Modern Reproductive Technologies to Improve Cattle Production

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The Grand Challenge

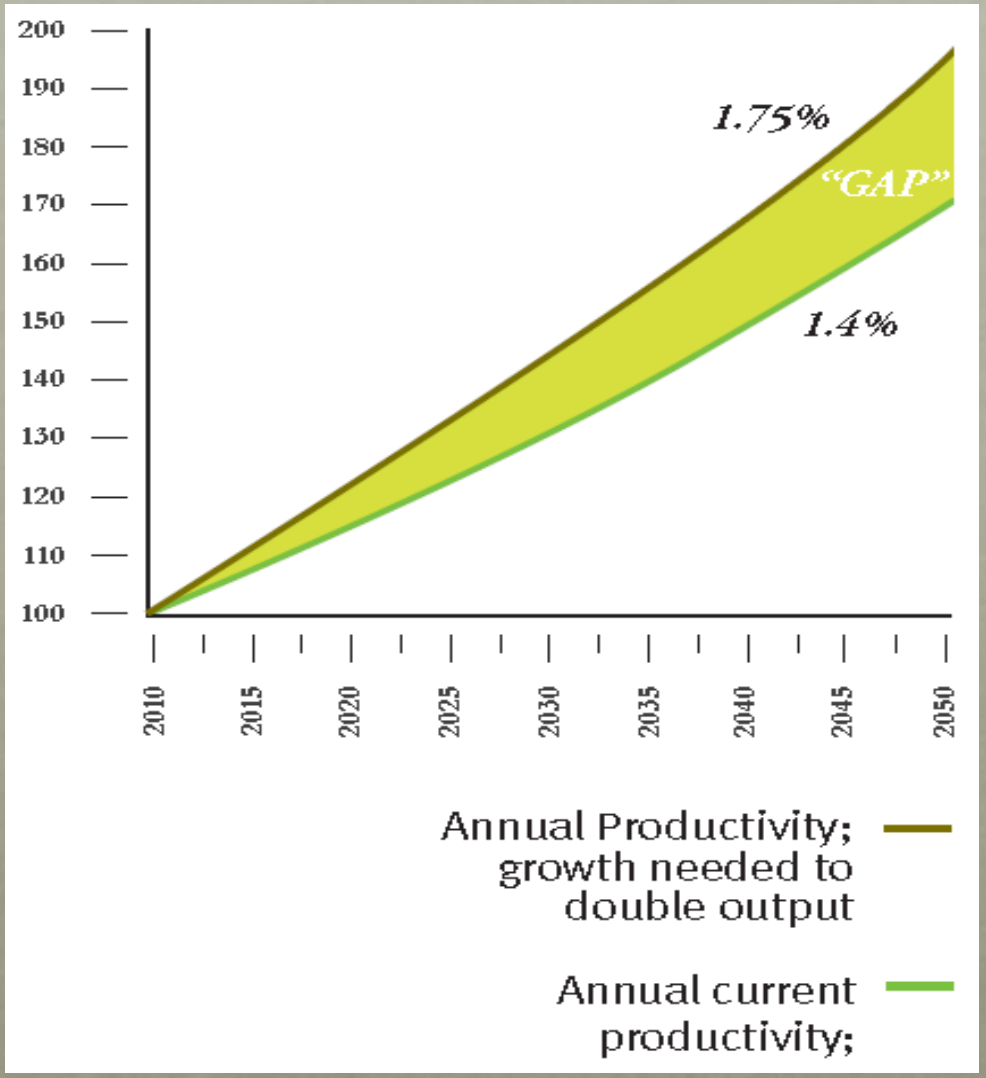
How to feed the world in 2050?

HUMAN POPULATION GROWTH

Population change	Number of years	Time period
1 billion to 2 billion	123	1804 to 1927
2 billion to 3 billion	33	1927 to 1960
3 billion to 4 billion	24	1960 to 1974
4 billion to 5 billion	13	1974 to 1987
5 billion to 6 billion	12	1987 to 1999
6 billion to 7 billion	13	1999 to 2012

THE FOOD GAP

- 9.6 billion people expected by the year 2050 (Searchinger et al., 2014)
- Food availability must double between now and then

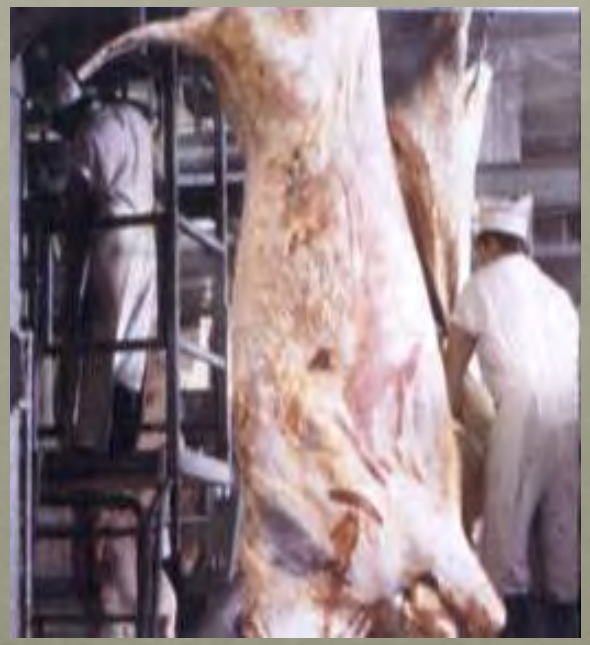


Closing the Food Gap

- Decrease pre-harvest food losses
- Decrease post-harvest food losses
- Increase total food production

