Comparison of slow freezing versus vitrification for human ovarian tissue cryopreservation and xenotransplantation

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CONTENTS

Introduction

1. Cancer patients have been increasing steadily.
2. Chemotherapy and radiotherapy for the cancer patients can be induced ovarian deficiency, premature ovarian failure and infertility.
3. Young women who had cancer treatment are not only can preserve their fertility but also their ovary can be restored endocrine function.

Purpose

1. To demonstrate superior method btw slow freezing and vitrification.
2. To establish the safety and effectiveness of hOT cryopreservation and transplantation using xenotransplantation model.

Slow freezing medium

* Reagent

Medium 199 – Sigma Aldrich, catalog# M4530, 500ml
DMSO – Sigma Aldrich, catalog# D2650, 100ml
SSS(Serum substitute Supplement) – Irvine Scientific, catalog# 99193, 100ml

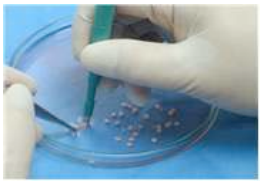
* Method

Solution 1 : 5% HSA(or SSS) supplemented M199(medium)
Solution 2 : 10% DMSO + 5% HSA(or SSS) supplemented M199
Solution 3 : 12.5% DMSO + 5% HSA (or SSS) supplemented M199

Slow freezing process

Method

1

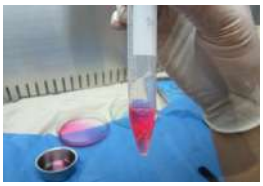


1. Tissue selection

2

2. Tissue cutting

3



3. Add sol. 1, 2, 3

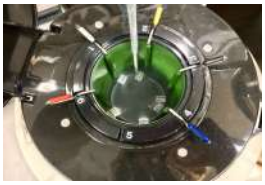
4

4. Slow freezing setting

5

5. Seeding process

6



6. Store in the LN tank

Vitrification medium

* Reagent

HEPES(1M) – Gibco, 15630-080, 100ml
Ethylene Glycol
DMSO(dimethyl sulphoxide) – Sigma Aldrich, catalog# D2650, 100ml
Sucrose – Sigma Aldrich, catalog# S1888, 500g
SSS(Serum substitute Supplement) – IrvineScientific, catalog# 99193, 100ml

Vitrification medium

* **Equilibration Solution(ES)**

65ml HEPES
supplemented 7.5ml
Ethylene Glycol
7.5ml DMSO
20ml SSS(or SPS)
(Total concentration: 7.5% EG, 7.5% DMSO, 20% SSS in solution)

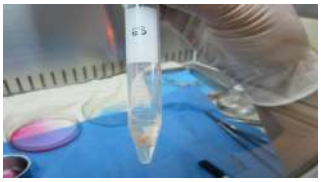
* **Vitrification Solution(VS)**

27.5ml HEPES
17.12g Sucrose
20ml Ethylene glycol
20ml DMSO
20ml SSS(or SPS)
(Total concentration: 20% EG, 20% DMSO, 20% SSS, 0.5M sucrose in solution)

