



# Studbook Registration of Horse Clones: Historic, Scientific and Rationale Basis.



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# 2003, the year of equine cloning

## A Mule Cloned From Fetal Cells by Nuclear Transfer

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Science, 29 may 2003

### brief communications

## A cloned horse born to its dam twin

A birth announcement calls for a rethink on the immunological demands of pregnancy.

Several animal species, including sheep, mice, cattle, goats, rabbits, cats, pigs and, more recently, mules<sup>1</sup> have been reproduced by somatic cell cloning, with the offspring being a genetic copy of the animal donor of the nuclear material used for transfer into an enucleated oocyte. Here we use this technology to clone an adult horse and show that it is possible to establish a viable, full-term pregnancy in which the surrogate mother is also the nuclear donor. The cloned offspring is therefore genetically identical to the mare who carried it, challenging the idea that maternal immunological recognition of fetal antigens

Our cloning procedure was relatively efficient, as one live foal was produced from four pregnancies, although there was high developmental failure from the cleavage stage to blastocyst (8 of 467 and 14 of 286 developed in the male and female cell lines, respectively) and early implantation. This success was aided by advances in assisted reproduction in the horse<sup>2</sup>, particularly at the oocyte-activation stage, when protein synthesis and phosphorylation must both be inhibited<sup>3</sup>, and in the refinement of the zona-free manipulation technique<sup>4,5</sup>.

The remarkable birth of a live foal from its genetically identical recipient is at odds



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Nature, 7 august 2003



***2005 the year of cloning Horse Champions,  
Birth of Pieraz-Cryozootech-Stallion, the clone of a castrated horse  
champion***





# *Horse Cloning, the calendar*

- 1996 birth of DOLLY, the sheep
- 2001 foundation of CryozooTech
- 2003 first clone of equids in Italy and in USA
- 2005 first commercial cloning of champion horses and their registration in a studbook
- 2007 first approval of a cloned stallion in a regular studbook
- 2007 WBFSH acknowledges the existence of clones and proposes rules for registration and approval of clones.
- 2008 first birth of a foal out of a cloned stallion
- 2009 first birth out of a cloned mare
- 2012 FEI decides to accept clones and offspring in FEI competitions.

# *One idea: restaure the breeding capacity of castrated champions*



- **Obstacles:**

- **Confusion with human cloning**
- **Confusion with GMO**
- **Mistrust in science**
- **Animalist ethic**

