EMBRYO COLLECTION
technique and evaluation of the factors affecting the recovery rate

DENIS NECCHI, DVM, Dipl. ECAR

Past Assistant Professor in Equine Reproduction Utrecht University

denisnecchi@me.com

EMBRYO COLLECTION:
tools

- FLUSHING MEDIA
- LONG STERILE SLEEVS
- LUBE non - spermicidal
- CATHETERS
- 50 ml SYRINGE
- Y CONNECTIONS
- FILTERS
- STEREOMICROSCOPE

EMBRYO COLLECTION: Method

1. Preparing the Donor
2. Introduction of the uterine catheter
3. Embryo recovery: the flushing
4. Treatments after the flush
5. Factors affecting the recovery rate

THE DAY OF FLUSHING BEFORE STARTING

SCAN THE MARE FOR:

- Estimate the uterine volume
- Unexpected double ovulations
- Endometritis
- Mucometra/Pyometra
- Quality of the CL's

1) PREPARING THE DONOR

Hygiene is crucial for donor and embryo

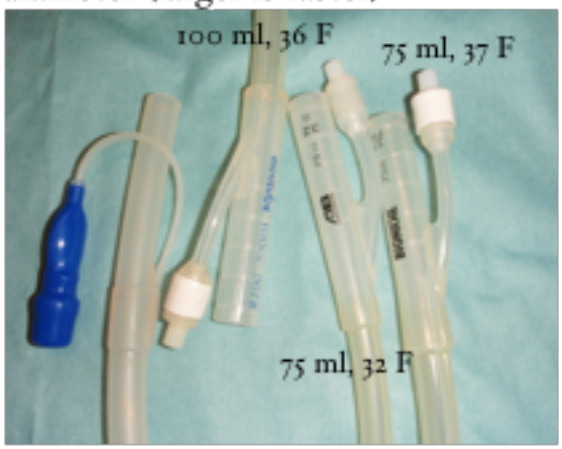
Wash with soap, rinse, soap, rinse3-4 times or till is clean

Administer Detomidine IV (if necessary)

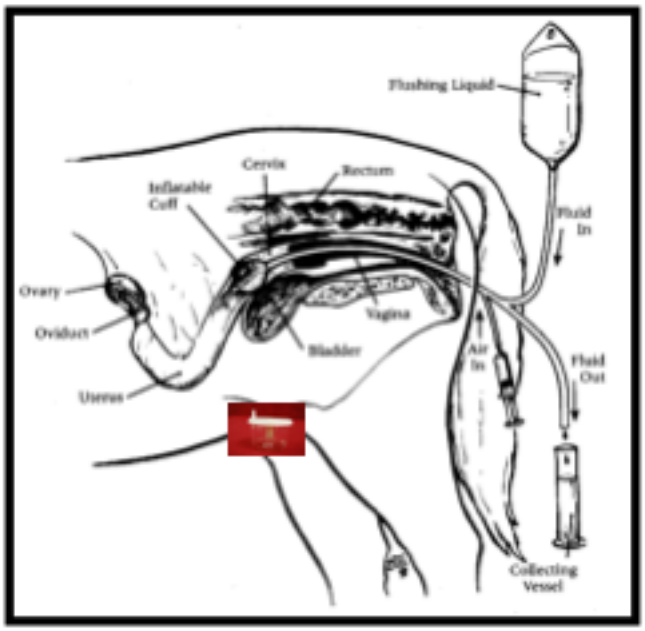
Old mares: very small dose of xylazine 20% (0,8-1 ml) helps the uterine contractility

Uterine Flushing Catheter

- Silicone (no plastic and no latex)
- Balloon cuff: 75 - 100 ml (the cervix must to be totally sealed)
- Open distal tip with 2 or more side ports
- 80 -150 cm long (longer require less connection tubes)
- Diameter 32-37 F, 7-9 mm internal diameter (larger is faster)



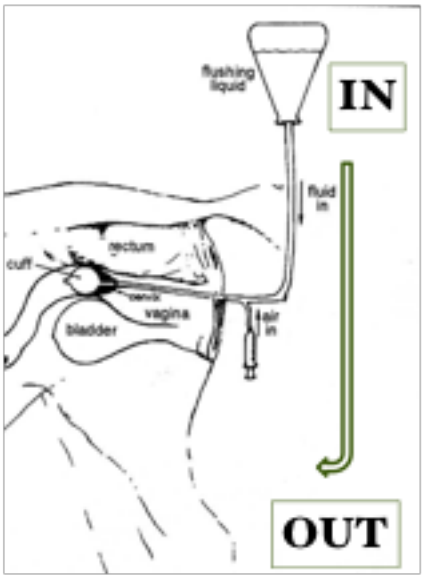
2) INTRODUCTION OF THE FLUSHING CATETHER

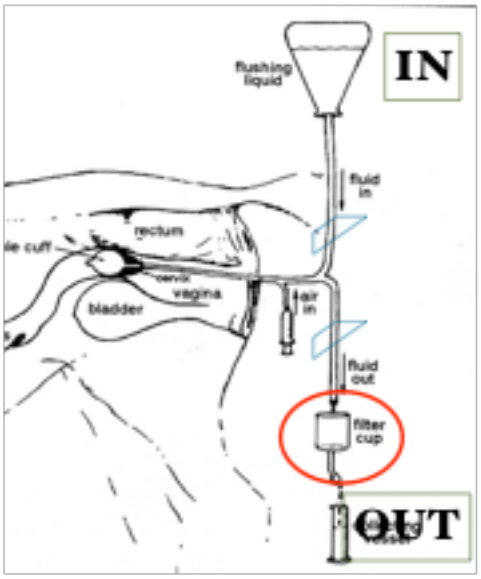


3) EMBRYO RECOVERY : flushing

ONE WAY: The uterus is filled up, the fluid is recovered in the same bottle.

2 WAYS or CLOSED SYSTEM: the fluid is immediately filtered

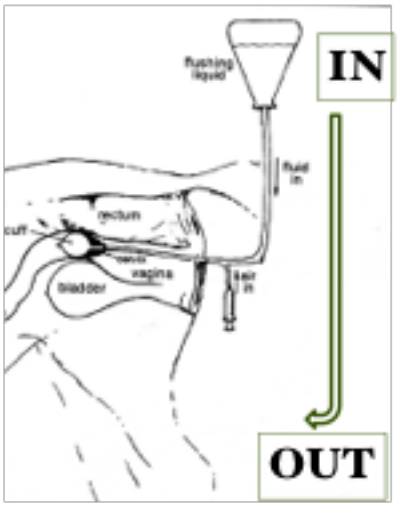




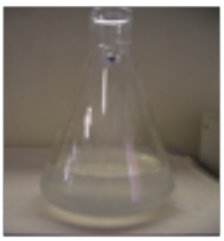
Fluid out: invert the bottle

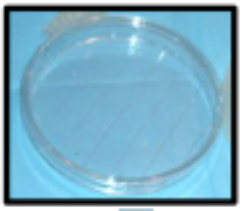
3) EMBRYO RECOVERY: flushing

ONE WAY: The uterus is filled up and the fluid is recovered in the same bottle.



