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The Science and Politics of Cloning: What the News Was All About

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This month, researchers at the Oregon Health and Science University published a ground-breaking paper describing the derivation of human stem cells from cloned human embryos. Despite the minor irregularities that were discovered in the figures (the paper had been rushed to publication after a mere four days of review), the work represents a significant scientific achievement, for the first time presenting credible evidence that human beings have been cloned.

There has been a surprising lack of uproar over this result, perhaps in part due to the less-than- frank reporting of what the researchers actually accomplished. Nature referenced the controversial result somewhat obliquely, stating, "Human stem cells created by cloning." The Scientist was even more misleading, casting the result as an *improvement* on cellular reprogramming by saying, "New Stem Cells on the Block: By reprogramming human fibroblasts into pluripotent stem cells with somatic cell nuclear transfer, scientists have come up with a viable alternative to iPSCs [induced pluripotent stem cells]." The popular news media followed suit, with Reuters, CBS News, Fox News and Businessweek all announcing that scientists had produced human stem cells from human skin cells by cloning, conveniently ignoring the *human embryos* that existed briefly in between.

Let's be clear: Cloning is cloning. This paper reports the generation of cloned human embryos, *human beings at the earliest stages of life*. The researchers used a total of 380 human eggs from at least 10 paid female donors to generate cloned embryos, 68 of which survived the seven days required to reach the blastocyst stage of development, before being destroyed to produce human stem cell lines. It is impossible to know how many of the clones produced were healthy human embryos. From the poor survival rate of the clones to seven days and the generally poor success for cloning other animals, it seems likely that many of the cells produced by cloning were defective in critical ways. But in the worst case scenario, 380 embryonic human beings gave their lives for the 18 stem cell lines this work generated.

Which stem cells are the best for research and for medical therapies?

Some scientists will undoubtedly argue that cloning is a necessary alternative to induced pluripotent stem cells (iPSCs) produced by reprogramming. Many commentators have been quick to point out that there are differences between iPSCs and embryonic stem cells (ESCs). And they are at least partially correct. Multiple groups have reported subtle differences between iPS and ES cells.[i] In contrast, others have found that the two cell types differ only slightly or are essentially "indistinguishable."[ii] Comparisons of stem cell lines produced in multiple labs have concluded that differences between *laboratory protocols*, not between the cells themselves, are responsible for much of the variation observed

between iPSCs and ESCs.[iii] The bottom line appears to be that iPSCs are roughly as different from ESCs as different ESC lines are from each other, and that better standardization of protocols for generating and culturing pluripotent stem cells would reduce this variation considerably.

Debate over the subtle differences between iPSCs and ESCs tends to obscure the well- documented abnormalities of ESCs themselves. ESCs are often referred to as the "gold standard" of pluripotency, yet this moniker grossly misrepresents the actual properties of ESCs. It is inarguably clear that ESC lines are abnormal compared to cells found within the inner cell mass of intact embryos.[iv] Multiple studies have concluded that ESC lines are quite variable, with many having 'restricted' potency and/or strong developmental biases.[v] Moreover, ESCs have repeatedly been shown to be genetically and epigenetically unstable, accumulating mutations that convert them into cancer cells.[vi] The same is also likely to be true for iPSCs,[vii] although perhaps to a lesser extent. In light of the evidence, it is hard to see the ESC "gold standard" as anything other than seriously tarnished.

In addition to the concerns regarding variable potency, unnatural epigenetic state and proclivity to accumulate mutations, ESCs are also "foreign" tissue that would be rejected by the immune system if transplanted to an adult patient. Consequently, most scientists agree that "autologous" or patient-matched stem cells would be preferable for therapeutic treatments. The two ways of obtaining patient-matched pluripotent stem cells are reprogramming and cloning. Thus, it is most appropriate to compare iPSCs to ESCs derived from nuclear transfer (NT-ESCs). And it is clear that NT-ESCs are at least as problematic for therapeutic applications as both iPSCs and ESCs, if not more so.

