CLONING DOLLY: How and Why

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EARLY DEVELOPMENT

In Vitro fertilized embryo

Two-cell stage

Four-cell stage

Eight-cell stage

Late blastocyst

Early day 0

Early day 2

Late day 2 to early day 3

Late day 3

Day 3 to day 6
DIFFERENTIATION:

- The developmental events between the fertilized zygote and the mature organism.
- Specialisation of cells to fulfill specific functions, i.e. kidney, skin, brain, muscle, etc.
- What are the mechanisms controlling differentiation.
August Weissman (1834 -1914)

- The fertilised egg contains all of the genetic determinants to form a complete individual (1898)
- During development this genetic material is divided with each cell division such that each cell type contains only the genetic determinants required for that function.
Wilhelm Roux (1850 - 1924)

• Killed a single blastomere at the 2-cell stage, this resulted in the production of a half embryo (1888).
• This appeared to support Weismann’s theory
Hans Adolph Eduard Driesch (1867 - 1941)

• Dissociated 2-cell sea urchin embryos by vigorous shaking (1892)
• Anatomically whole embryos developed however they were dwarfed
CONCLUSION: at least in the early stages of development, genetic determinants are not divided among the blastomeres

- McClendon (1910) - isolated frog blastomeres
- Gudrun Ruud (1925) - salamander blastomeres
PRIMITIVE CLONING:

- Jacques Loeb (1859 -1924). Using parthenotes noted that enucleate portions of cytoplasm did not divide until a nucleus was present (1894).
- Hans Spemann (1869 - 1941). Constricted Salamander eggs (1928)
• Spemann contemplated the results of these experiments and wished that he could place the nucleus of a more differentiated cell into an egg cytoplasm deprived of its own nucleus. He referred to this manipulation as a “fantastical experiment” (1938)
WHAT IS NUCLEAR TRANSFER?

• The reconstruction of an embryo by the transfer of genetic material from a donor cell to a recipient egg from which the genetic material has been removed
THE PROCESS OF NUCLEAR TRANSFER

1. Culture cells
2. Unfertilised egg/MII oocyte
3. Enucleation
4. Transfer donor nucleus
5. Activation
6. Culture
7. Transfer to surrogate

Gestation
NUCLEAR TRANSFER IN FROGS.

• 1952, Briggs and King report the production of normal tadpoles using blastula nuclei.

• 1962, Gurdon reported the production of adult Xenopus using tadpole epithelial cells as nuclear donors.

• In further experiments, Gurdon produced swimming tadpoles but no adults from adult keratinocytes.
NUCLEAR TRANSFER IN MAMMALS.

- 1986. Sheep 8-16 cell blastomeres. Willadsen
- MII oocyte
- Chromosomes
- Enucleation
- Oocyte/cell couplet
THE CLONING PROCESS
Enucleation of the recipient egg
NUCLEAR TRANSFER IN MAMMALS:

- Originally restricted to embryonic blastomeres.
- Development related to stage of development of embryo and species.
- Developmental stage related to MZT.
- Embryo Multiplication limited by cell number.

MII OOCYTE

ENUCLEATION

FUSION
NUCLEAR TRANSFER FROM SOMATIC CELLS:

• **WHY**
  • Studies on nuclear equivalence.
  • Cloning from adult animals.
  • Genetic modification.

• **HOW**
  • Find a cell type which works for NT
  • Modify NT Procedure.
NUCLEAR TRANSFER FROM SOMATIC CELLS:

- Megan & Morag
- Born July 1995
- 1st mammals produced by NT from a cultured differentiated cell line
- Quiescent cells used as nuclear donors
ISOLATION OF AN EMBRYO DERIVED CELL LINE (SEC1)

Isolated from the embryonic disc of a single day 9 ovine embryo
ISOLATION OF PRIMARY FOETAL FIBROBLASTS (BLWF1)

EVISCERATED DAY 26 FOETUS
ISOLATION OF ADULT MAMMARY EPITHELIAL CELL LINE (OME)

