

Oocytes and Assisted Reproductive Technologies (ARTs) in the horse

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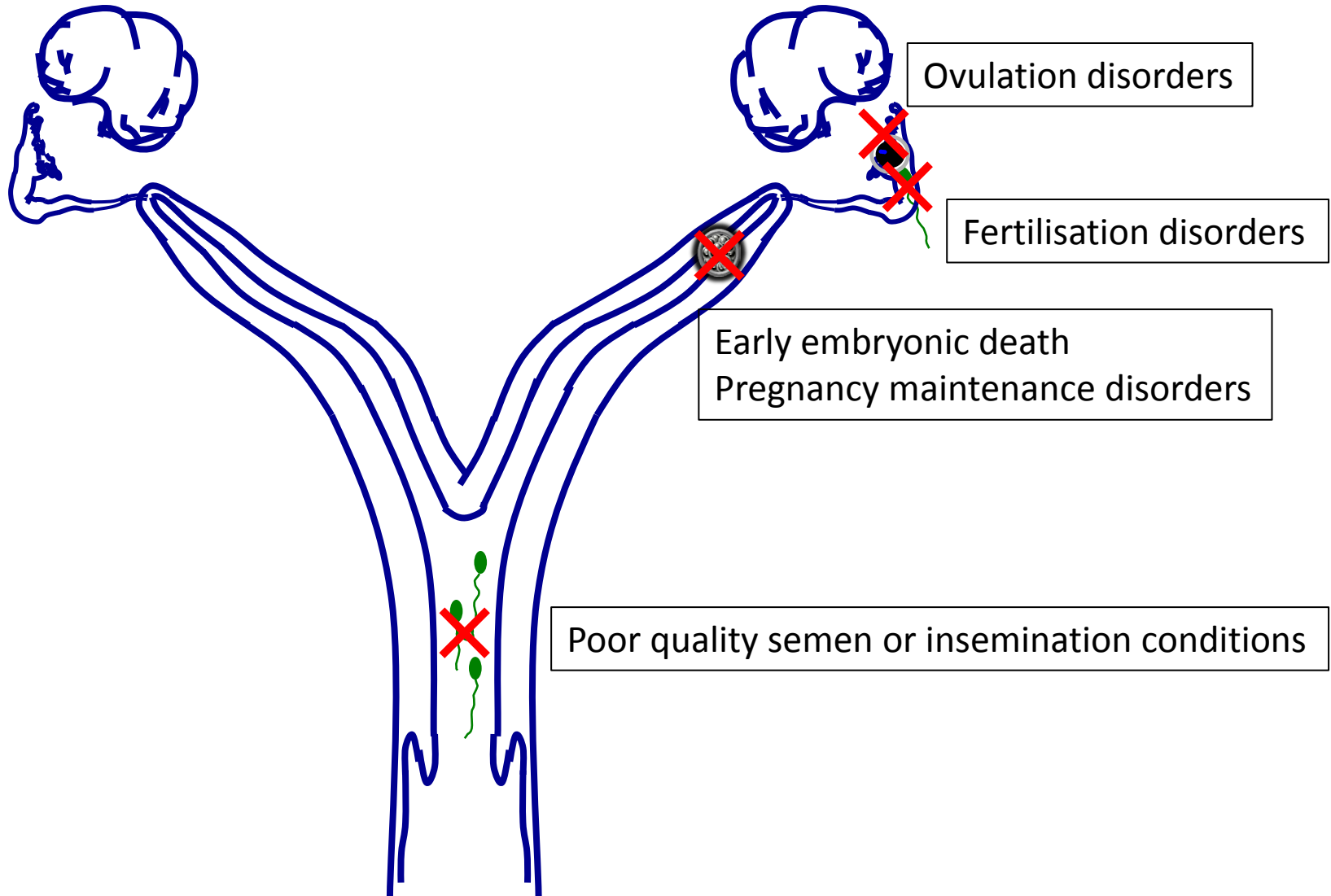
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Plan

- Introduction
- Oocyte collection, evaluation maturation
- Use of oocytes
 - Intra-follicular oocyte transfer
 - Intra-oviductal oocyte transfer
 - Intra-cytoplasmic sperm injection (ICSI)
 - Nuclear transfer (cloning)
- Conclusion

Introduction



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Oocyte collection ex vivo

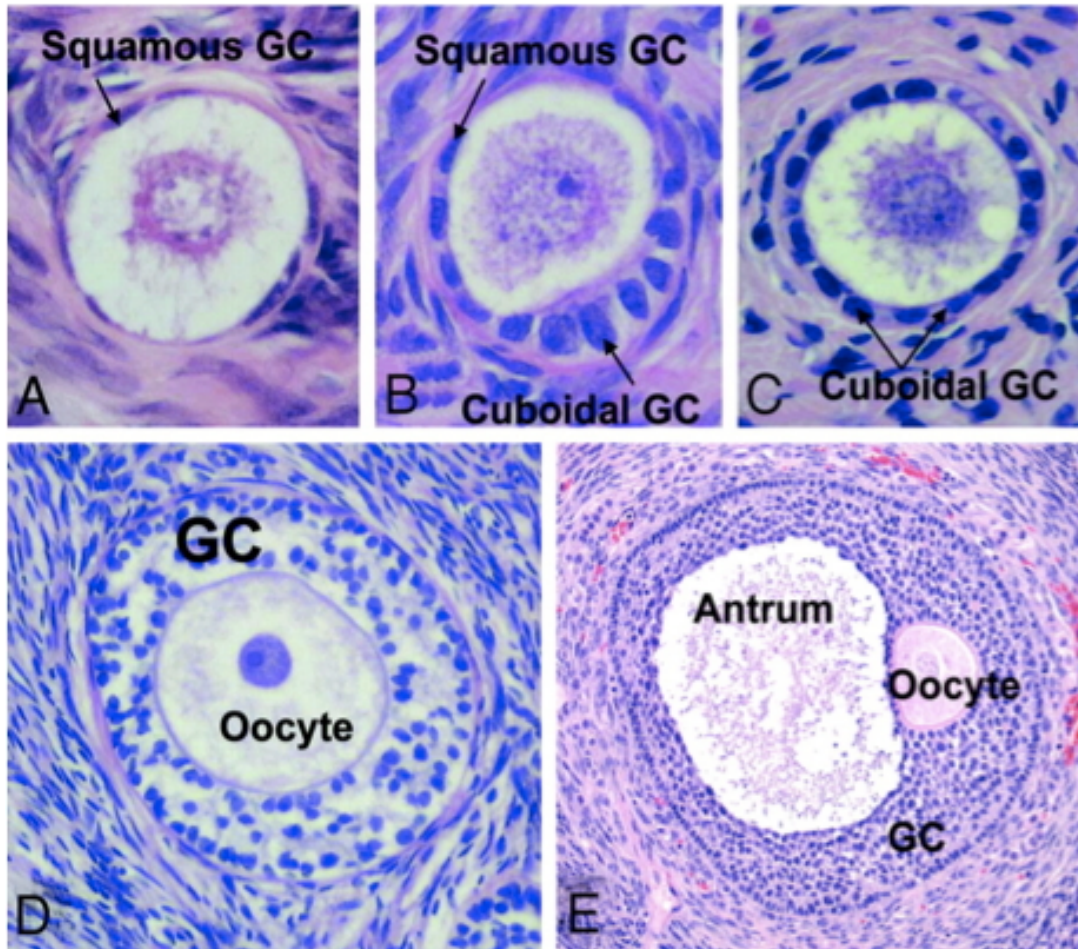
- Handling and transport of ovaries
 - excised ovaries kept in PBS, or saline+ antibiotics
 - temperature 20 to 30°C => ok for up to 3 hours



