ASSISTED REPRODUCTIVE TECHNIQUE
Ovum Pick Up and ICSI

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INTRODUCTION
Assisted Reproductive Techniques (ART)

- Artificial Insemination (AI) with fresh, cooled or frozen-thawed semen
- Embryo Transfer (ET) with fresh, cooled or frozen embryos
- Ovum Pick Up (OPU) and Oocyte Transfer (OT)
- Ovum Pick Up and Intracytoplasmic Sperm Injection (ICSI)
- Ovum Pick Up and In Vitro Fertilization (IVF)

INTRODUCTION
IVF and ICSI

- Many efforts concentrated on conventional IVF
- IVF successful in other species but not in horses
- Only two foals reported to be born from IVF (Palmer et al., 1991; Bezard 1992)
- Application of ICSI to the horse has overcome the barrier of inefficient IVF:
  Squires et al. (1996) Cochran et (1998)
  McKinnon et al. (2000) Galli et al. (2002)

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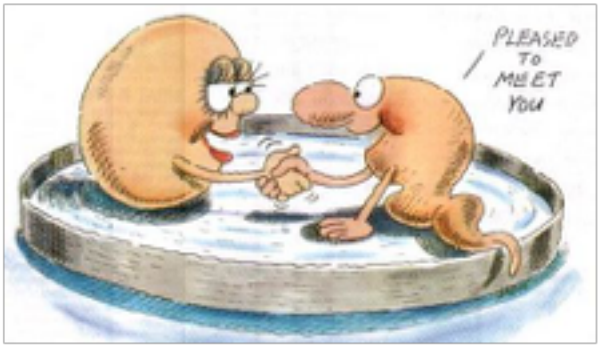
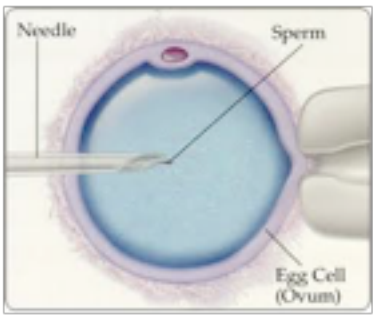
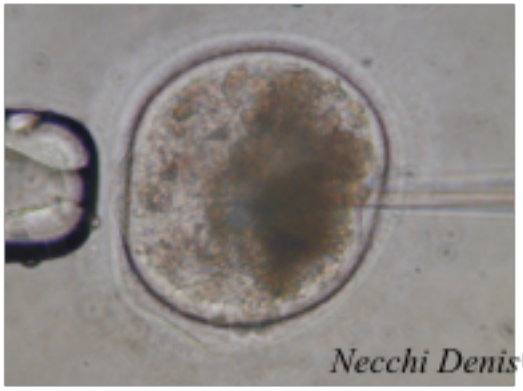
IVF



ICSI





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INTRODUCTION

PROCEDURE:

1. Oocyte collection
2. Oocyte search
3. Oocyte maturation
4. Sperm Preparation
5. ICSI
6. Embryo Maturation → Embryo Transfer
   Embryo Maturation → Embryo Freezing

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1) OOCYTE COLLECTION

MATERIALS

- Good ultrasound machine, with biopsy guide
- Modified endovaginal probe
- OPU aspiration pump
- 50 ml tube incubator
- 12 Gauge coaxial double lumen needle

