New tools in reproduction technologies

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Semen technologies

Embryo technologies

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Introduction

Why reproduction technologies?

Demanded by the farmer:

- high quality genetic products
- offspring of desired gender
- products with high fertility
-
-

Reproduction technologies are needed in order to fulfil this demand.

Introduction

Farmer : *to increase fertility / to choose gender of offspring*

- Highly fertile semen
- Sexed semen

Breeding industry: *to enhance genetic improvement*

- Increase selection intensity by creating a higher number of offspring per bull dam
- Decrease generation interval by increasing speed of embryo production

→ semen and embryo technologies

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Semen technologies

- *Semen sexing*
- *Semen release technologies*

Embryo technologies

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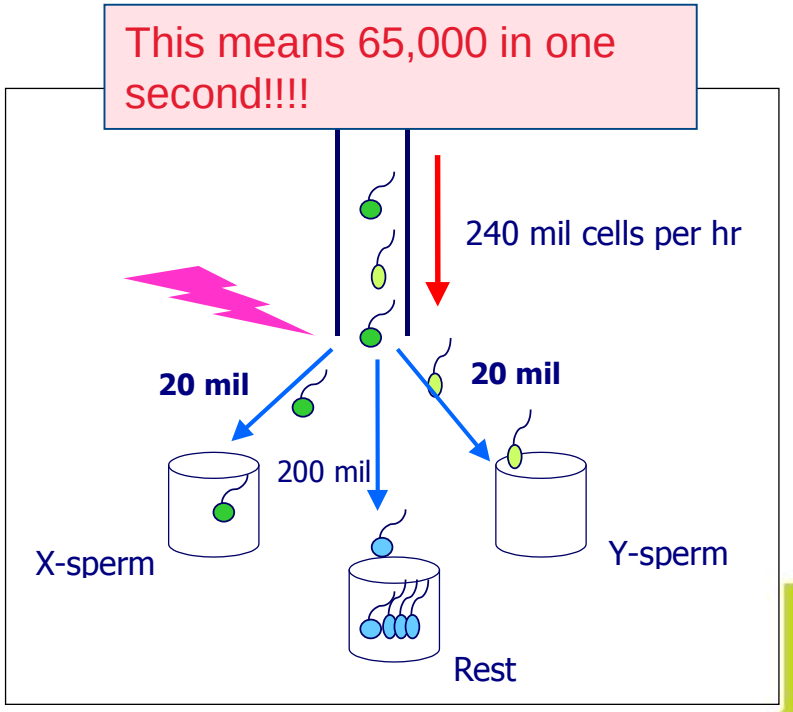
Semen technologies

Semen sexing

Semen sexing

- DNA staining
- Difference between X and Y bearing cells: 3.8%
- Sorting by flowcytometry
- 7 doses per hour per flowcytometer.
- Accuracy 90-95%
- Dosage: 2 mil sexed v 15 mil conventional



Semen sexing

World wide spread of laboratories

Use in both livestock management and breeding programs

- female calves from the best cows

Overall decline in NRR56-points: -13.6%

- 2/3 (-8.6%) due to low dosage
- 1/3 (-5.0%) due to sorting

Frijters et al 2009

Semen sexing

Field trials with conventional semen not predictive for fertility sexed semen

- The sensitivity of bulls for low dosage and sorting can differ

Fertility results of sexed semen can be improved by optimizing dosage and sorting process

- More research is needed

Currently, closely monitoring results of sexed semen & bull selection is the best way to improve fertility

- Decline NRR56 from -13.6 to -10% in The Netherlands.

Semen technologies

Sperm release technologies

Slow release semen

Basic principle:

Improved fertility by extended survival of semen in the uterus (days); heat detection becomes less critical

Norway: SpermVital

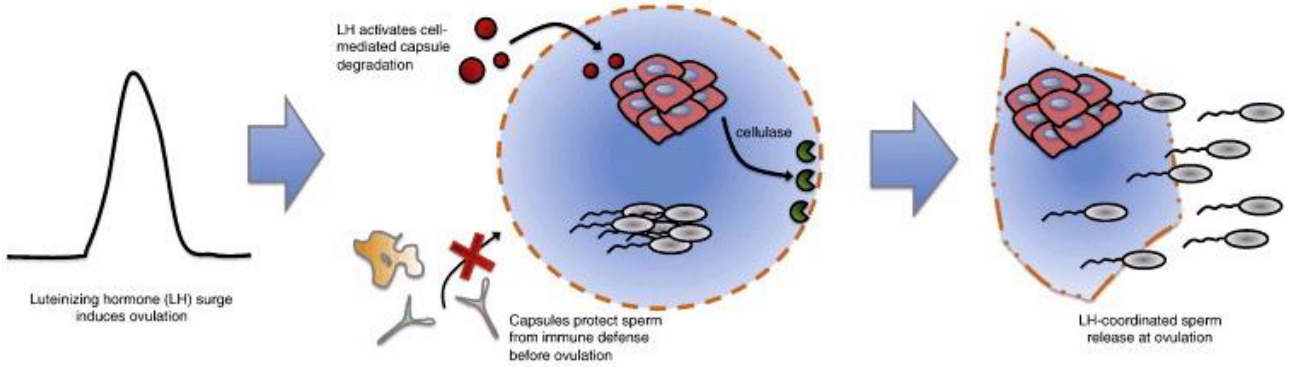
Developed by Geno (Breeding organisation) and SINTEF (research Inst.).
Marketing through BioKapital

- Semen captured in a matrix
- Slow release of sperm cells in the uterus after AI

LH induced release of semen

Switzerland:

Developed by Swissgenetics (Breeding organisation) and University of Basel, ETH Zurich and Agroscope Liebefeld-Posieux



