MODERN REPRODUCTIVE TECHNOLOGIES TO IMPROVE CATTLE PRODUCTION

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The Grand Challenge

How to feed the world in 2050?
# Human Population Growth

<table>
<thead>
<tr>
<th>Population change</th>
<th>Number of years</th>
<th>Time period</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 billion to 2 billion</td>
<td>123</td>
<td>1804 to 1927</td>
</tr>
<tr>
<td>2 billion to 3 billion</td>
<td>33</td>
<td>1927 to 1960</td>
</tr>
<tr>
<td>3 billion to 4 billion</td>
<td>24</td>
<td>1960 to 1974</td>
</tr>
<tr>
<td>4 billion to 5 billion</td>
<td>13</td>
<td>1974 to 1987</td>
</tr>
<tr>
<td>5 billion to 6 billion</td>
<td>12</td>
<td>1987 to 1999</td>
</tr>
<tr>
<td>6 billion to 7 billion</td>
<td>13</td>
<td>1999 to 2012</td>
</tr>
</tbody>
</table>
• 9.6 billion people expected by the year 2050 (Searchinger et al., 2014)

• Food availability must double between now and then
CLOSING THE FOOD GAP

- Decrease pre-harvest food losses
- Decrease post-harvest food losses
- Increase total food production
• Meat, milk, and eggs provide essential sources of protein (amino acids), minerals, and vitamins

• Livestock numbers (FAOSTAT3, 2014):
  • Poultry  20.96 billion head
  • Sheep & Goats  2.08 billion head
  • Cattle  1.42 billion head

• Food production from cattle:
  • 635.5 million tons of milk
  • 63.6 million tons meat
Artificial Insemination (AI)
- Genetically superior males produce a large number of offspring

Embryo Transfer (ET)
- Genetically superior females produce a large number of offspring

Overall purpose of AI & ET is genetic improvement
# The Power of Genetics

## U.S. Dairy Cattle Industry

<table>
<thead>
<tr>
<th></th>
<th>1965</th>
<th>2000</th>
<th>2014</th>
<th>Change</th>
</tr>
</thead>
<tbody>
<tr>
<td>M head of dairy cows</td>
<td>15.0</td>
<td>9.2</td>
<td>9.2</td>
<td>-35%</td>
</tr>
<tr>
<td>M kg of milk produced</td>
<td>56,445</td>
<td>76,342</td>
<td>93,657</td>
<td>+66%</td>
</tr>
<tr>
<td>Kg of milk produced per cow</td>
<td>3,775</td>
<td>8,274</td>
<td>10,117</td>
<td>+168%</td>
</tr>
</tbody>
</table>
THE POWER OF GENETICS

• Improved genetics has reduced the carbon footprint of the US dairy cattle industry


• 2007 carbon footprint only 37% of 1944 carbon footprint (per 1 billion Kg of milk produced)
  - 10% of land; 21% of animals
  - 23% of feedstuffs; 35% of water
INCREASING PRODUCTION OF CATTLE-DERIVED FOODS

• Three basic options to introduce new genetic resources to enhance productivity:

• 1. importation of live animals
  • expensive; risk of illness

• 2. importation of semen
  • Relatively inexpensive; no possibility for purebred animals without access to purebred females

• 3. importation of embryos
  • Complete genetic package; minimal health risk to importing country; passive immunity protects offspring
ARTIFICIAL INSEMINATION

- Highly impactful and widely utilized reproductive biotechnology
- One ejaculate diluted with semen extender can yield dozens of pregnancies
- Semen cryopreservation allows widespread distribution
• First US calf resulting from AI with frozen-thawed semen was born May 29, 1953 – named “Frosty”

• Today in the US ~90% of dairy cows and 12% of beef cows undergo AI
  • 23.6 M units dairy semen
  • 2.6 M unites beef semen
• Pre-determining the sex of a calf could be advantageous

