Vitrification of immature equine oocytes

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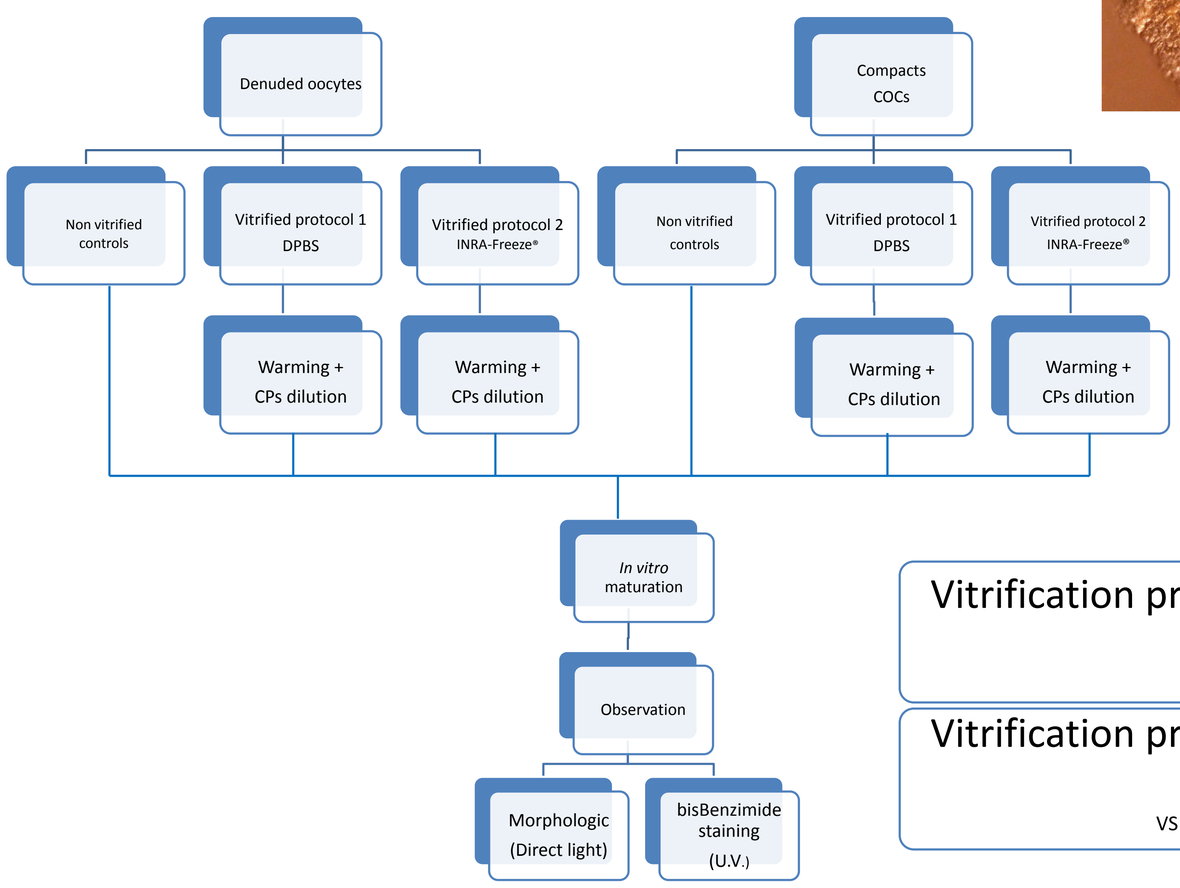
Introduction:

