Embryo Transfer (ET) in Cattle
Embryo Transfer in Cattle

- Breed regulations (e.g., Joint Dairy Breeds)
  - DNA profile and parentage of donor
- Industry regulations (e.g., IETS, CETA)
  - Freezing codes, forms, standards, CE
  - Certification program for embryo export
- Canadian Food Inspection Agency
  - Accreditation of certified practitioners
  - Export and import regulations
Applications

- Genetic Improvement
  - Slower than by AI
- Planned matings
  - Commercial value of offspring
- Disease Control
- Research
Embryo Transfer and the Practitioner

- Sound knowledge of reproductive physiology
- Good technical skills
- Attention to detail
- Time (group practice)
- May only set up for ET practitioner
Donor Cow

- Genetically superior
- Valuable offspring
- Reproductively and physically sound
- Good body condition and nutritional state
- >50-60 days postpartum
Success Rates
Averages/attempt

- 8-12 ova and embryos
- >60% fertilized
- 4-8+ transferable embryos
- Pregnancy rate 40-75%
- 2-6 pregnancies
  - 50:50 sex ratio

Procedure can be repeated after a normal estrus
However:

- 5-24% of collections produce no viable embryos
- ~64% of collections produce < average no. of viable embryos
- ~30-40% of collections produce ~70% of the viable embryos
Superstimulation

- Follicle stimulating hormone
  - Salvages follicles that would otherwise become atretic
Superstimulation

- Start treatment at days 8-12 of the cycle
  - Days 8-10 are optimal
- Check for CL before starting
Superstimulation

- Can start at any stage of the cycle if a CIDR or PRID is used
  - Provide progesterone
Optimize results

- Aspirate follicles ≥1 cm diameter 24-48 hr prior to treatment
- Use estradiol to inhibit the dominant follicle
  - A new wave will begin in ~4 days
Other Hormones Used

- Prostaglandins (PGF) to eliminate luteal tissue
Other Hormones Used

- GnRH, hCG, or LH to enhance ovulation
  - May not be necessary
Examples of Regimens

- Goal is to produce superovulation without:
  Overstimulation
### Example - Superovulation and recipient schedule:

<table>
<thead>
<tr>
<th>Date</th>
<th>Time</th>
<th>Donor</th>
<th>Recipients*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wed Nov 19</td>
<td>AM</td>
<td>3 cc Estrumate</td>
<td></td>
</tr>
<tr>
<td>Wed Nov 22</td>
<td>AM</td>
<td>3 cc Estrumate</td>
<td></td>
</tr>
<tr>
<td>Wed Nov 24</td>
<td>PM</td>
<td>Check for heat</td>
<td>Check for heat</td>
</tr>
<tr>
<td>Thu Nov 25</td>
<td>AM&amp;PM</td>
<td>Check for heat</td>
<td></td>
</tr>
<tr>
<td>Fri Nov 26</td>
<td>AM&amp;PM</td>
<td>Check for heat</td>
<td></td>
</tr>
<tr>
<td>Sat Nov 27</td>
<td>AM&amp;PM</td>
<td>Check for heat</td>
<td></td>
</tr>
</tbody>
</table>

If donor showed heat by Nov 26:

| Thu Dec 02 | PM    | Check for LH | Aspirate or break large follicles? |
| Fri Dec 03 | AM    |              |                                     |
| Sat Dec 04 | AM    | 3.0 cc Follitropin |                           |
| Sun Dec 05 | PM    | 3.0 cc Follitropin |                       |
| Mon Dec 06 | AM    | 3.0 cc Follitropin |                           |
| Mon Dec 06 | PM    | 3.0 cc Follitropin |                           |
| Tue Dec 07 | AM    | 1.0 cc Follitropin |                           |
| Tue Dec 07 | PM    | 1.0 cc Follitropin |                           |
| Wed Dec 08 | AM&PM | Check for heat | Check for heat            |
| Thu Dec 09 | AM&PM | Check for Luteal 150-200 µg GnRH at onset of heat** (usually in AM). Allow 12 hours after onset of heat. | Check for heat          |
| Fri Dec 10 | AM&PM | Check for heat | Check for heat            |
| Thu Dec 16 |       |            | Implant embryos          |

*Optimal: 7-10 day synchronization programs can be used for recipients.

