Biotechnologies applied to Equine Reproduction: State of art and challenges

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Botucatu – Strategic location
Stud Farms and Reproduction Centers

Distâncias de Botucatu para as Principais cidades do Estado:
- Aeroporto de Viracopos 167 km
- Araçatuba 282 Km
- Araraquara 150 Km
- Bauru 91 Km
- Franca 319 Km
- Marilia 187 Km
- Piracicaba 120 km
- Porto de Santos 303 km
- Presidente Prudente 373 Km
- Ribeirão Preto 240 km
- Rio Claro 119 Km
- São Paulo (Capital) 224.8 Km
- São José do Rio Preto 290 Km
- São José dos Campos 311 Km
- Sorocaba 150 km
HORSE INDUSTRY IN BRASIL

6 MILLION
(Cattle farms)

1.5 million pure breed

1.500.000 Employments
OTHER SMALL HORSE BREEDS IN BRAZIL

- Campolina – 50.000
- Arabian - 35.000
- Brasilian Jumping Horse - 20.000
- Thorougbred- 15.000
- Lusitano -10.000
Biotechnologies in the routine of horse reproduction

- Embryo Transfer
- Cool and Shipped Semen
- Frozen Semen
Biotechnologies in the routine of horse industry - Annual Birth

| Cooled and shipped semen         | USA       | 150.000  |
|                                  | EUROPE    | 100.000  |
|                                  | BRASIL    | 40.000   |

| Frozen semen                     | FRANCE    | 10.000   |
|                                  | HOLAND    | 2.000    |
|                                  | BRASIL    | 3000     |
|                                  |           | (200 - 3 years ago) |

| Embryo transfer                  | BRASIL    | 20.000   |
|                                  | USA e Argentina | 12.000   |
|                                  | EUROPE    | 5.000    |
ET FOALS BORNED - MANGALARGA MARCHADOR

Número de Produtos Registrados a partir de TE (Mangalarga Marchador)

2013
>
9000 foals

Goretti, 2012
WHAT MOVIES THE REPRODUCTION MARKET IN MM and QH in Brazil?

- Stallions with several owners
- Important stallions
- Important mares (more foals and commercialization of oocytes)
- Mares in competition
- Old mares
Advantages of the biotechnology for sport horses

- Have products from different stallions (Frozen semen and Embryos)
- Prolong athletic life
- Increase the chances to have a good sport horse from old mares
Challenges and obstacles that were overcome in sperm preservation over the last 5 years:

- Freeze semen from Stallions that do not resisted to freezing process.
- Past >large % Mangalarga bad freezers.
- Very low fertility of frozen epididimal sperm.
REPLACEMENT OF GLYCEROL PER AMIDES

LESS TOXICAL CRIOPROTECTORES
(STUDIES IN BRAZIL 1999-2004)
EFFECT OF DIMETHYLFORMAMIDE ON SPERM POST THAW TOTAL MOTILITY-COMPARISON BETWEEN BREEDS

ALVARENGA ET AL. 2004
Percentual of stallions that freezes well (TM>40%) using DF or GLY

- GLI: 21/55
- DF: 44/55
BOTUCRIO NEW EXTENDER

- Combines AMIDES with AMINOACIDS present in plants that resist better to winter

- Better for stallions that semen do not freeze well (Mangalarga)

- In general improves fertility even from good freezers.
Post–thaw motility of semen frozen with Botucrio, EDTA–Lactose and INRA extender.
Figure 1. Mean percentage membrane integrity (MI), total (TM) and progressive motility (PM) of frozen thawed semen cryopreserved using Ghent extender vs. BotuCrio before (control) and after receiving the feeding additive Equi Sperfor Plus (ES+).
Freezing semen from epididymus—Why???

- Last chance—Unexpected loss of a breeding stallion.
- However, fertility of cryopreserved epididymal semen used to be much lower than that of cryopreserved ejaculated semen.
- One recent Brazilian report revealed a high pregnancy rate (69%; 9 of 13) epididymal semen preserved in Botu-Crio extender.
FREEZING EPYIDIDIMAL SPERM 200 STRAWS = 50 -100 FOALS
New techniques to improve semen fertility
Stallions do not become sires because of reproductive capability!!!!
Centrifugation techniques > Separation of sperm with better motility and integrity
EquiPure™

This method has been used successfully with several commercial stallions.
Low-Dose Insemination
NEW CHALLENGES

SEX  SEMEN

IN VITRO FERTILIZATION

CLONNING > NUCLEAR TRANSFER
Why and how to determine the fetal sex?
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.
SEX DETERMINATION WITH EMBRIONIC CELL COLLECTED JUST BEFORE TRANSFER
NEW ASSISTED TECHNIQUES APPLIED TO HORSE REPRODUCTION
INTRA-CITOPLASMATIC SPERM INJECTION
ICSI

Applications

Stallions with low number frozen doses
ExtremalIly poor quality semen
ICSI - technique

MICROSCÓPIO E MICROMANIPULADOR
ICSI - technique
What is clonning?

NUCLEAR TRANSFER
Biopsy from the skin
Insert single donor cell

Electrofusion

Cell division
CLONNED HORSES:

Idaho Gem
May 2003
University Idaho, EUA
Gordon Woods, Dirk Vanderwall e Kenneth White

1º MULE

Outros 2 muares também nasceram no mesmo ano
Embryo recipient was the cell donor (Borned from her self)
3º CLONE

Comercial Programm

May 2005

Paris Texas (Quidan Revel)

Texas A&M e Cryozooted

Dra K. Hinrichs & Dr. E. Palmer
PARIS TEXAS =
QUIDAM DE REVEL (SPTZ)

45 días

7 meses

2005 Cryozootech + TAMU
SMART LITTLElena

Alex, Bogy, Camby, Dave e Eli

2006 TAMU
Clones Mangalarga in Brazil
July 2013
MUITO OBRIGADO PELA ATENÇÃO!!!!