

The clone's heritage

Identification of cellular reprogramming error in Penta the heifer redirects animal reproduction studies

Francisco Bicudo

On that cold winter's morning of June 30, 2002, Brazil got up early to celebrate the two goals scored by Ronaldo “the Phenomenon” Nazário against Germany, winning the country its fifth World Cup championship. A few days later, on July 11, Brazil would accomplish another memorable feat, this time in the scientific arena. At the Veterinary Hospital of São Paulo State University (Unesp) in the city of Jaboticabal, weighing 42 kilograms, the first Brazilian clone originating from adult cells was born – Penta the heifer, her name an unmistakable tribute to the Brazilian soccer team. The two previous clones had been produced from fetal or embryonic cells, which reprogram more easily.

But euphoria quickly turned into disappointment. Penta died within a month – on August 12. A consequence of cellular reprogramming errors – when genes express erratically or not at all – during the cloning process. In the Brazilian heifer's case, the manufacturing error had to do with her immunological system, which produced too few antibodies. “The thymus, which is the organ responsible for activating the defense system in newborns, did not function properly. Penta fell victim to a series of

infections and ended up dying of sepsis,” says Joaquim Mansano Garcia from the Department of Veterinary Medicine and Animal Reproduction at the Unesp School of Agrarian and Veterinary Sciences (FCAV/Unesp).

When their grieving was done, Penta's demise motivated the Unesp researchers to try and understand the risks involved in the cellular reprogramming process. It also led them to a more thorough investigation of what causes genes to misexpress or simply not express at all – as if they were “switched off” – when an embryo is obtained through cloning techniques. The consequences of these deviations can include malformations and respiratory, neurological, immunological or bone problems, not to mention premature aging. Any of these can result in death, as not only in Penta's case, but also another notorious barnyard animal: Dolly the ewe, the first living being to be successfully cloned in a laboratory, at the Roslin Institute in Scotland in 1996.

Dolly lived to the age of six. She was simultaneously young, considering the average lifespan of sheep, and old, as she was cloned from an adult cell whose chromosomal tips – known as telomeres – were already shortened. “There is an apparent contradiction that needs to be resolved. If we use normal genetic ma-

terial, from healthy donors, then why do these aberrations and misexpressions occur? Is it possible to overcome them? These are questions asked by many laboratories around the world, and that we've also decided to investigate,” Garcia reveals.

The researcher also explains that living beings produce enzymes called DNA methyltransferases, responsible for transferring methyl groups to DNA. This mechanism, in turn, provides cells with greater stability and protection, helping them establish more balanced relationships with their environment. Over the past 10 years, studies by the Unesp research team have been able to confirm that cells must contain adequate amounts of methyl in order to minimize or eliminate the risk of reprogramming errors – when there is too little or too much, silenced genes and unsuccessful cloning become more likely. “We know that methylation levels can be modified by the environment, but we still have no means to intervene, in the laboratory, to try to correct or control these changes”, says Garcia.

