Effect of equilibration temperature and time on feline ovarian tissue vitrification

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The entire members of Felidae are currently classified as endangered except the domestic cat (Felis catus) making it an excellent model for conservation studies. Vitrification of feline ovarian tissue is an emerging conservation technique suitable in field conditions however, not yet standardized. Thus, the aim was to establish a suitable vitrification protocol for feline ovarian tissue in field condition. Feline ovarian tissue fragments were punched with biopsy punch (1.5mm diameter) and divided into 4 groups. Group 1 was directly placed in culture (Fresh control - FC), while the other three were placed on 30G needles (4 fragments/needle) and vitrified using 3 protocols (A, B, C). Protocol A involved two step equilibrations for 10min each at 4°C and then vitrification [1]. Protocol B involved three step equilibrations for 14min in total at room temperature [2], while protocol C was the same with protocol B except the equilibration timings which were reduced by half. Fragments were warmed and placed in culture [1] for six days. Follicular morphology, cellular proliferation (expression of Ki-67, MCM-7) and apoptosis (expression of caspase 3) were evaluated. Data were analysed using Chi square. Proportions of morphological intact follicles were higher in FC (P = 0.0001) and protocol C (P = 0.0383) in comparison to the other protocols at the sixth day of culture. Generally, most follicles remained at primordial state which was confirmed by the low expression of ki-67, MCM-7 markers. In conclusion, protocol C, which has lower equilibration time at room temperature, can be used for vitrification of feline ovarian tissue. [1] Mouttham L., 2016, Cryobiology, 73, 187-95; [2] Amorim C.A., 2013, Human Reproduction, 28, 2146-56.