**Of questionable benefit.
Example - Superovulation controlling the estrous cycle with progesterone or progestagen and eliminating the dominant follicle with estradiol 17β.

Superovulation and recipient schedule:

<table>
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<tr>
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<th>Time</th>
<th>Donor</th>
<th>Recipients*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thu</td>
<td>PM</td>
<td>Insert CIDR or PRID</td>
<td>2 cc Estrumate (prostaglandin)</td>
</tr>
<tr>
<td>Tue</td>
<td>AM</td>
<td>2.5-6.0 mg estradiol 17β or 1 mg estradiol benzoate</td>
<td></td>
</tr>
<tr>
<td>Wed</td>
<td>AM</td>
<td>4.0 cc Folltropin</td>
<td></td>
</tr>
<tr>
<td>Sat</td>
<td>AM</td>
<td>3.0 cc Folltropin</td>
<td></td>
</tr>
<tr>
<td>Mon</td>
<td>AM</td>
<td>2.0 cc Folltropin</td>
<td></td>
</tr>
<tr>
<td>Tues</td>
<td>AM</td>
<td>2.0 cc Folltropin</td>
<td></td>
</tr>
<tr>
<td>Wed</td>
<td>AM</td>
<td>1.0 cc Folltropin</td>
<td></td>
</tr>
<tr>
<td>Thu</td>
<td>AM&amp;PM</td>
<td>Check for heat</td>
<td></td>
</tr>
<tr>
<td>Fri</td>
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<td>Check for heat</td>
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<td>Check for heat</td>
<td></td>
</tr>
<tr>
<td>Fri</td>
<td>AM</td>
<td>Implant embryos</td>
<td></td>
</tr>
</tbody>
</table>

Check to be certain the CIDR or PRID device is in place at least twice daily (there will be a string hanging from the cow); in the unlikely event that the device is lost, replace it immediately. There may be some vaginal discharge because of the device but this will clear soon after its removal.

*Optimal, ovulation synchronization programs can be used for recipients.

**50-100 mg progesterone is also injected; the CIDR or PRID can be inserted on this day. Alternatively, GnRH could be injected with device insertion rather than using estradiol.

***Of questionable benefit.
Breeding

- Use good-quality semen and a competent inseminator
- AI ~12 and 24 hr after the onset of estrus
Recipients

- Synchronize cycle to be within 1 day of donor
  - Treat with PGF 12-24 hr before donor
    - In estrus 60-72 hr after PGF but donor in estrus 36-48 hr after PGF
Recipients

- Healthy
- Good condition and nutrition
- Cows ≥50-60 postpartum
- Normal and cycling
- Grown out well and able to raise the calf
Embryo Collection

- Day 6-8 (usually 7) after onset of estrus
- Check ovaries for response
- Epidural anesthetic
- Strict hygiene
Media

- Flushing and embryo holding media are available commercially
  - Contain antimicrobials and a protein or surfactant to prevent embryos from sticking
Equipment - Catheters
Technique

- The catheter is inserted as far up the horn as possible
- Withdrawing the stylet gradually allows the catheter to be passed further
- The cuff is inflated with air to seal off the horn
Technique

- 25-50 ml fluid are flushed into the horn and then withdrawn
- Each horn is flushed 6-8 times
Alternative Technique

- Flow-through method
Post-flush

- >2 large doses of PGF 12-24 hr apart 1-2 days after the flush
- Induces estrus and prevents multiple-fetus pregnancies
Filtering the Flush Media
Embryo Searching

- Swirl and empty filter contents into a petri dish
- Rinse filter with a stream of flush fluid
Embryo Searching

- Room temperature
- Search at 10-15X
- Dish is scored on the bottom to facilitate searching
- Swirl dish between searches
Handling Equipment

20 gauge catheter

Micropipette tip
Embryo evaluation

- Embryos put in holding medium
- Evaluate at 50-100X
Embryo Stage

Figure 1. The scale for stage of development is based on the age of the embryo expressed as the number of days after the zygote was first observed in standing heat (day 0). In commercial embryo transfer embryos are usually collected on days 8-9 (morula to blastocyst).
Embryo Evaluation

