Institut National de la Recherche Agronomique

Centre Val de Loire

Genotyping and cryopreservation of equine embryos:

New developments

Florence GUIGNOT

INRA, PRC, F-37380 Nouzilly, France

64th Annual meeting of the European federation of animal science

Nantes, France August 26-30, 2013

Genotyping

- Definition:

  ☞ process of determining the genetic constitution (genotype) of an individual by examining their DNA sequence

- Application: PGD Preimplantation genetic diagnosis

  ☞ Refers to genetic status of embryo prior to implantation

  ☞ Identifies several genes, related to:

  * specific genetic disease,
  * sex,
  * coat colour,
  * sporting capacity .... etc

PGD methods

- Biopsy:

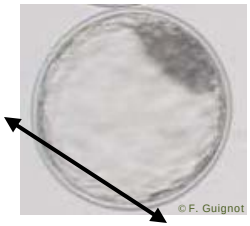


Figure 1 Biopsy by section of bovine blastocyst (Day 7)

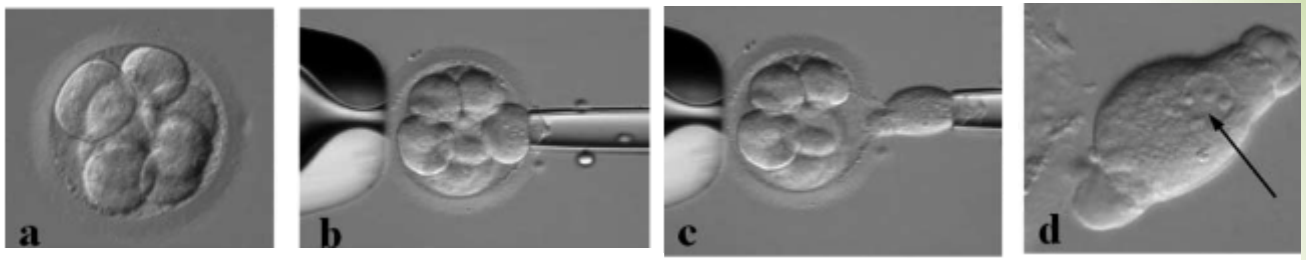


Figure 2 Blastomere biopsy of a human cleavage-stage embryo. (a) Eight-cell embryo, day 3 postfertilization; (b) embryo on holding pipette (left), with biopsy pipette (right) breaching the zona pellucida; (c) blastomere removal by suction; (d) biopsied blastomere with a clearly visible single nucleus (indicated by arrow).

Ogilvie et al, 2005

- Whole genome amplification
  - ☞ large amount of DNA is available
- Polymerase chain reaction (PCR)
  - ☞ to amplify gene(s) of interest

PGD in equine: in the past

Early blastocysts (<300 µM), biopsy with microblade, fresh transfer, sex determination

- First report: Huhtinen et al, 1997 (n=14)
  - ☞ 3 pregnancies (21%), 2 live female foals as predicted

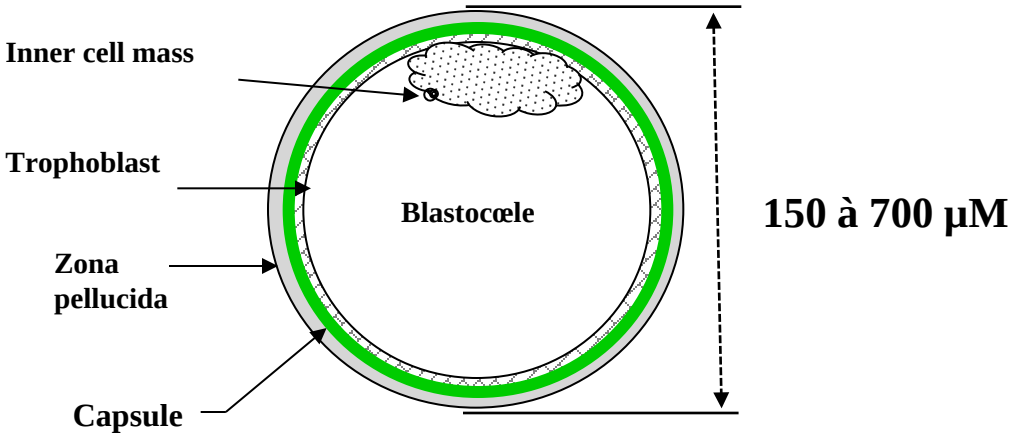
- Following reports:

Seidel et al, 2010 (n=15)
- ☞ 6 pregnancies at DG 14 (40%)

Guignot et al, 2013 (n=11)
- ☞ 2 pregnancies, DG 14 (18%);
- ☞ efficiency and accuracy of sex diagnosis: 100% (n=50)

Equine embryo peculiarities

- Blastocyst size at collection (Day 7)
  - ☞ large and variable, compared to bovine/goat/ewe embryos



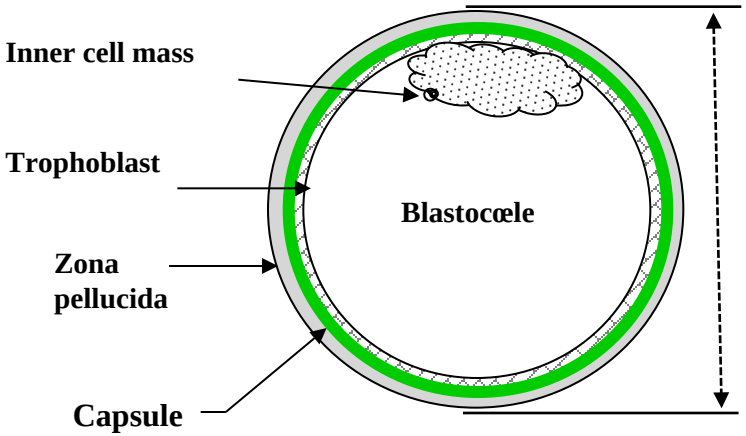
Equine blastocyst

- Capsule
  - ☞ acellular membrane, composed of mucine-like glycoprotein, present between Day 7 and Day 20

Equine embryo peculiarities

- Blastocyst size at collection (Day 7)