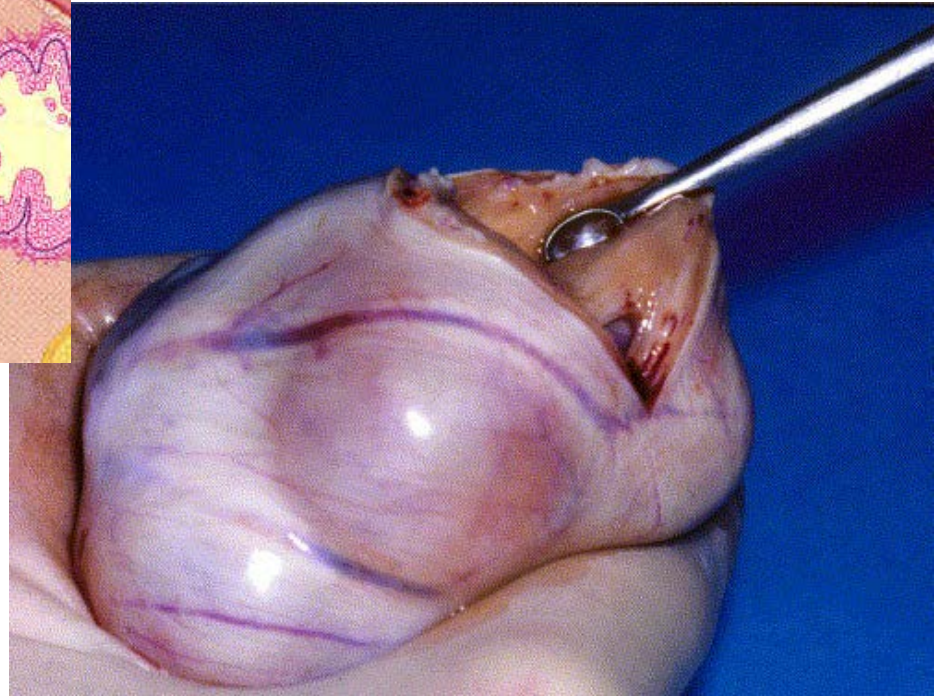
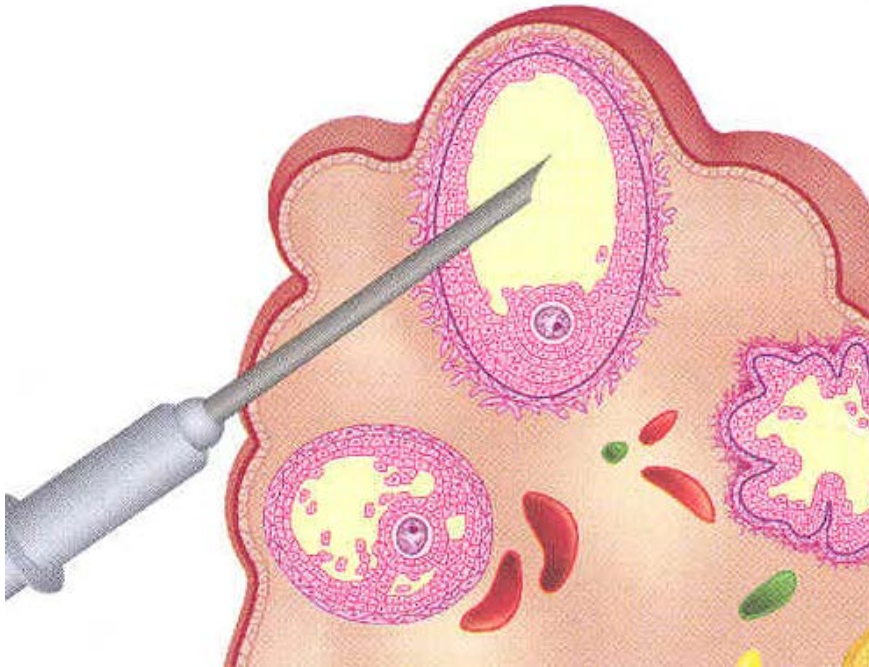
Oocyte collection ex vivo



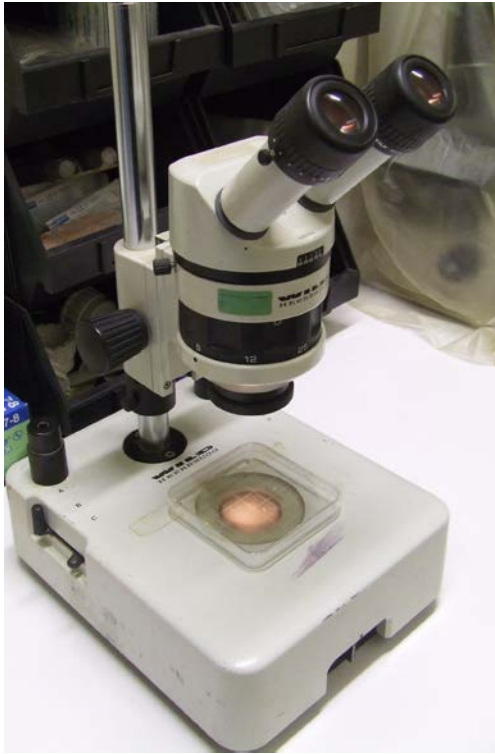
Oocyte collection ex vivo



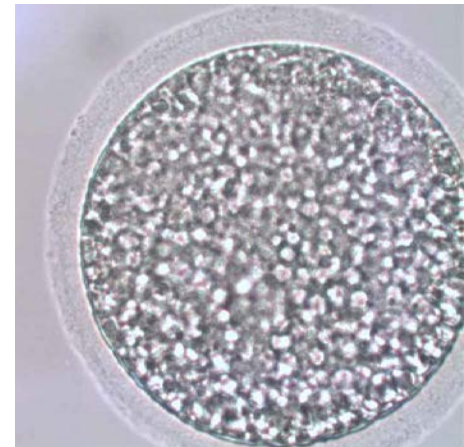
Oocyte collection ex vivo



Oocyte collection ex vivo



- Oocyte retrieval under stereomicroscope
- Average 6 follicles present per ovary
- Recovery rate 3-5 oocytes/ovary
- Heterogenous population of oocytes
- Time consuming !



