

# Evaluation of frozen thawed embryo outcome after transportation to a long-term storage facility versus in house storage facility

A. Seabaugh, J. E. Pabon, R. K. Srivastava

Fertility Center and Applied Genetics of Florida, Inc, Sarasota & Naples, FL

## ABSTRACT

**Objective:** The objective of this study was to evaluate the outcomes of frozen embryo transfers (FET) on the embryos that were cryopreserved and transported to a long-term storage facility and then brought back later for FET cycles as compared to the outcomes of embryos that were cryopreserved and thawed in house. This comparison was carried out as further quality control of our procedures for remote site embryo banking. **Design:** Retrospective comparative study. **Materials and Methods:** All the embryos that were used for this study were cryopreserved at blastocyst stage and the protocol for freezing and thaw were a modified protocol as described by us earlier (ASRM 2004, abstract # P-58). Frozen embryos were stored in liquid nitrogen (LN2) and monitored. For transportation to our chosen remote site cryo laboratory (Reprotech Ltd, Roseville, MN) dry shippers were used that were previously saturated with LN2 and embryos were shipped in dry shipper by overnight courier. For QC purpose these shippers contain a cryoguard indicator vial. Vial content is green as long as temperature inside the shipper is -120oC and if for some reason temperature falls, it will turn red. These shippers are good to maintain the temperature for at least a week and they are checked intermittently for any leaks or deviations from the required storage temperature. **Results:** We have analyzed 18 FET cycles that were cryopreserved at our facility and sent for long-term storage to use later for FET. A total of 63 blastocysts thawed and 47 blastocysts were transferred from September 2003 until March 2005. Out of 18 FET, 12 cycles had a positive HCG test and eight are clinically pregnant (44%). Clinical pregnancy is defined as the presence of fetal heart beat by ultrasound. Our in house clinical pregnancy rate for FET during the period evaluated was 40%. **Conclusion:** Based on these data we conclude that there is no difference in clinical pregnancy rate for the embryos that were sent for long-term storage versus in house storage, if a strict QC is maintained throughout storage and transportation. Sending embryos to a cryobank for the storage could be an attractive option for ART centers where space constraint is a problem. Remote storage out sources the daily inventory and LN2 monitoring work. A significant liability in storing these preimplantation embryos for long-term are also outsourced.

## INTRODUCTION

Long-term storage of embryos generated by ART laboratories in a remote cryobank could be an attractive option for many ART laboratories where space constraint is a problem. It has some other advantages as well because it can significantly eliminate the inventory maintenance and continuous liquid nitrogen monitoring. We have evaluated this option of sending the embryos to a remote cryobank keeping in consideration the stringent quality control procedures practiced during these transfers and storage. We also compared the clinical pregnancy rate obtained after transferring these embryos in frozen embryo transfer (FET) cycles after bringing them back to our laboratory with our ongoing clinical pregnancy rate in FET cycles of those embryos that remained in our laboratory.

## MATERIALS AND METHODS

All the embryos that were used for this study were cryopreserved at the blastocyst stage and the protocol for freezing and thaw were modified protocol as described by us earlier (Srivastava et al, 2004, Veeck et al, 2004). Only expanding, expanded, hatching or hatched blastocysts with well-defined inner cell mass and a cohesive trophectoderm were frozen on day 5, 6 and 7. Frozen embryos were stored in liquid nitrogen (LN2) and monitored. For transportation to our chosen remote site cryo laboratory (Reprotech Ltd, Roseville, MN) dry shippers were used that were previously saturated with LN2 and embryos were shipped in dry shipper by overnight courier. For QC purpose these shippers contain a cryoguard indicator vial. The vial content is green as long as the temperature inside the shipper is less than -120oC and if for some reason the temperature rises, it will turn red. These shippers are good to maintain the temperature for at least a week and they are checked intermittently for any leaks or deviations from the required storage temperature.

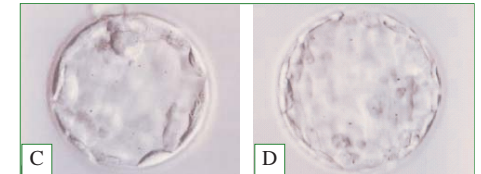
## TABLE:

### Comparison of clinical pregnancy rate between remotely stored embryos and in-house stored embryos

Total FET cycles analyzed when frozen embryos were sent earlier to cryobank and later brought back to our laboratory: (From 09/03 to 02/05):	18
Total blastocysts thawed:	63
Total blastocysts transferred:	47
Number of FET cycles with positive HCG:	12
Number of cycles with clinical pregnancy:	8
Clinical pregnancy rate:	44% (8/18)
Clinical pregnancy rate in FET cycles when frozen embryos stored in our laboratory (09/03 - 02/05)	40% (13/32)



**FIGURE:** (A) A blastocyst before freezing on day 6 that was sent to a cryobank for long-term storage, note the well-developed inner cell mass (ICM) and trophectoderm. (B) Same blastocyst after thaw retained the morphological features and implanted.



**FIGURE:** (C) One of the expanded blastocyst frozen on day 6 and stored in the laboratory cryostorage. (D) Same blastocyst was thawed for frozen embryo transfer. Morphological features are mostly retained post thaw. This blastocyst also implanted successfully.

## SUMMARY AND CONCLUSIONS

1. Clinical pregnancy rate did not differ for the embryos that were stored in a remote cryo bank versus in house storage.
2. Storing embryos in the cryobank for long-term storage could be a feasible alternative that can substantially eliminate the space constraint, inventory maintenance and complexities associated with LN2 storage and monitoring.
3. In our opinion long-term remote cryostorage diminishes the potential liability associated with this activity.

## REFERENCES

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2. Veeck LL, Bodine R, Clarke RN, Berrios R, Libraro J, Moschini RM, Zaninovic N, Rosenwaks Z (). High pregnancy rates can be achieved after freezing and thawing human blastocysts. *Fertil Steril* 2004; 82: 1418-1427