Cloning humans
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Why the apparent haste to clone humans?

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The recent desperation to clone human embryos may be seriously undermining accepted ethical principles of medical research, with potentially profound wider consequences

In her editorial in the February 2005 issue of this journal, Nikola Biller-Andorno questioned whether the effort and resources that have been invested in debates about cloning at the United Nations might have been somewhat disproportionate, if a binding universal agreement on reproductive cloning cannot be reached.[1] Although most of the overt disagreement has centred around ‘‘therapeutic’’ cloning, rather than the potential use of nuclear transfer for reproduction, it is none the less clear that the delay and ultimate failure to date in achieving consensus on the former has also increased the likelihood of the latter becoming a foreseeable reality in the absence of a legally binding global convention. Whilst the much heralded promise of therapies has now been severely undermined by scandals of fraud,[2] the available evidence from various primate studies[3–5] and the history of similar work with other mammalian species[6 7] have provided little reason to doubt that human reproductive cloning might be possible in principle (albeit grossly inefficient and untenably risky). I completely agree with Dr Biller-Andorno’s appeal that we need to ‘‘foster a genuine, worldwide discourse on bioethical issues’’ and not let our debate get completely derailed by vested interests, whether politically or economically motivated. If we don’t, we can probably expect dire consequences for the future of biomedical research and its impact on society at large.

Prior to the United Nations’ discussions regarding a ban on human cloning in October 2004, the Human Fertilisation and Embryology Authority (HFEA) in Great Britain announced that they had granted their first licence to clone human embryos by nuclear transfer, [8] though no other applications had apparently been made following the legalisation of restricted human cloning over three years previously. Bizarrely, the ultimate aim of this cloning licence was ostensibly to use patient matched embryonic stem cells to treat people with diseases such as type 1 diabetes,[9] despite the recognition that ‘‘transfer of immunologically identical cells to a patient is expected to induce the same rejection’’ in such autoimmune diseases. [10] The granting of their second licence was then announced barely a week before a United Nations working group was expected to begin meeting to finalise the text of a declaration on human cloning. This is curious since Britain’s representative to the United Nations had previously made it clear that any attempt to ban or unreasonably restrict cloning for research purposes would not be supported anyway, while asserting that ‘‘the United Kingdom is totally opposed to human reproductive cloning’’. Nevertheless, members of the divided House of Commons Science and Technology Select Committee subsequently questioned whether there should be ‘‘a total prohibition of any form of reproductive cloning’’, despite also acknowledging ‘‘that research in developing reproductive cloning would very likely involve experimentation that is highly unethical’’. Following initial correspondence with the HFEA in which I had raised several questions about the wisdom and timing of their decision to grant even the first of these licences,[11] the authority published a report on their website, describing the background to their decision. Among the various questions raised, I had specifically asked to know the justification for performing research of a preliminary nature with

cloned human embryos before conclusively demonstrating the superior therapeutic prowess of embryonic stem cells derived by nuclear transfer or validating the rationale for the proposed work in animal studies. Here I attempt to explore broader ethical and scientific issues arising from the response to this question.

CURRENT PROGRESS TOWARDS THERAPEUTIC CLONING

From what I can gather,[12] it appears that the support for human cloning rather than animal research has been primarily based on the results of only two papers.[13 14] However, the full implications of both of these papers with regard to purported therapeutic applications may be readily questioned, so the insistence that ''no further animal work is needed''[12] is therefore hard to substantiate. In the case of the so called ''proof of principle'' paper by Rideout et al,[13] the authors were unable to perform therapeutic cloning according to their own definition of the procedure as the genetic defect (Rag2 deficiency) that caused the immune disorder was not actually cured by genetic engineering of embryonic stem cells cloned from the original mice. Instead, it was corrected using cells from newborn or adult clones.[13] In other words, ''adult'' or tissue stem cells cured the mice with the original disorder, not embryonic stem cells derived by nuclear transfer. By contrast, the cells derived from cloned embryos were attacked by white blood cells called natural killer cells in the recipient mice. Apparently this occurred because the stem cells derived from cloned embryos displayed abnormally low levels of proteins called MHC class I molecules, which are required for self recognition by the immune system. Therefore, the only way that anything resembling therapeutic cloning could be achieved was by genetically engineering mutant mice with the original immune disorder (Rag2 deficiency) so their natural killer cells would also be removed.[13] To artificially create new humans without natural killer cells is clearly out of the question in terms of treating existing human patients, while it seems few people would currently advocate reproductive cloning for spare parts. So, aside from further demonstrating that cloned animals must at least reach a fetal stage of development before they can be dependably used as compatible tissue donors,[15] exactly what principle did this prove?