It is entirely undisputed that live-born cloned animals are epigenetically abnormal.[viii] These abnormalities do not arise as a consequence of gestational irregularities, but are seen at early embryonic stages.[ix] And direct examination of NT-ESC lines shows that, like the embryos they are derived from, cloned ESCs are also clearly "abnormal" when compared to ESCs derived from fertilization.[x] Specifically, NT-ESCs carry epigenetic "memory" of the adult cells from which they are derived.[xi] The same kind of "bias" (i.e., a restriction in potency) is also seen in iPSCs,[xii] and (as noted above at 5) in ESCs derived from fertilization, albeit to a lesser extent than either iPSCs or NT-ESCs.

In conclusion, *all* pluripotent stem cell types, (ESCs, iPSCs and NT-ESCs) are abnormal, laboratory-created cells. They *all* differ significantly from cells found in intact embryos, and they differ in subtle ways from each other, as well. They are *all* prone to genetic instability, accumulating mutations that convert them to cancer cells. While iPSCs and NT-ESCs are "patient matched," thereby avoiding the

significant complications of immune rejection, both of these cell types carry epigenetic "memory" of the tissue from which they were derived that limits their pluripotency. Yet in some cases, even ESCs derived from fertilization exhibit poorly understood restrictions in potency that bias them to produce only specific derivatives. There is not a perfect cell type in the bunch.

Which stem cells should we pursue?

Given the compromises and risks associated with all pluripotent stem cell lines, is it possible to decide which route to pursue, or must research go forward on all fronts until a clear winner emerges? The problems that have already been identified with all three types of pluripotent stem cells are *fundamental* (i.e., unlikely to be resolved by simple improvements in technology or more research) and of roughly equal magnitude for ESCs, iPSCs and NT-ESCs. Any superiority that emerges is likely to involve subtle advantages for specific applications, rather than a clear advantage overall.

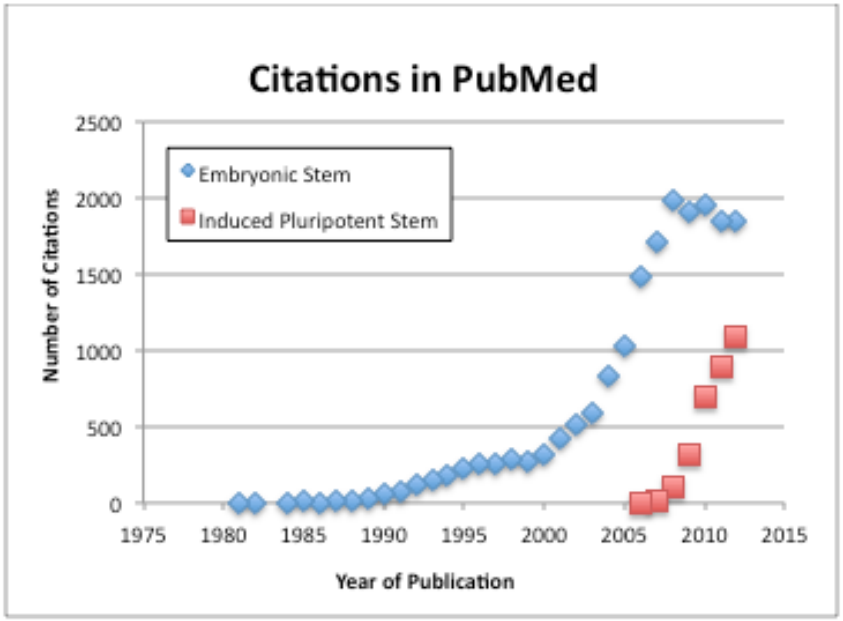
In contrast to the equally compromised scientific credentials of all pluripotent cell types, one contestant emerges as the clear victor on the field of ethics. Induced pluripotent stem cells do not require the production and subsequent destruction of human embryos. They do not require the exploitation of women to obtain eggs. They would not compromise patient health and safety by immune incompatibility. This does not make iPSCs "ideal," by any means, but it makes them ethically superior to either conventional or cloned ESCs.

iPSCs are also clear victors on a technical front. Reprogramming is a relatively simple procedure. It does not require researchers to have specialized training or an established relationship with an obstetrics practice to obtain human eggs and embryos. Scientifically, reprogramming offers a large number of technical advantages that allow researchers to have greater control of the reprogramming process, compared to human cloning. Because of these desirable features, iPS technology has advanced at a phenomenal pace (see Figure 1). Indeed, since the advent of reprogramming, the number of papers reporting on research using ESCs exclusively has remained flat, while the number of papers using iPS technology has increased a thousandfold. Researchers are voting with their feet. And increasingly, their footsteps are turning to cellular reprogramming as the way forward for pluripotent stem cell research.