Kemmer et al, 2011

- Semen captured in a capsule
- Release of sperm cells after LH surge

Content

Introduction

Semen technologies

Embryo technologies

- *MOET & OPU-IVP*
- *Genomic Selection / genotyping embryos*

Future

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Embryo transfer technologies

Used by breeding industry to enhance genetic improvement

Increase the number of offspring of per bull dam by:

- Multiple ovulation and embryo transfer (MOET)
- Ovum pick-up followed by in vitro embryo production (OPU-IVP)

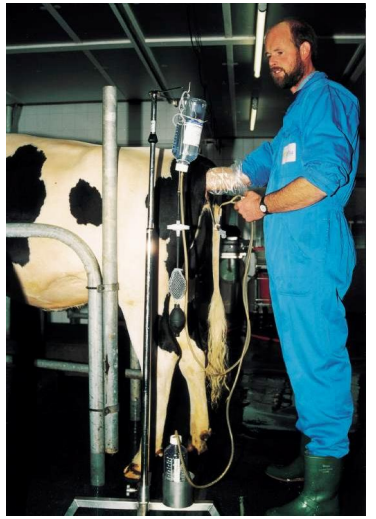
MOET

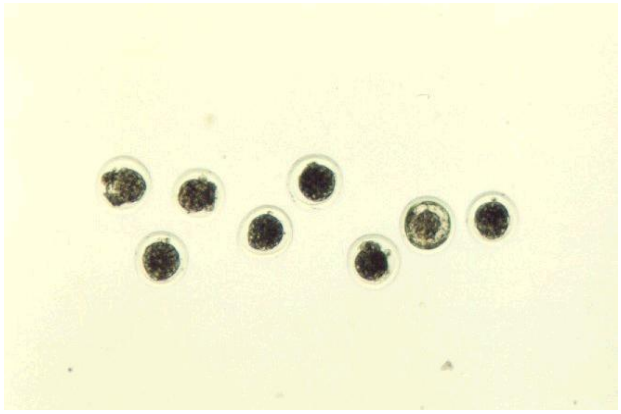
Since early 1980

Hormonal treatment to induce multiple ovulation

Embryos collected 7 days after heat

5-6 embryos per session; every 5 to 6 weeks





OPU-IVP

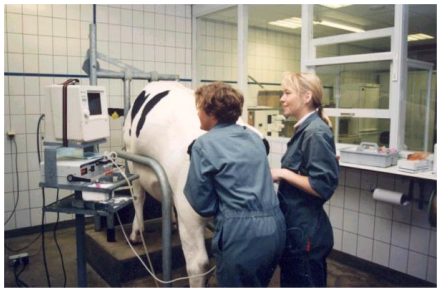
Since early 1990

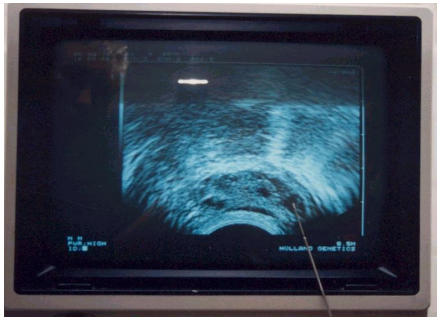
Collection of immature oocytes

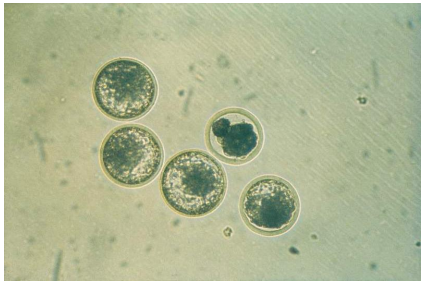
Follicle aspiration (ultrasonography)

Maturation	IVM	(1 day)
Fertilisation	IVF	(1 day)
Culture	IVC	(6-7 days)