• Dairy farmers may prefer heifers, whereas beef producers may prefer bulls

• Fetal sexing (via ultrasound) and embryo sexing (via PCR) do NOT pre-determine the genetic sex of a calf

• Sperm sexing should allow farmers to produce calves of the desired genetic sex
Sperm sexing

- expose sperm to DNA-specific fluorescent dye
- sperm with an X chromosome will fluoresce more than sperm with a Y chromosome (Y chromosome has less DNA)

bovine karyotype

Eldridge (1985)
Sperm sexing via flow cytometry

1. A piezoelectric crystal is undulated approximately 90,000 times/second, which breaks the stream into droplets at a particular point in time. The location of the last-attached droplet in the stream is highly controllable.

2. An X- or Y-bearing sperm is compared to a preset sort criteria.

3. After a time delay, the insertion rod is charged.

4. A charge is applied at the time the cell reaches the last attached drop.

5. The charged droplets are deflected as they pass between continuously charged plates.

6. Particles not meeting the criteria pass straight down to waste.
FLOW CYTOMETER
• expect an AI pregnancy rate of ~80% of whatever is normal for that particular bull with un-sexed sperm (Seidel, 2014)

• Example: if “normal” AI pregnancy rate for a bull is 60%, then expect 48% pregnancy rate with sexed sperm (60% x 80%)

• 2 M units of sexed semen sold annually (Seidel, 2014)
• SexedULTRA™ is a recent development; patent pending (Vishwanath et al., 2016)

• Three changes to procedure:
  • extend ejaculate with buffered holding medium
  • adjust concentration of extended sperm cells
  • catch sorted sperm cells in a medium containing an antioxidant
Table 6. Effect of increasing dose rates of sex sorted semen on field fertility. Sex sorted semen compared with XY method at 2.1 million and Conventional (15 million). Data produced in collaboration with GGI, Germany.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Number of inseminations</th>
<th>56 day NRR (%)</th>
<th>Relative fertility (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>XY method</td>
<td>1292</td>
<td>56.3&lt;sup&gt;A&lt;/sup&gt;</td>
<td>87%</td>
</tr>
<tr>
<td>SU 2.1 mill</td>
<td>1245</td>
<td>59.2&lt;sup&gt;A&lt;/sup&gt;</td>
<td>92%</td>
</tr>
<tr>
<td>SU 3 mill</td>
<td>1328</td>
<td>60.7&lt;sup&gt;AB&lt;/sup&gt;</td>
<td>94%</td>
</tr>
<tr>
<td>SU 4 mill</td>
<td>1182</td>
<td>65.0&lt;sup&gt;B&lt;/sup&gt;</td>
<td>100%</td>
</tr>
<tr>
<td>Conv (15 mill)</td>
<td>50,143</td>
<td>64.5&lt;sup&gt;B&lt;/sup&gt;</td>
<td></td>
</tr>
</tbody>
</table>

Data from cows and heifers. NRR results with different superscripts are significantly different P < 0.05.

Vishwanath (2014)
Efficient use of AI necessitates that estrus and/or ovulation be synchronized.

Two basic approaches exist:
- Synchronization of estrus
- Synchronization of ovulation

Both approaches utilize administration of exogenous hormones:
- typically use the same hormones the cow herself naturally produces
SYNCHRONIZATION OF ESTRUS

- Prostaglandin F2α (and analogues)
- Progesterone (and analogues)
SYNCHRONIZATION OF ESTRUS

- Combination of prostaglandin F2α and progesterone
- Farmer must detect estrus!
SYNCHRONIZATION OF OVULATION

• Developed to overcome difficulties with detection of estrus
• Farmer substitutes exogenous hormones for labor to detect estrus
• Facilitates timed artificial insemination (TAI); detection of estrus not needed
SYNCHRONIZATION OF OVULATION

- More than 15 different protocols!
- Approved protocols do not utilize any estrogen products (e.g., estradiol-17β, estradiol benzoate)
- See web sites of:
  - Dairy Cattle Reproduction Council
  - Beef Reproduction Task Force
OVSYNCH

