VAKGROEP VERLOSKUNDE, VOORTPLANTING EN BEDRIJFSDIERGENEESKUNDE
ONDERZOEKSGROEP VOORTPLANTING GEZELSCHAPSDIEREN

RECENT PROGRESS IN VITRIFICATION, OOCYTE AND EMBRYO CULTURE IN HORSES, CATS AND CATTLE

Ann Van Soom – 5 september 2019 - Wageningen, The Netherlands

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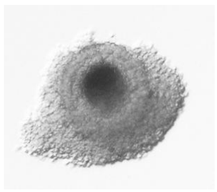
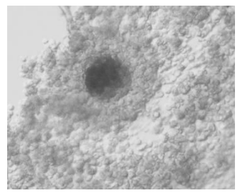
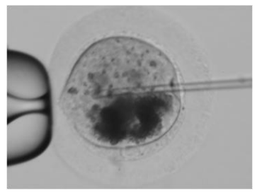
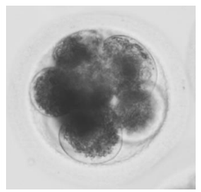
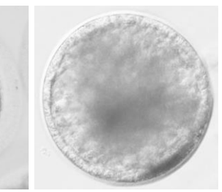
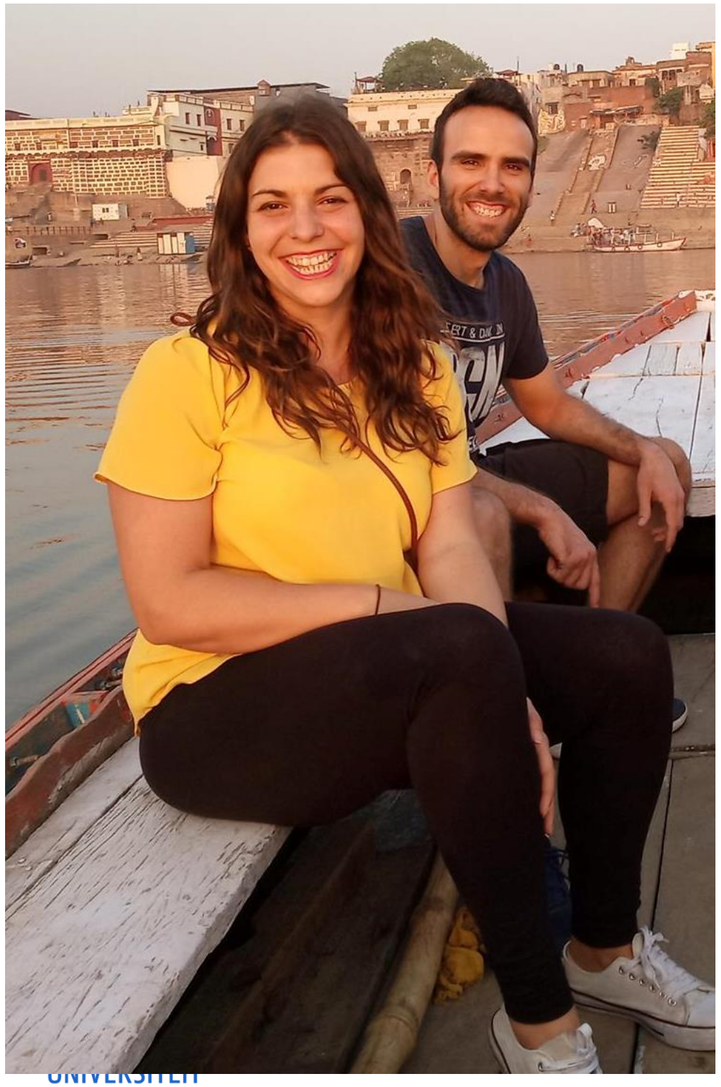
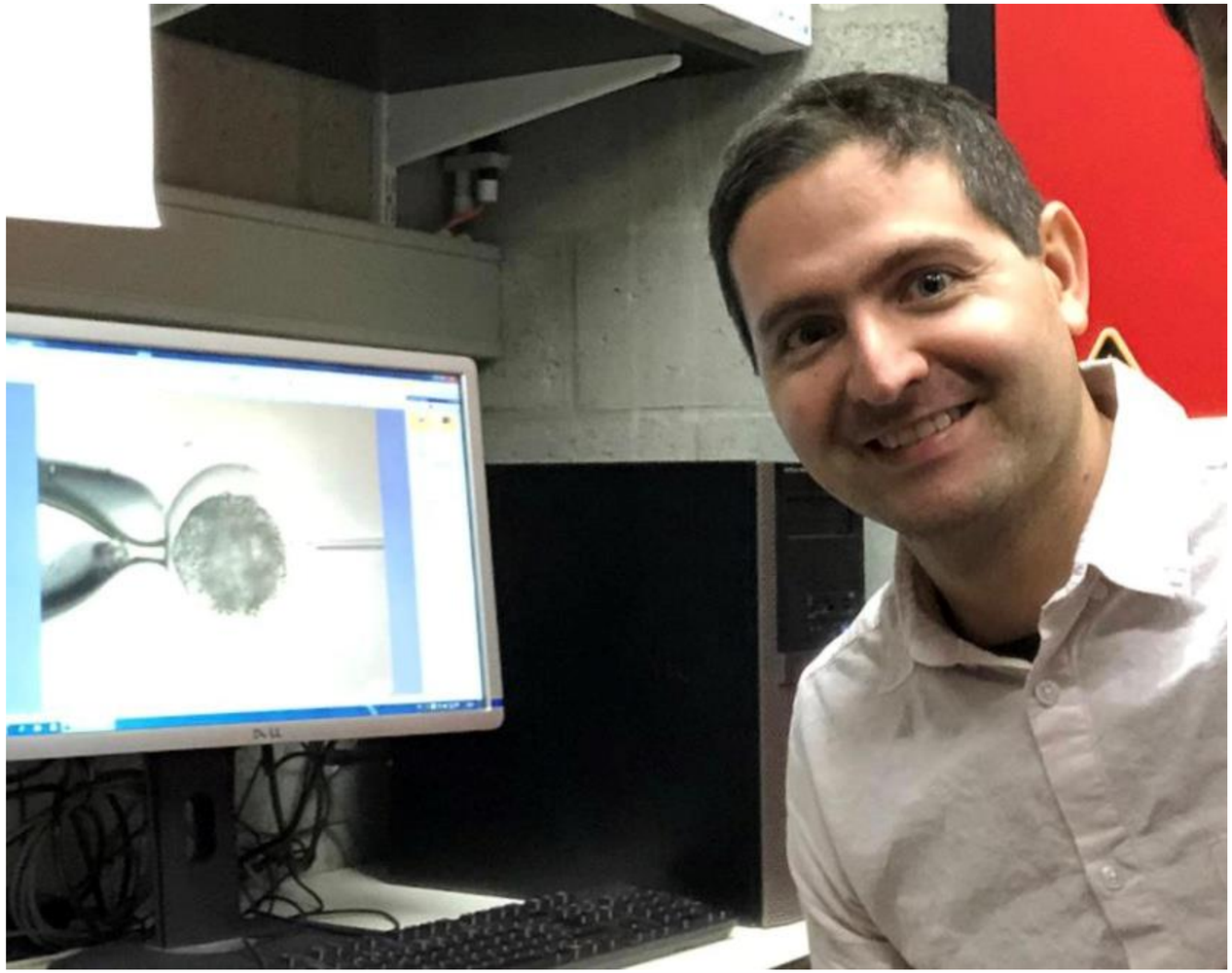







NEREA ORTIZ ESCRIBANO- KATRIEN SMITS



FELINE SNOECK – DANIEL VELEZ



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RECENT PROGRESS IN VITRIFICATION

ALASKAN WOOD FROG FREEZES OVER SLOWLY

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CRYOPRESERVATION TO STOP TIME

Oocyte/embryo

Water

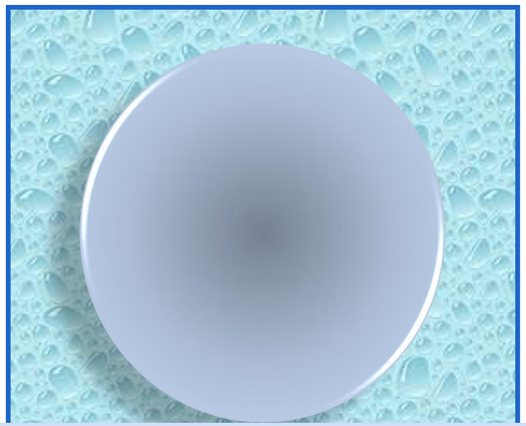
Cryoprotectants (CPA)

Low CPA
Slow cooling

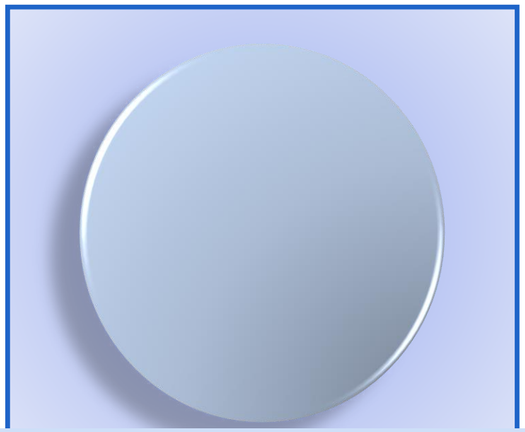
High CPA
Rapid cooling

SLOW FREEZING

VITRIFICATION



Ice crystals form in the medium



No ice crystals form in the medium

Human Reproduction Update, Vol.23, No.2 pp. 139–155, 2017
Advanced Access publication on November 4, 2016 doi:10.1093/humupd/dmw038

human reproduction update

Oocyte, embryo and blastocyst cryopreservation in ART: systematic review and meta-analysis comparing slow-freezing versus vitrification to produce evidence for the development of global guidance

Laura Rienzi[1,*], Clarisa Gracia[2], Roberta Maggiulli[1], Andrew R. LaBarbera[3], Daniel J. Kaser[4], Filippo M. Ubaldi[1], Sheryl Vanderpoel[5,6], and Catherine Racowsky[4]

WIDER IMPLICATIONS: Data from available RCTs suggest that vitrification/warming is superior to slow-freezing/thawing with regard to clinical outcomes (low quality of the evidence) and cryosurvival rates (moderate quality of the evidence) for oocytes, cleavage-stage embryos and blastocysts. The results were confirmed by cohort studies. The improvements obtained with the introduction of vitrification have several important clinical implications in ART. Based on this evidence, in particular regarding cryosurvival rates, laboratories that continue to use slow-freezing should consider transitioning to the use of vitrification for cryopreservation.

IN HUMAN OOCYTE VITRIFICATION

Clinical pregnancy rates do not differ between fresh (48 %) vs vitrified (49 %) oocytes BUT:

1. Denuded *in vivo* matured oocytes are used
2. Vitrified-warmed oocytes are often subjected to ICSI

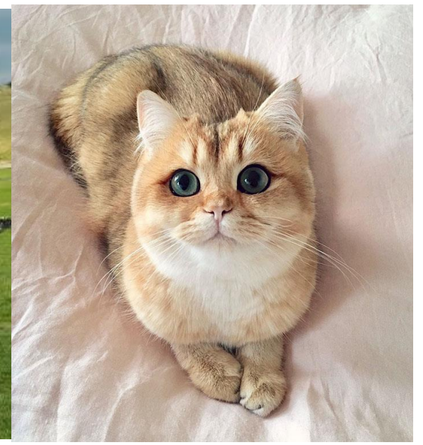
Cobo and Diaz, 2011

WHY OOCYTE VITRIFICATION IN ANIMALS?

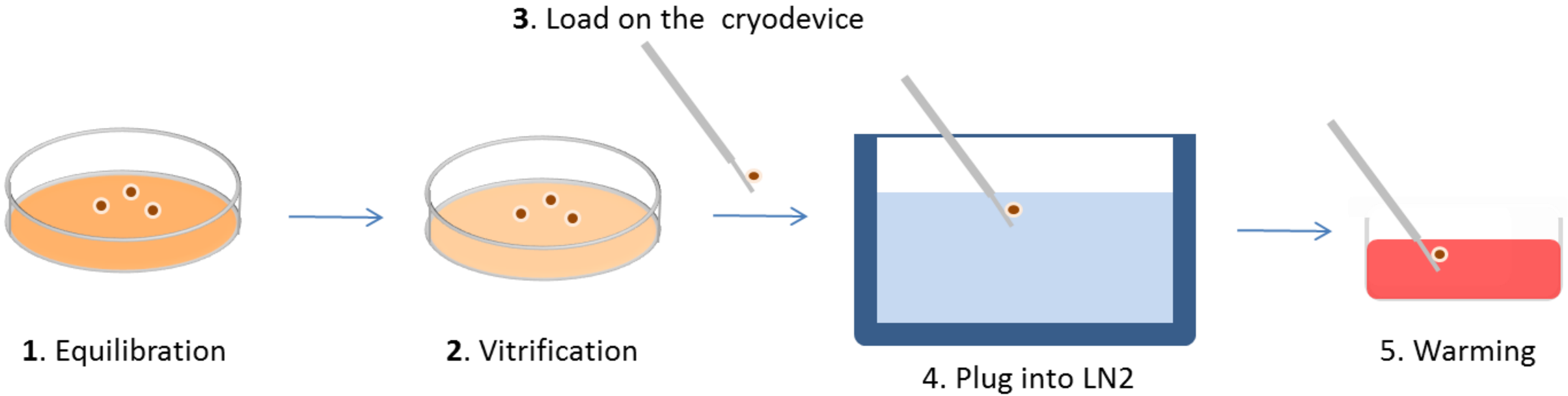
Rescue gametes from dead animal

Oocyte banking for research

Superior female genetics preservation



VITRIFICATION STEPS



15-20 % CPA

30-40 % CPA
0.5-1 M sucrose

VITRIFICATION OR GLASS FORMATION

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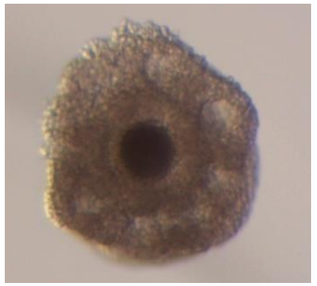
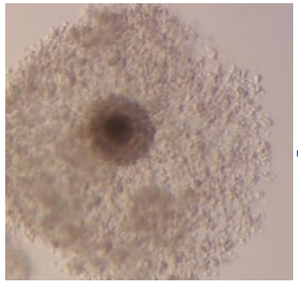
OOCYTE AND EMBRYO CULTURE IN CATTLE AND HORSES

ASSISTED REPRODUCTION IN CATTLE

Ovary puncture

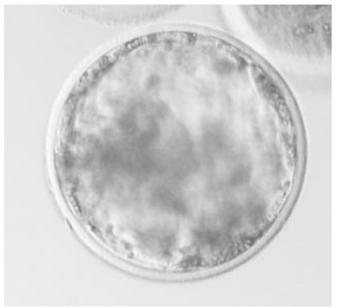


Immature



Mature

In vitro fertilization



Embryo

Embryo transfer

Offspring

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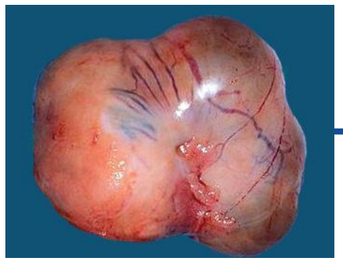
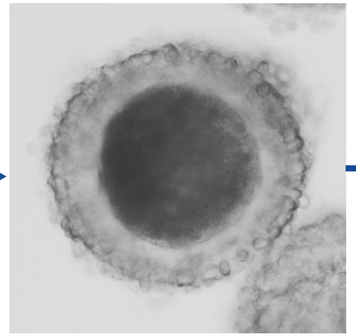
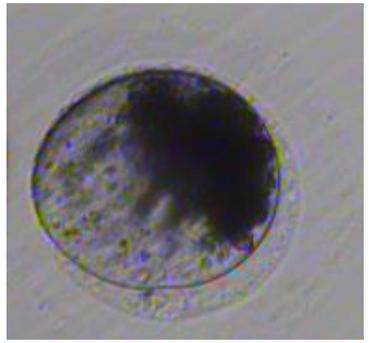
ASSISTED REPRODUCTION IN HORSES



