VAKGROEP VERLOSKUNDE, VOORTPLANTING EN BEDRIJFSDIERGENEESKUNDE
ONDERZOEKSGROEP VOORTPLANTING GEZELSCHAPSDIEREN

STATE OF THE ART IN EQUINE ASSISTED REPRODUCTIVE TECHNOLOGY (ART)

Katrien Smits - 29 august 2019

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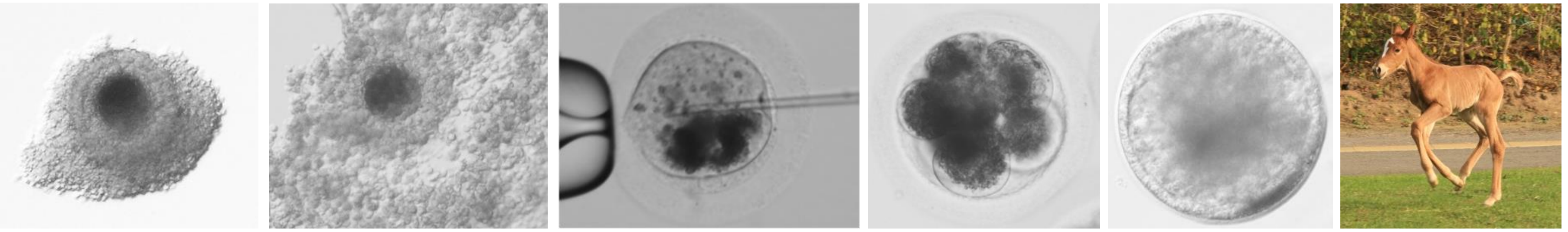
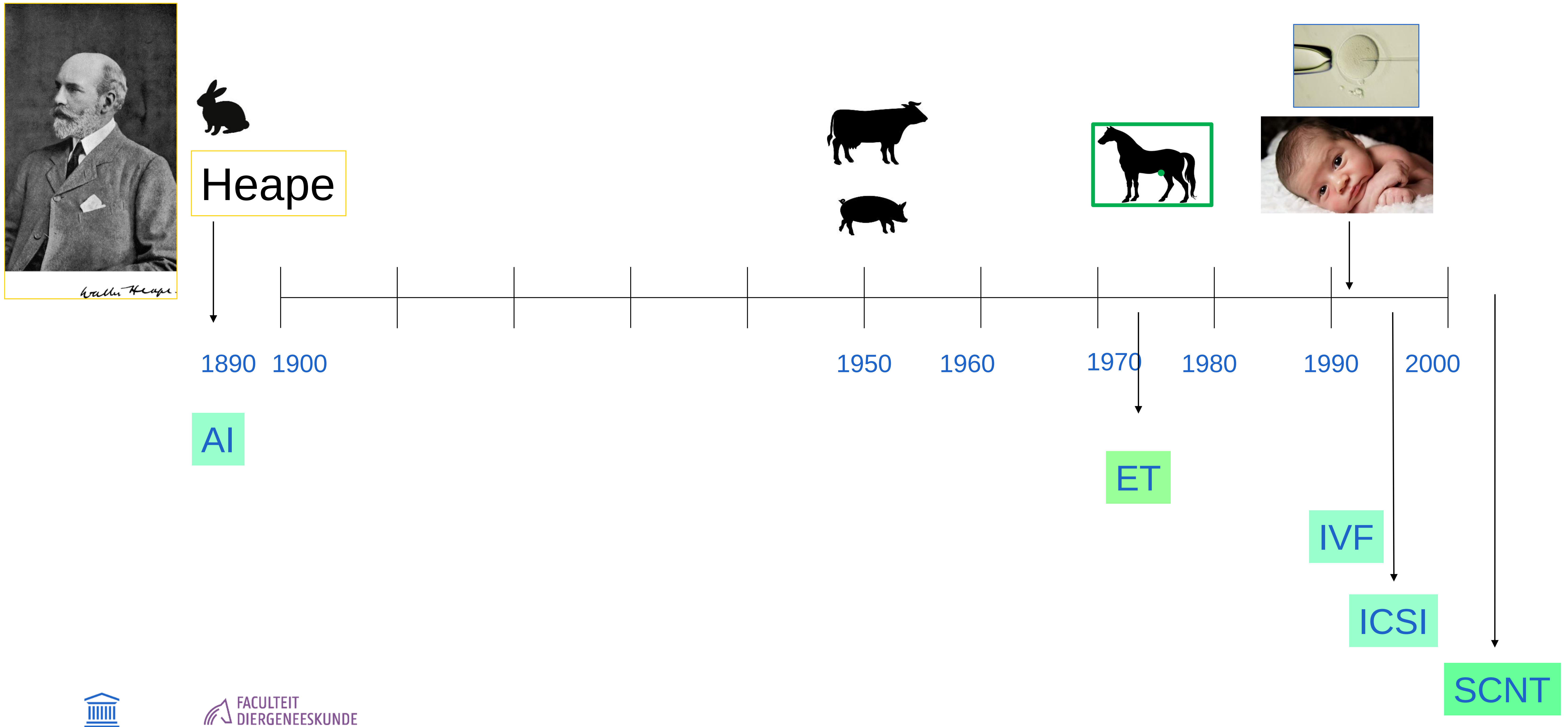


EAAP

HISTORY OF ART



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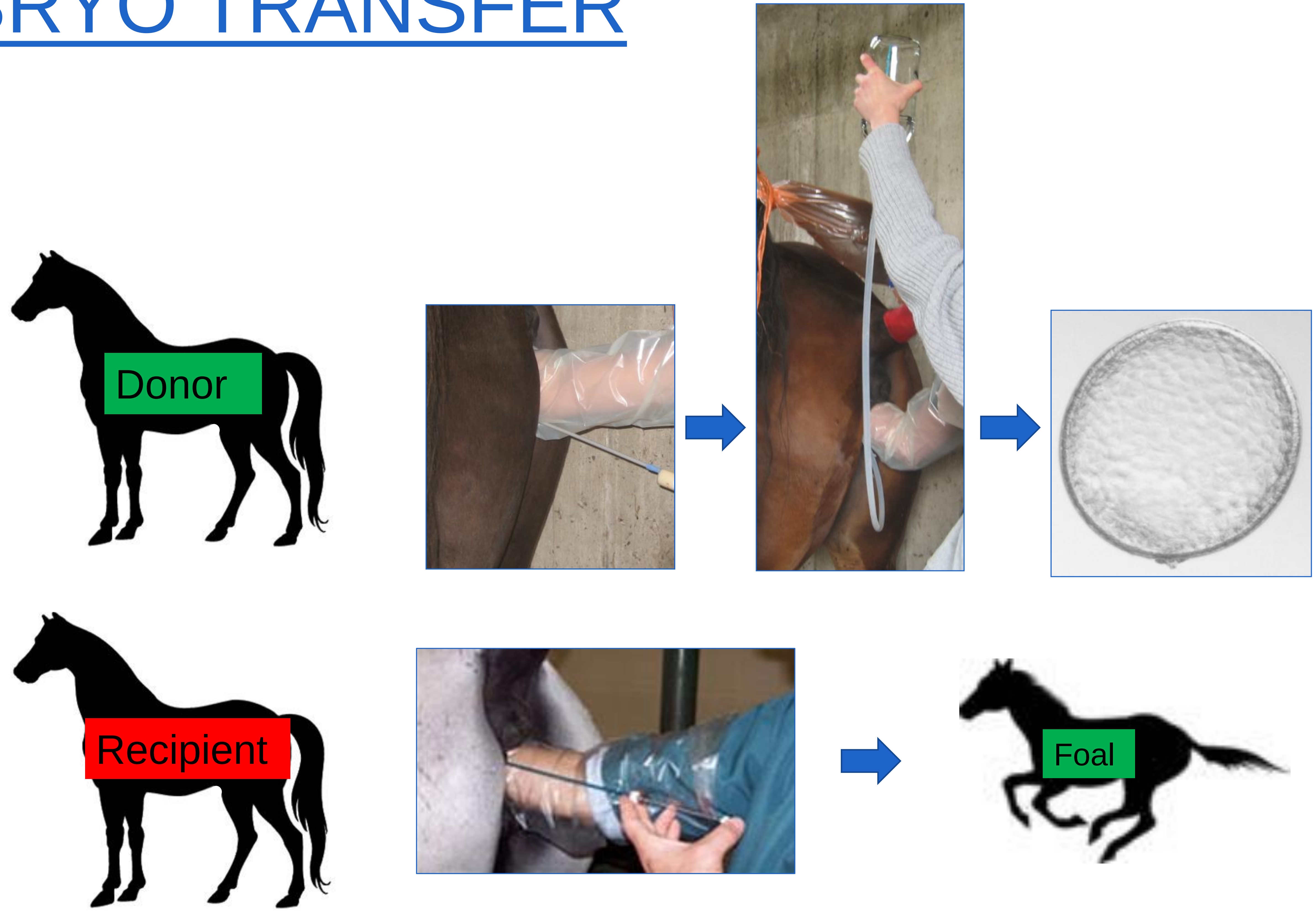
A mare with recurrent pregnancy loss

EMBRYO TRANSFER



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EMBRYO TRANSFER

- Success rate:
  - Embryo recovery ~75%
  - Pregnancy rate ~75%

50% recipient pregnancy rate per cycle

- Application in mares which :
  - cannot carry foal to term
  - are in competition
  - are wanted for multiple foal production

MOTHER OF ALL ART - EMBRYO TRANSFER (ET)

- *In vivo* derived embryos (fresh or frozen)
- *In vitro* produced embryos (fresh or frozen)
- Cloned embryos (fresh or frozen)

EMBRYO TRANSFER

- Limitations:
  - Requires genital tract to support embryo development until D7
  - Requires a full dose of good quality semen
  - Superovulation is problematic