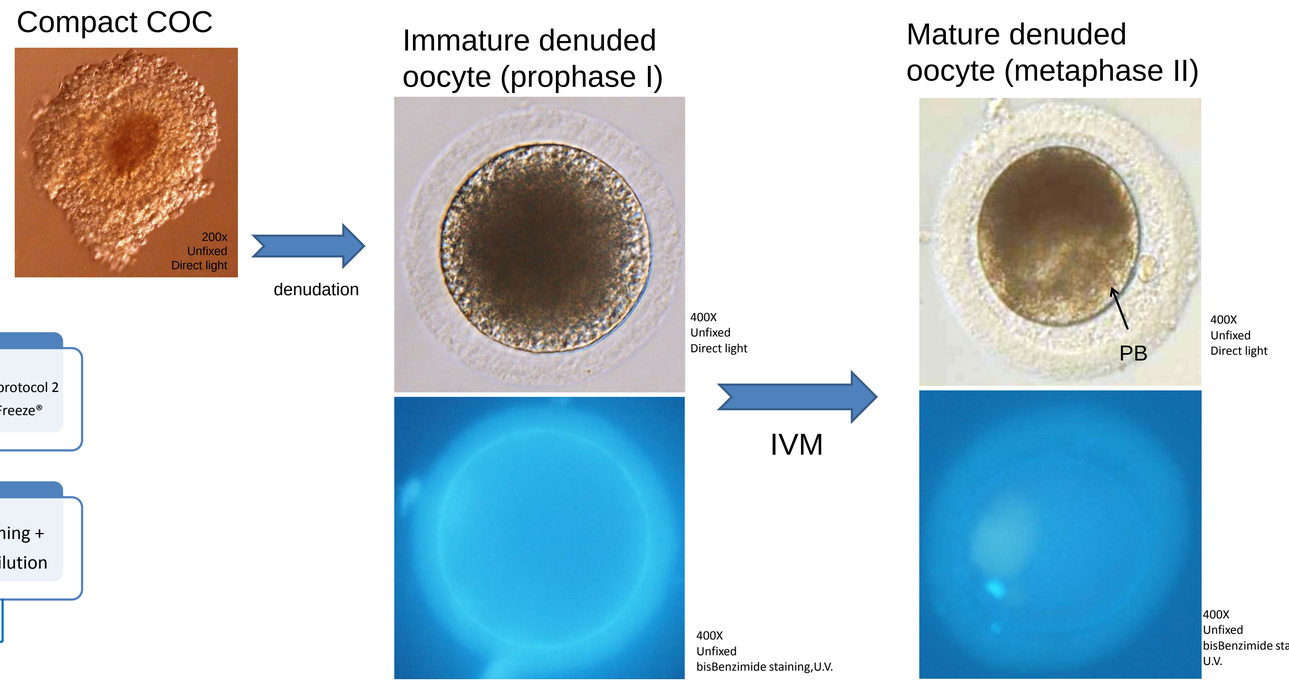
Vitrification is a method of cryopreservation. It implies solidification without crystallization during cooling & warming. This is obtained by an infinite increase of viscosity: solidified liquid has the same lack of internal molecular movements as in a crystalline solid.
Absence of ice crystal means no dilatation, no solution effect, no mechanical injuries. This can be obtained after a short time exposure to high concentration of cryoprotectants (CPs) associated with very fast cooling & warming.
This method was chosen as a gold standard for the equine immature oocyte conservation. Oocytes are usually retrieved surrounded by layers of granulosa cells forming the Cumulus-Oocyte-Complex (COC). These cellular layers, the large size, the low membrane permeability and the high lipid content of the equine oocyte interfere with the transmembranar flux of water and CP. These characteristics can compromise the survival of the cell after cryopreservation and limit the success of biotechnology of reproduction in horses.

Objective:

Investigate the impact of vitrification in DPBS or INRA-Freeze® based media and denudation on the oocyte's ability to reach metaphase II (maturation rate) after IVM.

