INFLUENCE OF DIFFERENT VITRIFICATION SOLUTIONS ON THE SURVIVAL OF SHEEP OVARIAN TISSUE

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Introduction: Over the past decades, advances in cryopreservation techniques and protocols for ovarian tissue is rapidly becoming a more widely offered technique by many medical centers and veterinary medicine around the world. Several studies of ovarian tissue cryopreservation of livestock animals have reported the feasibility of applying both slow freezing and vitrification methods. Vitrification is a fairly recent alternative method of cryopreservation and, compared slow freezing, is quicker and cheaper.

Methods: Ovarian tissue from 8 endangered Chuyi breed were transported to the laboratory at 36° C, dissected into smaller pieces (2.0x1.2x1 mm) in the L-15 with 5% FCS. The pieces were equilibrated sequentially (5%, 10% and 20% 10 min each) in six vitrification solution (VS): VS1: 20% DMSO + 20% EG + 0,5 M Sucrose; VS2: 20% DMSO + 20% PROH + 0,5 M Sucrose; VS3: 20% EG + 20% PROH + 0,5 M Sucrose; VS4: 20% DMSO + 20% EG + 0,5 M Sucrose + 10% FCS; VS5: 20% DMSO + 20% PROH + 0,5 M Sucrose + 10% FCS; VS6: 20% EG + 20% PROH + 0,5 M Sucrose + 10% FCS, then were vitrified by using the super-cooling ultra-rapid vitrification (SCURV) method in the Vit-Master™ (MTG, Germany). After thawing the pieces were equilibrated sequentially in the solution 0,75 M Sucrose + 10% FCS+ L-15 (10 min, 37°C) than L-15 + 10% FCS (15 min, twice), and the media for in vitro culture TCM-Hepes supplemented 10% ESS, 100 Lig/mL penicillin-streptomycin, 50 Lig/mL gentamicin and 2 mM L-Glutamine, 5 Lig/mL FSH, 5 Lig/mL LH.

Results: The viability assay was performed by light microscopy after hematoxylin and eosin staining of tissue sections after 7 days in vitro culture. The highest percentage of viable follicles was observed in the groups VS4 and VS5. The normal primordial, primary and secondary follicles in these groups were 58±3.3, 37±1.9, 29±1.6 and 52±3.1, 30±2.2, 25±1.4 respectively, but was significantly different from fresh control (98.2±1.1) ($P < 0.05$).

Conclusion: Using a solution containing VS4 and VS5 were the most efficient for vitrifying sheep ovarian tissue.