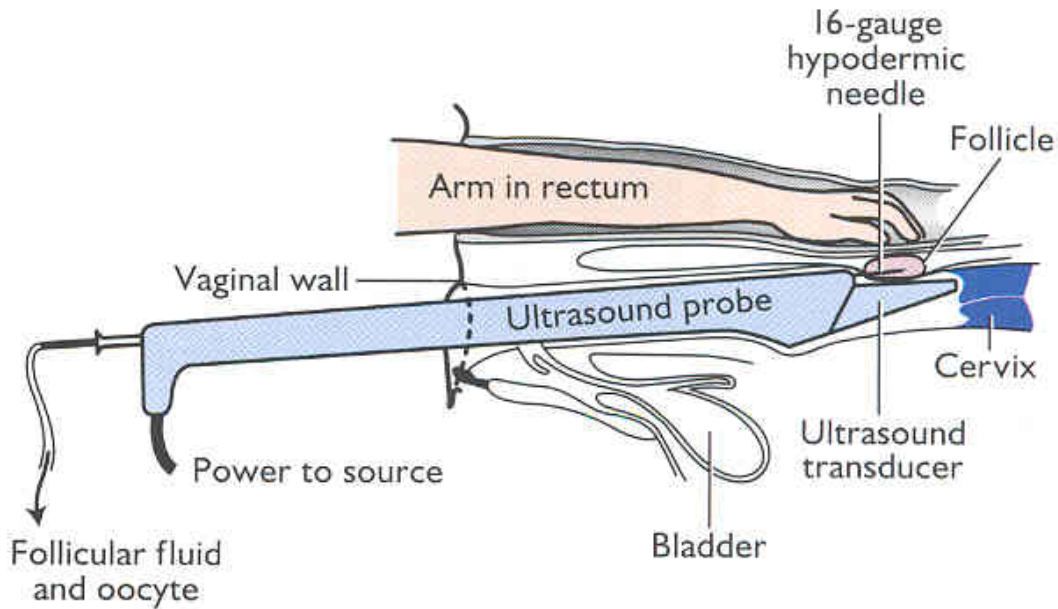
Oocyte collection in vivo

- Direct puncture through the flank
- Only for large (preovulatory) follicles



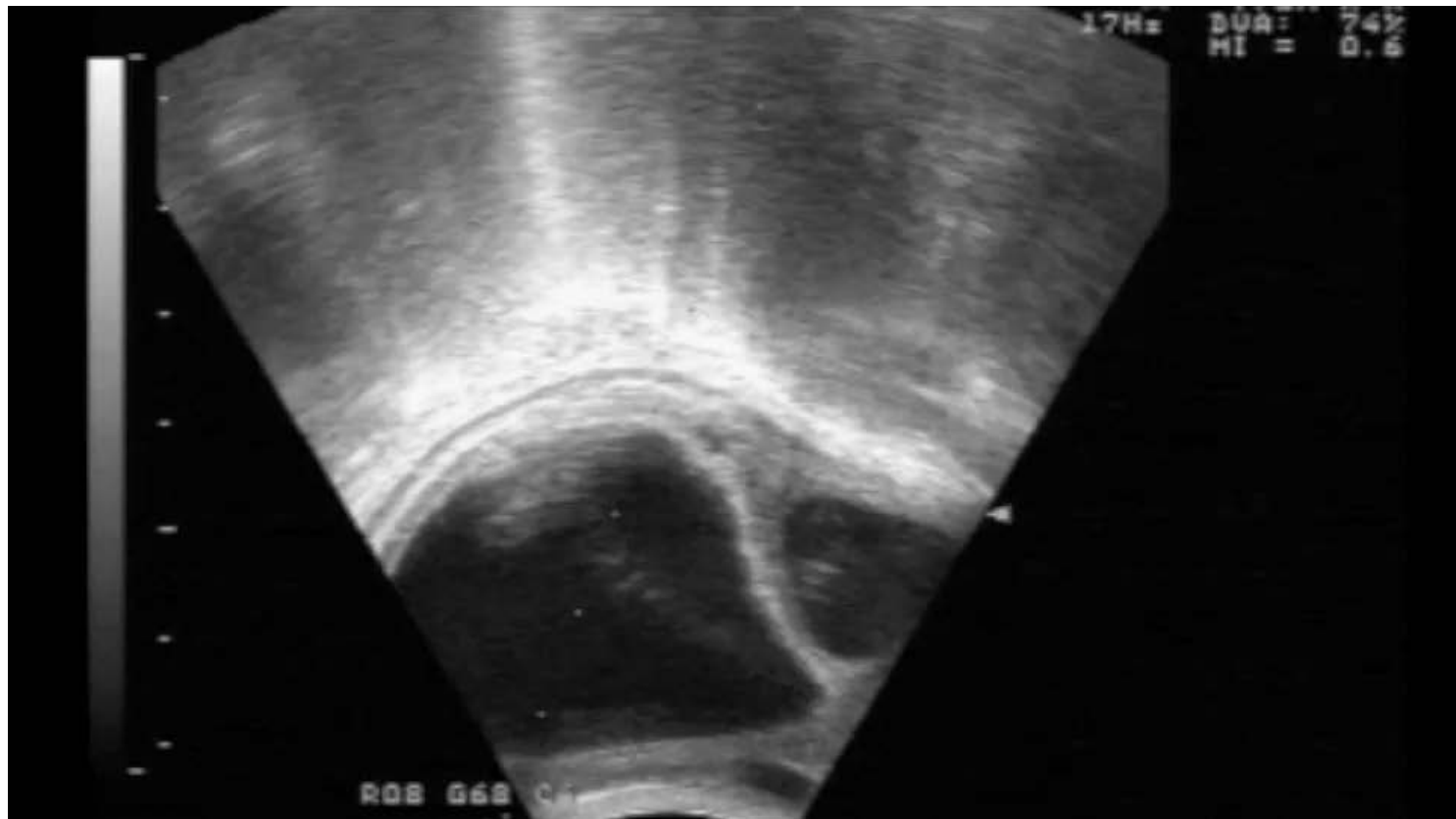
Oocyte collection in vivo

- Collection *in vivo* : Ultrasound guided aspiration or Ovum pick up (OPU)