A mare with cervical lacerations

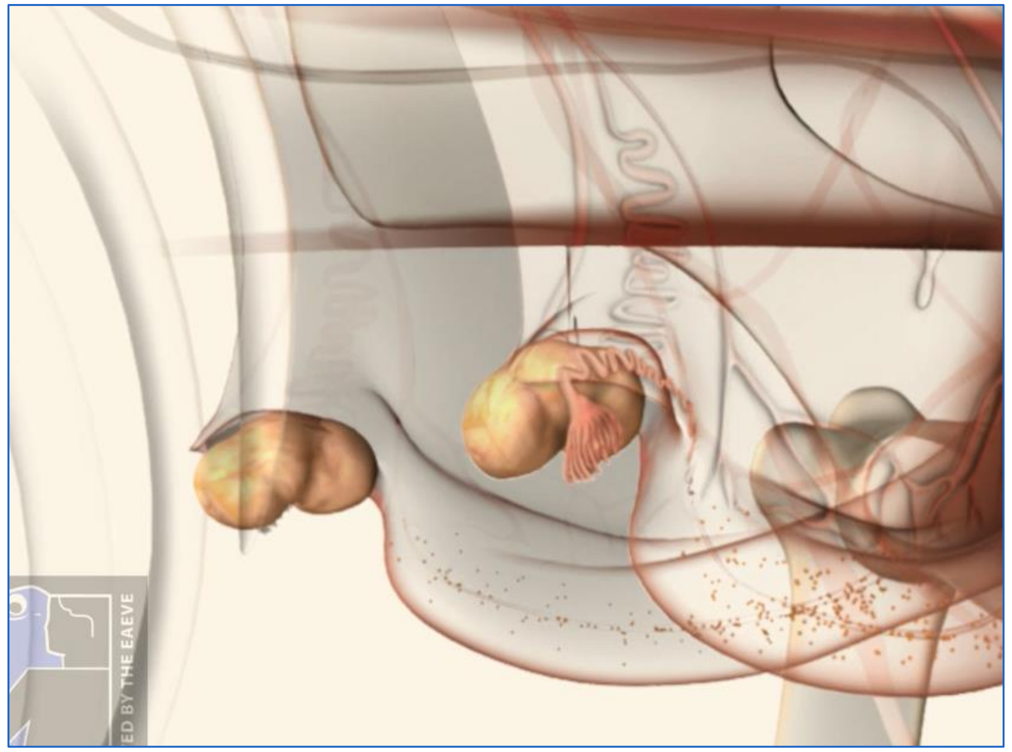
OOCYTE RECOVERY

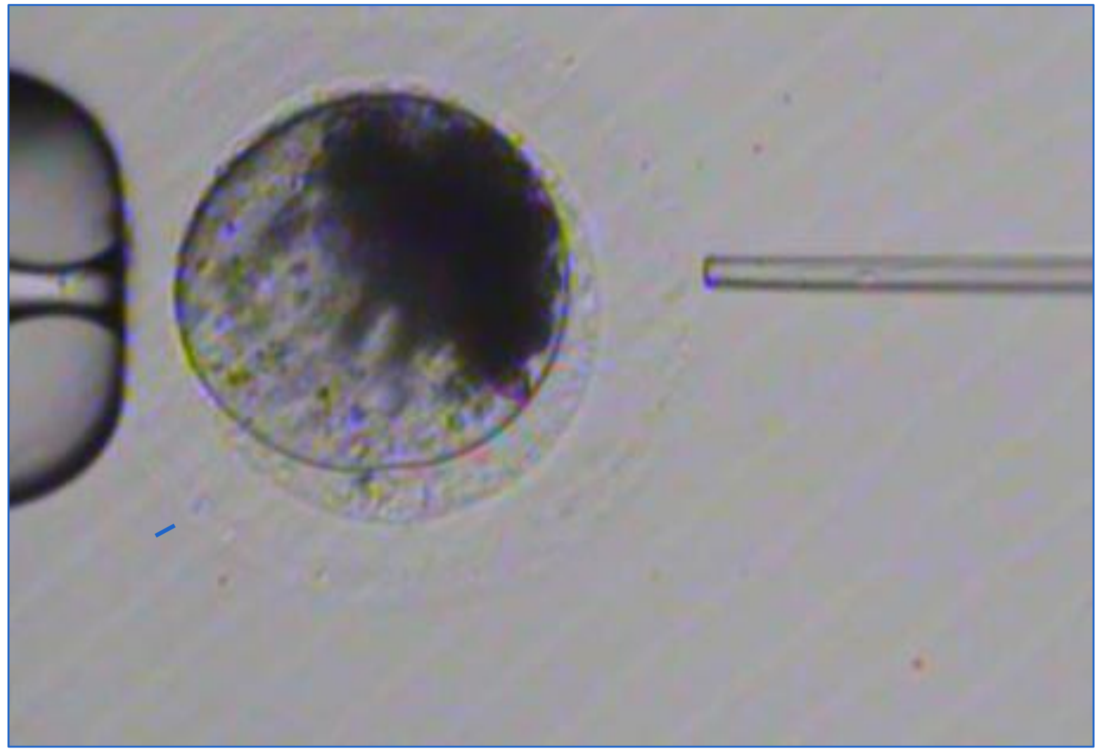
– Oocyte recovery: mature or immature

⇩

– Fertilization and embryo development

in vivo | *in vitro*





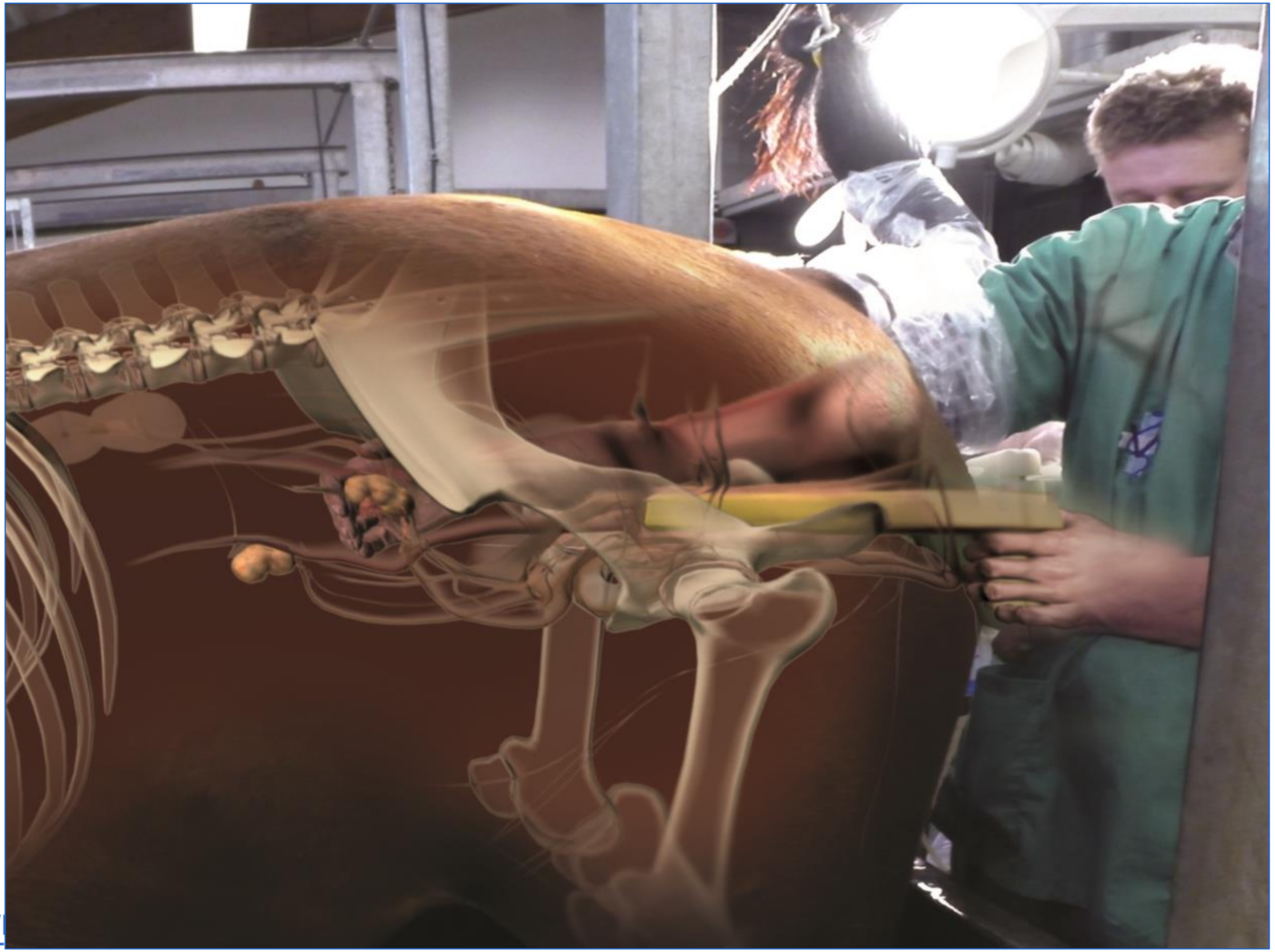
OOCYTE RECOVERY

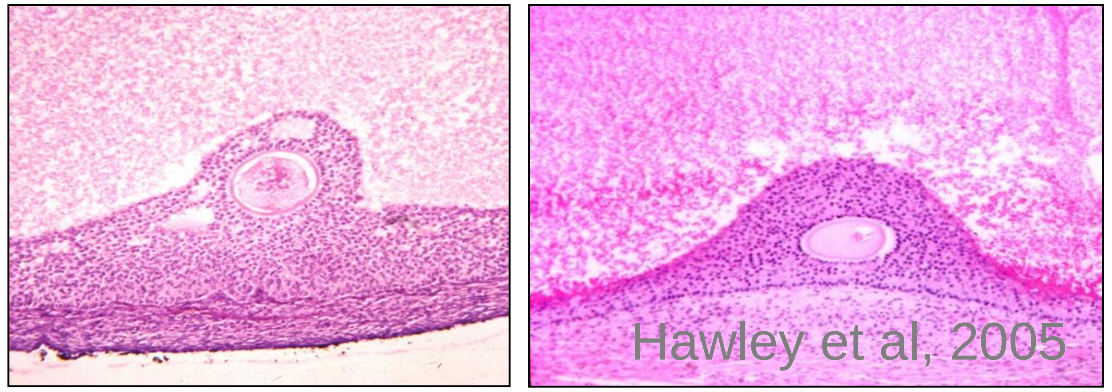
– **Mature oocyte** : flank aspiration

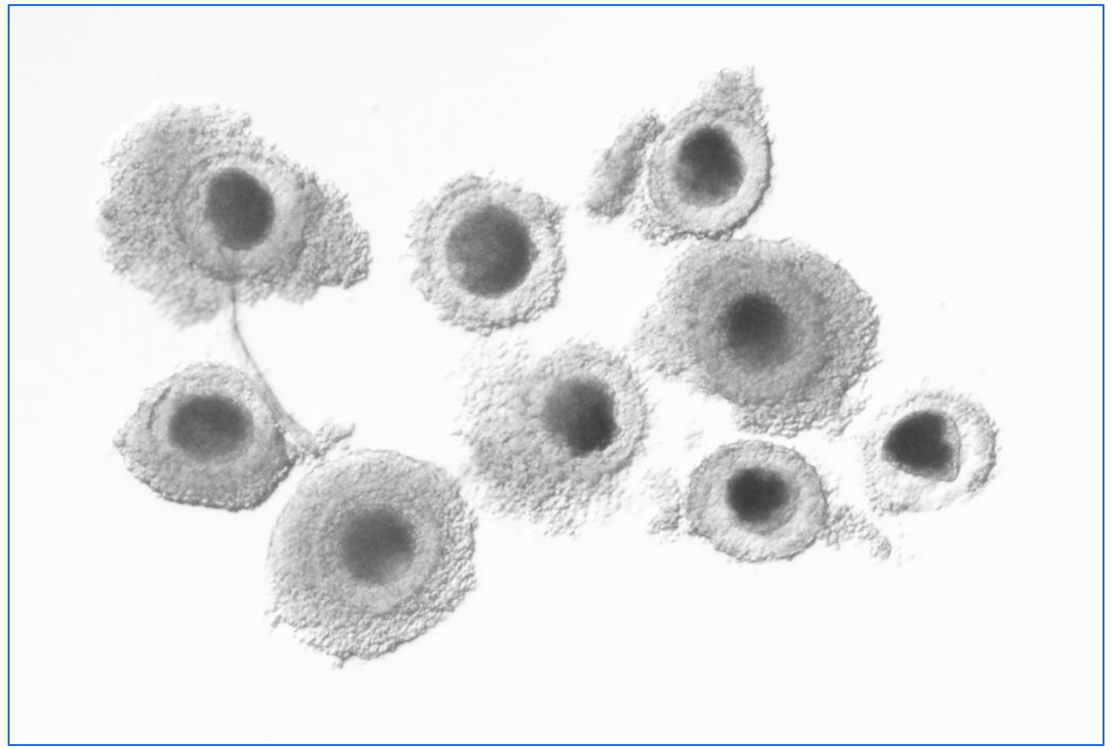
www.FoalInMare.com

OOCYTE RECOVERY

– **Immature oocytes** : Ovum Pick Up (OPU)