ANIMAL SOURCE FOODS

- 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



REPRODUCTIVE TECHNOLOGIES FOR CATTLE

- 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

	1965	2000	2014	Change
M head of dairy cows	15.0	9.2	9.2	- 35%
M kg of milk produced	56,445	76,342	93,657	+ 66%
Kg of milk produced per cow	3,775	8,274	10,117	+ 168%

The Power of Genetics

- Improved genetics has reduced the carbon footprint of the US dairy cattle industry
- Capper et al (2009) compared 1944 US dairy industry versus 2007 US dairy 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

ARTIFICIAL INSEMINATION

- 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

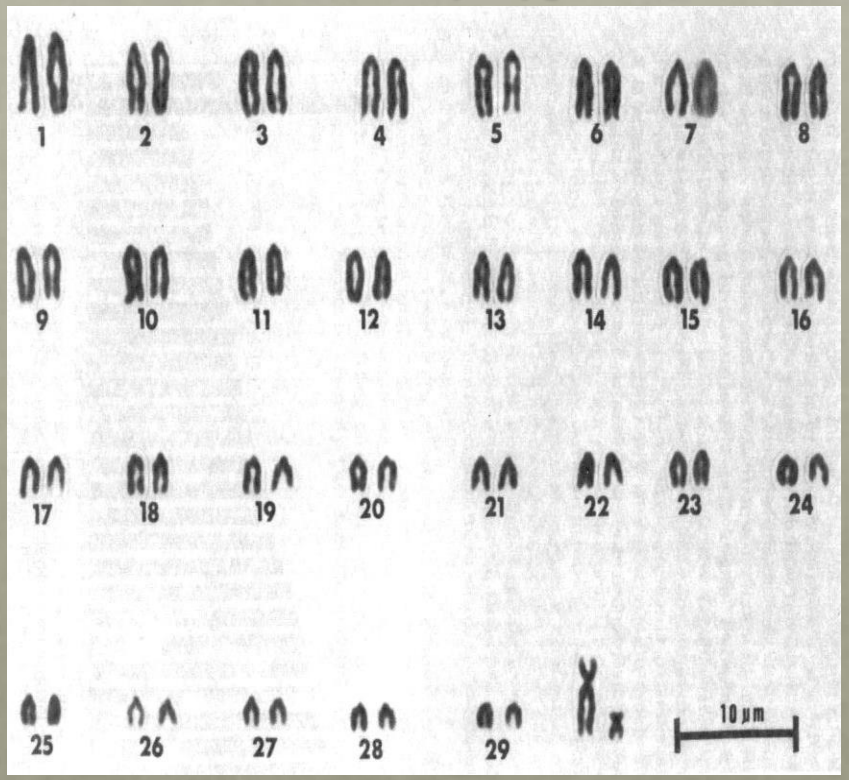
SPERM SEXING - MAKING AI BETTER?