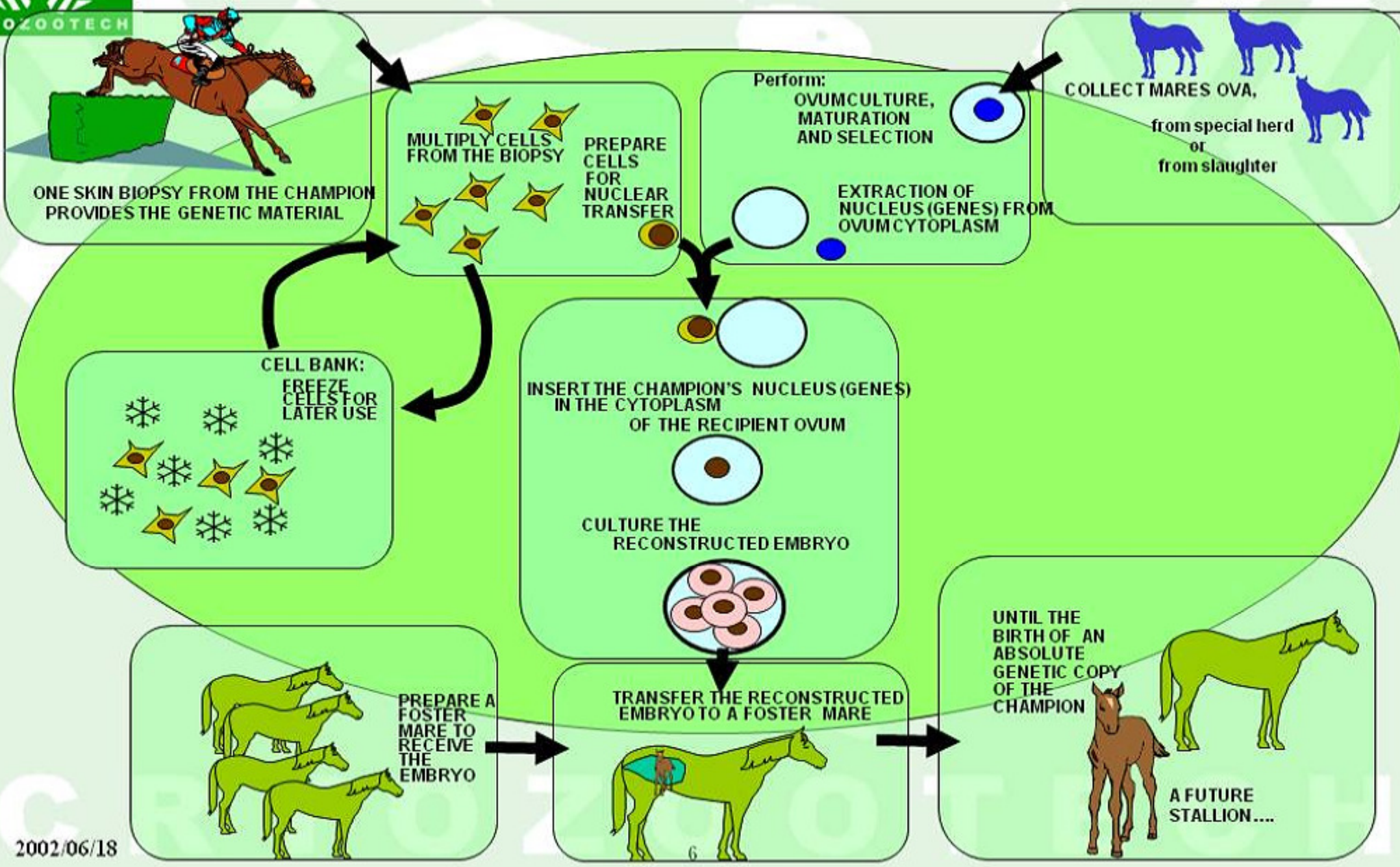
Animal welfare

Animal or species « dignity »...

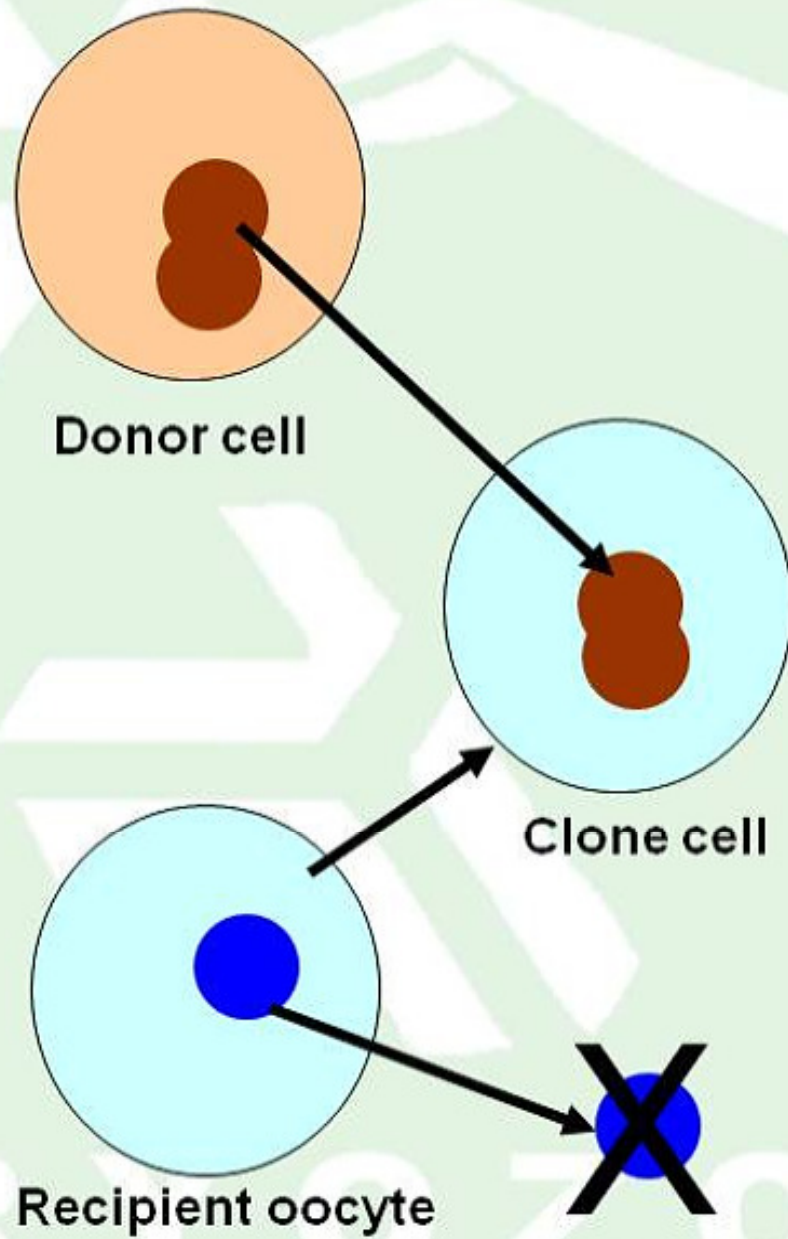
- **Opposition to the business aspect of the company**
- **The « politically correct »**
- **The fear of novelty**