The authors of this paper insist that the problems they encountered using stem cells from cloned embryos were host dependent, arguing that the failure of engraftment was due to elevated natural killer cell activity in the original mutant mice and not due to altered gene expression patterns in the transplanted stem cells.[13] One might then question the suitability of using such a Rag 2 knockout mouse strain in order to demonstrate the supposed general efficacy of therapeutic cloning. However, no direct evidence was provided to demonstrate how the mutant mice in question had increased numbers of circulating natural killer cells, though previously available data suggests that any such additional levels might vary considerably. As such data is pivotal to the authors' conclusions, it is strange that such data has not been shown. This is particularly odd since the paper in question contained only four figures (compared to an average of seven figures for most other papers in the same issue of the journal, not to mention additional supplementary material published online), so the lack of data shown was clearly not due to space constraints. This is even more curious given that successful engraftment of haematopoietic progenitor cells derived from embryonic stem cells had previously been described in similarly immunodeficient mice.[16 17] By contrast, there is abundant evidence that the cloning process can produce altered patterns of gene expression,[18–24] so it has yet to be demonstrated unequivocally that such epigenetic defects would not be responsible for problems associated with the transplantation of stem cells derived from cloned embryos (possibly due to

misexpression of genes affecting an immune response). In conclusion, various questions regarding the ‘‘proof of principle’’ paper describing therapeutic cloning[13] (discussed further in supplementary material available online at http://www.jmedethics.com/ supplemental) should make one exceedingly cautious about extrapolating its implications for future therapies without further confirmatory evidence.

Although the latter paper by Barberi et al1 [4] was able to demonstrate successful engraftment in parkinsonian mice of dopaminergic neurons derived from embryonic stem cells following nuclear transfer, it appears that similarly successful engraftment was achieved with neurons derived from regular embryonic stem cells. This is presumably because such cells were transplanted into the brain, an organ that is already well known to be an immune privileged site. Since the embryonic stem cells derived by nuclear transfer appeared no more effective therapeutically than other embryonic stem cells, and the derivation of the few embryonic stem cell lines by nuclear transfer that proved to be suitable for use in this study was itself highly inefficient,[25] the support for cloning provided by this paper appears questionable. Indeed, even Ian Wilmut subsequently commented that ‘‘in the treatment of diseases within the central nervous system cells from cloned embryos seem likely to offer less advantage’’.[10] In addition, it is debatable whether the limited time frame of this study (up to eight weeks) and the examination of limited numbers of recipients (six mice for each of the two cell lines) was sufficient to eliminate recognisable risks of teratoma formation[26] or carcinogenesis, especially given the unpredictably elevated chances of tumour progression associated with epigenetic aberrations.[27] Whereas the developmental competence of the ‘‘cloned’’ embryonic stem cells in the paper by Barberi et al.[14] was previously assessed by studying their incorporation into chimeric progeny,[28] this provided only limited evidence regarding the extent to which gene expression was unperturbed (and corresponding experiments on humans would be considered unethical anyway). Moreover, work with embryonal carcinoma cells[29] has shown that studies in which cells are injected into preimplantation embryos would still fail to address the potential of such cells to form aggressive tumours when injected into mature animals.

Aside from the study by Rideout et al, support for cloning with genetic modification (for both therapeutic[12] and apparently also reproductive purposes[30]) is supposedly based on a single report of successful gene targeting in human embryonic stem cells by Thomas Zwaka and James Thomson.[31] However, it would appear that even in this study, a large number of random insertions (with potential pathological consequences) may have occurred but were not detected. I am therefore unsure why it is concluded elsewhere that ‘‘there is little chance of a gene landing in the wrong place and causing problems’’[30] based on the data in this paper. On the other hand, more efficient gene targeting has been described in human adult stem cells[32] while Zwaka and Thomson have subsequently urged caution in the use of embryonic stem cells, based on their observations of aneuploid cells in culture.[33] This begs the question of why anyone would want to propose using stem cells from cloned embryos as the preferred route for gene therapy. So, where might any recent urgency to clone human embryos come from?

