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OSMOTIC CHALLENGE OF BOVINE EARLY PRE-ANTRAL FOLLICLES WITH DIFFERENT CRYOPROTECTANT AGENTS

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Significant advances in cancer diagnosis and treatments have stimulated interest in fertility preservation strategies as chemical or ionizing radiotherapy often threatens future reproduction. Ovarian tissue cryopreservation currently is the only option for preserving the reproduction potential of pre-pubertal girls and women whom cannot undergo hormonal stimulation, ovarian tissue cryopreservation currently is the only option. 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 preantral follicles (PAFs) (and subsequent *in vitro* culture (IVC), maturation (IVM) and fertilisation (IVF)) might therefore represent an interesting alternative. Existing protocols are based on protocols for freezing embryos and oocytes . However, in order to improve follicular survival after cryopreservation, it is essential to develop a protocol for follicles specifically. Indeed, follicles are quite different from both embryos and oocytes, if only because they are composed of two different cell types (namely the oocyte and the surrounding (pre-)granulosa cells). In order to provide a biophysical base for choosing optimal cryoprotectant agents (CPAs) that avoid severe volume changes and formation of intracellular ice crystals, in this experiment, two-day-old isolated bovine PAFs were osmotically challenged by exposing them to different concentrations of cryoprotectant agents (CPAs). Briefly, isolated bovine early PAFs were exposed to either ethylene glycol (EG) or dimethyl sulfoxide (DMSO) in different final concentrations: 1 M, 2 M, 3 M, 4 M and 5 M at room temperature, and photographed at different time points (every half minute between 0 and 5 minutes) after the onset of exposure to calculate their volume over time (5 - 10 follicles per CPA and per concentration). Although there was a high variability in the individual response of the follicles to this CPA challenge, all follicles showed a typical 'shrink/swell' curve. Analysis with two-way ANOVA showed no interaction between the type of CPA and the respective concentrations. This means that volume differences in time between the minimum and maximum for both EG and DMSO were uniform across concentrations. Across all concentrations, time until the post stimulus maximum (i.e. the maximum volume to which follicles re-expand after shrinkage) appeared significantly longer in the EG group (P = 0.04), indicating that bovine early PAFs are less permeable to EG than DMSO. To our knowledge, this is the first time that isolated bovine early PAFs were osmotically challenged by exposing them to different concentrations of penetrating CPAs. This has provided us with some basic insights in follicular permeability to CPAs. These insights are a first step in the design of cryopreservation protocols for isolated early PAFs specifically.

KEYWORDS: bovine preantral follicle, cryoprotectant agent, DMSO, EG, follicle permeability, osmotic challenge