  ☞ large and variable, compared to bovine/goat/ewe embryos



Equine blastocyst

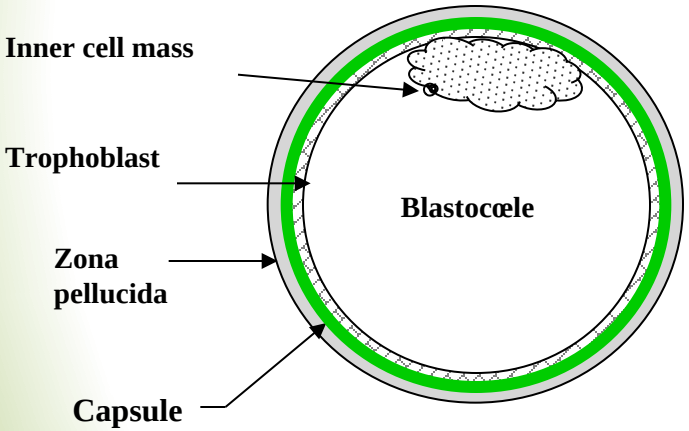
- Capsule

  ☞ acellular membrane, composed of mucine-like glycoprotein, present between Day 7 and Day 20

PGD in equine: new developments

- Biopsy by cell aspiration

  ☞ using a micropipette attached to a Piezo Drill



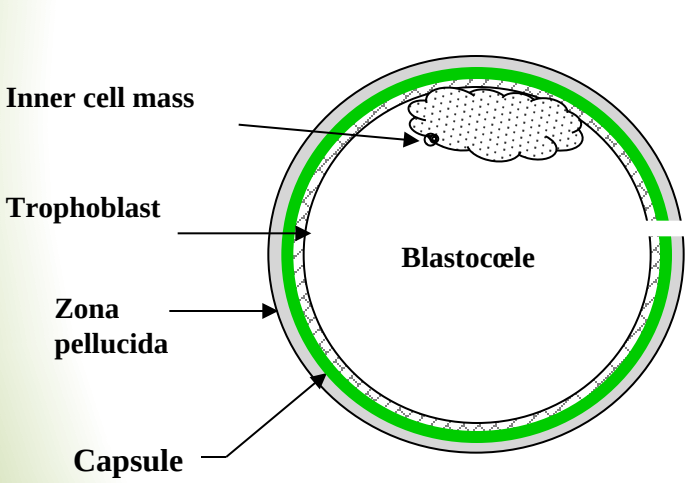
Equine blastocyst

piezo

PGD in equine: new developments

- Biopsy by cell aspiration

  ☞ using a micropipette attached to a Piezo Drill



Equine blastocyst

Illustration of cells aspiration

- Holding & aspiration pipettes

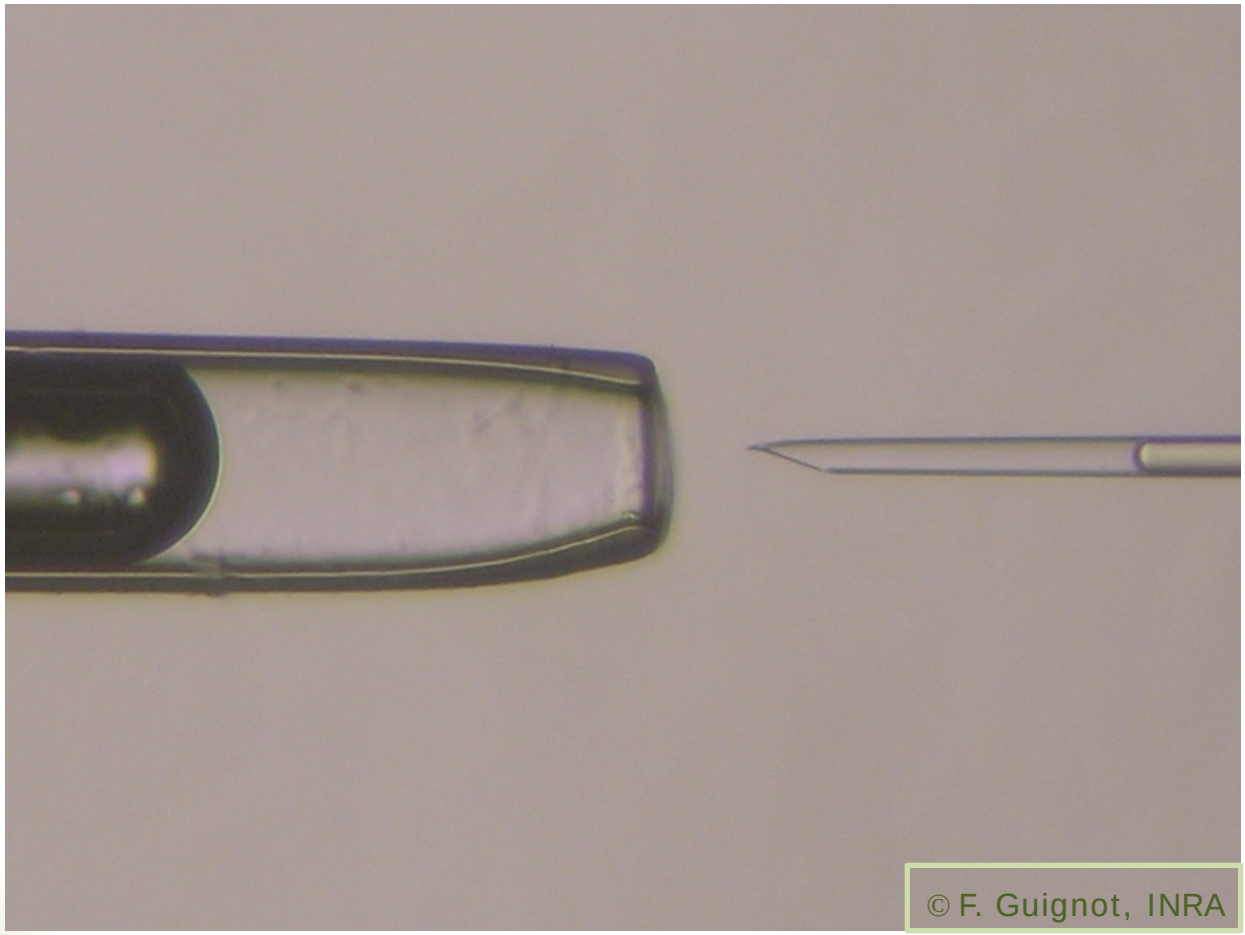


Illustration of cells aspiration

- Equine blastocyste (388 µM, Day 7)