- 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

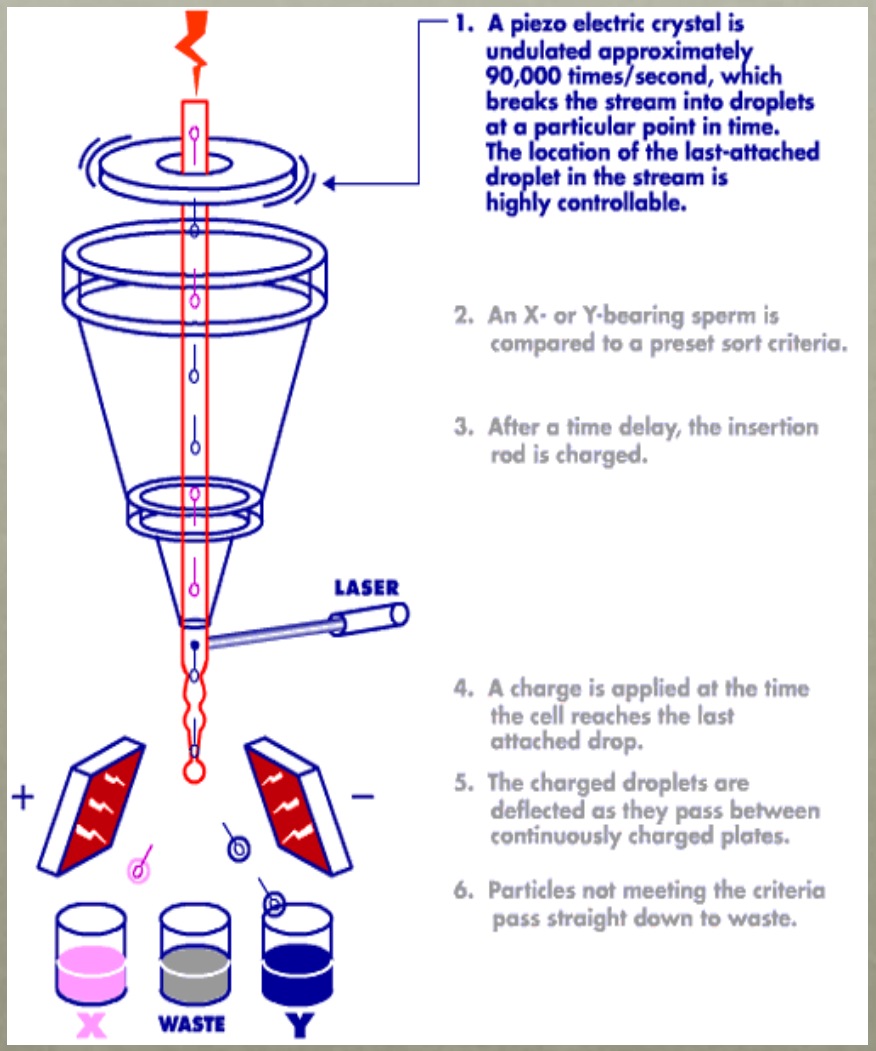
- expose sperm to DNA-specific fluorescent dye
- sperm with an X chromosome will fluoresce more than sperm with a Y chromosome (Y chromosome has less DNA)

bovine karyotype



Eldridge (1985)

SPERM SEXING VIA FLOW CYTOMETRY



FLOW CYTOMETER

SPERM SEXING

- 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)

SPERM SEXING – SEXED ULTRA™

- 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

SPERM SEXING – SEXED ULTRA™

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.

Treatment	Number of inseminations	56 day NRR (%)	Relative fertility (%)
XY method	1292	56.3^{A}	87%
SU 2.1 mill	1245	59.2^{A}	92%
SU 3 mill	1328	60.7^{AB}	94%
SU 4 mill	1182	65.0^{B}	***100%***
Conv (15 mill)	50,143	64.5^{B}	

Data from cows and heifers. NRR results with different super-scripts are significantly different $P < 0.05$.

Vishwanath (2014)

