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COMMENTARY

James A. Byrne · John B. Gurdon

Commentary on human cloning

Introduction

Human cloning refers to the production of genetically identical humans. This cloning is possible via either "embryo splitting" or "nuclear transfer". Embryo splitting involves the separation of an early human embryo into two or more parts. Each of these parts has the potential to develop into a blastocyst (late embryo), which, if implanted, can develop into a child. This is how genetically identical monozygotic twins are created. Artificial embryo splitting has been successfully implemented in various mammals including sheep (Willadsen, 1981), cows (Willadsen, 1989), mice (Agrawal and Polge, 1989) and monkeys (Chan et al., 2000), but has been performed only to the pre-implantation stages in humans (Hall et al., 1993). Recently the American Society for Reproductive Medicine declared that human cloning by artificial embryo splitting was an ethical procedure to increase the number of implantable human blastocysts used in certain infertility treatments (ASRM, 2000). However, embryo splitting can produce only a limited number of cloned individuals as the early embryo can be separated only a limited number of times, and the procedure is not able to produce a "clone" of an adult that already exists. The other method for producing cloned humans, nuclear transfer, does not suffer from these limitations, and the rest of this article will focus on human cloning achieved via this technique.

Nuclear transfer (or more specifically somatic cell nuclear transfer) is a conceptually simple procedure. The nuclear material is removed from an egg, a somatic cell nucleus is inserted into that enucleated egg via microinjection or electrofusion, and the resulting reconstituted zygote is activated. The reconstituted zygote has the potential to divide into a blastocyst, and if implanted, develop into a child genetically identical to the nuclear donor. There are two fundamentally distinct types of human cloning by somatic cell nuclear transfer: reproductive cloning and therapeutic cloning. The objective of reproductive cloning is to produce a child genetically identical to an individual. This has been suggested as a last resort when an infertile couple are unable to conceive a biologically related child via any other method (Zavos, 2001). The objective of therapeutic cloning is to produce embryonic stem cells that are genetically identical to a patient. These stem cells could then be differentiated into precursor replacement cells to treat one of a variety of degenerative diseases from which the patient might suffer (Gurdon and Colman, 1999). Several scientific and related ethical issues surrounding both types of human cloning are addressed in this commentary. However, many of the religious (Turner, 1997) and moral arguments (Pence, 1998) that have been associated with human cloning, are beyond our present scope.

Reproductive human cloning

The first Vertebrate reproductive cloning (nuclear transfer) experiments were on Amphibia. Initial success involved using embryonic donor cell nuclei (Briggs and King, 1952), but it was soon discovered that differentiated cell nuclei could also result in cloned offspring, proving that there was no loss of genetic material as differentiation occurred (Gurdon, 1962). Nuclear transfer work progressed into the use of mammals in the 1970s (Bromhall, 1975) and 80s (Willadsen, 1986), and resulted in the conception of the first mammal cloned from an adult cell nu-

J. A. Byrne · J. B. Gurdon (✉)
Wellcome CRC Institute, Tennis Court Road
Cambridge CB2 1QR
and Department of Zoology, University of Cambridge, UK
e-mail: j.gurdon@welc.cam.ac.uk
Fax: +44 1223 334185

cleus in 1996. The birth of the cloned sheep "Dolly" was announced in Nature in 1997 (Wilmut et al., 1997) and sparked worldwide discussion about the possibility of cloning humans. To date, sheep (Wilmut et al., 1997), cattle (Cibelli et al., 1998), mice (Wakayama et al., 1998), goats (Baguisi et al., 1999), and pigs (Betthauser et al., 2000; Onishi et al., 2000; Polejaeva et al., 2000) have all been cloned from differentiated cells. Suggesting that somatic cell nuclear transfer may eventually be successful in all mammals, including humans. However, the majority of scientific opinion is opposed to the reproductive cloning of humans in view of the developmental, morphological, and physiological problems observed in mammals that had been cloned (Jaenisch and Wilmut, 2001).