# What is horse nuclear transfer ?



# *transmission de l'AND nucléaire et cytoplasmique au clone*



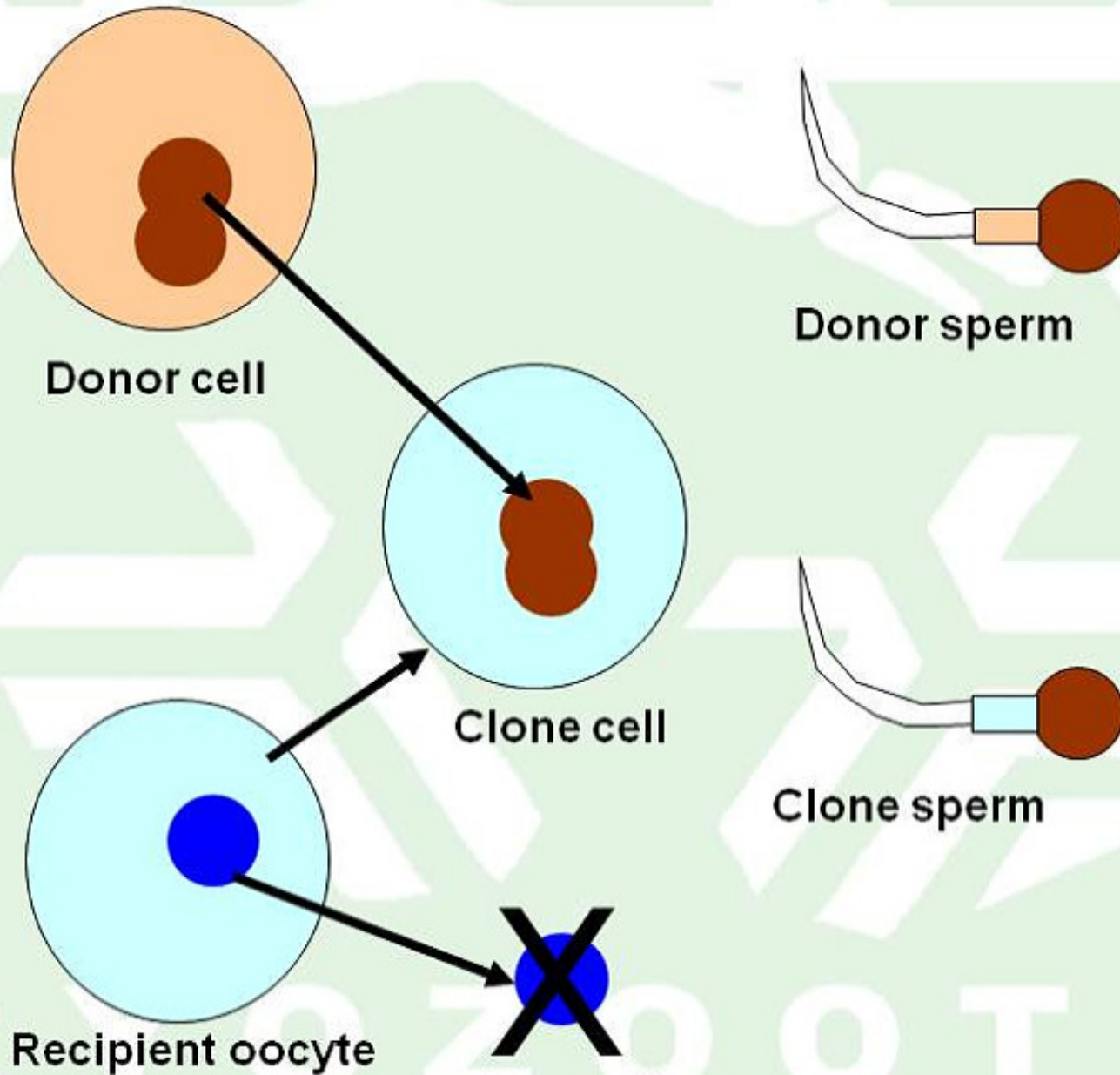


# *What can be different between a clone and its model?*

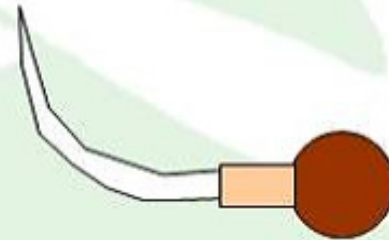
- The phenotype of a clone can differ from the phenotype of the original for multiple reasons:
  - imperfect reprogramming or epigenetic differences,
  - mitochondrial DNA,
  - environmental factors.



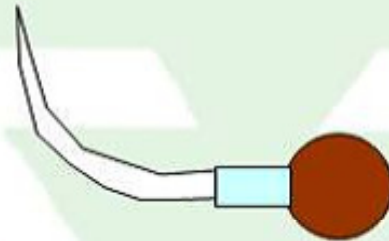
# *transmission de l'AND nucléaire et mitochondrial au spermatozoïde*



