

# ASSISTED REPRODUCTIVE TECHNIQUE: EMBRYO TRANSFER

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## EMBRYO TRANSFER

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- ◆ refers to the procedure for collecting a fertilized ovum (embryo) from a donor mare and transfer into a **synchronized** recipient mare
- ◆ First ET, involving rabbits, reported more than a century ago (1890)
- ◆ Developed for use in horses in the early '70s (1974, Japan)
- ◆ Started for commercial use in the late '80s



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## E.T. ADVANTAGES (1)

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1. Foals from sport mares in training
2. More than one foal from valuable mares, potentially 5,  
(according to legislation)

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## E.T. ADVANTAGES (2)

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3. 2 years old mares
4. Mares with pathologies or abnormalities causing sub-fertility or  
Early Embryonic Loss

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## E.T. ADVANTAGES (3)

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5. Old sub-fertile mare
6. No risk at parturition for the donor
7. Late foaling mares

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## E.T. DISADVANTAGES

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- ◆ High costs
- ◆ Technical expertise needed
- ◆ Expectations of clients disattended by results
- ◆ Detrimental effect of multiple flushings on donor endometrium
- ◆ More than one recipients per donor would guarantee more results

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# EMBRYO TRANSFER EXPECTATIONS

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- ❖ Stallion/ semen fertility x Donor mare fertility

$\% \text{ Embryo Recovery (50-70\%)*}$

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- ❖  $\% \text{ Embryo Recovery} \times \% \text{ successful transfer}$

$\% \text{ Recipient in foal at 14 days (50-70\%)*}$

\* Texas A&M data

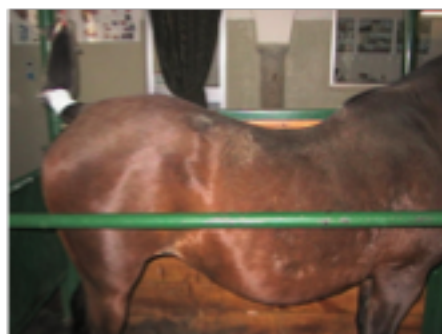
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# SELECTION DONOR/RECIPIENT

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- ❖ General physical examination
- ❖ Reproductive History
- ❖ Conformation of the mares back
- ❖ Conformation of the perineal area



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# SELECTION DONOR/RECIPIENT

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## COMPLETE A BREEDING SOUNDNESS EVALUATION

- Size and tone of the uterus
- Size, tone and shape of the cervix
- Status of the estrous cycle
- Uterine abnormalities  
Fluid, cysts, air,
- Ovarian abnormalities  
i.e. tumors



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# SELECTION DONOR/RECIPIENT

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## EXAMINATION OF THE VAGINA

- ❖ Use a sterile disposable speculum
- ❖ Visualization of the vagina:  
Persistent hymene  
Vaginal varicose veins  
Inflammation  
Pool of fluid / mucus / urine
- ❖ Visualization of the cervix:  
Adhesions  
Lacerations or tears

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# DIAGNOSTIC AIDS

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- ❖ Bacteriology
- ❖ Cytology
- ❖ Uterine Biopsy

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# SELECTION OF THE DONOR MARE

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- ❖ Breed registry guidelines: is Embryo Transfer allowed or not for the mare?
- ❖ Broodmare sport horse
- ❖ Value of the mare
- ❖ Potential value of the foal
- ❖ Costs
- ❖ Is she a good candidate?