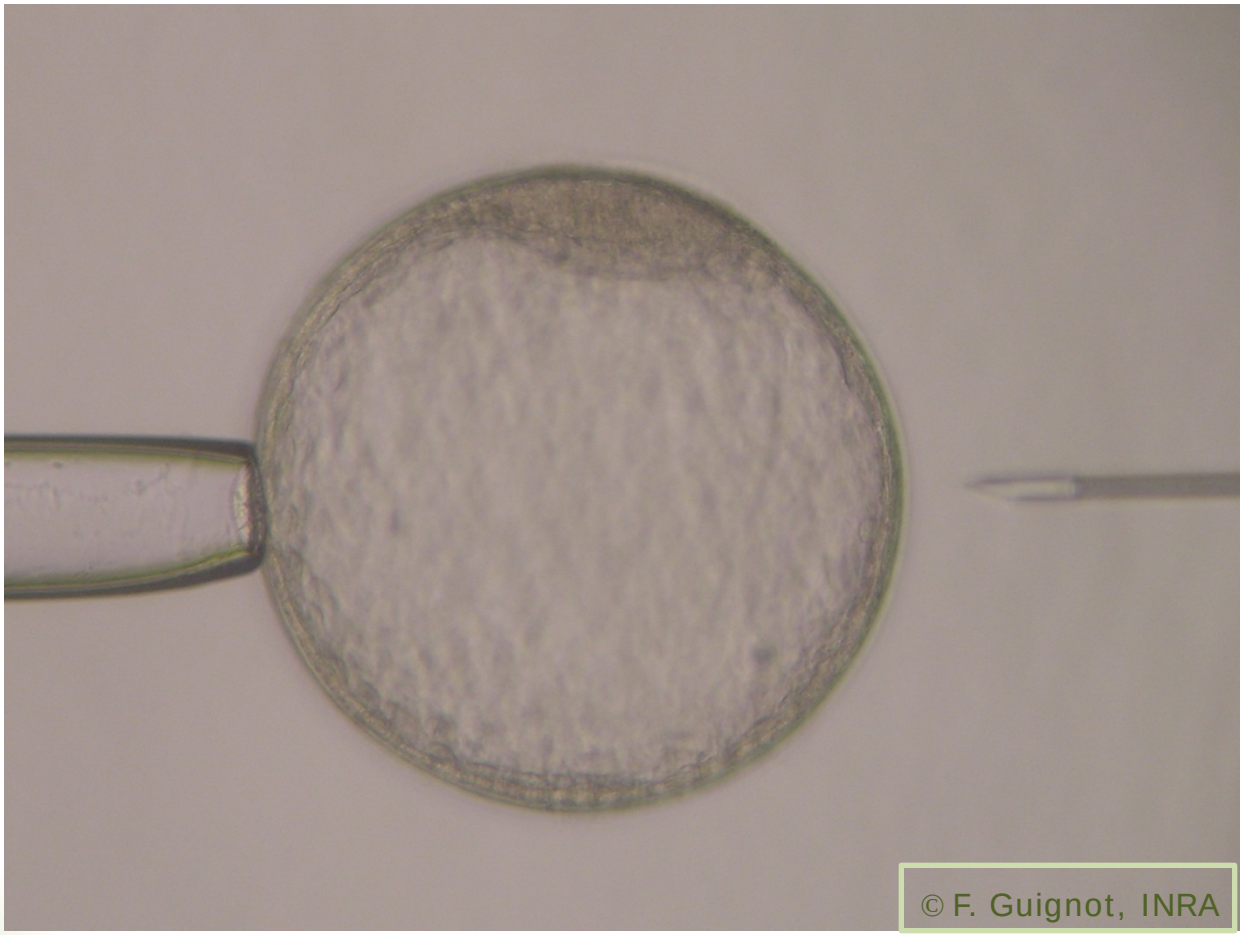


Illustration of cells aspiration

- Introduction of aspiration pipette

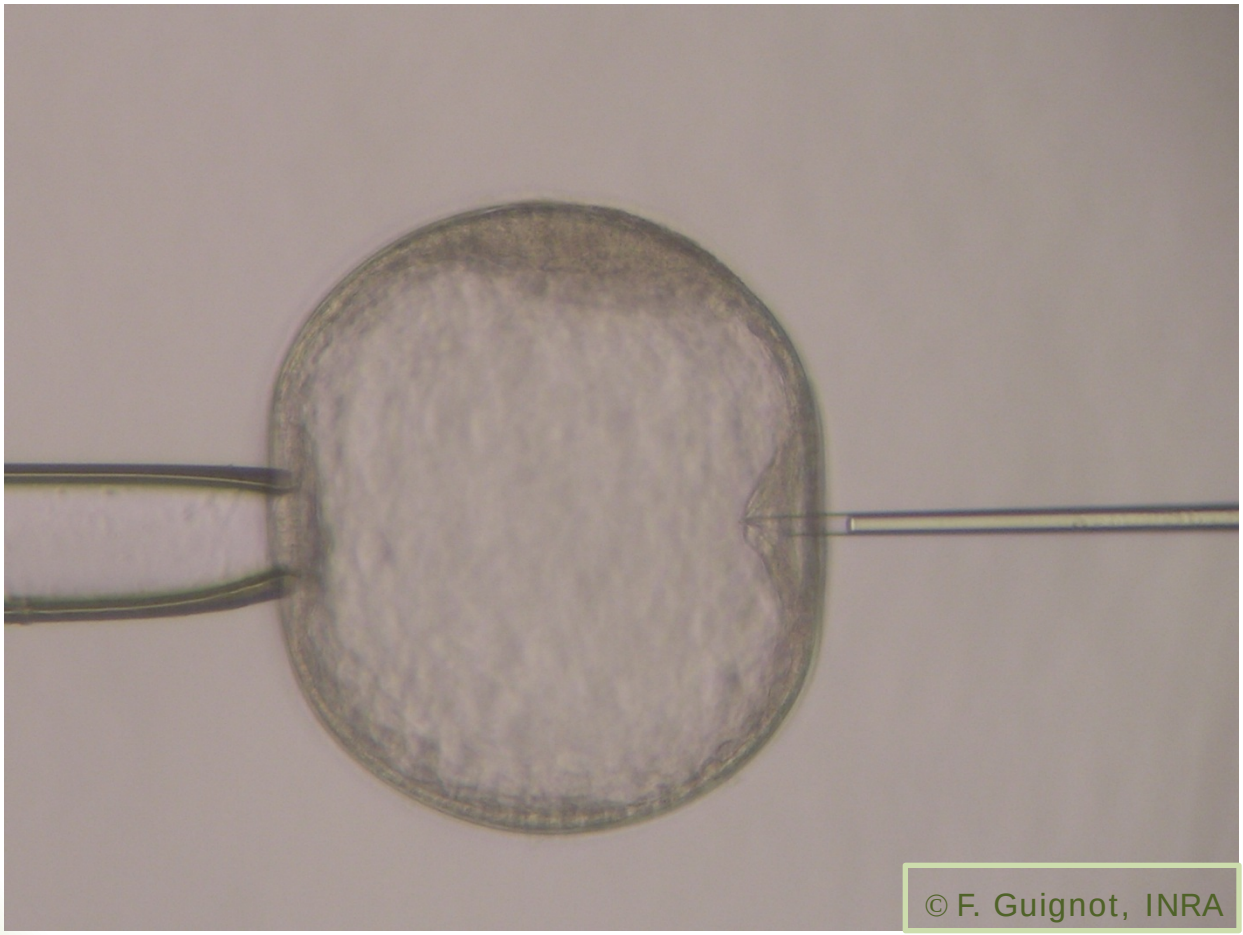