Unfertilized

Unfertilized, degenerate
Embryo Evaluation

Early development, probably dead
Embryo Evaluation

Morulae

Early blastocyst

Blastocyst
Embryo Evaluation

Expanded blastocyst

Hatched blastocysts & blastocyst
Embryo Evaluation

Code 1: Excellent morula

Code 1: Good morula
Embryo Evaluation

Code 2: Fair morula

Code 3: Poor morula
Embryo Evaluation

- Even poor quality embryos can achieve pregnancies but at a lower rate than better quality embryos.
- Only excellent or good embryos (code or grade 1) survive freezing well.
Embryo Washing

- Usually washed by 10 passages through holding medium (HM)
- Embryos for export must be treated with trypsin to remove IBR virus etc. from zona pellucida
  - 5 washes in HM, 2 in Trypsin solution, 5 washes in HM
- Transfer or freeze <3 hr after collection
Embryo Transfer

Each embryo is drawn into a straw and trapped in fluid between two air pockets.
Embryo Transfer
Embryo Transfer

- Recipient receives epidural anesthetic and perineum is cleaned
- Embryo is deposited as deep as possible without causing damage into horn ipsilateral to the CL
Freezing Embryos

- Very common
  - Insufficient recipients
  - Sale, including export
  - Convenience

- Lose <10% of good or excellent embryos due to freezing damage
Freezing Embryos

- Embryo deposited into freezing solution
  - 1.5 M ethylene glycol cryoprotectant
- Straw filled ~fresh transfer
Freezing Embryos

Straw with labeled plug

Labeled goblet and cane
Cell Freezers

- Alcohol bath
- Liquid nitrogen vapor
  - Some also freeze in the neck of a LN$_2$ tank
Freezing Embryos

- Seed liquid at -6.5 to -7°C
  - Touch forceps or swab dipped in LN₂ to bottom and top liquid columns
- Once all solution has crystallized
  - Cool at -0.5°C/min to -35°C
- Plunge into LN₂
- Load onto canes and store in LN₂
Seeding
Seeding
Crystallized: Ready to Start Cooling to \(-35^\circ C\)
Plunging into Liquid Nitrogen
Transferring Frozen Embryos

- Thaw in air 5-10 sec then
- Thaw in 30°C water for 30 sec
- Dry straw before loading it into ET gun
- Transfer to recipient immediately
Questions?
Determining the Sex of Embryos

- Biopsy taken of the embryo
- Y-chromosome detected by DNA sequence amplification using polymerase chain reaction (PCR)
Determining the Sex of Embryos

- This system is used commercially.
- Embryo biopsy may be replaced by the use of semen that has been sex-sorted by flow cytometry.
In Vitro Production (IVP) of Embryos

- Transvaginal ultrasound-guided follicular aspiration to obtain oocytes
  - Ovum pick up (OPU)
In Vitro Production of Embryos

- Oocytes are aspirated, matured and fertilized
- Embryos are cultured to the blastocyst stage
In Vitro Production of Embryos

- OPU can occur every 1-2 weeks without adverse effects
- OPU and IVP of embryos 4X every 60 days can produce 3-4X as many calves as traditional ET over the same time period
- Annual genetic progress can be 10-30% higher than for conventional ET, esp. if >1 bull is used with each in vitro fertilization
Additional Advantages of In Vitro Embryo Production

- It can be used in:
  - Cows pregnant up to 90-150 days
  - ‘Problem” cows
  - ET donors between regular flushes
  - Heifers >8-mos-old

- Fetal sex selection can be facilitated if sexed semen is used
  - Results using frozen-thawed sexed semen are not optimal
Disadvantages of In Vitro Embryo Production

- High cost
- IVP embryos are more fragile and difficult to freeze successfully
- Higher rates of fetoplacental abnormalities, and fetal and calf mortality
- Occasional very large calves
- Slightly higher ratio of male calves unless sexed semen is used
Cloning

- Nuclear material from a donor blastomere or somatic cell inserted into an enucleated oocyte
- Electrofusion
- Culture and implantation of the resulting embryo
Show-Jumping Champion Gem Twist

- Was a gelding and is now a stallion!
  - A clone was produced from a frozen flap of his skin taken before he died
  - His clone, Gemini, will be used for breeding
Problems with Cloning in Cattle

- High incidence of fetoplacental abnormalities and neonatal mortality e.g.,
  - High birth weight, hydropic conditions of the fetal membranes, prolonged gestation, embryonic death, abortion, still birth, dystocia
  - Cardiopulmonary, metabolic and musculoskeletal abnormalities
Questions?

"Holy great mother of God, I've been cloned!"