1-2 embryos per session; twice a week



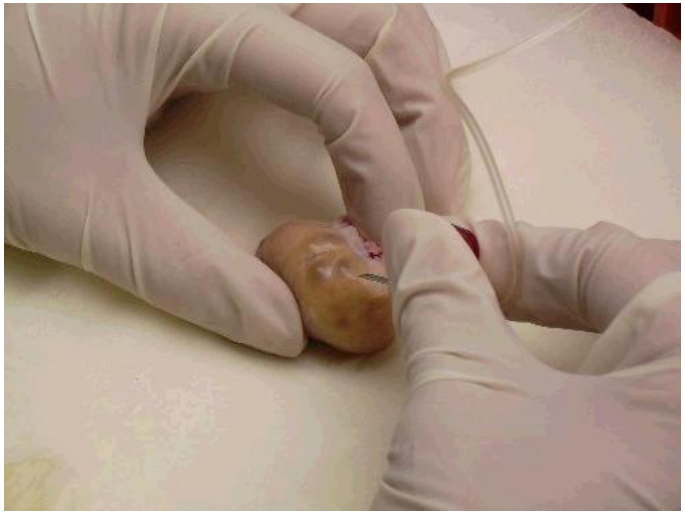




IVP and genetic recovery

Ovaries obtained from slaughtered cows

Follicle aspiration and oocyte collection in laboratory



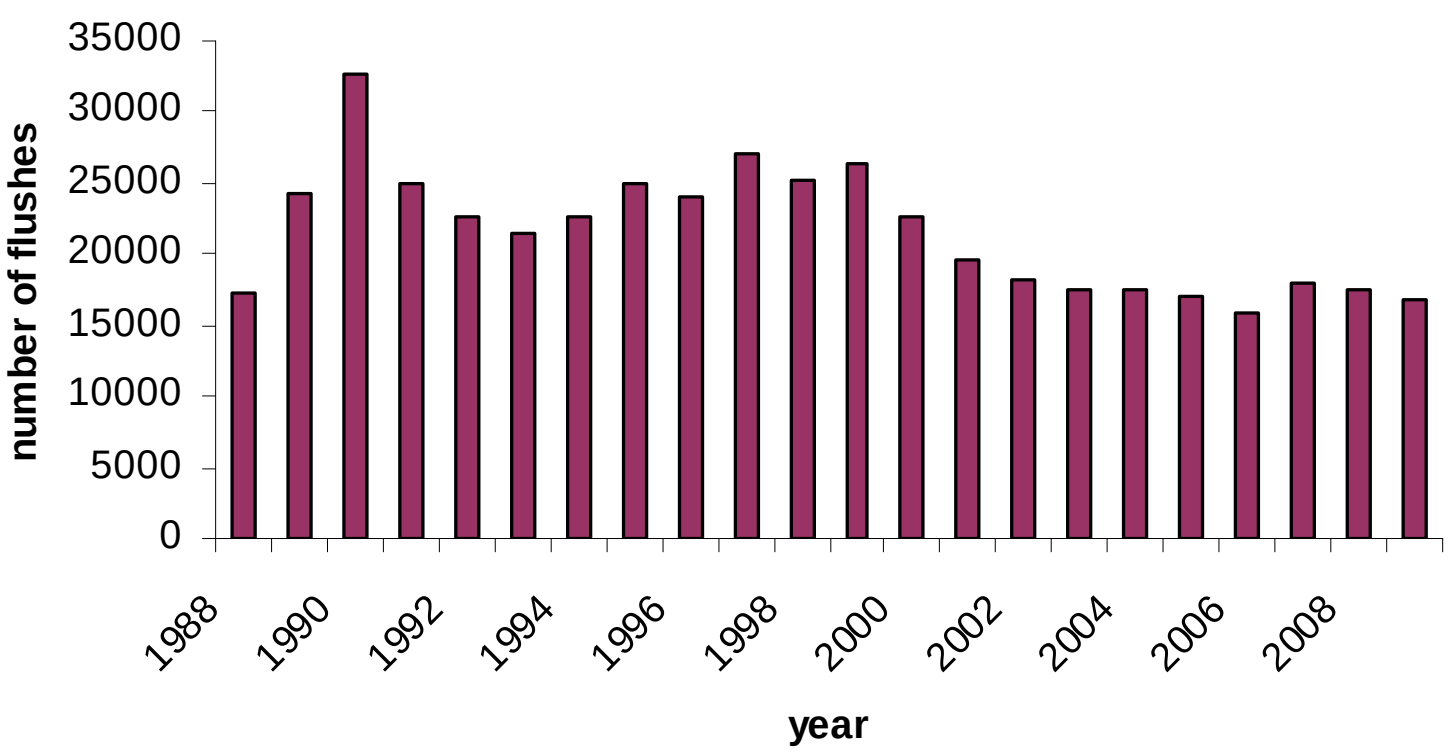
Statistical Data of Bovine Embryo Transfer Activity in Europe

AETE

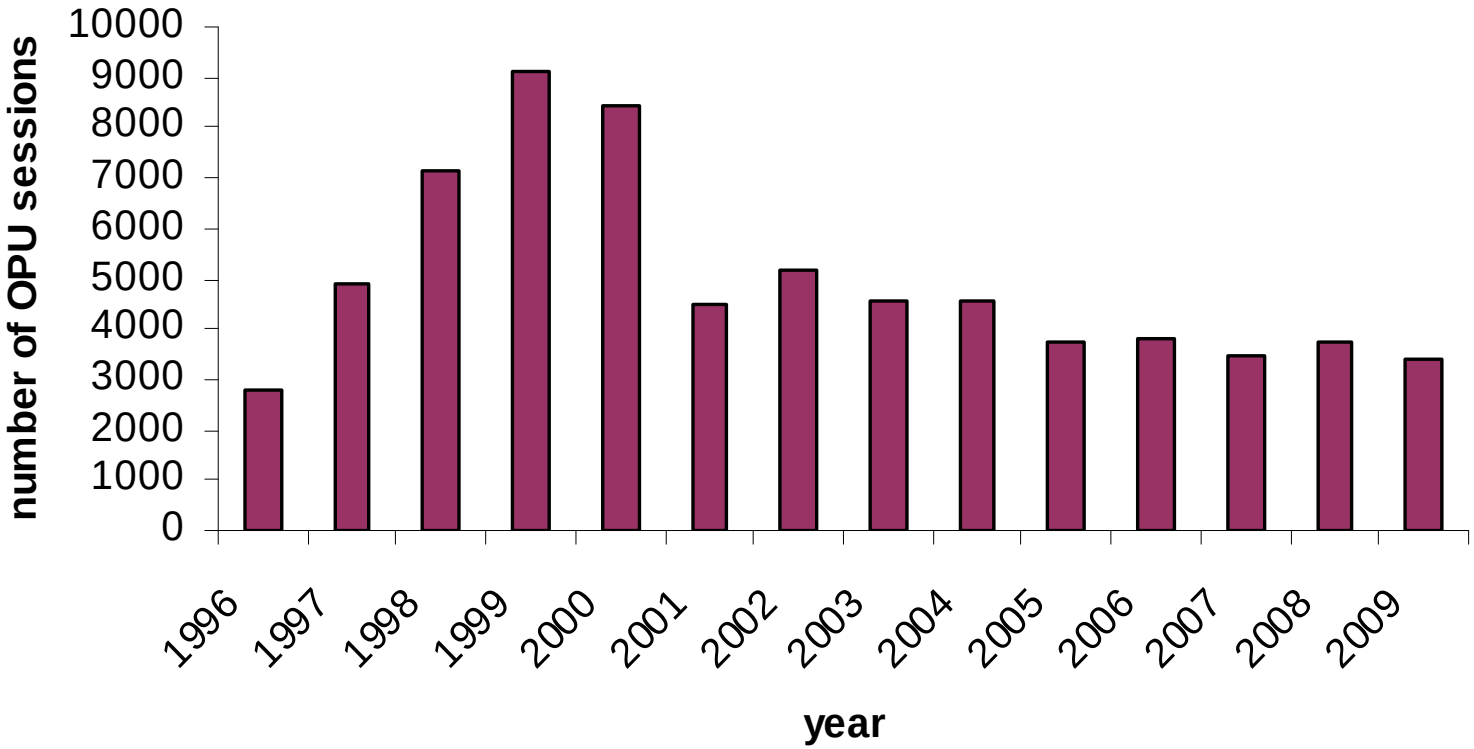
Association Européenne de Transfert Embryonnaire

