Comparison of the survival rates of ovarian tissue after slow freezing and vitrification by assessing histological structure and estradiol production during in-vitro culture

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Objectives

Our study was designed to identify a cryopreservation technique, which ensures better surviving of ovarian tissue. The aim is to compare effectiveness of slow freezing (SF) and vitrification (VIT), followed by in-vitro culture and histological analysis.

Materials and methods

All reagents were purchased from Sigma Aldrich SRL (Milan, Italy), unless other specified.

Cortical tissue was isolated from pubertal ovine ovaries, transported from the local slaughterhouse. Slivers (1*5*5 mm) were randomly allocated into six groups (n=5 in each): 1-non frozen control, 2-SF protocol, 3-VIT protocol, 4-non frozen control for in-vitro culture (IVC), 5-SF protocol for IVC, 6-VIT protocol for IVC.

The cryoprotectants used in SF protocol were 1.5M ethylene glycol (EG) and 0.1M sucrose (SUC). Vials with samples were thawed in a water bath at 37ºC and then washed in phosphate buffered saline containing 0.75M EG and 0.25M SUC. In VIT protocol cryoprotectants were 2.5M dimethyl sulfoxide, 2.5M EG and 0.5M SUC. Warming performedn at 37ºC in McCoy's 5a medium contained 0.5M SUC and then washed in the medium with 0.25M SUC.

For the histological analysis pieces of tissue were fixed in 4% paraformaldehyde, then dehydrated in series of ethanol and embedded in paraffin. The samples were sectioned (5 μm) and stained with hematoxylin and eosin. Follicles in the tissue were assessed by criteria established in our laboratory (Martelli et al., J Mol Endocrinol, 2006) and classified into three quality groups: intact, partially damaged and degenerated. Cortical strips were cultured in McCoy's 5a medium for 6 days at 37ºC and 5% CO2 with medium changed every 2 days. Then culture medium was analysed for the content of estradiol (E2) by ELISA assay (DRG, Marburg, Germany).

Results

The proportion of normal follicles showed significant difference between SF (total number of follicles counted=177) and VIT groups (total number of follicles=223): 27,96% vs 19,36% ($p<0,001$, χ^2 test). After the in-vitro culture, 84 and 69 follicles in total were counted for the SF and VIT groups, respectively. In this case, a higher percentage of intact follicles after slow freezing also has been shown: 21,87% vs 16,52% ($0<0,001$, χ^2 test).

The mean E2 concentrations for days 1,3 and 6 of in-vitro culture after SF protocol were 3,1 pg/ml; 11,4 pg/ml and 12,1 pg/ml, which were 20% lower, than values for non-frozen control (3,7 pg/ml; 13,6 pg/ml and 14,6 pg/ml). However, the difference of E2 concentration from the non-frozen control

was even greater for the VIT group, where the values were more than 50% lower: 1,8 pg/ml; 5,9 pg/ml and 7,6 pg/ml. An increase of E2 concentrations during the in-vitro culture was observed, which proved tissue recovering after cryopreservation.

Conclusions

Slow freezing is ensuring better morphological structure of ovarian cortex than vitrification. More specifically, a higher number of morphologically healthy follicles could be seen and a better production of estradiol during in-vitro culture of ovarian slivers was present.

KEYWORDS
Cryopreservation
Ovarian tissue