

# EXPERIMENTAL STUDIES ON OVARIAN CRYOPRESERVATION AND TRANSPLANTATION

Akademisk avhandling  
som med vederbörligt tillstånd av Medicinska Fakulteten,  
Sahlgrenska Akademin vid Göteborgs Universitet  
för avläggande av doktorsexamen i Medicinsk Vetenskap  
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av  
Milan Milenkovic

Avhandlingen baseras på följande arbeten:

- I. Whole sheep ovary cryopreservation: evaluation of a slow freezing protocol with dimethylsulphoxide.**  
Milenkovic M., Wallin A., Ghahremani M., Brännström M.  
*Journal of Assisted Reproduction and Genetics*. 2011;28:7-14.
- II. The human postmenopausal ovary as a tool for evaluation of cryopreservation protocols towards whole ovary cryopreservation.**  
Milenkovic M., Ghahremani M., Bergh A., Wallin A., Mölne J., Fazlagic E., Eliassen E., Kahn J., Brännström M.  
*Journal of Assisted Reproduction and Genetics*. 2011;Feb 25. *Epub ahead of print*
- III. Viability and function of the cryopreserved whole rat ovary: comparison between different cryoprotectant concentrations and protocols.**  
Milenkovic M., Díaz-García C., Wallin A., Brännström M.  
*In manuscript*
- IV. Ovarian cortex transplantation in the baboon: comparison of four different intra-abdominal transplantation sites.**  
Díaz-García C., Milenkovic M., Groth K., Dahm-Kähler P., Olausson M., Brännström M.  
*Submitted, Human Reproduction*

Handledare:  
Prof. Mats Brännström

Biträdande Handledare:  
Med. Dr. Pernilla Dahm-Kähler

Opponent  
Prof. Pasquale Patrizio  
Yale University, New Haven, USA



UNIVERSITY OF GOTHENBURG

## ABSTRACT

Milenkovic, Milan, 2011. **Experimental studies on ovarian cryopreservation and transplantation.** Institute of Clinical Science, Department of Obstetrics and Gynecology, the Sahlgrenska Academy of Göteborg University, SE-413 45 Göteborg, Sweden.

Around 8% of all cancer victims are below 40 years of age and the survival after cancer treatment during childhood and reproductive years has increased considerably to be around 80% today. The clinical field of fertility preservation has emerged to enable cancer patients that are treated with potentially gonadotoxic chemotherapy-radiotherapy during childhood or reproductive ages, to preserve their fertility. In prepubertal girls and women of reproductive age, where immediate IVF is not an option, ovarian cryopreservation and later re-transplantation is today the only fertility option. Today 13 live births have been reported worldwide after ovarian cortex cryopreservation and avascular re-transplantation some years after the woman has been declared disease-free. However, the effectiveness of the method of ovarian cryopreservation is low. This thesis investigates several models to be used in improvement of ovarian cryopreservation protocols, including whole ovary cryopreservation, and in addition studies different transplantation sites for avascular cortex transplantation in a non human primate species.

The ovine ovarian ovary was used to examine a slow freezing method with the cryoprotectant dimethylsulphoxide (DMSO). Sheep ovaries were cryopreserved in liquid nitrogen and after thawing several viability tests were used. It was shown that the presence of DMSO was advantages for steroid and cyclic AMP output during in vitro perfusion and in cultured ovarian cells. Light microscopy showed well preserved tissue in the DMSO group after perfusion and a higher density of small follicles as compared to ovaries cryopreserved without of CPA. This study shows that the sheep ovary is a suitable method for further studies on whole ovary cryopreservation, including comparisons of different cryopreservation protocols.

The human postmenopausal ovary was evaluated as a tool for further cryopreservation research in the human. Naturally cryopreservation of human ovaries is aiming at preserving premenopausal ovarian ovaries or ovarian tissue. However, this study on post menopausal ovary shows that the aged ovary can be used as a valuable tool for the research, with special emphasises on the function of the stroma and the vascularity. The study showed that human post menopausal ovaries could be effectively cryopreserved in DMSO and that the stroma secreted androgens during in vitro perfusion. Electron microscopy showed a well-preserved morphology in these human ovaries.

The rodents are commonly used in reproductive physiology research and there is a large knowledge about the ovarian function and folliculogenesis in these species. The present study developed a technique for cannulation of the vasculature to the rat ovary and cryopreservation of the rat ovary by either vitrification or slow freezing. The cryoprotectant used was DMSO in high and low concentration. The result of the study indicated that a whole rat ovary can successfully be cryopreserved and that the DMSO concentration of 1.5 M is optimal when evaluating a secretion of steroids and viability of primordial follicles after cryopreservation.

Cryopreserved ovarian cortex tissue can either be transplanted back to an orthotopic or a heterotopic site. The live births reported in the human have all been from the orthotopic site but there are no comparative studies of different transplantation site in primate species. This study used the baboon as a model to compare different heterotopic intraabdominal transplantation sites. It was found that transplantation of the omentum was of advantage compared to transplantation to the pelvic wall or the pouch of Douglas. After a lag phase of 2-3 months the freshly transplanted ovarian tissue showed signs of growth of a large follicle and cyclicity of the animals.

In summary, the study presents several useable models for viability tests after whole ovary cryopreservation. These models can be explored in further research in the area. In a primate species, the omentum has been found a suitable heterotopic ovarian site. This finding can be used also in a human situation where orthotopic ovarian cortex transplantation is impossible because of anatomical or pathophysiological reasons.

**Key words:** ovary, cryopreservation, transplantation, animal models.

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