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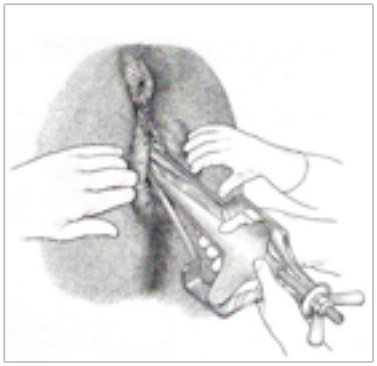
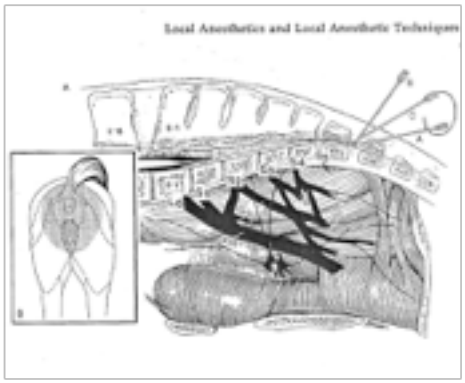
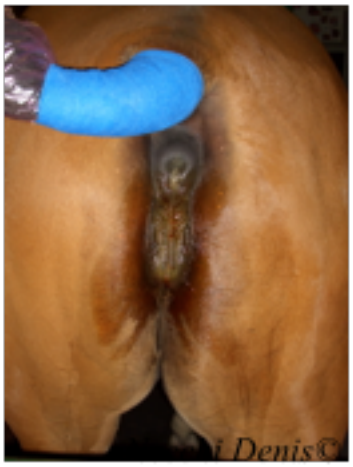
1) OOCYTE COLLECTION

THE DONOR MARE MUST HAVE A GOOD NUMBER OF FOLLICLES

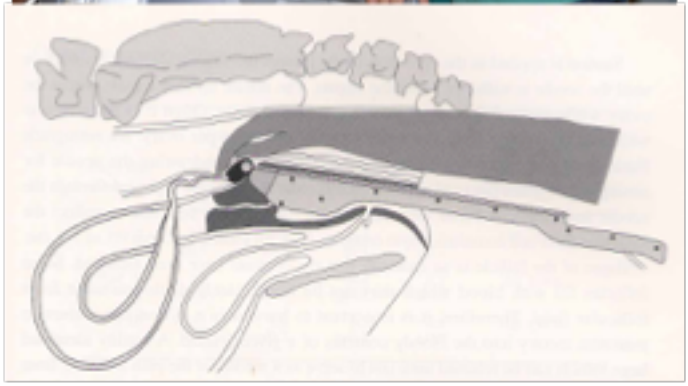
- Sedated with detomidine
- High epidural analgesia (6-8ml)
- Rectum emptied
- Speculum is inserted and the vagina is washed
- The US probe is inserted in the vagina