European Embryo Transfer Association

MOET


35000
30000
25000
20000
15000
10000
5000
0
number of flushes
1988
1990
1992
1994
1996
1998
2000
2002
2004
2006
2008
year

OPU-IVP


number of OPU sessions
10000
9000
8000
7000
6000
5000
4000
3000
2000
1000
0
1996
1997
1998
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2002
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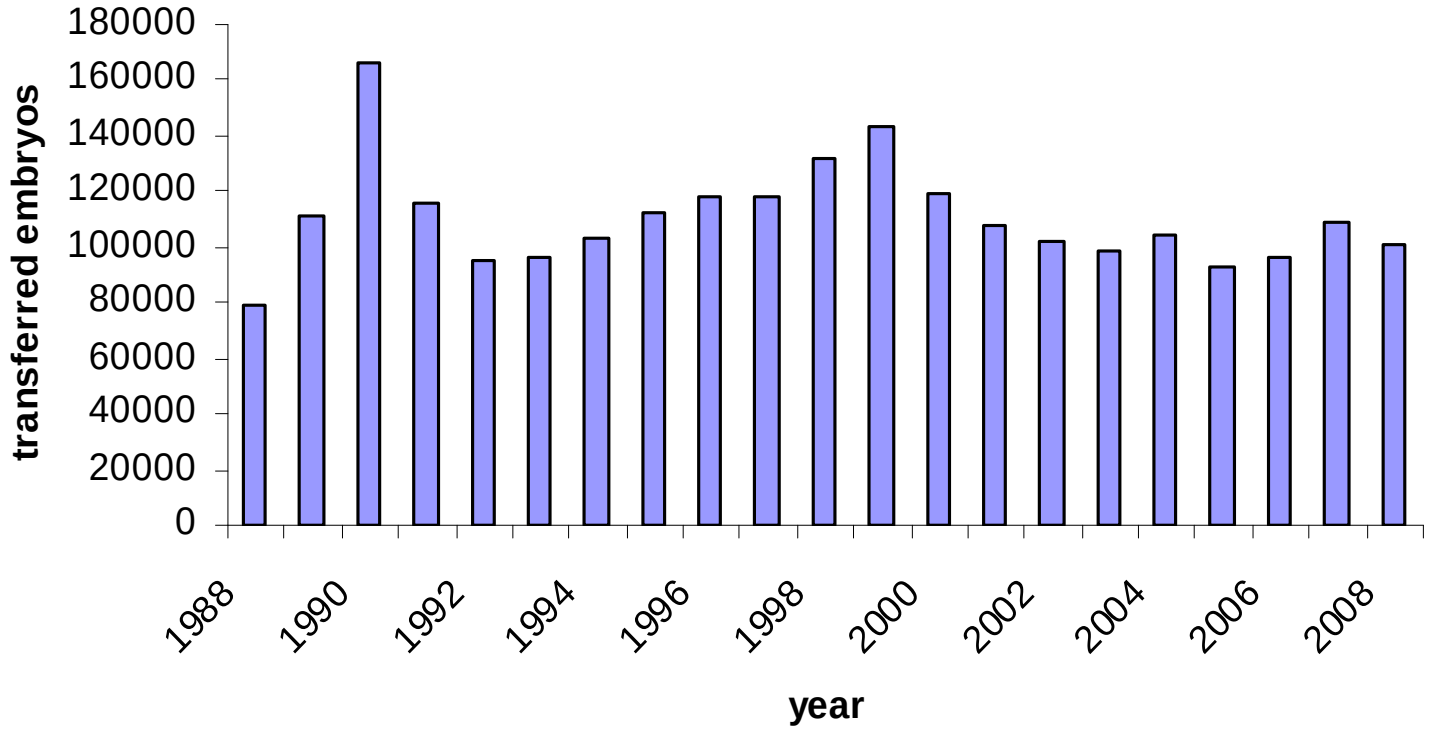
2007
2008
2009
year

Embryo transfers (MOET + IVP)