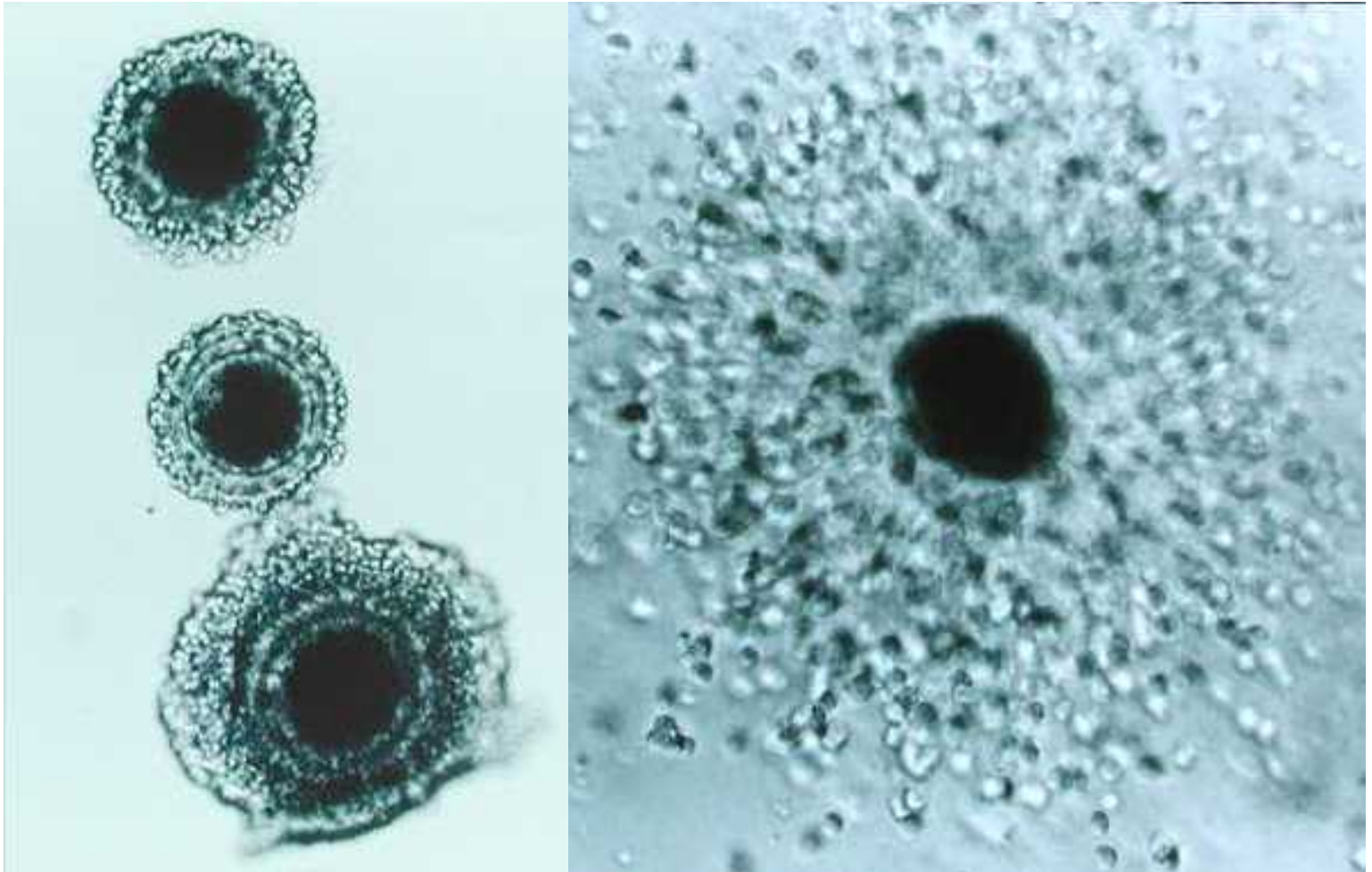


Senger, 2003

Oocyte collection in vivo

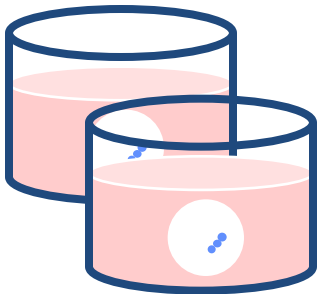


Oocyte collection in vivo



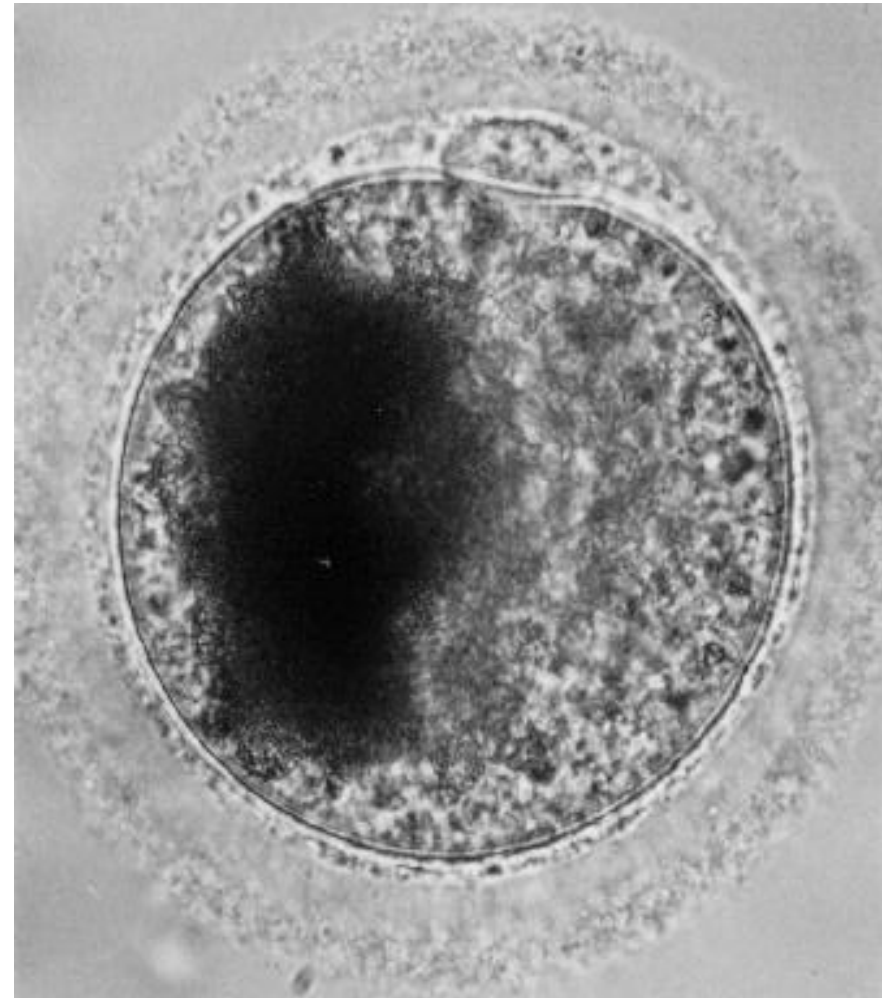
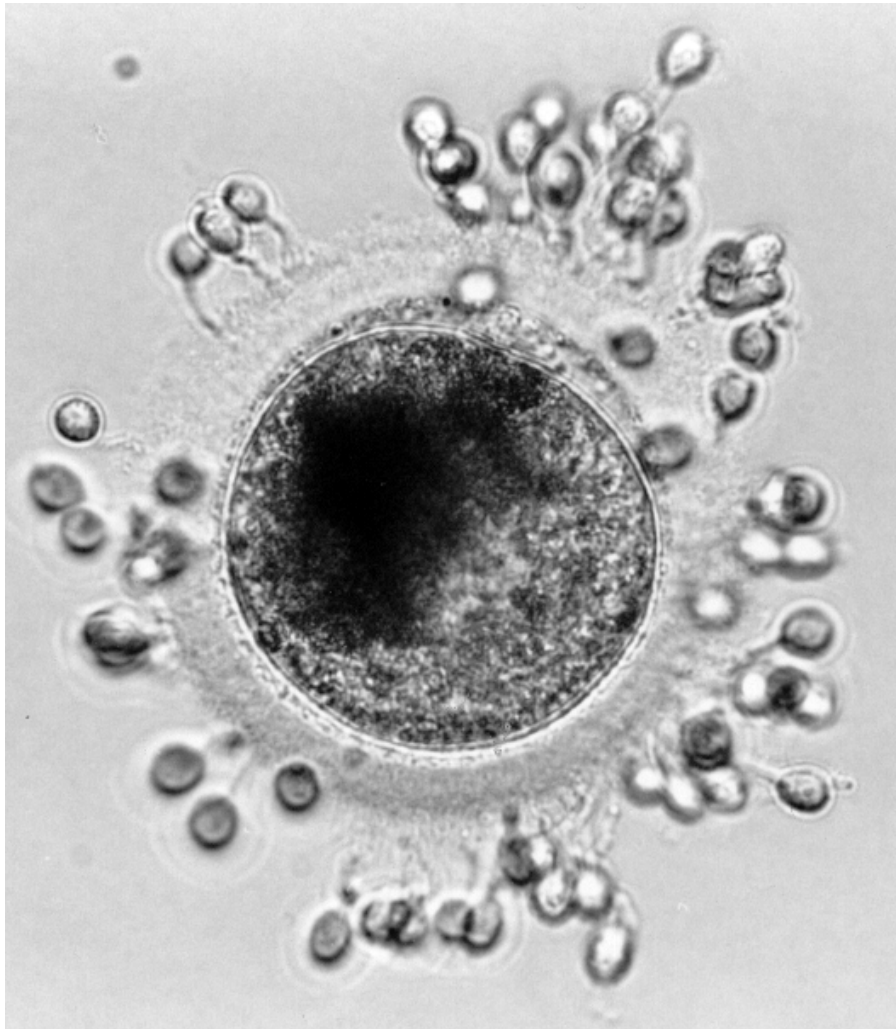
In vitro Maturation

500 μ l TCM199
+FCS + EGF
 \Rightarrow 30 hours
 \Rightarrow incubator
at 38,5 $^{\circ}$ C with 5% CO₂

