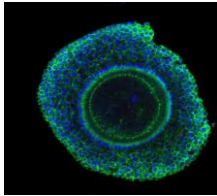


In vitro production, cryopreservation and transfer of horse embryos



Tom Stout

Department of Equine Sciences,
Utrecht University



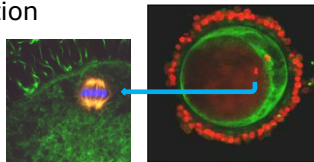
Why in vitro fertilization?

- Chronic sub-fertility in mares
 - Unable to recover (normal) embryos
- Mares in sport
- Mare – euthanasia / death
 - genetic salvage
- Too few viable sperm available
 - Dead stallion (epididymal sperm?)
 - Sexed-sorted sperm (?)
 - Sub-fertile stallion (acquired)



In vitro embryo production

- In vitro maturation
- ICSI
(Cloning: SCNT)
- In vitro embryo culture
- Not trivial procedures!
 - Laboratory expertise & experience



➡ Transport to lab?



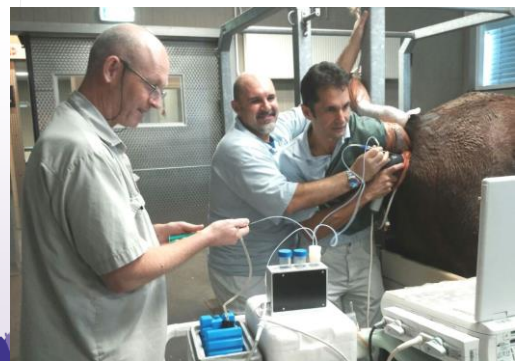
Ovum pick-up (OPU)

Double lumen needle: 12g 'Scrape'; not just aspirate



Mare preparation

- Enough follicles (>8mm) ≥12?
Spring / Autumn
- Out-patient <1 h
- Disease tests? CEM/EIA
- Sedation: Epidural or intestinal relaxation
- NSAIDs / antibiotics (sport mares – doping)
- Well tolerated – post-OPU discomfort
No permanent ovarian damage
(Velez et al, 2012)



Immature follicles



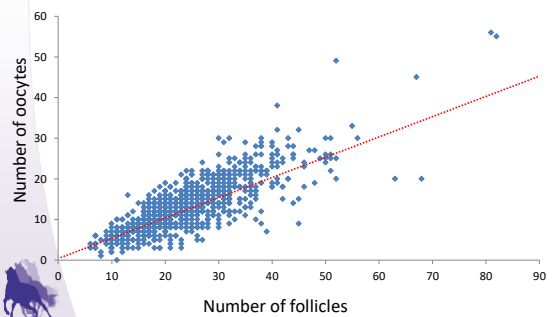
Multiple follicles



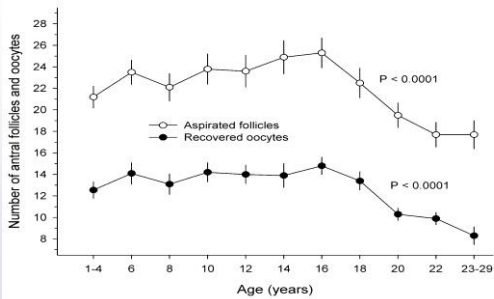
Sept 2014 – July 2019

- 1337 commercial OPUs
 - ET flush medium + heparin (10000 IU/L) / oocyte recovery medium
 - Flush each follicle 5-10 times
 - Mean no. Follicles: 23.8 (6-82)
 - Oocyte recovery: 13.3 (0-56)
 - Efficiency = 56% (0-100%)
- Mare (age) effect (Claes et al 2016)

Oocyte recovery (879 OPUs)



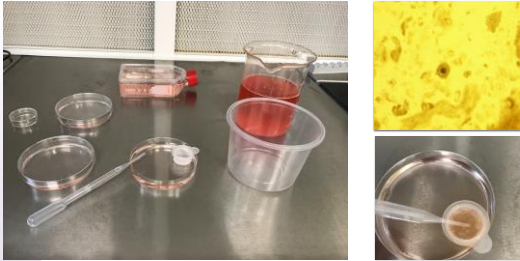
Mare age affects oocyte recovery



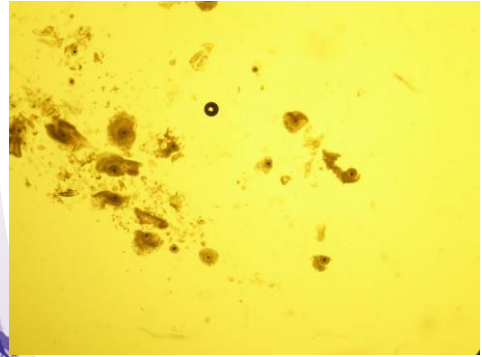
Oocyte searching



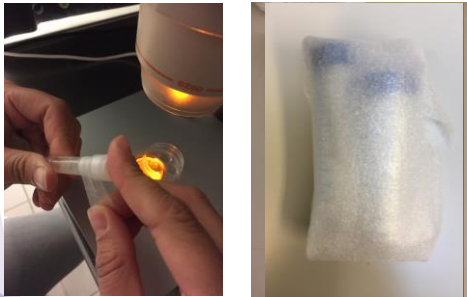
Oocyte searching



Filter, wash, transfer to petri dish



Packaging for transport



H-SOF or embryo holding medium

Packaging: Organ transport box



All materials pre-incubated at 25°C

Packaging: Organ transport box



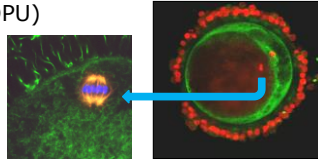
- For < 24h - oocytes arrive at >20°C
- Delay (48h) - 23 OPU's / 11 embryo's (0.48)

Transporting oocytes?