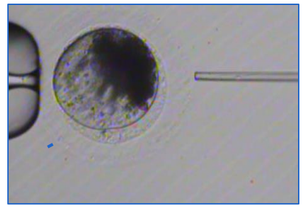
Ovary puncture



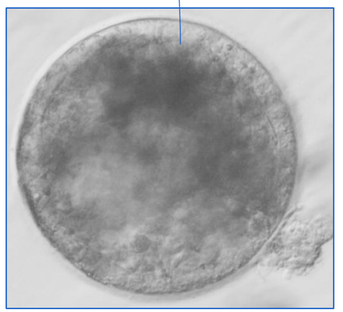
Immature



Mature



ICSI



Embryo

Embryo transfer

Offspring

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FACTORS FOR VITRIFICATION SURVIVAL

- CPA-concentration and properties
- Size of the cells (cryoprotectants and cooling speed)
- Size of device (cooling speed)
- Cell-cell interaction
- Lipids in membrane/cytoplasm

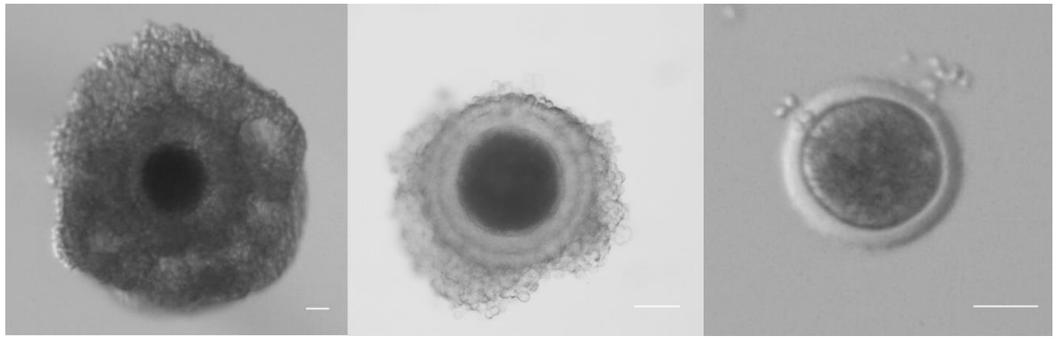
FACTORS FOR VITRIFICATION SURVIVAL

- CPA-concentration and properties
- Size of the cells (cryoprotectants & cooling speed)
- Size of device (cooling speed)
- Cell-cell interaction
- Lipids in membrane/cytoplasm

SIZE OF THE CELLS

SPECIFICS OF OOCYTES

- Large cell (120 µm)
- Germinal vesicle (immature) or metaphase II (mature)
- Surrounded by zona pellucida
- Can be surrounded by cumulus cells



Cumulus oocyte complex | Corona Radiata oocyte | Denuded oocyte

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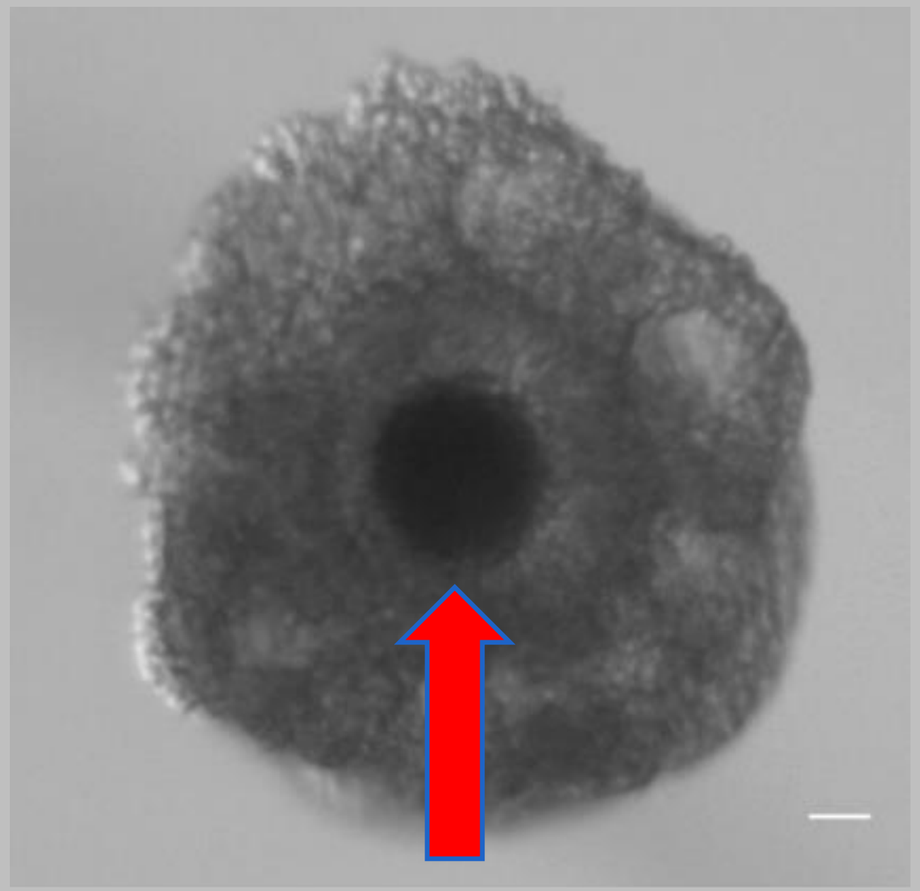
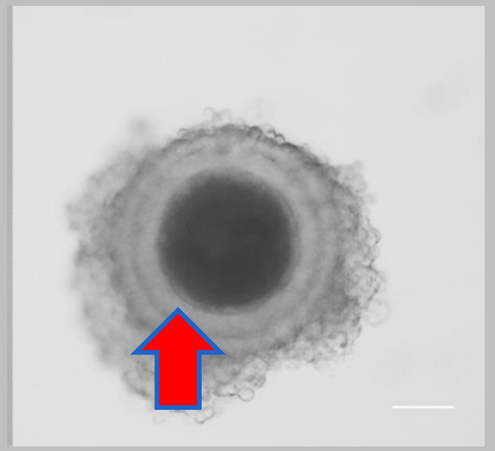
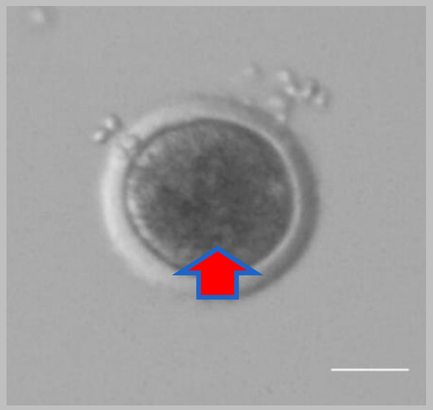
CRYOPROTECTANTS PENETRATE IN OOCYTES







Theriogenology 86 (2016) 635–641

Contents lists available at ScienceDirect

Theriogenology

journal homepage: www.theriojournal.com

Role of cumulus cells during vitrification and fertilization of mature bovine oocytes: Effects on survival, fertilization, and blastocyst development

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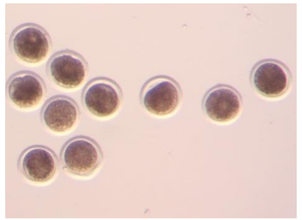
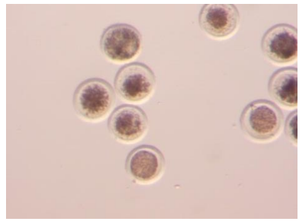
[a] Faculty of Veterinary Medicine, Department of Reproduction, Obstetrics and Herd Health, Ghent University, Merelbeke, Belgium
[b] Department for Reproductive Medicine, Ghent University Hospital, Ghent, Belgium
[c] Animal Breeding and Genomics Centre, Wageningen UR Livestock Research, Wageningen, The Netherlands

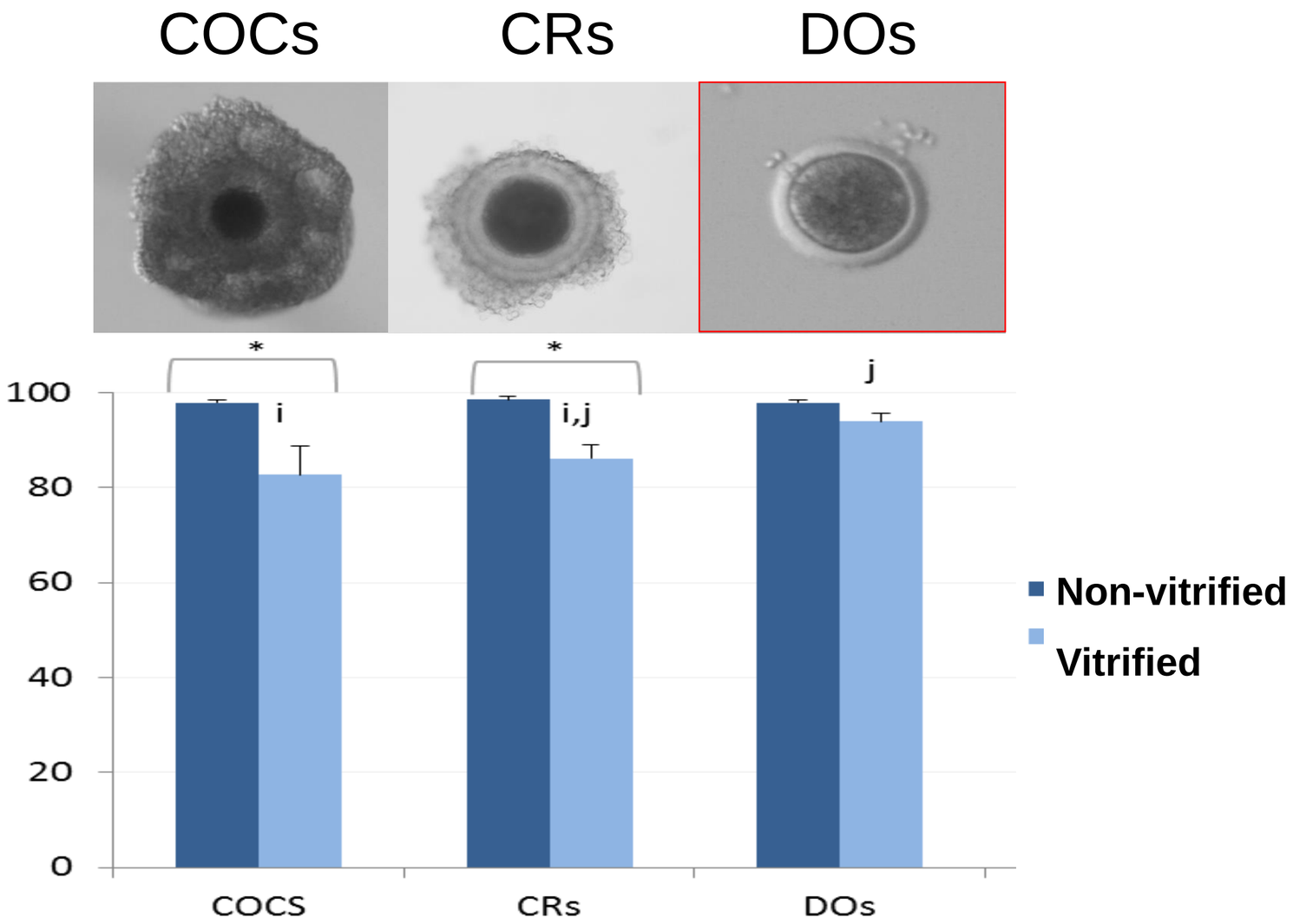
SURVIVAL OF OOCYTE VITRIFICATION

Alive





Dead



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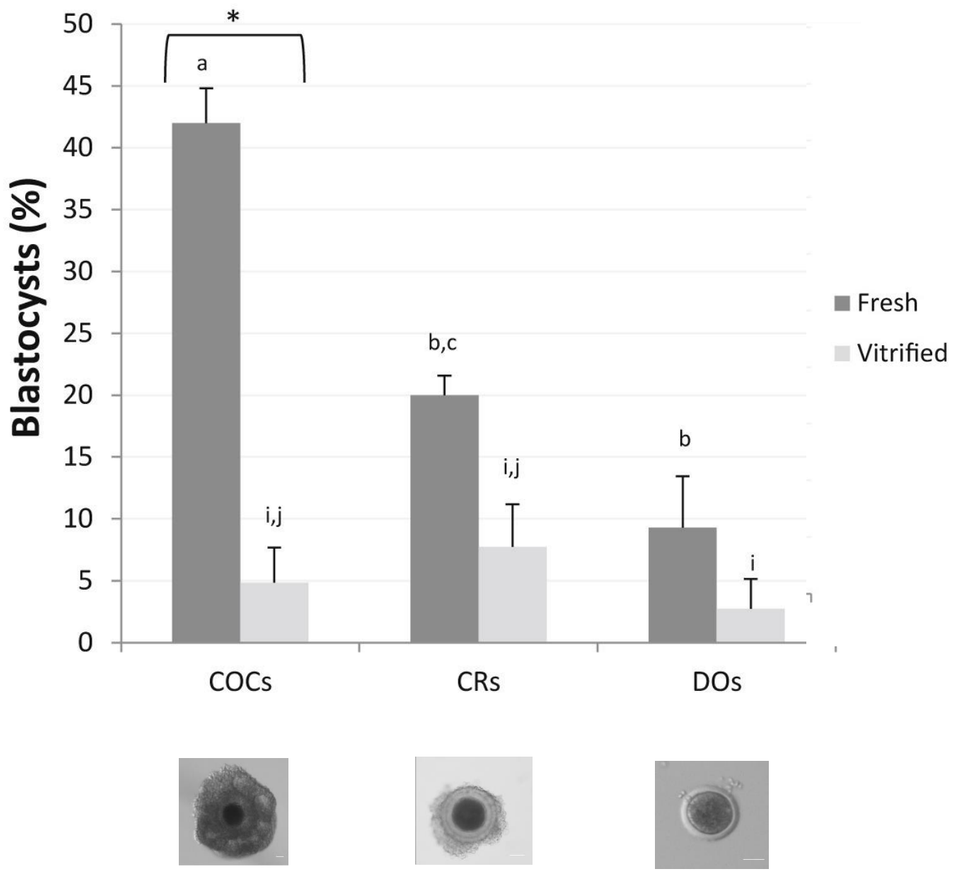
DOWNSIDE OF DENUDING OOCYTES (DOS)

- Maturation rates decrease
- Fertilization rates decrease
- Cleavage rates decrease
- Blastocyst rates decrease