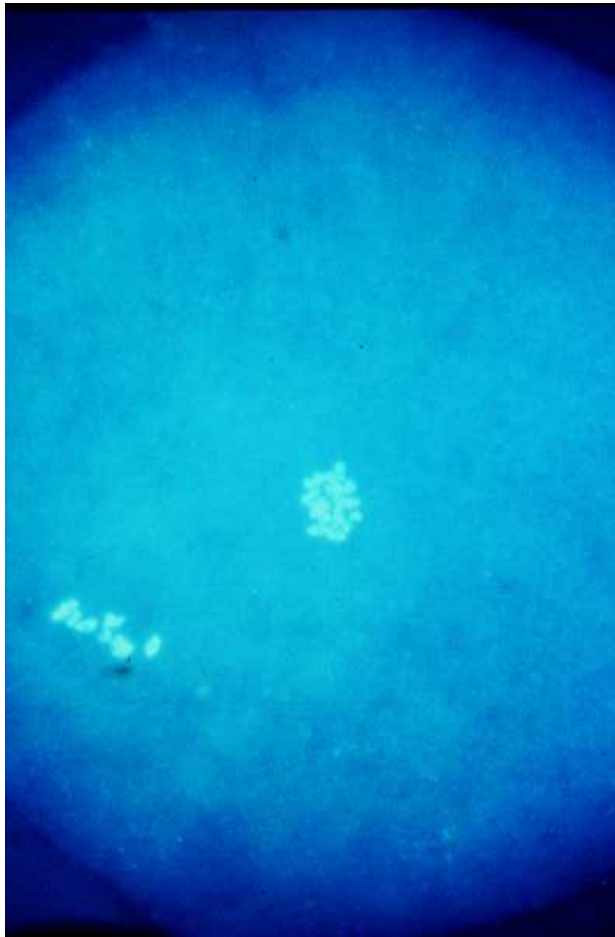


Metaphase II

In vitro Maturation



In vitro Maturation

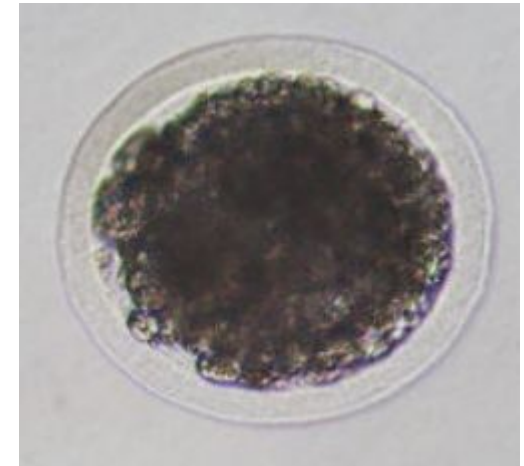


- Expected nuclear maturation rates approx 40-70%
- Nuclear maturation \neq Cytoplasmic maturation
 - mRNA production
 - cytoplasmic changes
 - ...

Hoechst 33342 – DNA staining

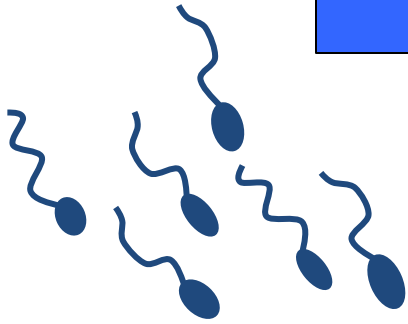
In vitro Maturation

- Nuclear maturation rates = Metaphase II rate
- Cytoplasmic maturation ??????????

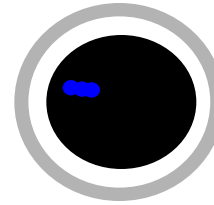


Best assessed by the oocyte's ability to yield an embryo !!!