Illustration of cells aspiration

- Aspiration pipette inside the blastocoele

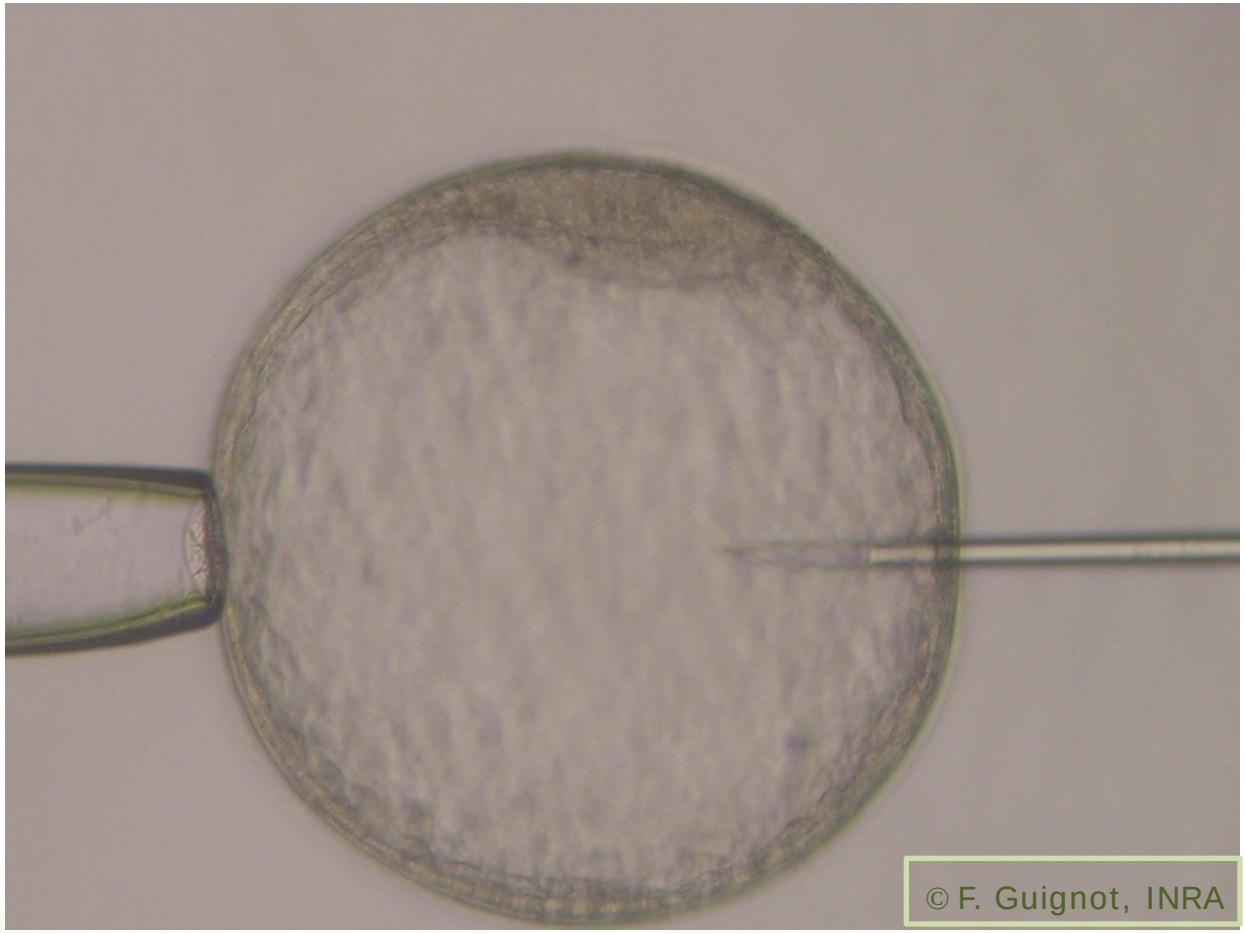