- But **rescue** possible …



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Ortiz-Escribano et al. 2016

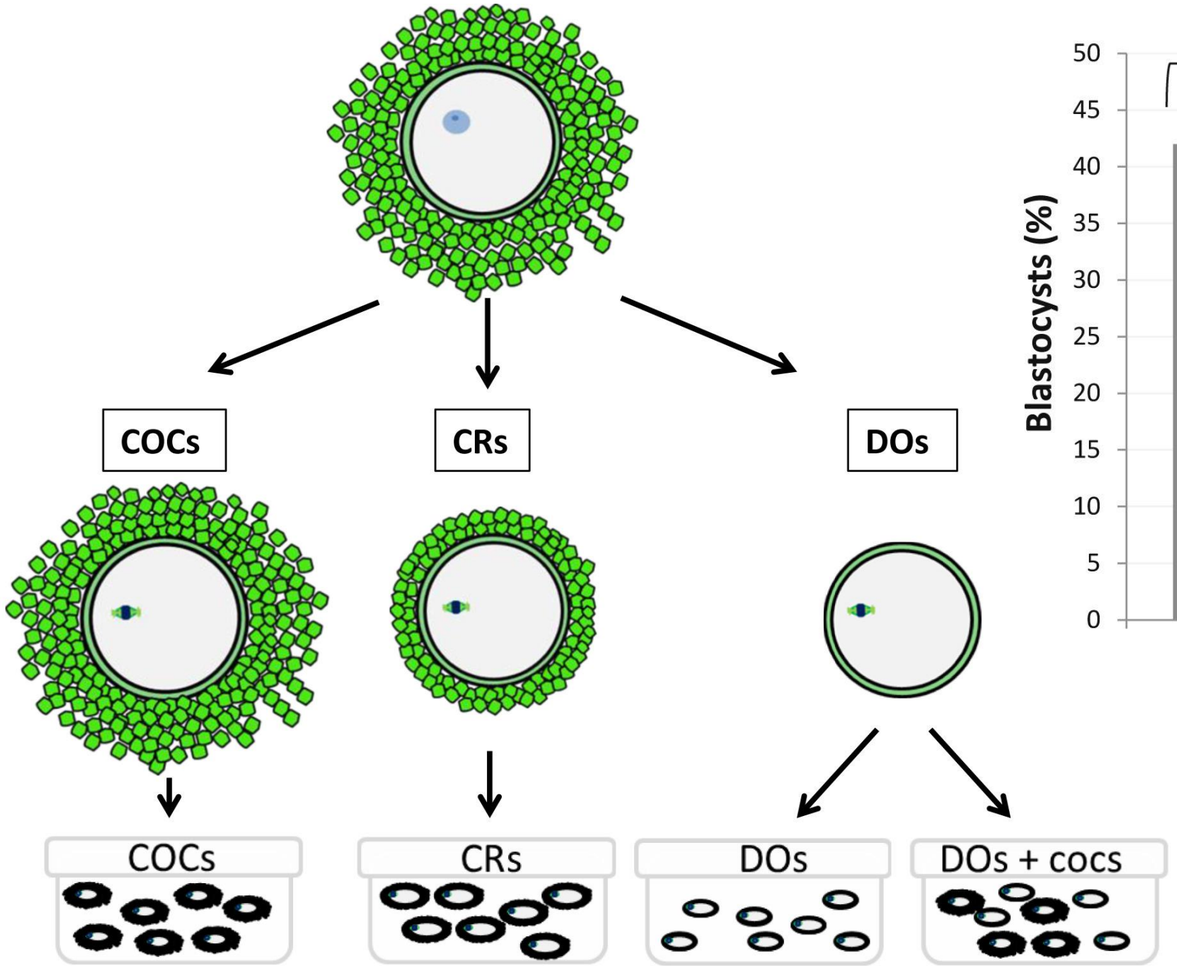
RESCUE OF DENUDED OOCYTES BY COCS

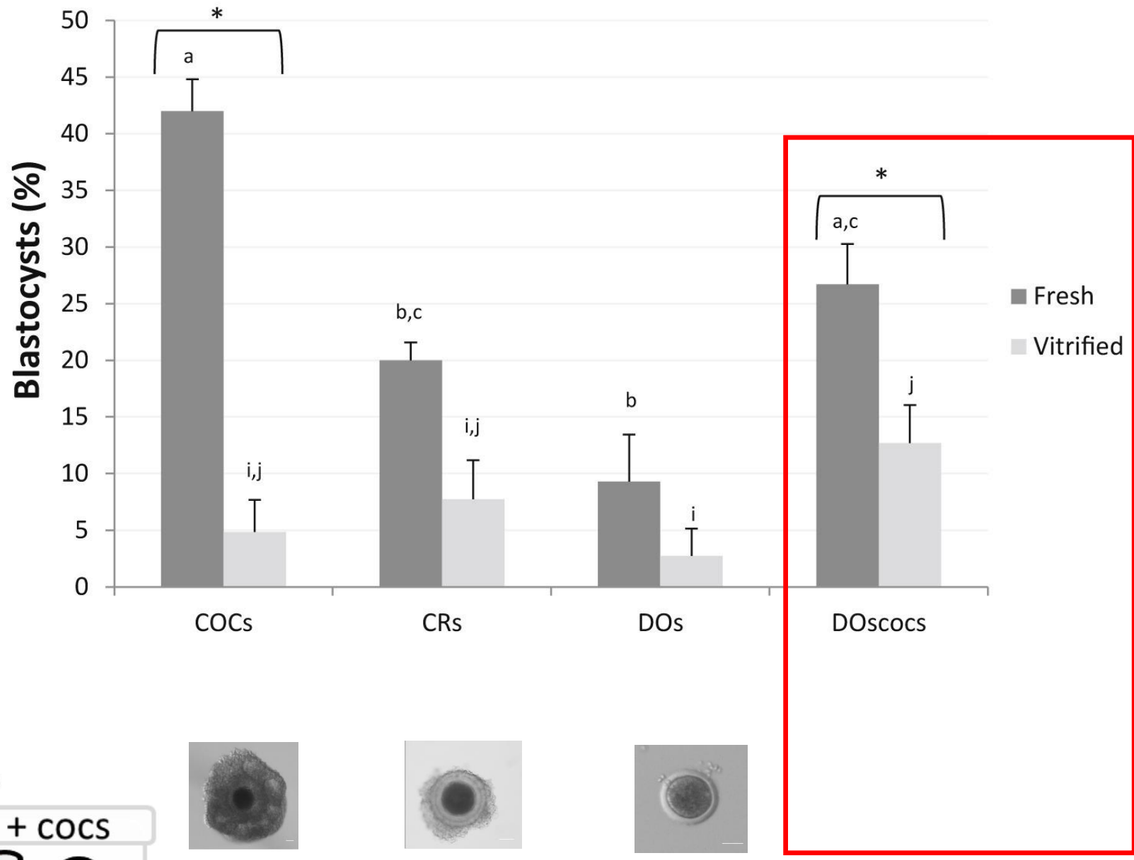
COCs, Maturation

Denudation
(complete, partial or not)

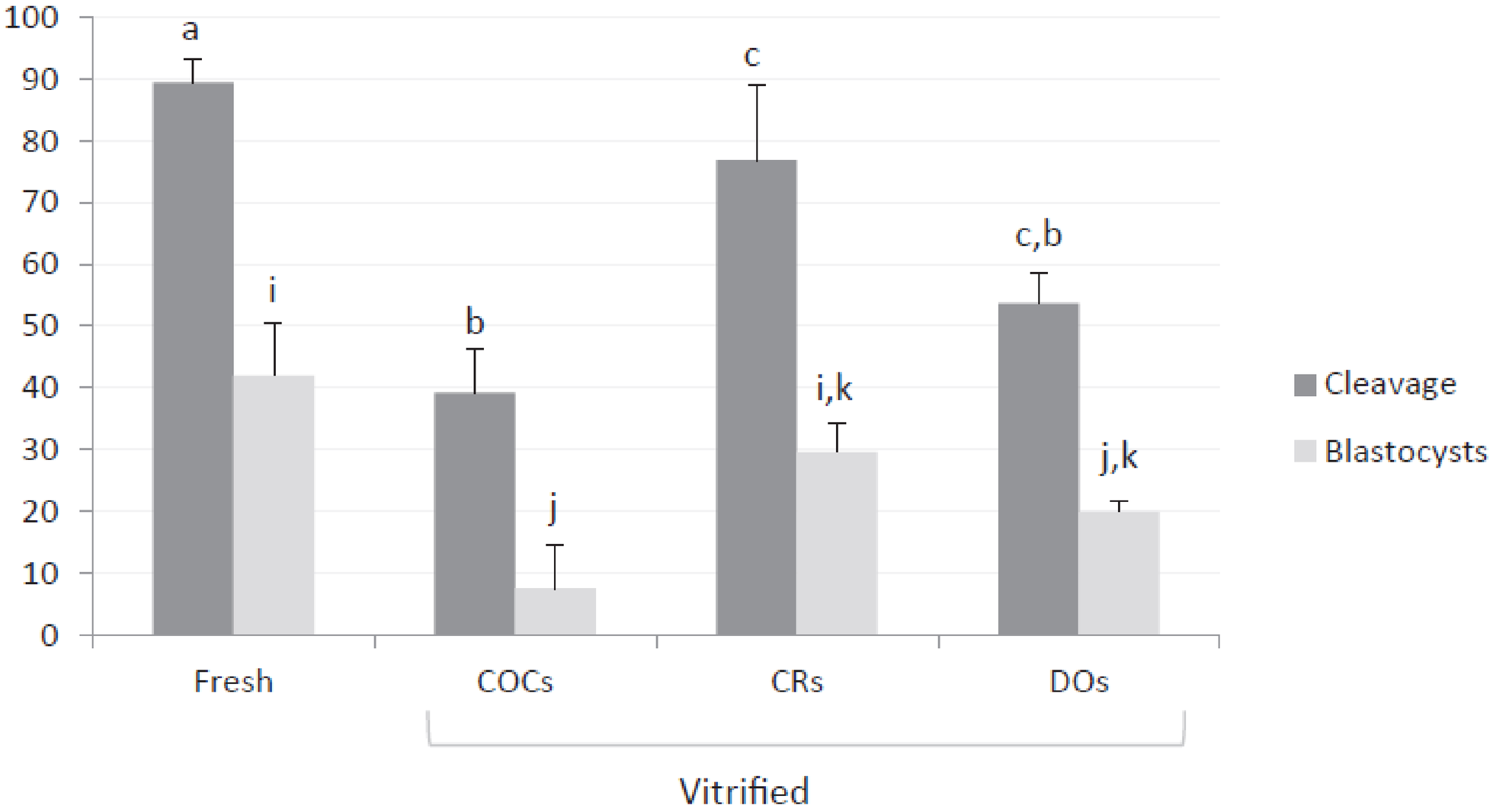
Vitrified-warmed/
or not vitrified

Fertilization,
denudation → culture
or assessment





PARTHENOGENETIC ACTIVATION



Ortiz-Escribano et al. 2016

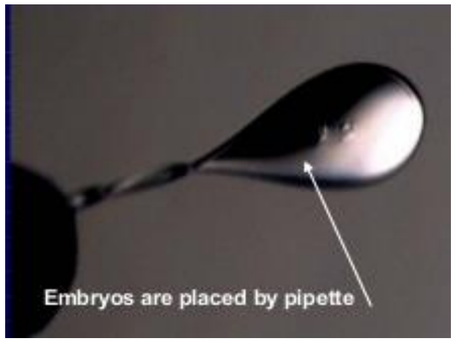
CONCLUSION

- Vitrification of mature bovine oocytes is feasible, preferably with corona radiata (8% blastocysts)
- Vitrified denuded oocytes can be rescued for IVF by coincubation with mature fresh COCs (13% blastocysts)

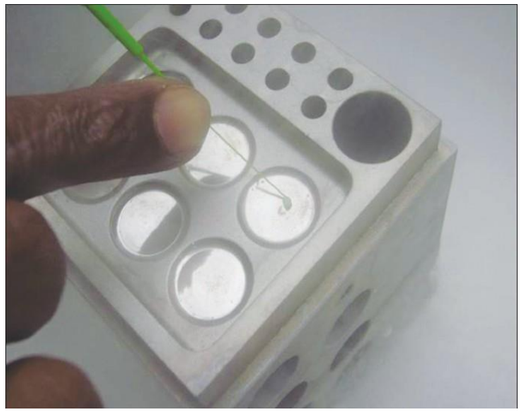
SIZE OF THE DEVICE

SIZE OF DEVICES

Open Pulled Straw (OPS)

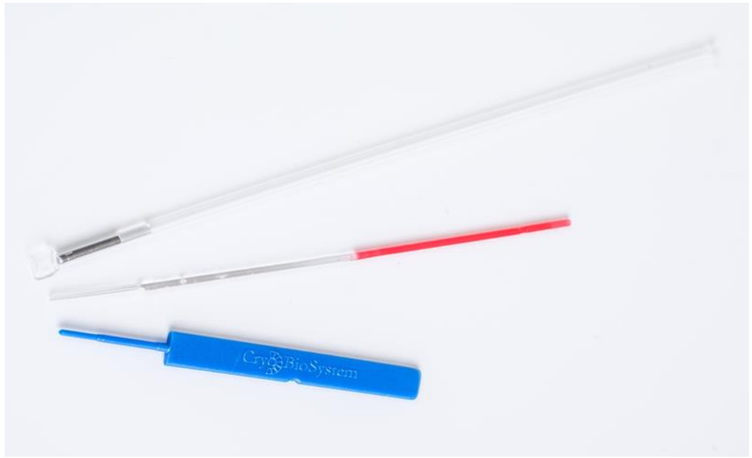


Cryoloop

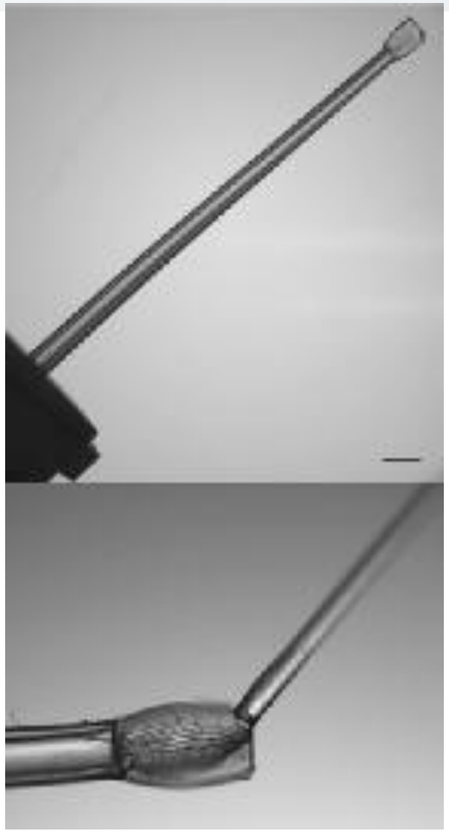


Solid surface vitrification

Cryotop



High security vitrification



Spatula vitrification

Equine Veterinary Journal

Equine Veterinary Journal ISSN 0425-1644
DOI: 10.1111/evj.12747

An improved vitrification protocol for equine immature oocytes, resulting in a first live foal

N. ORTIZ-ESCRIBANO[†]*, O. BOGADO PASCOTTINI[†], H. WOELDERS[‡], L. VANDENBERGHE[†], C. DE SCHAUWER[†], J. GOVAERE[†], E. VAN DEN ABBEEL[§], T. VULLERS[¶], C. VERVERS[†], K. ROELS[†], M. VAN DE VELDE[†], A. VAN SOOM[†] and K. SMITS[†]