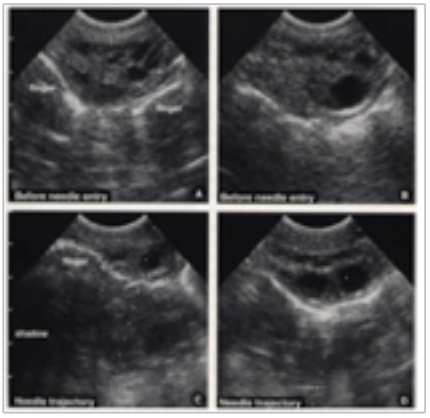






1) OOCYTE COLLECTION





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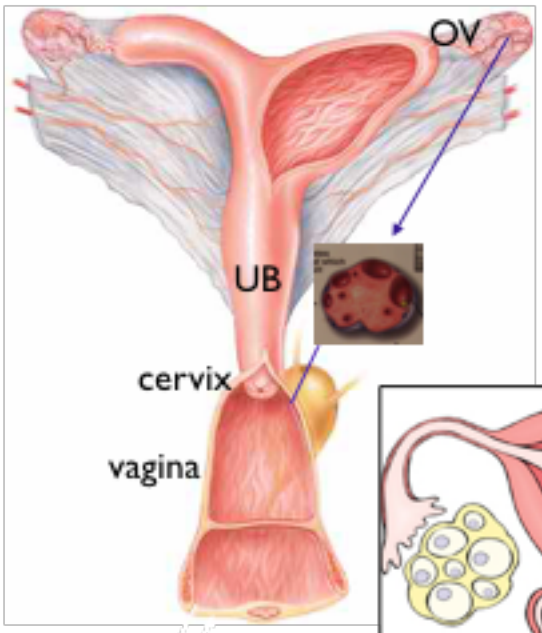
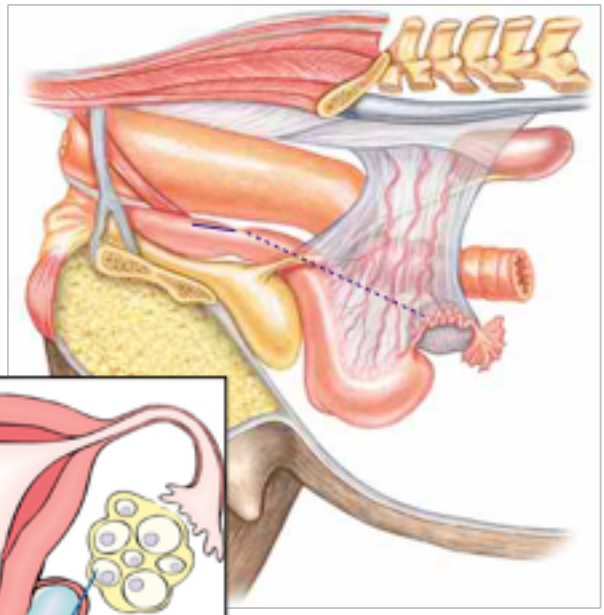
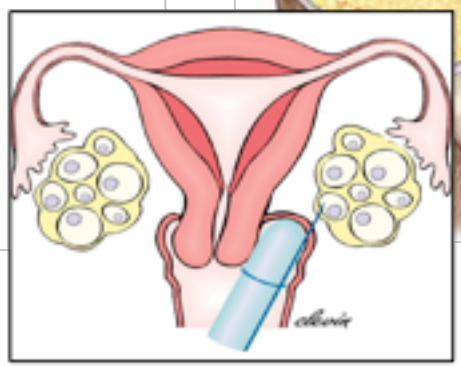
1) OOCYTE COLLECTION







Fluid out

Fluid in

Only the needle is going through the vaginal wall

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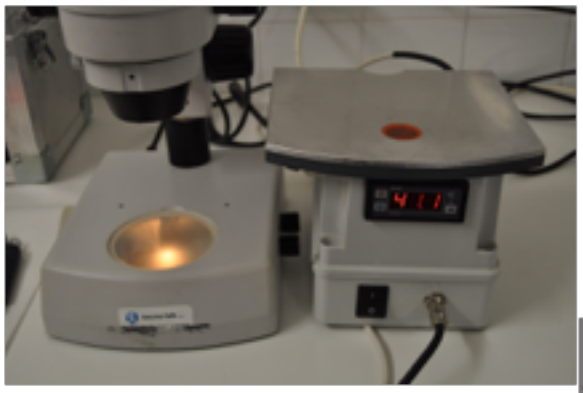
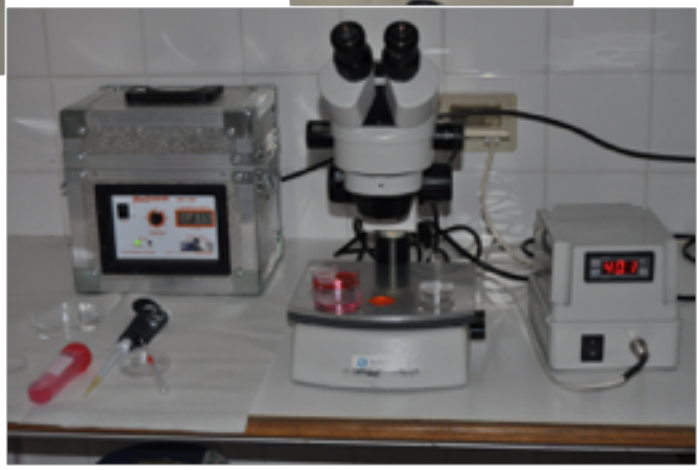
2) OOCYTE SEARCHING