Illustration of cells aspiration

- Cells aspiration

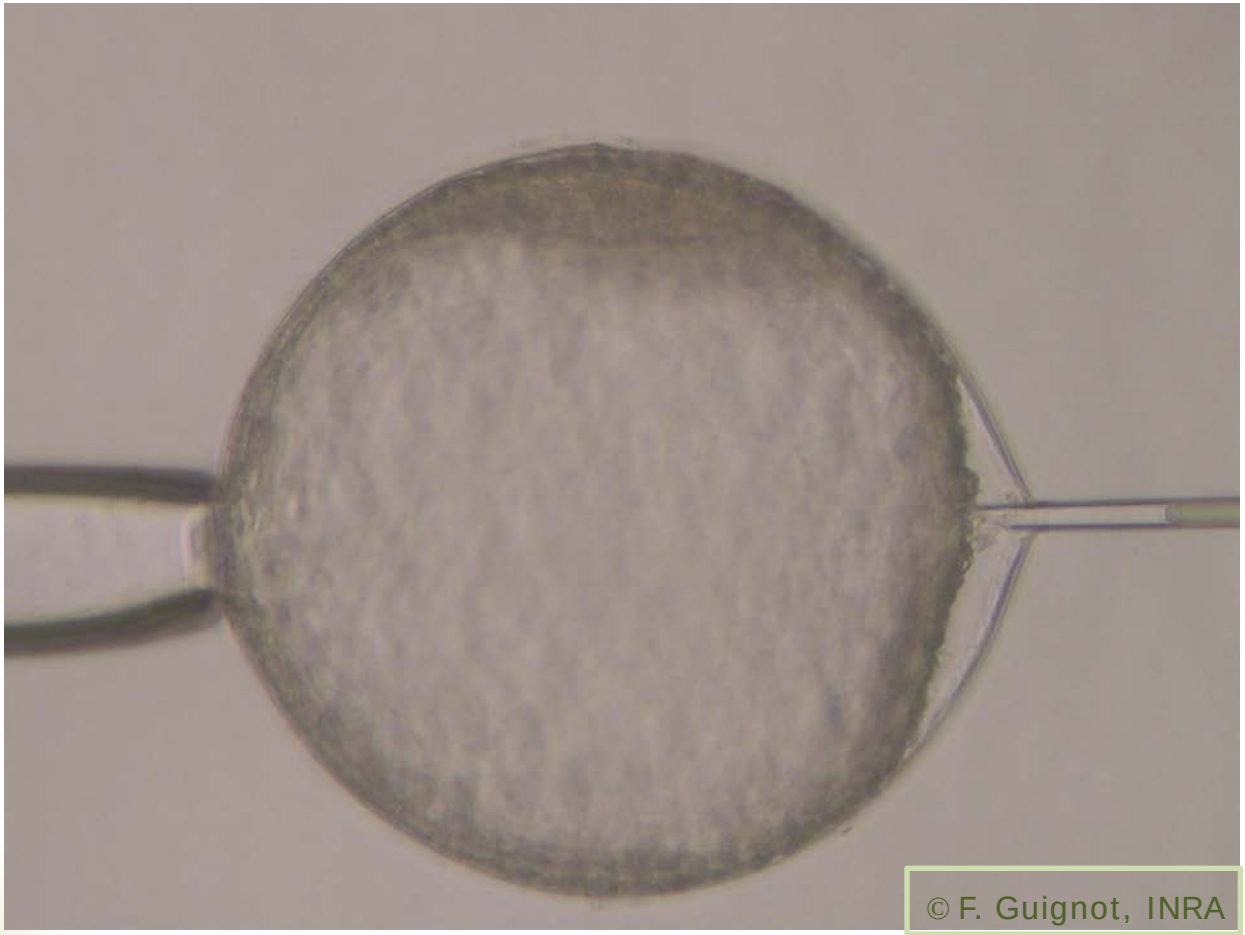
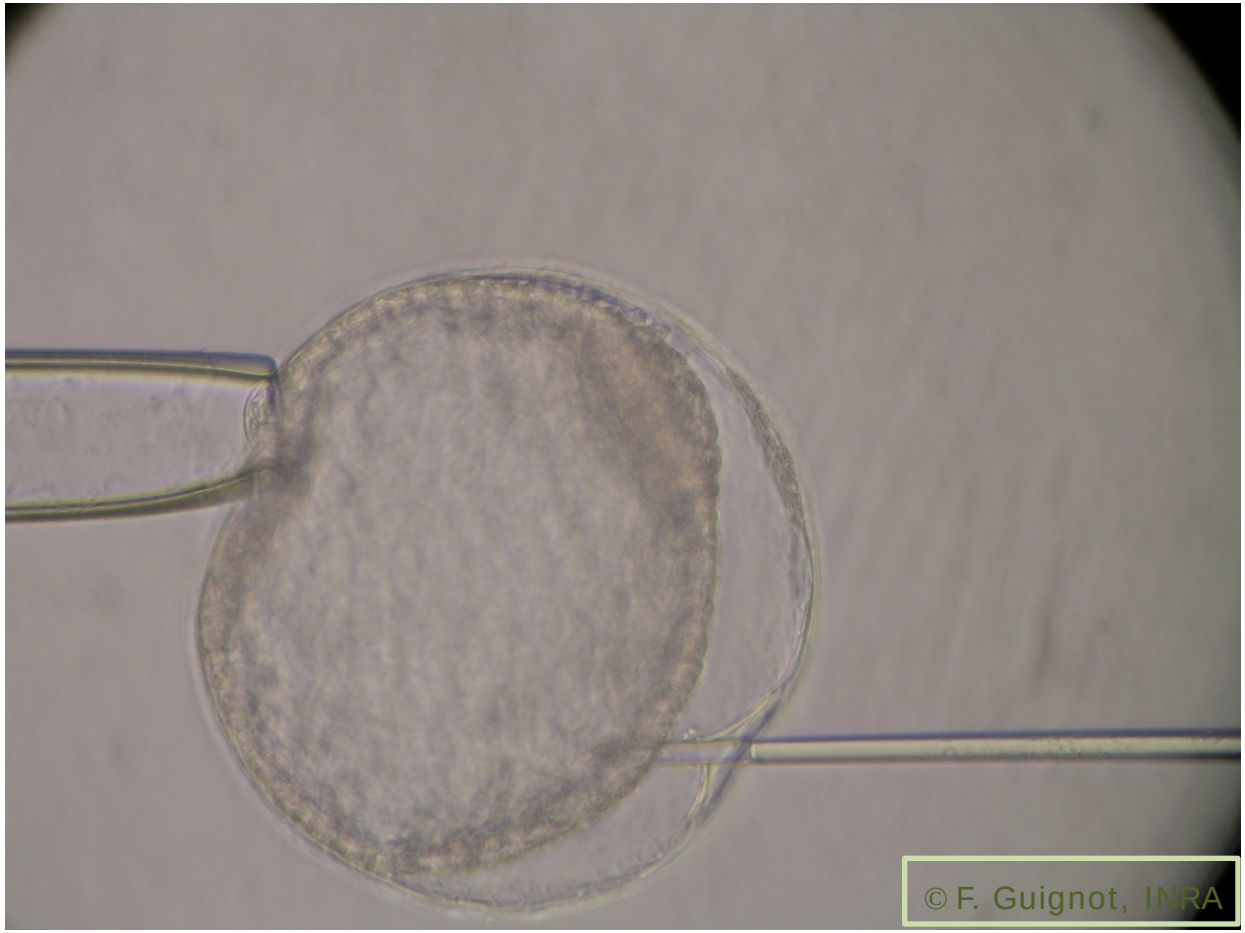