# *Can we recognise the semen of a clone from the semen of his model ?*

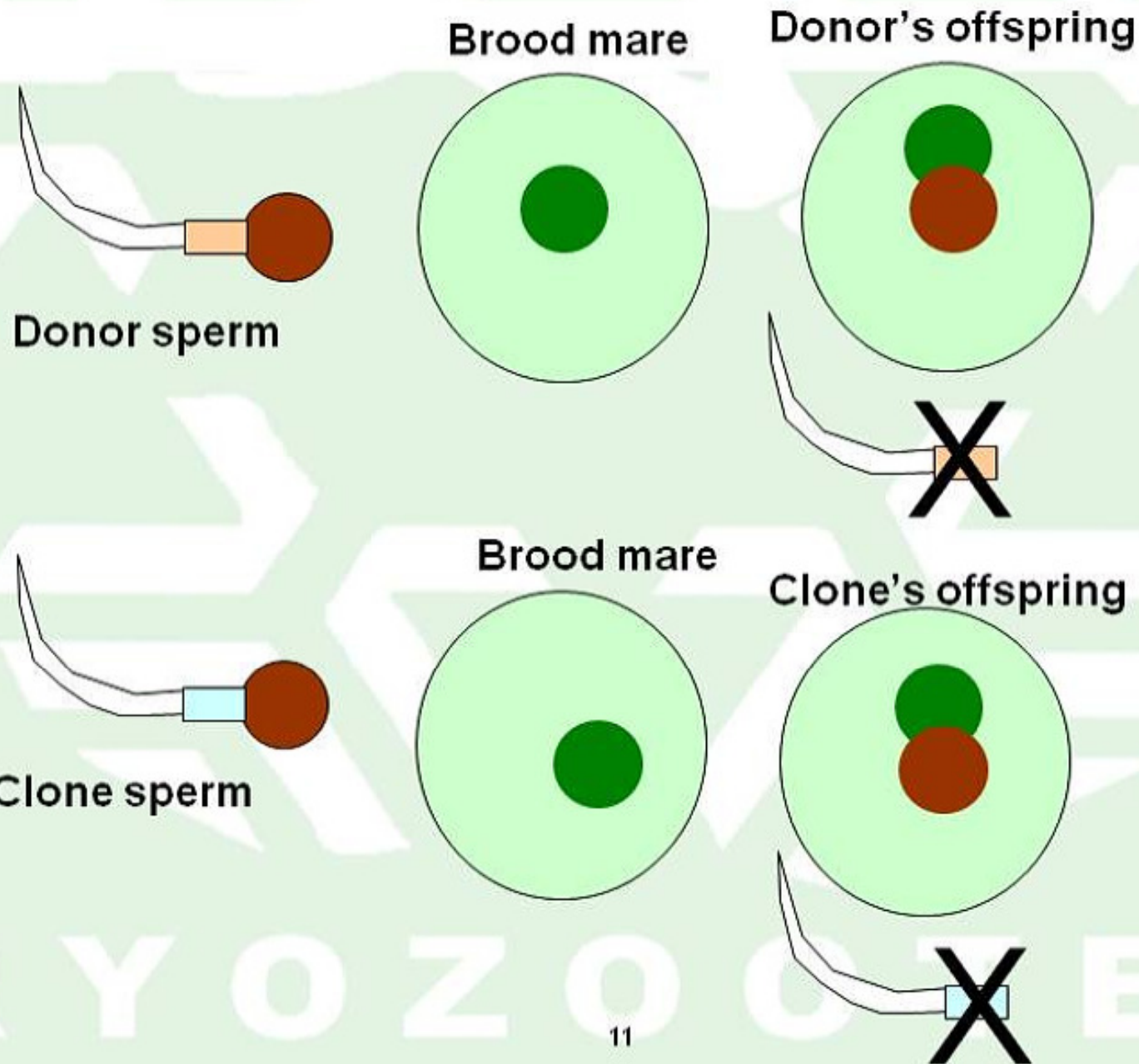


Donor sperm



Clone sperm

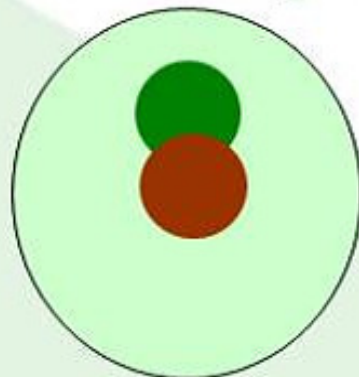
*As a breeding animal, the clone is 100% equal to donor*



*Difference between the offspring  
of a clone or of the model ?*

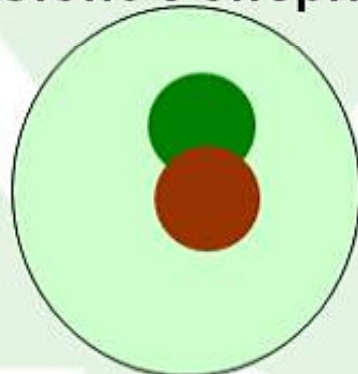
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Donor's offspring



**No difference**

Clone's offspring





## *Can we detect that a horse is a clone?*

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- If we do not know the identity of the model **NO!**  
No specific difference between clones and control
- If you suspect that a horse is the **clone of a known horse**, you can compare the DNA to that of the suspected model: **nuclear DNA should be identical.**

# Cloning makes value: a technique for high value animals

All these horses are world champions in their respective discipline



Their value at the top of their career is between 1 and 5 million \$



They are geldings, and their value will be nul when they have finished their sporting career

