Vitrification of immature equine oocytes: investigation of volume variations during the process

Gatez C.[1], Ectors F.J.[2], Farnir F.[3], Beckers J-F.[1] and Deleuze S.[4]

1. Laboratory of animal Reproductive Physiology , Department of functional Sciences, FMV, ULg - 2. GIGA Murine Transgenic Platform, ULg
3. Biostatistics and bioinformatics, Department of animals productions, FMV, ULg - 4. Department of clinical Sciences, FMV, ULg.

❑ Introduction:

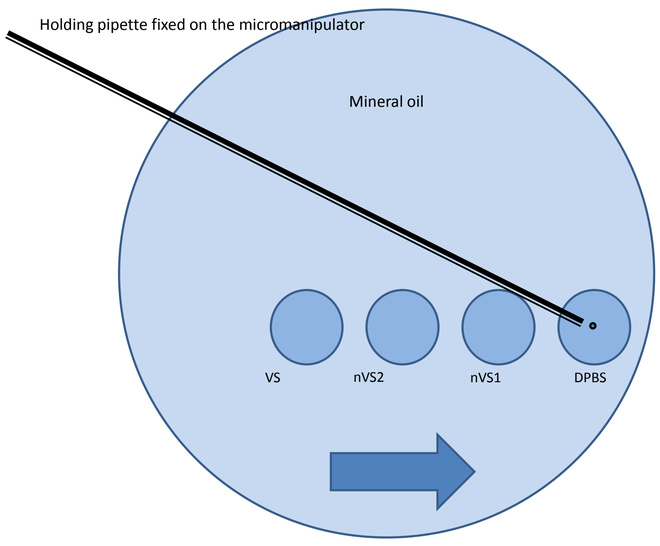
Vitrification is a cryopreservation method based on an increase to infinite viscosity (no crystallization) obtained after short time exposures to high concentration of cryoprotectants (CPs) associated with very fast cooling and warming.
Crystallization of intracellular water induces disorganisation of cell organites and cell death. For these reasons, when oocytes are vitrified, the dehydration and CPs incorporation have to be optimized for a better cell survival. Actually the large size, the low membrane permeability and the high lipid content of the equine oocyte will interfere with the transmembrane flows of molecules. The bad cryopreservation results are exacerbate by difficulties to collect equine ovary in slaughterhouse and to recover oocytes by follicle scraping.

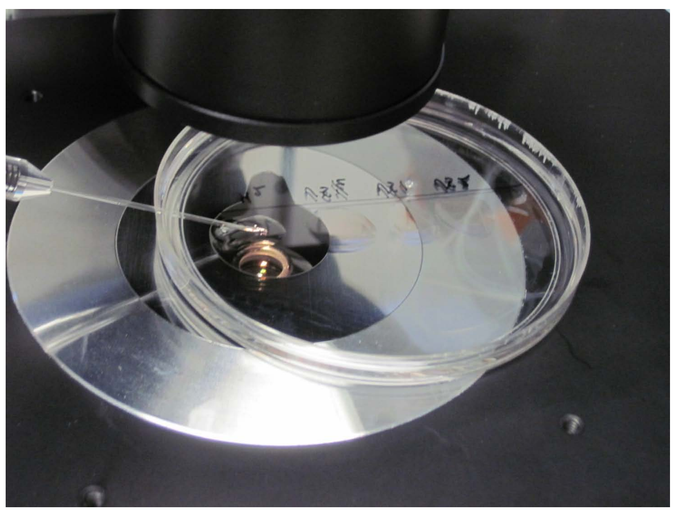
❑ Objective:

This study was conducted to evaluate denuded oocyte volume variations during two protocols of vitrification based on dimethyl sulfoxyde, ethylene glycol and sucrose. Base solution of the first one was a usual saline solution (PBS), and for the second one a commercialized solution for slow freezing of equine sperm and containing glycerol and egg yolk plasma (INRA-Freeze®).

❑ Material and methods:

Oocyte volume was extrapolated from surface measurements of microscopic photographies taken every 30 seconds during expositions to CPs solutions.
These observations are performed on the lid of a 10 cm petri dish. Fifty microliters drops of successive CPs solutions were aligned one after the other. Oocytes are maintained with a holding pipette fixed on the micromanipulator. The petri dish is manually moved in order to place the oocyte during exposition times in the successive CPs solutions.