Stereomicroscope 10 - 40x
Heating system, with orange filter lens
Disposables (Petri's Dish)
Transportable incubator

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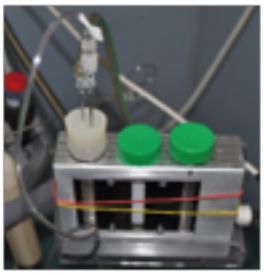
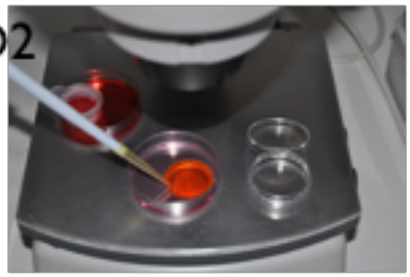
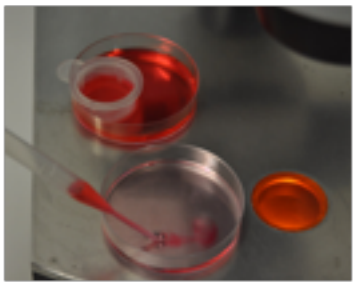
2) OOCYTE SEARCHING




+ CO2

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3) OOCYTE MATURATION

Recovered oocytes are matured in vitro for 24 hours in the incubator

The day after, only the oocytes reaching metaphase II are in vitro fertilized by ICSI and in vitro cultured up to the blastocyst stage

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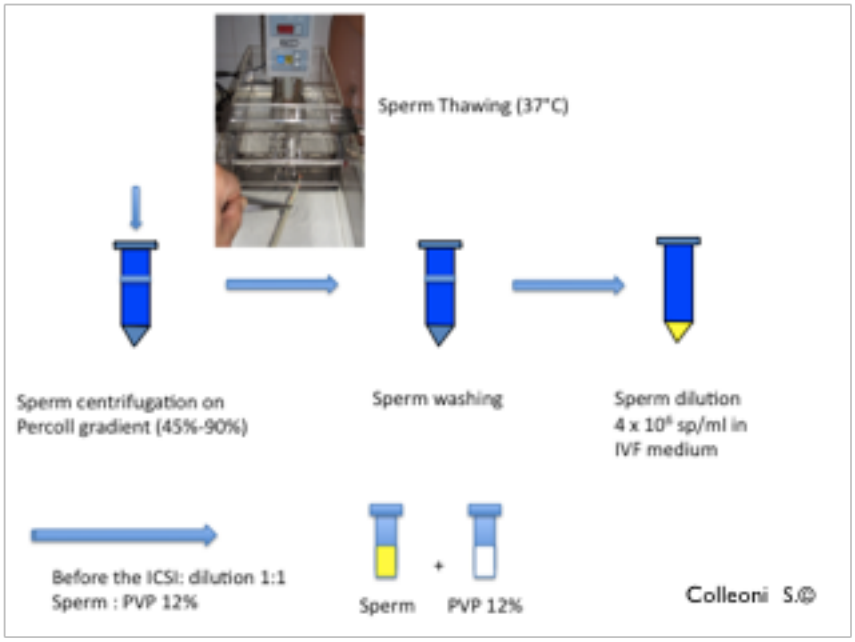
4) SEMEN PREPARATION