Control of Estrus and Ovulation for AI

- 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

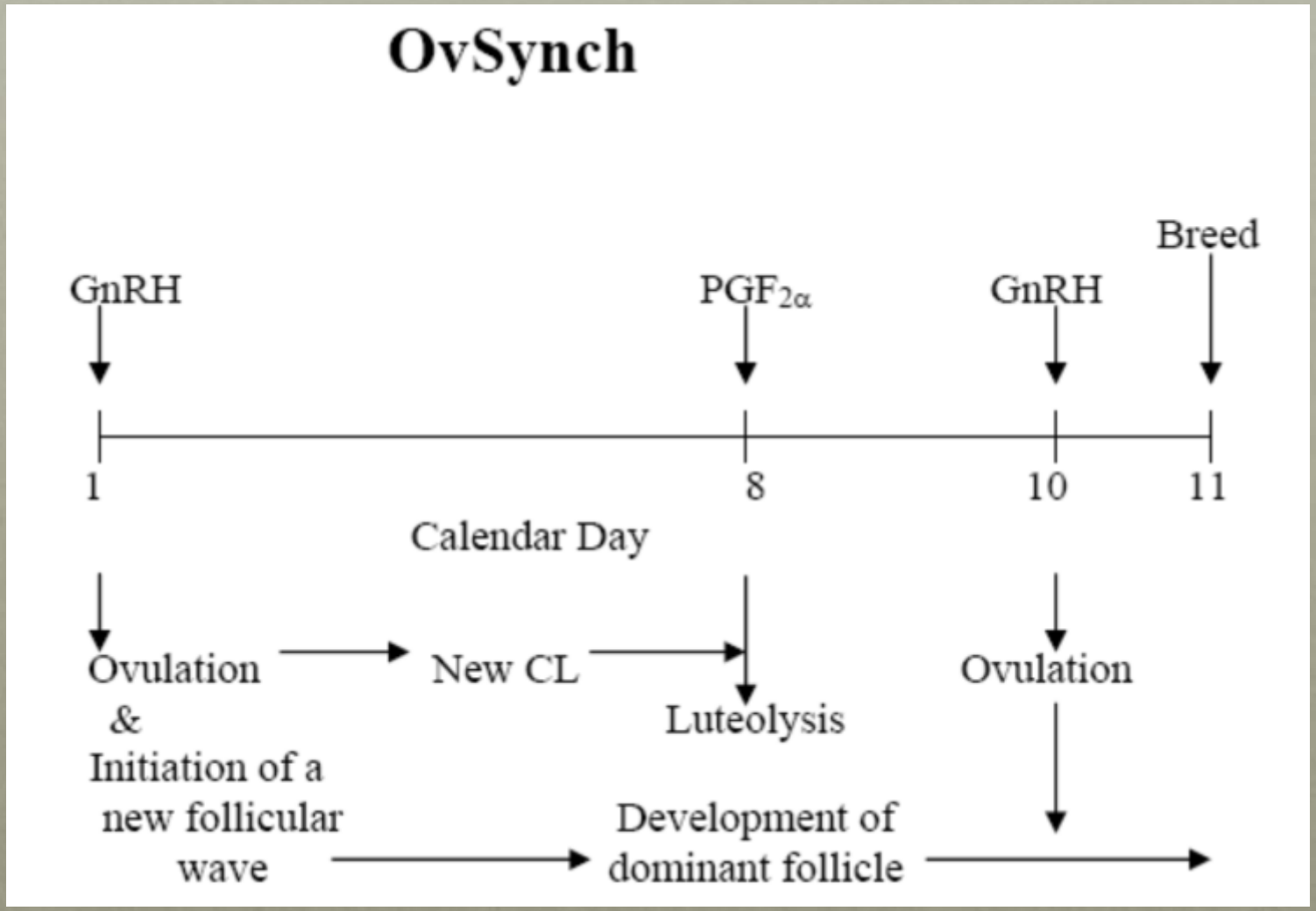
SYNCHRONIZATION OF OVULATION

- 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


OvSynch
GnRH
PGF2α
GnRH
Breed
1
8
10
11
Calendar Day
Ovulation
&
Initiation of a
new follicular
wave
New CL
Luteolysis
Development of
dominant follicle
Ovulation

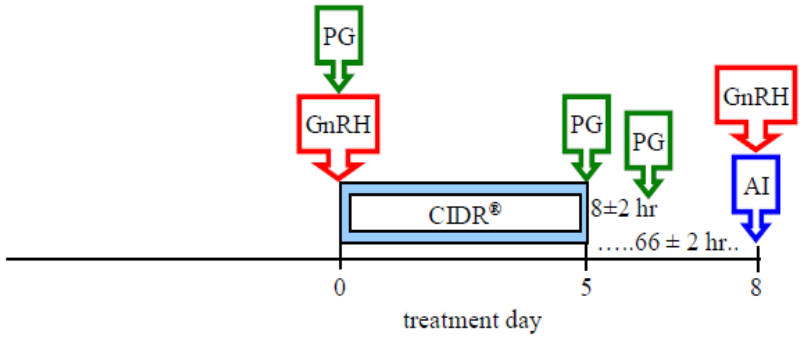
PG 5-DAY CO-SYNCH + CIDR

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 — Cystorelin®, Factrel®, Fertagyl®, OvaCyst®, GONABreed®

PG — estroPLAN®, Estrumate®, In-Synch®, Lutalyse®, ProstaMate®

n Task Force

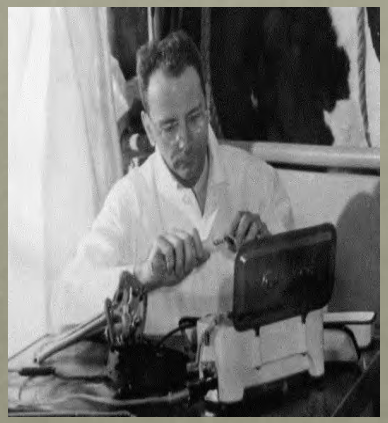
EMBRYO TRANSFER

- first successful mammalian embryo transfer (ET) occurred on April 27, 1890 (more than 125 years ago!)

EMBRYO TRANSFER

- 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)

- Willett, EL, WG Black, LE Casida, WH Stone and PJ Buckner. 1951. Successful transplantation of a fertilized bovine ovum. Science 113(2931):247.