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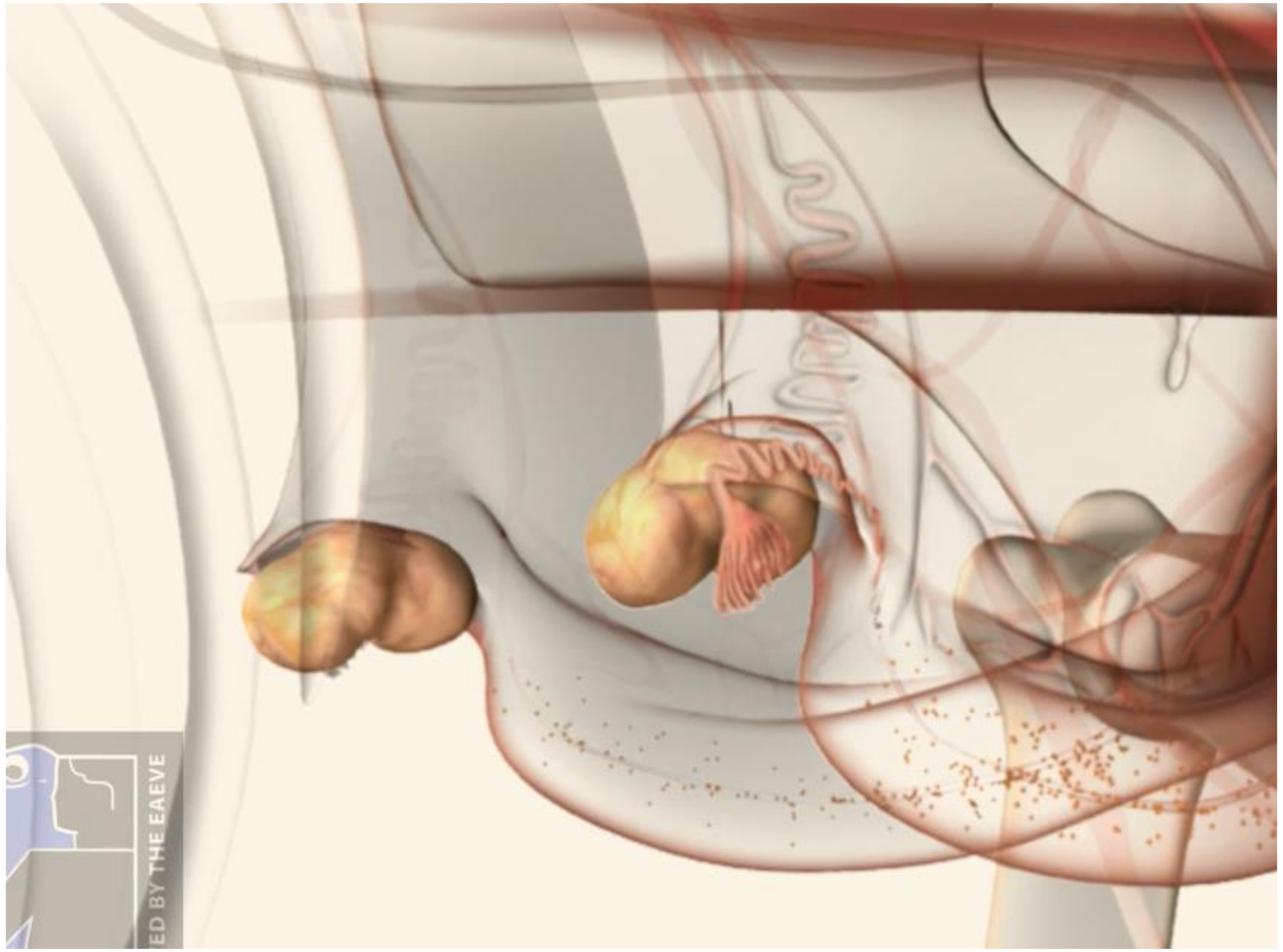
OOCYTE RECOVERY EFFICIENCY

- 1 mature oocyte
- Recovery rate : 80%
- Timing cycle, hCG
- Embryo/oocyte collection: 0,33

- ~10 immature oocytes
- Recovery rate : 50-60%
- *In vitro* maturation needed
- Embryo/OPU : 1

OOCYTE TRANSFER (OT)

- Transfer of a mature oocyte to the oviduct of an inseminated recipient mare
- *In vivo* fertilisation
- Clinical application
  - Argentina and USA
- Limited in Europe:
  - Surgery of recipient mare
  - Multiple pregnancies



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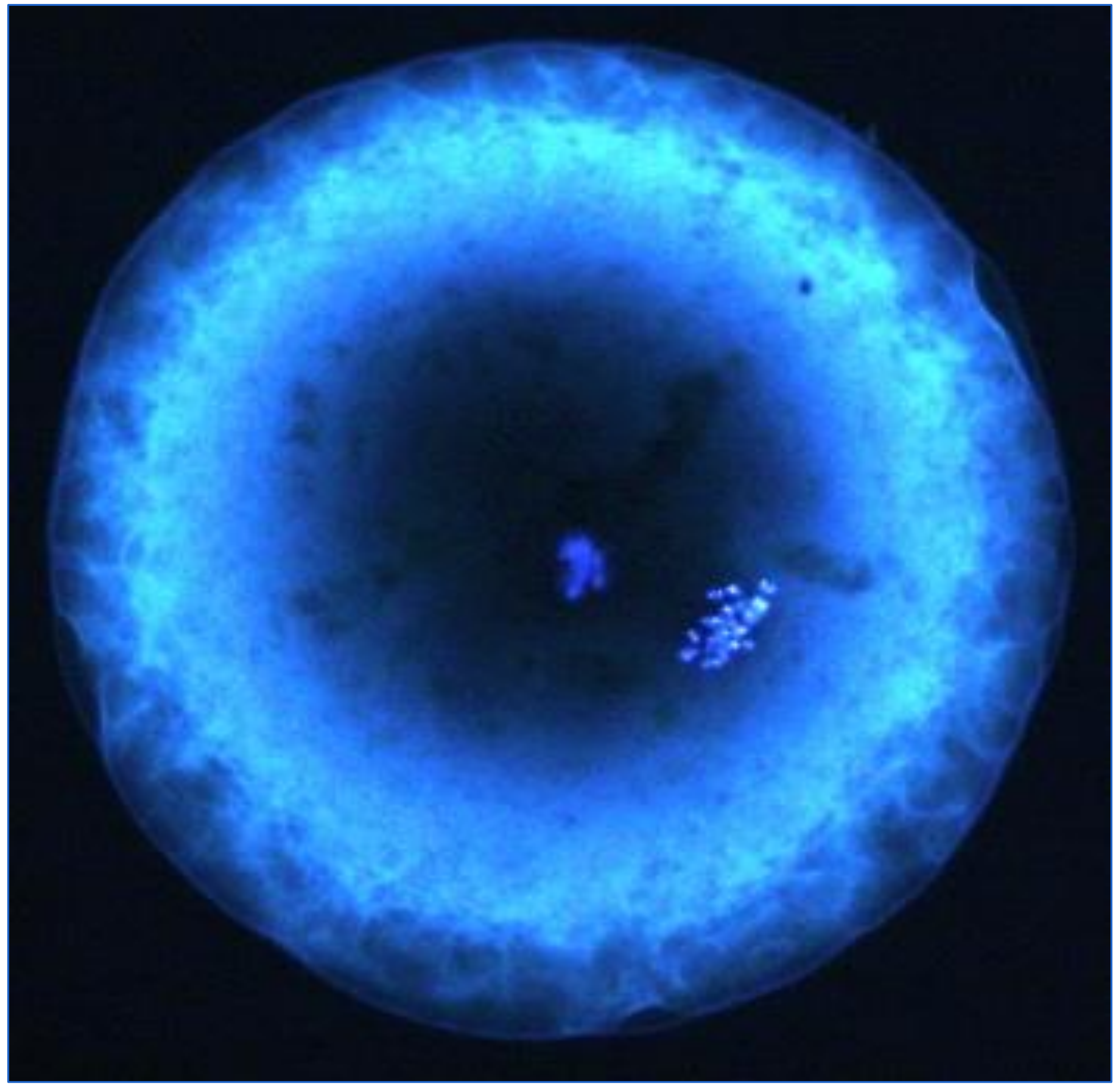
www.FoalInMare.com

OPU - ICSI

– *In vitro* maturation



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OPU - ICSI

– Intracytoplasmic sperm injection (ICSI)

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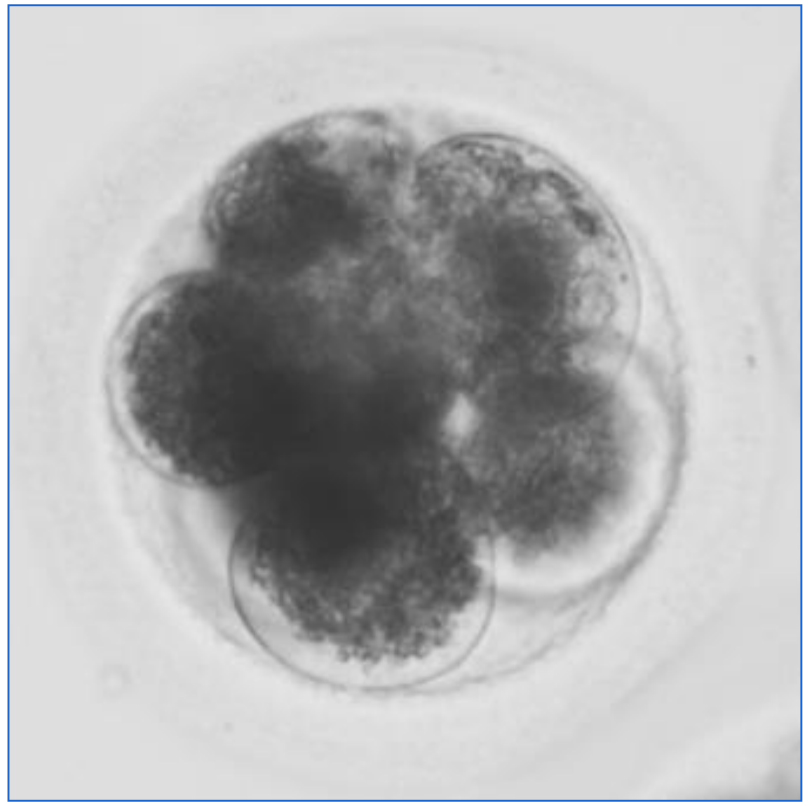
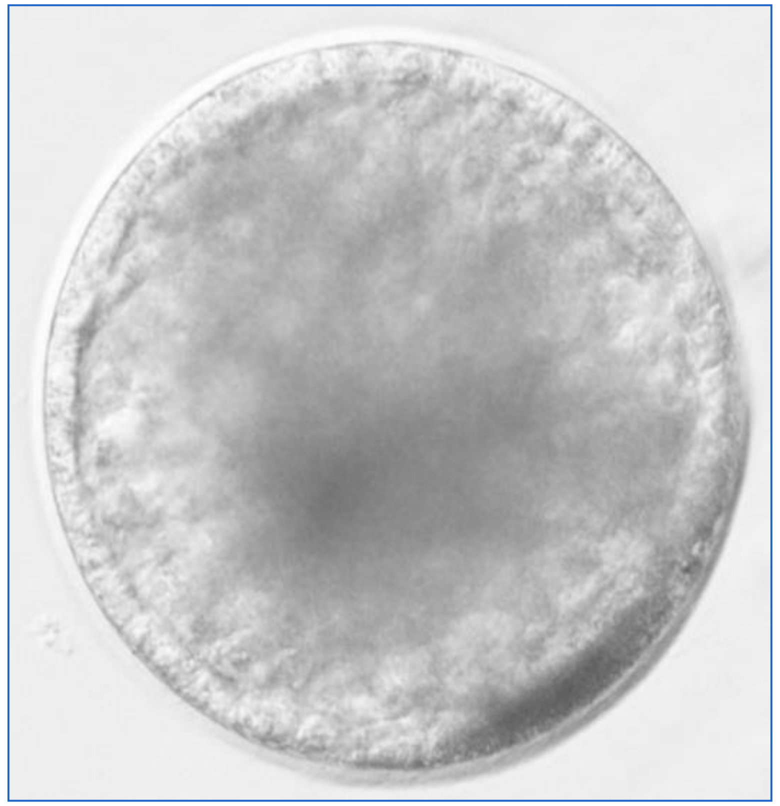
OPU - ICSI