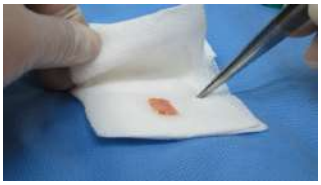
Vitrification process

Method

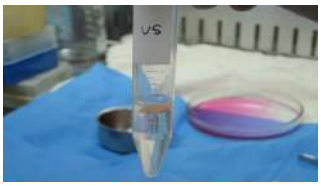
1
2
3



1. ES treatment



2. Remove solution

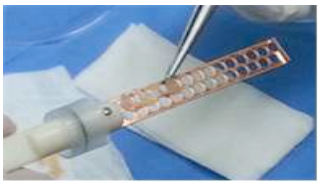


3. VS treatment

4

4. Remove solution

5

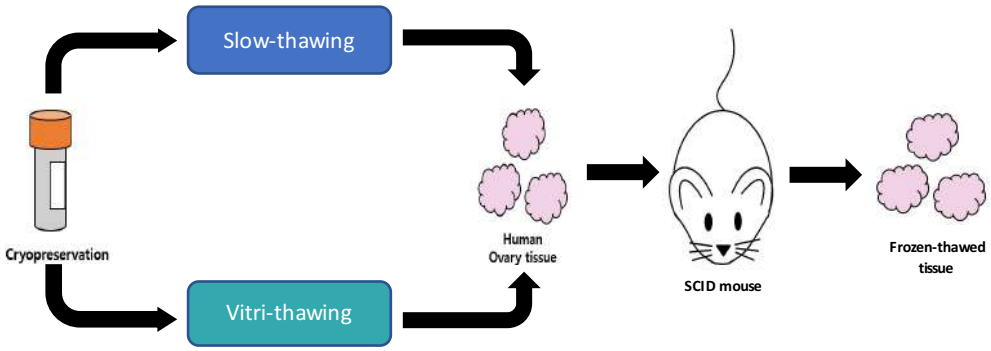


5. Put tissues on strainer

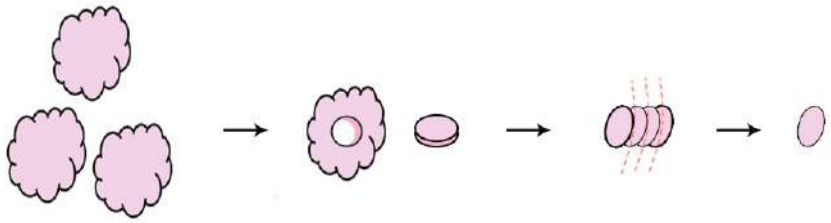
6

6. Plunge into LN

Process



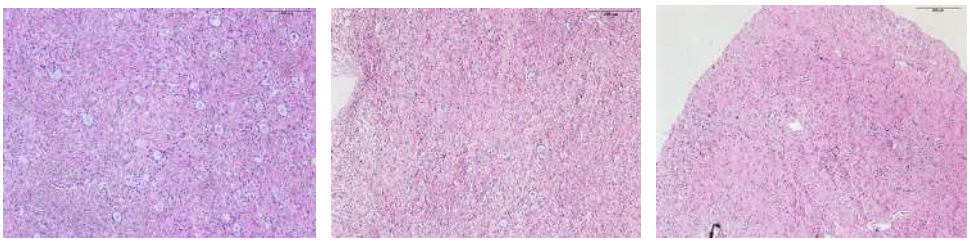
Process



Human ovarian tissue | Punctured with diameter 4mm | Sectioning (2µm) | Final tissue

Hematoxylin & Eosin stain

Results

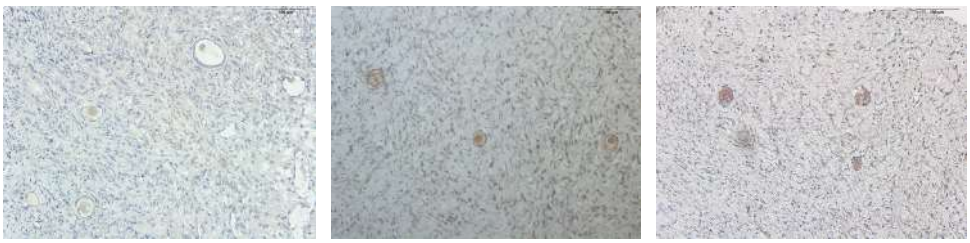


Fresh | Slow-thawed | Vitri-thawed

Follicle growth with Hematoxylin and eosin staining after thawing process.

γ-H2AX

Results

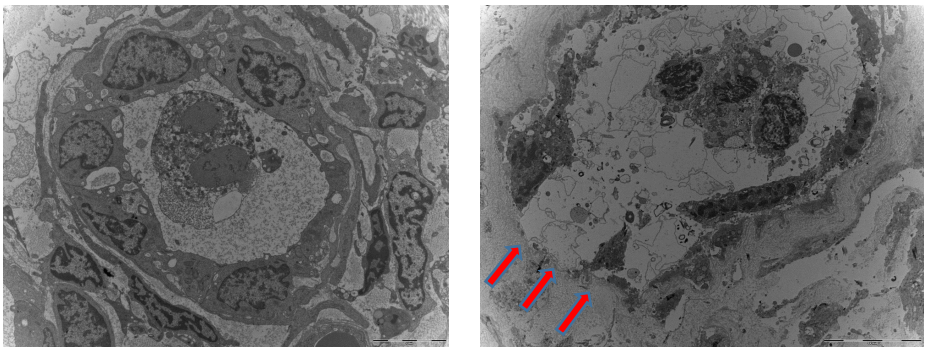


Fresh Slow-thawed Vitri-thawed

Histological featues of double-strand breaks in the deoxyribonucleic acid.