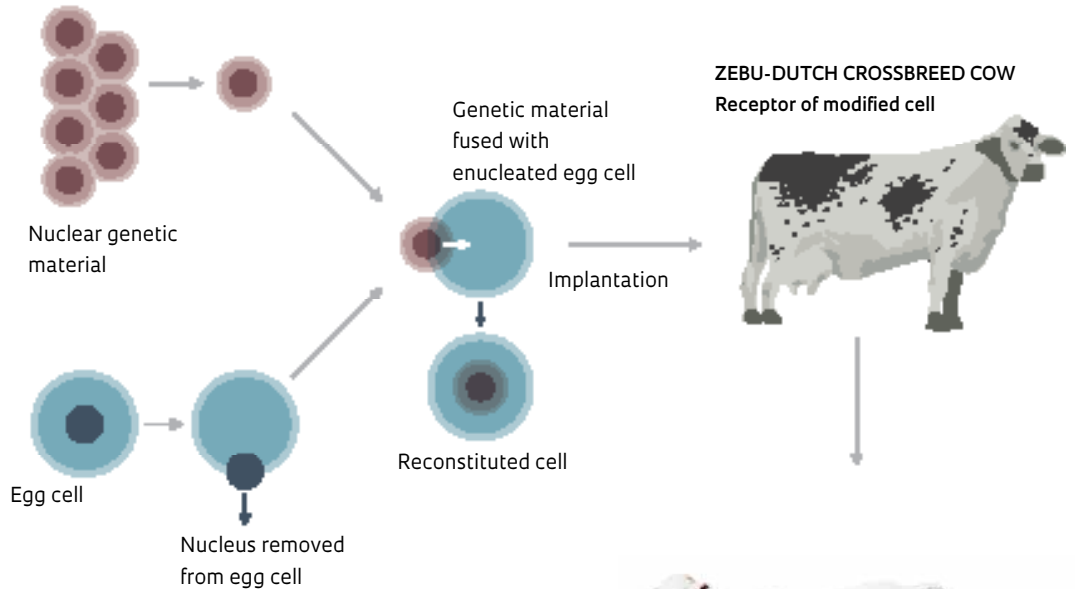
Precisely because of the relationships established with the environment and the potential problems they can create for cloning, the Unesp researcher

Breeding a champion

NELORE COW,
origin of clone



DUTCH COW,
egg donor



reminds us that it's also necessary to monitor and investigate the cell culture system and the conditions of the donated egg cell that will generate the embryo. If such elements are handled or preserved incorrectly or carelessly, reprogramming errors can ensue. With this in mind, Garcia is currently coordinating a comparative analysis of DNA methylation in fetuses and placentas at 60 days of gestation, obtained through natural (*in vivo*) fertilization, artificial (*in vitro*) fertilization, and cloning. The goal is to identify whatever distinct evolution pathways and differences may exist among the three situations, as relates to genetic reprogramming. Initial results of the work should come to light by the end of the year [2008].

Despite her short lifetime, Penta has certainly helped Brazilians sophisticate their knowledge on cloning. She was born from the DNA of a somatic cell (with 46 chromosomes) removed from the tail of a Nelore cow (*Bos indicus*, Indian herd) and injected into an egg cell donated by a Dutch cow (*Bos taurus*). However, although the nucleus belonged to the Indian mother, 97% of Penta's genetic code resembled that of her European mother. "It was an expected result, but we were antici-

pating more of a mix. Penta confirmed that, in cloning, the cytoplasmatic material prevails over the nucleus," Garcia confirms.

The Unesp researchers revealed in August 2002 (*Pesquisa FAPESP* issue No. 78) that although they applied the same technique that was used to produce Dolly, the cloning of Penta was innovative in its use of strontium chloride combined with the drug ionomycin to activate the reconstituted egg cell. In previous experiments, ionomycin had been used with the compound 6-DMAP (6-dymethylaminopurine). According to Garcia, strontium chloride is more advantageous because it can more accurately reproduce the effects of normal fertilization by a sperm cell.

The researcher believes that, in addition to widening the perspectives for genetic improvement of bovine livestock in Brazil, studies like the ones that resulted in Penta's cloning can serve as a tool for the study of mitochondria, known as the energy reservoirs responsible for cell respiration. "Changes in the functions of this organelle," says Garcia, "can compromise cellular metabolism and – depending on the intensity – create susceptibility to degenerative diseases that also manifest in humans, such as Alzheimer's disease." ■



PENTA

PROJECT

Study of the Function and Heritage of Mitochondrial DNA (mtDNA) in Cattle: An Animal Model Produced with Nelore – No. 98-11783-4 (1999-2004)

GRANT MECHANISM

Thematic Project

COORDINATOR

Joaquim Mansano Garcia (Unesp Jaboticabal)

INVESTMENT

R\$875,415.17

FROM OUR ARCHIVES

Penta, the champion clone
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