The first problem observed in mammalian cloning is a consistently low efficiency of reconstituted eggs developing to parturition (birth). Typically, to get one cloned animal to parturition, approximately one hundred eggs must be enucleated and reconstituted with donor somatic cell nuclei, either by electrofusion (Wilmut et al., 1997) or microinjection (Wakayama et al., 1998). Thus nuclear transfer from adult or specialized cells is usually only 1% efficient. Even the highest efficiency observed in reproductive mammalian cloning from adult donor cell nuclei does not exceed 3% (Wakayama et al., 1998). If human reproductive cloning suffers from this low efficiency, then large numbers of human eggs would be needed to generate a single child. However, other human IVF procedures can also require large numbers of eggs, with 10–15 eggs being removed from the ovary at each operation, and commonly several such operations are required (on average) for a successful pregnancy to result (Elder, 2000). So the inefficiency argument against reproductive human cloning becomes somewhat weakened if one accepts IVF as an acceptable procedure to conceive a human child.

The second scientific argument against reproductive human cloning is the frequency of developmental abnormalities that have been observed in various mammals that have been created by somatic cell nuclear transfer. The applicability of this animal data to humans has been debated recently at a National Academy of Sciences conference in Washington, DC (NAS, 2001). Scientists at the conference who oppose reproductive human cloning pointed out that approximately a third of the mammals cloned have developmental abnormalities (Wilmut, 2001a), most commonly a collection of defects referred to as LOS (large offspring syndrome), where the offspring is born oversized with disproportionately large internal organs, and often also has respiratory, circulatory and other problems (Young et al., 1998), and that the same abnormalities would probably occur following human somatic cell nuclear transfer. Scientists also observe that there is currently no molecular technique available that could screen the entire genome for incompletely reprogrammed genes following nuclear transfer, and that any one of the 30,000 plus human genes could be incorrectly reprogrammed following nuclear transfer (Jaenisch and Wilmut, 2001). Conversely, scientists who support reproductive human cloning suggest that many of the defects observed in animal cloning are due to poor culture conditions, and that culture conditions have been improved and optimized for human embryos and cells over the 23 years of IVF and other assisted reproductive technologies (Zavos, 2001). They also note that LOS appears to be correlated to incorrect imprinting of the *IGF2R* gene (Young et al., 2001) and that this gene is not imprinted in humans or other primates (Killian et al., 2001), suggesting that these species may be safer to clone. The difference in incidence of LOS defects following human and nonhuman IVF provides empirical evidence supporting this hypothesis (Young et al., 1998). Also, the Rhesus monkeys that have been cloned by nuclear transfer of embryonic nuclei have shown no developmental or physiological abnormalities (Meng et al., 1997). This evidence suggests that humans and other primates may be less subject to defects after cloning than artiodactyls (sheep, cows, pigs) and rodents, but the evidence is not conclusive.

The incidence of congenital developmental abnormality in cloned mice is 12%, and in cloned goats it is nearly 38% (Wilmut, 2001b). Many have therefore concluded that the risk in artiodactyls and rodents is roughly 30%. However, this estimate is highly variable between experiments and species (Wilmut, 2001b). If Killian's hypothesis is correct, the incidence of developmental abnormalities following human somatic cell nuclear transfer may be significantly less than 30%. The incidence of developmental abnormality following natural sexual reproduction is 3% (Waitzman et al., 1994) and is significantly higher when the maternal age is over 40. It is clear that many potential parents accept these risks to conceive a child. If human cloning is banned as a reproductive technique solely due to the risk to the child, then we may find ourselves in the untenable position of having banned other currently accepted reproductive techniques that suffer equal or higher risks. Legislation would have to be carefully worded, especially if the intent is not to ban therapeutic human cloning, a method that uses the same somatic cell nuclear transfer technology to produce cloned embryos.

