

Successive fertility following optimized perfusion and cryopreservation of whole ovary and allotransplantation in a premature ovarian failure rat model

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Introduction: Whole ovarian cryopreservation and transplantation (WOCP&TP) with vascular reanastomosis represents an exciting new technique aimed at improving the efficacy of ovarian tissue cryopreservation and fertility restoration. Studied showed that perfusion which was commonly used to expose the ovaries to cryoprotectants may cause substantial structural damage that limits the ovarian function. The aim of this study was to optimize perfusion, and to explore the possibility of restoring ovarian function and natural fertility after WOCP&TP to a premature ovarian failure (POF) rat model. We also investigated the effects of cryopreservation on offspring of rats after WOCP&TP.
Materials and methods: Sixteen rat whole ovaries were divided into two groups: the optimized group and the fresh group. The whole ovaries were perfused in a mode of linear increasing concentration of DMSO at a constant speed of 0.35ml/min for 20 minutes before cryopreservation. With the aid of a Planer controlled rate freezer, a slow-freezing cryopreservation protocol was utilized. After thawing, the cryoprotectants were washed out by reversing the concentration gradient by perfusion. The whole ovaries were observed in morphology and immunohistochemistry after cryopreservation and thawing. Healthy mature Lewis rats aged 8-10 weeks weighing 180-200g were used as donors and recipients for allotransplantation. To establish animal models of chemotherapy induced ovarian failure, 20 rats received a loading dose of intraperitoneal Cyclophosphamide(CTX) (50mg/kg) followed by daily intraperitoneal CTX injection of 8 mg/kg for 14 consecutive days. The ovarian function was assessed by blood hormonal levels and vaginal smears. Fertility restoration was quantified by live birth rate after mating and appearance of offsprings.
Results: The rates of non-apoptotic follicles in optimized group were about 80%, with no statistical differences in fresh group. Of the 20 recipient rats, fourteen began to recover ovarian function after 2 weeks of transplantation, with normal hormone levels after 4 weeks of transplantation. Four in 14 rats were pregnant and delivered live births. One rat was given second time pregnancy and delivery live birth. The second generation of young rats gave birth of the third generation of rats with no abnormalities.
Conclusions: High rates (70%) of restoration of ovarian function and live birth rates (20%) were obtained following WOCP&TP to a CTX-induced POF rat model utilizing optimized perfusion, which hold promise for the preservation of fertility in women. Cropservation could not affect the nature of successive generations.

Keywords : whole ovary, perfusion, cryopreservation, fertility restoration, premature ovarian failure

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