CRYOPRESERVATION OF BOVINE OOCYTES BY VITRIFICATION USING MICRODROPLET METHOD

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CONTENTS

1. Introduction

- The ability of freezing sperm and embryos has been technical feasible and a widely practical procedure in the world. However, the ability of cryopreservation oocytes have been much more difficult and resulted in low survival rate. It applies for nuclear transfer and cloning technologies and conservation of genetic resources.

…Introduction

- Oocytes and embryos can be cryopreserved using slow-cooling or vitrification techniques.
- In Vietnam, cryopreservation of bovine oocytes is a new method.

2.Materials and methods

Materials

- Oocytes (COCs) were collected from Ovaries obtained from a slaughterhouse
- TCM-199 medium + 5% CS
- Ethylene glycol
- Sucrose
- Liquid nitrogen

…Methods

1) Preparation and maturation of oocytes

- Collect cumulus-oocyte-complexes (COCs) from ovaries follicles 2 to 6 mm in diameter.
- Choose good oocytes with compact cumulus cell multilayer (grade A).
- Maturation of oocytes in TCM-199 medium + 5% CS for 24h at 38.5^{o}C under 5%CO_2 .

…Methods

2) Pre-equilibration

Matured oocytes with good quality.

Transfer oocytes into pre-equilibration consists of 1M ethylene glycol (EG) in TCM 199 medium + 20% CS for 20 min.

…Methods

- 3)Freezing

  Then 5-8 oocytes were transferred into vitrification medium consisting of 5.5M EG and 1.0 M sucrose in TCM 199 medium + 20% CS.

  After 30sec, 6µl of vitrification solution containing 5-8 oocytes was dropped directly in liquid nitrogen. The vitrified micro drops were transferred to a cryotube to store in liquid nitrogen.

…Methods

- 4)Thawing, dilution, culturation in vitro for 3h.

The vitrified micro drops were exposed to dilution medium containing 1.0 M Sucrose in TCM199 + 20% CS. Then they were cultured for 3h at 38.5^{o}C under 5% CO2 in air. Oocyte survival was morphologically evaluated with the color and re-expansion of cumulus cell multilayer.

3.Results

- Total of 914 oocytes collected from ovaries derived slaughterhouse.
- 555 (60.72%) COCs classified grade A were cultured in TCM199 + 5% CS for 24h.
- The maturation rate in this experiment was 58.56 % (325 maturated COCs /555 COCs).
- After preservation for a week, oocytes were thawing and culturing in vitro for 3h. Oocytes with light color and re-expanded cumulus cell multilayer are evaluated survival.

4.Discussion and conclusion

- Cryopreservation of oocytes by vitrification is now successful but still low survival rate. Microdroplet-a satisfactory results.
- Advantages of microdroplet method

  not induce ice crystals formation

  pass quickly subphysiological temperature occurred chilling injury .

Problem:

Chemical toxicity

Osmotic shock

...Discussion and conclusion

- In conclusion, we applied successfully microdroplet method.
- we achieved 47.08% survival oocytes after freezing and thawing.