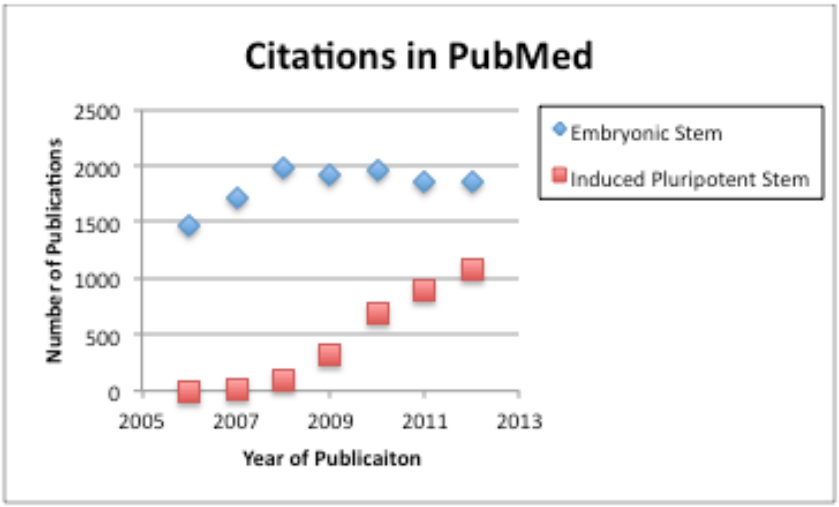
Which brings us back to the question of why the news of human cloning was so anticlimactic. It may well be that the public has simply accepted the concept of therapeutic cloning. Or that the reporting of this result was so oblique, the public simply didn't catch on. But the notably faint praise that greeted this result from the

scientific community may also reflect the fact that the field has already moved on. As one leading stem cell researcher said, "Given the difficulty of obtaining human oocytes, and the controversial nature of the research, embryonic stem cells aren't likely to ever be the preferred tool of regenerative medicine." And that would be even more so the case for *cloned* human embryonic stem cells.

Figure 1. (A) Mouse embryonic stem cells (ESCs) were described in 1981, with human ESCs isolated in 1998. The Bush administration funding policy announced in 2001, that for the first time allowed the use of federal funds for human embryonic stem cell research, was associated with a rapid increase in the number of publications in this field.



(B) After reaching a peak in 2008, the number of publications exclusively involving ESCs (animal and human; not iPSCs) has been flat or slightly declining, while the number of papers involving iPSCs has increased a thousandfold since they were first described in 2006.



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References

[i] Induced pluripotent stem cells and embryonic stem cells are distinguished by gene expression signatures. Chin MH, Mason MJ, Xie W, Volinia S, Singer M, Peterson C, Ambartsumyan G, Aimiuwu O, Richter L, Zhang J, Khvorostov I, Ott V, Grunstein M, Lavon N, Benvenisty N, Croce CM, Clark AT, Baxter T, Pyle AD, Teitell MA, Pelegrini M, Plath K, Lowry WE. Cell Stem Cell. 2009 Jul 2;5(1):111-23.; Persistent donor cell gene expression among human induced pluripotent stem cells contributes to differences with human embryonic stem cells. Ghosh Z, Wilson KD, Wu Y, Hu S, Quertermous T, Wu JC. PLoS One. 2010 Feb 1;5(2):e8975.

[ii] iPSCs are transcriptionally and post-transcriptionally indistinguishable from fESCs. Wang YF, Li J, He YZ, Yu HQ, Li Y, Gu XD, Li W, Li HW. Front Biosci. 2012 Jan 1;17:1659-68.; Genome-wide analysis reveals the unique stem cell identity of human amniocytes. Maguire CT, Demarest BL, Hill JT, Palmer JD, Brothman AR, Yost HJ, Condic ML. PLoS One. 2013;8(1):e53372.; Comparative proteomic analysis of human somatic cells, induced pluripotent stem cells, and embryonic stem cells. Kim SY, Kim MJ, Jung H, Kim WK, Kwon SO, Son MJ, Jang IS, Choi JS, Park SG, Park BC, Han YM, Lee SC, Cho YS, Bae KH. Stem Cells Dev. 2012 May 20;21(8):1272-86.