Transmission Electron Microscope

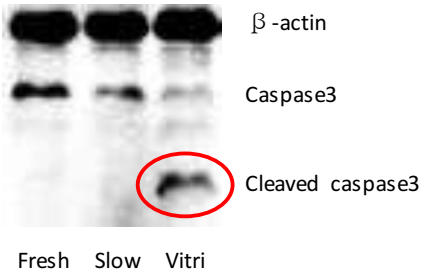
Results



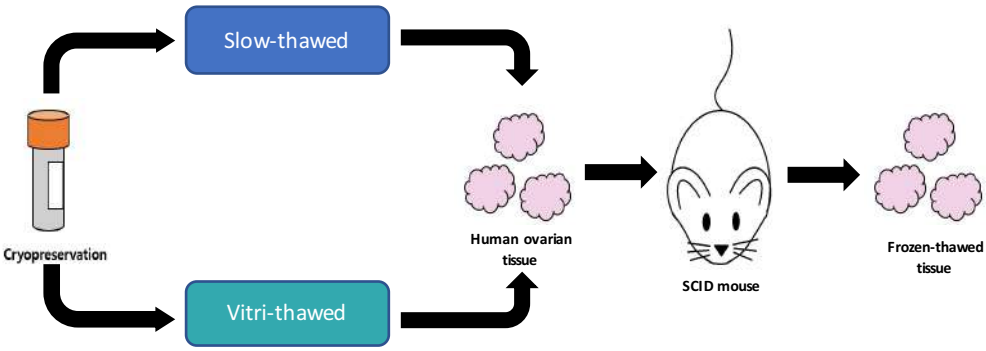
Slow-thawed primordial follicle Vitri-thawed primordial follicle

Western blot

Results



Process



Xenotransplantation

∗ Objective

The purpose of this study was to compare the cryopreservation methods and establish safety and effectiveness of human ovarian tissue transplantation using xenotransplantation model.

∗ Material

Ovarian tissues were obtained from 15 patients who underwent benign ovarian surgery with informed consent and IRB approval (IRB No.: ED11138)

Human ovarian tissues were equally divided and prepared for the slow freezing and vitrification.

Frozen thawed human ovarian tissues were transplanted into back muscle of about 100 SCID mice (1st 40+2nd 51) 4 weeks after cryopreservation, which were ovariectomized prior to the xenograft.