Illustration of cells aspiration

- Blastocoele contraction



PGD in equine: new developments

- Result of pregnancy
  - ☞ fresh transfer

DG16: 75% (n=8, <300 µM) 29% (n=7, >300 µM)	Seidel et al, 2010
Heartbeat: 37.5% (n=8, < & >300 µM)	Guignot et al, 2012
Birth: 25% (n=12, >300 µM)	Choi et al, 2010

- Genotyping efficiency & accuracy

☞ Sex	100% (n=8) & 100% 82% (n=28) & 100%	Choi et al, 2010 Guignot et al, 2013
☞ SCN4A [1]	75% (n=8) & 100%	Choi et al, 2010
☞ PPIB [2]	87% (n=8) & 100%	

[1] responsible for hyperkalemic periodic paralysis (Rudolph et al, Nature Genetics, 1992)
[2] responsible for equine regional dermal asthenia (Tryon et al, Genomics, 2007)

PGD in equine: in the future

- Other genes of interest to test on embryo biopsy

☞ hereditary diseases:

* JEB (skin disease: junctional epidermolysis bullosa) in draft horses

* HERDA (skin disease: dermal astenia) & GBED (glycogen branching enzyme deficiency) in Quarter horses

* SCID (lake of immune system) in Arabian horses

* CLCN1 (congenital myotonia; neuromuscular channelopathy affecting skeletal muscles)

☞coat color genetics

* Alezan / Tobiano / Sabino / Overo / Black/ Appaloosa / cream dilution /.....

☞ Others

* parental control

* DMRT3 gene in Trotters (control of limb movement)

Cryopreservation

- Definition:

  ☞ process of cooling & storing at very low temperature to maintain cells/tissues/organs viability.

- Application to embryo:

  ☞ >90% of water

  ☞ important membrane surfaces

  ☞ different developmental stages (2 cells / morulae / blastocysts)

  ☞ peculiarities: example in equine

  * capsule
  * large blastocoelic cavity

Cryop. in equine: in the past

- First birth: Yamamoto et al, 1982
  - ☞ 1 male foal (< 300µM, Day 6, slow freezing)

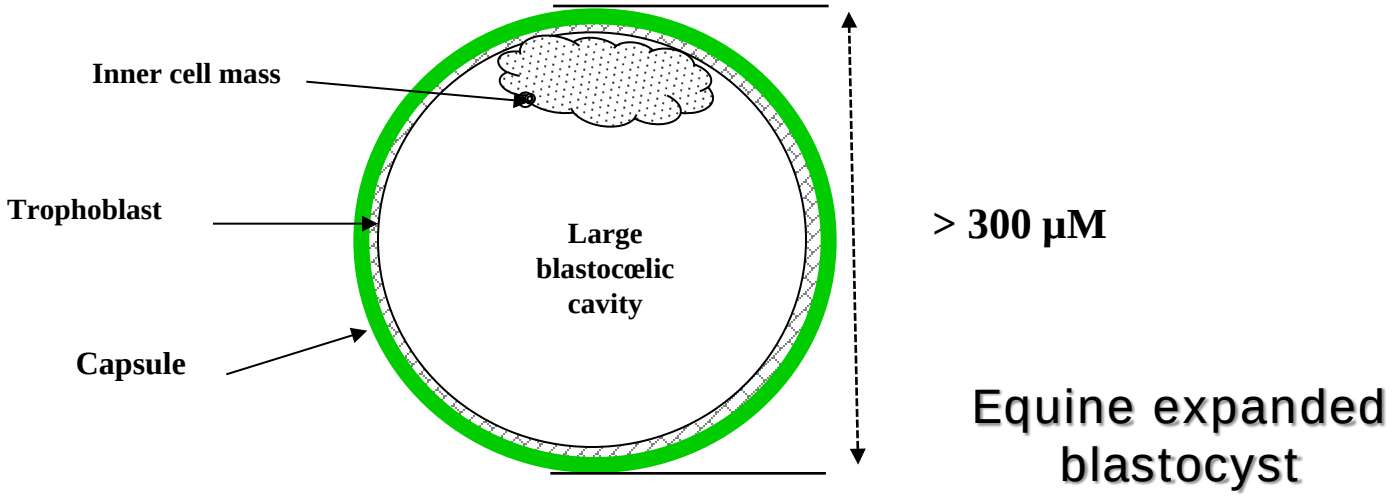
- Following reports: (1985-2009)

  < 300 µM
  - ☞ at DG 16: 60-80% of pregnancy (slow freezing & vitrification)
  - ☞ at DG 55/60: 55% (slow freezing) & 40% (vitrification)

  > 300 µM
  - ☞ at DG 16: 40-60% of pregnancy (slow freezing) & 0% (vitrification)
  - ☞ at DG 30/50: 10-14% (slow freezing)

>300 µM embryo peculiarities

- Large blastocoelic cavity
  - ☞ large amount of fluid: significant impact on embryo survival post-thaw



- Equine embryonic capsule
  - ☞ physical barrier between embryo and endometrium / cryoprotectants

Cryop. : new developments

- Piezo drill

  ☞ to facilitate the pipette penetration through capsule

- Aspiration of the blastocoelic fluid

  ☞ to reduce the blastocoelic volume before cryopreservation like in human (Vanderzwalmen et al, 2002)