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## MANAGEMENT OF THE DONOR: Crucial Points

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- ❖ The cycle of the donor set the rhythm
- ❖ Good hygiene practices
- ❖ Choices of stallion and semen type
- ❖ Accurate monitoring of cycle and time of ovulation
- ❖ Allow the donors to foal naturally after 2-3 years of embryo transfer

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## SELECTION OF THE RECIPIENT(S)

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General conditions and reproductive health are crucial:

- ❖ 3-10 years of age
- ❖ Good size, 450 – 600 kg
- ❖ Good body conditions
- ❖ Healthy
- ❖ Maiden or not Maiden?
- ❖ “3 foaling max”
- ❖ Good behavior, broken and easy to handle
- ❖ Good perineal conformation

**Acquisition of quality recipient remain one of the most important component of successful ET program**

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# MANAGEMENT DONOR/RECIPIENT

## Synchronization

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- ❖ Induce ovarian activity or shortening of the transitional phase
- ❖ Regular cycling mares
- ❖ Try to obtain the best reproductive situation
- ❖ Daily ultrasound examination when in estrous
- ❖ Induce ovulation with hCG or GnRH implant
- ❖ AI with fresh, cooled or frozen semen
- ❖ Determine the time of ovulation, accuracy is important
- ❖ **Day of ovulation = day 0**

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# ESTROUS SYNCRONIZATION

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## - 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

- 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 days

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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# DAY OF COLLECTION

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## When we do the uterine flushing?

- ❖ 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  $\mu\text{m}$
- ❖ Best freezing results with embryos smaller than 250  $\mu\text{m}$  (day 6.5)

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# DAY OF COLLECTION

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- ❖ DAY OF OVULATION: Day 0
- ❖ DAY 6,5-7: (morula / early blastocyst) is small, can be still in the oviduct, difficult to visualize. Freezing / vitrification
- ❖ DAY 7-8: (blastocyst) normal routine, larger and easier to visualize / manipulate
- ❖ DAY 8,5-9: (expanded blastocyst) can be too big and fragile.  
Old mares: the embryo can reach the uterus later.  
A.I post-ov / frozen semen: late fertilization

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# SYNCRONIZATION

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STAGE OF THE RECIPIENT THE DAY OF TRANSFER:

The aim is to have the recipient mare that has ovulated 1 day prior (+1) or 0-3 days after (0,-1.-2-3) the donor

On the day of transfer, the recipient should be on DAY 5 OR DAY 6 POST - OVULATION (Day 0= Ovulation)

Preferences in recipient mares:

Day 5/6   Day 4   Day 7   Day 2-3   Day 8

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## THE PROCEDURE : Uterine flushing

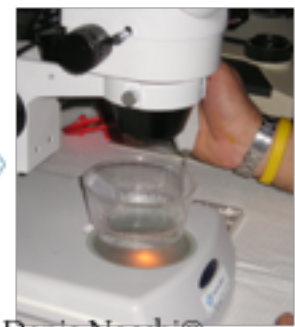
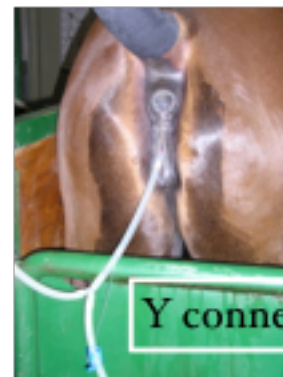
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- ❖ Clean the donor: Hygiene is crucial
- ❖ Introduction of the flushing catheter: passed through the cervix, balloon is inflated with 50-90 ml air

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# EMBRYO RECOVERY: flushing

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



# UTERINE FLUSHING

fill the uterus with 1-1,5 liters of pre-warmed solution



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# IDENTIFICATION AND MANIPULATION

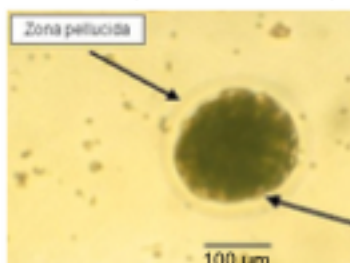
- ❖ Once identified move the embryo in the holding media:
- ❖ washing and grading (from 1-4)



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# SIZE AND STAGE OF THE EMBRYO