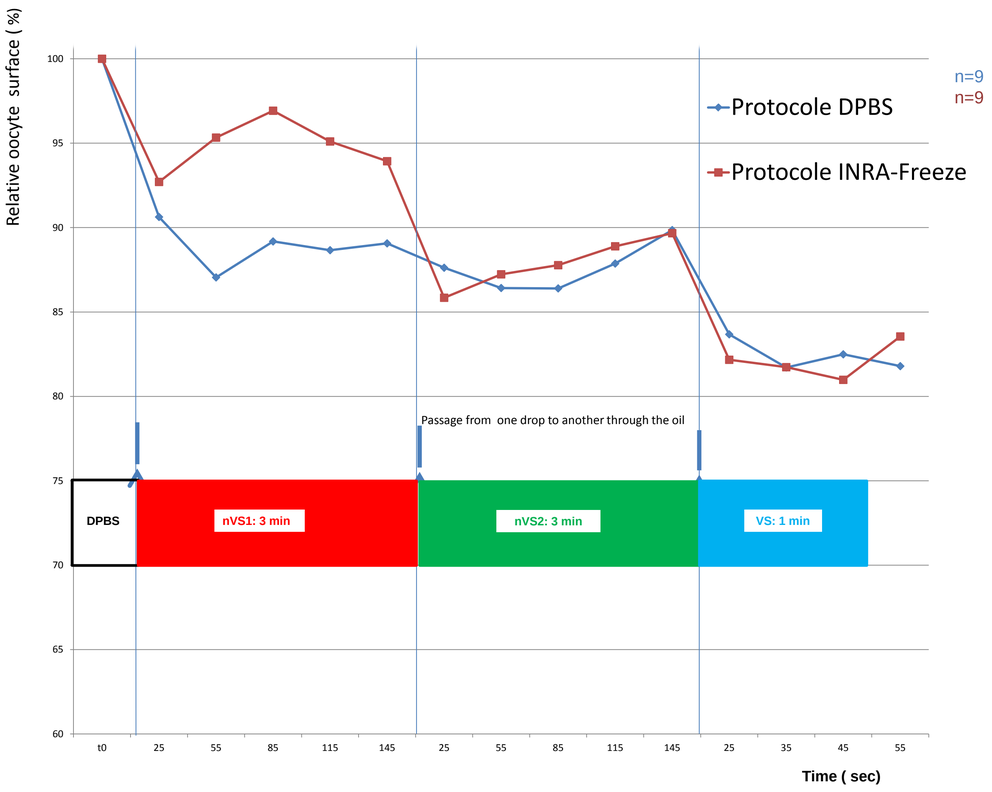
Vitrification protocol 1 based on DPBS+ 10%FCS:
nVS1 : DMSO 5% + EG 5% 3 min
nVS2: DMSO 10% + EG 10% 3 min
VS: DMSO 20% + EG 20% + Suc 0,5M 1 min

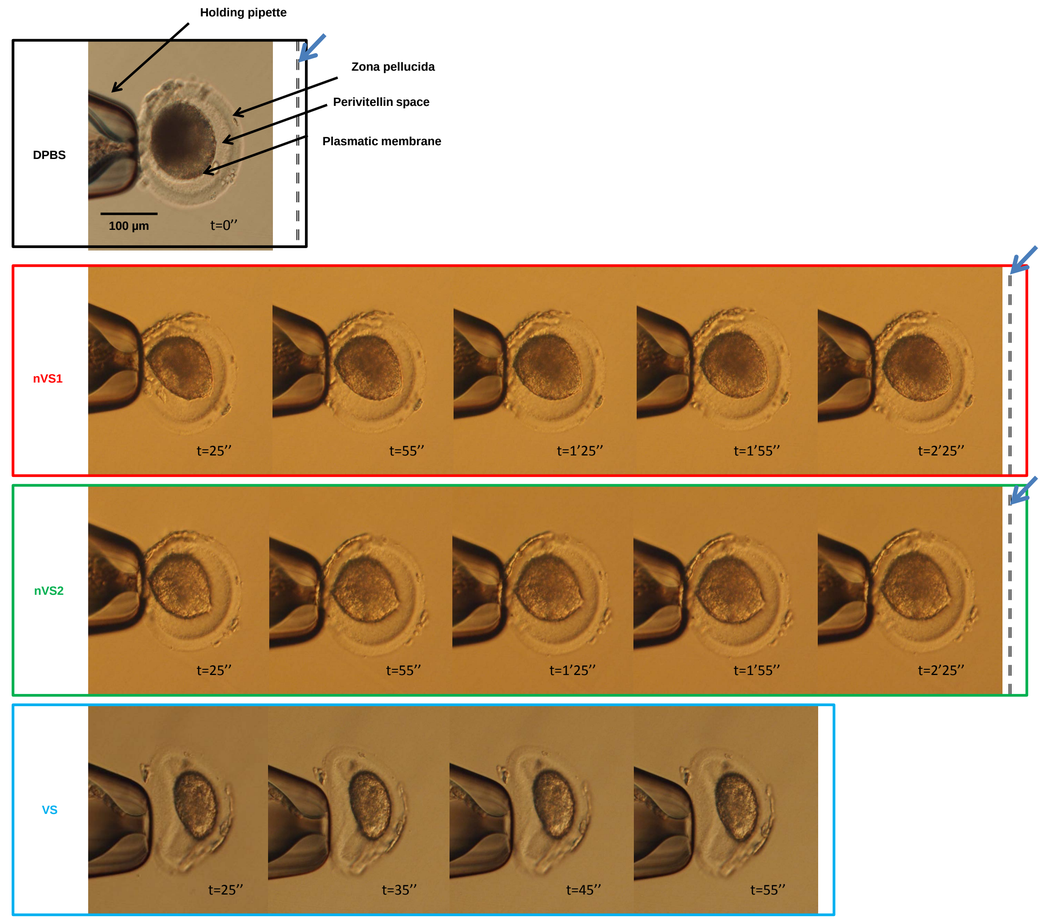
Vitrification protocol 2 based on INRA-Freeze®:
nVS1 : DMSO 3,75% + EG 3,75% 3 min
nVS2: DMSO 8,75% + EG 8,75% 3 min
VS: DMSO 18,75% +EG 18,75% +Suc 0,5M 1 min

Solution for equine sperm slow freezing (ready-to-use) containing **INRA96®**
+ egg yolk plasma (2%) + glycerol (2.5%)

❑ Results:

We observed a rapid size reduction immediately after immersion into the solution illustrating the osmotic effect (outflow of water) followed by a small recovery of volume corresponding to a partial inflow of CPs across the membrane.





Microscopic photographies compilation of the same oocyte during the vitrification protocol (320x)
Passage from one drop to another through the oil

❑ Conclusion:

Oocyte volume decreased significantly during the two protocols (about 30%), whereas reduction was significantly more important with the DPBS as base solution (P=0.04). The lower membrane permeability observed with the INRA-Freeze® as base solution could be explained by the presence of glycerol or egg yolk plasma in the medium.