Therapeutic cloning in humans

Therapeutic cloning involves the creation of a cloned blastocyst, genetically identical to a patient who suffers from a degenerative disease. That blastocyst (basically a ball of cells) can then be cultured into an embryonic stem cell line, which excludes most of the blastocyst cells, except for the inner stem cells that become immortalized. A stem cell is defined as a cell that can proliferate indefinitely and differentiate into a wide variety of cell types. The embryonic stem cells obtained from a cloned blastocyst are undifferentiated and can then be

made to differentiate into precursor cells, which can be injected back into the patient to cure or treat the symptoms of the degenerative disease (Gurdon and Colman, 1999). Because the cells are genetically identical to the patient, they would not elicit the immune rejection response that tissue transplants normally face. Diseases that could potentially be treated by this procedure include heart disease, diabetes, Parkinson's and most other degenerative diseases. The main opposition to this research stems from the fact that this procedure uses cloning to create a human embryo, and that this embryo is then destroyed to obtain the embryonic stem (ES) cells. This has led opponents of this research to suggest the use of various alternatives, including noncloned ES cells, adult stem cells, and *in vitro* dedifferentiated stem cells.

1. Noncloned stem cells

Noncloned ES cells are derived from normally fertilized (rather than cloned) embryos, and thus this procedure is preferable to individuals and groups opposed to any form of human cloning. The problem with embryonic stem cells derived from noncloned embryos is that they would not be genetically identical to a patient and would require strong immunosuppressive drugs with their subsequent cost, inconvenience, and side effects (Gurdon and Colman, 1999).

2. Adult stem cells

Adult stem cells that are found in bone marrow and some other tissues have been isolated and encouraged to proliferate. Various pro-life groups have suggested that stem cell research should be restricted to these cells only, as this does not involve the destruction of human embryos or cloning techniques. However, adult stem cells have some fundamental disadvantages when compared to embryonic stem cells. They are hard to isolate and have restricted proliferation potential; furthermore, the range of cells they can be differentiated into is limited (McKay, 2000). There is a lack of identified stem cells for most tissues. There have been reports of adult stem cells transdifferentiating, for example, from a haematopoietic (bone marrow derived) fate into a neural fate (Brazelton et al., 2000; Mezey et al., 2000), and continued research into adult stem cells is certainly recommended, but the fact remains that by far the greatest therapeutic potential lies with embryonic stem cells.

3. *In vitro* dedifferentiated stem cells

The ideal situation would be to obtain embryonic stem cells by directly dedifferentiating normal body cells *in vitro*. While not yet achieved, research into nuclear reprogramming is currently being performed precisely for this reason. This situation would have all the therapeutic benefits of undifferentiated genetically identical embryonic stem cells without the ethical problem of having to destroy a human embryo.

The alternatives to therapeutic human cloned ES cells are either unfeasible with today's technology (*in vitro* dedifferentiated stem cells) or of relatively limited therapeutic potential (adult stem cells, noncloned ES cells). Embryonic stem cells obtained by transfer of nuclei from adult cells to enucleated eggs offer the greatest therapeutic potential, with today's technology, for tissue replacement therapy (McKay, 2000).

Overview of human cloning

The scientific debate around reproductive human cloning centres on the right of an infertile individual or couple to reproduce without governmental interference, set against the right of the child not to be exposed to an excessively high risk of developmental abnormality. What is regarded as "excessive risk" for the child is clearly subjective, with different potential parents in different situations inevitably having different perceptions of what is an acceptable risk to conceive a biologically related child. If the risk of developmental abnormality and/or perinatal death following human somatic cell nuclear transfer could be proven to be lower than that 3% observed from sexual reproduction, a significant proportion of the scientific opposition would cease, but opposition would no doubt remain from religious and other ethical quarters. The prudent suggestion would be to perform extensive primate nuclear transfers before an informed scientific decision could be reached either for or against this reproductive technology. There is currently insufficient empirical evidence to convincingly establish that the technology is intrinsically safe or unsafe (from the potential offspring's perspective) in either humans or other primates.

The scientific debate regarding therapeutic human cloning revolves around the therapeutic benefits against the ethical cost of destroying the early cloned embryo. Many allocate to the early embryo the status of an individual with fundamental human rights and consider the destruction of that embryo equivalent to murder (Shenfield et al., 2001). Several details should be considered when debating the issue. The early mammalian embryo is a ball of cells without even a rudimentary nervous system, and the division of this ball of cells into two or more parts results in two or more monozygotic twins. Thus whether this early embryo can yet be classified as an "individual" is questionable. Abortion legislation in most countries has already established that the rights and choices of grown adults supersede the rights of the early embryo. Most embryos ($>70\%$) that result from natural sexual reproduction do not implant into the

uterine endometrium. If each of these embryos has fundamental human rights, this would make premeditated attempts at pregnancy by natural sexual reproduction the logical equivalent of mass murder. The ethical considerations basically come down to our society's value system. Which is of greater value, the life of an adult or child dying from a degenerative disease, or a 5-day-old embryo that is little more than a ball of cells?

