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Nuclear transfer results in inherently unstable offspring

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Although nuclear transfer technology has been used to produce live clones in several species, including cattle, sheep, goats, pigs and mice, only a small percentage of nuclear transfer embryos develop to term. A team from the Whitehead Institute for Biomedical Research, Cambridge, Massachusetts, and the Department of Anatomy and Reproductive Biology, University of Hawaii, analyzed imprinted gene expression in mice cloned by nuclear transfer and compared it to expression in the original embryonic stem (ES) cell population. The aim was to try to correlate gene expression with both survival and foetal overgrowth.

They found that the epigenetic state of the ES cell genome was extremely unstable and that variation in imprinted gene expression occurred in most cloned mice - even in those derived from ES cells of the same clone. But the most intriguing finding was that a small number of the mice survived to adulthood despite widespread dysregulation of their genes (Science 2001, **293**:95-97).

Lead researcher Rudolph Jaenisch from the Whitehead Institute reported: "This suggests that even apparently normal clones have subtle aberrations of gene expression that are not easily detected in the animal clone. Contrary to previous thought, mammalian development may be rather tolerant to epigenetic abnormalities." He thought that lethality might be the outcome only with cumulative effects of loss of normal gene regulation at multiple loci.

The findings have given the opponents of human cloning some of their most powerful ammunition to date and have led to the general opinion that even seemingly healthy clones might have subtle genetic abnormalities with unknown consequences. Jaenisch has warned in the past that attempts to clone humans are "irresponsible and dangerous". He has been joined by Ian Wilmut, head of the Roslin Institute in Edinburgh, UK, the centre that produced the first mammalian embryonic stem cell clone, Dolly the sheep.

Commenting on Jaenisch's latest work Wilmut said: "I think this shows that any attempt to [clone humans] at the moment would include many late abortions, birth of children who would die, and, probably worst of all, the birth of children who would survive with perhaps gross abnormalities."

Jaenisch argued that the research demonstrated that cloning based on currently available techniques is an imprecise and variable procedure, irrespective of the species in which it is performed. In his work, cloned mouse embryos were produced by transferring low passage ES cell nuclei into enucleated oocytes. These were then transferred into surrogate mothers and delivered by Caesarian section 19.5 days later.

Total RNA was isolated from placentas and organs of the newborn clones. Northern blots were then used to quantify H19 and Igf2 expression in both the cloned and normal neonates. Control levels of expression were derived by using placentas from normal pups and placentas derived from normal zygotes cultured in vitro until blastocyte stage before transfer to surrogate mothers. Although some clones expressed both genes at the normal levels, most showed aberrant expression levels.

The differentially methylated region (DMR) upstream of H19 was also analyzed and showed the extreme variability of the alterations. As expected, the DMR was highly methylated in the placentas that had silenced the H19 gene and partially unmethylated in those that showed H19 expression. But there

was no difference in methylation levels between control and cloned off spring at the Igf2r DMR region. This showed that epigenetic alterations at one imprinted loci did not necessarily predict changes at other loci.

They then tested whether the altered expression of imprinted genes observed in cloned animals correlated with foetal overgrowth and neonatal mortality. The majority of pups cloned by nuclear transfer had increased birth and placental weights and displayed a number of changes in the expression of several imprinted genes, regardless of their postnatal survival.

Publication of Jaenisch's research coincided with the announcement that a US company claims to be close to achieving its goal of cloning the first human being. Brigitte Boisselier, director of the company, Clonaid, and leader of the project told USA Today that the first clone would be produced "very soon". But, she refused to be drawn on a more precise date and to disclose the stage that the project had reached.

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