



**Karolinska  
Institutet**

**Institutionen för klinisk vetenskap, intervention och teknik,  
Karolinska Institutet**

# Clinical grade vitrification of human ovarian tissue for fertility preservation

**AKADEMISK AVHANDLING**

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av

**Mona Sheikhi**

MSc

*Huvudhandledare:*

Professor Outi Hovatta  
Karolinska Institutet  
Inst. För Klinisk vetenskap, intervention och  
teknik, CLINTEC

*Fakultetsopponent:*

Professor Mats Brännström  
Göteborg Universitet  
Enheten för obstetrik & gynekologi

*Bihandledare:*

Docent Monalill Lundqvist  
Karolinska Institutet  
Inst. För Klinisk vetenskap, intervention och  
teknik, CLINTEC  
Enheten för obstetrik & gynekologi

*Betygsnämnd:*

Professor Anna Wedell  
Karolinska Institutet  
Inst. för molekylär medicin och kirurgi

Professor Angelica Linden-Hirschberg  
Karolinska Institutet  
Inst. Kvinnors och barns hälsa  
Enheten för obstetrik & gynekologi

Docent Agneta Lukinius  
Uppsala universitet  
Inst. För genetik & patologi

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## ABSTRACT

Cryopreservation of human ovarian tissue is one approach to preserve fertility for women who can be predicted to undergo premature ovarian failure as a consequence of chemotherapy, radiotherapy or genetic disorders. It is the most suitable option for pre-pubertal girls and for many young women to store oocytes. To date, auto-transplantation of frozen- thawed cortical tissues has resulted in birth of 24 healthy children, worldwide. Cryopreservation can be performed using slow freezing or vitrification. Vitrification is known as solidification without formation of lethal intracellular ice crystals. The aim of this thesis was to further develop methods for cryopreserving follicles in human ovarian tissue of women who have a risk of losing their fertility.

In the first study, we systematically compared two cryopreservation methods for human ovarian cortical tissue, slow freezing and vitrification. Cryoprotectants we used for slow freezing were either 1,2- propanediol (PrOH)- sucrose or ethylene glycol (EG)-sucrose. For vitrification, we used solutions containing a combination of the cryoprotectants dimethyl sulphoxide (DMSO), PrOH, EG and polyvinylpyrrolidone (PVP). Light microscopy (LM), transmission electron microscopy (TEM) and post-thaw tissue culture were carried out to evaluate the structure and the viability of the follicles. The follicles were well preserved and the ovarian stroma showed better morphological integrity after vitrification. In the second study, we developed a clinical grade vitrification of human ovarian tissue. Ovarian tissue was vitrified in a closed system without any direct contact with liquid nitrogen using a non-toxic and sterile cryotube. Vitrification solutions used contained a combination of cryoprotectants DMSO, PrOH, EG and PVP. The morphology of the follicles in the vitrified tissue showed well -preserved structures as verified by LM, TEM and also after post -thaw culture. The system used is compatible with the European tissue directive and the Swedish tissue law. In the third article, we studied the occurrence of apoptosis in vitrified tissues. We used Terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) assay and DNA fragmentation analysis, which showed no incidence of apoptosis in follicles or stroma after using either vitrification solution (i.e., a combination of DMSO, PrOH, EG and PVP, or EG and bovine serum albumin (BSA)). In the fourth one, we performed a study to simplify our earlier described closed vitrification procedure. Permeating cryoprotectants used in vitrification solutions consisted either of a combination of DMSO, PrOH, EG or EG only. Ovarian tissue was vitrified in closed sealed tubes containing either of the vitrification solutions. Morphological analysis (LM and TEM) showed that oocytes, granulosa cells and stroma were equally well preserved when either of the vitrification solutions was used. No apoptosis was observed in primordial and primary follicles using immunohistochemistry for active caspase- 3. Conclusion: We present here new vitrification procedures that can be performed in a clinical setting. The morphology of follicles in the ovarian tissue as evaluated by using LM and TEM proved to be normal after the procedures. Ultra-structural analysis by TEM used in this study is the best-known method to evaluate cryoinjury. We have developed a new effective clinical grade method for cryo-storage of human ovarian tissue.

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