THE CASE FOR RESEARCH CLONING

Of course, recognising the limited progress toward making therapeutic cloning a reality, it is becoming more common to advocate the use of nuclear transfer in humans as a research tool,[30] creating cell cultures from embryonic clones of patients in an attempt to

avoid invasive and risky biopsies that might normally be required to study how particular cells could be affected in disease or how they might respond to drugs.[30] However, such an approach appears to be seriously flawed because of the significant variation in gene expression between clones. Consequently, greater variability in placental and fetal development has been reported in bovine fetuses cloned from the same nuclear donor compared to half siblings resulting from IVF or artificial insemination.[34] In fact, according to a recent meeting review,[35] Ian Wilmut also described cloned offspring as being more variable than siblings, in addition to repeatedly asserting that there is no currently available or foreseeable way to reliably predict the developmental performance of cloned embryos and determine a priori how their gene expression patterns might be altered.[6 36 37] In a previous article, he also pointed out that aberrant gene expression caused by cloning could invalidate studies that are aimed at identifying subtle differences in drug metabolism between genotypes.[6] Unfortunately, the additional variation in gene expression resulting from nuclear transfer is likely to confound the interpretation of such experiments. The use of cloned embryos for such research becomes even more questionable when the genetic differences of interest are those thought to affect relatively late onset conditions with a variable penetrance, rather than those that are truly congenital in nature. Indeed, Ian Wilmut had previously highlighted the epigenetic instability inherent to embryonic stem cell lines and stressed the importance of studies to detect possible defects ''throughout a life span''.[36] As I am presently unaware of any animal studies that demonstrate the feasibility of such research with human embryos, I am therefore left wondering what the real rationale behind such work might be.

Surprisingly, it appears that these difficulties have subsequently been overlooked by some advocates of cloning in their writing for the general public.[30] In lobbying the United Nations prior to a prospective final decision on the issue of human cloning, it was claimed that cells from cloned embryos would not be subject to the same developmental defects as cloned animals.[38] However, this paradoxical assertion conflicts with numerous published studies showing that the potential for cloned embryos to develop normally is limited by epigenetic defects in the embryos themselves.[22 24 39 40] Consequently, the few individuals which make it to term and present abnormalities are therefore likely to represent a minority of embryos in which nuclear reprogramming is most successful and epigenetic dysregulation is least severe (discussed further in supplementary material: see website address above). Furthermore, as Rudolph Jaenisch and his coworkers have clearly demonstrated, cloning by nuclear transfer introduces a host of new defects in gene expression in both embryonic and extra-embryonic tissues, which cannot be accounted for simply in terms of artefacts resulting from artificial in vitro culture conditions.[19] Although it has recently been shown that some embryonic stem cell lines derived from cloned or fertilised mouse embryos may appear to be transcriptionally and functionally indistinguishable,[41] one should note that the selected cell lines that were described in this study were those already shown previously to support life following injection into chimeric embryos. However, this paper[41] neglects to highlight the low overall rate at which the resulting chimeric embryos actually survived to term,[13 42 43] and corresponding experiments on humans to assess the behaviour of human embryonic stem cells would obviously be considered unethical. It is therefore hard to envisage why epigenetic defects might only present problems for reproductive cloning but not therapeutic cloning.