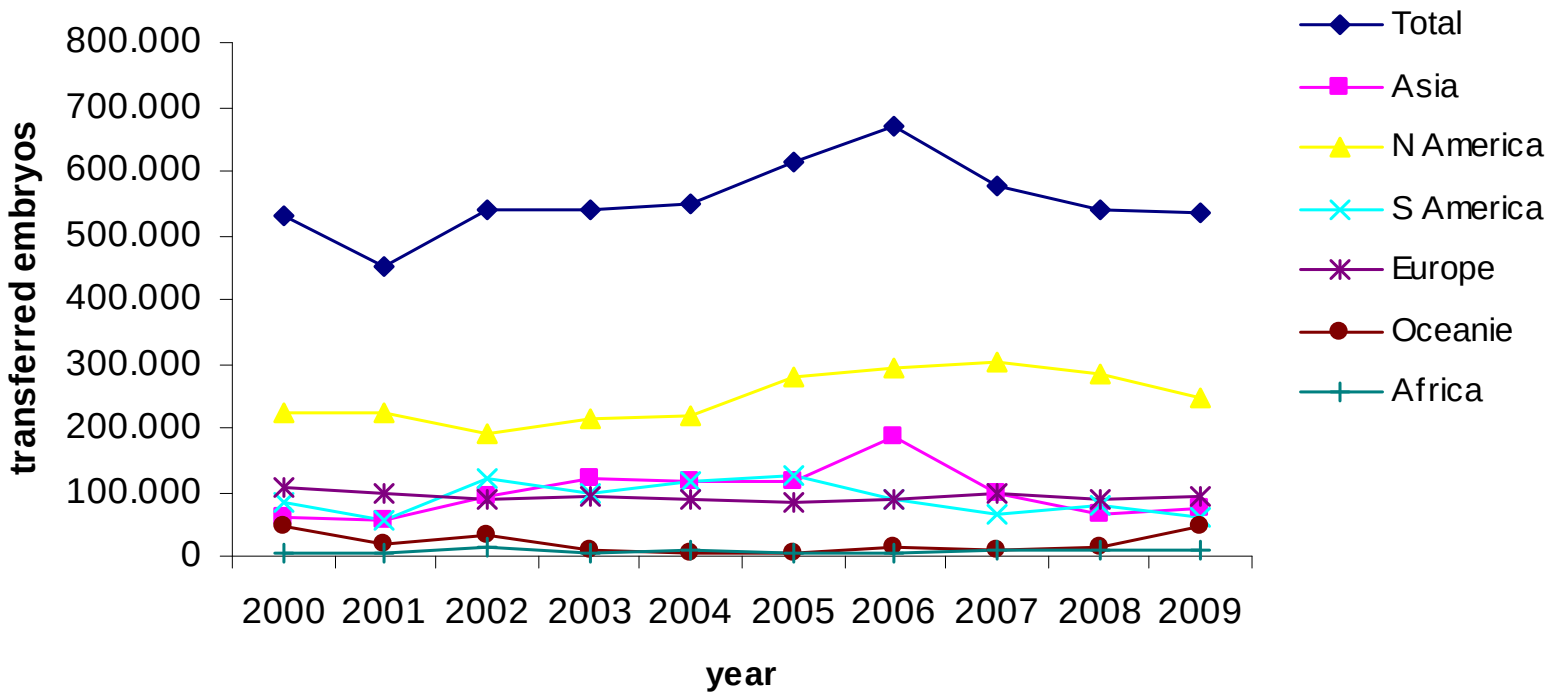


Other species, embryo transfers

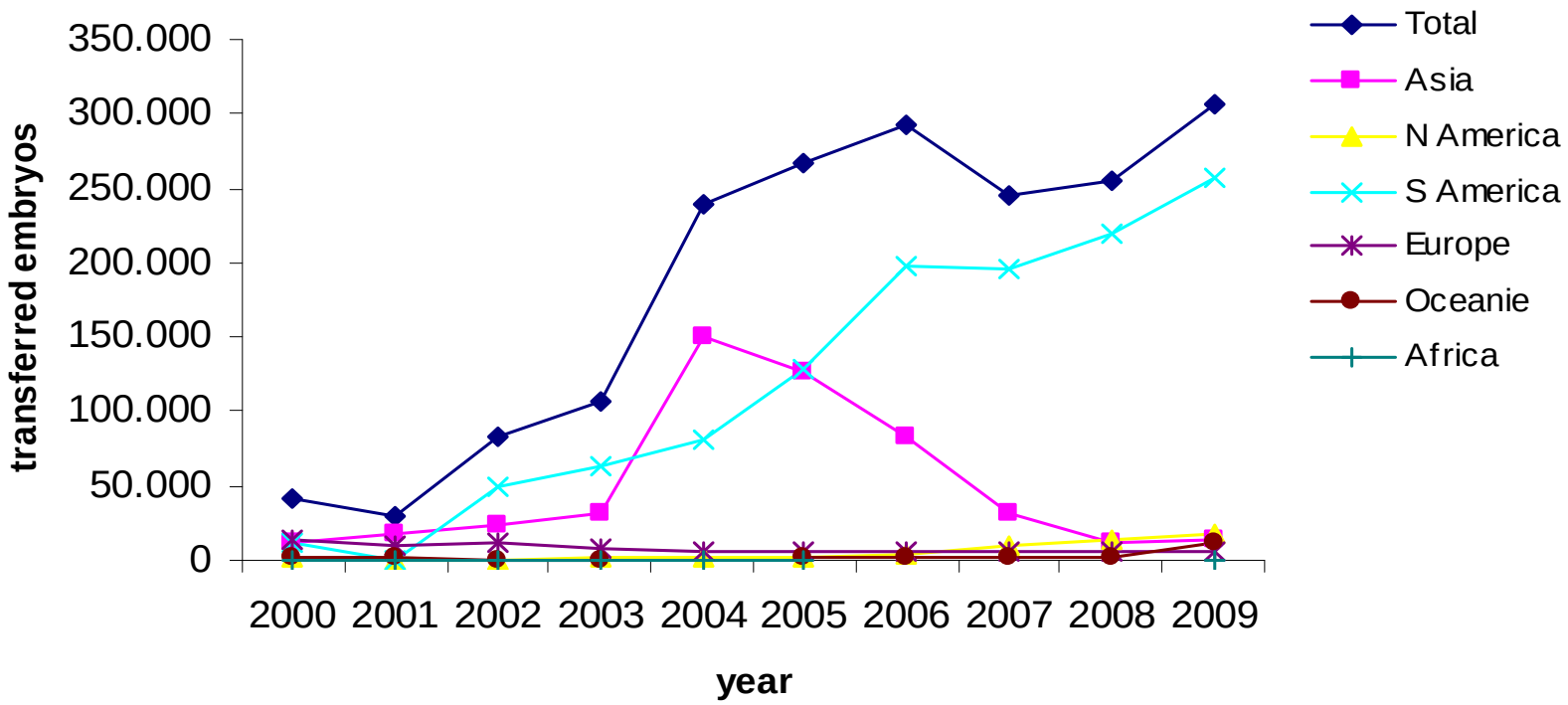
Species	Transfers (2009)
Sheep	143
Swine	20
Goat	-
Equine	1,037
Bovine	100,678