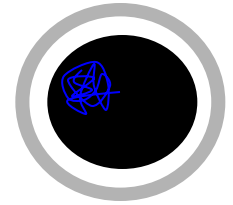
In vitro fertilization



Spermatozoïds



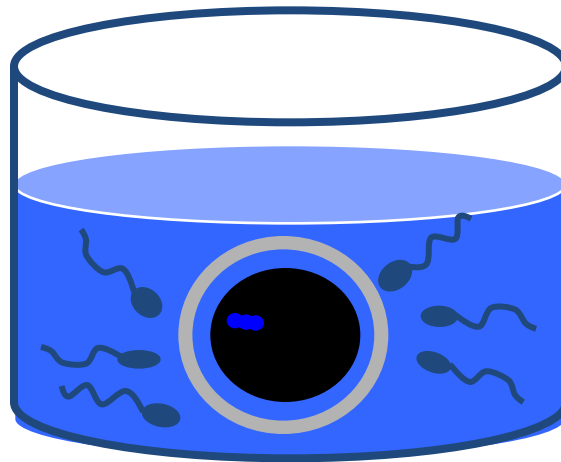
Mature
Oocyte



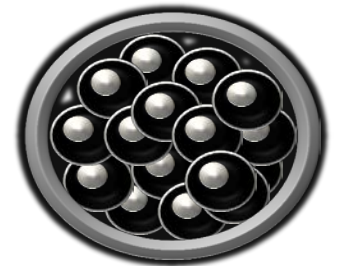
Immature
Oocyte

In vitro Capacitation

IVM

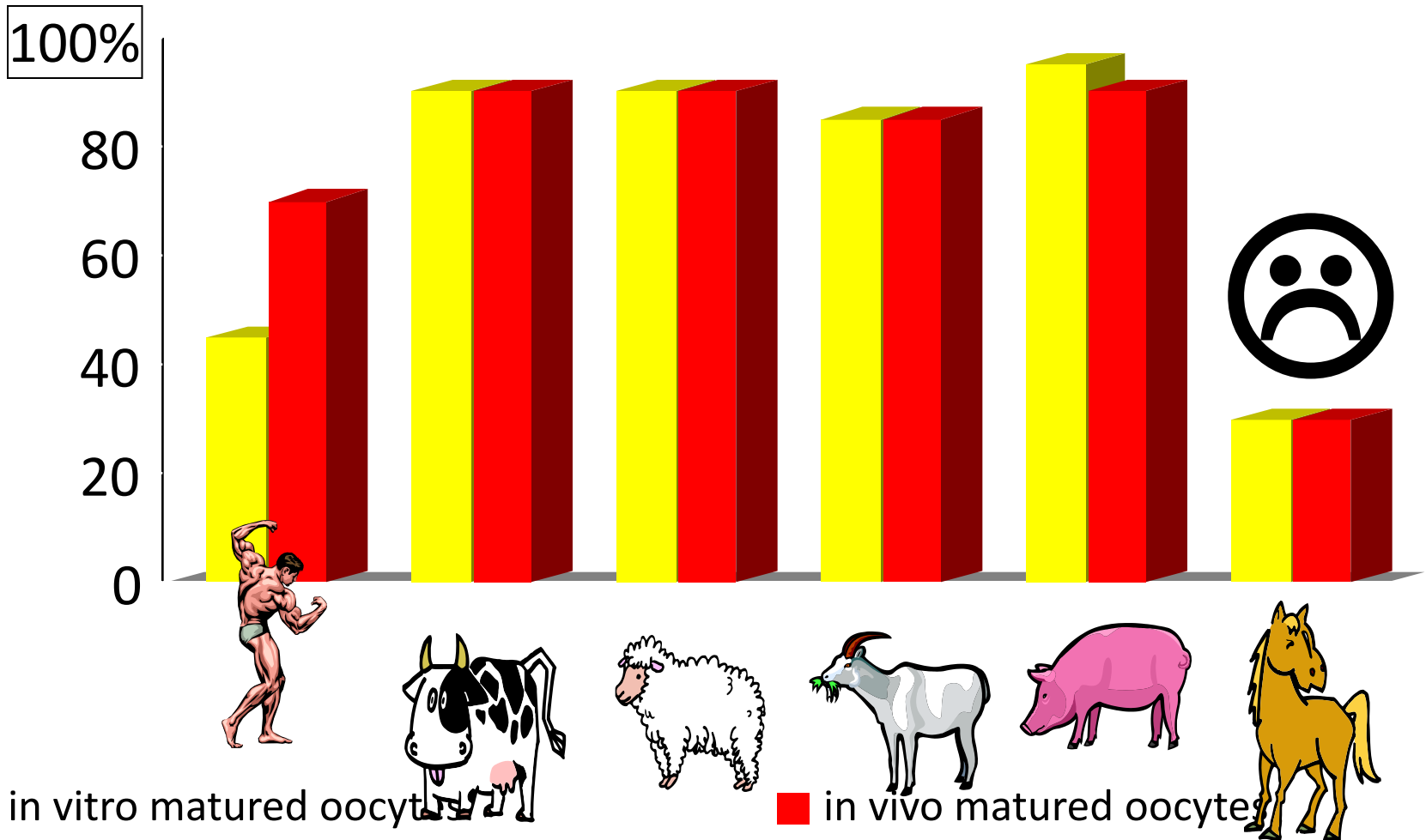


IVF



IVC

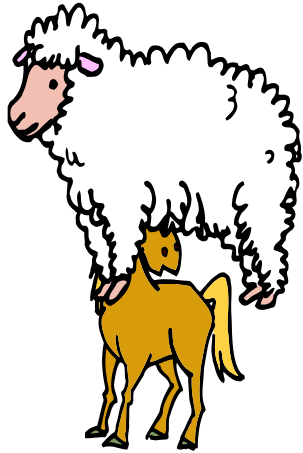
In vitro fertilization



In vitro embryo culture



IVC



35-45%



27-38%
(4-16%)

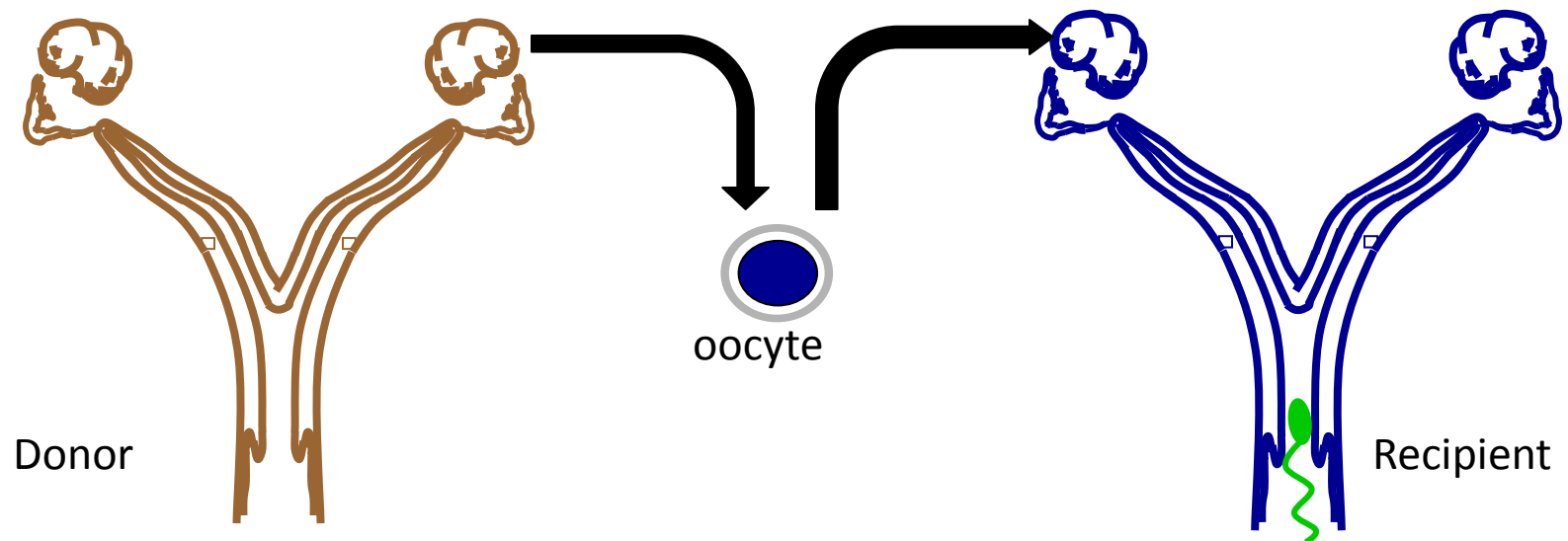


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Intra-Follicular Oocyte transfer

- Intra-Follicular Oocyte transfer (IFOT)



Intra-Follicular Oocyte transfer

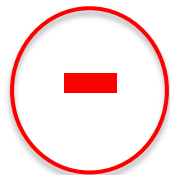


- Intra-Follicular Oocyte Transfer (IFOT):
 - immediately (in vivo matured) : 12.8% embryo coll^o rate
 - after 30h in vitro maturation: 5.5% embryo coll^o rate
 - increased rate of haemorrhagic follicles
 - Immediate IFOT might be an alternative to the absence of superovulation !

Intra-Follicular Oocyte transfer



- ✓ reasonably easy
- ✓ low costs
- ✓ « forced superovulation » (Donor = Recipient)
- ✓ potential field application



- ✓ Oocyte from recipient can not be removed !!!
- ✓ Risk of foal born from recipient
- ✓ Increased anovulatory haemorrhagic follicles incidence

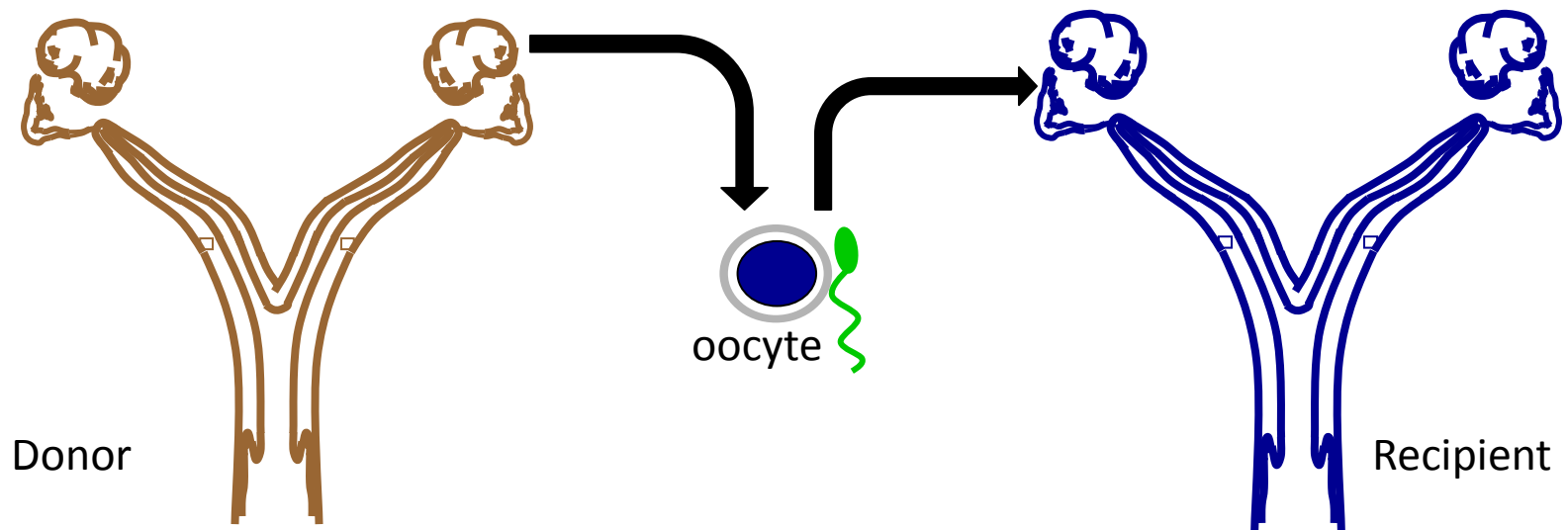
Still a lab procedure !

