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Reproductive Cloning and its Inefficiency

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Welcome to our first Topic of the Month (TOM).

To introduce this feature, we thought it would be fitting to launch with an outline of a controversial issue overshadowing the stem cell research field: Cloning.

This literature listed below provides an overview of reproductive cloning and its inefficiency. The listed reviews cover the history of reproductive cloning and give an idea both of the actual numbers of live clones obtained in the different systems and their pathologies. While reproductive cloning may establish itself in animal husbandry and preservation of endangered species and possibly even resurrect extinct species, its application to humans is currently rejected by most. As there is no reproductive cloning debate without a therapeutic cloning debate, and vice versa, we include two reviews that give a glimpse of the advances in therapeutic cloning.

Special thanks to Nature Publishing Group and the New England Journal of Medicine for their authorization to post the full-text articles by Wilmut et al, Rhind et al, and Hochedlinger & Jaenisch.

First, the paper that started the media hype:

Viable offspring derived from fetal and adult mammalian cells. Wilmut I, Schnieke AE, McWhir J, Kind AJ, Campbell KH (1997). *Nature* 385:810.

After the euphoria abated, dissenting minds dared to raise the question: Could Dolly be an imposter? Indeed, it seemed very difficult to reproduce the feat of mammalian cloning with nuclei from differentiated cells as donor DNA.



Dolly the sheep. From the Roslin Institute website (www.roslin.ac.uk).

Nuclei originating from embryonic cells appeared to be a much more efficient material, which raised the question of possible contamination of Dolly's donor material with nuclei from rare somatic stem cells.

This gnawing issue was resolved a few years later by an elegant study from Hochedlinger and Jaenisch. The authors demonstrated that by using nuclei from terminally differentiated peripheral T cells and B cells, which carry the genomic rearrangement for their individual receptors, they obtained cloned mice that carried the same gene rearrangement as the original donor nucleus in each somatic cell.

Monoclonal mice generated by nuclear transfer from mature B and T donor cells. Hochedlinger K, Jaenisch R (2002). *Nature* 415:1035.

With the feasibility of reproductive mammal cloning being established, the following short list of reviews provides an overview of the current situation and gives a glance at the first steps of therapeutic cloning.

The first half-century of nuclear transplantation. Gurdon JB, Byrne JA (2003). *Proc Natl Acad Sci U S A* 100:8048.

Gurdon and Byrne give a summary of the struggles of nuclear transplantation since Briggs and King performed the first nuclear transplants on frogs 50 years ago.

Somatic cell nuclear transfer. Wilmot I, Beaujean N, de Sousa PA, Dinnyes A, King TJ, Paterson LA, Wells DN, Young LE (2003). *Nature* 419:583.

A concise review on the problems afflicting reproductive cloning.

Cloned lambs--lessons from pathology. Rhind SM, King TJ, Harkness LM, Bellamy C, Wallace W, DeSousa P, Wilmot I (2003). *Nat Biotechnol* 21:744.

This short review focuses on the physical ailments observed in the clones.

Human cloning: can it be made safe? Rhind SM, Taylor JE, DeSousa PA, King TJ, McGarry M, Wilmot I (2003). *Nat Rev Genet* 4:855.

An in-depth review on the profound molecular problems occurring during reproductive cloning, resulting in aberrant gene expression (embryonic and extra-embryonic) and abnormal development. Contains a practical table comparing the cloning efficiencies between species and pathologies in the different affected organs.

Nuclear transplantation, embryonic stem cells, and the potential for cell therapy. Hochedlinger K, Jaenisch R (2003). *N Engl J Med* 349:275.

A comprehensive review that covers reproductive cloning, the problems of epigenetic reprogramming, the practical difficulties of therapeutic cloning, and, succinctly, the potential of adult stem cells for therapy.

Therapeutic cloning in the mouse. Mombaerts P (2003). *Proc Natl Acad Sci U S A*. 100 Suppl 1:11924.

A short review on the first frail steps of therapeutic cloning, in the mouse, raising a gloomy outlook for human application, with a prognostic price tag.

Additional papers of interest:

Abnormal gene expression in cloned mice derived from embryonic stem cell and cumulus cell nuclei. Humpherys D, Eggan K, Akutsu H, Friedman A, Hochedlinger K, Yanagimachi R, Lander ES, Golub TR, Jaenisch R (2002). *Proc Natl Acad Sci U S A* 99:12889.

Humpherys et al. compared gene expression with microarrays between cloned mice produced by nuclear transfer from ES and cumulus cell nuclei. The group found a common set of genes abnormally expressed in

the two clone types, when compared to controls, but also genes that are differentially expressed between the two clone types.

Incomplete reactivation of Oct4-related genes in mouse embryos cloned from somatic nuclei. Bortvin A, Eggan K, Skaletsky H, Akutsu H, Berry DL, Yanagimachi R, Page DC, Jaenisch R (2003). *Development* 130:1673.

The authors show that Oct4 and 10 Oct4-related genes were not expressed properly in cloned blastocysts derived from cumulus cells, while these genes were expressed normally in ES cell-derived cloned blastocysts and control embryos. Moreover, the expression efficiency of these 11 genes correlated with efficiency of the embryonic development. Failure to express these genes appropriately may underlie the low efficiency of cloning with somatic nuclei.

Molecular correlates of primate nuclear transfer failures. Simerly C, Dominko T, Navara C, Payne C, Capuano S, Gosman G, Chong KY, Takahashi D, Chace C, Compton D, Hewitson L, Schatten G (2003). *Science* 300:297.

Simerly et al. shed light on the current impossibility to clone primates. They show that two proteins, NuMA, a matrix protein responsible for spindle assembly, and HSET the centrosomal kinesin, are absent after nuclear transfer, suggesting that the removal of the nuclei depleted the primate oocytes of these proteins crucial for correct chromosomal segregation. While cleavage continued, chromosomes segregated unevenly, producing aneuploid embryos. In other mammalian species both proteins are not exclusively concentrated on the mitotic spindle and removal of the chromosomes may leave sufficient levels of these proteins behind for cell division to occur properly.

Cloning livestock: a return to embryonic cells. Wells DN, Oback B, Laible G (2003). *Trends Biotechnol* 21:428.

The authors argue that cloning with ES cell nuclei may prevail in the future for the production of livestock, as it is more efficient than cloning from somatic nuclei, and since ES cells have a higher longevity in culture and allow better gene insertion via homologous recombination than primary somatic cells.

If you are interested in more details on animal cloning you will find a good resource in the Web site of the Roslin Institute, where it all began <http://www.roslin.ac.uk>.

And to finish this TOM, welcome to the latest recruits to the ballet of the clones:

Pregnancy: a cloned horse born to its dam twin.

Galli C, Lagutina I, Crotti G, Colleoni S, Turini P, Ponderato N, Duchi R, Lazzari G (2003). Nature 424:635.

A mule cloned from fetal cells by nuclear transfer.

Woods GL, White KL, Vanderwall DK, Li GP, Aston KI, Bunch TD, Meerdo LN, Pate BJ (2003). Science 301:1063.