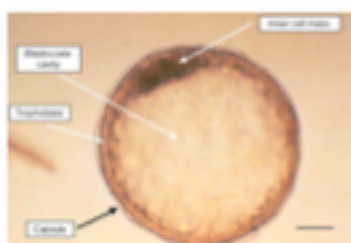
Morula: day 6,5; 150- 200  $\mu\text{m}$



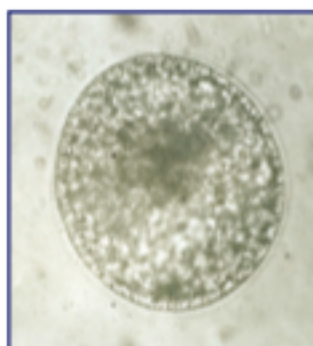
Early blastocyst : day 7; 150-200  $\mu\text{m}$



Blastocyst 200-400  $\mu\text{m}$ : day 7,5-8



Expanded Blastocyst: day 8-9  
400  $\mu\text{m}$ - 1mm



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# Day of collection and embryo size

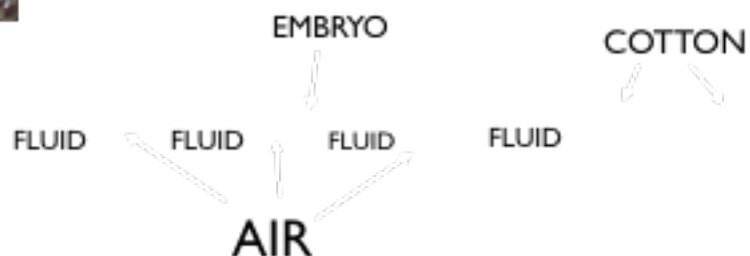
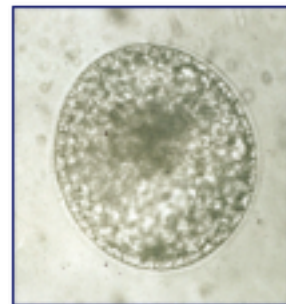
Day Post-ov	# embryos	Mean (mm)	Range (mm)
6	121	0,208	0,132-0,756
7	144	0,406	0,136-1,460
8	142	1,132	0,120-3,980
9	41	2,22	0,730-4,520

The diameter doubles every day

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Peter Daels

# LOAD THE EMBRYO



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# EMBRYO TRANSFER

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*SELECT THE RECIPIENT IN  
CASE WE HAVE MORE THAN  
ONE AVAILABLE:*

- ❖ Size of the donor
- ❖ Days after ovulation
- ❖ Quality of the CL (doppler)
- ❖ Ultrasound uterine appearance
- ❖ Uterine and cervix tone

*Always ultrasound the recipient immediately prior the transfer*

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# EMBRYO TRANSFER

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- ❖ Administer  
Butorphanol+detominide
- ❖ Empty the rectum
- ❖ Start to wash the perineal  
area of the recipient
- ❖ Perform the transfer in the  
cleanest way possible



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## 5. TREATMENT AFTER TRANSFER

- ❖ more severe decline in P4 with smaller embryos, some drop below the critical point
- ❖ P4 supplementation does not result in an increase in pregnancy rate
- ❖ P4 supplementation is associated with a decline in P4 endogenous secretion in 10% of mares
- ❖ If started, the treatment should not be stopped before 90-100 days or 50-70 days with visible secondary CL's
- ❖ REGUMATE®: 0,044 mg/kg orally SID, from the transfer to 90-100 days. Stop treatment gradually in 5-10 days

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## PREGNANCY CHECK



**11-12 Days:**  
can be early but in time  
to recycle the donor



**14 Days**  
Normal or if open at 12;  
add one day for frozen  
or small embryos

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# ET Pregnancy rates

PR	EVALUATION	COMMENTS
≥ 90%	OUTSTANDING	Difficult to consistently achieve with large numbers
80-90%	EXCELLENT	Achievable with effort
70-80%	VERY GOOD	Solid goal
60-70%	FAIR	need to improve
<60%	MARGINAL	Need remedial help