BIOPSY
FROM MAMMARY GLAND OF PREGNANT EWE (6 YEARS)
Mothers leg
SCOTS boffins yesterday revealed they had bomb- ed by naming the world's first cloned sheep after busty singing star Dolly Parton. Boftins at the Roslin Institute, near Edinburgh, said because the sheep was created using a cell from another sheep's udder — they decided to call her Dolly after the star with the huge boobs. Dr Ian Wilmut said: "Dolly is derived from mammary gland cells and the team could not think of a more impressive set than Dolly Parton's."
SUCCESES OF SCNT

- SHEEP 1996
- CATTLE 1998
- MICE 1998
- GOATS 1999
- PIGS 2000
- GAUR 2000
- MOUFLON 2001
- CAT 2002
- RABBIT 2002
- BANTENG 2003
- RAT 2003
- MULE 2003
- DEER 2003
- HORSE 2003
- DOG 2005
- FERRET 2006
- WOLF 2007
- CAMEL 2009
- IBEX 2009

SUCCESSES OF SCNT
MAJOR OBJECTIVES OF CLONING IN ANIMALS

• ANIMAL PRODUCTION
  • Preservation of Genome.
  • Multiplication of Elite Animals.
  • Preservation of Rare Breeds.
  • Reproduction of Sterile Animals.
  • Reproduction of Diseased Animals.
  • Research on Embryo Development.
  • Research on Aging.

• GENETIC MODIFICATION
  • Biopharmaceuticals.
  • Nutraceuticals.
  • Xenotransplantation.
  • Alteration of Production Traits.
  • Disease resistance.

• ANIMAL PRODUCTION.
  • Companion Animals.
GENETIC PRESERVATION
Multiplication Of Elite Animals.

• Example…high milk producing dairy cow.

• Picture from Wells et al.
GENETIC PRESERVATION
ENDERBY ISLAND COW
REPRODUCTIVELY COMPROMISED ANIMAL

• Dairy animals abandoned on island South of New Zealand.
• Underwent 20 generations of inbreeding.
• Modified diet, phenotype.
• Culled due to habitat destruction.
• Saved 1 female “Lady” (age 7) & semen from 10 bulls.
• Refractive to AI (6 years).
• OPU/IVF – single Bull Calf.

• Cloned from Granulosa Cells
GENETIC PRESERVATION

Other examples:

- CLONED WILDCAT AND OFFSPRING.
  Audubon Center. USA.
  - REASON. Age problems. Disease Problems.

- CLONED CHAMPION QUARTER HORSE
  Viagen, USA.
  - REASON. Gelded
GENETIC PRESERVATION

• Other examples:

*EUROPEAN MOUFLON.* Genetic rescue of an endangered mammal by cross-species nuclear transfer using post-mortem somatic cells closely related sheep eggs

*GAUR.* Cloned using cattle eggs.
ANIMAL PRODUCTION
Companion Animals

• REASONS:
• Favourite pet.
• Selection of working dogs/blind/epilepsy etc

• PROBLEMS
• May not be identical
GENETIC MODIFICATION

• OBJECTIVES OF GENETIC MODIFICATION

To produce a stable and heritable change in the genome.

• Add genes.
• Remove genes.
• Modify genes or their control sequences.
THE PROCESS OF NUCLEAR TRANSFER

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4. Transfer donor nucleus
5. Activation
6. Culture
7. Transfer to surrogate

Gestation
All Animals are Transgenic

**Microinjection**

**Nuclear Transfer**

- Green sheep
- Yellow sheep
Instant Production Flocks/Herds

**Microinjection**
- G0 Founders
  - Breeding
    - G1 Production founder
      - G2 Production flock
        - 44 months - sheep
        - 78 months - cows

**Nuclear Transfer**
- G0 Production flock
  - 18 months - sheep
  - 33 months - cows
USES OF GENETIC MODIFICATION

• Biopharmaceuticals.
• Nutraceuticals.
• Xenotransplantation.
• Alteration of Production Traits.
• Disease Resistance.
• Models of Human Disease
BIOPHARMACEUTICALS

- MILK
- URINE
- PLASMA
- SEMEN
<table>
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<th>PROTEIN</th>
<th>CATEGORY</th>
<th>FIELD</th>
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<tr>
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Nuclear Transfer Beyond Dolly

Polly

- The world’s first transgenic cloned lamb
- Born in July 1997
POLLY, HOLLY, MOLLY & OLLY
NT LAMBS
TRANSGENIC FOR ECSOD

PPL JULY 1998
NT LAMB TRANSGENIC FOR CALCITONIN:
a neuroactive peptide for preventing osteoporosis
TRANSGENICS FOR DISEASE PREVENTION: Gene Addition.