– *In vitro* culture

Cleaved embryo



Blastocyst



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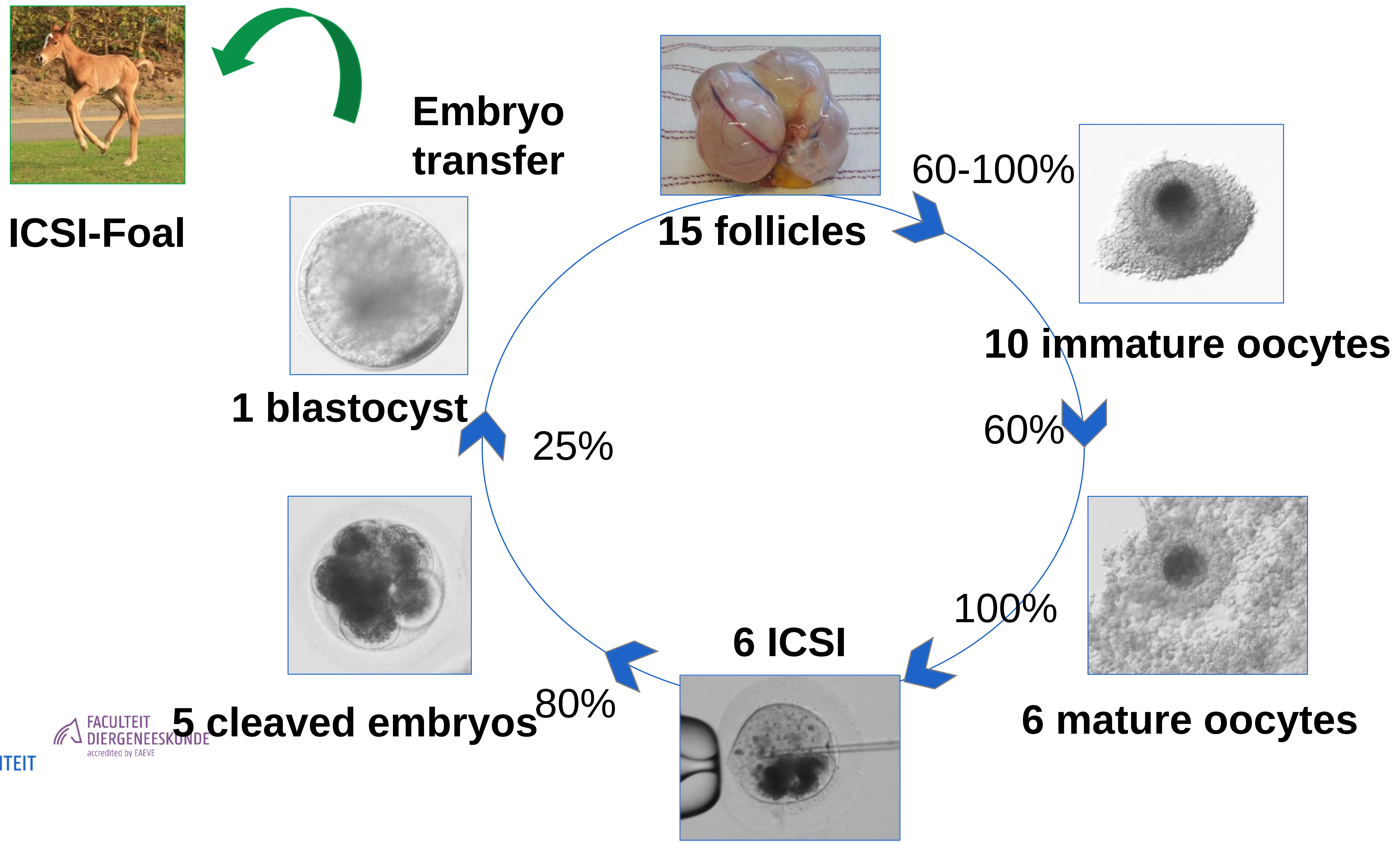
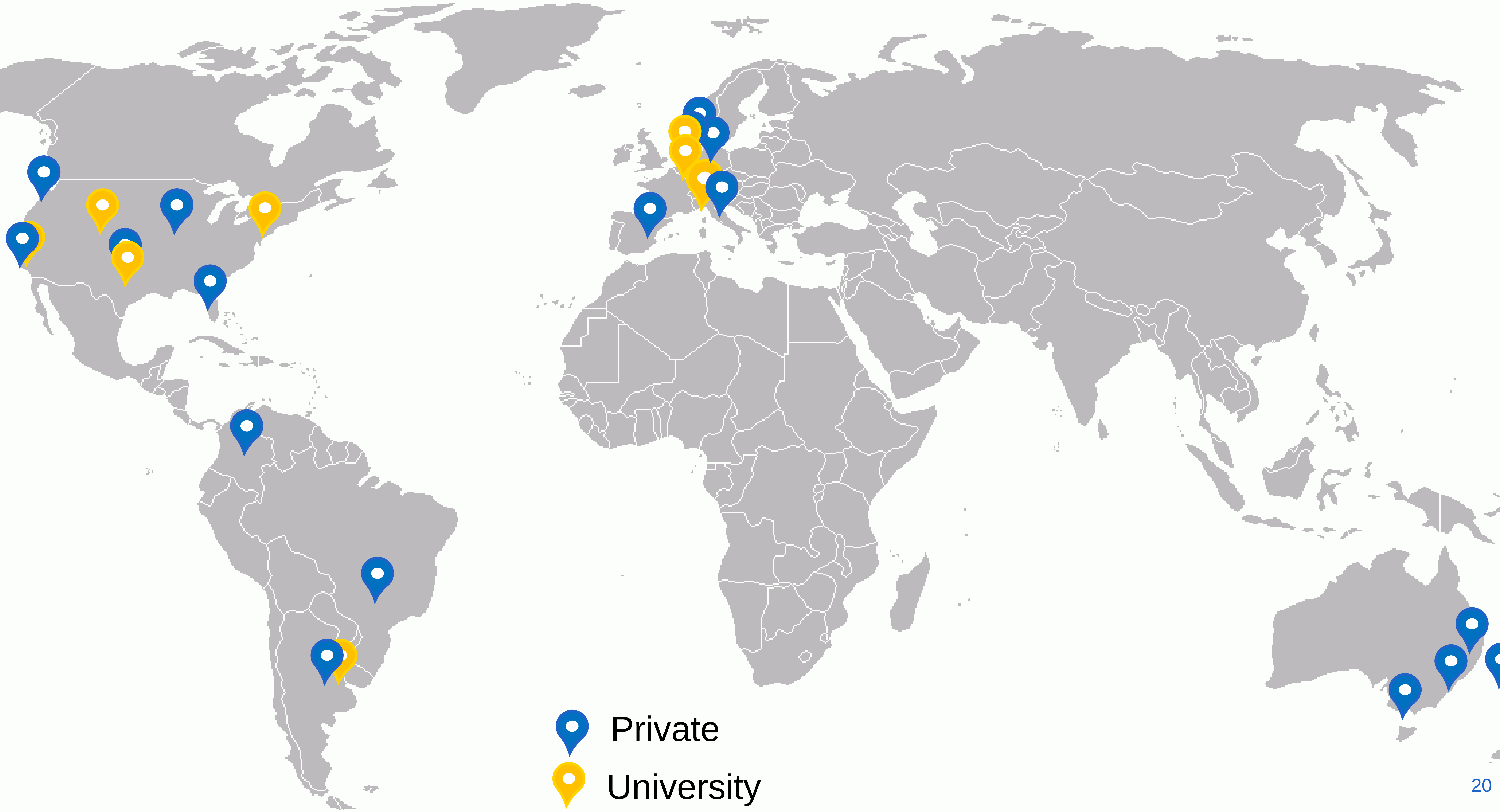
WHAT TO EXPECT FROM OPU-ICSI?



CANDIDATES FOR ICSI?

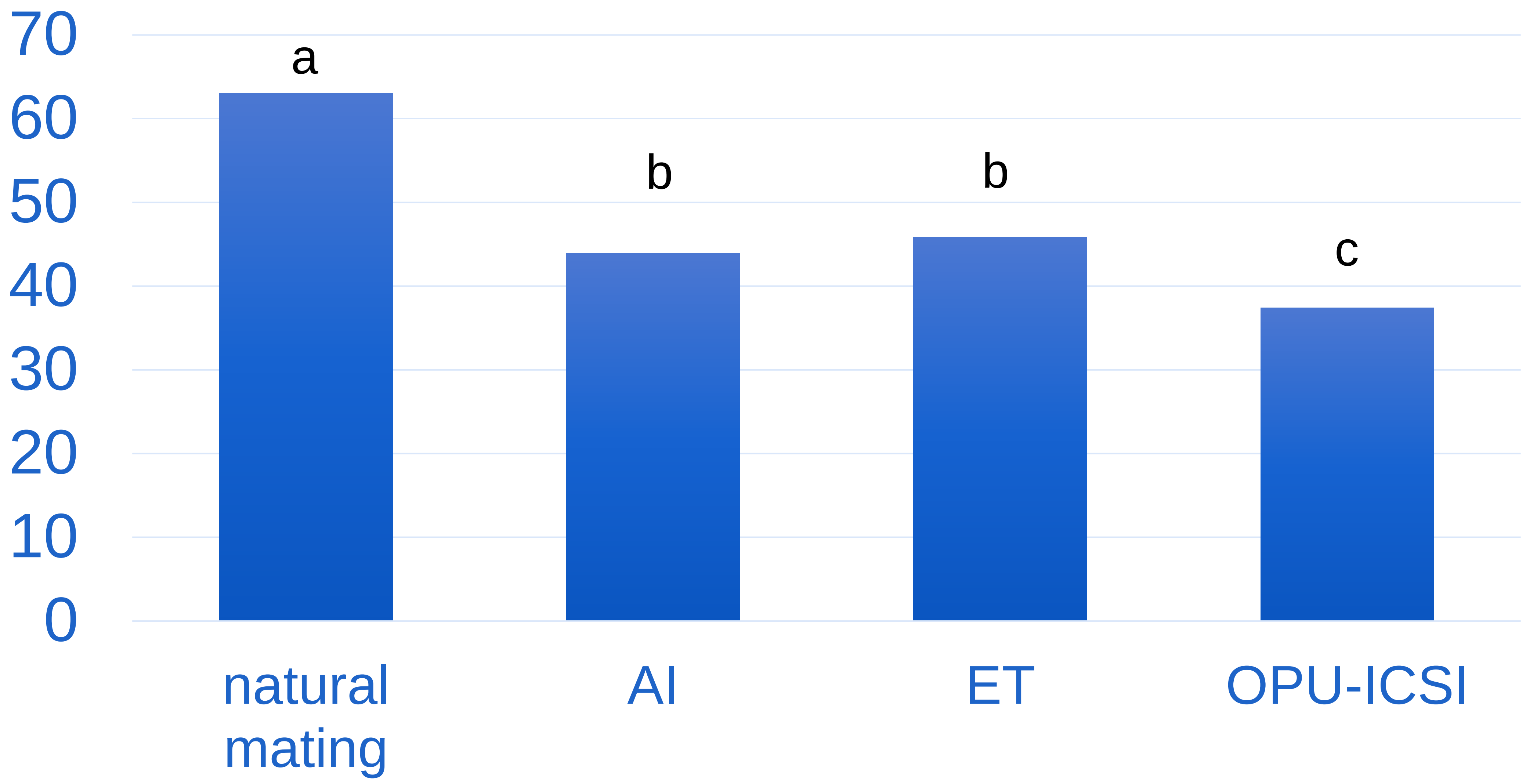
- High value mares with
  - Cervical problems
  - Chronical endometritis
  - Blocked oviducts
  - …
- High value stallions with
  - Subfertility
  - Limited availability of (frozen) semen
  - …..