OLD: decantation and siphoning





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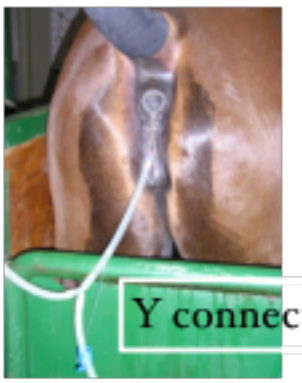
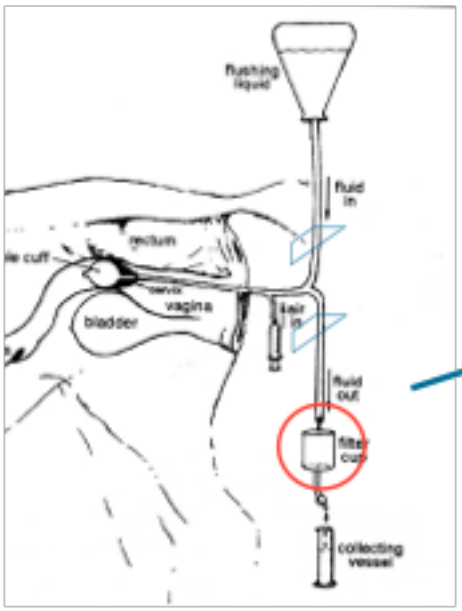
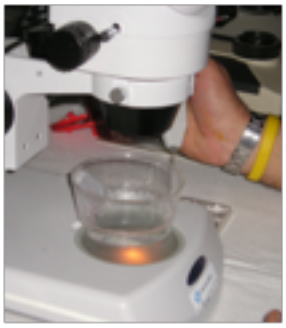
3) EMBRYO RECOVERY: flushing

2 WAYS or CLOSED: the fluid out is going directly through the filter







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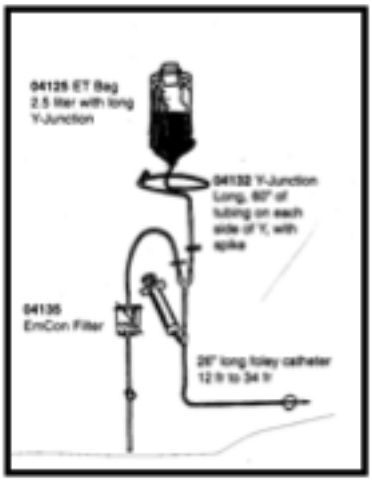
3) EMBRYO RECOVERY : flushing

FLUSH EQUIPMENT:

Complete Flush Kit

All components provided

Disposable



FLUSH MEDIA:

- 'Complete' Flush Media

EmCare™ - ICP, ViGro™ - AB Technology/Bioniche, -Euro Flush- IMV
EquiPro™ - Minitube

Dulbecco's Phosphate Buffered Saline (DPBS)

plus antibiotics and fetal/newborn calf serum

- Lactated Ringer's solution + FCS

3) EMBRYO RECOVERY : flushing

STANDARD LAVAGE METHOD:

- preload tubing and catheter with media
- fill the uterus with 1-2 liters
- massage the uterus after each filling and during emptying
- repeat fill-empty cycle 3-6 times (6-9 liters). Consider that can be the only cycle.
- fluid recovery may be monitored by transrectal ultrasonography
- when at least 90% of the fluid has been recovered, close the clamps, deflate the ballon and open clamps

3) EMBRYO RECOVERY : flushing

TRICKS TO EMPTY THE UTERUS:

- if no return put in more flushing solution
- if the catheter plugs, rotate and/or pull it backward
- It is not necessary to completely empty the uterus at each siphoning
- during the last flush, deflate cuff 50% and gently advance it into the uterus
- massage the uterus and/or push down the catheter with the arm in the rectum
- tranrectal ultrasonography
- give oxytocin

be sure the catheter cuff is not inside the cervical canal

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3) EMBRYO RECOVERY: FILTERS

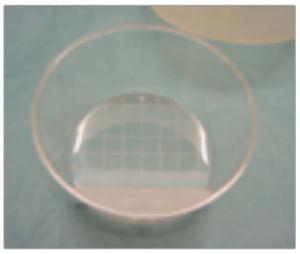
1) READY TO SEARCH: - directly on the bottom of the filter cup

MINITUBE

Filter in the middle, unscrew at the end. The search grid is on the bottom

EZ WAY FILTERS

Side filter
Open at the end
Close with the cap
Search grid on the bottom



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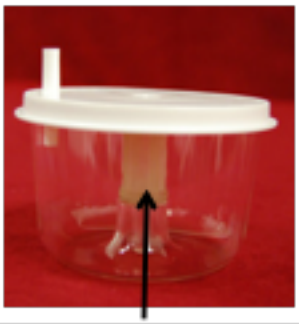
3)EMBRYO RECOVERY: FILTERS

2) WITHOUT GRID: need extra steps
- The filter content must be moved to a search dish with grid
- Filter must be rinsed

VCI E.T. LARGE VOLUME FILTER



Filter in the middle
Fluid always present
no dehydration
of the embryo
Heavy fluid vortex

EMCON FILTER

- Filter on the bottom

If totally emptied embryo dehydration

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FLUSHING MEDIA

Ringer Lactate vs other flushing media

ADVANTAGES:

CHEAPER: up to 10-12 liters per flushing

5 liters bags

Less waste of money if embryo found at the beginning

No bubbles and foam

DISADVANTAGES

Does not contain ab

Does not contain albumin and FCS

Embryo could be sticked to flushing catheter and tubes

pH and osmolarity extremely variable

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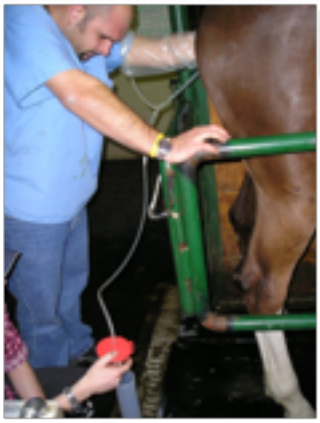
3)EMBRYO RECOVERY:

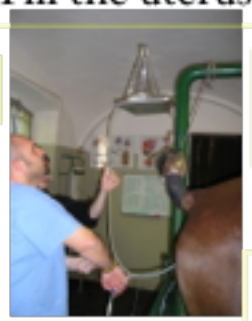
The extra flush (CSU)

Perform 3 uterine lavage



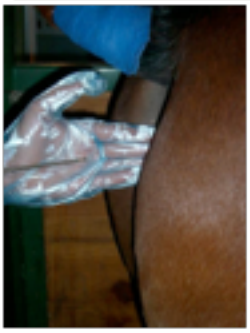
20 U.I. of oxitocin I.V.

Fill the uterus



Wait 5 min.

Recover the solution



Embryo found: Handling, lavage and....

No embryo

Search the embryo

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EXTRA FLUSH PROCEDURE (CSU)

RETROSPECTIVE STUDY:

- 208 embryo flush attempt
- 89 embryos collected during the first 3 lavages (42,8%)
- 30 embryos collect on the extra flush
- total of 119 positive flushing on 208 (57,2% overall embryo collection rate

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3)EMBRYO RECOVERY: Next day re-flush

IN SOME CIRCUMSTANCES THE DONOR CAN BE RE FLUSHED

- Fertile mare
- Great cycle
- Excellent semen
- Perfect ovulation, no PMIE

RETROSPECTIVE STUDY CSU: 3 embryos on 31 re-flush (9,7%)

- Questionable: only one or last cycle?

4) TREATMENT AFTER THE FLUSHING

ALWAYS GIVE PROSTAGLANDIN TO THE DONOR

Even if you got the embryo/s

Causes luteolysis

Allows to return in estrous

Induce uterine contraction

Prevent unwanted pregnancy

.....even if is the last flushing for the mare

DONOR MARE REMAIN PREGNANT AFTER FLUSH

- embryo not recovered or only one
- PGF not administered, if given to the owner write on the mare's chart
- Sometimes PGF might not work: if is the last flushing of the season check the mares 20-30 days later
- ADMINISTER PGF DURING FLUSHING: Helps with the uterine contraction and IT'S DONE

EMBRYO COLLECTION: Method

1. ~~Preparing the Donor~~
2. ~~Introduction of the uterine catheter~~
3. ~~Embryo recovery: the flushing~~
4. ~~Treatments after flush~~
5. Factors affecting the recovery rate

DAY OF COLLECTION
When to Collect?

