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VITRIFICATION OF ISOLATED BOVINE PRE-ANTRAL FOLLICLES USING A COMMERCIAL VITRIFICATION KIT

BUS A, MERCKX E, ANDRIES S, LEROY JLMR, BOLS PEJ

Gamete Research Centre, Veterinary Physiology and Biochemistry, Department of Veterinary Sciences, University of Antwerp, Universiteitsplein 1, B-2610 Wilrijk, Belgium

Significant advances in cancer diagnosis and treatment have stimulated interest in fertility preservation strategies as chemicals or ionizing radiotherapy often threaten future reproductive capacity. Ovarian tissue cryopreservation currently is the only option for preserving the reproduction potential of pre-pubertal girls and women whom cannot undergo hormonal stimulation. However, there is a huge concern regarding the possible presence of malignant cells in the retrieved ovarian tissue, which could lead to cancer reintroduction after reimplantation of the frozen-thawed tissue strip. Cryopreservation of isolated early pre-antral follicles (PAFs) (and subsequent *in vitro* culture, maturation and fertilization) might therefore represent an interesting alternative. We hypothesized that isolated bovine PAFs can be vitrified on a routine basis using a commercially available vitrification kit.

Mechanically isolated PAFs (Ø 30-70µm) were cultured in DMEM/Ham's F12 (38,5°C, 5% CO_2). At day 2 of culture, follicles with an intact basal membrane and intact connection between oocyte and surrounding granulosa cells were selected for vitrification using the IrvineScientific® Vitrification Kit for human oocytes and embryos (Alere Health BV. Tilburg, NL). After placement of 2 follicles in a drop of culture medium on a 90mm petri dish, the drop was merged into the first drop of equilibration solution (ES; 7,5% DMSO, 7,5% EG; 1 min). Then follicles were transferred to the second drop of ES (1 min) and vitrification solution (VS; 15% DMSO, 15% EG, 0,5M sucrose; 30 s). Follicles were loaded on a capillary rod (HSV Kit, Groupe I.M.V. Technologies) that was placed into a straw and quickly plunged into LN_2. Follicles were thawed the same day. After merging the capillary rod in a drop of thawing solution (TS; 1,0M sucrose; 1 min), follicles were transferred to a drop of dilution solution (0,5M sucrose; 4 min) and to 2 drops of washing solution (4 min each). Follicles were cultured individually in a 96-well plate (38,5°C, 5% CO_2). Viability was assessed one day post warming using neutral red [1].

In total, 56 follicles (2 replicates) were vitrified, 36 were cultured after thawing. One day post thawing, 16/21 follicles (76%) stained positively for neutral red.

A large loss of follicles occurred, as the small size and shrinkage of follicles during exposure to ES, VS and TS, makes them difficult to retrieve. Gaining expertise with handling follicles greatly improves the efficiency. In conclusion, PAFs can be successfully vitrified using the IrvineScientific® Vitrification Kit, although additional trials will be necessary to improve the efficiency.

[1] Langbeen A, Jorssen EPA, Granata N, Fransen E, Leroy JLRM, Bols PEJ. Effects of neutral red assisted viability assessment on the cryotolerance of isolated bovine preantral follicles. J Assist Reprod Genet 2014; 31**:** 1727-36.