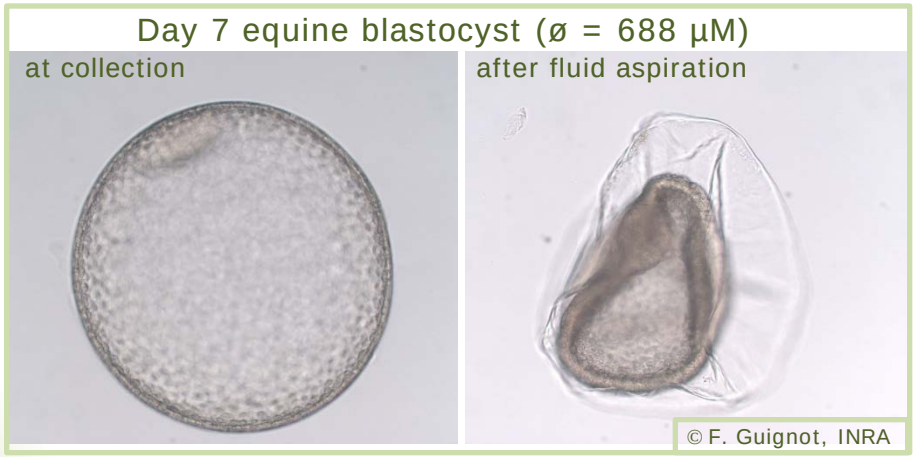


Illustration of fluid aspiration

- Glass pipette attached to a Piezo drill

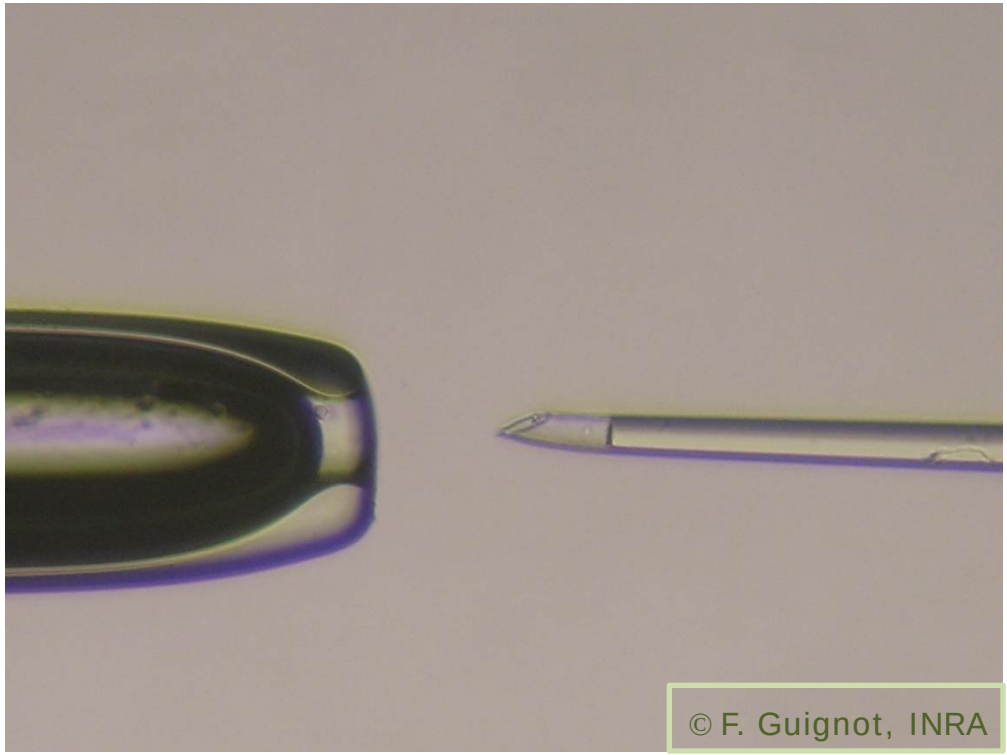


Illustration of fluid aspiration

- Introduction of aspiration pipette

Illustration of fluid aspiration

- Introduction of aspiration pipette

Illustration of fluid aspiration

- Introduction of aspiration pipette

Illustration of fluid aspiration

- Introduction of aspiration pipette

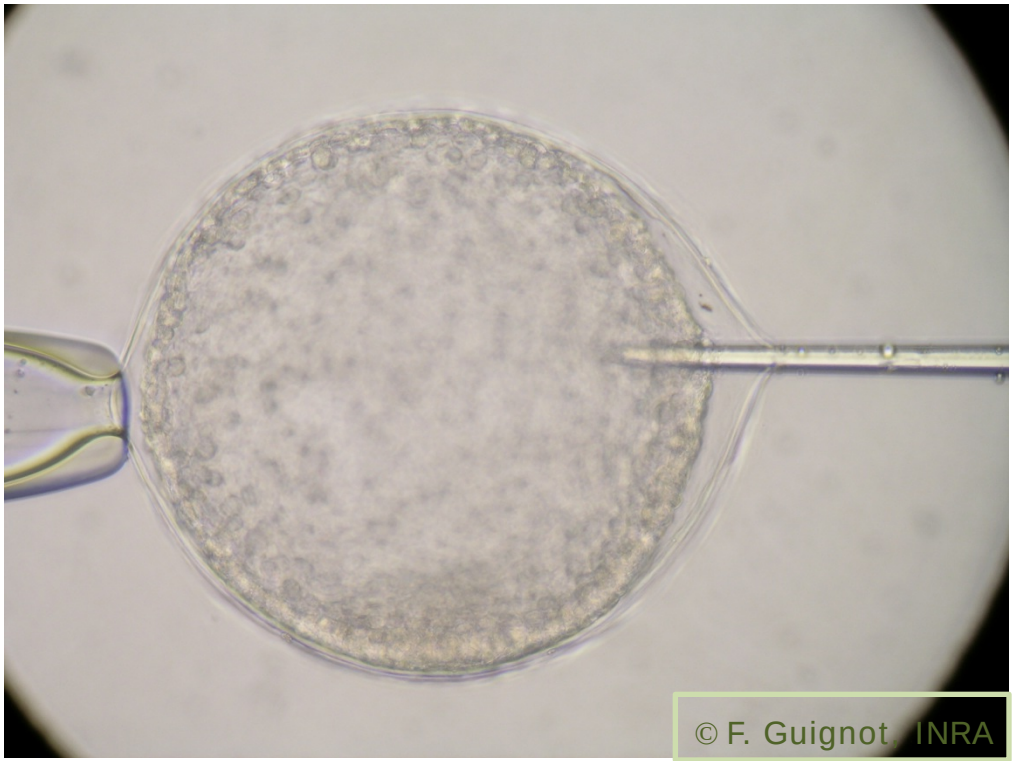


Illustration of fluid aspiration

Illustration of fluid aspiration

Illustration of fluid aspiration

Illustration of fluid aspiration

Illustration of fluid aspiration

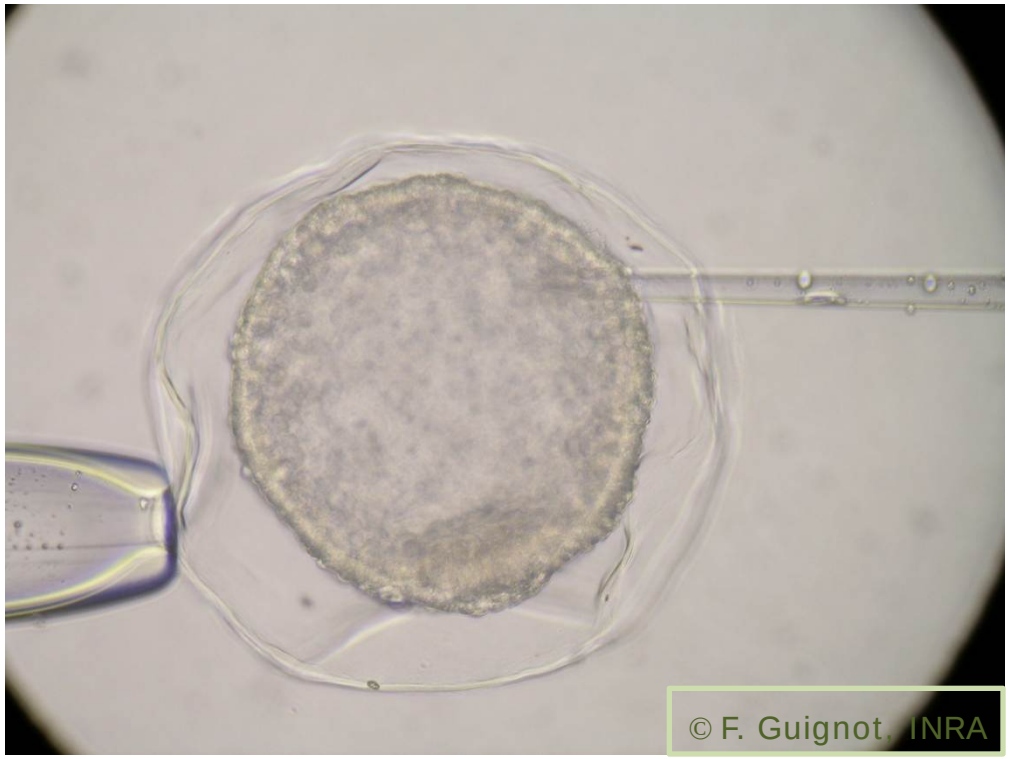


Illustration of fluid aspiration

Illustration of fluid aspiration

Illustration of fluid aspiration

Illustration of fluid aspiration

Cryop. : new developments