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[‡]Animal Breeding and Genomics Centre, Wageningen UR Livestock Research, Wageningen, the Netherlands
[§]Department for Reproductive Medicine, Ghent University Hospital, Ghent, Belgium
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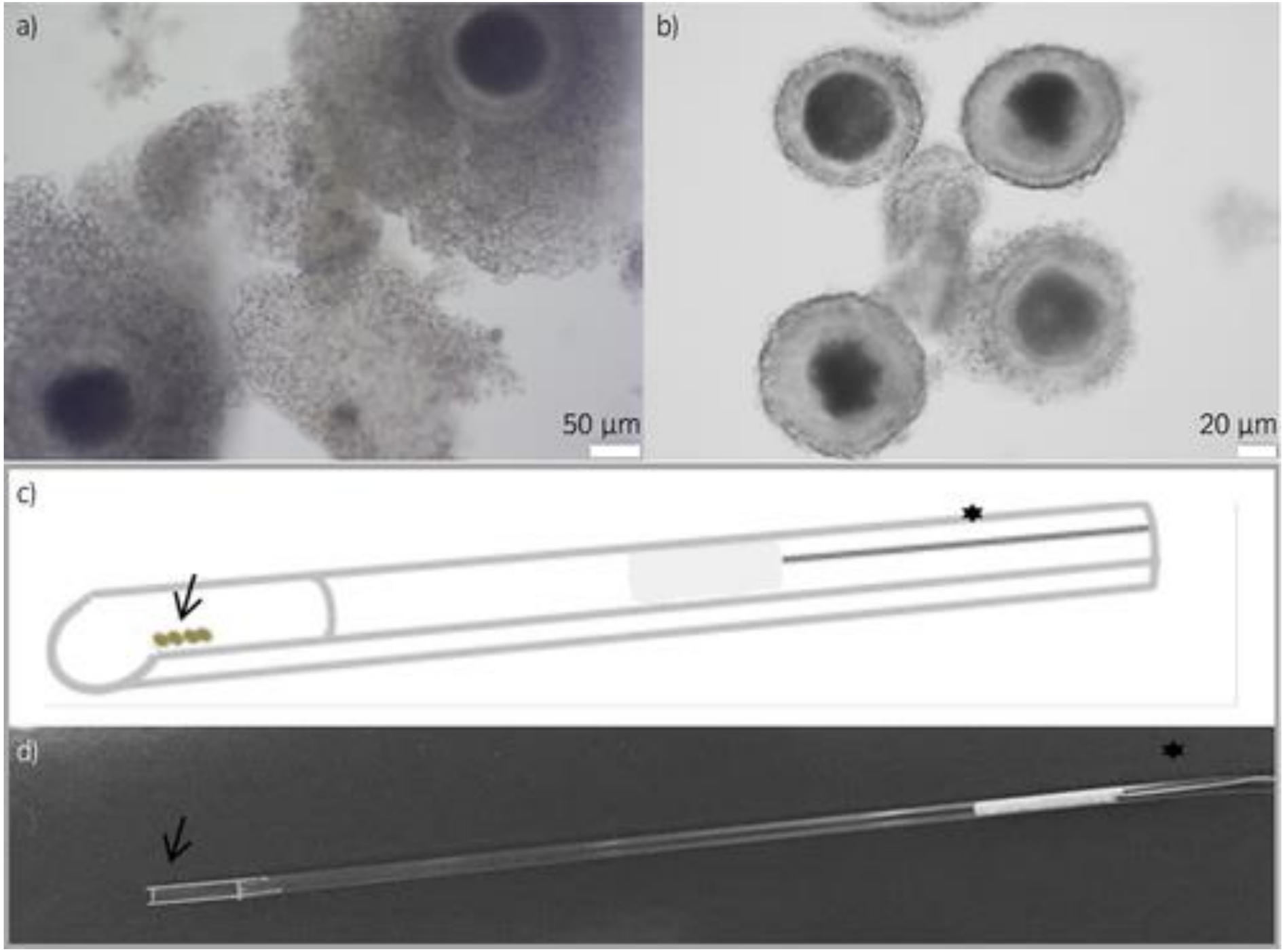
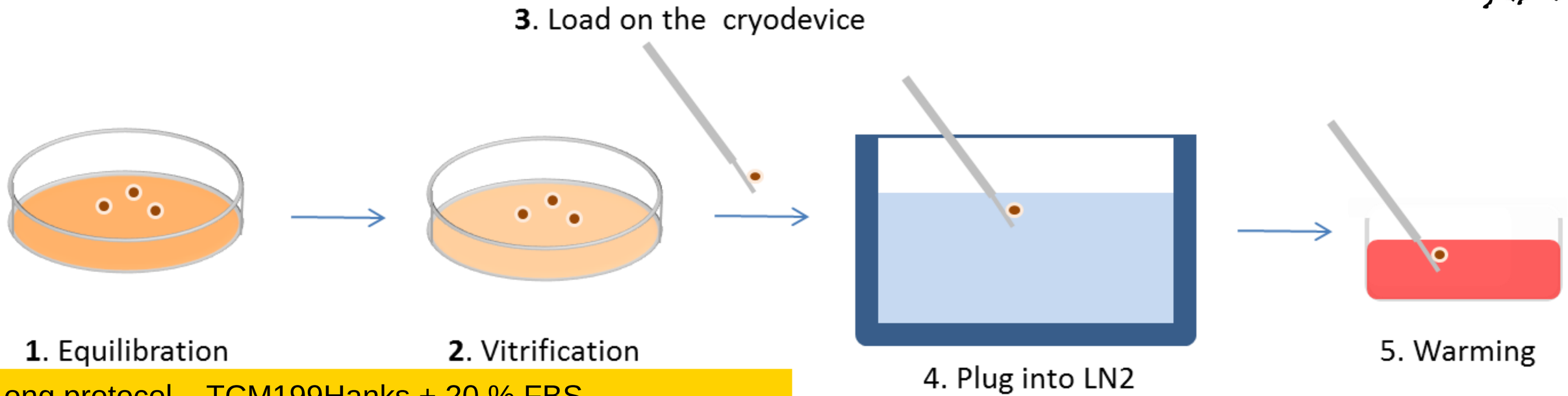
*Correspondence emails: nerea.ortizescribano@ugent.be or ortizescribanonerea@gmail.com; Received: 24.10.16; Accepted: 06.08.17


a)
50 µm
b)
20 µm
c)
d)

LONG AND SHORT PROTOCOL



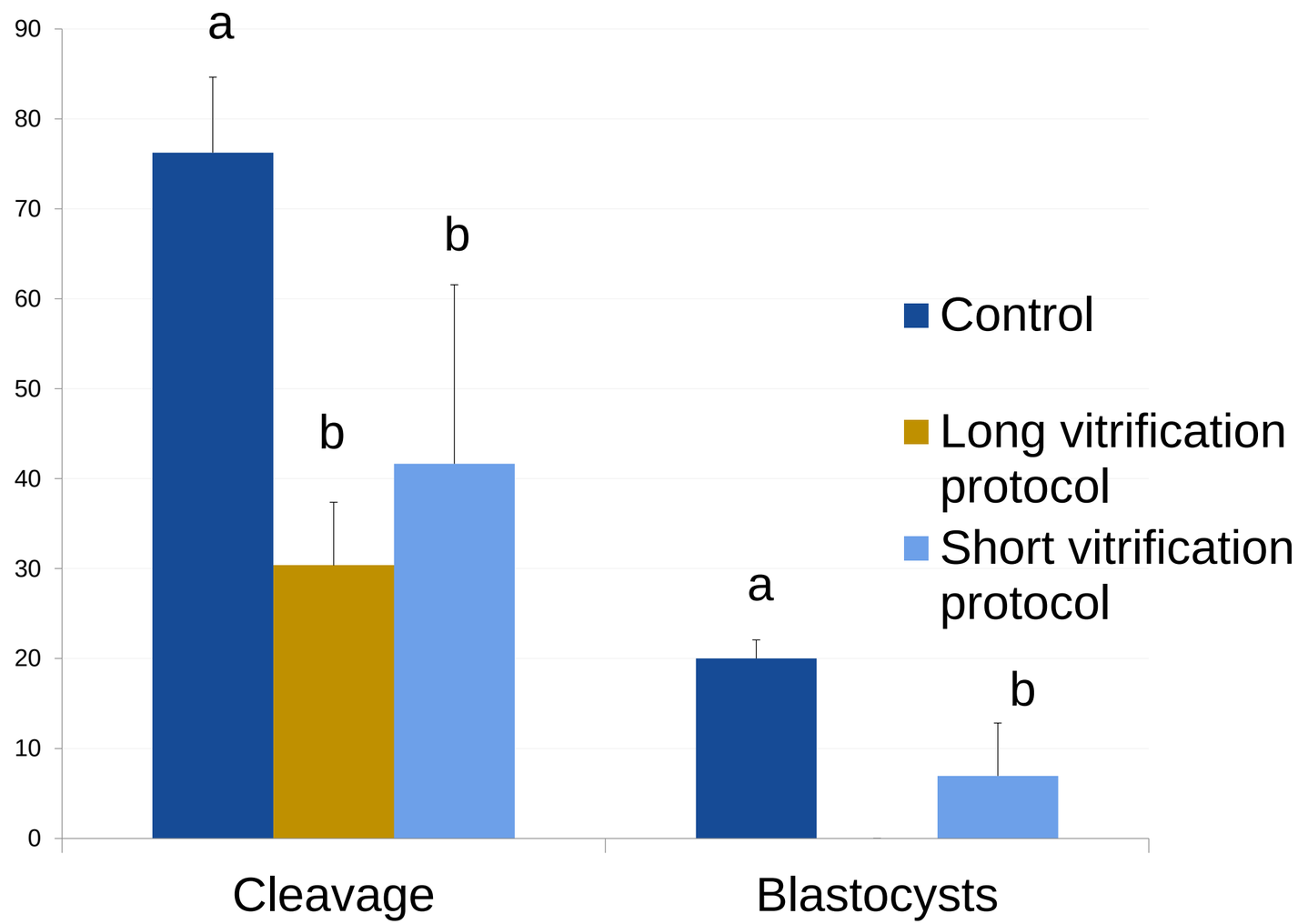
Long protocol – TCM199Hanks + 20 % FBS	
7.5 % ethylene glycol 7.5 % dimethyl sulfoxide **10 MINUTES**	15% ethylene glycol, 15% dimethyl sulfoxide 0.5 M sucrose **1 MINUTE**

Adapted from Kuwayama et al. 2005

Short protocol –TCM199Hanks + 0.014 % BSA	
10 % ethylene glycol 10 % dimethyl sulfoxide **25 SECONDS**	20% ethylene glycol, 20% dimethyl sulfoxide 0.5 M sucrose **15 SECONDS**

Adapted from Tharasanit et al. 2006

LONG VS SHORT PROTOCOL WITH EQUINE CORONA RADIATA OOCYTES



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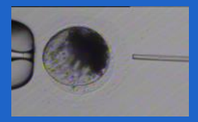
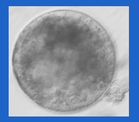
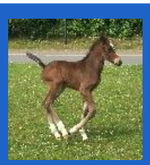
VICSI May 12 2017

EFFICIENCY OF OOCYTE VITRIFICATION IS LOW

				
Fresh	146	80 (55%)	16 (20%)	10 (60%)
Vitrified	179	72 (40%)	5 (7%)	1 (20%)

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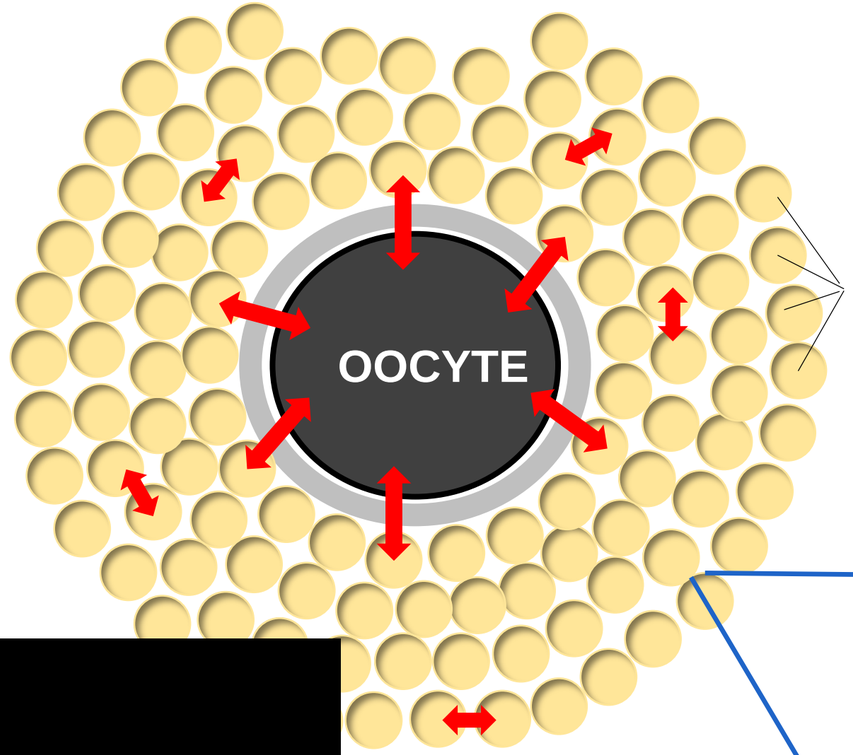
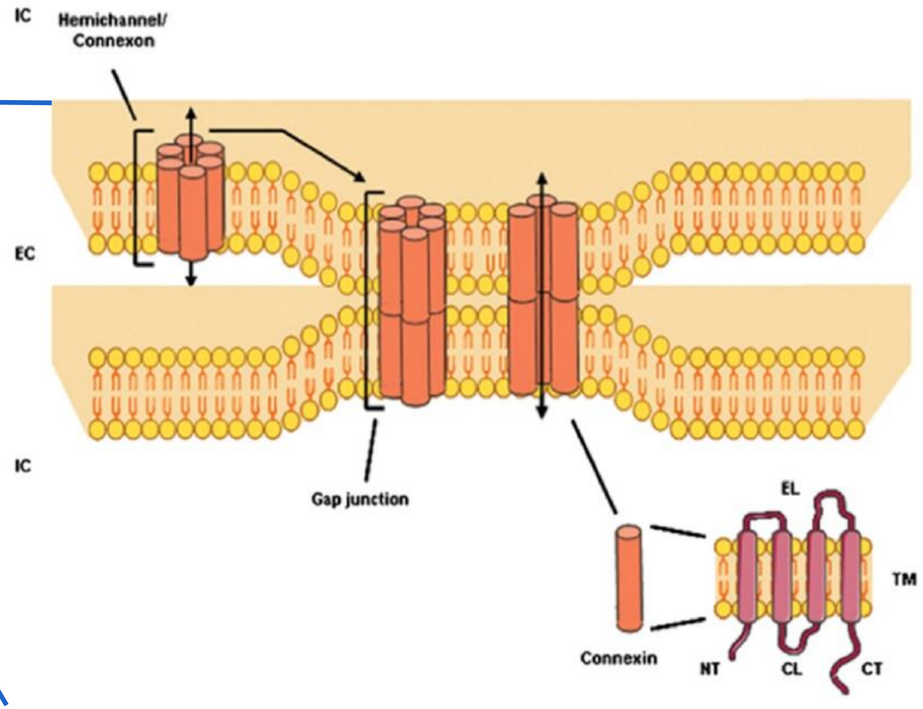
CONCLUSION