In summary, the risks associated with reproductive human cloning have not been conclusively established. Perhaps future research will establish the safety of the procedure for both mother and child, but other ethical and religious objections would almost certainly remain. With our current level of scientific and technological skills, therapeutic human cloning has the greatest medical potential in comparison to its suggested alternatives. Not to proceed with this therapeutic research is equivalent to turning our backs on one of the greatest medical advances of our time and condemning millions of adults and children to a premature death or a life of intense misery and suffering. Is this the brave new world we wish to live in?

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References

Agrawal, K.P. and Polge, C. (1989) A protocol used for splitting mouse embryos into two halves. Indian J Exp Biol 27(7):607–610.

ASRM (2000) Embryo splitting for fertility treatment. American Society of Reproductive Medicine. Birmingham, Alabama, USA.

Baguisi, A., Behboodi, E., Melican, D.T., Pollock, J.S., Destrempes, M.M., Cammuso, C., Williams, J.L., Nims, S.D., Porter, C.A., Midura, P., Palacios, M.J., Ayres, S.L., Denniston, R.S., Hayes, M.L., Ziomek, C.A., Meade, H.M., Godke, R.A., Gavin, W.G., Overstrom, E.W. and Echelard, Y. (1999) Production of goats by somatic cell nuclear transfer. Nat Biotechnol 17(5):456–461.

Betthauser, J., Forsberg, E., Augenstein, M., Childs, L., Eilertsen, K., Enos, J., Forsythe, T., Golueke, P., Jurgella, G., Koppang, R., Lesmeister, T., Mallon, K., Mell, G., Misica, P., Pace, M., Pfister-Genskow, M., Strelchenko, N., Voelker, G., Watt, S., Thompson, S. and Bishop, M. (2000) Production of cloned pigs from in vitro systems. Nat Biotechnol 18(10):1055–1059.

Brazelton, T.R., Rossi, F.M., Keshet, G.I. and Blau, H.M. (2000) From marrow to brain: expression of neuronal phenotypes in adult mice. Science 290(5497):1775–1779.

Briggs, R. and King, T.J. (1952) Transplantation of living nuclei from blastula cells into enucleated frogs' eggs. Proc Nat Acad Sci USA 38:455–463.

Bromhall, J.D. (1975) Nuclear transplantation in the rabbit egg. Nature 258(5537):719–722.

Chan, A.W., Dominko, T., Luetjens, C.M., Neuber, E., Martinovich, C., Hewitson, L., Simerly, C.R. and Schatten, G.P. (2000) Clonal propagation of primate offspring by embryo splitting. Science 287(5451):317–319.

Cibelli, J.B., Stice, S.L., Golueke, P.J., Kane, J.J., Jerry, J., Blackwell, C., Ponce de Leon, F.A. and Robl, J.M. (1998) Cloned transgenic calves produced from nonquiescent fetal fibroblasts. Science 280(5367):1256–1258.

Elder, K. (2000) In Vitro Fertilization. Cambridge University Press, Cambridge.

Gurdon, J.B. (1962) The developmental capacity of nuclei taken from intestinal epithelium cells of feeding tadpoles. J Embryol Exp Morph 10:622–640.

Gurdon, J.B. and Colman, A. (1999) The future of cloning. Nature 402(6763):743–746.

Hall, J.D., Engel, D., Gindoff, P.R. et al. (1993) Experimental cloning of human polyploid embryos using an artificial zona pellucida, in: Fertility and Sterility 60(Suppl.):S1 (The American Fertility Society conjointly with the Canadian Fertility and Andrology Society, Program Supplement, 1993 Abstracts of the Scientific Oral and Poster Sessions, Abstracts 0-001).