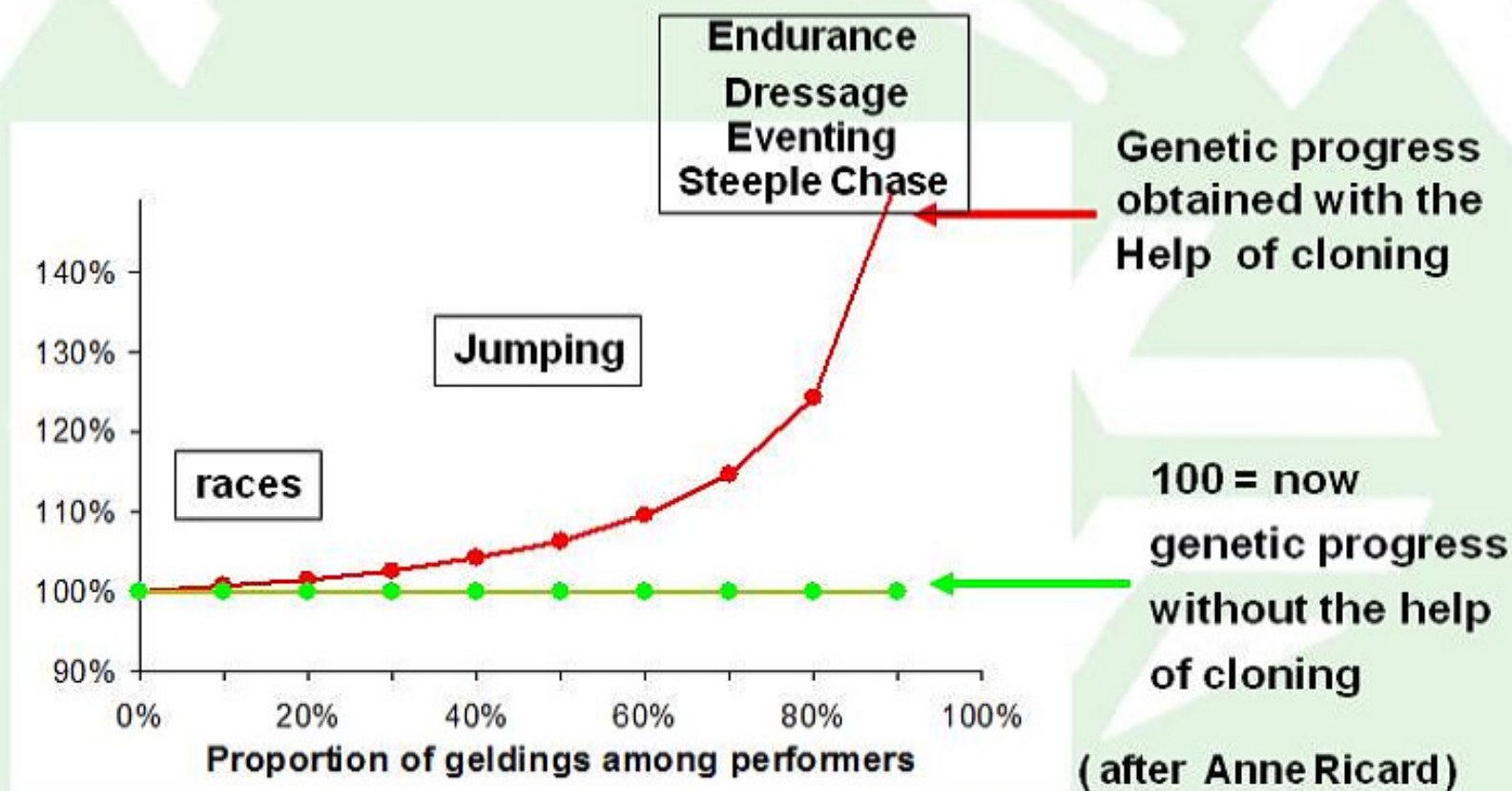
Gender of the 15 best world performers 2001				
Discipline	gender			
	gelding	mare	stallion	?
Endurance	9	4	0	2
Steeple-Chase	15	0	0	0
Dressage	12	0	3	0
Jumping	7	2	6	0
Eventing	13	0	0	2



Their value as a stallion could be more than 2 million \$  
 $200 \text{ services} \times 2000 \$ \times 5 \text{ years} = 2 \text{ million } \$$

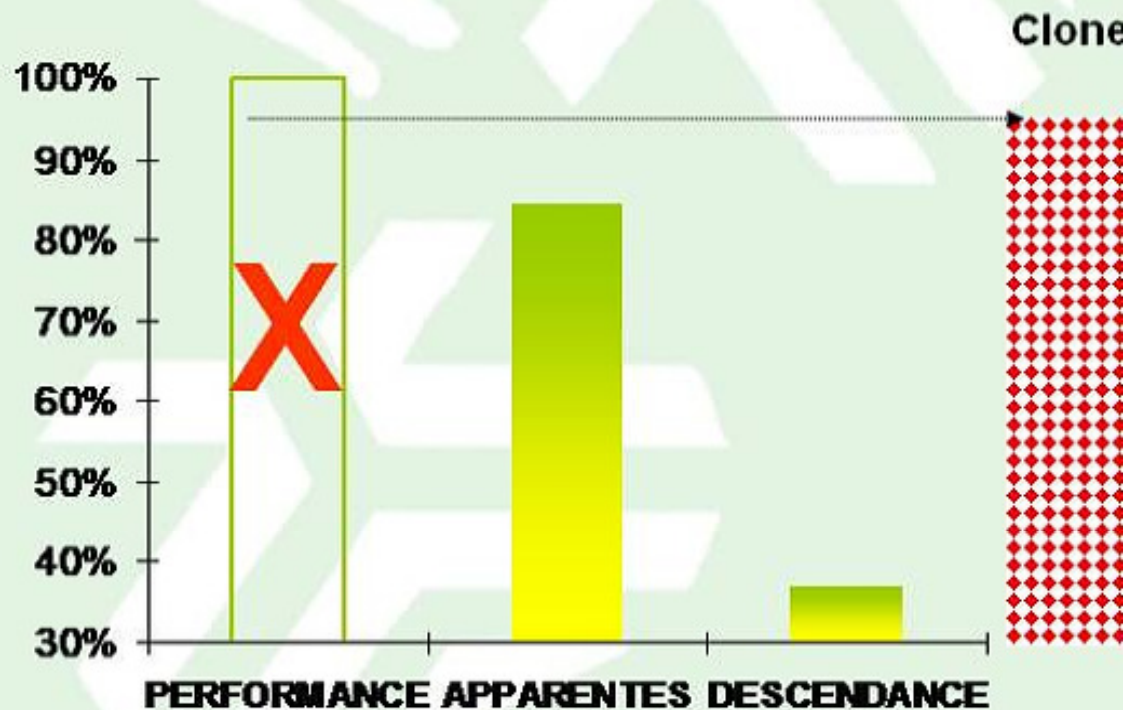
# Cloning, a way to accelerate genetic progress