- Embryo in the uterus 5,5-6,5 days post-ovulation
- Embryo grows very fast once it is into the uterus
- Pregnancy rates lower with embryos > 1000 µm
- Best freezing results with embryos smaller than 250 µm (day 6.5)

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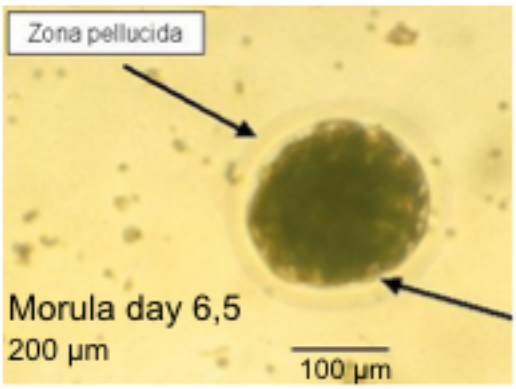
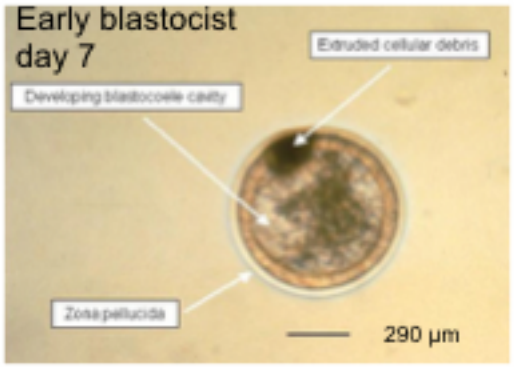
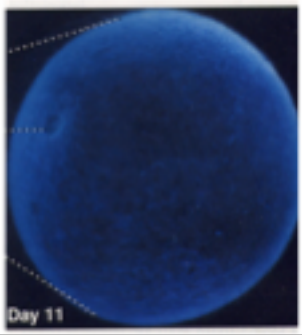
SIZE OF THE EMBRYO

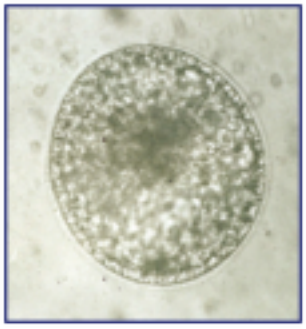




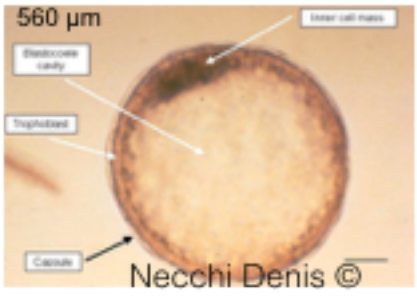
Expanded Blastocist



Blastocist day 8,5 = 1000 µm



Blastocist (day 7,5-8)



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Abstract

Evaluation of reproductive parameters in a commercial equine embryo transfer program☆

J.C.F. Jacob[a], G.O. Santos[a], J.P. Oliveira[a], M.O. Gastal[b], E.L. Gastal[c,*]

[a] Department of Reproduction and Animal Evaluation, Federal Rural University of Rio de Janeiro, Seropedica, RJ, Brazil
[b] Department of Dairy Science, University of Wisconsin, Madison, WI, USA
[c] Department of Animal Science, Food and Nutrition, Southern Illinois University Carbondale, 1205 Lincoln Drive, MC 4417, Carbondale, IL 62901, USA

DAY OF COLLECTION AND RECOVERY RATE:

DAY 6: 42%; DAY 7: 61%; DAY 8: 66%; DAY 9: 59%; DAY 10: 56%

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DAY OF COLLECTION:
Summary

- DAY 7-8 post-ov: normal E.T. routine, larger and easier to visualize/ manipulate
- DAY 6,5-7: embryo is small, can be still in the oviducts, difficult to visualize. Vitrification/Freezing
- Day 8.5-9:
  risk that embryo is too large and fragile, need transfer with A.I. pipette.

  TAKE HOME MESSAGE:

  - the embryo can reach the uterus later in old mares

  - A.I. Post-ovulation/Frozen semen: add 4-12 hours to fertilization and start of embryo development

  WHAT IS DAY 7 ? Same day next week

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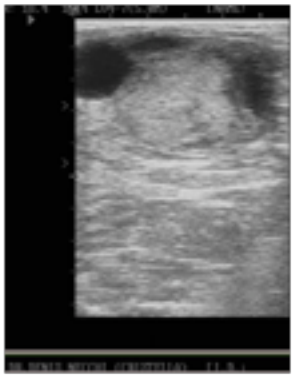
DAY OF COLLECTION: Planning



- Ovulation seen this morning:
  (Donor mare examined daily) the next week same day or next week plus one day
  BUT IN THE MORNING
- Ovulation early today :next week same day afternoon or same day plus one in the morning
- Ovulation in the afternoon: next week same day plus one morning or afternoon

If double ovulations 24 hours asyncronous :
8 days after the first ovulation Necchi Denis ©

WASHING PROCEDURE

- Destinate containers only for E.T.
- Eliminate all the disposables
- Immediately rinse catheter and reusables with tap water
- Use inorganic soap (CONTRAD 2000)
- Rinse for 5 to 10 times with distilled water
- Once dried sterilize in autoclave

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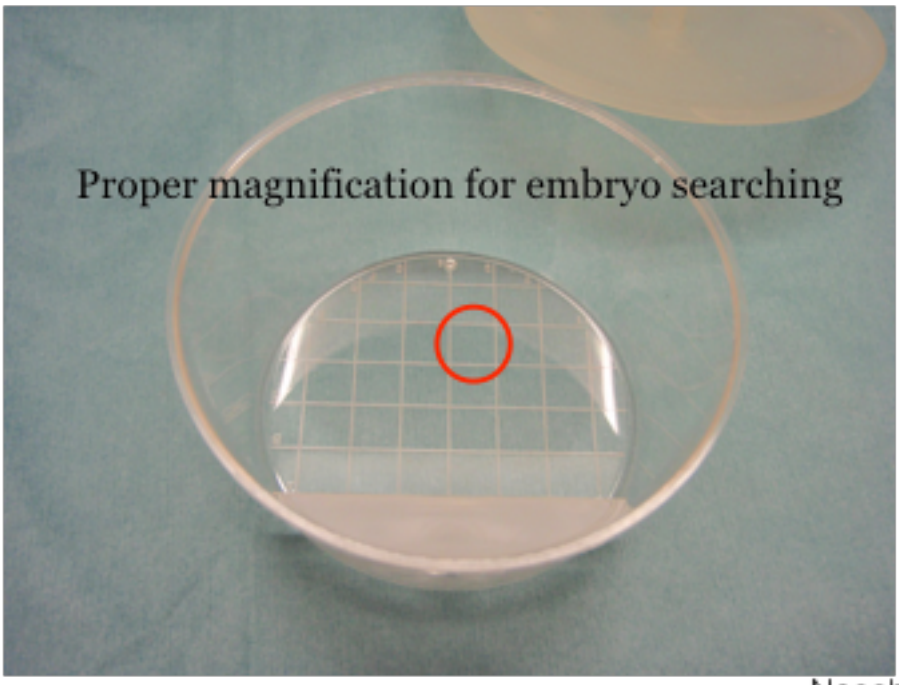
IDENTIFICATION EVALUATION AND MANIPULATION OF EQUINE EMBRYOS

IDENTIFICATION: MICROSCOPE

- Good quality stereomicroscope
- Enough vertical space for filter
- Magnification 10-40x, zoom 0,7-4 x
- Mirrored light
- Hand support (not necessary but helps)
- Trinocular (camera)
- Micrometer?