Xenotransplantation

Method

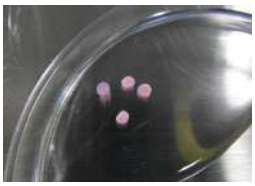
1

1. SCID mouse

2

2. Respiratory anesthesia

3



3. Human ovarian tissue

4

4. Ovariectomy

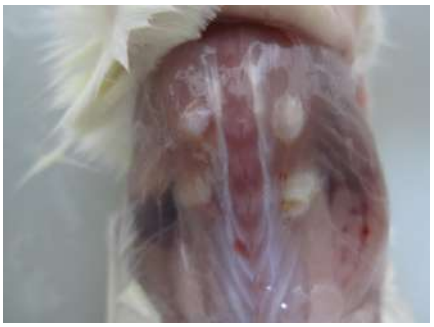
5

5. Xenotransplantation

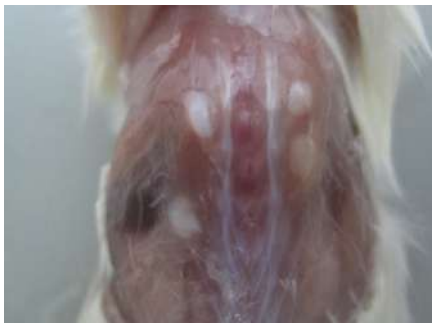
6

6. Tissue retreival

Tissue retrieval

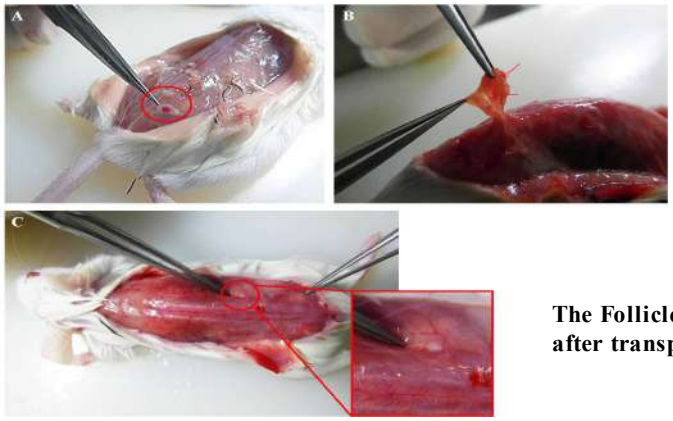


Slow freezing



vitrification

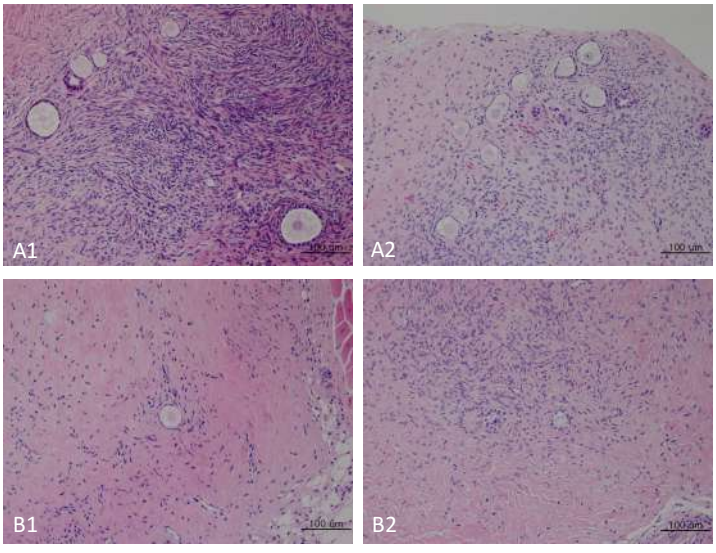
Tissue retrieval



The Follicle growth and angiogenesis after transplantation

Hematoxylin & Eosin stain

Results

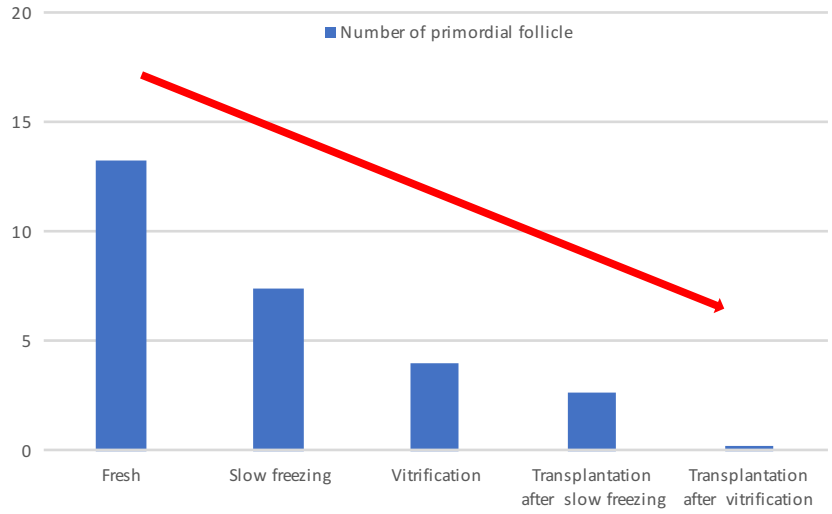


Follicle growth with Hematoxylin and eosin staining after transplantation.