ONE SINGLE STRAW IS DIVIDED IN 4-8 PORTIONS
CAN BE USED FOR 4-8 OPU COLLECTIONS

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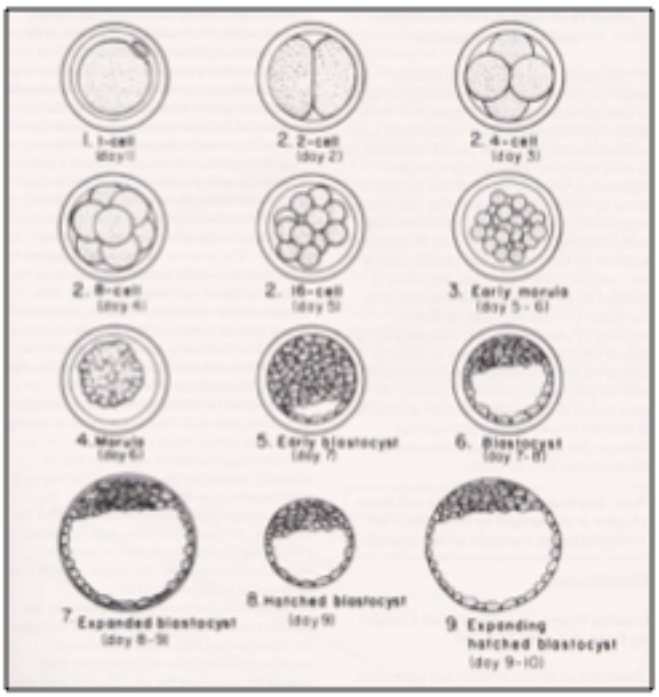
6) EMBRYO MATURATION

ONLY EMBRYOS REACHING, 7-9 DAYS AFTER ICSI , THE BLASTOCYST STAGE ARE TRANSFERRED AS FRESH OR FROZEN FOR LATER USE/ EXPORT



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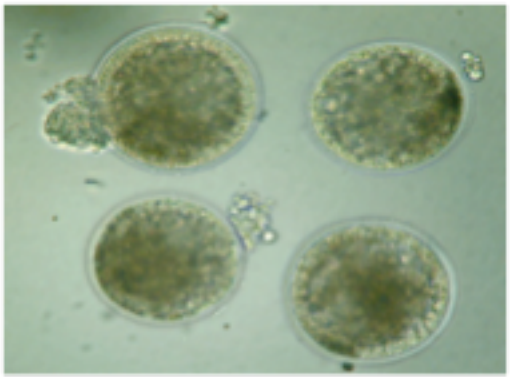
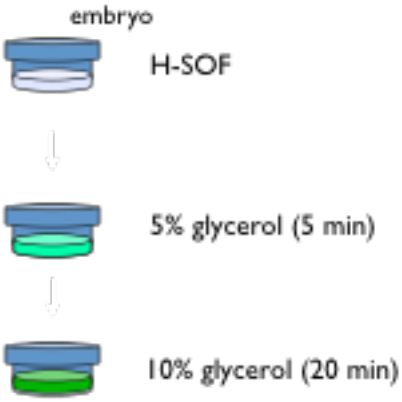
EMBRYO FREEZING

FREEZING METHOD





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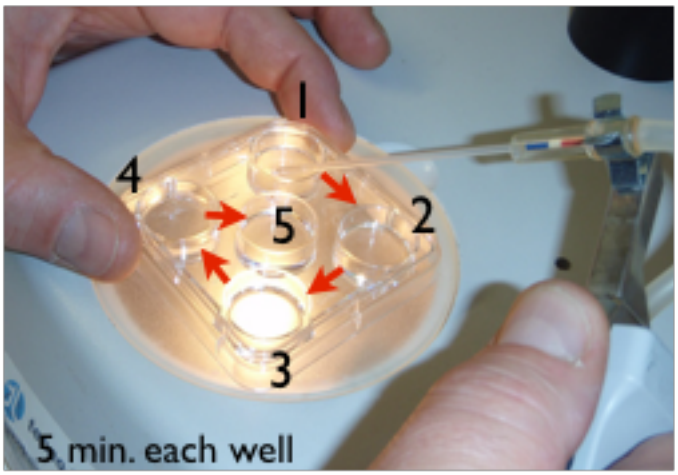
EMBRYO THAWING / WASHING