CUMANO


Private
University

COMPARATIVE REPRODUCTIVE EFFICIENCY

– ‘= having at least one D45 pregnancy per attempt (%)’ (Cuervo-Arango et al, 2019)



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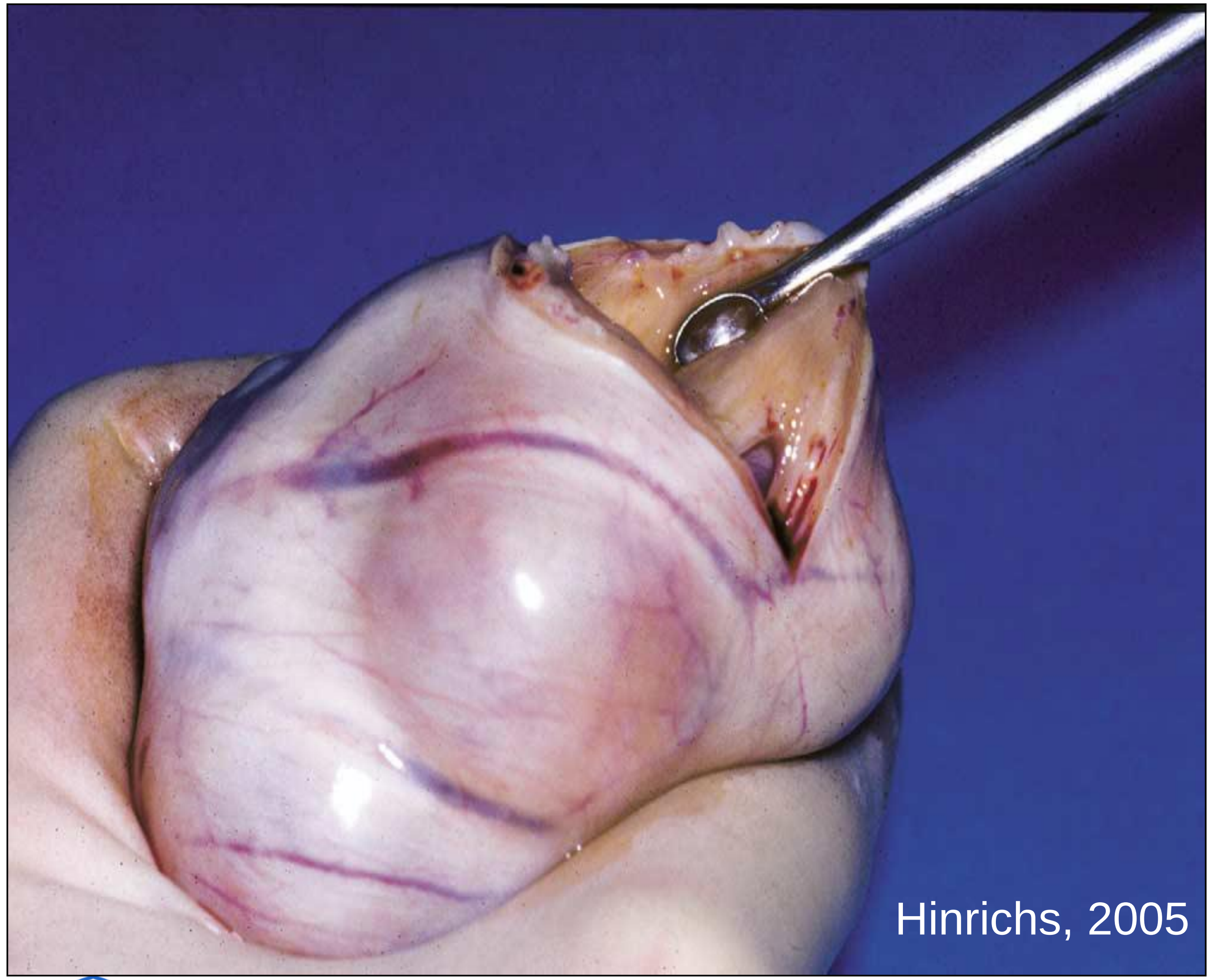
A dead mare

POST MORTEM OOCYTE COLLECTION - ICSI

– Excision of ovaries, transport to lab within 6-12 hours

– Scraping

Aspiration



Hinrichs, 2005

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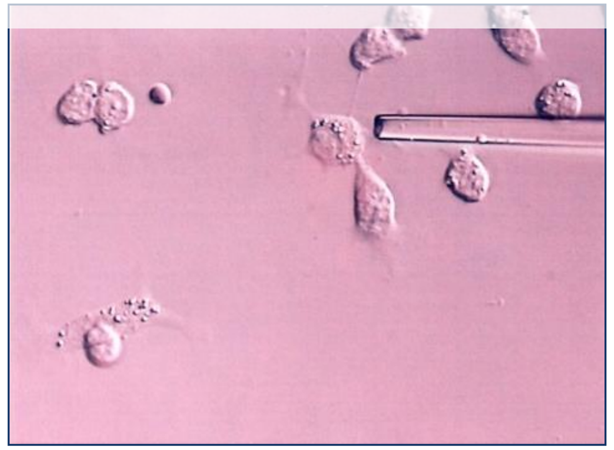
CLONING OR NUCLEUS TRANSFER

Skin biopsy from donor horse

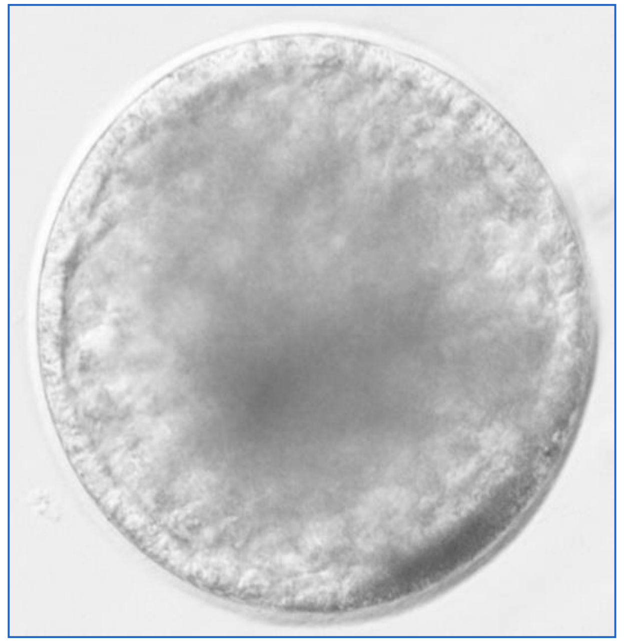


Culture of somatic cells

Collection of horse oocytes

Enucleation of mature oocyte

Transfer of donor nucleus to recipient oocyte : fusion and activation by electrical pulse



Culture of the embryo to the blastocyst stage

Intrauterine transfer to recipient mare
Pregnancy is monitored by ultrasound

Gambini and Maserati, 2017

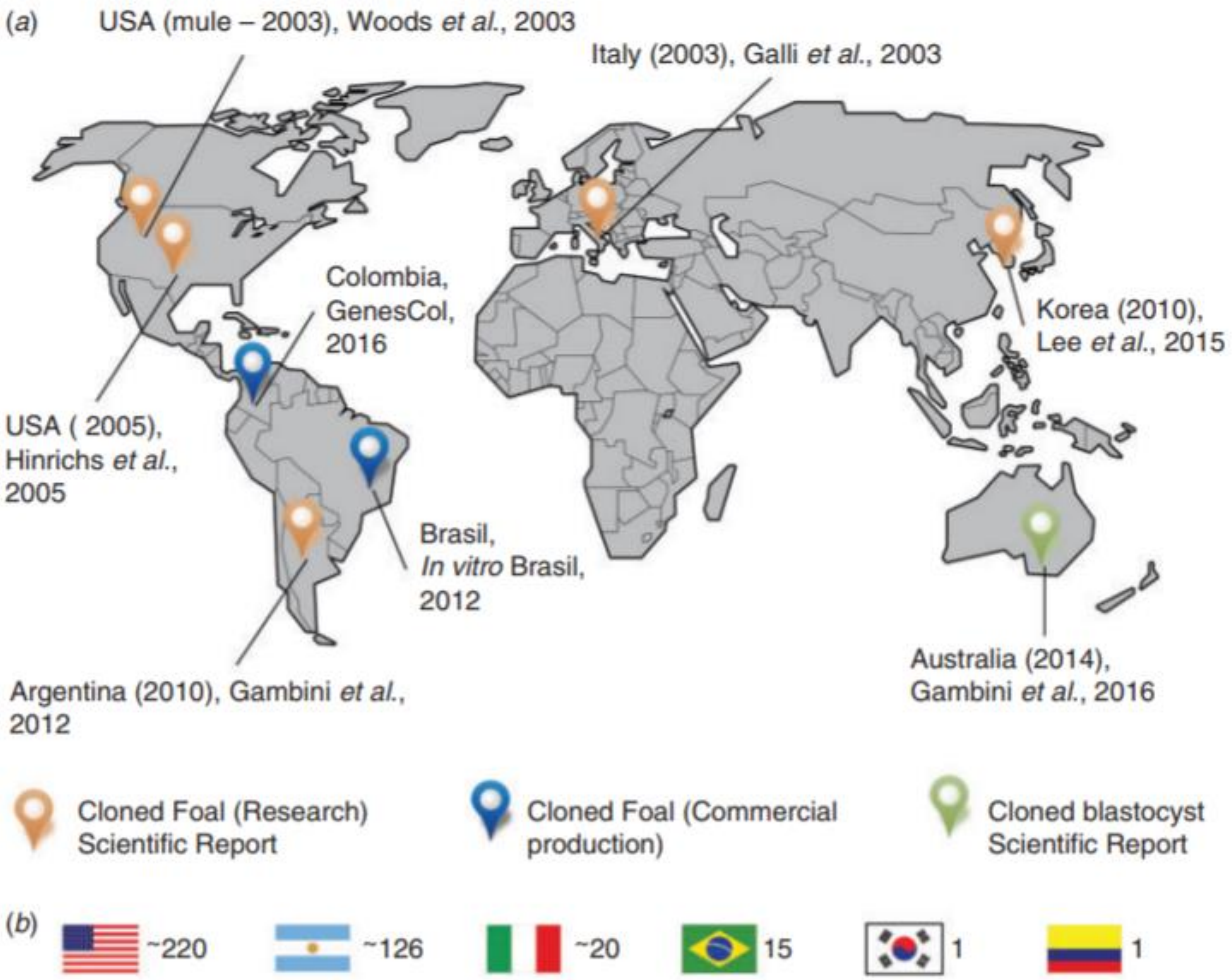
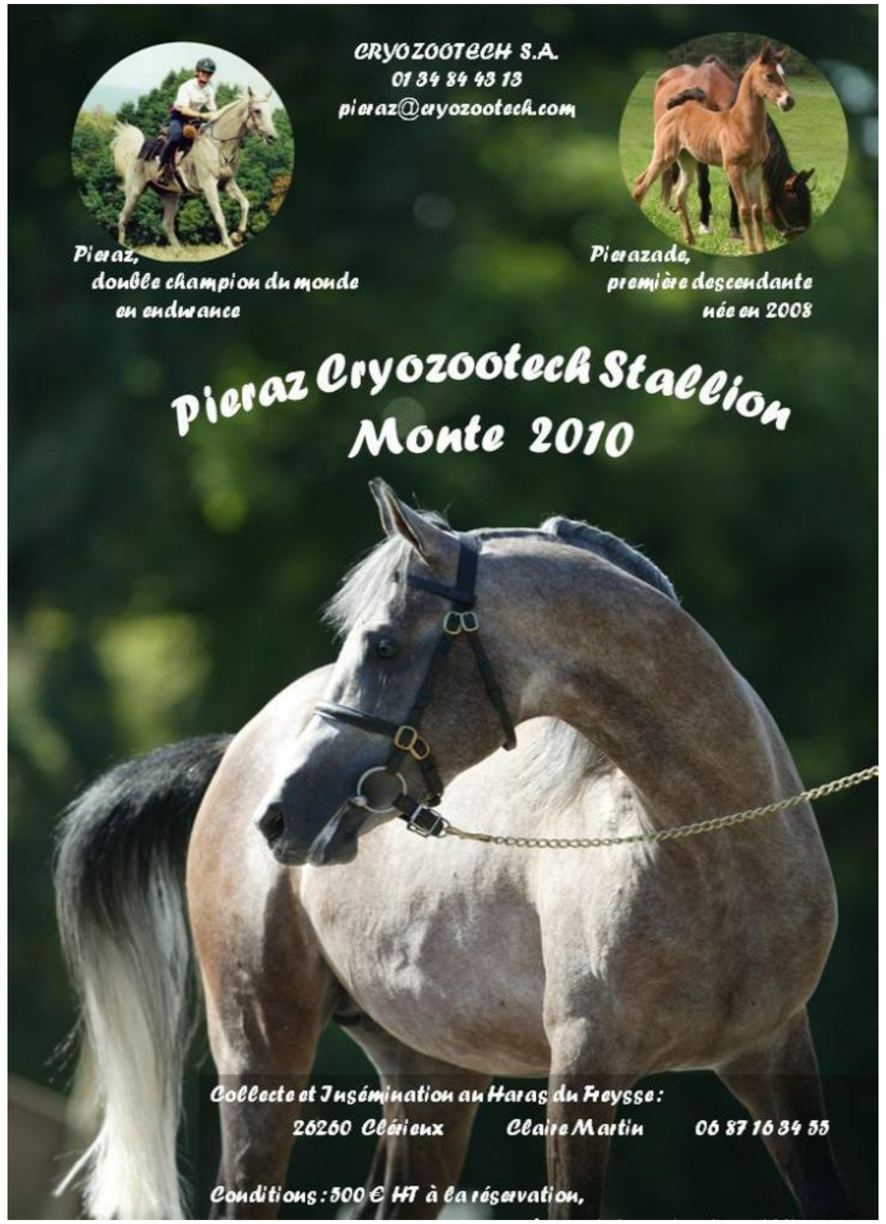


