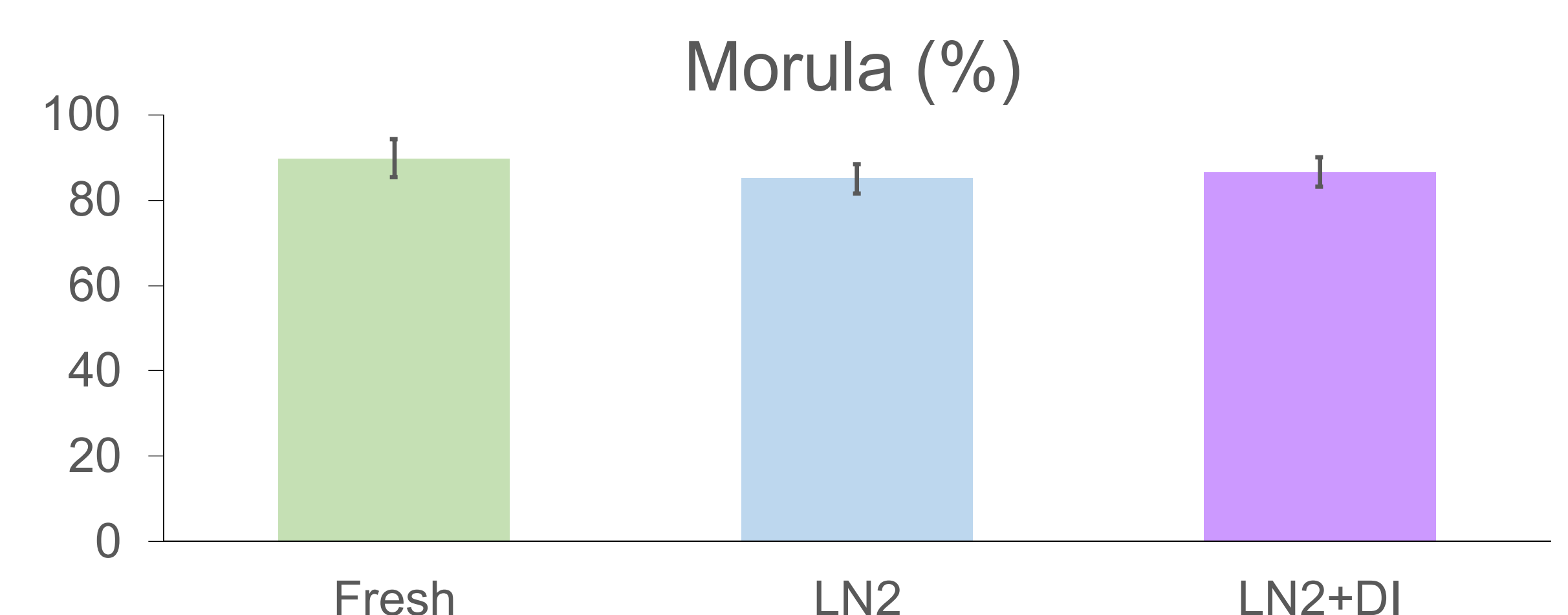


Figure 3: Percent morula development of two cell embryos fertilized in vitro with fresh sperm, frozen sperm cryopreserved in LN<sub>2</sub>, and frozen sperm stored on dry ice for 1 week.

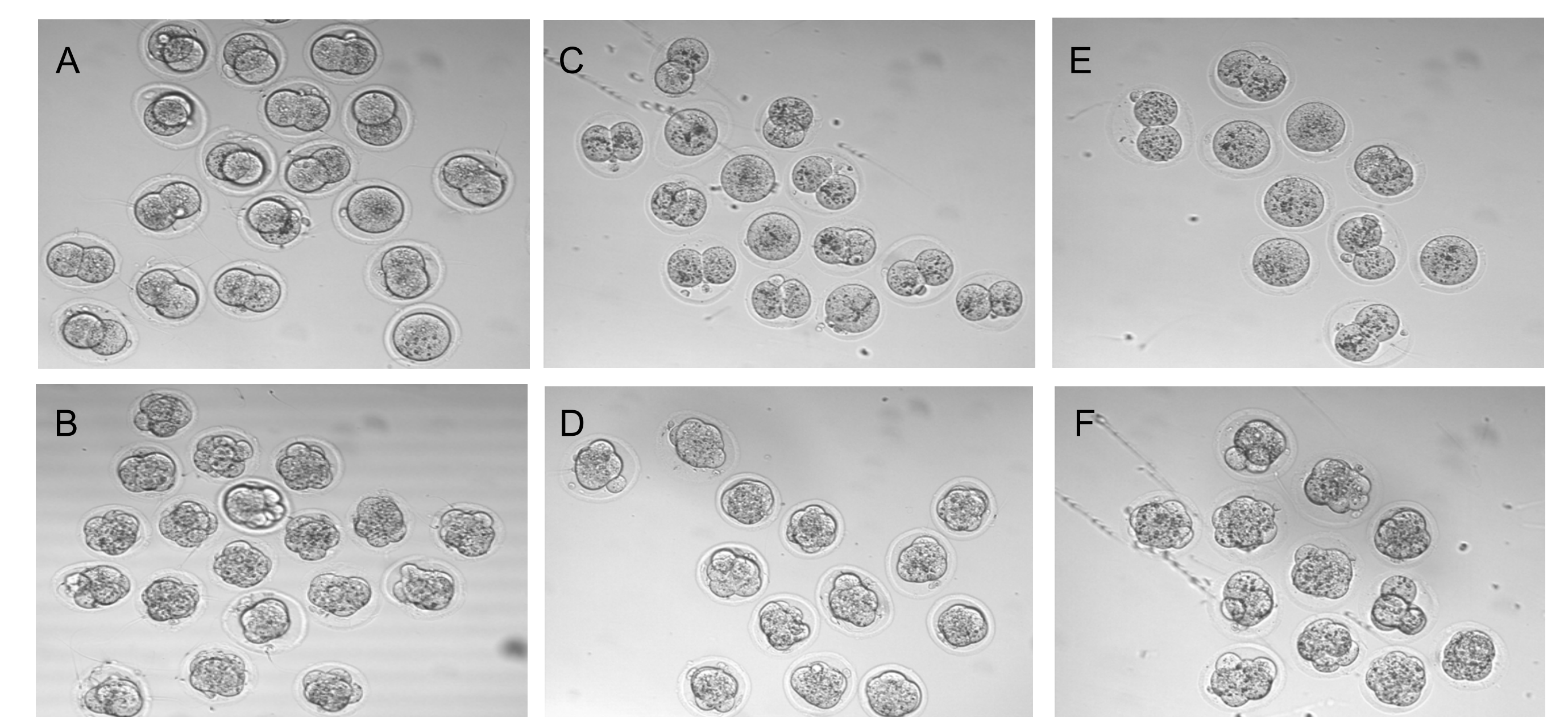


Figure 4: Representative images of 2-cell and morula stage embryos resulting from oocytes fertilized with freshly collected (A, B), LN<sub>2</sub> (C, D), or dry ice stored (E, F) C57BL/6 sperm.

## REFERENCES

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