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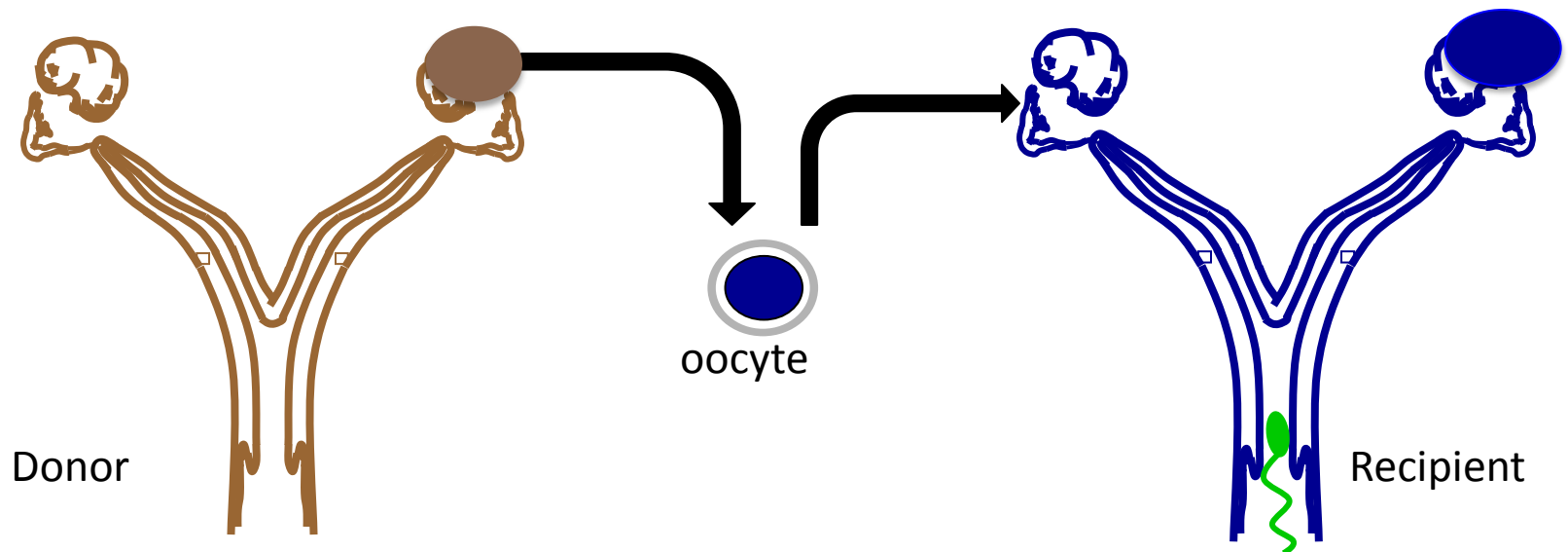
Intra- oviductal Oocyte transfer

- Gamete intra-fallopian transfer (GIFT)



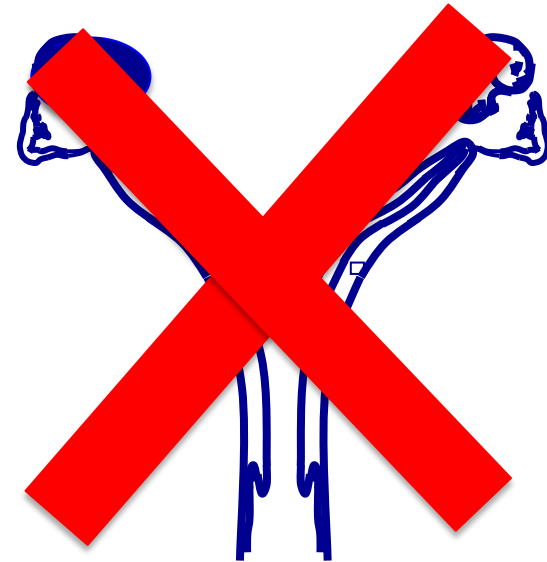
Intra- oviductal Oocyte transfer

- Intra-Oviductal Oocyte transfer (OT)

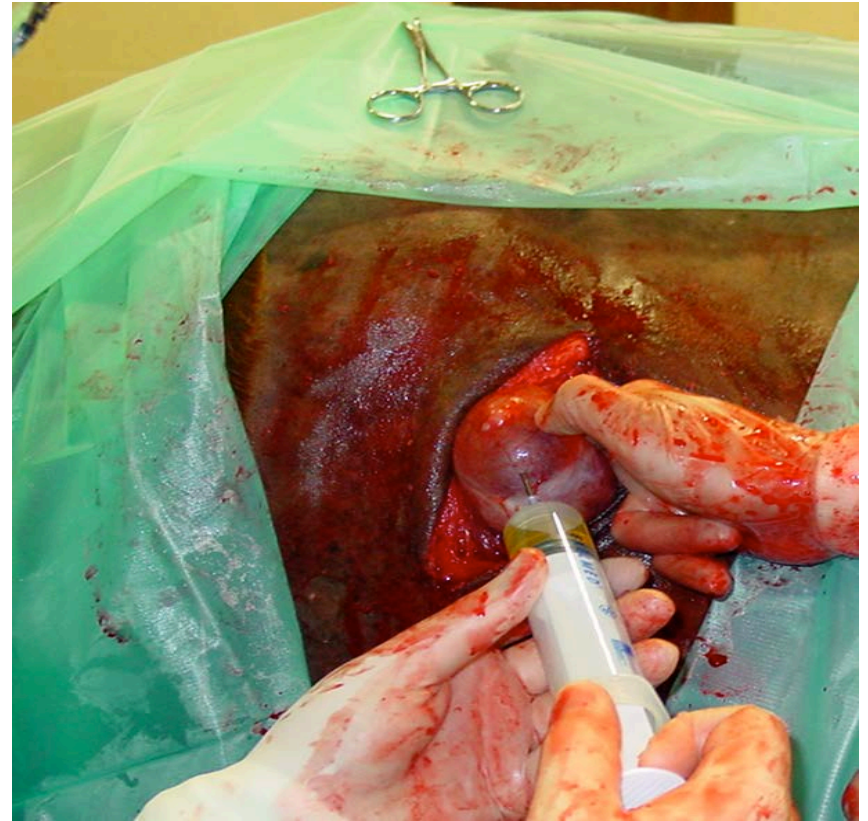


Oocyte transfer

- Risk of foal originating from the recipient's oocyte!! (rather than the donor's)
 - Nil if oocyte has been recovered
 - Very low if follicle from recipient thoroughly aspirated
 - Aspiration incomplete: risk +/- 30%
- => DO NOT USE that mare !!!!



Oocyte transfer



Oocyte transfer



Oocyte transfer

- Transfer of a single in vivo matured oocyte: (24h after ovulation induction with hCG + 16hours IVM) : +/- 73 to 83% pregnancy rate
- Transfer of IVM (post-mortem obtained) oocytes: 7 to 15% pregnancy rate
- Age of mare is a major factor



Oocyte transfer

- Indicated for :
 - mares dying suddenly (oocyte collection post-mortem): +/- 15% pregnancy rate
 - infertile mares suffering from pathologies that prevent embryo transfer: +/- 80% pregnancy rate (if preovulatory in vivo matured oocyte, but age = negative factor!)



