Effect of cryopreservation in the imprinting pattern of gametes

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PROJECT PROPOSAL
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INTRODUCTION

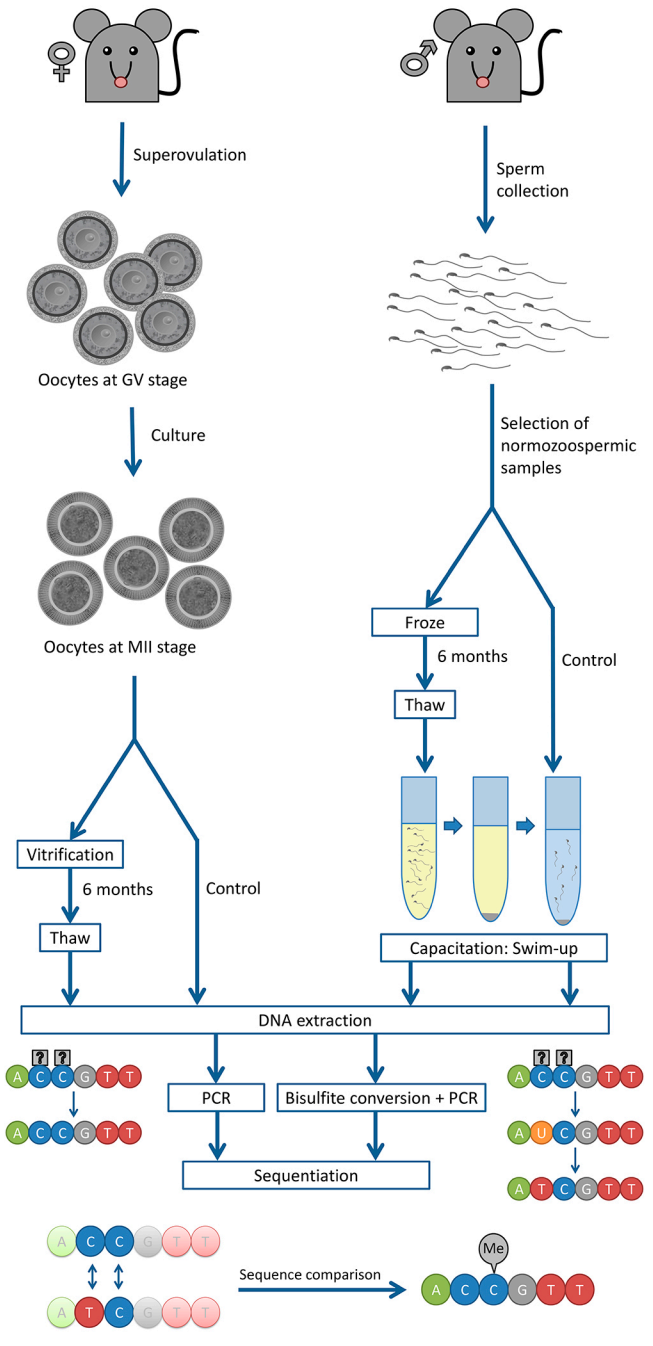
Assisted Reproductive Technology (ARTs) is the technology that includes all fertility treatments in which both eggs and sperm are handled according Centers for Disease Control and Prevention. This concept includes treatments as fertility medication, artificial insemination, *in vitro* fertilization and surrogacy. However, some studies report an increased incidence of imprinting disorders due to the use of ARTs.

In particular, cryopreservation and the consequent thawing of the gametes is a widely employed procedure in fertility techniques. It is known that cryopreservation causes structural and functional alteration in gametes, and is supposed to cause DNA alterations and epigenetic mutations. This is due to the effect of both toxic cryopreservatives and stress for the technique that could disturb the activity of DNA methyltransferases (DNMT), which establish methylations marks such as imprinting.

OBJECTIVES

1. Perform a comparative analysis of the methylation pattern of *Ndn*, *Magel2*, *Mkrn3*, *Ube3A* and *Kckn9* genes of mouse oocytes vitrified and non-cryopreserved.
2. Perform a comparative analysis of the methylation pattern of *Ndn*, *Magel2*, *Mkrn3*, *Ube3A* and *Kckn9* genes between fresh sperm, and frozen and thawed sperm.

METHODS



STATISTICAL ANALYSIS

Each experiment will be repeated 3 times. Statistical analyses between cryopreserved and non-cryopreserved gametes will be done using unpaired Student's t–test; $P < 0.05$ will be considered statistically significant. Statistical significance for multiple tests will be corrected using the Bonferroni correction.

STAGES AND DEVELOPMENT OF TASKS

Methods	Year 1	Year 2	Year 3
1. Oocyte collection.	✓		
2. Analysis of non-cryopreserved oocytes.	✓		
3. Cryopreservation of oocytes and their analysis.	✓	✓	
4. Sperm collection.		✓	
5. Analysis of non-frozen sperm.		✓	
6. Freezing and subsequent analysis of sperm.		✓	✓
7. Methylation patterns determination.		✓	✓
8. Determination of mutations in methylation patterns caused by cryopreservation.		✓	✓
9. Statistical analysis.		✓	✓

EXPECTED RESULTS

GENES	Imprinted allele	Expected mC in Controls	Cryopreservation effect	
			Oocytes	Sperm
Ube3A	Paternal	6	-	↓
Kcnk9	Paternal	11	-	↓
Ndn	Maternal	6	↓	-
Magel2	Maternal	9	↓	-
Mkrn3	Maternal	17	↓	-

Hypermethylation is not expected because the disturb in DNMT activity would only involve an activity decrease.

TRANSLATIONAL RELEVANCE OF THE PROJECT

This project will offer us information that will allow us to perform other future projects as:

- The effect of cryopreservation in the imprinting pattern of mice embryos.
- The effect of cryopreservation in the imprinting pattern of human gametes and embryos.

Assuming that all this research will show us the implication of gamete and embryo cryopreservation on imprinting diseases, we will be able to develop a platform of preimplantational or prenatal diagnosis.

Also it could provide us information about Birk-Barel disease which have been recently described and it is caused by imprinting defects in *KCNK9* gene.

REFERENCES

[1] Cheng, K.-R., Fu, X.-W., Zhang, R.-N., Jia, G.-X., Hou, Y.-P., & Zhu, S.-E. (2014). Effect of oocyte vitrification on deoxyribonucleic acid methylation of H19, Peg3, and Snrpn differentially methylated regions in mouse blastocysts. Fertility and Sterility, 102(4), 1183–1190.e3
[2] Eroglu, A., and Layman, L. C. (2012). Role of ART in imprinting disorders. Seminars in Reproductive Medicine, 30(2), 92–104
[3] Trapphoff, T., El Hajj, N., Zechner, U., Haaf, T., & Eichenlaub-Ritter, U. (2010). DNA integrity, growth pattern, spindle formation, chromosomal constitution and imprinting patterns of mouse oocytes from vitrified pre-antral follicles. Human Reproduction, 25(12), 3025–42
[4] Valcarce, D. G., Cartón-García, F., Riesco, M. F., Herráez, M. P., & Robles, V. (2013). Analysis of DNA damage after human sperm cryopreservation in genes crucial for fertilization and early embryo development. Andrology, 1, 723–730

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