ANNIE. Resistant to Staphylococcal Mastitis

Wells at al, USDA, USA
TRANSGENICS FOR DISEASE PREVENTION: Gene removal/Knockout

World’s First Targeted (Gene KO) Cloned Sheep (Cupid and Diana).

PRP KO.
Denning et al (2001)
Nature Biotech 19, 559
Gene Targeting Candidates:

<table>
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<tr>
<th>Gene</th>
<th>Species</th>
<th>Objective</th>
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<td>PrP (scrapie/BSE)</td>
<td>Sheep/cows</td>
<td>Disease Research</td>
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<tr>
<td>Milk protein</td>
<td>Cow</td>
<td>Less allergenic milk</td>
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<tr>
<td>α 1-3 gal transferase</td>
<td>Pig</td>
<td>Better xenograft</td>
</tr>
<tr>
<td>Bovine serum albumin</td>
<td>Cow</td>
<td>Replacement with HSA</td>
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<tr>
<td>Bovine Ig locus</td>
<td>Cow</td>
<td>Replacement with human IG locus</td>
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</table>
XENOTRANSPLANTATION:

 +/- GENETIC MODIFICATION  

 NUCLEAR TRANSFER

 DIFFERENTIATE

 UNDIFFERENTIATED CELLS

 +/- GENETIC MODIFICATION

 ORGANS
OTHER APPLICATIONS

- Disease models i.e. Cystic Fibrosis
- Development
- Aging
- Differentiation
- Reproduction
- Agriculture
- Genetic Preservation
- Cellular De-differentiation – Stem cell isolation
LOSSES & ABNORMALITIES

INEFFICIENT OR INEFFECTIVE REPROGRAMMING

EMBRYO CULTURE EFFECTS

RECIPIENT CELL EFFECTS

DONOR CELL EFFECTS

DONOR CELL CULTURE EFFECTS

Dolly the cloned sheep kills a lamb — and EATS it!

By MIKE FOSTER / Weekly World News

EDINBURGH, Scotland — A frightened scientist says Dolly the cloned sheep has killed a young lamb — and eaten it!

What’s more, the world’s first cloned mammal has exhibited other strange behavior, such as chasing a young child, biting a keeper and staring menacingly at frazzled scientists.

“When you do something to anger her, she looks at you with those intense red eyes —

WIN AT SLOTS

Slot Jackpots!

CONCERNED

Rev. Bob Tweeny

eyes full of hate,” said a researcher involved in the cloning project.

Dolly’s eerie antics — including the “cannibalism” episode — have not been mentioned by scientists at the Roslin Institute in Edinburgh, where the historic cloning took place.

“They have a vested interest in portraying Dolly as a normal, lovable animal just like the sheep she’s a genetic duplicate of,” said the whistle-blowing researcher, who requested anonymity. “But nothing could be further from the truth.”

Dolly first began to show signs of abnormality about two months ago. “A keeper was giving her a bath, which she doesn’t seem to enjoy very much,” recalled the researcher.

“When his back was turned, she showered him over, then ripped his face, drawing blood.

“Another time I brought my 8-year-old daughter to see Dolly in her pen. She was terrified and was looking forward to seeing this famous creature and telling all her friends at school about it. “But as she tried to pet Dolly, the animal suddenly charged at her, trying to sink its teeth into my daughter’s leg.

“We had to use a cattle prodd to get Dolly off her.”

But the final straw came three weeks ago when a month-old lamb was left in Dolly’s pen overnight. The researcher said: “We looked in on the lamb the next day, the tiny lamb was half eaten — and the wool around Dolly’s mouth was soaked in blood.”

Since Dolly has been kept in isolation from other animals, zoologists say Dolly’s behavior is highly unusual. “Although rains can be violent during the mating season, I’ve never heard of one sheep killing another for food,” declared one animal expert.

“Perhaps a clone simply has no soul.”

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