Fig. 3. (*a*) Worldwide distribution of reports of the first equine clones in different countries. (World map image from https://commons.wikimedia.org/wiki/File:BlankMap-World-1985.png, accessed 8 October 2017; licensed under the Creative Commons Attribution-Share Alike 3.0 Unported license, modified.) (*b*) Estimated number of cloned horses produced in different countries by 2016.

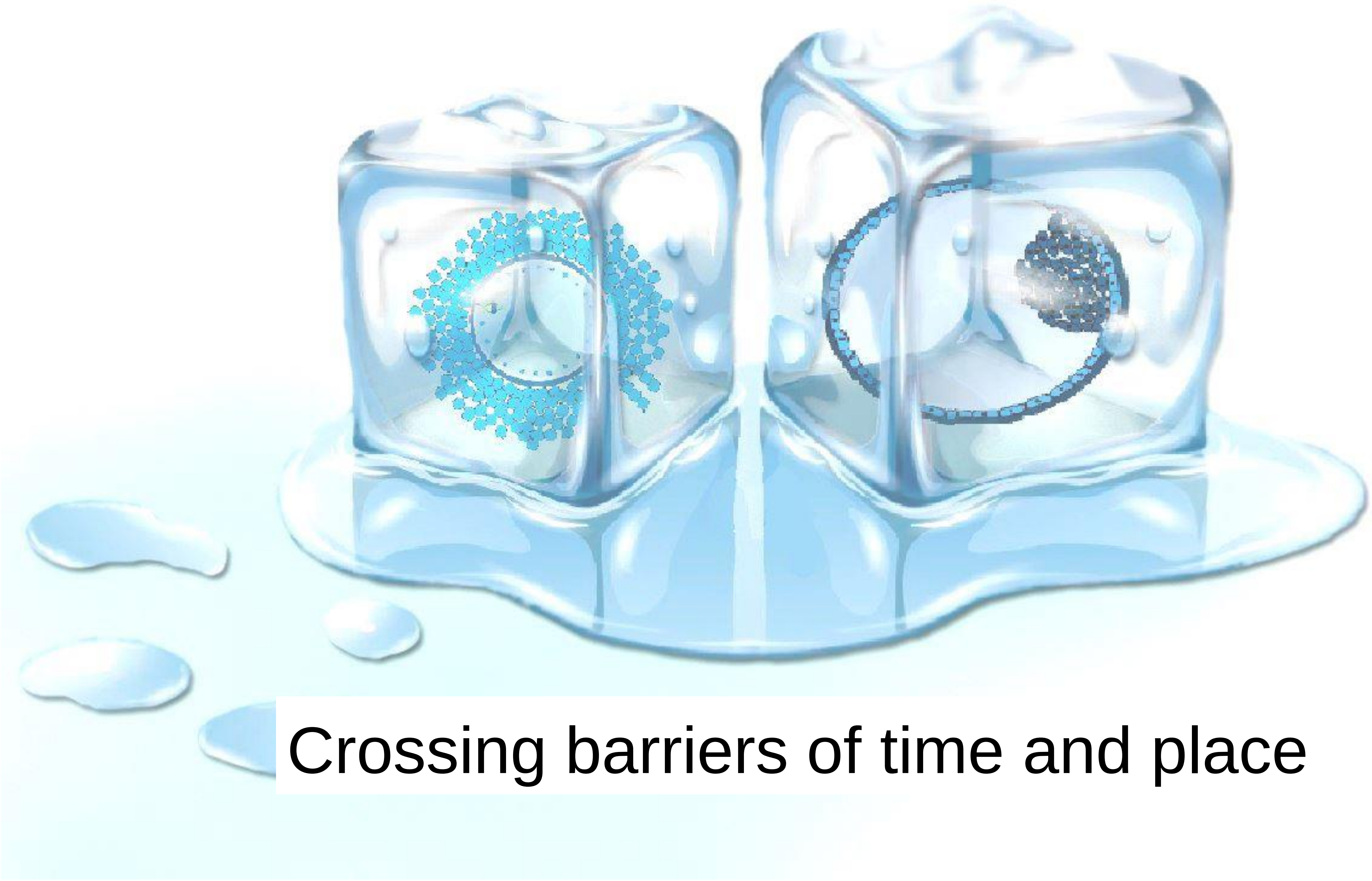
Gambini and Maserati, 2017


SOUTH AMERICAN
LOOK SOUTH
TOURS
ARGENTINA, POLO
Argentine polo superstar Adolfo Cambiaso clones 100 of his favourite horses


CRYOZOOTECH S.A.
01 34 84 43 13
pieraz@cryozootech.com
Pieraz,
double champion du monde
en endurance
Pierazade,
première descendante
née en 2008
Pieraz Cryozootech Stallion
Monte 2010
Collecte et Insémination au Haras du Freysse :
26260 Clérieux
Claire Martin
06 87 16 34 55
Conditions : 500 € HT à la réservation,

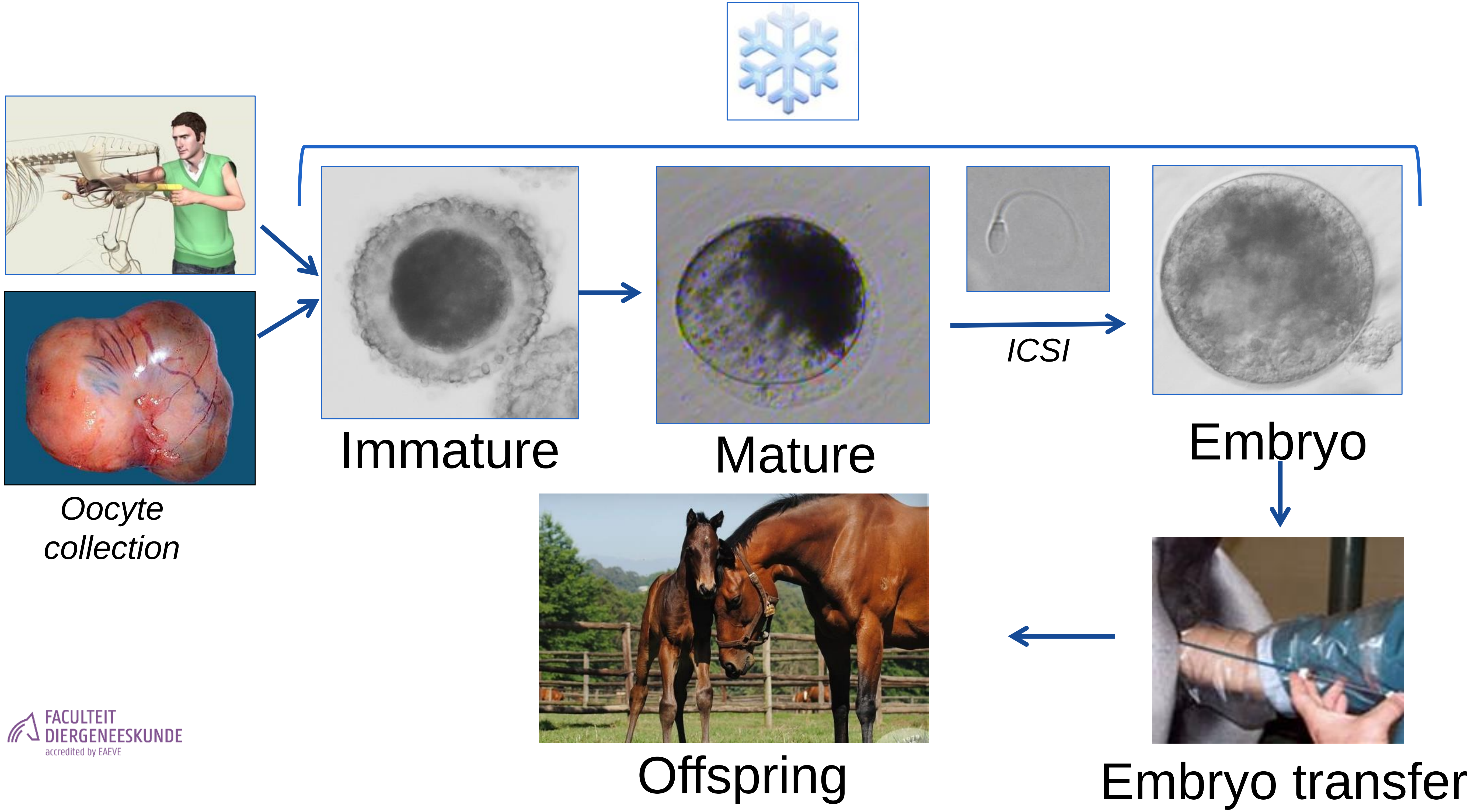
CLONING: LIMITATIONS

- Availability of equine oocytes
- Efficiency
  - Embryo production
  - High pregnancy loss
  - Perinatal problems
- Registration of clones