IDENTIFICATION
EZ-WAY FILTER



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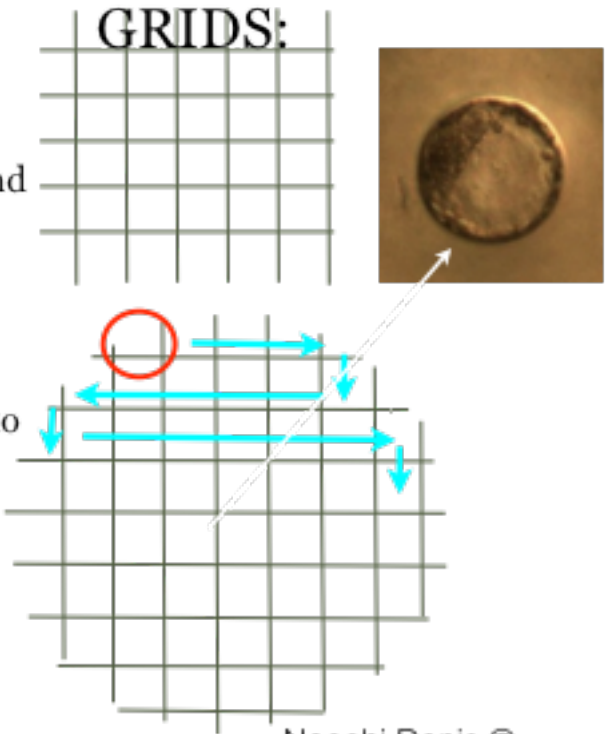
IDENTIFICATION

- Start at one square magnification
- Squares are identified with letters and numbers
- Embryo diameter 1/3 to 1 width of grid line
- Gently move the filter, embryo tent to go in the middle
- Bring bottom into focus
- no embryo start again with double magnification



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IDENTIFICATION & MANIPULATION WHAT ELSE?

- HOLDING MEDIA OR WASHING SOLUTIONS: are different from flushing medium (higher protein concentration, nutrients and antibiotics)
- EM-CARE, Vigro,IMV, Syngro, Equi-pro holding medium), 6-20-50 ml. Kept +5 °C
- Syngro can be stored between 2°C and 30°C

IDENTIFICATION & MANIPULATION WHAT ELSE?

- Multiwell dish (5-12 wells)
- Sterile straws (0,25 ml crystal clear + short straws)
- Sterile straws 0,5 ml for large embryos
- micromanipulator (roller) or syringe/straws adaptors

MANIPULATION: WASHING THE PUPPY

- The goal is to move the embryo from the first well to the last, removing as many debris as possible during the procedure
- Pass through minimum 4-6 baths
- Evaluation and grading at the beginning, during or at the conclusion of the wash steps
- Use 0,25 short straws to move the embryo

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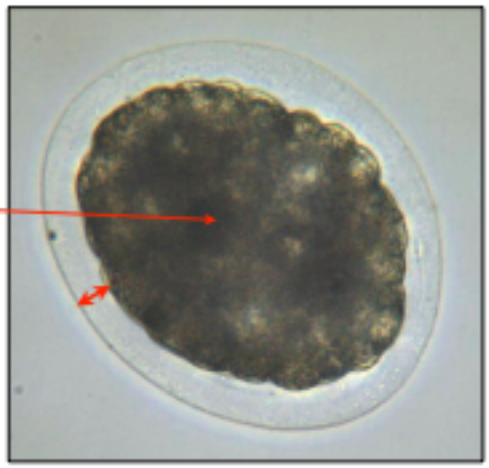
MORULA DAY 6-6,5

- Size 150-200 µm
- Solid mass of bastomeres, initially large and individually identifiable. Later it becomes compact aggregate of smaller blastomeres.
- Thick zona pellucida



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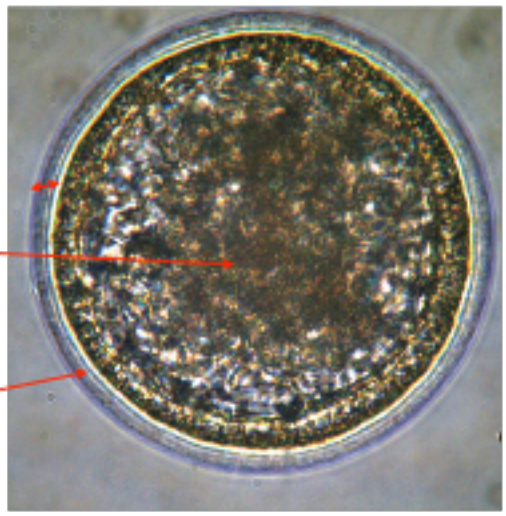
EARLY BLASTOCYST: DAY 6,5 - 7

- Size 150-300 µm
- Thick zona pellucida, becoming thinner because the embryo is growing and stretching the zona
- Blastocele cavity beginning to form between blastomeres
- Capsula identifiable below the zona



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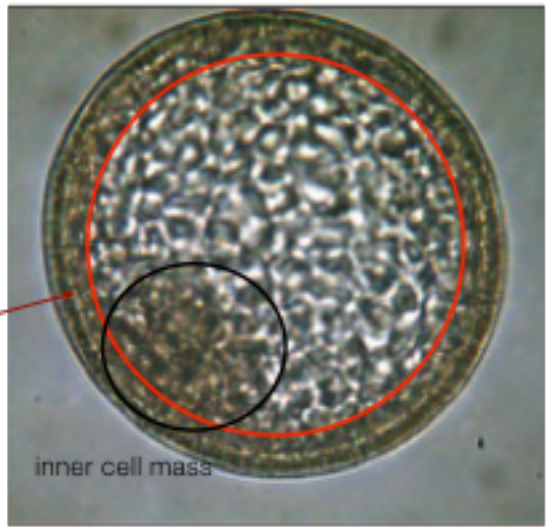
BLASTOCYST: DAY 7-8

- Size 200-500 µm
- blastocele cavity surrounded by a layer of trofoblast cells
- Inner cell mass develops
- Capsule evident between trophoblast layer and zona pellucida
- Zona pellucida thinner



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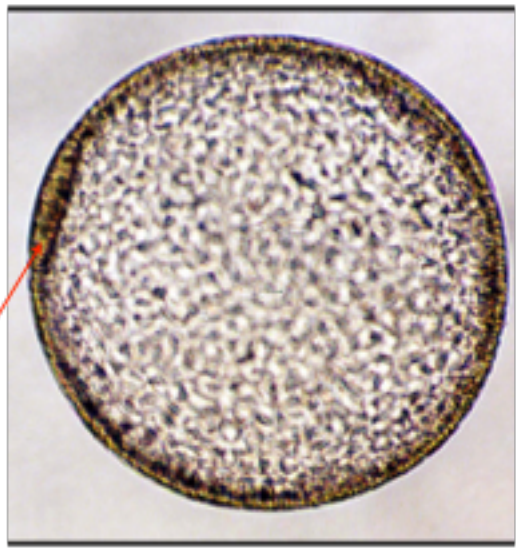
EXPANDED BLASTOCYST: DAY 8-9

- Size 500 - 3000 µm
- Large blastocele cavity fully developed
- Capsula is the external layer, zona may have been shed
- Capsula can be adherent or slightly detached
- Inner cell mass visible