- selecting stallions in a wider population of performers will allow a higher selection rate, and consequently more rapid genetic progress





# Annual genetic progress obtained with different strategies



## *Is a gelded champion a potential stallion?*

- All stud-books rely on selection and consider the sport result not only as a target, but also as a test
  - Genetic + environment → performance
  - Performance → Genetic test
- There is no champion without a potential
- E.T. was three years the 1st at WBFSH ranking.
  - If this does not mean that he has the required aptitude, why this ranking ?



*Multiply rapidly the top genetic*

## Pieraz-Cryozootech-Stallion

clone of Pieraz,

2X world champion in endurance

Born 2005....

And Production of 2009 breeding



**PIERAZ CRYOZOOTECH STALLION :**  
Naissances en région RHONE-ALPES



Agile des Collines



Alaska de Rivoiron



Akajou de Rivoiron



Alick de Chateaudu



PIERAZ CRYOZOOTECH STALLION



Alysée des Pras



Artémis de Clairebois



Anous de Freyssa



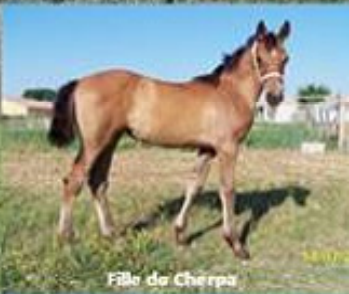
Axum de Charbesse



Fils de Roca



Végaz



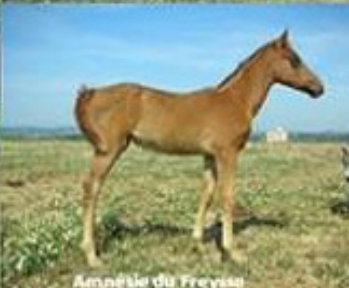
Fille de Cherpa



Atila et Pieraz Mize



Atox de Chateaudu



Amélie du Freyssa

CRYOZOOTECH



## *The market wants also cloning stallions and females :*

- **Cryozootech first claim: cloning castrated horses**
- **In 2008 the cell bank contained**
  - 22 geldings
  - 22 stallions
  - 32 mares
- **In 2008 the cumulated orders were**
  - 2 geldings (plus 4 Cryozootech)
  - 5 mares
  - 4 stallions