- administer GnRH (gonadotropin releasing hormone) after appropriate voluntary waiting period

- 7 days later, administer prostaglandin F2α (PGF)

- 2 days later, administer a second injection of GnRH

- perform TAI 16 hours after 2nd GnRH
OvSynch

GnRH

PGF$_{2\alpha}$

GnRH

Breed

1

8

10

11

Calendar Day

Ovulation

&

Initiation of a new follicular wave

New CL

Luteolysis

Development of dominant follicle

Ovulation
**Fixed-time AI (TAI)**
*for Bos Indicus cows only*

**PG 5-day CO-Synch + CIDR**
Perform TAI at 66 ± 2 hr after CIDR removal with GnRH at TAI. Two injections of PG 8 ± 2 hr apart are required for this protocol.

* The time listed for “Fixed-time AI” should be considered as the approximate average time of insemination. This should be based on the number of cows to inseminate, labor, and facilities.

- **GnRH**
- **PG**
- **CIDR**
- **TAI**

* Cystorelin®, Fectrol®, Fertagyl®, OvaCyst®, GONABreed®
* estroPLAN®, Estrumate®, In-Synch®, Lutalyse®, ProstaMate®

*Task Force*
• first successful mammalian embryo transfer (ET) occurred on April 27, 1890 (more than 125 years ago!)
• world’s first bovine ET calf was born on December 19, 1950 in Wisconsin (USA)

• project director: Elwyn Willett, PhD (American Foundation for the Study of Genetics)

THE POWER OF EMBRYO TRANSFER

US MILK PRODUCTION ALL-TIME RECORD

- Ever Green View - My 1326-ET
- 32,805 kg milk; 1,267 kg fat; 974 kg protein
The power of embryo transfer

Sommer and Youngs (2016)
MOET – MULTIPLE OVULATION AND EMBRYO TRANSFER

- selection of genetically superior donor females
- synchronization of estrus in donor and recipient females
- superovulation of donor females
- detect estrus - donors & recipients
MOET – MULTIPLE OVULATION AND EMBRYO TRANSFER

- insemination of superovulated donor females
- embryo recovery and subsequent embryo evaluation
- transfer to synchronous recipient females
## A. Beef Cattle Embryo Recovery Statistics

<table>
<thead>
<tr>
<th>Donor type</th>
<th>No. flushed</th>
<th>No. of TQE's</th>
<th>TQE/flush</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stimulated, unsorted semen</td>
<td>22,685</td>
<td>156,631</td>
<td>6.90</td>
</tr>
<tr>
<td>Stimulated, sex sorted semen</td>
<td>602</td>
<td>3,374</td>
<td>5.60</td>
</tr>
<tr>
<td><strong>TOTAL</strong></td>
<td><strong>23,287</strong></td>
<td><strong>160,005</strong></td>
<td></td>
</tr>
</tbody>
</table>

## B. Dairy Cattle Embryo Recovery Statistics

<table>
<thead>
<tr>
<th>Donor type</th>
<th>No. flushed</th>
<th>No. of TQE's</th>
<th>TQE/flush</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stimulated, unsorted semen</td>
<td>14,845</td>
<td>93,704</td>
<td>6.31</td>
</tr>
<tr>
<td>Stimulated, sex-sorted semen</td>
<td>598</td>
<td>2,811</td>
<td>4.70</td>
</tr>
<tr>
<td><strong>TOTAL</strong></td>
<td><strong>15,443</strong></td>
<td><strong>96,515</strong></td>
<td></td>
</tr>
</tbody>
</table>
MOET – MULTIPLE OVULATION AND EMBRYO TRANSFER

- Expect pregnancy rates of 55-65% with fresh embryos and 50-60% with frozen-thawed embryos
An alternative to MOET is the transfer of embryos produced in the laboratory via *in vitro* procedures.

