First Symposium of the European College of Animal Reproduction (ECAR)
4-6 July 2019, Vienna, Austria

THE USE OF ALGINATE BEADS FOR THE VITRIFICATION OF ISOLATED BOVINE PRE-ANTRAL FOLLICLES

BUS A, LEROY JLMR, BOLS PEJ

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

Fertility preservation is a concern in humans and animals, as the preservation of genetic material from endangered animal species or animals with important genetic traits will also greatly benefit from the development of alternative fertility preservation strategies. In humans, cancer treatments often threaten future reproductive capacity. Currently, ovarian tissue cryopreservation is the only option to preserve fertility for pre-pubertal girls and women whom cannot undergo hormonal stimulation. However, the reintroduction of malignant cells after reimplantation of a frozen-thawed ovarian tissue strip is a huge concern. Cryopreservation of isolated pre-antral follicles (PAFs) might therefore represent an interesting alternative. However, so far, individual follicle cryopreservation techniques, without hydrogel support, are labor-intensive and a substantial proportion of isolated follicles is lost during handling and after warming. A vitrification protocol, successfully used for non-embedded isolated PAFs, resulted in lower viability when PAFs were vitrified encapsulated in alginate beads. Therefore, we hypothesized that embedded isolated bovine PAFs (as a model for human), can be vitrified more efficient when a longer exposure time to cryoprotectantia (CPA) and/or higher CPA concentration is used.

Mechanically isolated bovine PAFs (Ø 30-70µm) were cultured in DMEM/Ham's F12 (38,5°C, 5%CO2). At D2 of culture, morphologically normal follicles (i.e. intact basement membrane and intact connection between oocyte and granulosa cells) were embedded in 2% sodium alginate beads and cultured in DMEM/Ham's F12 (38,5°C, 5%CO2). At D3, embedded follicles were vitrified using stainless steel mini mesh cups, following 4 different vitrification protocols (Group 1: Equilibration Solution1 (ES): 7,5% DMSO+EG - 2min, ES2: 7,5% DMSO+EG - 2min, Vitrification Solution (VS): 15% DMSO+EG - 30sec; Group 2: ES1: 7,5% DMSO+EG - 3min, ES2: 7,5% DMSO+EG - 3min, VS: 15% DMSO+EG - 3min; Group 3: ES1: 10% DMSO+EG - 2min, ES2: 25% DMSO+EG - 2min, VS: 40% DMSO+EG - 30sec; Group 4: ES1: 10% DMSO+EG - 3min, ES2: 25% DMSO+EG - 3min, VS: 40% DMSO+EG - 3min). Follicles were warmed the same day (1M sucrose - 1min, 0,5M sucrose - 4min) and cultured. On D4, viability was assessed by the non-invasive vital dye Neutral Red. Preliminary data using the 4 treatments described above, showed the following survival rates: Group 1 (n=16) 56,25%, group 2 (n=18) 88,89%, group 3 (n=15) 93,33%, group 4 (n=10) 0%.

We conclude that embedding allowed us to handle follicles smoothly without excessive manipulation, was less labor-intensive and reduced the loss of follicles. The protocol used for group 1 was successfully used with non-embedded isolated follicles in earlier experiments, but showed a lower viability when used with embedded follicles. A longer exposure time to CPA (group 2) and a higher CPA concentration (group 3) resulted in a higher viability as shown with NR staining. An even higher CPA concentration and longer exposure time (group 4) is detrimental for alginate beads and follicles. Beads fell apart and no follicles did survive the vitrification procedure. Based on the increased work efficiency, we consider it advantageous to optimize the protocol for the vitrification of embedded follicles to increase survival and maintain morphology after vitrification.