Crossing barriers of time and place

CRYOPRESERVATION IN THE HORSE



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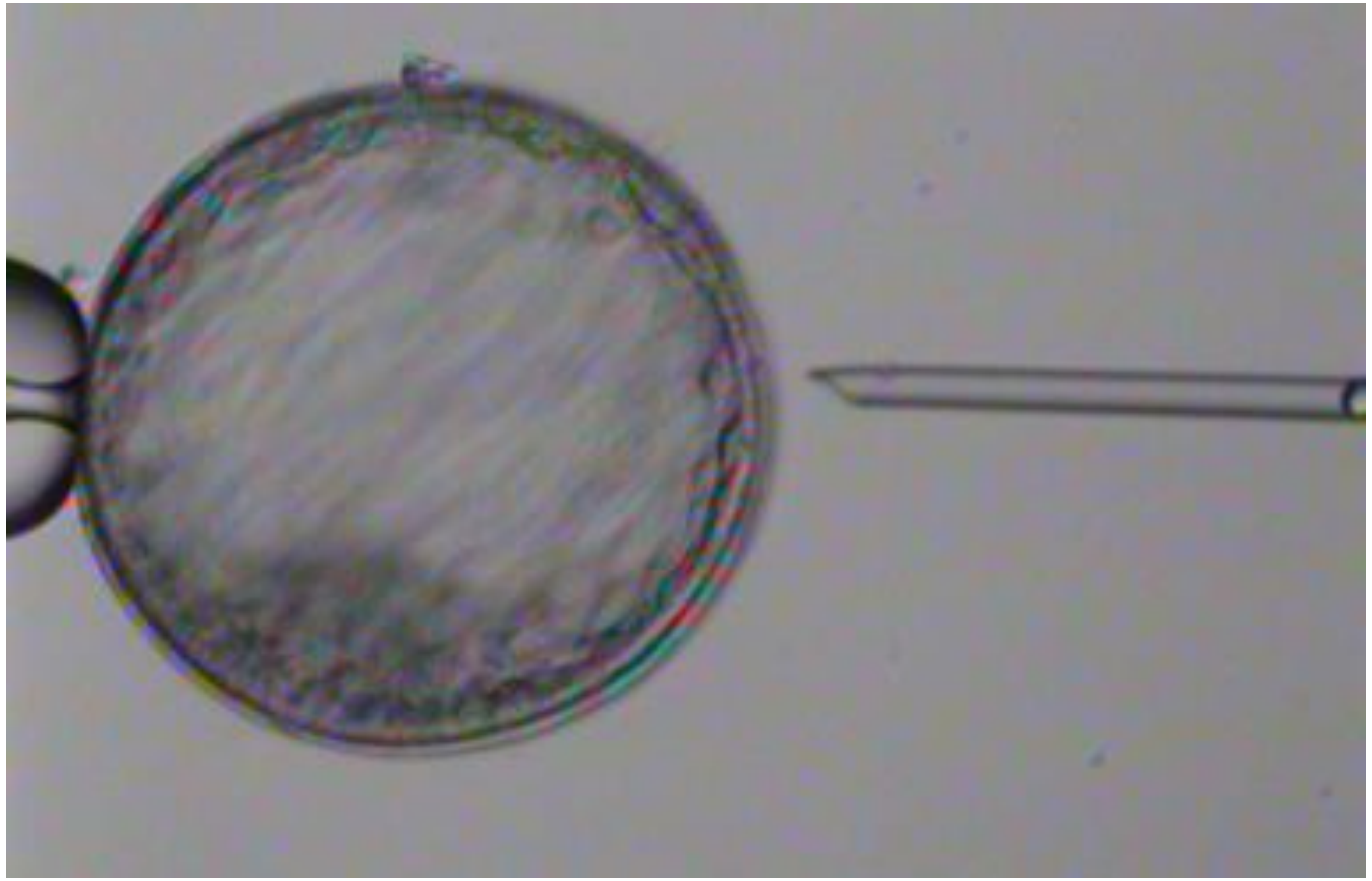
EMBRYO FREEZING

– *In vivo*

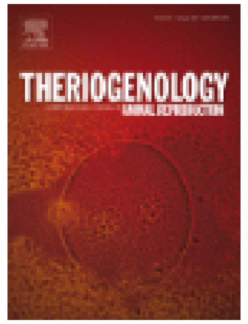


EMBRYO FREEZING

- *In vitro*
  - Pregnancy and foaling rate frozen embryos = fresh

ELSEVIER

Theriogenology
Volume 87, 1 January 2017, Pages 48-54



Research article

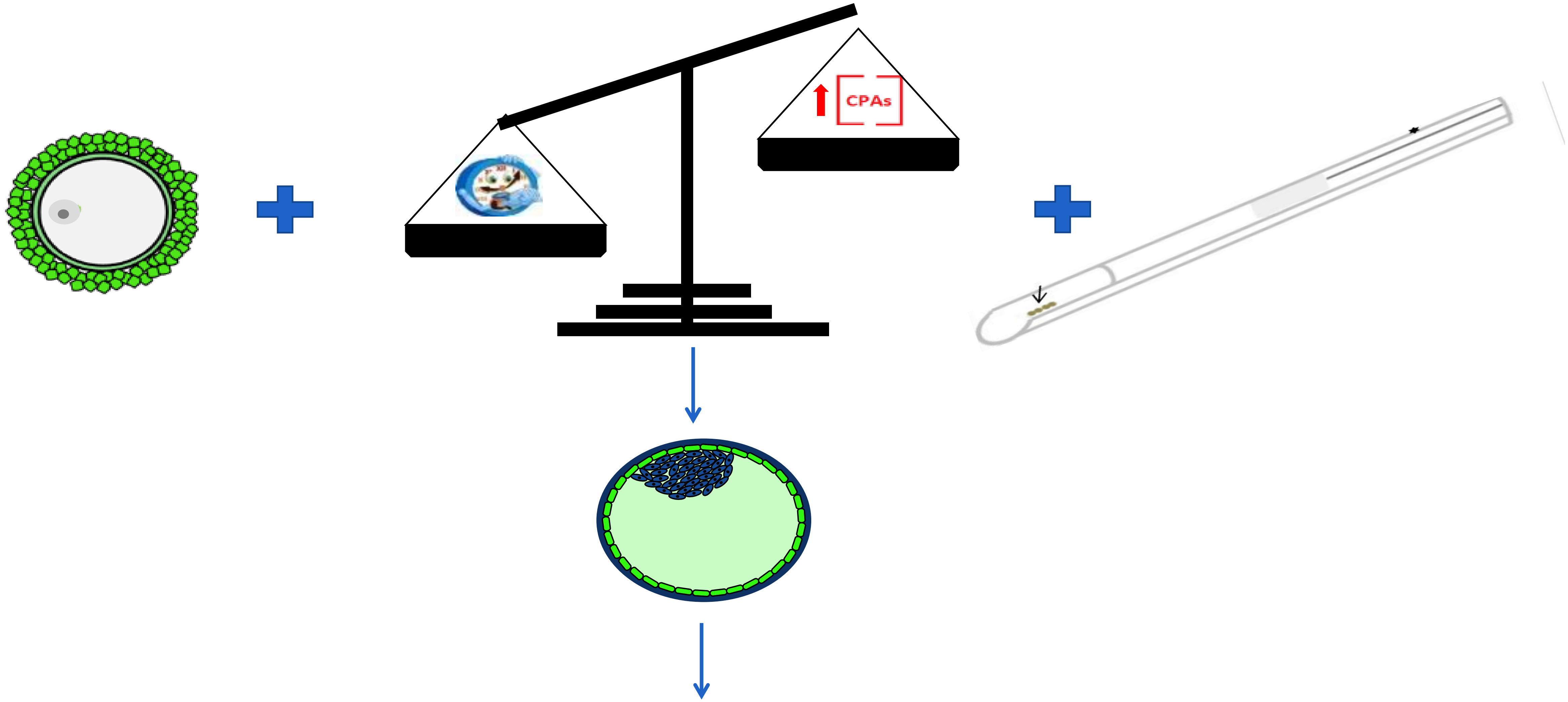
Vitrification of *in vitro*-produced and *in vivo*-recovered equine blastocysts in a clinical program

Young-Ho Choi, Katrin Hinrichs

⊞ Show more

https://doi.org/10.1016/j.theriogenology.2016.08.005

OOCYTE VITRIFICATION



VICSI, May12, 2017

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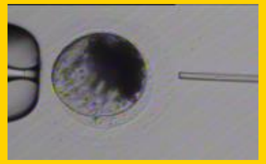
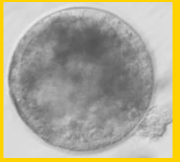
OOCYTE VITRIFICATION

– Limitation: efficiency

				
Fresh	146	80 (55%)	16 (20%)	10 (60%)
Vitrification	179	72 (40%)	5 (6,9%)	1 (20%)

– Applications
  – Increase flexibility of OPU-ICSI
  – Research
  – Genome resource banking

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GENE BANKING

– Interspecies embryo transfer and hybrids


Allen and Short, 1997

GENE BANKING

– ART in horse as a model for endangered species

nature
COMMUNICATIONS

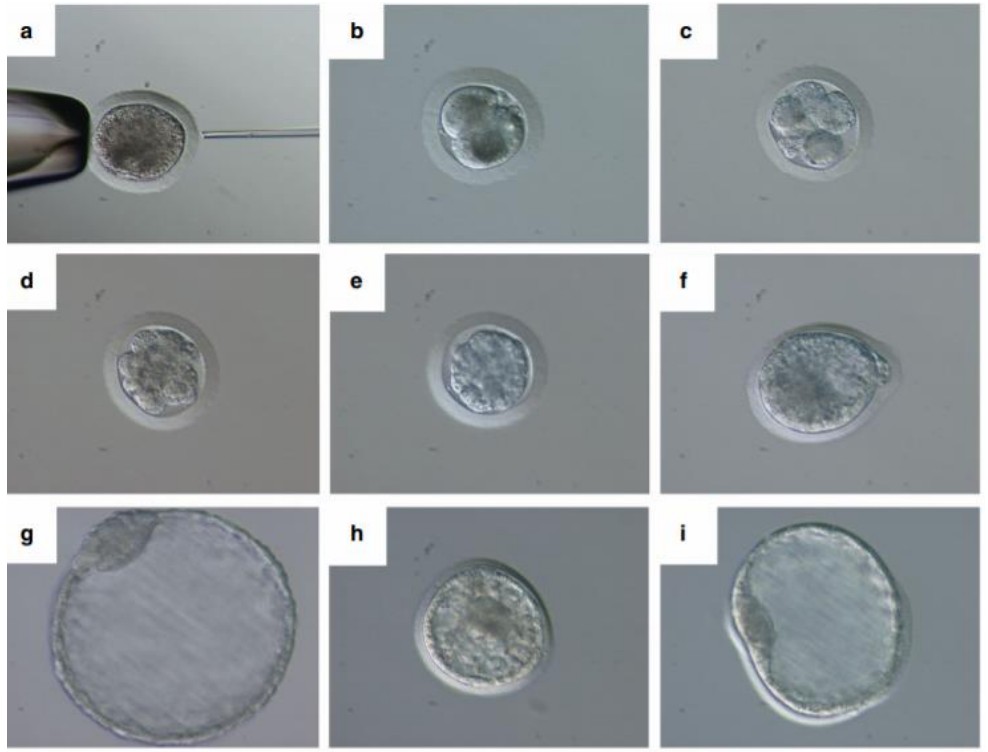
ARTICLE

DOI: 10.1038/s41467-018-04959-2

OPEN

Embryos and embryonic stem cells from the white rhinoceros

Thomas B. Hildebrandt[1,2], Robert Hermes[1], Silvia Colleoni[3], Sebastian Diecke[4,5], Susanne Holtze[1], Marilyn B. Renfree[6], Jan Stejskal[7], Katsuhiko Hayashi[8], Micha Drukker[9], Pasqualino Loi[10], Frank Göritz[1], Giovanna Lazzari[3,11] & Cesare Galli[3,11]



