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A COMPARISON BETWEEN 2D AND 3D SYSTEMS FOR THE VITRIFICATION OF ISOLATED BOVINE PRE-ANTRAL FOLLICLES

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Cancer treatments often threaten future reproductive capacity. Currently, ovarian tissue cryopreservation is the only option to preserve fertility for pre-pubertal girls and women who cannot undergo hormonal stimulation. However, the reintroduction of malignant cells after reimplantation of a frozen-thawed tissue strip is a huge concern. Cryopreservation of isolated pre-antral follicles (PAFs) might therefore represent a promising alternative. So far, individual follicle cryopreservation techniques, without hydrogel support, are labor-intensive and a substantial proportion of isolated follicles are lost during handling and after warming. Therefore, we hypothesized that isolated bovine (as a model for human) PAFs can be vitrified more efficient when encapsulated in alginate beads.

Mechanically isolated adult bovine PAFs (Ø 30-70µm) were cultured in DMEM/Ham's F12 (38,5°C, 5%CO_2). 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 (on average 3 per bead). At D3, embedded follicles were vitrified using stainless steel mini mesh cups while non-embedded follicles were vitrified individually using High Security Vitrification straws. Always the same vitrification protocol (2x2min 7,5%DMSO+7,5%EG, 30s 15%DMSO+15%EG+0,5M sucrose, LN_2) was used. Follicles were warmed the same day (1min 1M sucrose, 4min 0,5M sucrose) and cultured. On D4, viability was assessed by fluorescent marker calcein-AM and the non-invasive vital dye Neutral Red. Embedded follicles (n=41) showed a survival rate of 66,6% compared to 83,3% if non-embedded (n=41). 36,6% of the embedded follicles showed normal light-microscopic morphology compared to 75% of the non-embedded.

We conclude that, embedding allowed us to handle follicles smoothly without excessive manipulation, was less labor-intensive and reduced the loss of follicles. Regarding the increased work efficiency, but lower viability and higher proportion of follicles showing impaired morphology, we consider it advantageous to optimize the protocol for the vitrification of embedded follicles to increase survival.