- Take the embryo out
- In air for 3" and then in water at 22-25°C
- Glycerol (embryo frozen 10%) 1: 8%; 2: 6%; 3: 4%; 4: 2%
- # 5: holding medium



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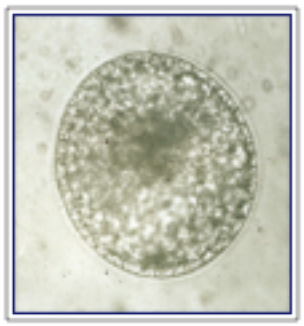
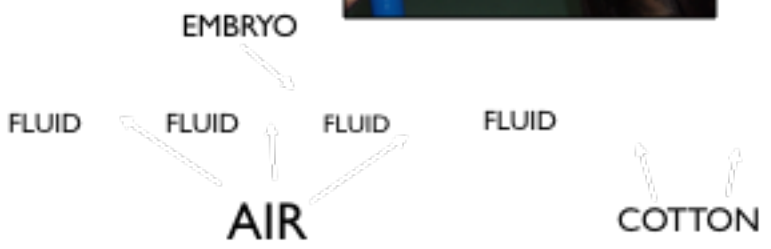
EMBRYO TRANSFER

The technique is the same for frozen/thawed or fresh embryos produced by ICSI





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RESULTS

2004-2009 (SBSI+Avantea)

2003- 2014 (Avantea Italy)

2014- 2016 (Utrecht University)

SBS SelectBreedersServicesItalia

Universiteit Utrecht

LTR AVANTEA LABORATORIO DI TECNOLOGIE DELLA RIPRODUZIONE

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AIM OF THE STUDY
(2004-2009 SBSI-Avantea)

- Impact of the donor mares age
- Impact of reproductive disorders
- Use of transitional versus cycling mares
- Use of performance vs non performance mares
- Use of frozen semen with poor post-thaw quality and/or with low fertility
- Mares fertility versus stallion fertility

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MATERIAL & METHODS

1. ***2004 - 2009 (From OPU to Birth)***
2. ***123 donor mares***
3. ***290 OPU sessions***
4. ***Frozen-thawed semen from 77 stallions***
5. ***OPU performed during the spring/fall transition or in diestrus in the absence of a dominant follicle (if at all possible)***

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RESULTS

SUMMARY DATA *(123 Donor Mares – 290 OPUs)*

1. ***Ovarian follicles aspirated = 4845***
   Recovered oocytes = 3150 (65.02% - 10.86/OPU)
   Oocytes → metaphase II and injected = 2029 (64.41%)
   Cleaved oocytes = 1189 (58.60%)
   Blastocysts = 171 (14.38% - 0.6/OPU)
   145 Blastocysts transferred → 81 pregnancies (55.86%)
   51 foals born (35% of the blastocyst, 63% of the pregnancy)
   20 pregnancies lost before day 50
   9 pregnancies lost after day 50
   1 pregnancy no feed back

2003-2014 AVANTEA

The world's first cloned horse, Prometea (2003)
has had a healthy foal (Pegasus 2008)

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OPU from 2010-2013 per breed

BREED	n.	n.	n.		%	%	n.		cleavage	n.	%/	%/	%/
donors	DONORS	OPU	follicles	oocytes	recovery	M II	cleaved	cleaved	rate %	embryos	oocyte	injected	cleaved
WARMBLOOD	79	140	2204	1594	72,32	1115	69,95	736	66,01	123	7,72	11,03	16,71
per OPU			*15,74*	*11,39*		*7,96*		*5,26*		*0,88*			
ARABIAN	69	264	4699	3282	69,84	1975	60,18	908	45,97	84	2,56	4,25	9,25
per OPU			*17,80*	*12,43*		*7,48*		*3,44*		*0,32*			
QUARTER	29	64	878	619	70,50	423	68,34	256	60,52	44	7,11	10,40	17,19
per OPU			*13,72*	*9,67*		*6,61*		*4,00*		*0,69*			