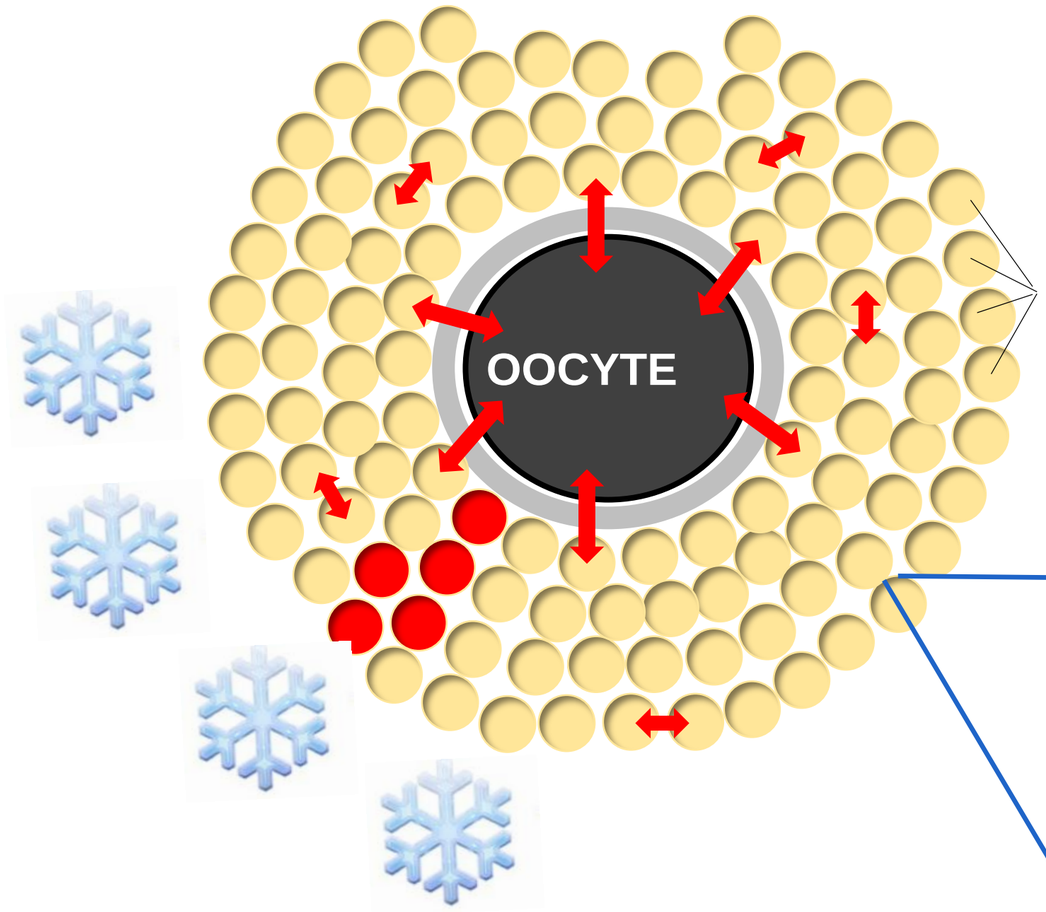
- Vitrification of immature horse oocytes is still not efficient (7% blastocysts), but can yield live foals
- The use of ICSI is beneficial since it circumvents fertilization problems
- Vitrification of mature equine oocytes may be more efficient (cfr. human), but is less practical in the horse

CELL-CELL INTERACTION

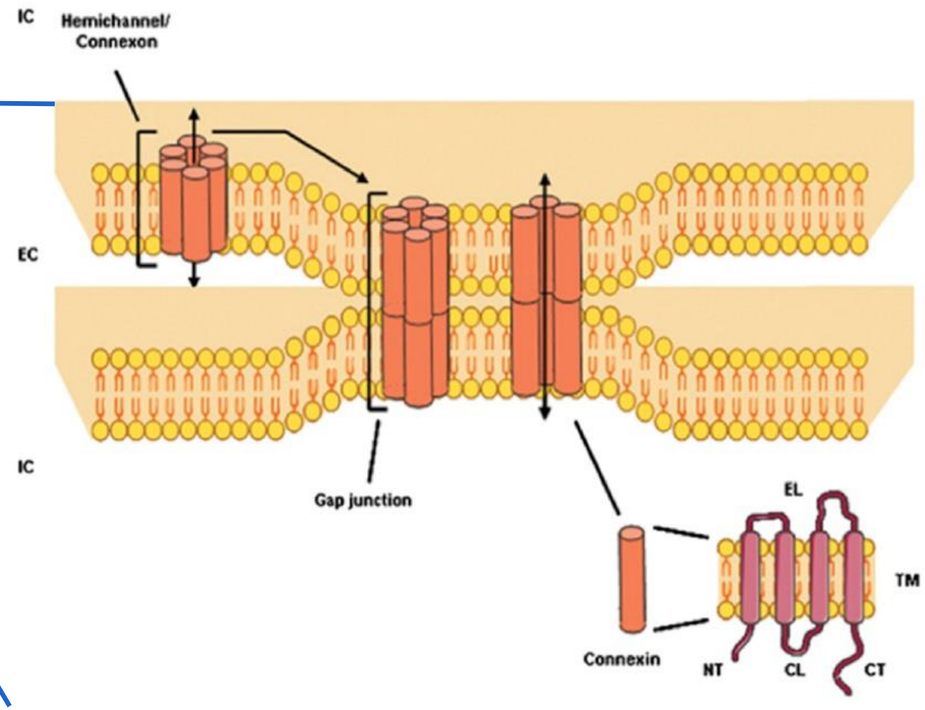


Cumulus cells

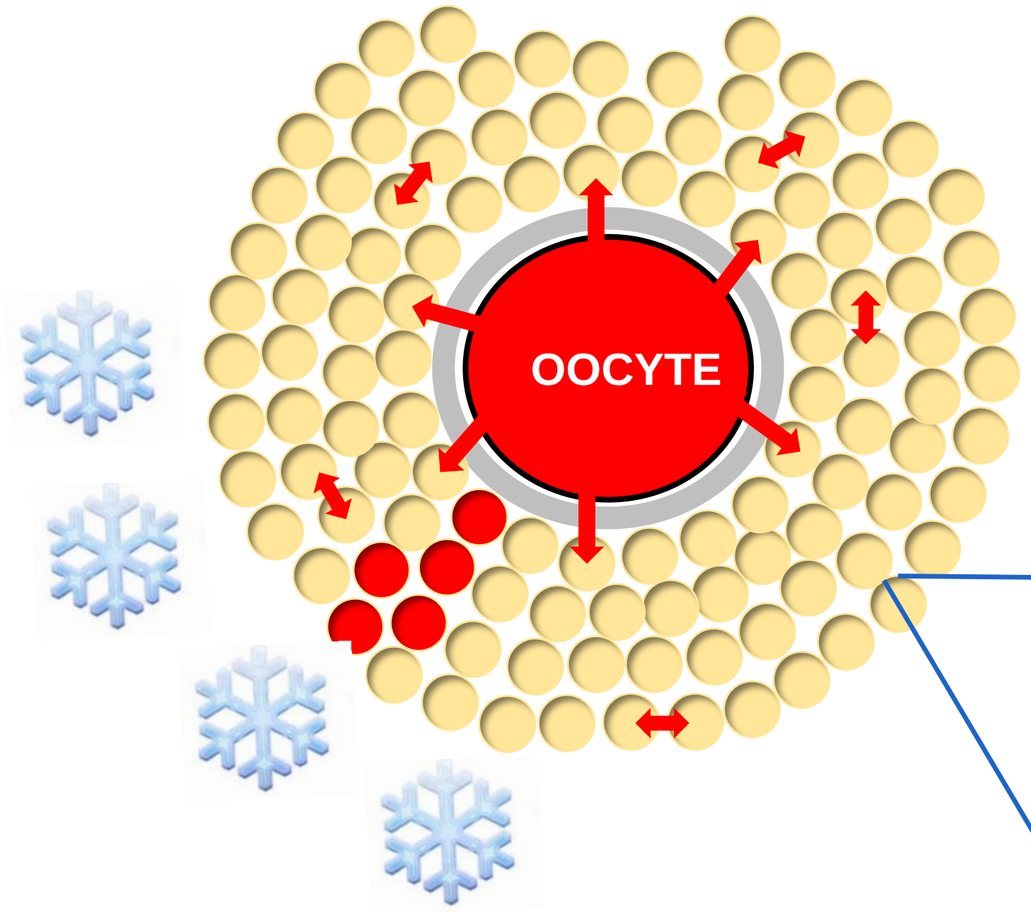


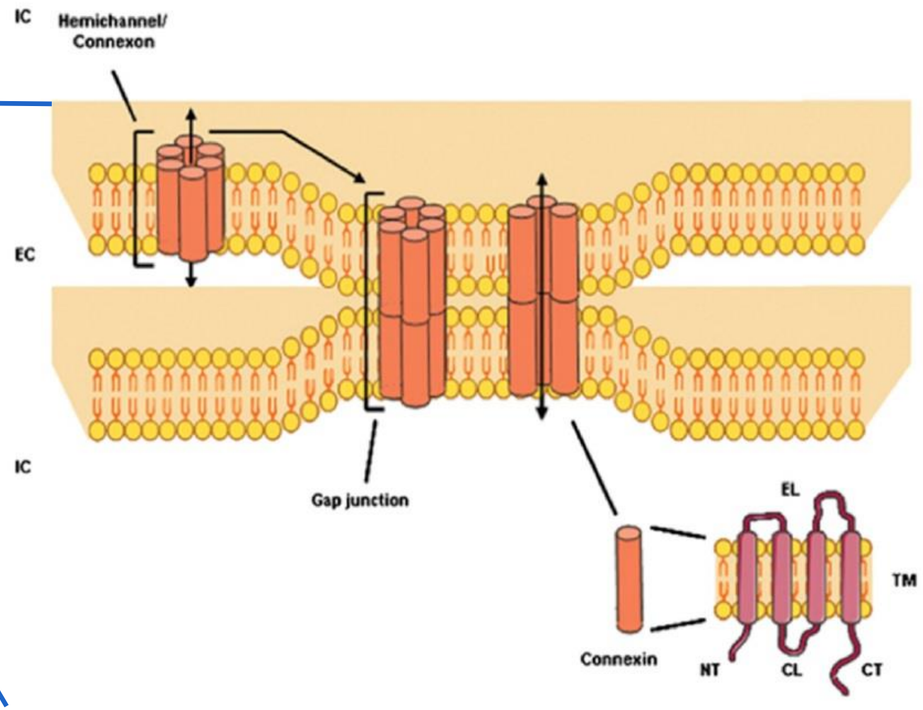


Cumulus cells



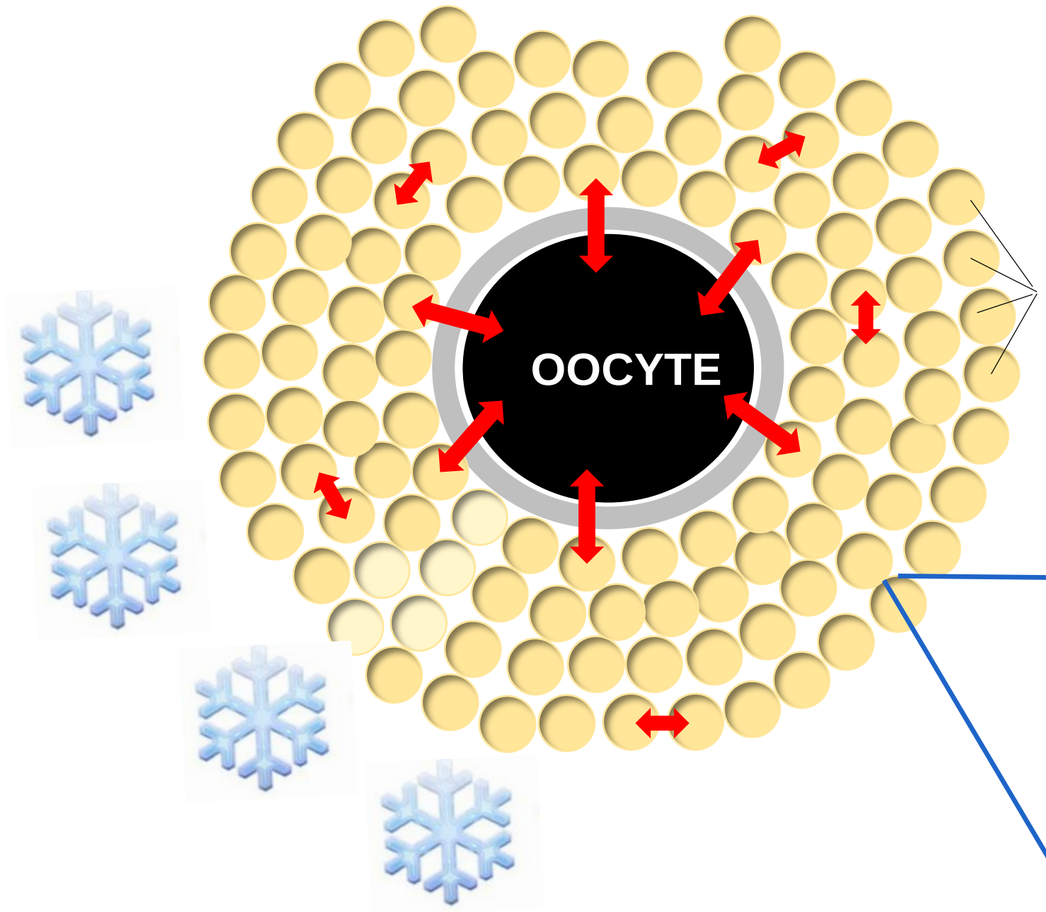
Apoptosis or cell death induced by vitrification





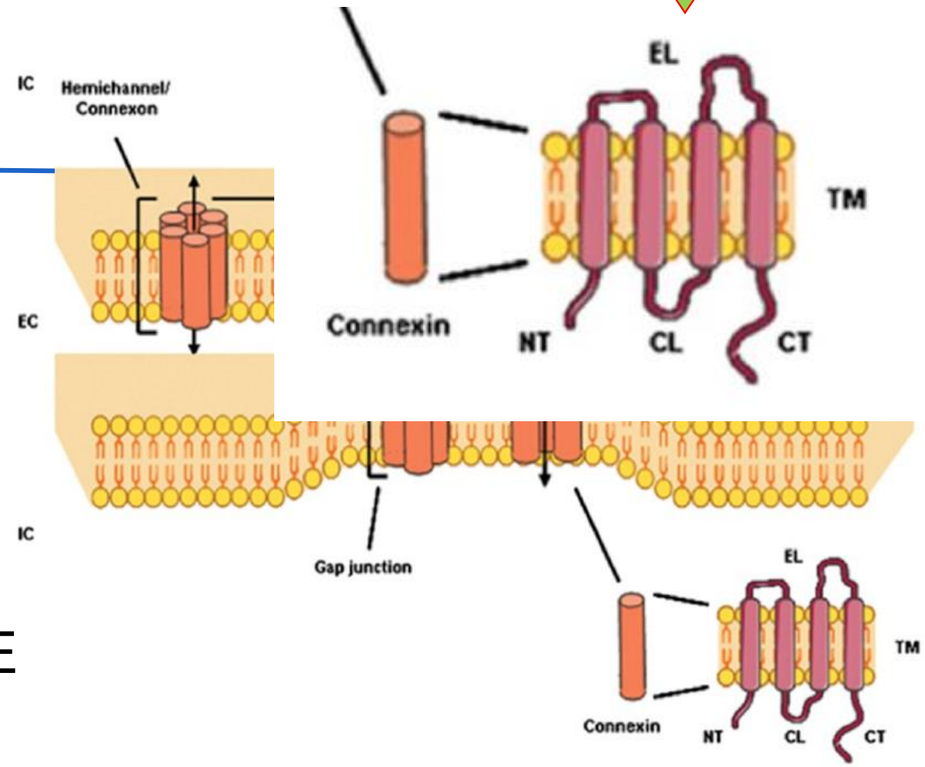
Connexins between cumulus cells may induce **apoptosis in neighboring cumulus cells,** even in oocyte

BLOCKING CONNEXIN CHANNELS WITH GAP26



Cumulus cells

Mimetic peptide Gap26



GAP26 may prevent apoptosis to occur in OOCYTE

Similar events in EMBRYO

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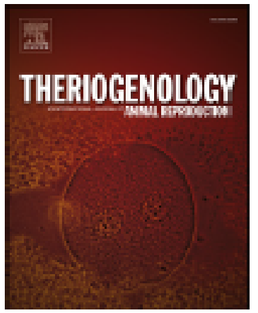
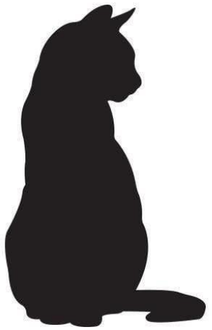