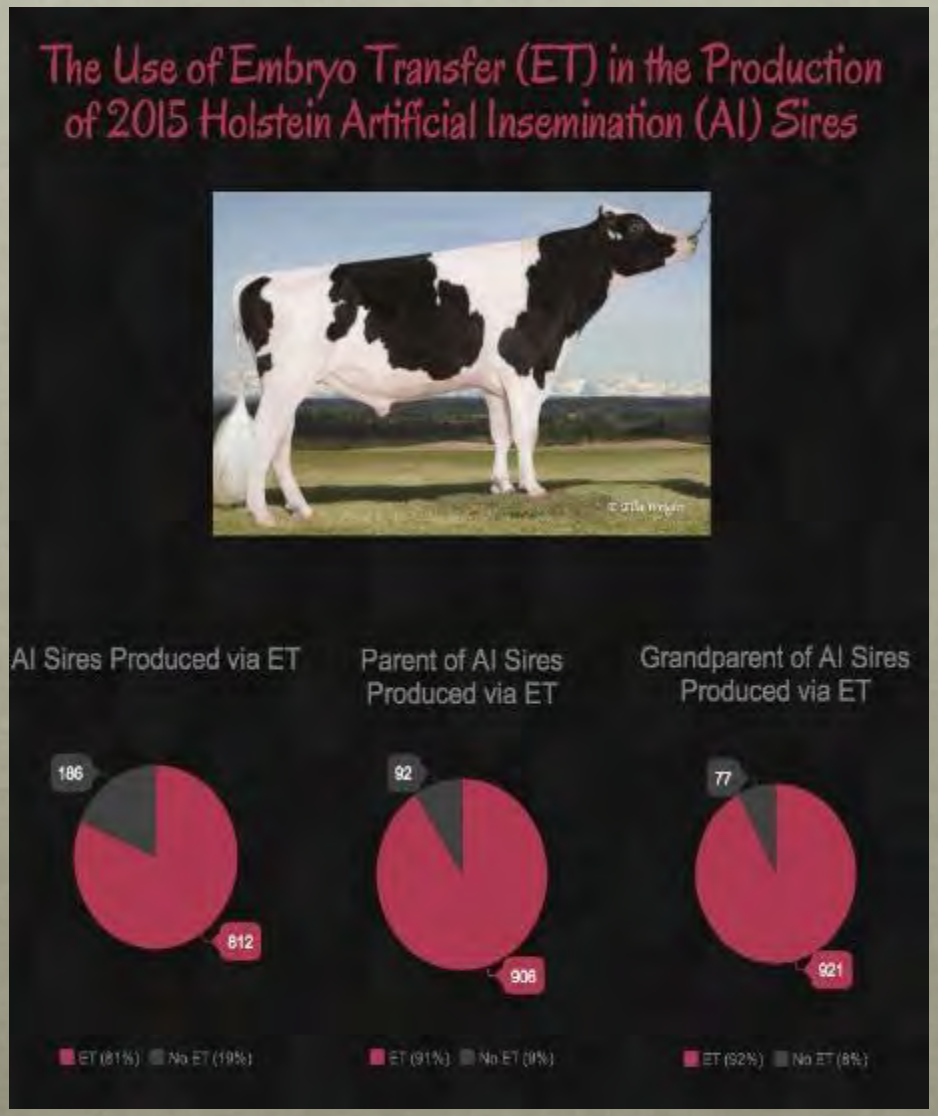


THE POWER OF EMBRYO TRANSFER

US MILK PRODUCTION ALL-TIME RECORD

- Ever Green View - My 1326-ET
- 32,805 kg milk; 1,267 kg fat; 974 kg protein

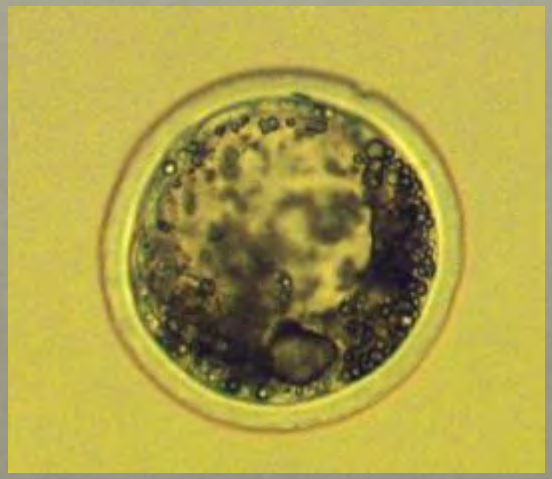
THE POWER OF EMBRYO TRANSFER



Sommer and Youngs (2016)