- Transport at 22°C for < 24h
 - Good for GV (immature) oocytes
 - MII oocytes do not tolerate holding well
- Our experience - drop below ~18°C
 - No effect on IVM / reduced blastocysts
- Oocyte cryopreservation
 - MII spindle damage / few blastocysts
 - But
 - 5 times (17 OPU's) - transport failed
 - 6 times (23 OPU's) - transport delayed

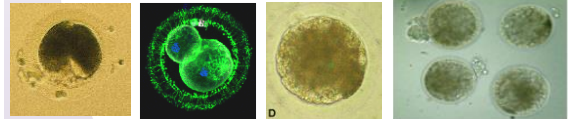
Oocyte maturation

- 24-28 h culture at 38.2°C
- Can be held for up to 24h prior to IVM
- Mature to MII
 - 57% (7.9 per OPU)
- Oocytes with polar body

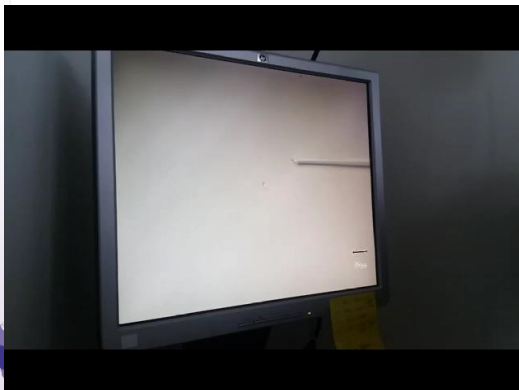


ICSI

- 1 motile sperm
- Inject at 6 or 12 o'clock
- Cleavage
 - 69.4% (5.5/OPU)
 - 7-8 day culture



ICSI



EMBRYO FREEZING

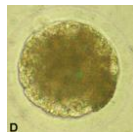
- Day 6-8 blastocysts
 - Late development ≡ poor quality
- 10% glycerol (2 steps)



Pregnancy rates fresh ≡ frozen IVP embryos
- small, no capsule

Embryo production: 1348 OPUs

- Day 6-8 blastocysts: 1872
 - mean = 1.39 (17.7% injected)
 - ≥1 embryo (62%)
 - 0 - 518 x (38%)
 - 1 - 361 x (27%)
 - 2 - 209 x (16%)
 - 3 - 126 x (9%)
 - ≥4 - 141 x (max. 10)
 - ~2.26 per success



(Excludes failed shipments / NB 7 x no ICSI)

Effect of transport on results (2015)?

	On-site immediate IVM	Oocytes shipped at 20°C
OPUs (mares)	202 (82)	158 (102)
Oocytes	1923 (9.5 per OPU)	2058 (13 per OPU)
Degenerated	216 (11.2%)	556 (27.0%)
MII	1400 (72.8%)	1210 (58.8%)



Utrecht University and Avantea (Galli et al, 2016: ISEET)

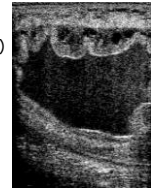
Effect of transport on results (2015)?

	On-site Immediate IVM	Oocytes shipped at 20°C
Cleaved	960 (68.6%)	853 (70.5%)
Day 6-8 blastocysts (cryopreserved)	238 (17%) 1.18 per OPU	182 (15%) 1.15 per OPU
Pregnancies	46/66 (69.7%)	28/46 (60.9%)

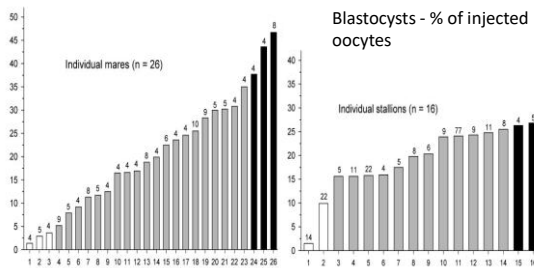
Utrecht University and Avantea (Galli et al, 2016: ISEET)

Factors affecting blastocyst production

- Stallion
 - Individual (2-26%)
 - *in vivo* fertility
 - Straw (Galli et al, 2016: ISSR)
- Mare
 - Individual (2-47%: 0.2-4.2 per OPU)
 - Follicle & oocyte number
 - Fertility status
 - Active endometritis

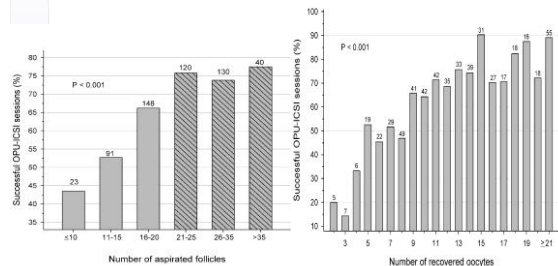


Individual animal effects: mare!



Mares and stallions used ≥ 4 times

Effect of follicle number on likelihood of an embryo



Pregnancies

Year	# transfers	Pregnancy
2015	66	65%
2016	195	53%
2017	137	75%
2018	251	73%
2019	475	76%

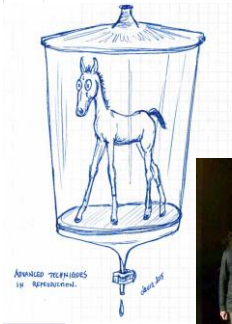


Conclusions

- OPU / ICSI
 - works well with transported oocytes
 - Sub-fertile & competing mares
 - Stallions - sub-fertile /expensive semen
- Areas for improvement
 - Longer term oocyte storage
 - More embryos of better quality
 - fewer pregnancy losses



KWPN stallion show
champion 2018!



Thank you for
your attention!