Oocyte transfer

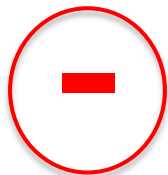


✓ in vivo embryo production from mares with oviductal or uterine disorders

✓ Bypasses the IVC step

✓ in vivo sperm selection and capacitation

✓ selected field applications



✓ Expansive +/-

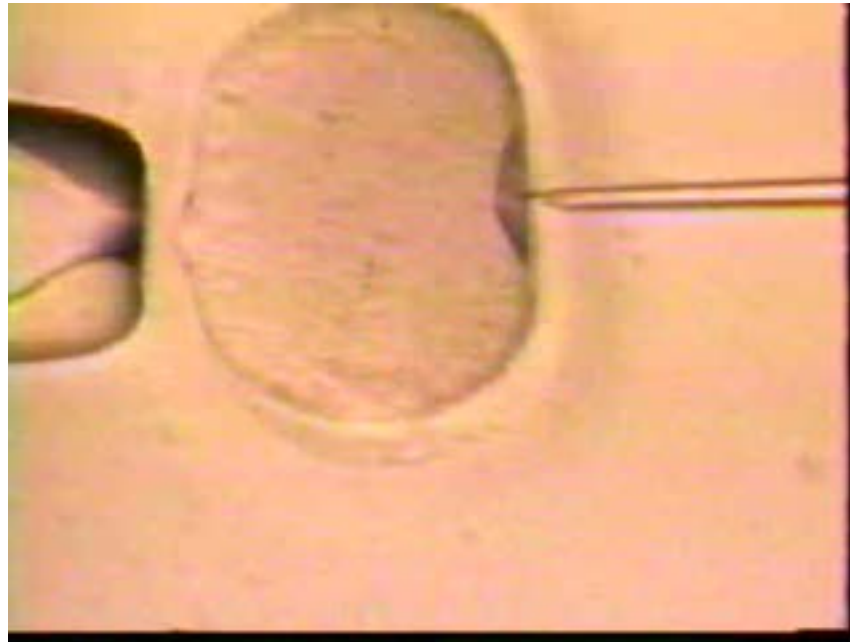
✓ Best with preovulatory oocyte (only 1 per cycle)

✓ Surgery

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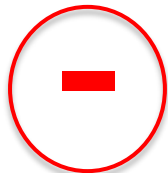
ICSI: Intra cytoplasmic Sperm injection



ICSI: Intra cytoplasmic Sperm injection



- ✓ Any type of semen (fresh, chilled, frozen)
- ✓ Bypasses the capacitation and acrosomal defects



- ✓ Expansive equipment and expertise
- ✓ Choice of THE « good » sperm cell
- ✓ High percentage of oocyte damage
- ✓ Chromosomal abnormalities
- ✓ Further embryonic culture still difficult

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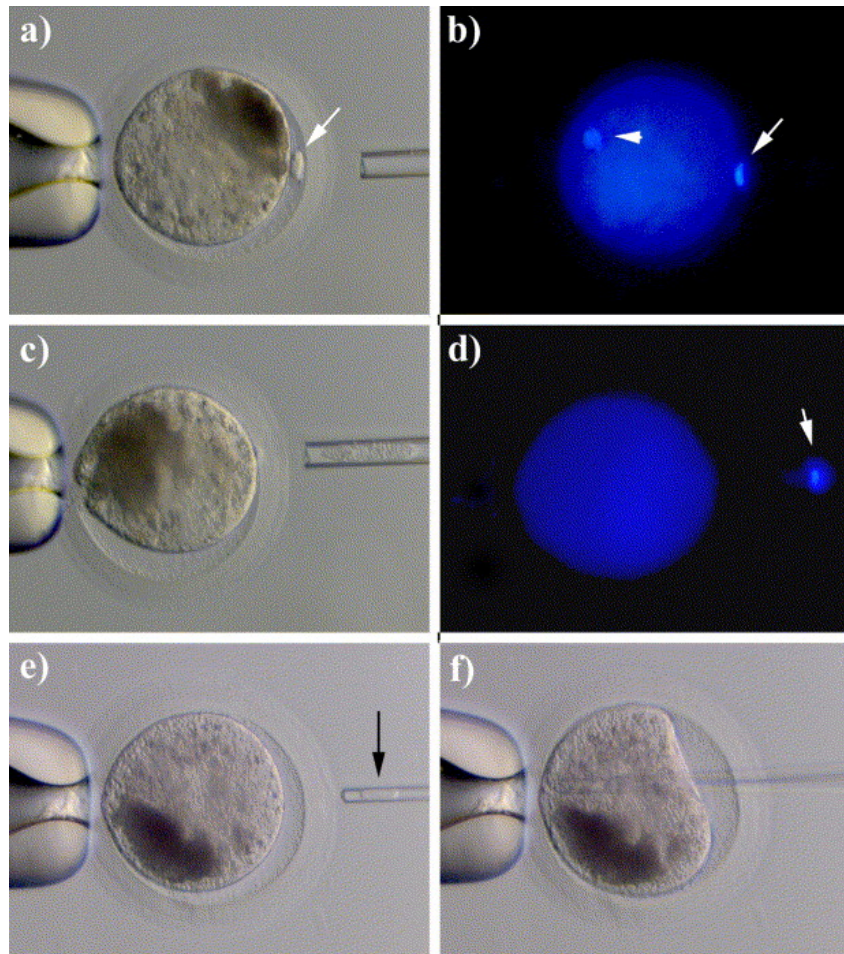
Nuclear transfer (Cloning)

- Technically similar to ICSI (gametes micro-manipulation)
- Culture of somatic cells (fibroblasts) from donor
- Enucleation of recipient cell (oocyte)
- Injection of donor cell nucleus into « empty » recipient cell
- Stimulation for multiplications, + IVC

Nuclear transfer (Cloning)

- First equine clone 2003
- Rescue genetics from valuable individuals
 - Mares
 - Stallions
 - **Geldings**
- Clones (males and females) have shown to be able to produce foals

Nuclear transfer (Cloning)

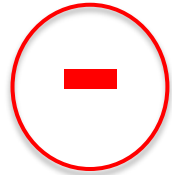


Nuclear transfer (Cloning)



- ✓ Clones from sterile, dead individuals
- ✓ Rescue of highly valuable genetics (geldings)
- ✓ Somatic cells cryopreserved (no time limit)
- ✓ Nuclear DNA=exact same => Clones should be used for reproduction

Nuclear transfer (Cloning)



- ✓ Equipment and expertise
- ✓ Expansive +++++
- ✓ Global success rate very low
- ✓ Cumulates all the difficulties from the other techniques (Availability of oocytes, IVM rates, Enucleation & transfer of donor DNA, Mitosis stimulation (early embryo), Embryo culture, Embryo transfer)
- ✓ Increased pregnancy loss rate & neonatal pathologies for first few weeks
- ✓ Potential concentration of genetic pool (no time limit)

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Conclusion

- Based on their economical value, or for emotional reasons, a few selected mares may benefit from ART's that require expertise and money! Access still limited
- Conventional IVF has recently been shown to achieve repeatable satisfactory results (next presentation with G. Goudet !)

Conclusion

- If research focuses on tools to optimally select oocytes (cytoplasmic maturation) and establishment of a satisfactory IVC procedure, in vitro embryo production might become available for a wider population of horses that would benefit the equine industry.

Conclusion

- A fully in vitro produced foal (IVM+IVF+IVC) seemed completely out of reach only a few years back; however,



Thank you !!

things are changing fast lately!