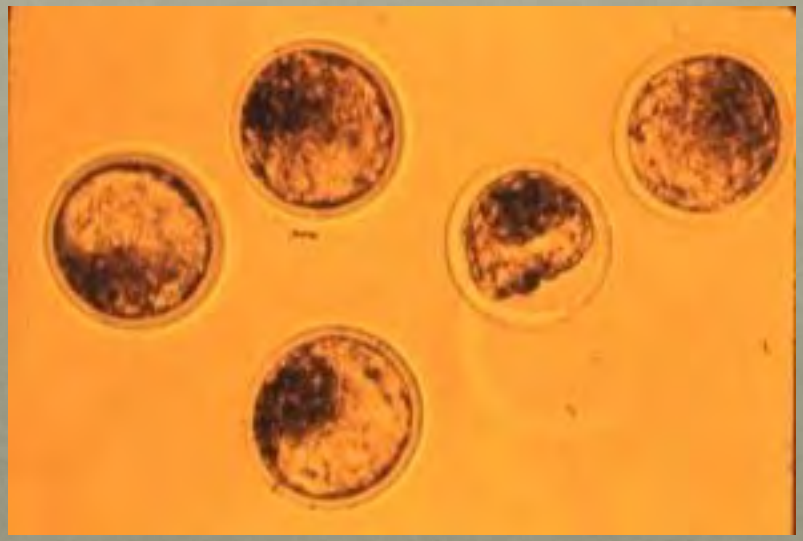
MOET – MULTIPLE OVULATION AND EMBRYO TRANSFER

- 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



ET INDUSTRY DATA - AETA

A. Beef Cattle Embryo Recovery Statistics

Donor type	No. flushed	No. of TQEs	TQE/flush
Stimulated, unsorted semen	22,685	156,631	6.90
Stimulated, sex sorted semen	602	3,374	5.60
TOTAL	23,287	160,005	

B. Dairy Cattle Embryo Recovery Statistics

Donor type	No. flushed	No. of TQEs	TQE/flush
Stimulated, unsorted semen	14,845	93,704	6.31
Stimulated, sex-sorted semen	598	2,811	4.70
TOTAL	15,443	96,515	

MOET – Multiple Ovulation and Embryo Transfer

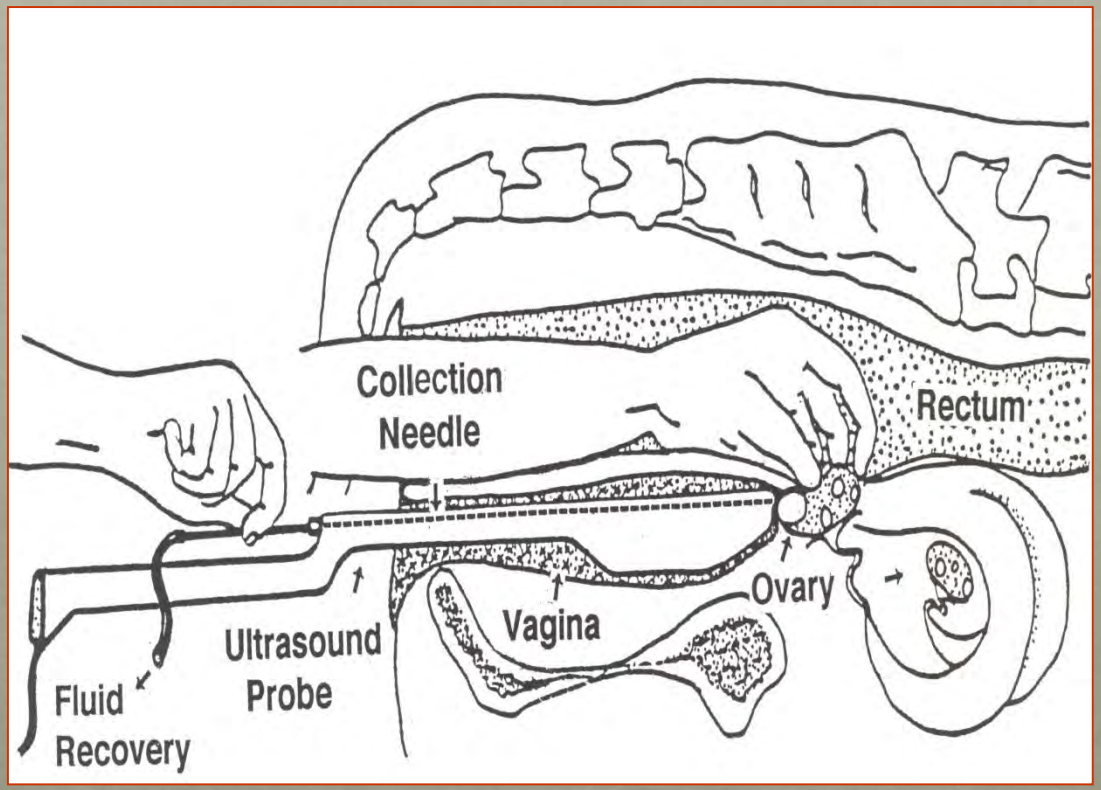
- Expect pregnancy rates of 55-65% with fresh embryos and 50-60% with frozen-thawed embryos

IN VITRO EMBRYO PRODUCTION

- 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

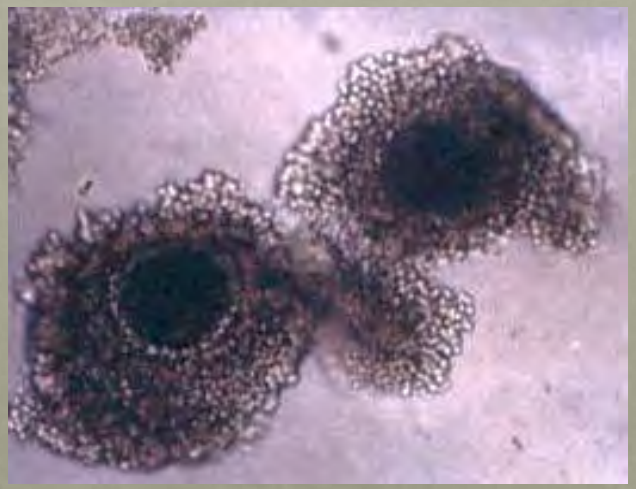
- ultrasound-guided ovum pick-up (OPU)