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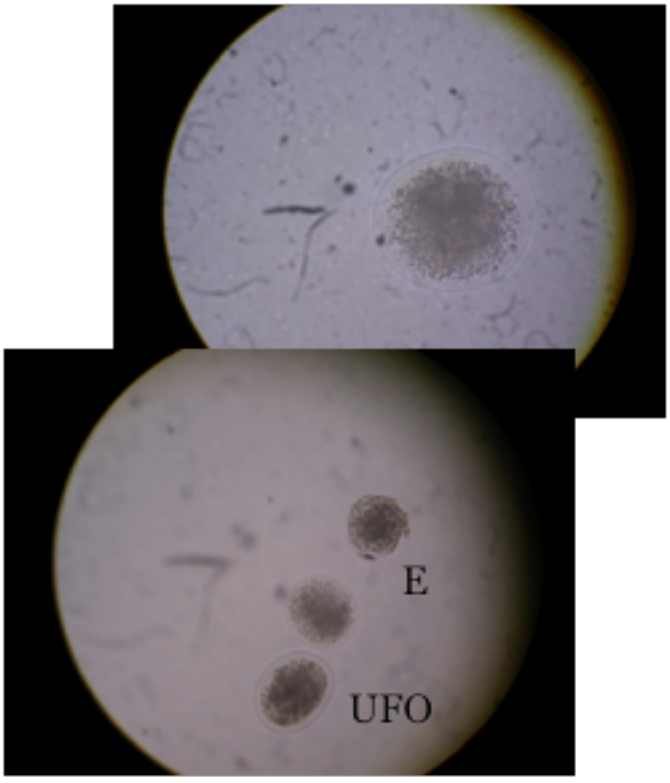
UNFERTILZED OOCYTE **UFO**

- Size 125-150 (similar to morula)
- Thick zona pellucida
- Mostly oval in shape
- Flat
- Membrane and cytoplasm may be degenerated



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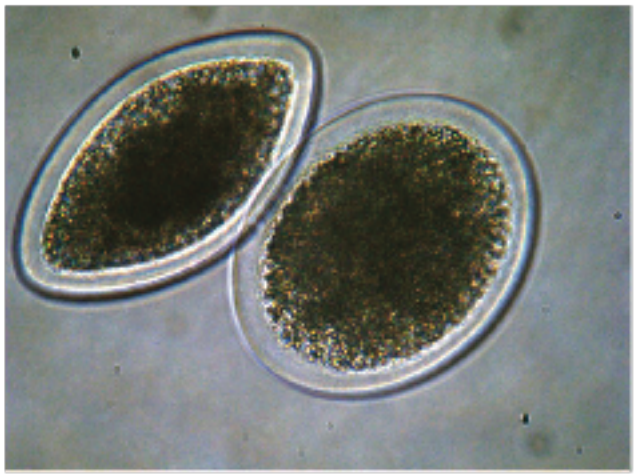
UNFERTILZED OOCYTE **UFO**

- Generally retained within the oviduct (Mares)
- If UFO and no embryo: keep looking, re-flush the mare
- They need a viable embryo to be trasported through the UTJ into the uterus
- DO NOT ROLL WHEN MANIPULATED (vs embryo)



A pair of UFOs

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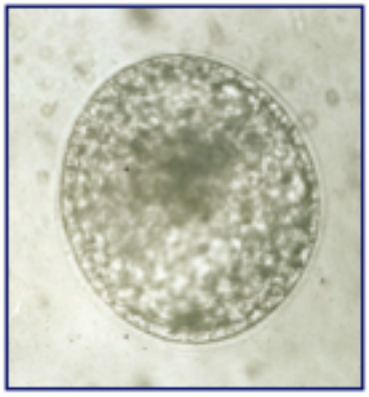
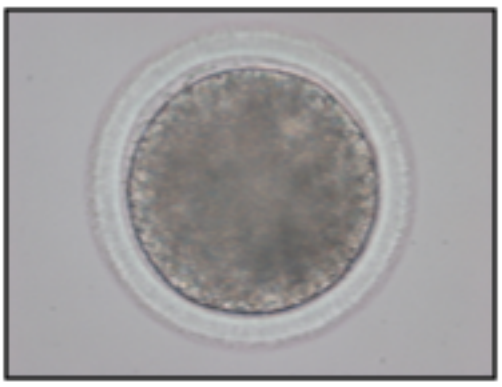
EMBRYO EVALUATION

MANUAL OF INTERNATIONAL EMBRYO TRANSFER SOCIETY:

- GRADE 1: EXCELLENT
  No abnormalities observed, spherical, cells uniform in size, color and texture; size and development appropriate for post-ovulation period. Intact zona pellucida





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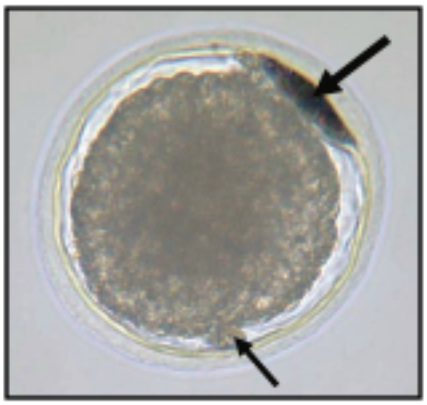
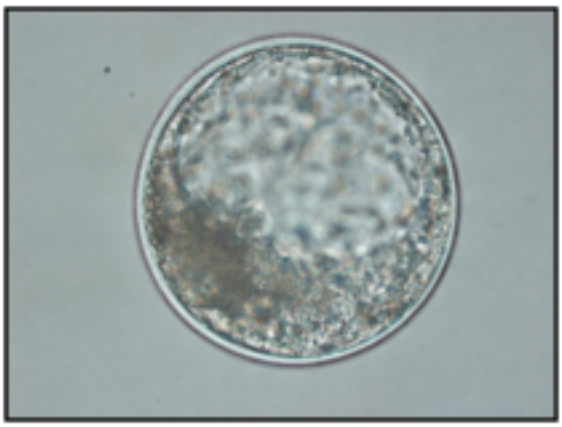
EVALUATION

- GRADE 2: GOOD Minor imperfections, i.e. few extruded blastomeres. Minors abnormalities in shape, size, colour and texture. Limited separation between layers





Arrows: extruded blastomeres

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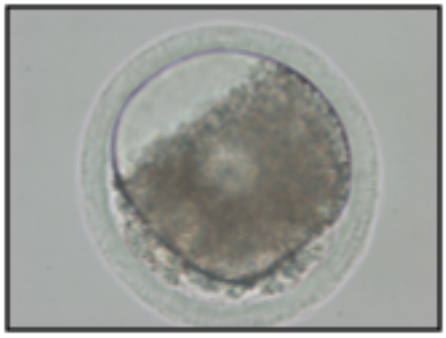
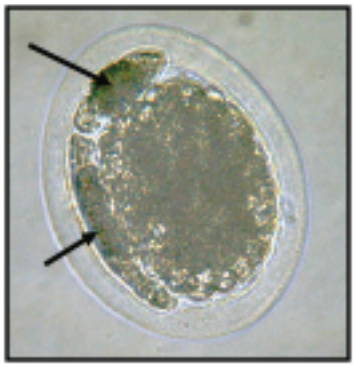
EVALUATION

- GRADE 3: POOR
  clear imperfections, larger percentage of extruded or degenerated blastomers, blastocele partially collapsed, moderate shrinkage of trophoblast from zona pellucida or capsule