Blocking connexin channels during vitrification of immature cat oocytes improves maturation capacity after warming



Féline Snoeck [a, *], Katarzyna Joanna Szymanska [b], Steven Sarrazin [a], Nerea Ortiz-Escribano [a], Luc Leybaert [b, 1], Ann Van Soom [a, 1]

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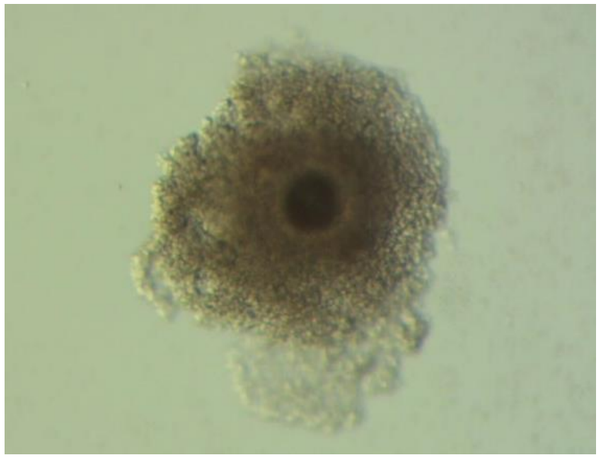
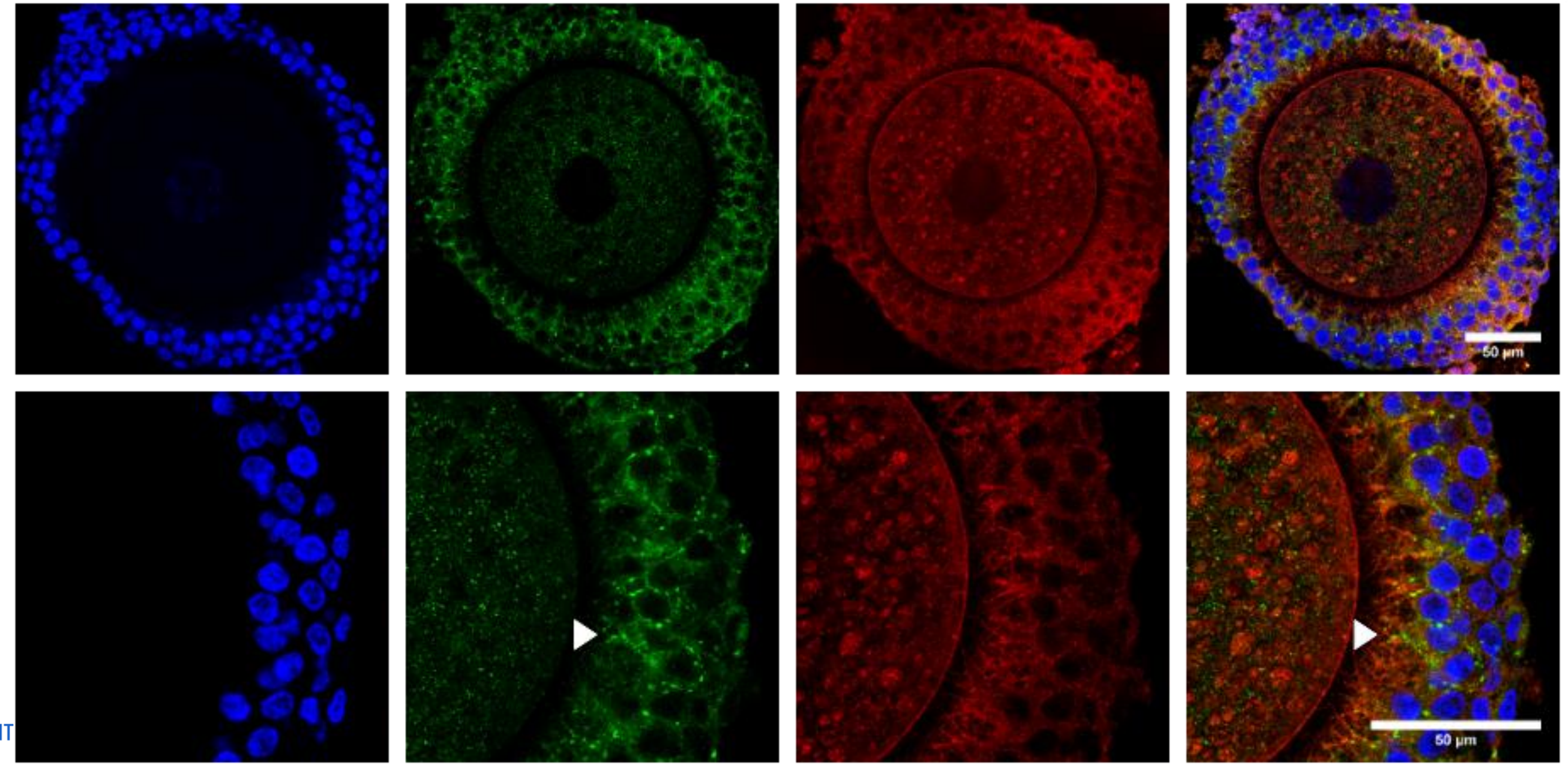
[b] Faculty of Medicine and Health Sciences, Department of Basic Medical Sciences - Physiology Group, Ghent University, Ghent, Belgium



PRESENCE OF CONNEXIN43 IN CAT OOCYTES



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VITRIFICATION OF IMMATURE CAT OOCYTES WITH GAP26

	Total no. of oocytes	Matured (%)	Cleaved (%)	Blastocyst (%)
Control	134	65 (49)	51 (38)	17 (13)
Vitrified-warmed	143	12 (8)	5 (3)	0 (0)
Vitrified-warmed with GAP26	139	27 (19)	13 (9)	3 (2)

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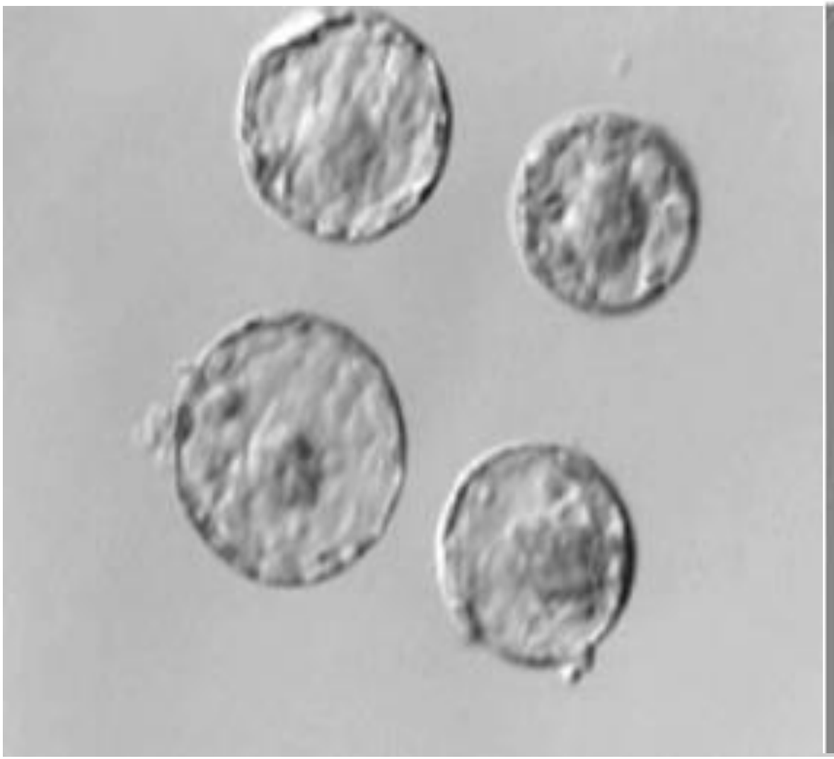
CONCLUSION

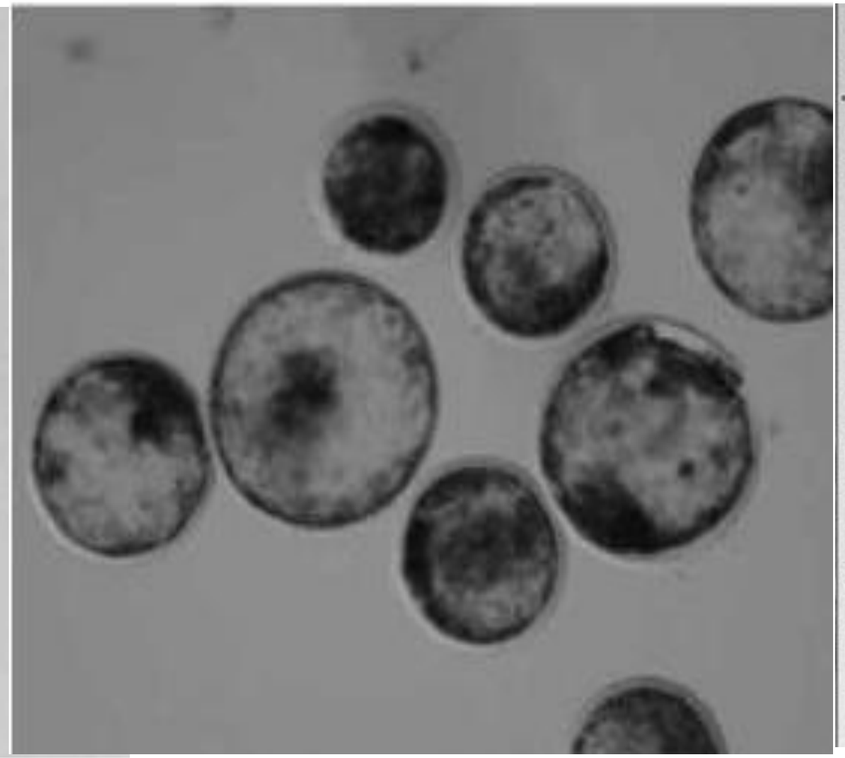
- Vitrification of immature cat oocytes is still in its infancy (2% blastocysts)
- Further research on cats in Belgium is not possible due to law on early cat spaying (prepubertal oocytes)

LIPIDS

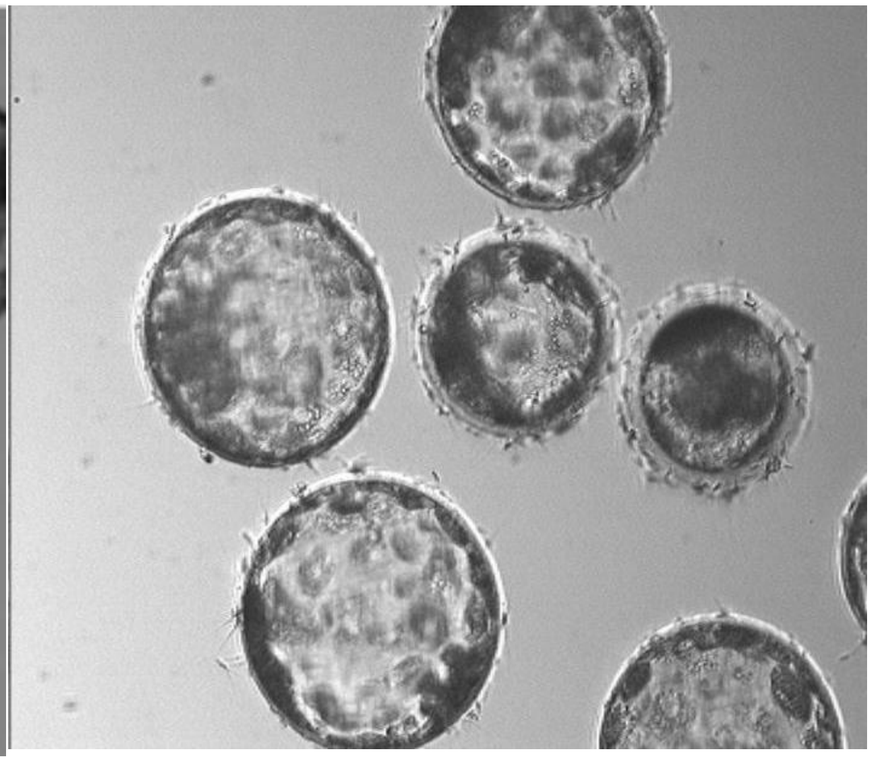
LIPID CONTENTS DIFFERS IN EMBRYOS FROM DIFFERENT SPECIES



Human



Bovine



Porcine

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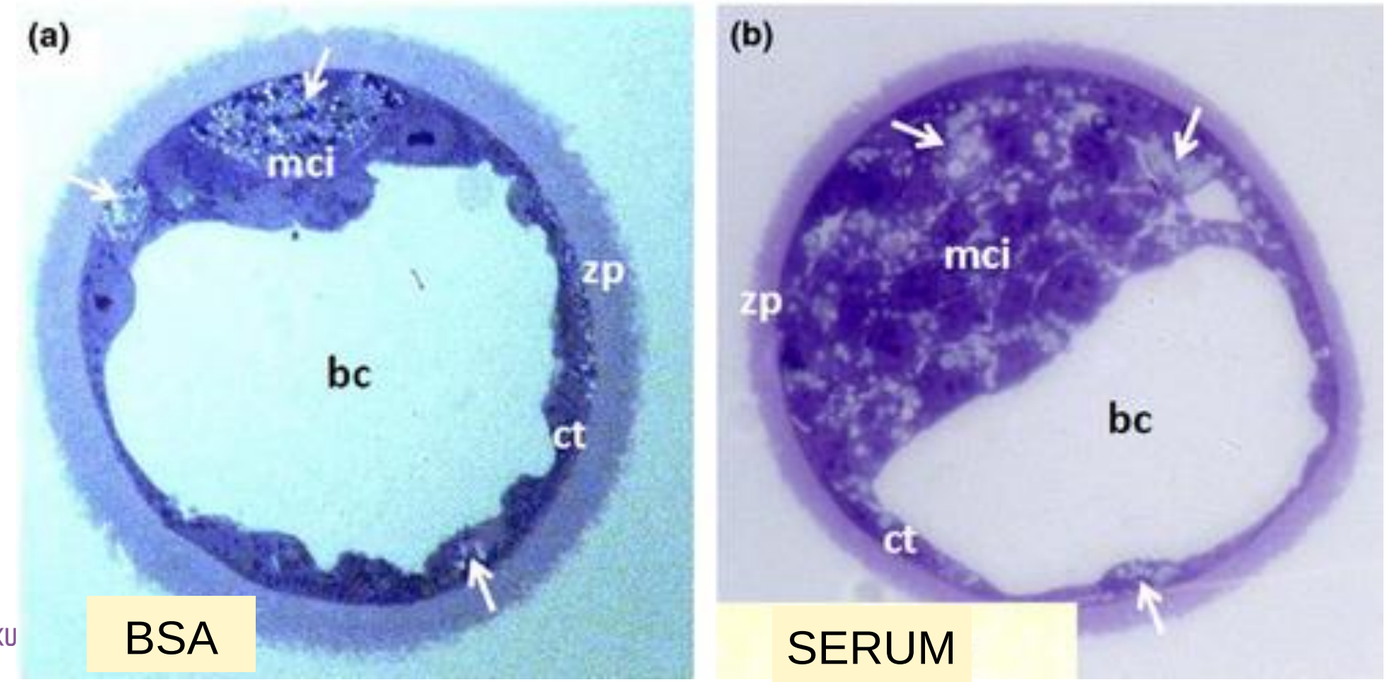
LIPID CONTENTS IN EMBRYOS IS INFLUENCED BY CULTURE MEDIUM

