



Equine Cloning

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- **Cloning** is the production of genetically identical individuals by non-sexual means

- Achieved in two ways:

- Embryo splitting

- Seen in identical twins

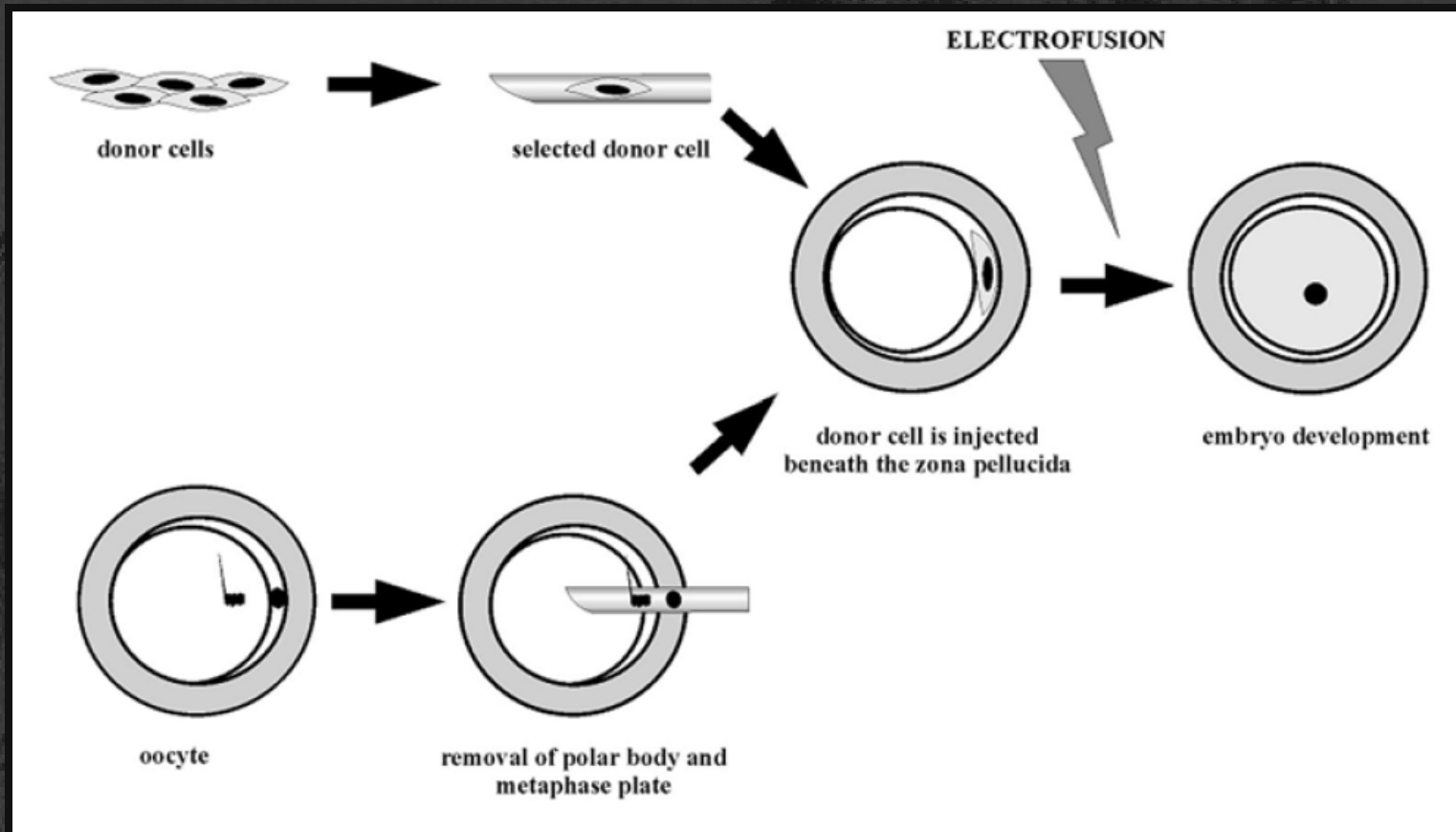
- Nuclear transfer

- Somatic cells from genetic donor
- Enucleated oocyte (chromatin removed)
- Fused by electric pulse or direct injection



Identical twin foals at CSU

Somatic cell nuclear transfer



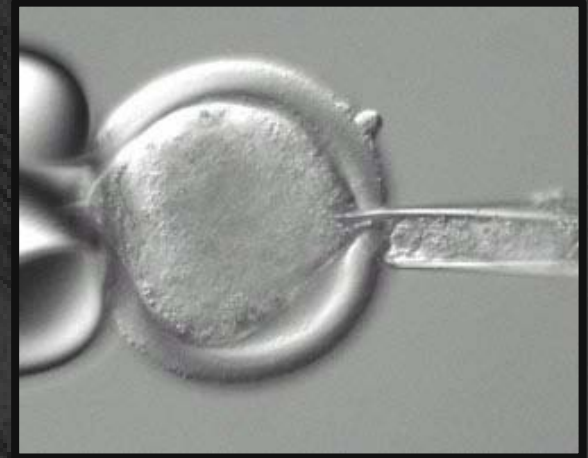
Donor cell

- Fibroblast most commonly used (subcutaneous connective tissue)
- Small tissue sample obtained by skin incision
- Cell-culture medium for transport to lab
- Cells proliferate and synchronise cell cycle (resting phase)