Arrows: large percentage of extruded blastomeres

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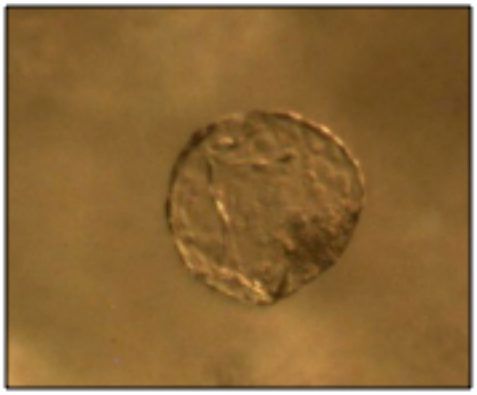
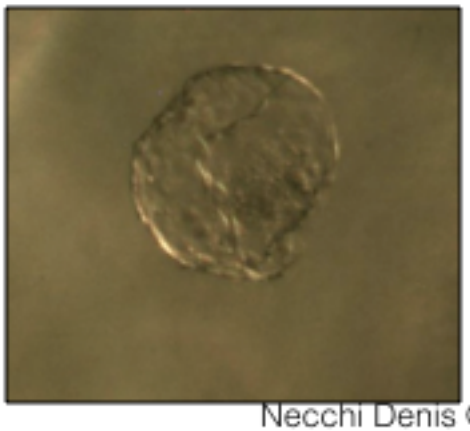
EVALUATION

- GRADE 4: DEGENERATED OR DEAD
  Severe problems easy to identify, high % of degenerate blastomeres; partially collapsed of blastocele, moderate shrinkage of trophoblast from zona pellucida or capsule





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ESTROUS SYNCRONIZATION

- NORMAL CYCLING MARES

- PGF : Shortening the luteal phase. Administer PG to the recipient 1-2 days after the donor. Ovulation aspected in 7-10 days la

- progesteron-type+ PGF : shortening transitional phase. Start with follicles >35 mm, for 10-14 days, ovulation aspected in 10-12 days.

LOW DOSE DESLORELIN: 50-100 μg, IM, q12 h
Works well in transitional mares (follicle ≥25mm)
Average duration treatment 3-5 day
Administer hCG to induce ovulation
GnRH agonist therapy is not effective in all the mares.
Mares tent to revert back into anaestrus after ovulation if the treatment is started too early

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REMINDERS
INTERVAL FROM PROSTAGLANDIN ADMINISTRATION TO NEXT OV

Mare with 35 mm follicle in diameter at the time of PGF:
14,5%: Ovulate within 48 hours without edema
75.4% : Ovulate after 48 hours with uterine edema
10,1% : regression without ovulation and emergence of a new follicular wave

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Burden et al (2014)

REMINDERS
INTERVAL FROM PROSTAGLANDIN ADMINISTRATION TO NEXT OV

- Duration from PGF to the next ovulation is 7 to 10 days
- Interval is **inversely proportional** to diameter of the largest follicle at time of treatment
- Mares with small follicles take longer to ovulate after PG

Follicle size (mm)	Interval (days)
10-14	9,6±0,2
15-19	8,6±0,2
20-24	7,6±0,2
25-29	6,7±0,4
30-35	4,9±0,4
≥35	unpredictable

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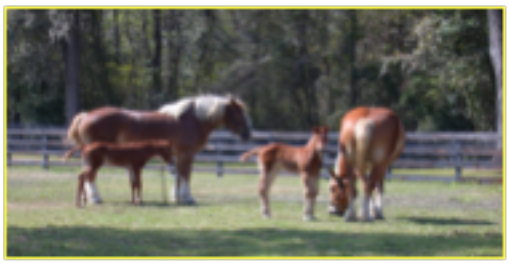
Loy et al, 1979
Burden et al, 2014

1. SELECT RECIPIENT

Recipient selection (age, day after ovulation, quality on day 5) significantly affected pregnancy and embryo loss rates (Carnevale et al., 2000).

To select the best recipient mare is the best way to increase your success in E.T. and.....
Profits are directly tied to success



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SELECT THE RECIPIENT

FACTORS THAT DISQUALIFY RECIPIENT :

- poor quality cycle
- ovulation within 2 days after receiving PG
- failure of ovulation or development of AHF
- no edema during the cycle
- fluid
- medical problems

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2. TREATMENT PRE-TRANSFER

- Acepromazine, Detomidine + Butorphanol or Xilazine 20% + butorphanol, relax the mare and the cervix
- NSAID: 10 cc of Flumixin Meglumine 15 minutes before the transfer, blocks PGF2α release (questionable effect)
- ANTIBIOTICS: not effective

2. TREATMENT PRE-TRANSFER

ACEPROMAZINE (2-2,5 ml), DETOMIDINE (0,4 - 1 ml), BUTORPHANOL (0,2-0,5 ml)

ADVANTAGES:

- The transfer is easier (maiden and fresh recipients)
- The transfer is safer (vet, embryo)
- Cervix is more relaxed

DISADVANTAGES:

- Perineum and vagina very relaxed, difficult to work on the cervix in maiden mares

DO IT, or when you will realize the need it could be too late

4. PERFORM THE TRANSFER

EQUIPMENT

- 0,25-0,5 ml straws
- Cassou Guns for 0,25 ml (Bovine) 0,5 ml (Equine)
- A.I. catheter for large "unexpected" embryos
- Sterile or non-spermicidal Lube
- Long sterile plastic sleeves

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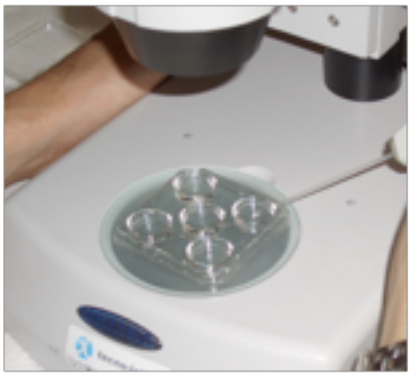
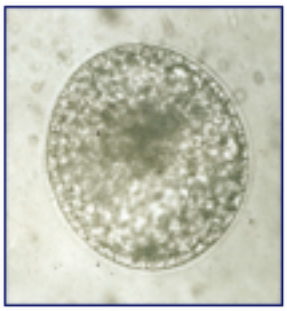
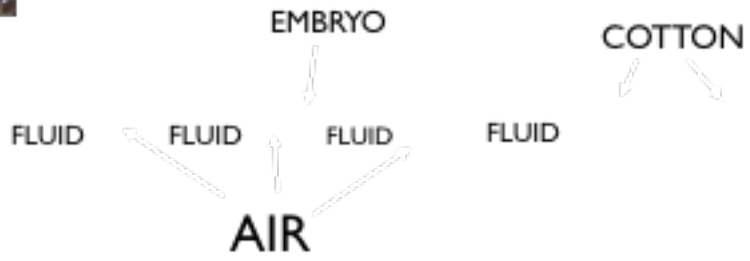
4. PERFORM THE TRANSFER







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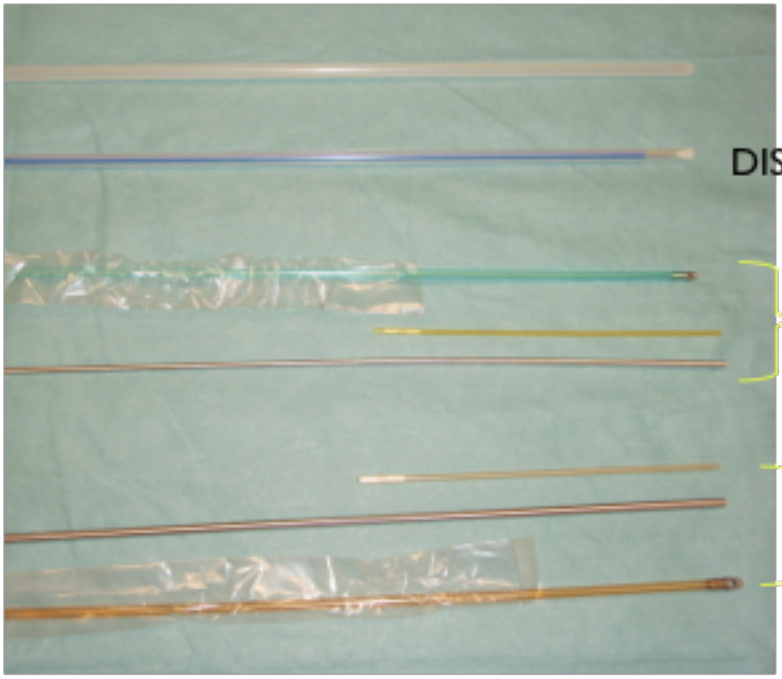
4. PERFORM THE TRANSFER