Meintjes et al. (1995)

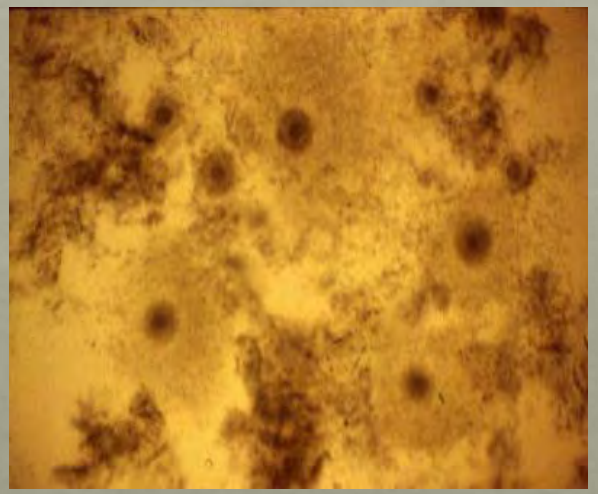
ultrasound monitor image

IN VITRO EMBRYO PRODUCTION



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

oocyte maturation



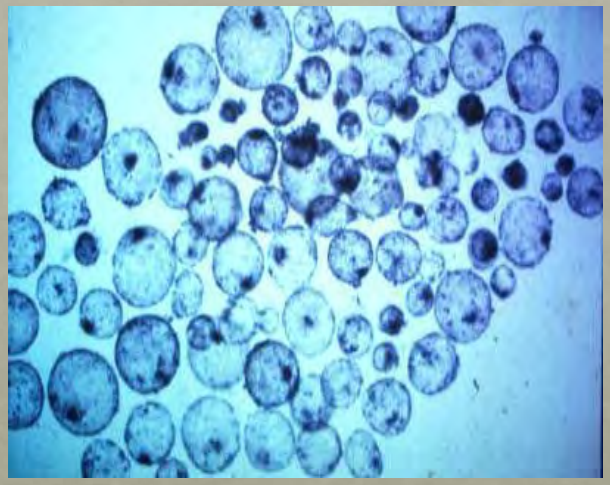
matured oocytes

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

ET INDUSTRY DATA - AETA

I. Oocyte collections (for in vitro fertilization)

Donor type	No. OPU sessions	Oocytes/OPU	No. of TQEs	TQE/OPU
Beef	4,896	21.5	25,332	5.17
Dairy	9,081	17.8	41,569	4.58

In vitro produced embryos transferred: 39,448 fresh + 4,874 cryopreserved = 44,322

IN VITRO EMBRYO PRODUCTION

- 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

IN VITRO EMBRYO PRODUCTION

- 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)

IN VITRO EMBRYO PRODUCTION

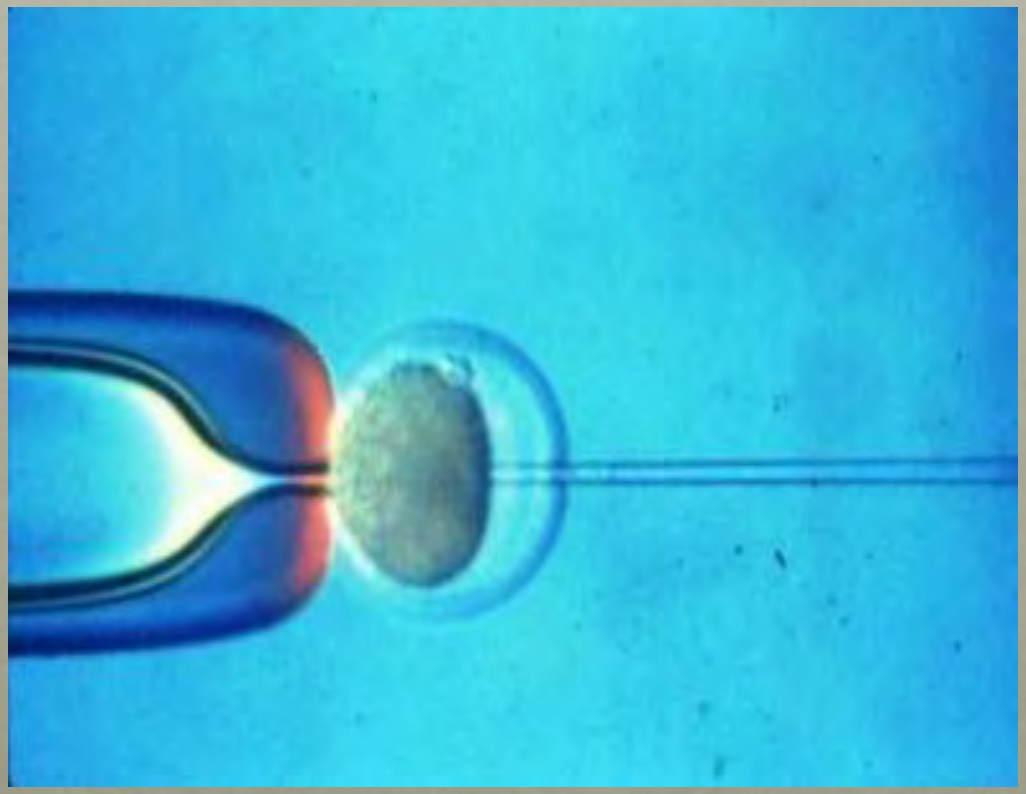
- Disadvantages of OPU/IVF:
- limitations on cryopreservation of in vitro produced embryos
- temperature regulation of oocytes during collection, maturation, and fertilization is crucial
- requires access to specialized laboratory