- Result of pregnancy

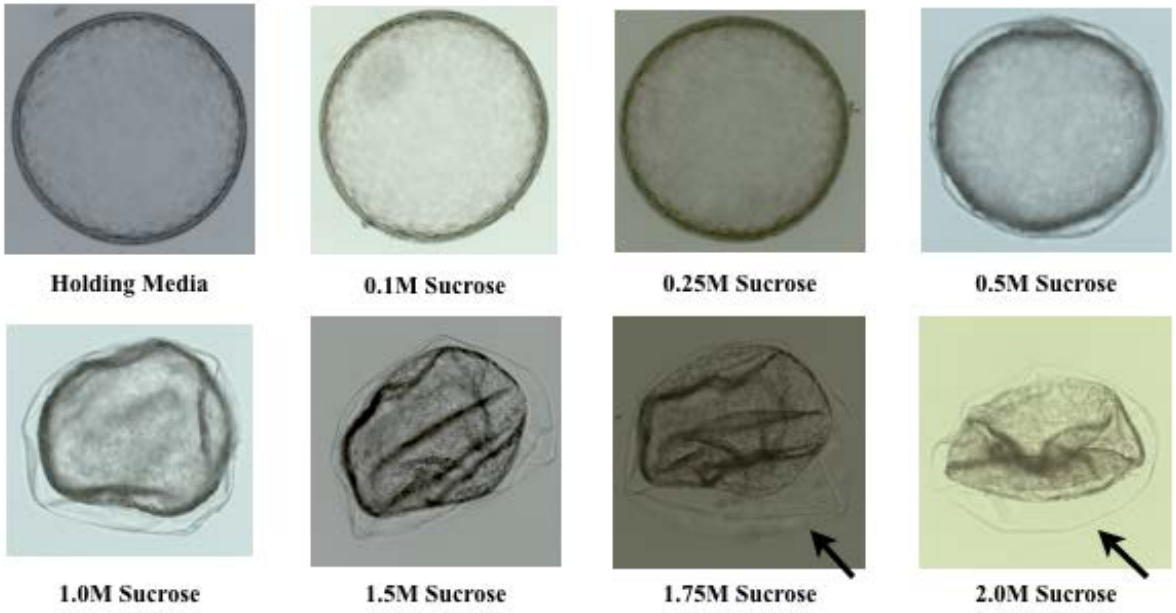
  ☞ embryo transfer post thaw

DG16: 47% (n=19, <300 µM)	Seidel et al, 2010
Heartbeat: 43% (n=7, < & >300 µM)	Reigner et al, 2013
71% (n=7, >300 µM)	Choi et al, 2011
Pregnancy in progress (>DG50): 57% (n=7) (<300 µM)	Guignot et al, 2013/14
Birth: 37% (n=8, <300 µM)	Troedsson et al, 2010

Cryop. in equine: in the future

- Easier process to collapse embryo
  - ☞ saccharide solutions

Image sequence of a 550 µM embryo dehydrating in sucrose at 21ºC.
(Arrows show where embryo folds and shape is irregular)



Brittany Foster, Thesis, University of Guelph, 2012

Conclusions

- Genotyping & cryopreservation in equine

* exciting new approachs

(Piezo drill & blastocoele collapsing)

☞ especially for expanded blastocyst

* widespread clinical use ?

☞ procedure should be simplified