Statistical Data of Bovine Embryo Transfer Activity in the world

Embryo transfers (MOET)

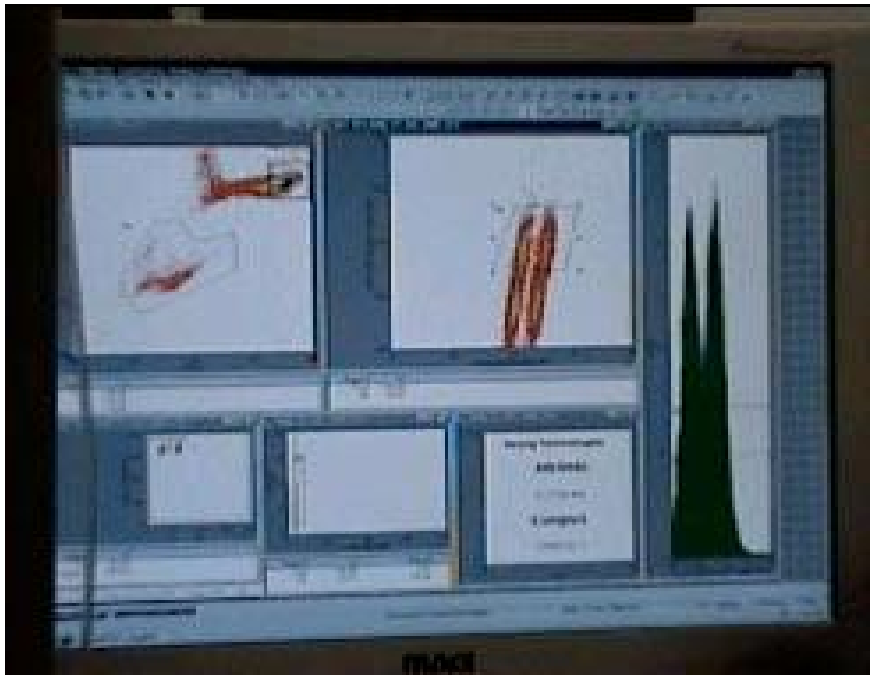


Embryo transfers (IVP)



Use of new technologies in embryo production

Sexed semen



Sexed semen in MOET

MOET

- # produced embryos in heifers slightly lower (≤10%)
- # produced embryos substantially lower in cows (≤ 50%)

Insemination strategy modified:

- delayed insemination moment; at 18 and 30 hrs v 12 and 24 hrs
- special straws with higher dosage; 5 mil v 2 mil

AI	Embryo development
2 straws of 2M	28.9 %
1 straw of 5M	39.0 %

(data from Transova; beef 2009)

Sexed semen in IVP

IVP

- # produced embryos affected (≤30%)

IVF process modified:

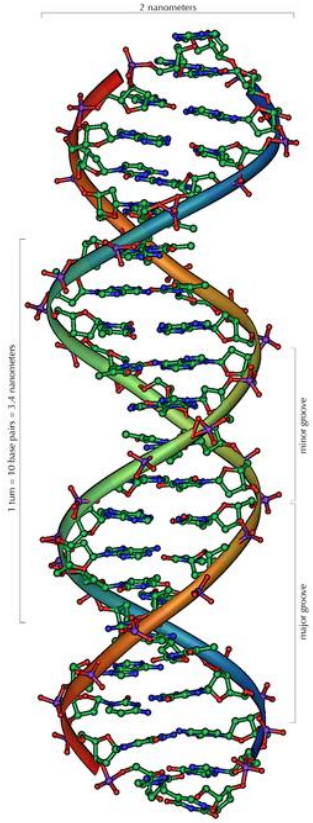
- modified semen processing
- selection of semen batches
- sorting strategy

Sorting strategy	Embryo development
Normal frozen	27.9 %
Sexed frozen	18.5 %
Reverse sort fresh	24.5 %

(data from Transova; beef and dairy 2009/2010)

