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## Equine Embryo Transfer

### 1. Introduction

General Introduction (i.e. what is ET)

Advantages and uses of embryo transfer

Changes in the ET Industry

Breed regulations (include a Table of most major breeds; ET, frozen embryos, etc.)

Per cycle ET Success = Embryo Collection Rate (50-60%) x Embryo Transfer

Pregnancy Rate (70-80%)

Goals of the Manual

### 2. History of equine embryo transfer

Livestock

Horses

Domestic horses as surrogates for endangered equids(?)

### 3. Reproductive Anatomy and Physiology of the Mare

Anatomy of the mare

Physiology of the estrous cycle

Physiology of early embryonic development and early pregnancy

### 4. Management of the donor mare

Selection of the donor

Evaluation of the donor (BSE)

Management and Day of breeding (fresh, cooled, frozen semen)

Palpation/ultrasound examinations relative to flush; daily vs every 6-8 hrs for frozen semen; BID if goal is to collect a small embryo at day 6.5 for cryopreservation

Induction of ovulation (hCG and deslorelin)

Donor mare management (PMIE, fluid, etc.)

Estrous cycle control (Lights, P&E, PGF, hCG, Deslorelin, Regumate)

Allow a mare to carry to term by approximately 10 years of age

Allowing mare to carry own every 3-4 years

Effect of repeated flushing on uterine health and embryo recovery

# flushes per year recommended

Fertility of mares after flushing (i.e. same season)

Problem mares (i.e. PMIE, etc.)

Maiden mares (young vs older)

Post partum mares (i.e. flushing on foal heat)

### 5. Superovulation

History

Techniques

EFSH

Optimal follicle size at onset...

Problems – same stallion vs. goal of different stallions  
Not every mare responds to FSH  
PAF's and HAF's

## **6. Embryo Collection**

Equipment (Box Table)  
Facilities (stocks vs stall, etc.)  
Procedure; (incl. clean out and wash up)  
Ultrasound prior to flush in problem mares (PMIE) for fluid detection  
Day of flush – options day 6.5, 7, 8, 9  
Fluid volumes relative to maiden, open and post-foaling mares  
Number of lavages per flush attempt  
Rectal manipulation of uterus to move fluid around (massage)  
Direct visualization of embryos in cup  
Looking for embryos after each lavage  
Techniques (Standard vs French, Fernando Rivera)  
Medications (oxytocin, sedation, buscopan, etc.)  
Reflush option (Extra flush same day standard; next-day reflush option;  
superovulation reflush ( $\geq 50\%$  embryo recovery relative to ovulation guideline)  
PGF after flush; why (luteolysis – clean up and avoid unwanted carry-own  
pregnancy); what happens if you do not; option to let mare carry

## **7. Factors affecting embryo recovery**

Donor age and reproductive status  
Day of recovery  
Number of flushes  
Stallion effects  
Number of ovulations (single vs. spontaneous multiple, superovulation)  
Effect of ovulation rate and side of multiple ovulations on recovery rate (Fernando Riera data)  
Synchronization of ovulations – embryo size and recovery  
Reflushing (same day, next day)

## **8. Embryo Handling**

Equipment – straws, dishes (size, round vs square)(Box Table)  
Search procedures  
Debris in dish (how to handle)  
Miscellaneous items in dish  
Swirling dish  
Embryo size expected  
Embryo morphology expected  
Hints regarding bubbles, etc. (swirl, let contents settle, then aspirate bubbles along edge)

## **9. Washing and holding embryos**

# and sizes of drops  
Types of holding media; how long to hold a fresh embryo  
Types of wash dishes (flat vs round bottom)  
Storage vessels (dishes vs straws)

## **10. Evaluation of embryos**

Morphology

Grade

Size

Lots of photographs and drawings

ET Log (flush and transfer logs)

## **11. Cooled Storage and Transport of Embryos**

When to cool (i.e. how many hours between flush and transfer)

Cooled embryo technique

Time limit for holding embryos

Media available (types; buffer systems, etc) Ham's F-10

Equipment

## **12. Cryopreservation of Embryos**

History of embryo freezing

Slow freeze vs Vitrification

Selection of embryos (flush days, embryo size, etc.)

Vitrification technique (supplies, method)

Storage of vitrified embryos

Warming and transfer

Pregnancy results

## **13. Management of Recipient mares**

What makes a good recipient

Selection – age, size, parity, temperament, physical health

History of mares (barren, maiden, foaling)

Examination schedule

Examination of recipients – 5 day check; pass system

Housing recipient mares

Synchronization options (new data from perla); general 'window' of synchrony (+1 to -3 or -4)

Line up recipient with embryo characteristics (fine tune)

Recipient:Donor Ration (3:1) for synchronization

Individual recipient for single donor (1:1) – how to manage

'Floating' recipient herd

Synchronization schemes

Optimal day(s) of transfer

Management after transfer (housing, hormones, etc.)

Use of non-cycling, ovariectomized, XO and pregnant mares as recipients

Using the donor mare as her own recipient (in the event of twin embryos)

## **14. Transfer Procedures**

Surgery (midline, flank) [Old school] vs Nonsurgical/transcervical

Speculum procedure (Allen and Wilshire)

Equipment for nonsurgical (Box Table)

Day of transfer

Medications (pre and post)

Prostaglandin release during transcervical transfer (p4 Graph)

Technique – details

The 'art of transfer'

### **15. Factors affecting pregnancy rates**

Age and reproductive status of donor mare  
Embryo age, quality and size  
Transfer technique, technician variability  
Recipient factors  
Expected pregnancy rates (day 16 vs day 50 vs foaling)  
Carry to term data (AQHA data)  
Twins/Triplets from transfer of a single embryo

### **16. Pregnancy examination after transfer**

Days of examination (11, 12, 14, 16, 25, etc)  
Relationship between embryo size at transfer and first day visible on ultrasound (graph)  
Percentage of truly pregnant recipients with embryos visible at 11, 12, 14, 16 days (graph)

### **17. Disease transmission with embryos**

### **18. International transport of embryos**

### **19. Miscellaneous**

Embryo micromanipulation (splitting)  
Embryo sexing

### **20. Future directions of equine embryo transfer**

Superovulation  
Early pregnancy factor – know when to flush  
Improvement in reproductive management of problem mares (PGE oviduct)  
Assisted reproduction  
Embryo biopsy for genetic diagnosis

## **Appendix 1: ET Equipment and supplies**

Sources

Catheters

Fluid types (LRS vs Complete vs old style PBS); osmolarity; pH; stability/shelf life; protein source (FCS, albumen vs PVA) to prevent embryos from sticking; ingredients (general); buffer systems (if any)

Y tubing

Filter cups – types (list and photos), how to use them (i.e. fill with fluid as per Fernando Rivera); direct visualization vs pour-off)

Search dishes (round vs square; size; gridded vs plain)

Microscopes and micrometer (types of scopes; magnification, glass – clear vs frosted; sources; new vs used)

Cleaning procedures (what can be re-used); autoclave; enzyme cleaning; gas sterilization).