Abstract Details

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Residual ethylene glycol (eg) and dimethyl sulfoxide (dmso) concentration in human ovarian tissue during warming steps of vitrification method and slow freezing method

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Study question:

Is there concern whether all the cryoprotectants, which may be toxic in the human body, can be washed out at the time of transplantation in vitrification and slow freezing methods?

Summary answer:
Around 10mg/g DMSO and 10mg/g EG in vitrification method whereas 0mg/g in slow freezing method remained in ovarian tissue after warming just before transplantation.

What is known already:
There have been 60 births after transplantation of cryopreserved ovarian tissue (Donnnez J and Dolmans MM, 2015): 58 using the slow freezing method, and two using the vitrification method. In the warming protocols of vitrification method, the concentration of cryoprotectants is four times higher and warming time is four times shorter than in slow freezing. We have discussed time, cost, histology of follicles and stroma, viability test, and follicular development in both methods; however, we did not examine the residual amount of cryoprotectants in human ovarian tissue after warming just before transplantation and did not discuss about safety of patients.

Study design, size, duration:
This study was approved by the ethics committee of our clinics. Four patients who cryopreserved by vitrification method had Gender Identity Disorder and one who cryopreserved by slow freezing method had uterine cancer. We measured the amount of cryoprotectants before warming (I), after TS (II), after DS (III), and after WS2 (IV) in the vitrification method, and before warming (I), after TS3 (II), and after WS2 (III) in the slow freezing method.

Participants/materials, setting, methods:
Four ovarian tissue (10x10x1mm) were cryopreserved by the vitrification method using 6.4 mol/l EG and DMSO (Kagawa et. al., 2009) and one ovarian tissue (8x4x1mm) was frozen by the slow freezing method using 1.5 mol/l DMSO (Isachenko V., et al., 2012). The concentrations of DMSO and EG were analyzed by Shimadzu Techno-Research Inc., Kyoto, Japan. Concentrations in the ovary tissues were quantified using GCMS-QP2010Ultra (Shimadzu) with InertCap Pure-WAX column (0.25mm×30m, 0.50µm thickness; GL Science).

Main results and the role of chance:
The maximum amount of DMSO and EG in vitrification method vs. slow freezing method was 130.0±8.2 mg/g and 112.5±15.0 mg/g vs. 70 mg/g and < 0.050 mg/g before warming,respectively. In the slow freezing method, most of the cryoprotectants were washed out and the amount reached zero after thawing. In the vitrification method, there was a 30 to 50% reduction in after each step in warming, however, never reaching undetectable limits. We found around 10mg/g DMSO and 10mg/g EG in ovarian tissue after warming just before transplantation. The amount of cryoprotectant of ovarian tissue when cryoprotectants were not used was <0.050 mg/g. We believe that cryoprtectants should be completely washed out when we transplant ovarian tissue into the human body after warming. Safety is crucial in clinical medicine.

Limitations, reasons for caution:
We found most of the residual cryoprotectants in ovarian tissue were washed out and approached the detection limit in the case of slow freezing method. However, in the vitrification method by Cryotissue kit, around 10mg/g DMSO and 10mg/g EG remained in ovarian tissue after warming just before transplantation.

Wider implications of the findings:
We do not know the risk of toxicity of the cryoprotectants in detail, however, we are afraid of toxicity for mother and child. So, we use slow freezing method in our human ovariantissue preservation enterprise ' HOPE" for fertility preservation.Further research for improvement of cryopreservation method are needed.

Trial registration number:
None.

Keywords:
fertility preservation
residual cryoprotectants
human ovarian tissue
cryopreservation
vitrification