THE CONTINUED NEED FOR RESEARCH IN OTHER SPECIES

At present, it is clear that cloning by nuclear transfer is still far from efficient, and the limited data currently available for therapeutic cloning from blastocysts[25] would appear to suggest that it is even less successful than reproductive cloning.[6] This contrasts with exaggerated claims that therapeutic cloning ''can help just about any condition in which there is lost or damaged cells'' such that ''the list is almost endless''.[44] Despite the current obstacles, it is nevertheless conceivable that something resembling part of the promise of therapeutic cloning may become feasible some time in the distant future. However, this would depend on considerable investment in further basic research and for now it remains unclear to me why this should necessarily require cloning human embryos, rather than those of other model organisms. The utility of mouse embryonic stem cells as a model system is demonstrated both by the fact that so many published differentiation protocols for human embryonic stem cells have been based on prior work with mouse stem cells, and by the potential to validate differentiation protocols by transplantation of cells into mature animals or studying the incorporation of such cells into developing rodent embryos. Indeed, it has been shown that the overall expression of markers of the pluripotent state is essentially similar in mouse and human embryonic stem cell lines, with most identifiable differences thought to reflect different stages at which stem cells had been harvested from the inner cell mass in different organisms or contamination with differentiated human cells.[45] As mouse stem cells provide a convenient model for studying differentiation requirements, we should be critical of the primary motivation underlying any apparent urgency to use cloned human embryos at this stage in the search for therapies. This is particularly true in light of the barely discussed phenomenon of host dependent tumorigenesis following transplantation of embryonic stem cells, which should make it clear that the safety of stem cells derived from human embryos cannot be determined by xenotransplantation.[26] It is therefore vital that significant risks should be properly eliminated in studies from other species before any therapeutic use of human embryonic stem cells can be considered. If, however, mouse stem cells prove to be unsuitable for a particular reason, then it is worth asking why anyone should be so keen to bypass the possible use of embryonic stem cells from other mammalian species.[46] Although I myself find it extremely hard to condone any unnecessary or inhumane animal experimentation, shouldn't we be even more critical of any intentional exploitation of human life when the rationale behind the proposed research appears questionable and remains to be validated by corresponding studies in other species?

Of course, there are various different views concerning the justification for experimentation on humans or other animals, which are suitably addressed elsewhere. Nevertheless, if one opposes vivisection on the grounds of its nonconsensual nature at an individual level and the assumption that one would not wish to be subjected to similar procedures, then one is unlikely to consider corresponding experiments on humans any more justifiable, even less so where one believes that the latter lives have greater potential value. Both the Nuremberg Code and the Declaration of Helsinki stipulate that any allowed experimentation involving human subjects should be capable of being supported by the relevant research literature and preceded by corresponding humane work in animals if necessary. For example, the Declaration of Helsinki states that ''research involving human subjects includes research on identifiable human material'' and stipulates that ''medical research involving human subjects must conform to generally accepted scientific principles, be based on a thorough knowledge of the scientific literature, other relevant sources of

information, and on adequate laboratory and, where appropriate, animal experimentation''. [47] Aside from concerns about wholesale disregard for nascent human life, one also wonders what meaning ''informed consent''might have if women might be expected to provide eggs for research on the assumption that such work is potentially life saving,[48] when the requisite evidence for this from animal studies is still sorely lacking and the risks to a woman's health can be potentially grave.[49–52] Indeed, one might have questioned the rush to create a World Stem Cell Hub under Woo-Suk Hwang's leadership, facilitating global recruitment of patients and exchange of cells derived by nuclear transfer, when it was still acknowledged that ''preclinical evidence is required'' to prove that transplantation of stem cells from cloned embryos ''can be safe, effective, and tolerated''.[53] Will ''informed consent'' embrace correcting the confusion caused to the average patient by the persistent public deception surrounding both procedures for obtaining human eggs for cloning[54] and fraudulent claims regarding the purported efficiency with which patient specific embryonic stem cell lines could be created by nuclear transfer?[2] Regardless of any potential therapeutic benefits of research using stem cells from embryos otherwise destined for destruction, if we should choose to permit both the creation and destruction of human life and potential exploitation of patients simply for the express purpose of pursuing currently unsubstantiated and questionable research with no obvious or immediate clinical applications, then we should recognise how this risks the crossing of an ethical boundary that was originally drawn to prevent further abuses of human rights previously associated with Nazi doctors. Even in Britain, this would be a striking departure from the presumed intention of the Human Fertilisation and Embryology (Research Purposes) Regulations 2001, that permits limited human embryo cloning in principle.[55] Nevertheless, this does not seem to have deterred proposals to use critically ill patients as test subjects for highly risky and premature experiments, [56] for which the long term consequences remain worryingly uncertain. I therefore pose the question, if we now allow such human experimentation without prior and thorough validation from humane work in other species, do we really know where we are going?