TRANSFER GUNS



A.I. CATHETER
(LARGE EMBRYOS)

DISPOSABLE GUN

Cassou gun 0,25 ml
IMV (bovine)

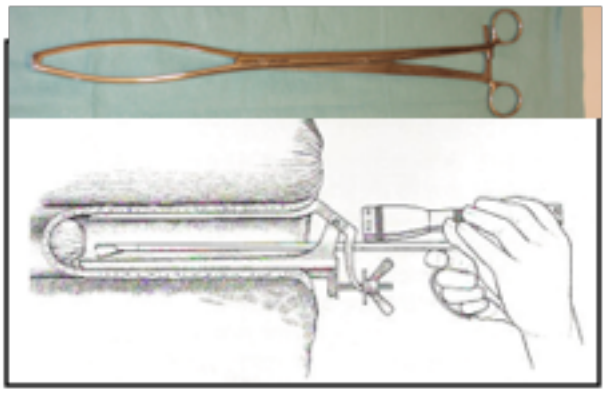
Cassou gun 0,5 ml
IMV (Day 8)

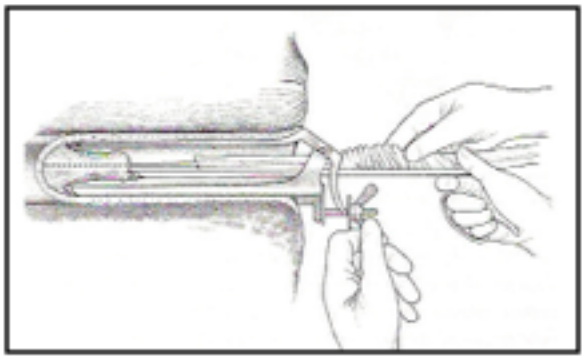
4. PERFORM THE TRANSFER

1. SURGICAL TRANSFER: obsolete, research, rarely used, not legal in most of the EU countries
2. NON SURGICAL TRANSFER WITH SPECULUM
3. NON SURGICAL TRANSFER: with or without rectal guide

NON SURGICAL TRANSFER WITH SPECULUM

- S.WILSHER, W.R.ALLEN; An improved method for nonsurgical E.T. in the mare. E.V.E; 02/04





ADVANTAGES

Reduced mechanical action on the cervix

No contamination of the vaginal canal

Higher pregnancy rate?

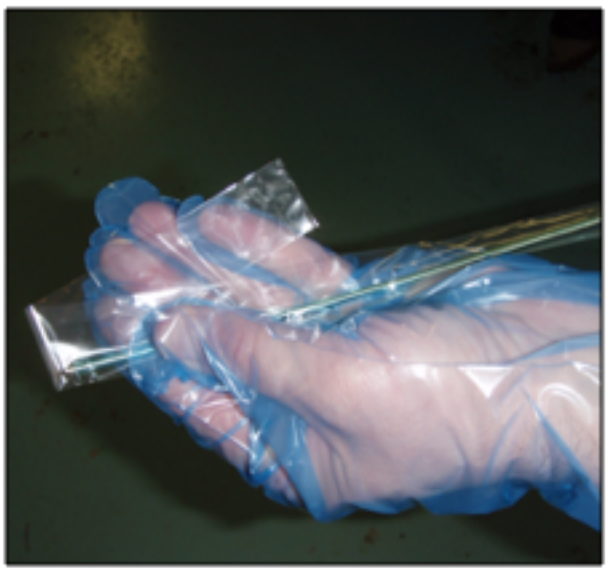
DISADVANTAGES

Epidural anesthesia ?

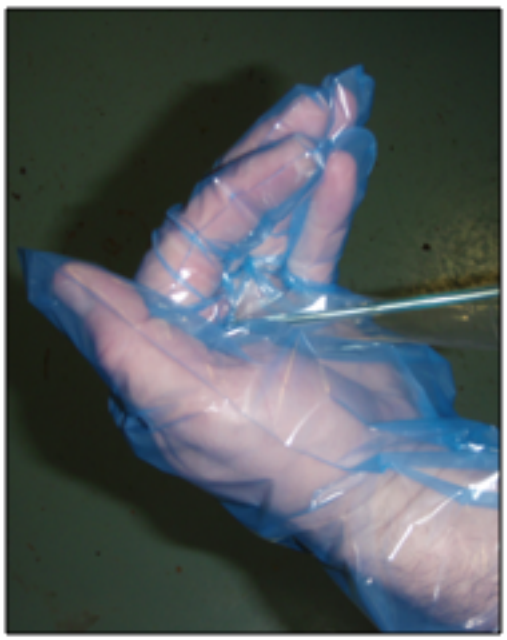
More air inside the vagina

More time and people

4. PERFORM THE TRANSFER



Bend the soft
hygienic plastic sheath

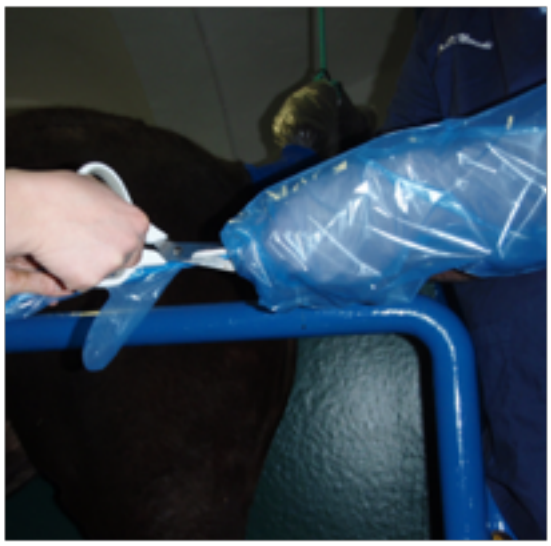


Protect the tip of the "PISTOLETTE"

Double sleeve technique

Necchi Denis ©

Double sleeve technique



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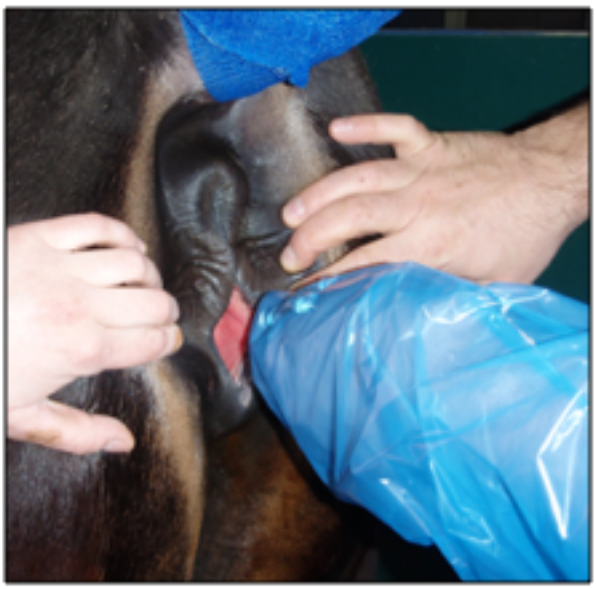
4.PERFORM THE TRANSFER