- Oocytes are harvested directly from the ovarian follicles of genetically superior cows.
- Oocytes are matured, fertilized, and cultured *in vitro*.
IN VITRO EMBRYO PRODUCTION

- ultrasound-guided ovum pick-up (OPU)

Meintjes et al. (1995)

ultrasound monitor image
IN VITRO EMBRYO PRODUCTION

immature oocytes – place into maturation medium and ship to the laboratory

oocyte maturation

matured oocytes
**IN VITRO EMBRYO PRODUCTION**

- **sperm capacitation**
  Photo courtesy of Dr. Robert A Godke, Louisiana State University, USA

- **placement of sperm with oocytes for co-incubation**

- **results after one week of in vitro culture**
  Photo courtesy of Dr. Charles Looney, OvaGenix, USA
## I. Oocyte collections (for in vitro fertilization)

<table>
<thead>
<tr>
<th>Donor type</th>
<th>No. OPU sessions</th>
<th>Oocytes/OPU</th>
<th>No. of TQE</th>
<th>TQE/OPU</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beef</td>
<td>4,896</td>
<td>21.5</td>
<td>25,332</td>
<td>5.17</td>
</tr>
<tr>
<td>Dairy</td>
<td>9,081</td>
<td>17.8</td>
<td>41,569</td>
<td>4.58</td>
</tr>
</tbody>
</table>

**In vitro produced embryos transferred:** 39,448 fresh + 4,874 cryopreserved = 44,322
Advantages of OPU/IVF may include:

- use semen from more than one bull per OPU session
- use one straw of semen to produce embryos from multiple OPU sessions (multiple donor females)
- produce embryos from recently deceased female
- more embryos per donor per year
In vitro embryo production

• in the US, a typical OPU session yields 4.67 transferrable quality embryos (TQEs)
  • when performed every other week, in vitro embryo production can result in 121 embryos/donor/year

• in the US, conventional MOET yields 6.74 TQE per donor
  • when MOET is performed every 45 days, flushing can result in 54 embryos/donor/year
Advantages of OPU/IVF (continued):

- circumvent any reduction in fertility when using sexed semen for AI of MOET donors
- ability to produce embryos from early pregnant (<100 days) females
- ability to produce embryos from early post-partum (<50 days) cows
- low cost (as low as $50/embryo)
Disadvantages of OPU/IVF:

- limitations on cryopreservation of in vitro produced embryos
- temperature regulation of oocytes during collection, maturation, and fertilization is crucial
- requires access to specialized laboratory
IN VITRO EMBRYO PRODUCTION

Portable oocyte incubator

Overnight shipment
Intracytoplasmic sperm injection (ICSI)

Injection of a single sperm into the cytoplasm of an oocyte
Intracytoplasmic sperm injection (ICSI)

- can use when male produces very few morphologically normal sperm
- preferred method of IVF in some species
  - horses
Intracytoplasmic sperm injection (ICSI)

Dr. Kazufumi Goto (Kagoshima, Japan) with Japanese black cattle produced via ICSI with dead sperm
• desired end result of AI, TAI, and ET programs is a confirmed pregnancy

• commonly used methods:
  • manual palpation (≥ day 32)
  • ultrasound (≥ day 28)
  • biochemical test for pregnancy-specific compound (e.g., BioPRYN®)
a practical, on-farm alternative to identify non-pregnant females before a pregnancy is confirmed is the FastBack™ protocol:
UNSUCCESSFUL MATINGS - OPTIONS

- re-synchronization protocols
SUMMARY

• Cattle producers have a variety of options to achieve successful reproduction in their herds:
  • Artificial insemination with sex-sorted semen
  • Synchronization of ovulation for timed artificial insemination
  • MOET
  • In vitro embryo production
  • Pregnancy testing / resynchronization
SUMMARY

• Successful reproduction is a prerequisite for meat and milk production

• Wise use of reproductive technologies can enhance global food security
¿PREGUNTAS?