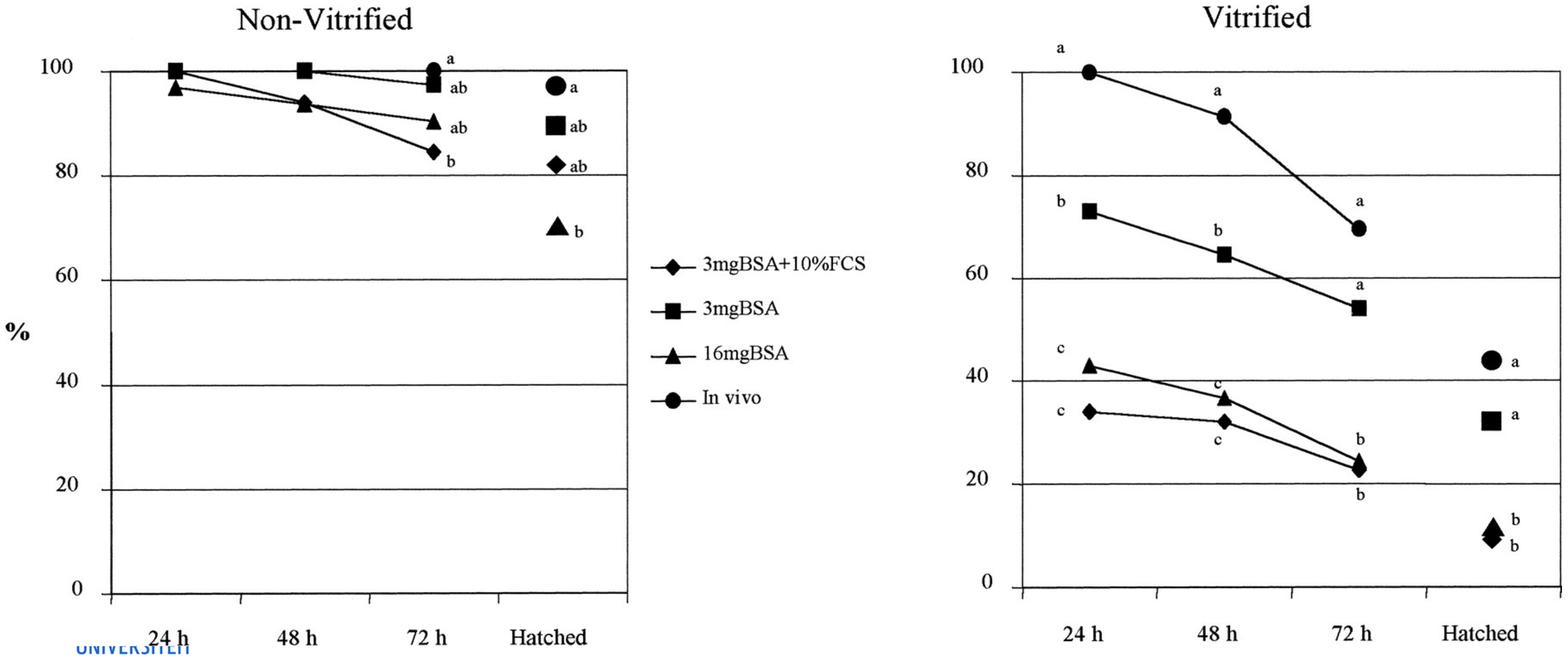
- Serum in culture medium is increasing lipid droplets
- BSA in culture medium is lowering lipid droplets



Ordonez-Leon *et al.* 2014

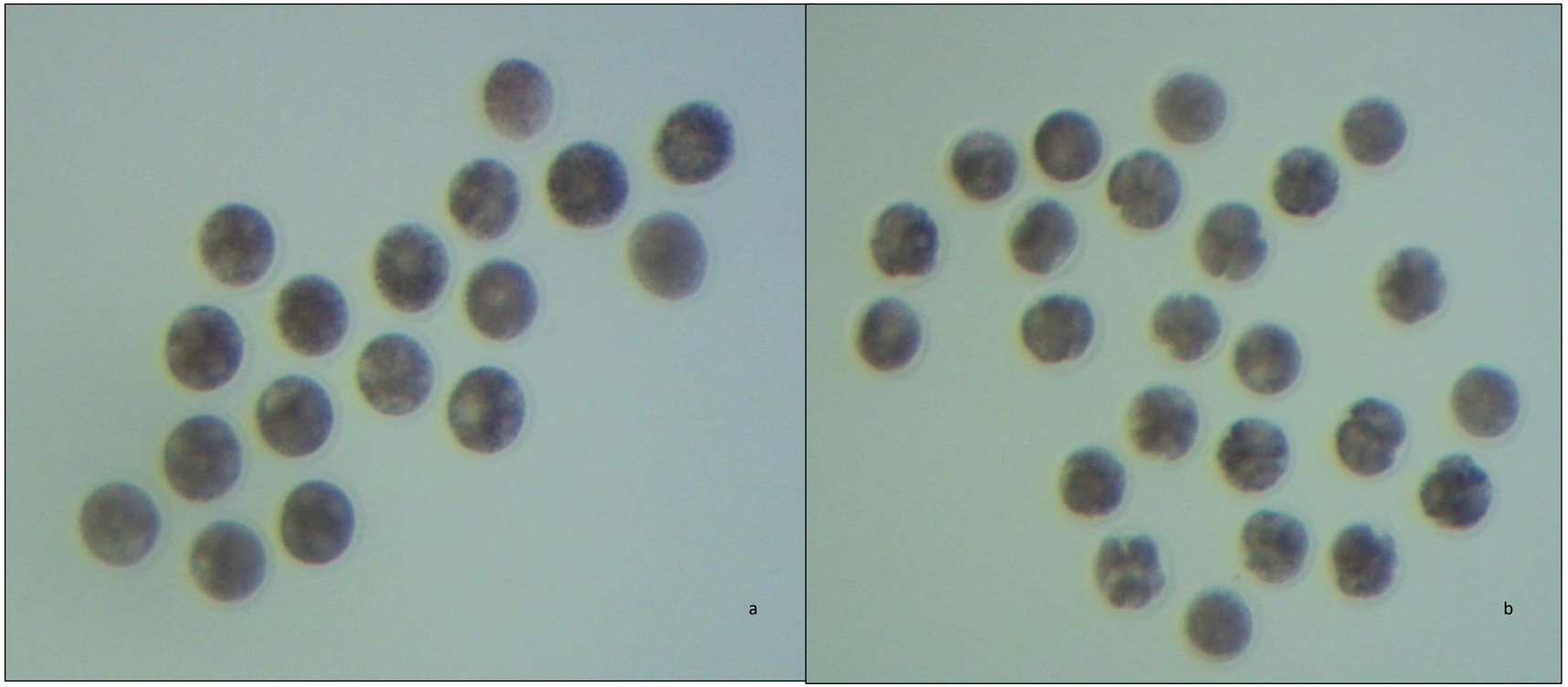
CULTURE MEDIUM AND EMBRYO FREEZABILITY

- Serum in medium is decreasing cryosurvival
- BSA in medium is <u>increasing cryosurvival</u>



Rizos et al, 2003

VITRIFIED-WARMED BOVINE OOCYTES CLEAVE LESS IF MATURED IN SERUM

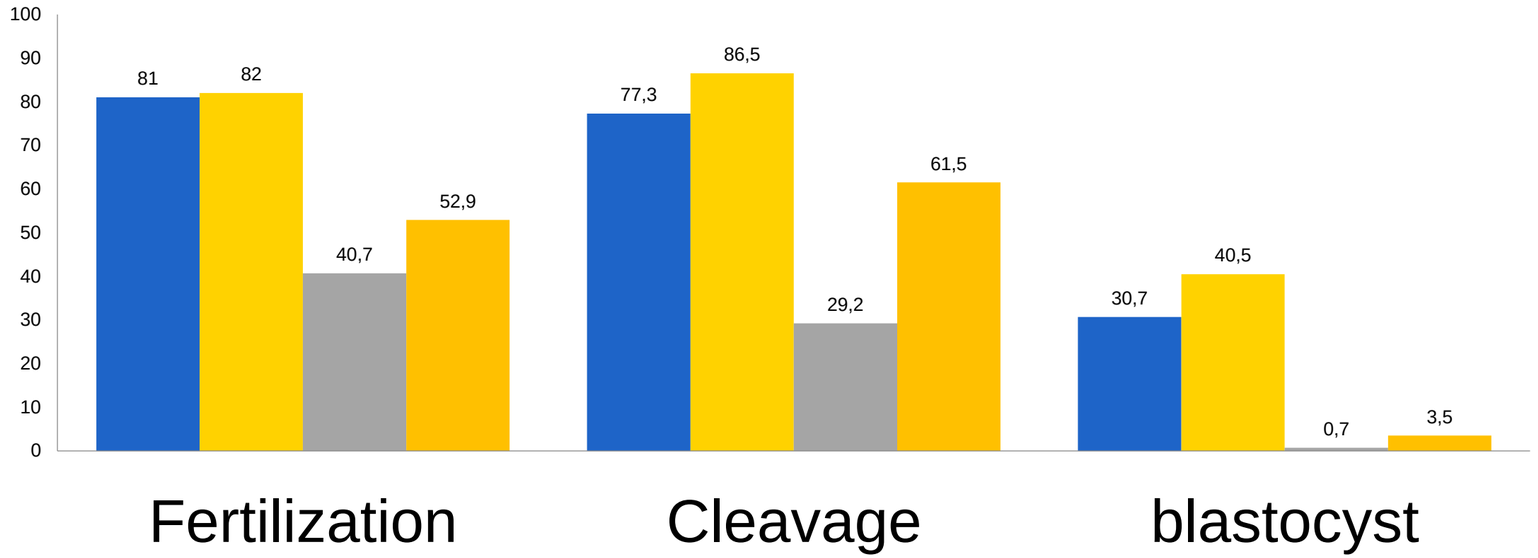


After maturation in serum

After serum-free maturation in EGF

Ortiz-Escribano, unpublished

VITRIFIED-WARMED OOCYTES PRODUCE LESS BLASTOCYSTS IF MATURED IN SERUM



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Ortiz-Escribano unpublished

Biology of Reproduction, 2017, 96(2), 288–301
doi:10.1095/biolreprod.116.144121
Research Article
Advance Access Publication Date: 24 January 2017

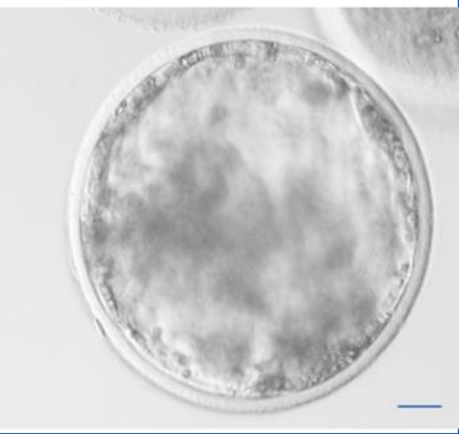
OXFORD

Research Article

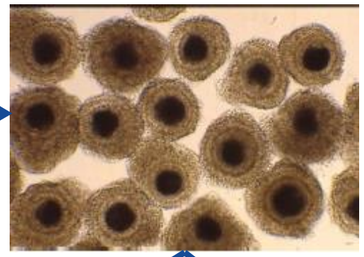
Blocking connexin channels improves embryo development of vitrified bovine blastocysts†

Nerea Ortiz-Escribano[1,*,‡], Katarzyna Joanna Szymańska[2,‡], Melissa Bol[2], Lynn Vandenberghe[1], Elke Decrock[2], Mario Van Poucke[3], Luc Peelman[3], Etienne Van den Abbeel[4], Ann Van Soom[1] and Luc Leybaert[2,*]

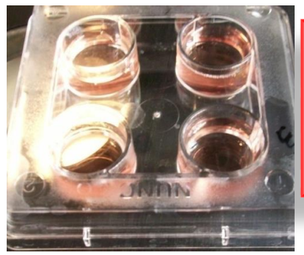
[1]Reproduction, Obstetrics and Herd Health, Ghent University, Merelbeke, Belgium; [2]Physiology group, Department of Basic Medical Sciences, Ghent University, Ghent, Belgium; [3]Department of Nutrition, Genetics and Ethology, Ghent University, Merelbeke, Belgium and [4]Reproductive Medicine, Ghent University Hospital, Ghent, Belgium



