**Non-Surgical Transfer:**
- The standard transfer technique for equine embryos
- Minimal equipment required
- Requires technical expertise
- Several variations
- Lots of opinions
- Lots of superstition

**Equipment for Non-Surgical Transfer:**
- Small embryos (< 1,000 µm) - transferred with a Cassou gun and sterile sheath/chemise
- Equipment List:
  - 0.25 ml straw
  - Straw connector
  - 1.0 ml syringe
  - Cassou gun
  - Sterile sheath and chemise

**Larger embryos (> 1,000 µm) - transferred with an AI pipette and sterile chemise**
- Equipment List:
  - AI pipette
  - 1.0 ml syringe
  - Sterile chemise

**Preparation of Recipient for Transfer:**
- Sedate (if needed)
  - Acepromazine (20 mg, IV) or
  - Xylazine (150 mg, IV)
- Non-steroidal anti-inflammatory drug
  - Flunixin meglumine (Banamine®) 500 mg, IV
- Wash, rinse and dry perineal area thoroughly
  - Swab vestibule as well to prevent contamination

**Load Embryo Into Straw:**
- Rinse straw with holding media
- Load ~ ½ straw with media
- Air gap(s)
- Load embryo in media
- Air gap(s)
- Load media until plug is wet
TRANSFER TECHNIQUES – SMALL EMBRYO

Load Embryo Into Straw:
- Embryo can be visualized within straw (if necessary)

TRANSFER TECHNIQUES – SMALL EMBRYO

Load Transfer Gun:
- Place straw into blue sheath
- Push straw to end of sheath with transfer gun
- Completely push gun onto sheath
  - Sheath will ‘split’ at end when fully on gun
- Lock sheath onto gun with blue ‘donut’

TRANSFER TECHNIQUES – SMALL EMBRYO

Transfer Step 1:
- Guard transfer gun and chemise within sterile gloved hand
- Apply small amount of sterile lube to hand
- Advance hand to cranial vaginal vault
- Identify external cervical os

TRANSFER TECHNIQUES – SMALL EMBRYO

Transfer Step 2:
- Place transfer gun at external cervical os
- Penetrate gun through chemise
- Gently advance gun through cervix
  - No not penetrate cervix with finger
  - Make absolutely sure gun is entirely through cervix and into uterus
  - Some manipulation may be necessary

TRANSFER TECHNIQUES – SMALL EMBRYO

Transfer Step 3:
- Withdraw gloved hand from vagina
- Apply more lubricant (non-sterile)
- Insert hand/arm into rectum
- Identify tip of transfer gun in uterus

TRANSFER TECHNIQUES – SMALL EMBRYO

Transfer Step 4:
- Straighten one uterine horn (manipulation per rectum)
- Gently slide transfer gun up horn
- Adjust tip of transfer gun to center of uterine horn lumen
  - Raise horn slightly with fingers
  - Lower transfer gun slightly with base of hand
TRANSFER TECHNIQUES – SMALL EMBRYO

Transfer Step 5:
- Slowly deposit embryo while slightly withdrawing gun
- Concept is to gently ‘lay embryo down’ in uterine lumen
- Withdraw gun from mare

Transfer Step 6:
- Take gun back into laboratory
- Rinse/flush tip of sheath with media
- Look through microscope to see if embryo is still present
  - Occasionally (~1 in 200 transfers) an embryo will still be in tip of sheath (transfer again)

TRANSFER TECHNIQUES

Transfer of Large Embryos (> 1,000 µm):
- Wash embryo
  - Use cut-off AI pipette (if needed)
- Load embryo into AI pipette
  - Aspirate small volume of media
  - Air gap
  - Embryo in media
  - Air gap
  - Media

TRANSFER TECHNIQUES

Transfer of Very Large Embryos (> 3,000 µm):
- Unexpected embryo size
  - i.e. Day 9 flush or late Day 8 flush
- Acquire sterile large-bore tube
  - i.e. outer sheath from culture instrument
- Load embryo into tube
- Protect with sterile chemise
- Transfer as previously described

TRANSFER TECHNIQUES

Option for Transfer – ‘Wilsher Forceps’:
- Grasping forceps modified for embryo transfer
- Attach to ventral aspect of external cervical os
- Used to pull cervical os caudally during transfer to straighten cervical folds, kinks, etc.
- Speculum needed
TRANSFER TECHNIQUES
Problems Encountered During Transfer:
- Cannot pass gun through cervix
  - Solution – stop procedure and select new recipient
- Cervical defect (laceration, adhesion) encountered
  - Solution – stop procedure and select new recipient
- Mare straining during transfer
  - Solution – sedate mare and/or administer Buscopan™
- Embryo detected when sheath tip rinsed post-Tx
  - Solution – Transfer embryo again (± same mare)

GRADING TRANSFER:
- Some veterinarians will assign a grade to the transfer attempt
  - i.e. easy, difficult, etc.
  - Opinion – grade of transfer is not well correlated with pregnancy outcome

PREGNANCY RATE POST TRANSFER
Factors Affecting Pregnancy Rate:
- Embryo quality
- Recipient quality
- Synchrony of recipient
- Donor mare age and status
- Skill of transfer technician
- Ease of transfer
- Other/Unknown factor(s)

PREGNANCY RATE POST TRANSFER
Expectations:
- Day 16 pregnancy rates

<table>
<thead>
<tr>
<th>Pregnancy Rate</th>
<th>Evaluation</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>≥ 90 %</td>
<td>Outstanding</td>
<td>Difficult to consistently achieve with large numbers of transfers</td>
</tr>
<tr>
<td>80 to 90 %</td>
<td>Excellent</td>
<td>Achievable with effort</td>
</tr>
<tr>
<td>75 to 80 %</td>
<td>Very Good</td>
<td>A solid goal</td>
</tr>
<tr>
<td>70 to 75 %</td>
<td>Good</td>
<td>Work on details</td>
</tr>
<tr>
<td>60 to 70 %</td>
<td>Fair</td>
<td>Need to improve</td>
</tr>
<tr>
<td>&lt; 60 %</td>
<td>Marginal</td>
<td>Need remedial help</td>
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</tbody>
</table>

PROBLEMS IN EQUINE EMBRYO TRANSFER
Common Problems
- Donor mare not cycling
- Mating induced endometritis
- Infectious endometritis
- ‘Old Maiden Mare’ Syndrome
- Poor fluid recovery (flush)
- ‘Dirty flush’ (debris)
- Semen issues – poor quality, cannot obtain semen, etc.
- Lack of enough good, cycling recipient mares
- Collection of a small-for-gestational-age embryo

Less Common Problems
- Failure of the embryo to leave the ET gun
- Failure of maternal recognition of pregnancy
- Twins after transfer of one embryo
- Inability to palpate donor mare
- Continued pregnancy in donor mare
- Fluid in uterus in pregnant recip.
- UFO recovered / no embryo
- Excessive debris adherent to embryo