CLONING FOR WHOSE BENEFIT AND AT WHOSE COST?

In her closing remarks, Dr Biller-Andorno suggested it is peculiar that bioethics should be associated in the minds of many people with cloning and embryos rather than questions of fair access to health-care systems for these embryos once they have grown into adult human beings. I completely agree that the latter should not be neglected by a focus on the former, yet I also wonder whether part of the difficulty in achieving international consensus on the use of cloning has also been a reflection of the extent to which its immediate benefits might only be for he wealthy. Public reactions to legal proceedings that might threaten the use of research cloning in Britain have shown how some insistence on such experimentation may be influenced primarily by economic interests, rather than genuine concern for patient welfare.[57] It is already apparent that reproductive cloning of animals can involve considerable sums of money[58] and the use of cloning for either reproductive or therapeutic purposes in humans is unlikely to be much cheaper, given the high price of human eggs.[59 60] Naturally, concerns about potential exploitation of women in developing countries would only be heightened by the HFEA's proposal to relax rules on importing human eggs,[60] subsequently leading to a European Parliament resolution that seriously questioned the role of the HFEA in facilitating a trade of human eggs from vulnerable Romanian women.[61] Aside from the physical discomfort more commonly associated with egg donation, this is

especially worrying because of the elevated risks of ovarian hyperstimulation syndrome following aggressive hormonal treatments [49–52] and previous concern that both these risks and the surgical risks associated with oocyte retrieval may not be given adequate attention when money becomes a dominant motive.[59]

Meanwhile, proposals to acquire human eggs for cloning by subsidising fertility treatment in developing countries[62 63] will appear highly suspicious wherever more pressing health issues than infertility predominate. Such concerns may be further reinforced by a growing demand for human eggs of the highest quality to be used in cloning research,[48] especially where egg donors for IVF already appear to be in short supply.[60]

Ian Wilmut has himself acknowledged that ''therapeutic cloning is unlikely to be practical for routine use'',[30] since the process would require an inordinate supply of eggs. It is no secret that the initial cloning of human embryos in South Korea claimed to have used at least 242 fresh oocytes donated by healthy women, from which only one embryonic stem cell line was purportedly derived.[64] However, it has since been concluded that as many as 2061 eggs from 129 women were used for such research over a three year period, without deriving any confirmed stem cell lines from cloned human embryos.[65] Despite these shortcomings, it still remains possible that production of human embryos by nuclear transfer may be improved by modified protocols using freshly obtained eggs from younger donors.[66] Nevertheless, development to the blastocyst stage (in order to derive embryonic stem cells) is not necessarily equivalent to full reprogramming [24] and the inability to predict which of the few such embryos might have relatively unperturbed gene expression also indicates that the required number of eggs for research or therapeutic applications would remain unpredictably large. So far, two novel solutions have been proposed to solve this problem: either using eggs from other mammals to perform nuclear reprogramming, or oocytes differentiated from embryonic stem cells. However, the currently available data fails to support either of these proposals as feasible alternatives at present [67] (discussed further in supplementary material: see website address above). Consequently, it is clear that the shortage of human eggs required for cloning is still a considerable obstacle that poses a potential threat to the welfare of poorer women (as pointed out by Nigeria's representative to the United Nations on 18th February 2005).[68] In conclusion, it would appear that a host of vested interests may have played a significant role in encouraging potentially profound misrepresentation of both the science surrounding cloning and its foreseeable clinical implications. This is considerably more worrying than previous concerns regarding the hasty manner in which some human cloning research had been prematurely publicised before peer review,[69] leading to fears that the image of science as a whole might be seriously threatened by the recent scandal surrounding falsified data in high profile human cloning papers.[70] Perhaps we would be wise to ask ourselves not so much whether the question of human cloning has deserved so much debate, but rather whether the seemingly biased nature of the debate so far has blinded many of us to its possible wider implications for the practice of medical research and its true beneficiaries.

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