Jaenisch, R. and Wilmut, I. (2001) Developmental biology. Don't clone humans! Science 291(5513):2552.

Killian, J.K., Nolan, C.M., Wylie, A.A., Li, T., Vu, T.H., Hoffman, A.R. and Jirtle, R.L. (2001) Divergent evolution in M6P/IGF2R imprinting from the Jurassic to the Quaternary. Hum Mol Genet 10(17):1721–1728.

McKay, R. (2000) Stem cells – hype and hope. Nature 406(6794):361–364.

Meng, L., Ely, J.J., Stouffer, R.L. and Wolf, D.P. (1997) Rhesus monkeys produced by nuclear transfer. Biol Reprod 57(2):454–459.

Mezey, E., Chandross, K.J., Harta, G., Maki, R.A. and McKercher, S.R. (2000) Turning blood into brain: cells bearing neuronal antigens generated in vivo from bone marrow. Science 290(5497):1779–1782.

NAS (2001) Scientific and medical aspects of human cloning. National Academy of Sciences. Washington, DC, USA.

Onishi, A., Iwamoto, M., Aklita, T., Mikawa, S., Takeda, K., Awata, T., Hanada, H. and Perry, A.C. (2000) Pig cloning by microinjection of fetal fibroblast nuclei. Science 289(5482):1188–1190.

Pence, G.E. (1998) Flesh of my flesh: The ethics of cloning humans. Rowman and Littlefield Publishing, Lanham, Maryland, USA.

Polejaeva, I.A., Chen, S.H., Vaught, T.D., Page, R.L., Mullins, J., Ball, S., Dai, Y., Boone, J., Walker, S., Ayares, D.L., Colman, A. and Campbell, K.H. (2000) Cloned pigs produced by nuclear transfer from adult somatic cells. Nature 407(6800):86–90.

Shenfield, F., Pennings, G., Sureau, C., Cohen, J., Devroey, P. and Tarlatzis, B. (2001) I. The moral status of the pre-implantation embryo. Hum Reprod 16(5):1046–1048.

Turner, R.C. (1997) Human cloning: Religious responses. Westminster John Knox Press, Louisville, Kentucky, USA.

Waitzman, N.J., Romano, P.S. and Scheffler, R.M. (1994) Estimates of the economic costs of birth defects. Inquiry 31(2):188–205.

Wakayama, T., Perry, A.C., Zuccotti, M., Johnson, K.R. and Yanagimachi, R. (1998) Full-term development of mice from enucleated oocytes injected with cumulus cell nuclei. Nature 394(6691):369–374.

Willadsen, S.M. (1981) The development capacity of blastomeres from 4- and 8-cell sheep embryos. J Embryol Exp Morphol 65:165–172.

Willadsen, S.M. (1986) Nuclear transplantation in sheep embryos. Nature 320(6057):63–65.

Willadsen, S.M. (1989) Cloning of sheep and cow embryos. Genome 31(2):956–962.

Wilmut, I. (2001a) Presentation made at the National Academy of Sciences. Washington, DC, USA.

Wilmut, I. (2001b) Somatic Cell Nuclear Transfer (Cloning) Efficiency, Roslin Inst, Midlothian, UK.

Wilmut, I., Schnieke, A.E., McWhir, J., Kind, A.J. and Campbell, K.H. (1997) Viable offspring derived from fetal and adult mammalian cells. Nature 385(6619):810–813.

Young, L.E., Fernandes, K., McEvoy, T.G., Butterwith, S.C., Gutierrez, C.G., Carolan, C., Broadbent, P.J., Robinson, J.J., Wilmut, I. and Sinclair, K.D. (2001) Epigenetic change in IGF2R is associated with fetal overgrowth after sheep embryo culture. Nat Genet 27(2):153–154.

Young, L.E., Sinclair, K.D. and Wilmut, I. (1998) Large offspring syndrome in cattle and sheep. Rev Reprod 3(3):155–163.

Zavos, P. (2001) Testimony before the House Subcommittee on Oversight and Investigation Hearing on Issues Raised by Human Cloning Research.