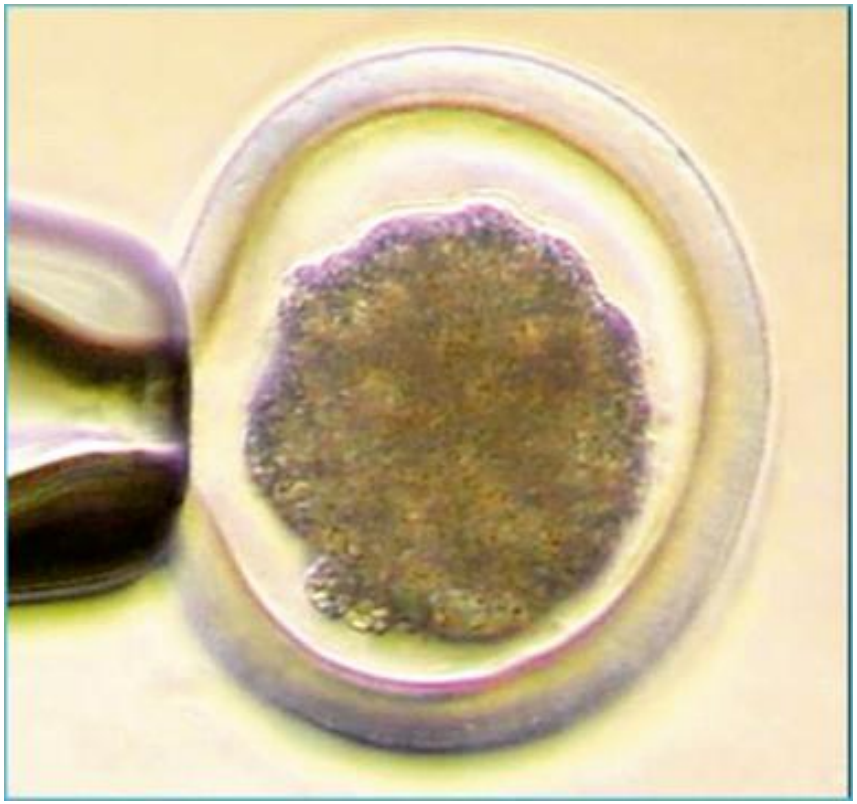
Use of new technologies in embryo production

Genomic Selection of embryos



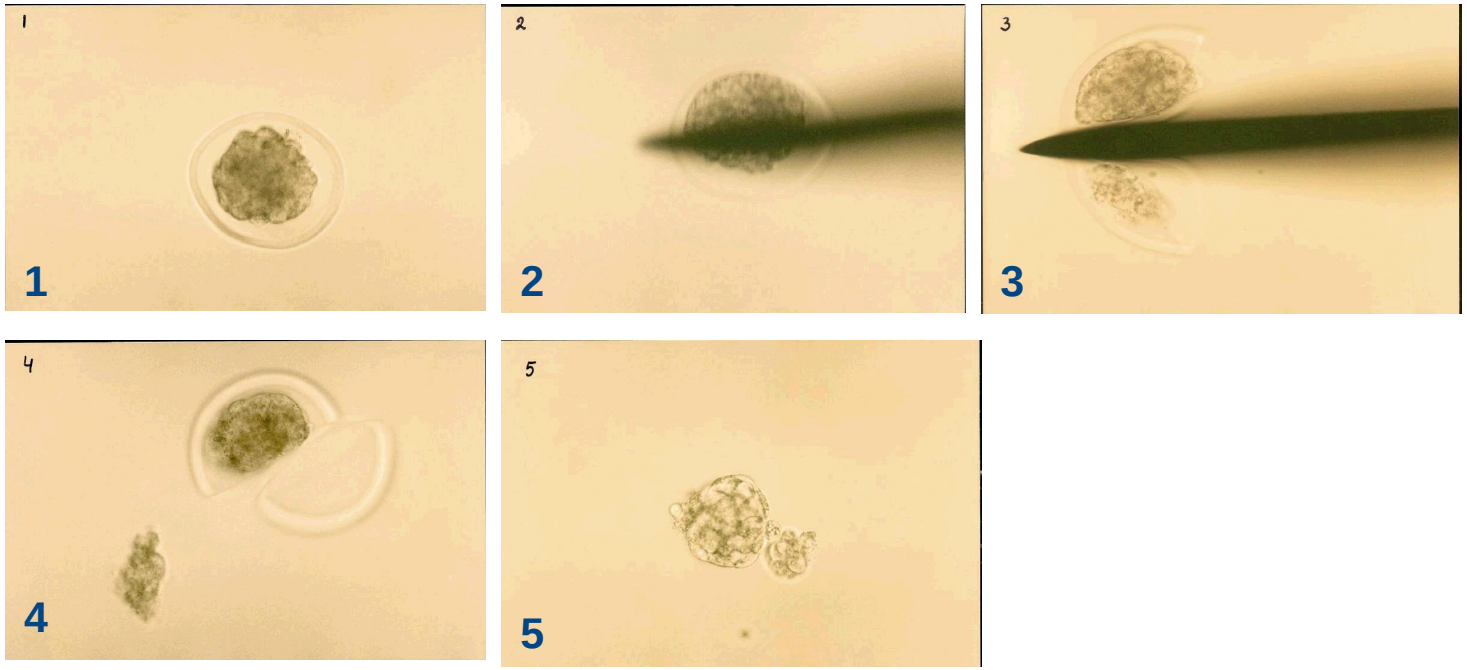
Which *bull calf* received the best genes from the parents?

Which *male embryo* received the best genes from the parents?