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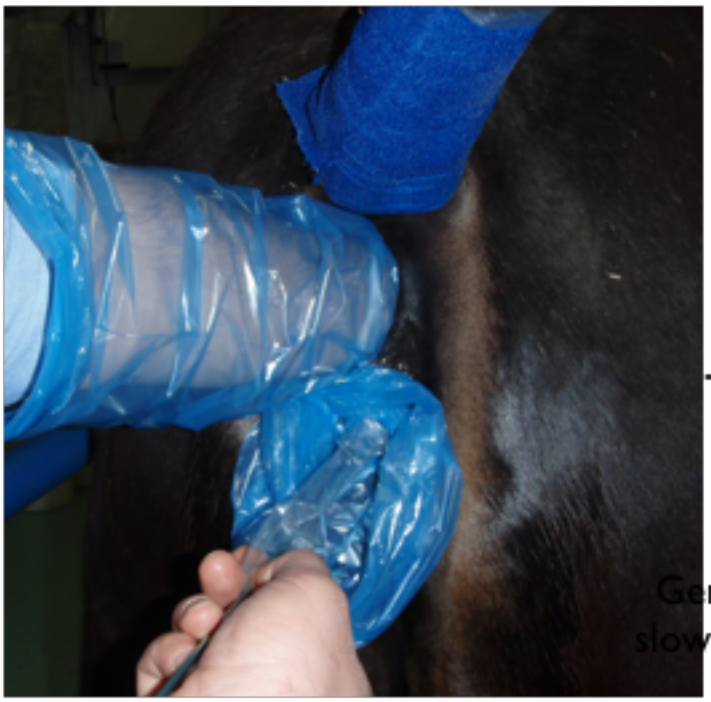
4. PERFORM THE TRANSFER



The sleeve left inside the vagina avoid contamination

<u>Arm inside the rectum:</u>
- confirm correct placement
- deep horn transfer

Gently deposit the embryo while slowing withdrawing the cassou gun

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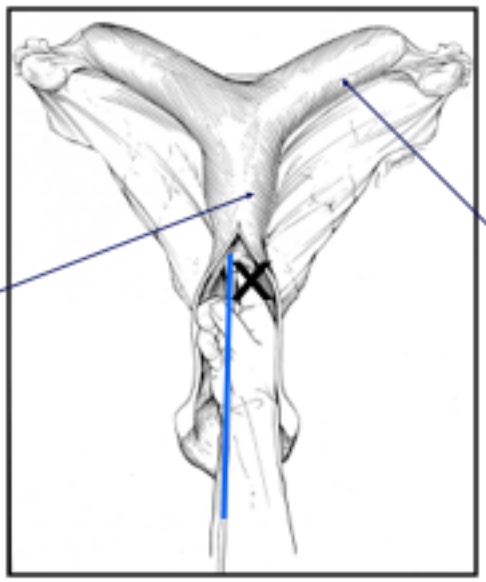
4. PERFORM THE TRANSFER

EMBRYO UTERINE PLACEMENT:
body *vs* horn



UT BODY

UT. HORN
rectally guided
↑ ox
↓ P4

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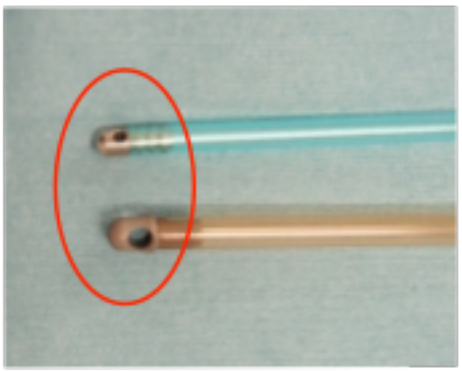
4. PERFORM THE TRANSFER

- AFTER THE TRANSFER ALWAYS RINSE OUT THE TIP OF THE CASSOU GUN INTO A PETRI DISH TO MAKE SURE THAT THE EMBRYO WAS NOT RETAINED AT THE TIP OF THE INSTRUMENT



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PREGNANCY RATE

PR	EVALUATION	COMMENTS
≥ 90%	OUSTANDING	Difficult to consistently achieve with large number of transfers
80-90%	Excellent	Achievable with effort
75-80%	Very good	A solid goal
70-75%	good	Work on details
60-70%	Fair	Need to improve
<60%	Marginal	Need remedial help

Necchi Denis ©

VITRIFICATION KIT

Vs1: 1,4 M glicerol
Vs2: 1,4 M glicerol + 3,6 M ethilen glicol
Vs3: 3,4 M glicerol + 4,6 M ethilen glicol
(high concentration of cryoprotector)
Diluent : 0,5 M galactose

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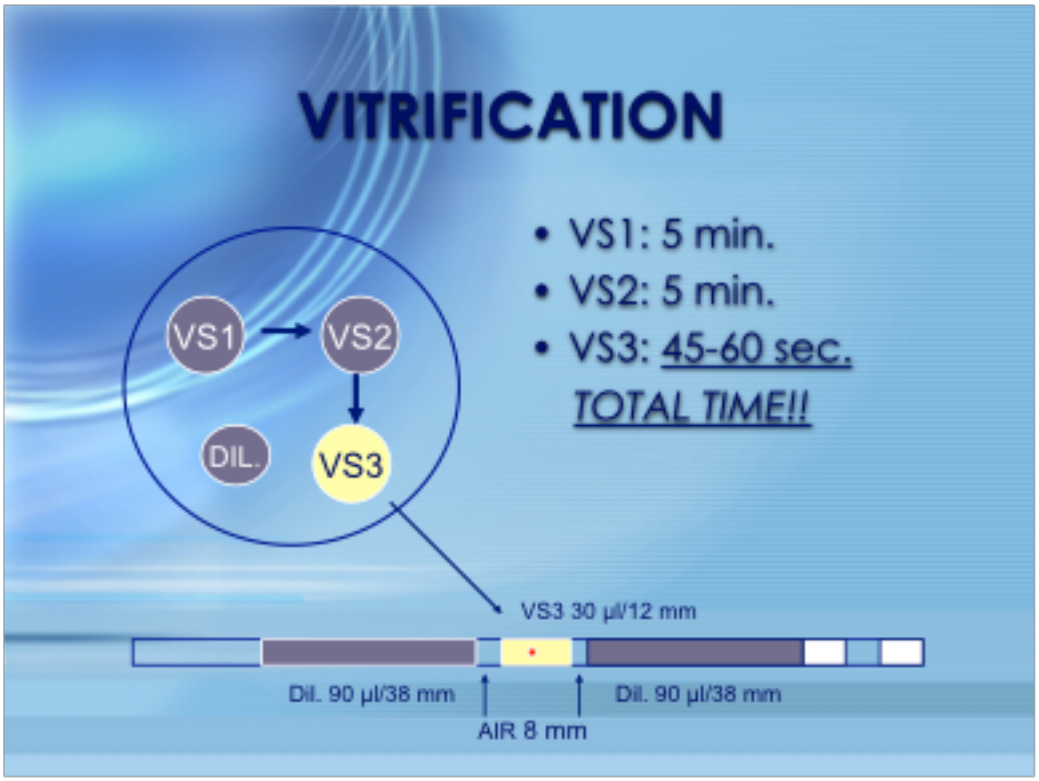

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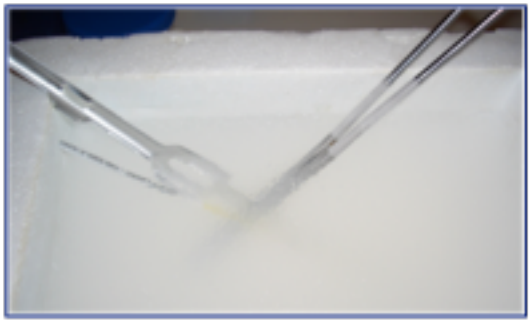
VITRIFICATION

- Plug the straw into a empty goblet without liquid nitrogen
- After one minute submerge the goblet and the straw
- Transfer in a storage tank



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