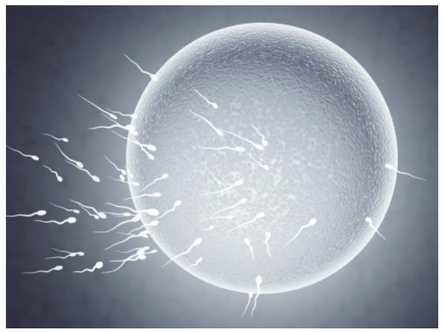
MATURATION MEDIA AND MIMETIC PEPTIDES

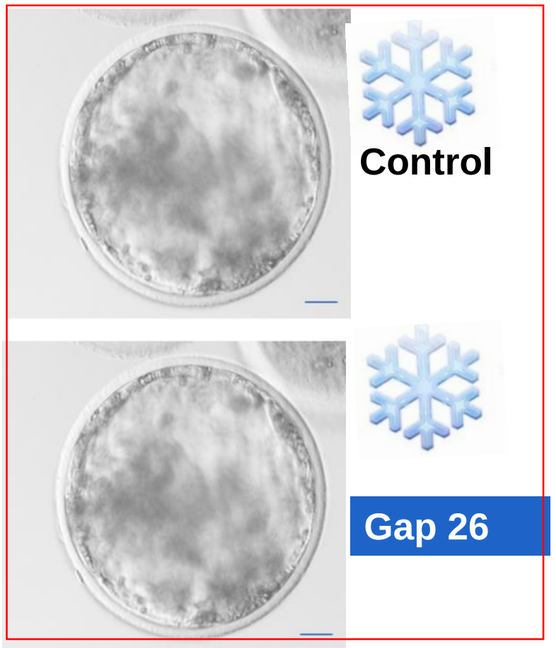


TCM 199
+ EGF



TCM 199
+ FBS





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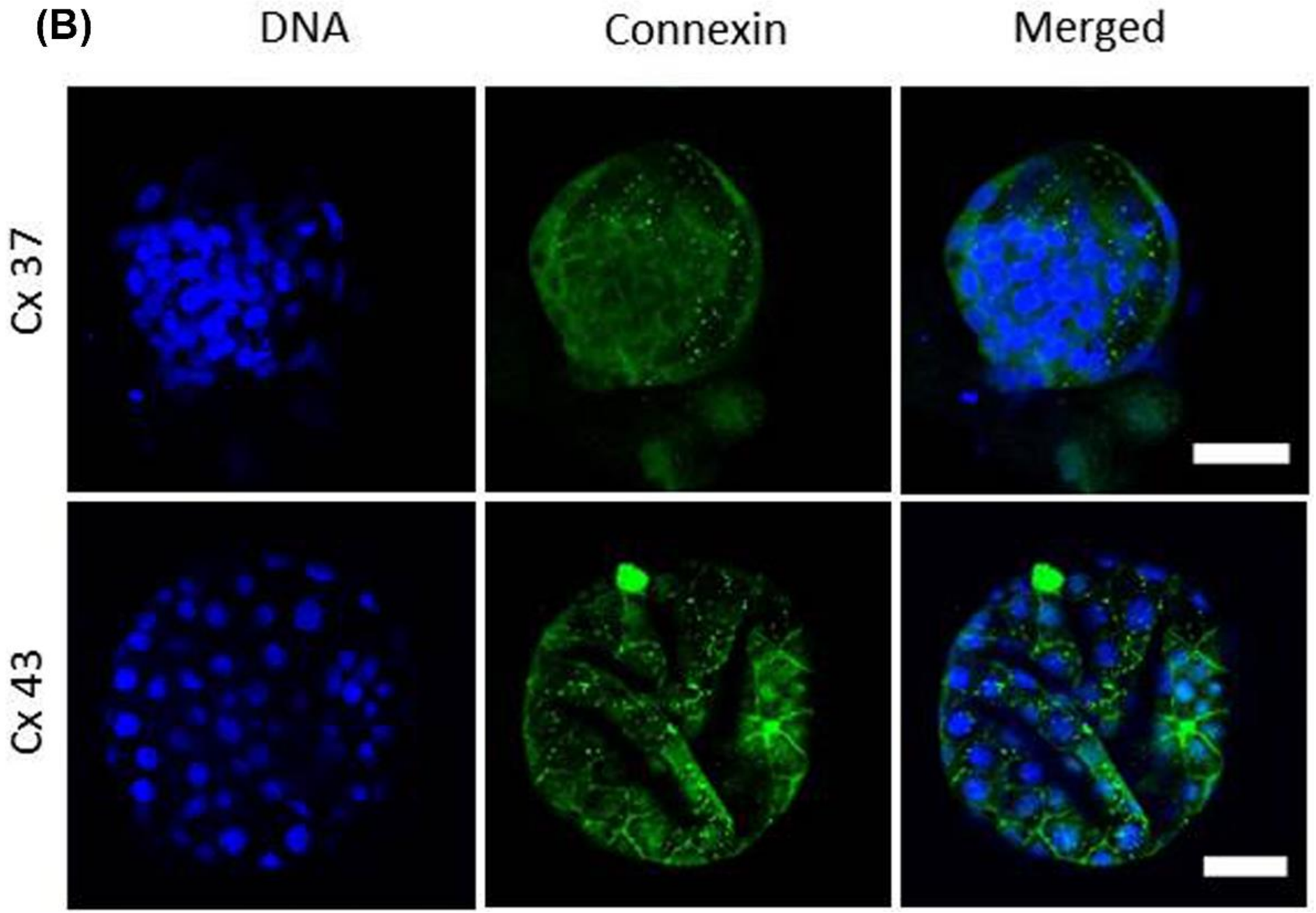
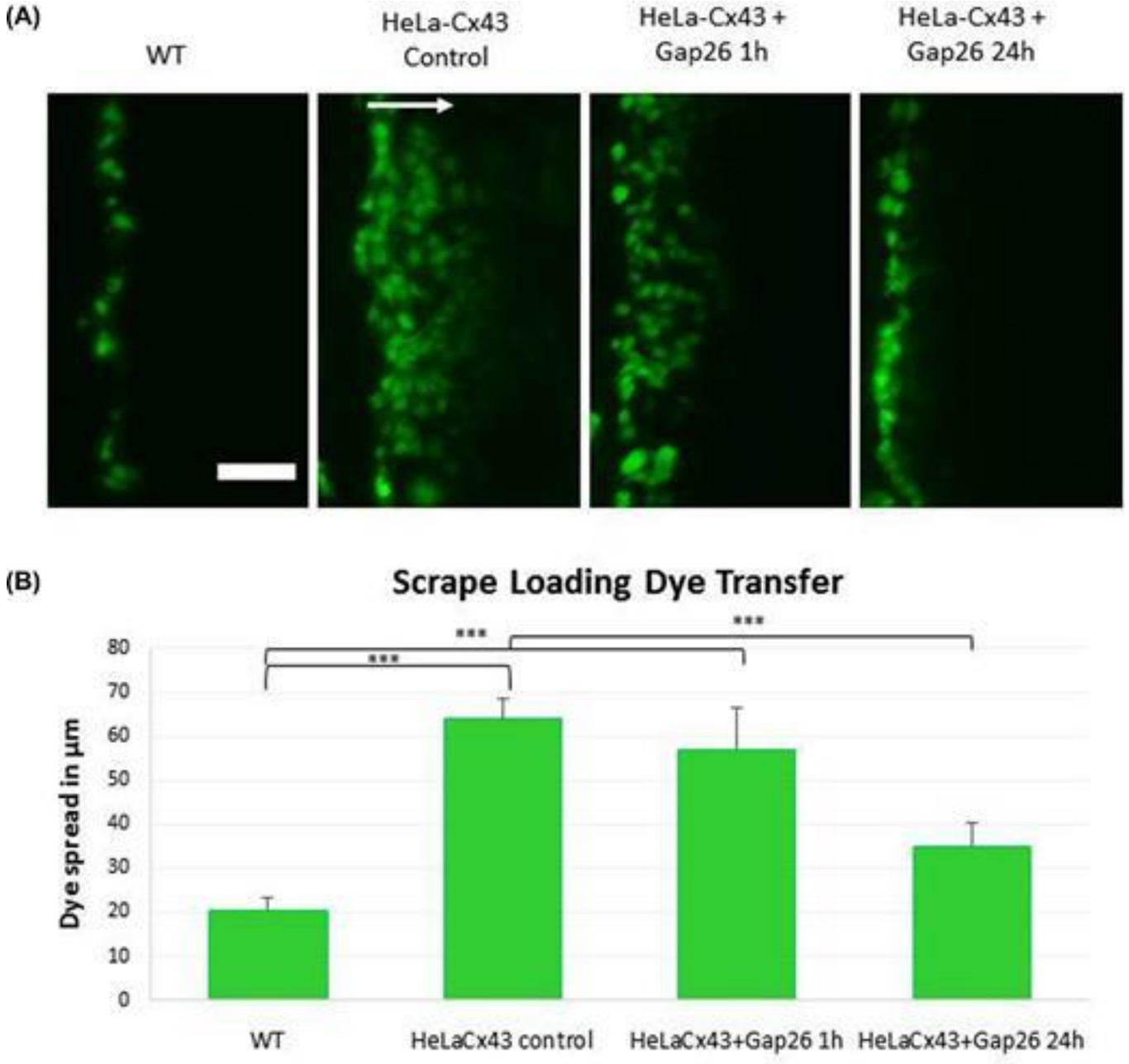
CONNEXIN IN BOVINE EMBRYOS

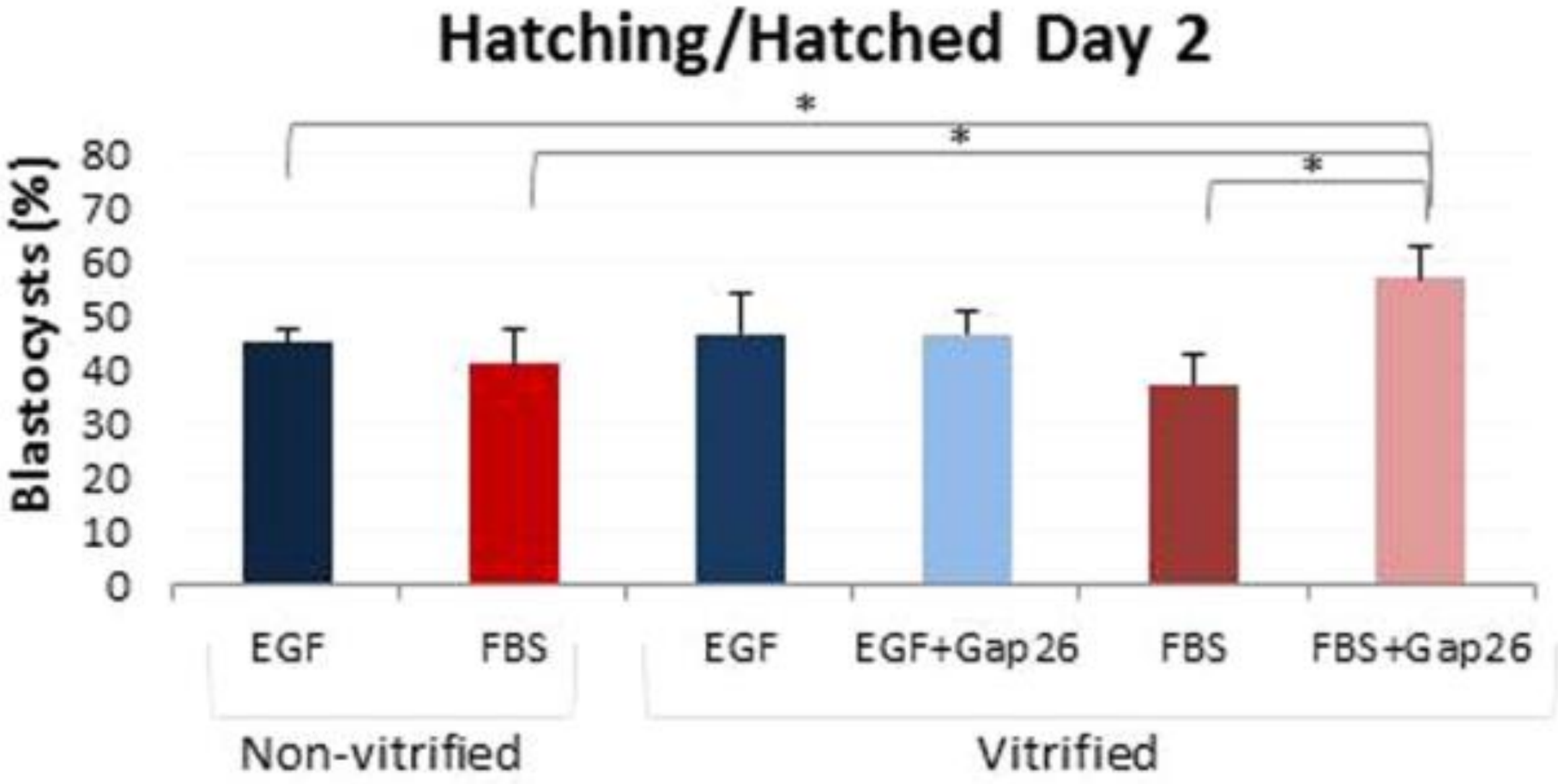


EFFECT OF GAP26 ON DYE SPREAD IN HELACELLS

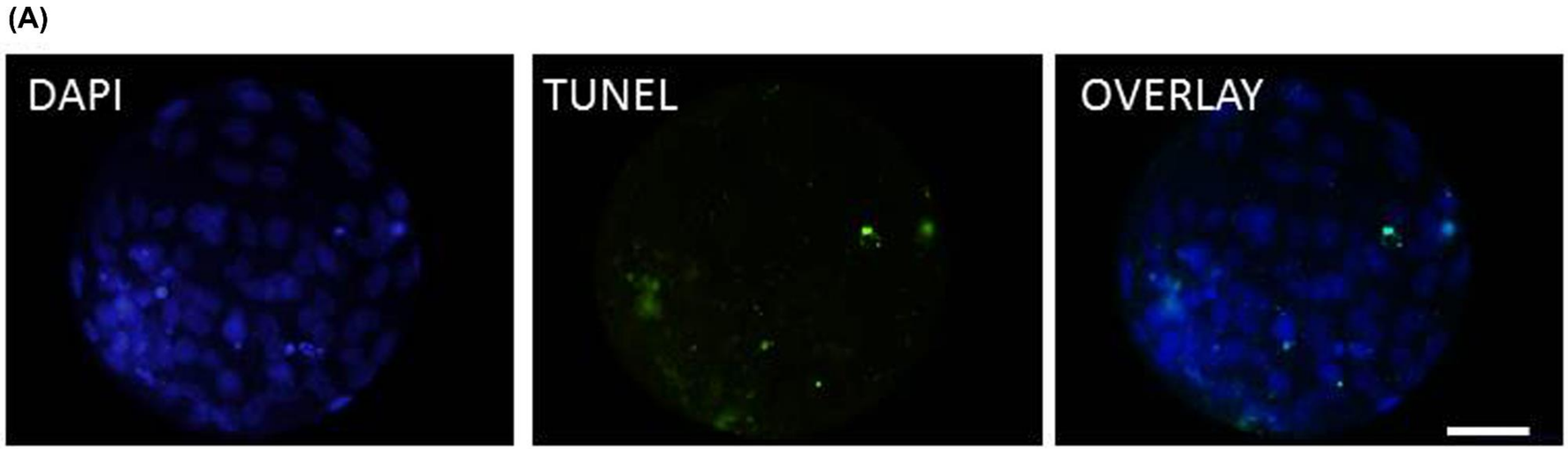


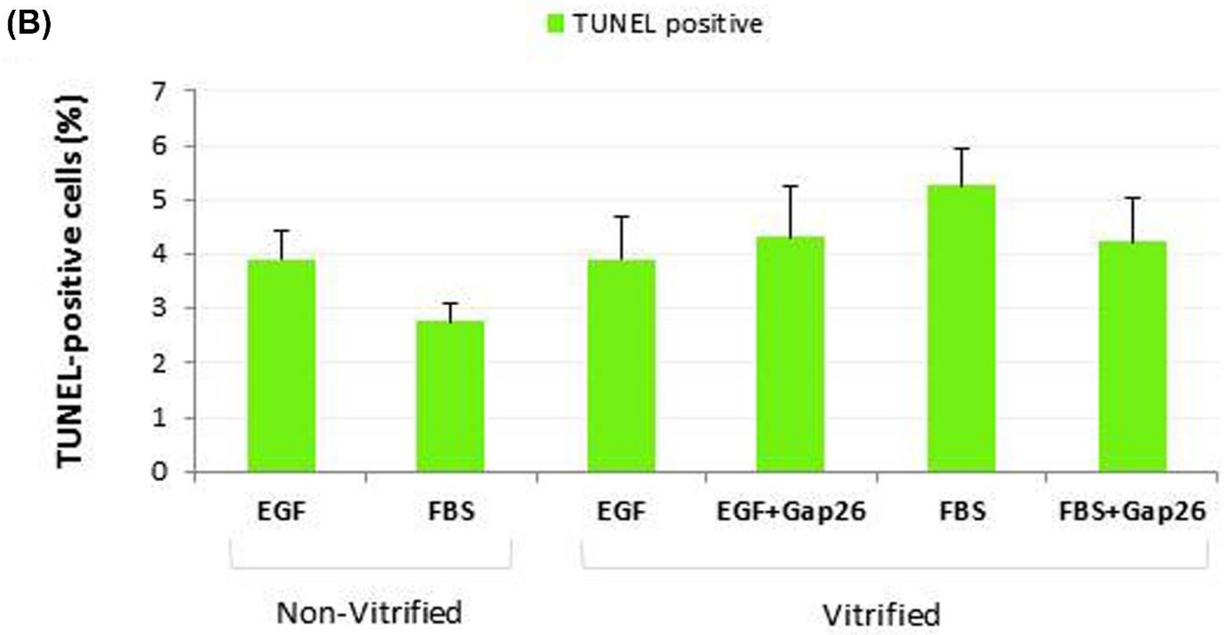
HeLa Cells

GAP26 IMPROVES HATCHING IN VITRIFIED FBS-MATURED EMBRYOS



BUT HAS NO EFFECT ON CELL DEATH….





Ortiz-Escribano et al. 2017

CONCLUSION

- Oocyte maturation in serum can decrease freezability of resulting bovine embryos
- This negative effect can be counteracted at the embryo stage by GAP26
- The positive effect exerted by GAP26 on hatching was not caused by decreased apoptosis

TAKE HOME MESSAGE

- Oocyte vitrification works a little in cattle, horse, cat…
- Human oocyte vitrification works very well!
- We can either learn from human :
  - *in vivo* matured oocytes,
  - ICSI
- We can use some tricks in domestic animal oocyte vitrification :
  - Gap26,
  - removal of cumulus cells
  - remove serum from maturation medium…

ACKNOWLEDGEMENTS

- Luc Leybaert, UZ Gent (Connexin- GAP26)

- Henri Woelders, Wageningen (Vitrification principles)

- Etienne Van den Abbeel, UZ Gent (human oocyte vitrification)

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