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AVANTEA

BREED EFFECT ON EFFICIENCY OF OPU/ICSI

	n.	n.	n.	n.	n.	n.	%	n.	% blast/	blastocyst
breed	stallions	mares	OPU	oocytes	injected	cleaved	cleavage	blastocysts	injected	/ OPU
WARMBLOOD	59	114	244	2507	1751	1162	66.36 a	204	11.65 a	0,84
per OPU				*10.27*	*7.18*	*4.76*		*0.84*		
ARABIAN	26	47	320	4073	2487	1069	42.98 b	100	4.02 b	0,31
per OPU				*12.73*	*7.77*	*3.34*		*0.31*		
QUARTER	20	20	78	758	529	324	61.25 a	60	11.34 a	0,77
per OPU				*9.72*	*6.78*	*4.15*		*0.77*		
TROTTER	7	14	28	373	188	155	82.45 c	29	15.42 a	1,04
per OPU				*9.21*	*6.71*	*5.54*		*1.04*		

Chi square test: numbers within columns with different letters differ ($p<0.05$).

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AVANTEA

OOCYTE SHIPPING FROM U.U. TO AVANTEA

	N°of OPU	N°of donors	Oocyte Recov.	% Deg.	MII Injec.	%MII	% Cleaved	% BI /Inj	embryo per OPU	pregnancy rate frozen
immediate	202	82	1923	11.23	1400	72,80	68.60	17.00	1.23	69.70
Holding 24 hr at 20 at°C	158	104	2058	27.02	1210	58,79	70.50	15.00	1.15	60.90

Universiteit Utrecht

AVANTEA

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Pregnancy rate with fresh vs. frozen clinical OPU-ICSI embryos

Type of embryo	No. of embryos	No pregn. at D 14 (%)	No pregn. at D 50 (%)
fresh	74	44 (59)	38 (51)
frozen	513	301 (59)	266 (52)

AVANTEA

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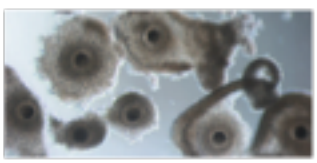

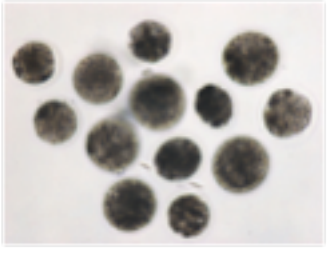

Effect of stallion fertility on embryo development

Stallion	No. Oocytes injected	No. Embryo cleaved	Cleavage Rate %	Total N° of Blastocysts	% Blast/ Cleaved	% Blast/ Inj
FERTILE	351	254	72.36 a	74	29.13 c	21.08 e
UNFERTILE	312	193	61.86 b	28	14.51 d	8.97 f

- Stallions may also significantly affect the outcome of embryo production in vitro
- Sorting for better quality sperm showed a tendency for producing more embryos in infertile stallions
- Low in vivo fertility stallion are also in general less fertile in vitro, but embryos can be produced

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Colleoni et al. 2012

UTRECHT UNIVERSITY RESULTS

- 527 OPU sessions
- 13,8 oocyte per OPU
- 55% recovery rate
- 52% of the OPU produced one or more embryo
- 1,8 embryos per OPU
- 1-8 range of embryos produced
- infertile mares were 4-6 times less likely to produce embryos
  - PR 2015: 66 T, 65%; 2016: 195 T, 53%; 2017: 137 T, 75%