A- Slow-thawed and transplanted
B- Vitri-thawed and transplanted

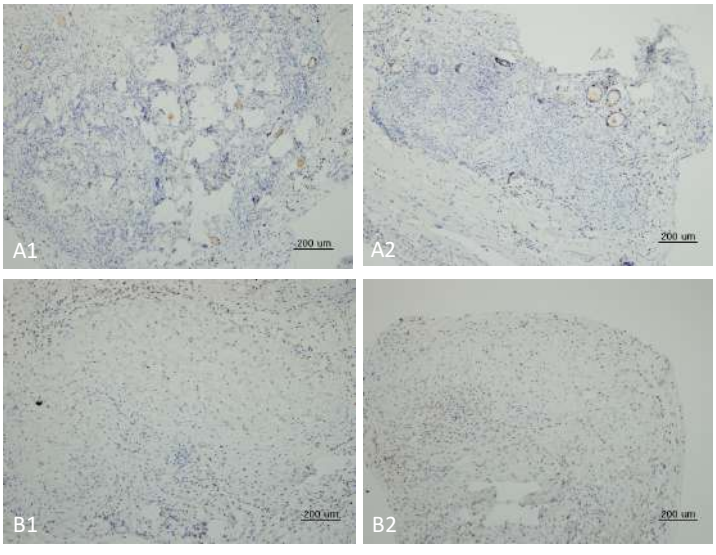
Ovarian follicle count

Results



Ki67

Results

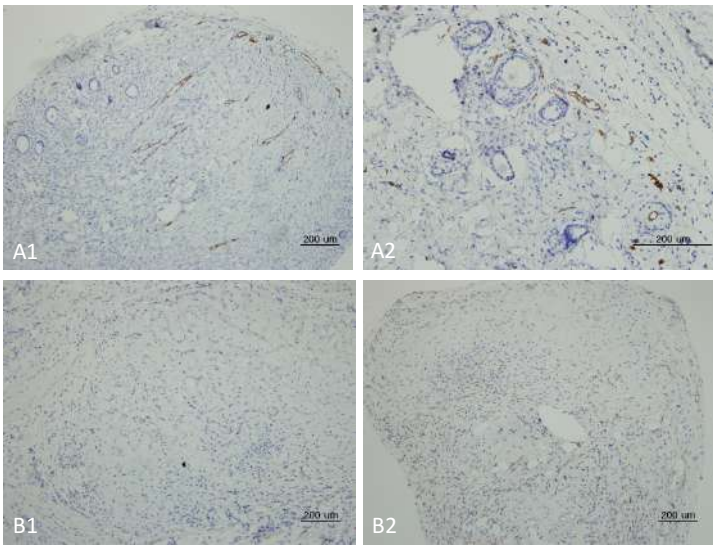


Histological featues of cell proliferation

A- Slow-thawed and transplanted
B- Vitri-thawed and transplanted

CD31

Results

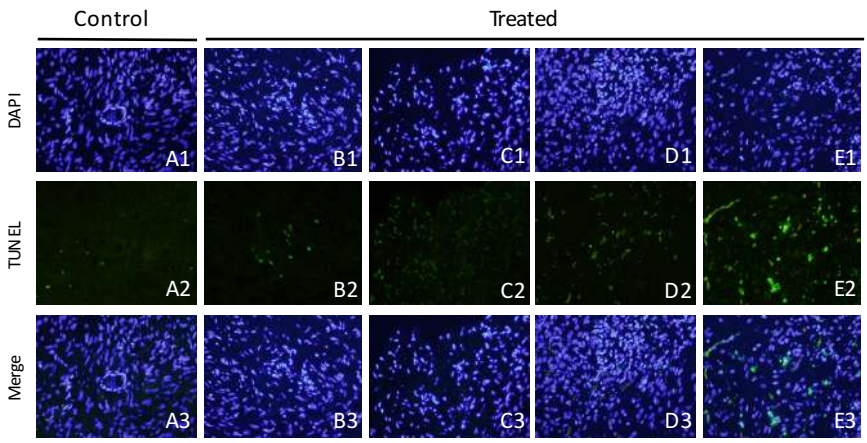


Histological featues of Angiogenesis

A- Slow-thawed and transplanted
B- Vitri-thawed and transplanted

TUNEL assay

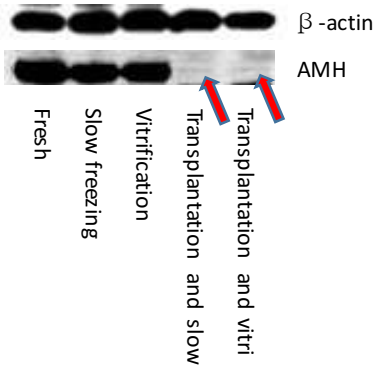
Results



A- Fresh tissue B- Slow-thawed C- Vitri-thawed
D- Slow-thawed and transplanted E- Vitri-thawed and transplanted

Western blot

Results



Conclusion

* Slow freezing for ovarian tissue cryopreservation was superior to vitrification in terms of the follicle growth and histologic feature of ovarian tissues after xenotransplantation.
* More research is needed to improve vitrification results in the future.
* We believe that this study will provide useful information for reproductive women with cancer who need cryopreservation for fertility preservation.

Thank you for your attention

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