Genotyping embryos

Embryo biopsy

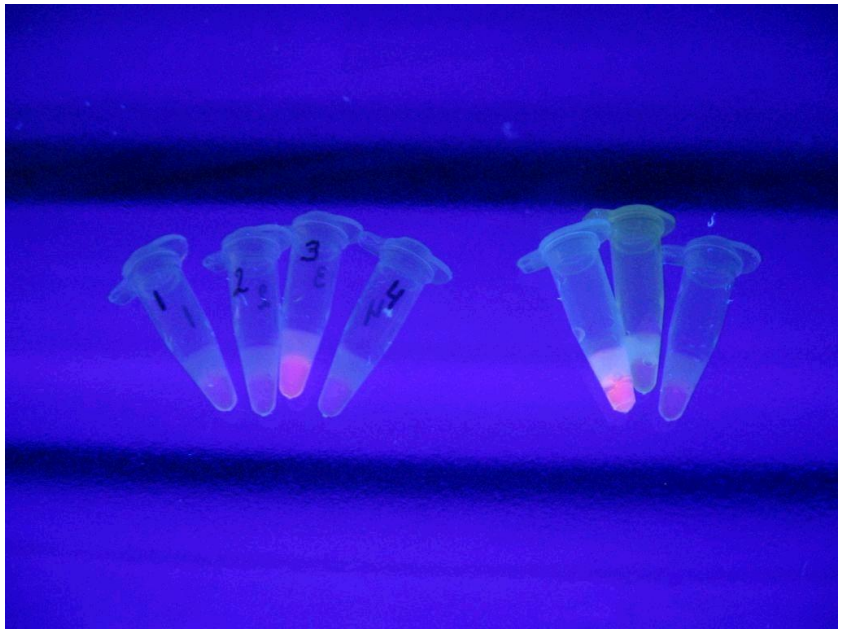


Embryo sexing

PCR Amplification Y DNA

Commercial service

0 - 10% reduction in pregnancy rate



Genotyping embryos

Main challenge: whole genome amplification (WGA)

Blood sample 5-10.10^6 cells v Biopsy 5-15 cells

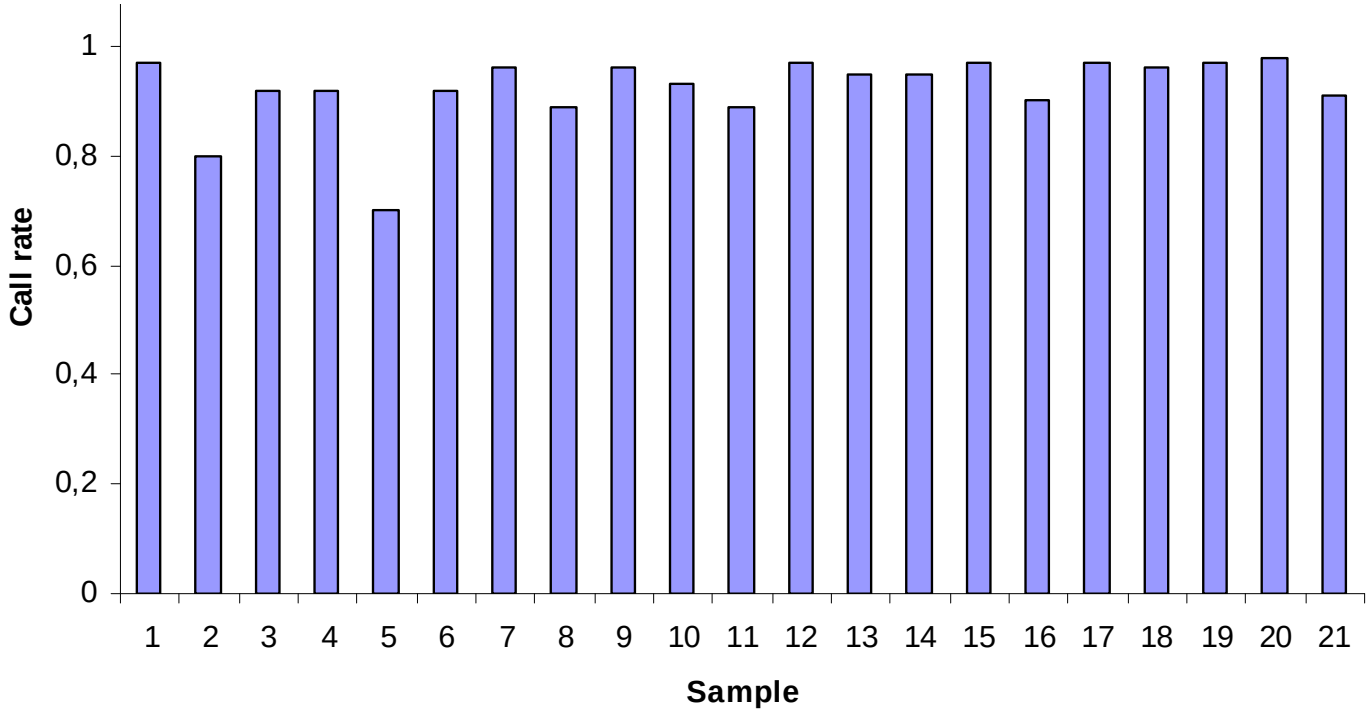
Call rate:

- presence of signals of all single nucleotide polymorphism (SNP)

Allel Drop out (ADO):

- expected heterozygous SNPs (both parents are genotyped) is measured as homozygous due to incomplete WGA.

Call rate



20 biopsies out of 21 (95%) above critical 0.8

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Future (short term)

Semen technologies

Slow / timed released version of sexed semen.

Embryo technologies

Embryo cloning in order to secure the birth of calf from a genotyped embryo.

(Re)introduction of prepuberal embryo production (at 2-4 months) in order to decrease in generation interval.

Future (long term)

Embryo technologies

Embryonic stem cell technologies in order to decrease in generation interval:

- Stem cell isolation from embryo (undifferentiated stem cells)
- In vitro production of gametes (differentiated stem cells)
- Production of new embryos by ICSI-IVP.

Velo genetics; gametes from foetus

(Georges and Massey, Theriogenology, 1991)

Whizzo genetics; gametes from stem cells

(Haley and Visscher, 1998)

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- Semen sexing has become a proven technology.
- Sperm release strategies can be helpful, mainly in livestock management.
- Both MOET and OPU-IVP are well established reproduction techniques; reliable and accepted by the farmers.
- Introduction of GS will shift application of embryo production technology towards OPU-IVP.
- Embryo genotyping currently introduced.
- Recent developments will increase the interest in prepuberal embryo production, cloning and stem cell technologies.

Questions?