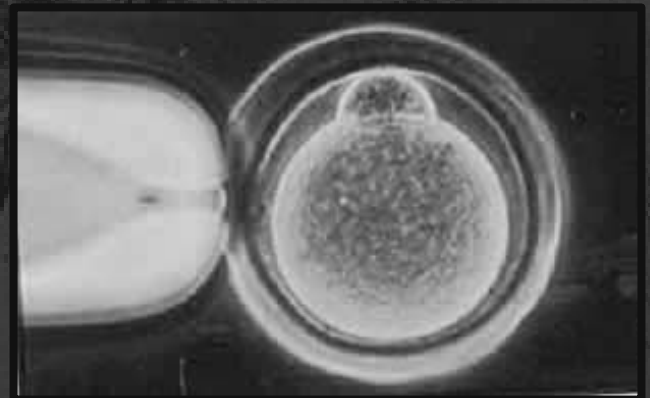
Cytoplast

- Quality a major factor
- Healthy MII oocytes with adequate cytoplasmic maturation
- Dominant pre-ovulatory or matured *in vitro*
- Metaphase plate and polar body removed
- Enucleation difficult in horses (high lipid content)
- Mechanical and chemical methods



Fusion

- Electric pulse
- Breaking donor cell membrane & injection into cytoplasm
- Sendai virus
- Piezo drill
- Micropipete





Activation

- Reprogramming of nucleus to convert cell function from fibroblast to embryonic cell
- Combination of drugs to
 - Increase intracellular calcium (mimic fertilisation)
 - Reduce meiosis-inhibiting factors
- Epigenetic modification – not completely understood
- Reprogrammation errors → high embryo mortality

Culture & Transfer

- Once activated, the reconstructed oocyte can be:
 - surgically transferred into oviduct of recipient mare
 - cultured *in vitro* to blastocyst stage and then transferred into uterus of recipient mare (as for normal ET)
- Rates of development to blastocyst stage are 3 to 10% (Galli et al. 2003, Lagutina et al. 2005, Hinrichs et al. 2006).



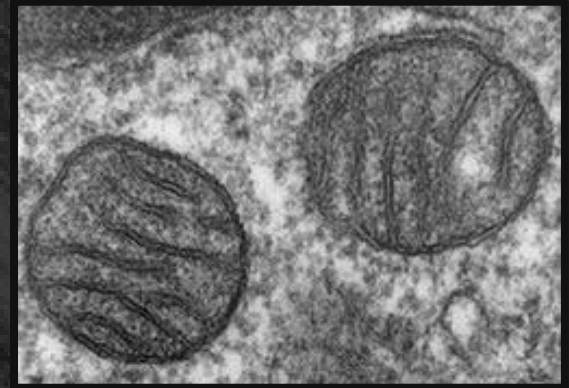
World's first cloned horse – Prometea – and her genetically identical mother (Italy, 2003)



Five foals produced by nuclear transfer from a single donor cell line

Resemblance

- Depends on three factors:
 - Epigenetic changes
 - Mitochondria
 - Embryo transfer
 - Environment
 - Individual variation in growth
- Deemed unlikely that clones will *perform* at same level as donor



Mitochondria



Progeny

- Progeny of clone = progeny of donor
- Progeny of female clone → different mitochondrial DNA
- Progeny of male clone → genetically indistinguishable from progeny of donor
- Epigenetic factors do not affect progeny

Cloning in practice

- **A method of genetic banking**
- High achieving performance horses
- Valuable horses that are castrated
- Valuable horses that are infertile
- Horses that die unexpectedly before reproducing
- Dearly loved pets
- Conservation



Sorraia horses



Przewalski's horse

Health

- Problems in other species – mostly resolved
- Horses appear healthy so far
- Increased incidence of crooked legs and thickened umbilicus
- Numbers too small for accurate data
- Effect of mitochondrial DNA uncertain



“Dolly” the cloned sheep



Some final thoughts...

- Efficiency limits *current* clinical use
- Ethics – just another ART?
- Potential for misuse and exploitation of cloning
- Reputation of donor horse
- Registration & competition

- Genetic **preservation** – not improvement!

References

- GALLI, C., LAGUTINA, I., CROTTI, G., COLLEONI, S., TURINI, P., PONDERATO, N., DUCHI, R. & LAZZARI, G. 2003. A cloned horse born to its dam twin - A birth announcement calls for a rethink on the immunological demands of pregnancy. *Nature*, 424, 635-635.
- GALLI, C., LAGUTINA, I., DUCHI, R., COLLEONI, S. & LAZZARI, G. 2008. Somatic cell nuclear transfer in horses. *Reproduction in Domestic Animals*, 43, 331-337.
- HINRICHS, K. 2006. Equine cloning. *Veterinary Clinics of North America-Equine Practice*, 22, 857-+.
- HINRICHS, K., CHOI, Y. H., LOVE, C. C., CHUNG, Y. G. & VARNER, D. D. 2006. Production of horse foals via direct injection of roscovitine-treated donor cells and activation by injection of sperm extract. *Reproduction*, 131, 1063-1072.
- JOHNSON, A. K., CLARK-PRICE, S. C., CHOI, Y. H., HARTMAN, D. L. & HINRICHS, K. 2010. Physical and clinicopathologic findings in foals derived by use of somatic cell nuclear transfer: 14 cases (2004-2008). *Javma-Journal of the American Veterinary Medical Association*, 236, 983-990.
- LANDIM-ALVARENGA, F. C., FERNANDES, C. B., DEVITO, L. G., DERUSSI, A. A. P., BLANCO, I. D. P. & ALVARENGA, M. A. 2008. New assisted reproductive technologies applied to the horse industry: successes and limitations. *Animal Reproduction (Belo Horizonte)*, 5, 67-82.
- TAYLOR, R. W. & TURNBULL, D. M. 2005. Mitochondrial DNA mutations in human disease. *Nat Rev Genet*, 6, 389-402.