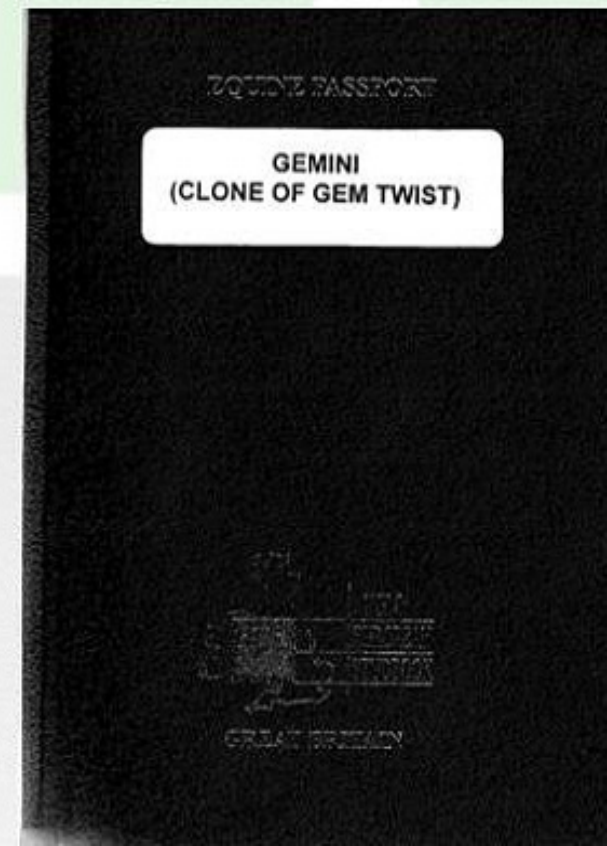
# *Law and social acceptation*

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- **Law:**
  - **Laws on animal experiments ( see GB, DK)**
  - **Laws on animal welfare, veterinary ethics**
  - **Laws on safety of human consumption**
- **Breeding rules**
  - **Internal rules of different studbooks (WBFSH, WAHO, Jockey Club)**
  - **EU rules for recognised studbooks**
- **Rules for competition and races**
  - **Jockey Club flat races and European Trotting Association prohibit clones and clone offspring.**
  - **FEI has published in 2012 a resolution accepting clones.**



# Clones are registered by ZANGERSHEIDE and ANGLO-EUROPEAN STUD BOOK



**2012 : KWPN is registering  
the 2 newborn clones  
of JAZZ,  
The World Top Dressage Stallion**



**REGISTRATION  
DOCUMENT**

For European Performance Horses  
P O Box 630 Haywards Heath West Sussex PO16 2WW  
01444 458840

**Full Studbook**



# Approval of the clones for breeding were delivered By Zangersheide and AES studbooks

Studbook Zangersheide V.Z.W.



## ATTESTATION

Je soussigné Jean Jacques Vandenberghe, Domein Zangersheide B-3620 Lanaken, agissant en qualité de Vice-président de l'ASBL Studbook Zangersheide certifie que l'étalon:

E.T. Cryozootech Z (Cl) - Z6567.06- par Espri FRh et Gracia est admis à la monte publique pour trente juments.

Fait à Lanaken le 10 mars 2009.

J.J. Vandenberghe  
Vice-président



# CRYOZOOTECH



## ***2012 State of Clones in EUROPE***

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- **18 clones were born and registered in European studbooks**
- **5 stallions have been approved and are used:**
  - **Pieraz, E.T., Quidam de Revel, Gem Twist, Chellano Z**
- **No clone or offspring has participated to competition, but FEI has accepted their participation.**



## *Should clones perform before being approved as breeding animals?*

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- **Genetic principles:**
  - **The genes already proven** by the performance of the donor
  - **Short interval of generations** for rapid genetic improvement.

## *Should clones be accepted in competition?*

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- International competitions do not need a registration in a stud-book.
- If they compete this will provide additional information on the genes.
- If they are forbidden , they will come anonymous in the international competition circuit.....
- Consequently (?) FEI has published in 2012 a resolution accepting clones.



## *Participation of clones to competitions and/or to public tests to become stallions ?*

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- **The breeding organizations (WBFSH) say**
  - clones in public tests before approval as breeding animals.
- **The Genetics says**
  - The genotype has already been proven by the model.
  - No competitions before breeding as this will increase the interval between generations,

# *Historical evolution of rules for AI and ET*

- **Successive steps**

- 1) Forbid
- 2) Accept with restrictions (quality , number, mention of « AI » or « ET »)
- 3) Suppress restrictions
- 4) Suppress mention of « AI » or « ET »

- **Each studbook has progressed (or stopped) at its own speed:**

- 1) Still forbidden : Thoroughbred
- 2) Restricted acceptance : i.e. Trotteur Français studbook
- 3) Full acceptance : most sport horse studbooks

- **It is suggested that the same evolution will occur for cloning**

# *Suggested logic rules for studbooks*

- **Accept or refuse clones**
- **If accepted,**
  - The mention of “clone of XX” should be added on passport and studbook.
  - The clone of a registered horse is automatically registered
  - The clone of an approved stallion is automatically approved.
  - The clone of a gelding
    - Can be approved on superior performances like a stallion can be approved for performances.
    - Otherwise the clone has to follow the normal selection process.



# *Suggested logic rules for competitions*

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- **Separate the case of clones and offspring of clones**
- **For clones**
  - Accept or refuse clones
  - If non registered horses are accepted , then clones are also accepted or they will compete anonymous...
- **For offspring of clones**
  - Accept as any other registered horse

## *FONTAINEBLEAU , August 27, 2012 Last Minute ! Clone offspring in foal contest and public sales*

- **ETHANOL VERT Z**, by E.T.CRYOZOOTECH and Qualité Verte by Calvaro is winner of Foal Contest, (class other than S.F.) organised by ADECSIF during the young Horses Championship.
- **ETHANOL VERT Z** is selected for the **FENCES** sales, the most pretigious sales for Sport Horses in France