Material and methods:





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

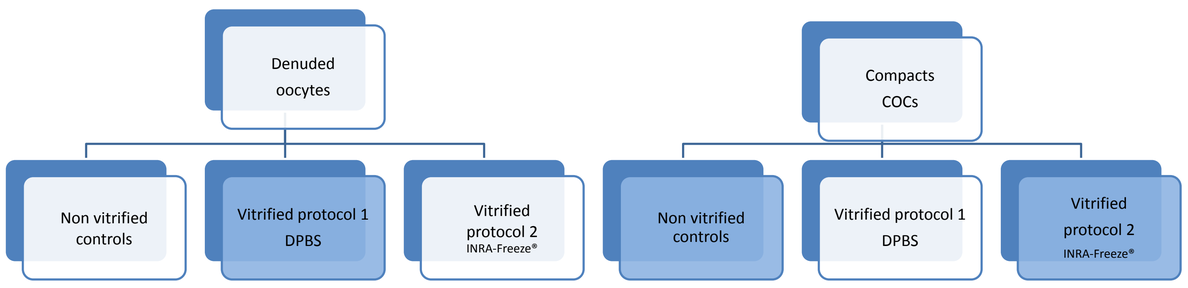
Vitrification protocol 2 based on INRA-Freeze®:

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®**

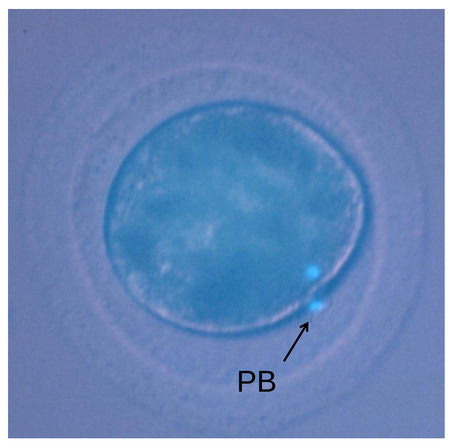
+ egg yolk plasma (2%) + glycerol (2.5%)

Results:



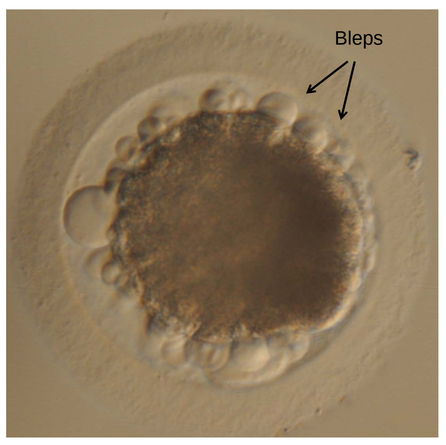
Observation U.V. n=94	% (n=7)	% (n=15)	**%** (n=14)	% (n=23)	% (n=21)	% (n=14)
Meta II	14 (1)	7 (1)	30 (4)	26 (6)	24 (5)	36 (5)

Observation Direct light n=142	% (n=10)	% (n=20)	**%** (n=22)	% (n=31)	% (n=30)	% (n=29)
Meta II	**20 (2)**	**10 (2)**	**41 (9)**	**42 (13)**	**17 (5)**	**31 (9)**



Oocyte in metaphase II:
Chromosomes can be observed as a fluorescent spot in the peripheric zone of the oocyte. The second spot located in the perivitellin space corresponds to the 1st polar body (PB).

400X
Unfixed
bisBenzimide staining, U.V. +direct light



Degenerated oocyte :
The plasmatic membrane has been desorganized and shows iatrogenic extrusions called "Bleps". Blebs are due to an excessive osmotic stress.

400X
Unfixed
Direct light

Discussion:

As previously described, non-vitrified denuded oocytes have a lower maturation rate than COCs. In our experiment, maturation rates following vitrification in INRA-Freeze® based medium were higher for both COCs and denuded oocytes. This suggests that the use of a mixture of CPs at lower concentrations decreases their toxicity. Adjunction of INRA-Freeze® to the medium somehow seems to have conteracted the deleterious effect of denudation on IVM.

Conclusion:

Further studies with increased numbers of oocytes are still needed to validate these results, identify the steps of the experiment where INRA-Freeze® is most beneficial and the mechanisms involved.