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)

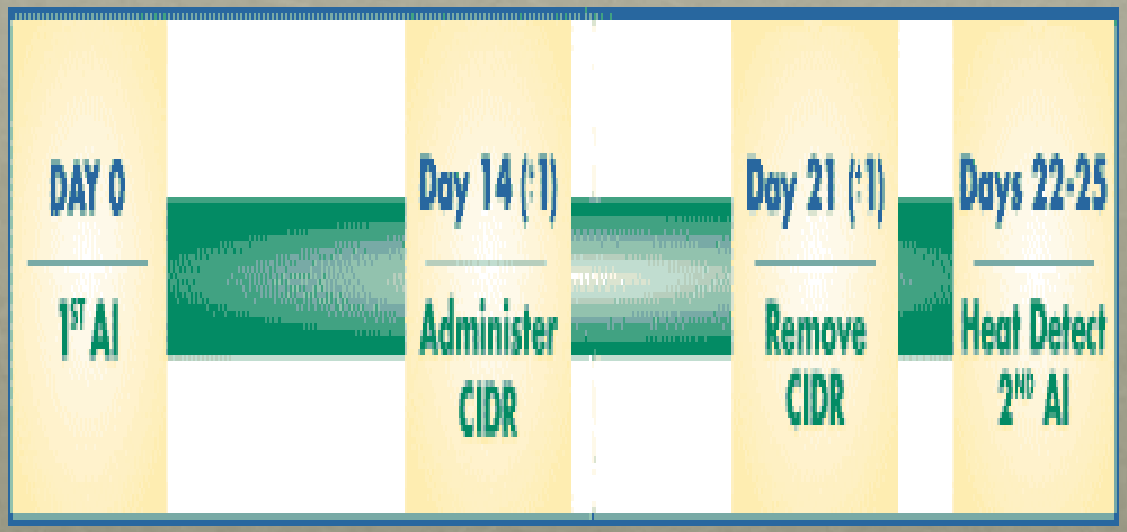
Dr. Kazufumi Goto (Kagoshima, Japan) with Japanese black cattle produced via ICSI with dead sperm

MEASURING SUCCESS

- 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®)

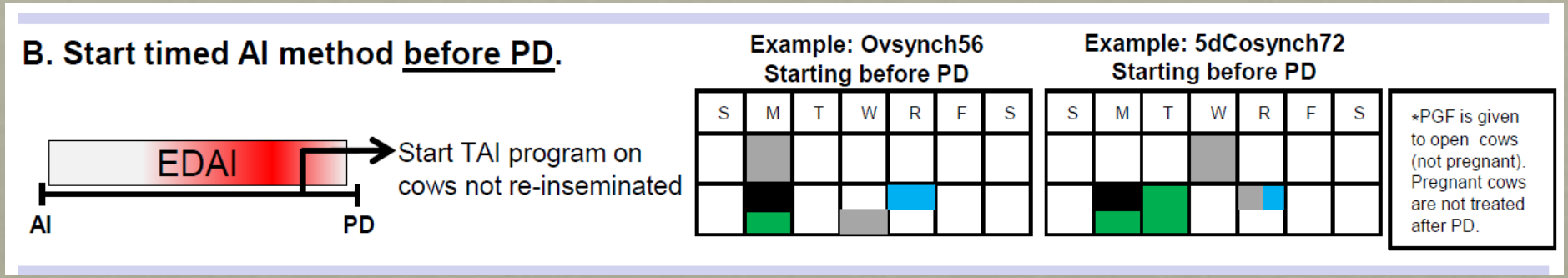
MEASURING SUCCESS

- 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 / resynchonization

SUMMARY

- Successful reproduction is a prerequisite for meat and milk production
- Wise use of reproductive technologies can enhance global food security

¿PREGUNTAS?