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## FACTORS AFFECTING E.T. SUCCESS

- ◆ Donor Mare (Age, Reproductive Status)
- ◆ Quality and Type of Semen (Fresh, Cooled, Frozen)
- ◆ Number of Flushing per Uterine Lavage
- ◆ Recipient Mare
  - ◆ Synchrony Recipient/Donor
  - ◆ Embryo (Age, Morphology, Grade)
  - ◆ Embryo Type (Fresh, Cooled, Frozen)
- ◆ Transfer Technique
- ◆ Pre or Post-Transfer Treatments
- ◆ Experience of Technician
- ◆ Number of transfers for the recipient

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# COOLED TRANSPORTED EMBRYOS

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- ❖ If recipient are not available Embryos can be shipped



Embryo+ holding medium

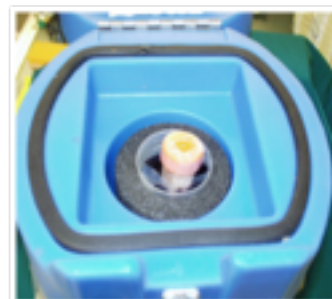
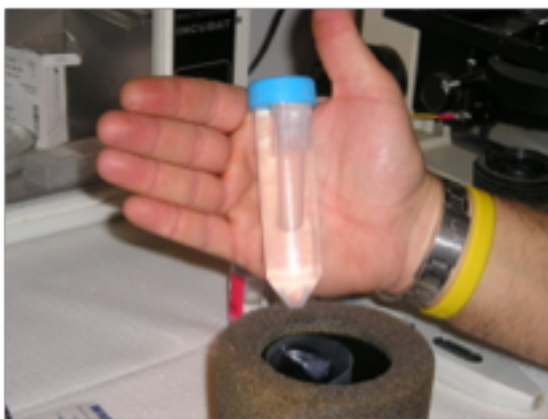
Ringer lactate

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# COOLED TRANSPORTED EMBRYO

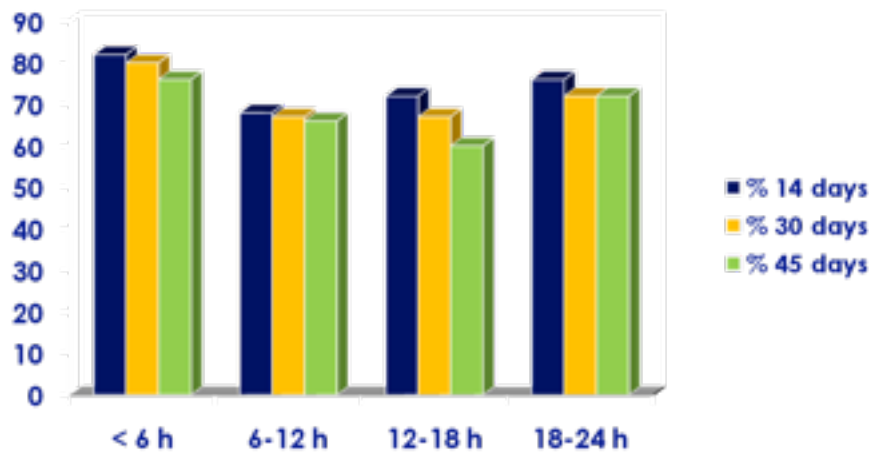
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Insert information of the Donor, stallion, owner

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## PREGNANCY RATES WITH SHIPPED EMBRYOS



KEROS: after 210 transfer

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## CRYOPRESERVATION

Small embryo (6-6,5 day after ovulation) can be stored in liquid nitrogen for later transfer

VITRIFIED: no ice crystal,  
transparent, good pregnancy rate

FROZEN: difficult for practitioners,  
require experience

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