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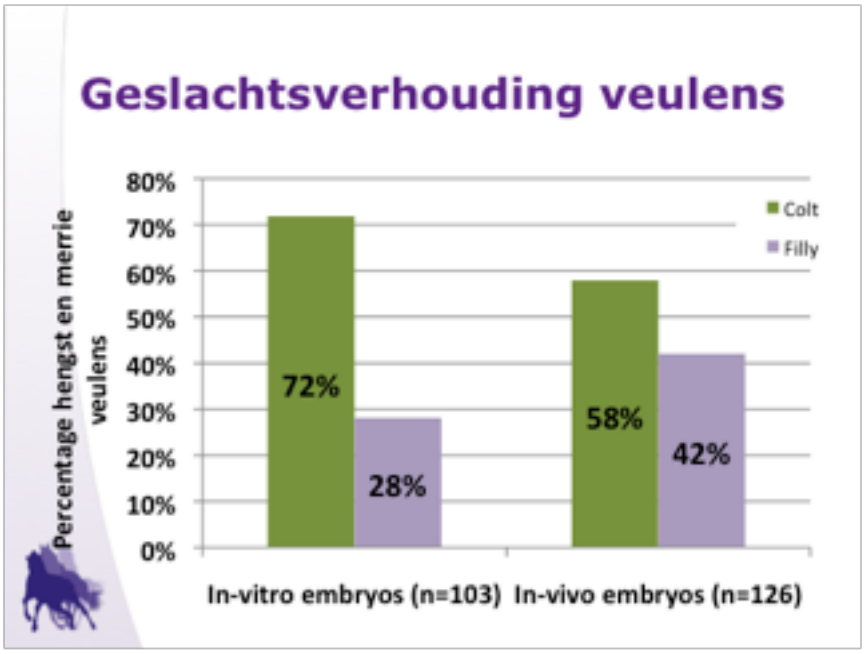

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CLINICAL CASES

BEST MARES
30 follicle, 22 oocytes 2 embryo
20 follicles, 15 oocytes 3 embryos
9 follicles, 4 oocytes, 1 embryo
10 OPU, 128 oocytes, 25 embryos, 2,5 embryos/opu

BUT…….
WORSE MARE
27 follicles, 17 oocytes no embryo
33 follicles, 29 oocytes, 7 blastocyst cleaved, no embryo

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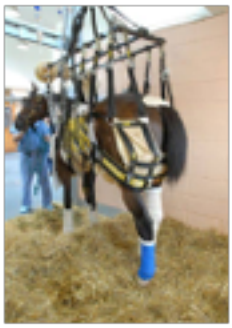
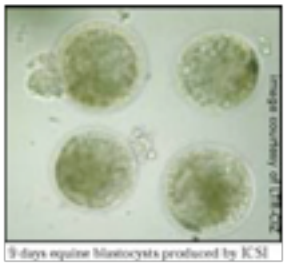
GENETIC SALVAGE

- Preferably do not wait till the mare is dead
- After euthanasia remove the ovaries from the carcass ASAP
- Keep the ovaries at 20-25 °C in an insulated box in a plastic bag (do not freeze or refrigerate the ovaries)
- Send the ovaries to the laboratory in the shortest time possible
- If courier is used, euthanize the animal in the afternoon and arrange delivery as early as possible next morning
- If feasible recover the oocytes from the ovaries and ship the oocytes in holding medium at 20°C in insulated box
- Provide the semen to be used at the same time





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CONCLUSIONS

- OPU-ICSI can be used as an alternative reproductive technique to produce foals.
- OPU-ICSI it's a technique that can be considered to save semen
- OPU-ICSI can be considered as a therapeutic last resort option to obtain offspring from problem mares
- OPU-ICSI can be successfully used on both cycling and transitional mares. In this study better results were obtained with transitional mares
- Embryos can be produced and frozen for later transfer, E.T. synchronization is not required

When using OPU-ICSI a higher pregnancy loss was observed for:

- Aged Mares
- Mares with Reproductive Disorders

Low oocytes quality?

- ICSI can be performed also in sick mares. Expensive and only for valuable mares

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