TAKE HOME MESSAGE

- ET, OPI-ICSI, cloning and embryo cryopreservation have reached efficiency that allows clinical application
- Main indications:
  - Female or male subfertility
  - ‘multiply genetics’
- ART in the horse presents a valuable model for conservation of genetics in (endangered) breeds/species

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BELGIUM
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[REFERENCES]

- A retrospective comparison of the efficiency of different assistedreproductive techniques in the horse, emphasizing the impact ofmaternal ageJuan Cuervo-Arango*, Anthony N. Claes, Tom A. Stout. 2019. Theriogenology 132, 36-44
- [Fernando L.Riera[a]Jaime E.Roldán[a]JoséGomez[a]KatrinHinrichs] 2016Factors affecting the efficiency of foal production in a commercial oocyte transfer program. Theriogenology 85 1053-1062

MILESTONES IN EQUINE EMBRYO TRANSFER

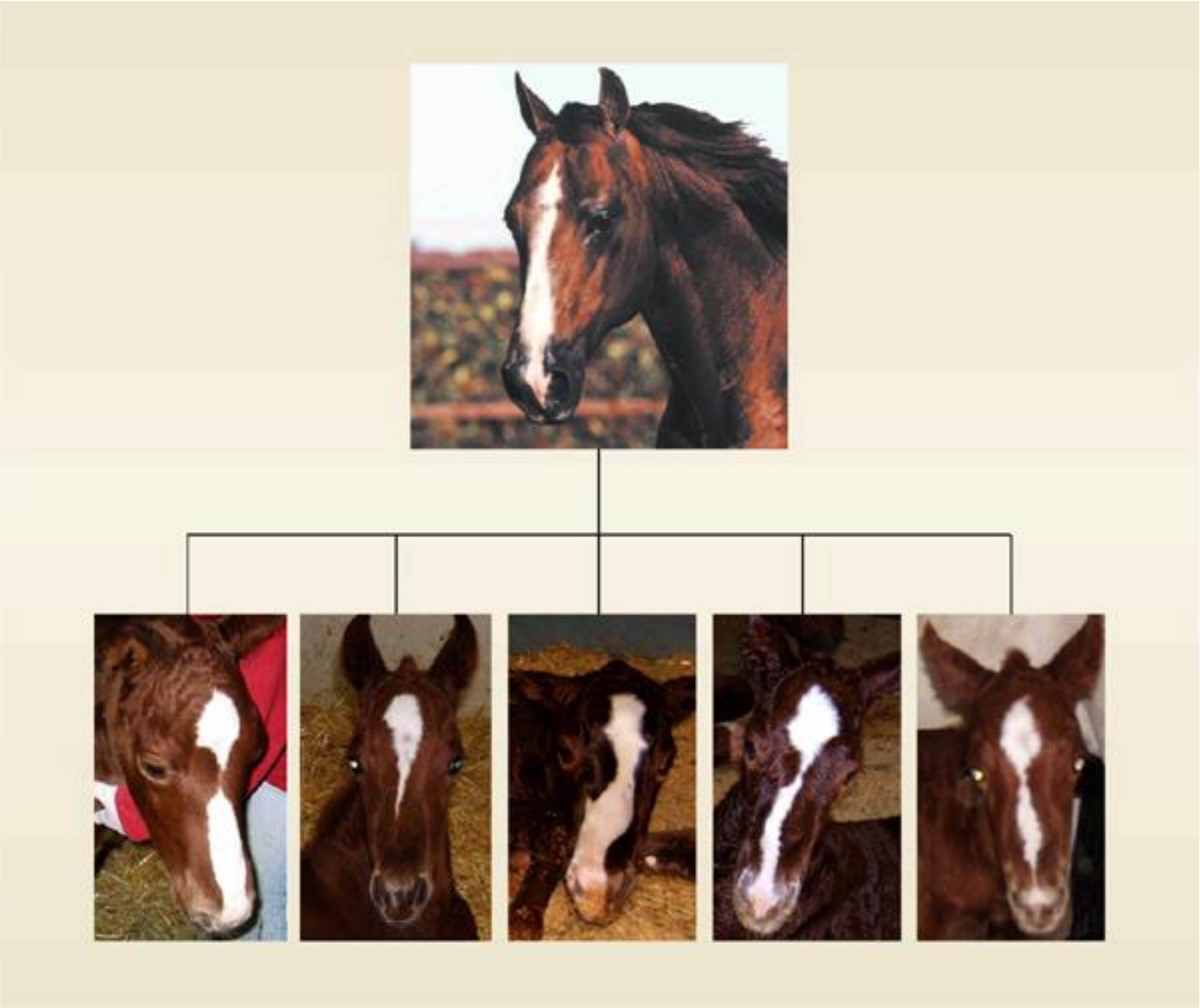
- 1974 The first foal produced by embryo transfer was born (Japan) 2
- • 1974 First report of successful superovulation of mares (Wisconsin)
- • 1976 Long-distance transport of equine embryos first reported (England)
- • 1982 Foal born following transfer of a frozen-thawed embryo (Japan)
- • 1984 Production of twins following bisection of an equine embryo (Colorado)
- • 1987 Technique for successful cooling of equine embryos reported (Colorado)
- • 1988 Birth of first foal following gamete intrafallopian transfer (Colorado)
- • 1991 Birth of first foal produced by in vitro fertilization (France)
- • 1996 First foal produced from intracytoplasmic sperm injection (Colorado)
- • 2002 Report of 2 foals born following transfer of vitrified oocytes (Colorado)
- • 2003 Birth of a mule foal produced by cloning (Idaho)
- • 2003 Birth of first horse foal produced by cloning (Italy)

Table 1. Some of the main breeds of horses cloned reported until 2016

Breed	Reference
Criollo Argentino	Gambini *et al.* (2012)
Polo pony	Gambini *et al.* (2014), Olivera *et al.* (2016), Kheiron[A], Crestview Genetics[A]
Haflinger	Galli *et al.* (2003), Lagutina *et al.* (2005)
Warmblood	Lee *et al.* (2015)
Arabian horse	Lagutina *et al.* (2005), Crestview Genetics[A]
Thoroughbred	Kheiron[A]
Jumping horse	Gambini *et al.* (2014), ViaGen[B], Kheiron[A]
Paso colombiano	GenesCol[A]
Paso fino	ViaGen[B]
Cutting horse	ViaGen[B]
Mangalarga marchador	*In vitro* Brasil Clonagem[A]
Campolina	*In vitro* Brasil Clonagem[A]
Bucking horse	ViaGen[B]
Barrel racing horse	ViaGen[B]
Quarter horse	ViaGen[B]

[A]Personal communication, June 2017.
[B]See 'Our Equine Client Stories' at http://www.viagen.com, accessed 8 October 2017.

Gambini & Maserati 2018



Equine Veterinary Journal

Equine Veterinary Journal ISSN 0425-1644
DOI: 10.1111/evj.12839

50 YEARS 1968-2018

Review Article: Celebrating 50 years of Equine Veterinary Journal

The development of in vitro embryo production in the horse

L. H. A. MORRIS

EquiBreed NZ Ltd, Te Awamutu, New Zealand.

Correspondence email: lee@equibreed.co.nz; Received: 16.09.17; Accepted: 22.03.18

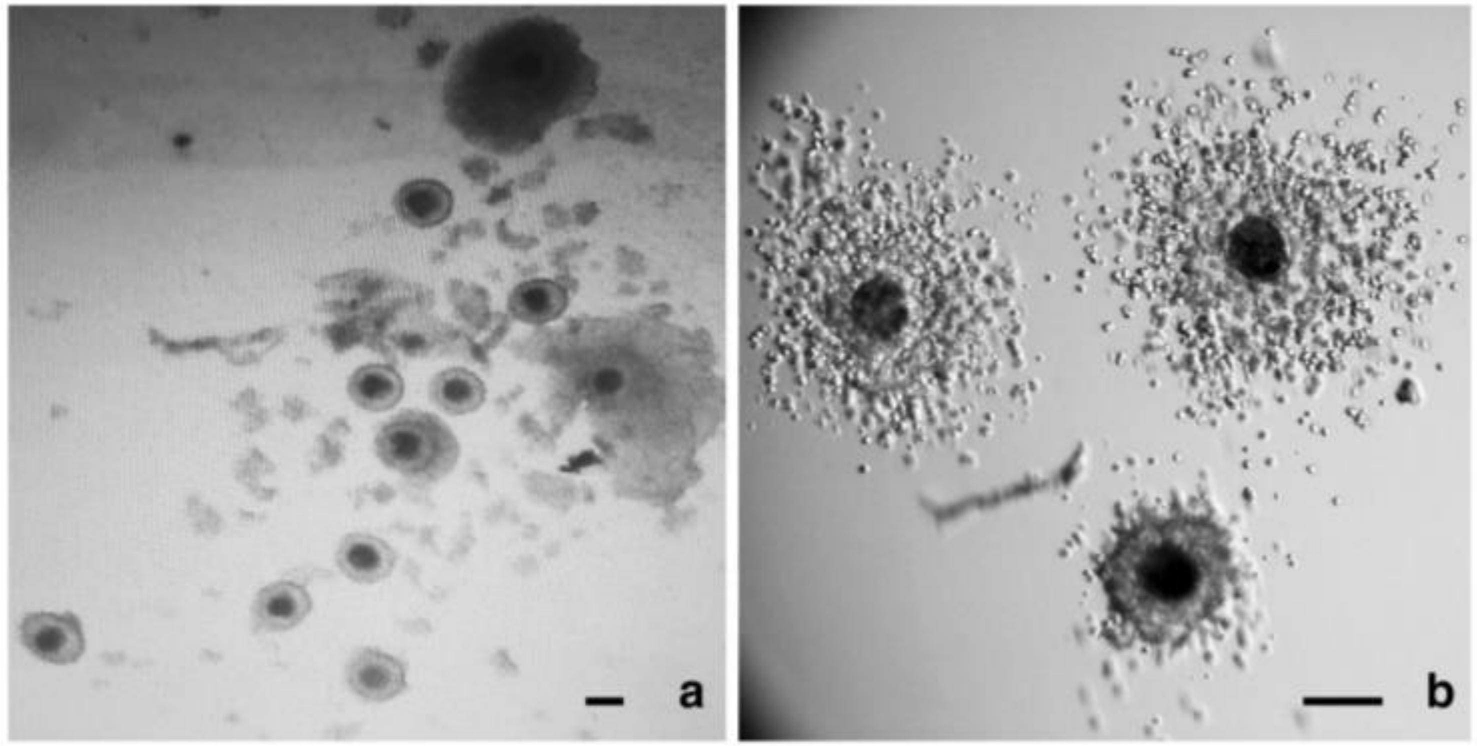


Fig. 1 Appearance of COCs on their initial recovery from immature follicles of the donor mare (**a**), showing the compact cumulus, typically three to four layers; and on recovery from the dominant stimulated follicle 30 h after IFOT (**b**) showing varying levels of cumulus expansion. Bar = approximately 150 µm

1978

1992 Steptoe and Edwards

Palermo et al.

1981-1986

1996-2001

1991