[iii] Lab-specific gene expression signatures in pluripotent stem cells. Newman AM, Cooper JB. Cell Stem Cell. 2010 Aug 6;7(2):258-62.; The quantitative proteomes of human-induced pluripotent stem cells and embryonic stem cells. Munoz J, Low TY, Kok YJ, Chin A, Frese CK, Ding V, Choo A, Heck AJ. Mol Syst Biol. 2011 Nov 22;7:550.

[iv] Gene expression profiles of human inner cell mass cells and embryonic stem cells. Reijo Pera RA, DeJonge C, Bossert N, Yao M, Hwa Yang JY, Asadi NB, Wong W, Wong C, Firpo MT. Differentiation. 2009 Jul;78(1):18-23.; The origins of human embryonic stem cells: a biological conundrum. Brink TC, Sudheer S, Janke D, Jagodzinska J, Jung M, Adjaye J. Cells Tissues Organs. 2008;188(1-2):9-22.; Tracking the progression of the human inner cell mass during embryonic stem cell derivation. O'Leary T, Heindryckx B, Lierman S, van Bruggen D, Goeman JJ, Vandewoestyne M, Deforce D, de Sousa Lopes SM, De Sutter P. Nat Biotechnol. 2012 Feb 26;30(3):278-82.; Different telomere-length dynamics at the inner cell mass versus established embryonic stem (ES) cells. Varela E, Schneider RP, Ortega S, Blasco MA. Proc Natl Acad Sci U S A. 2011 Sep 13;108(37):15207-12.; Tracing the derivation of embryonic stem cells from the inner cell mass by single-cell RNA-Seq analysis. Tang F, Barbacioru C, Bao S, Lee C, Nordman E, Wang X, Lao K, Surani MA. Cell Stem Cell. 2010 May 7;6(5):468-78.

[v] Diverse epigenetic profile of novel human embryonic stem cell lines. Lagarkova MA, Volchkov PY, Lyakisheva AV, Philonenko ES, Kiselev SL. Cell Cycle. 2006 Feb;5(4):416-20.; Distinct differentiation characteristics of individual human embryonic stem cell lines. Mikkola M, Olsson C, Palgi J, Ustinov J, Palomaki T, Horelli-Kuitunen N, Knuutila S, Lundin K, Otonkoski T, Tuuri T. BMC Dev Biol. 2006 Aug 8;6:40.; Distinct cardiogenic preferences of two human embryonic stem cell (hESC) lines are imprinted in their proteomes in the pluripotent state. Moore JC, Fu J, Chan YC, Lin D, Tran H, Tse HF, Li RA. Biochem Biophys Res Commun. 2008 Aug 8;372(4):553-8.

[vi] Trisomy 8: a common finding in mouse embryonic stem (ES) cell lines. Kim YM, Lee JY, Xia L, Mulvihill JJ, Li S. Mol Cytogenet. 2013 Jan 16;6(1):3.; Recurrent genomic instability of chromosome 1q in neural derivatives of human embryonic stem cells. Varela C, Denis JA, Polentes J, Feyeux M, Aubert S, Champon B, Piétu G, Peschanski M, Lefort N. J Clin Invest. 2012 Feb 1;122(2):569-74.; Recurrent chromosomal abnormalities in human embryonic stem cells. Spits C, Mateizel I, Geens M, Mertzanidou A, Staessen C, Vandeskelde Y, Van der Elst J, Liebaers I, Sermon K. Nat Biotechnol. 2008 Dec;26(12):1361-3.; Human embryonic stem cells reveal recurrent genomic instability at 20q11.21. Lefort N, Feyeux M, Bas C, Féraud O, Bennaceur-Griscelli A, Tachdjian G, Peschanski M, Perrier AL. Nat Biotechnol. 2008 Dec;26(12):1364-6. Recurrent gain of chromosomes 17q and 12 in cultured human embryonic stem cells. Draper JS, Smith K, Gokhale P, Moore HD, Maltby E, Johnson J, Meisner L, Zwaka TP, Thomson JA, Andrews PW. Nat Biotechnol. 2004 Jan;22(1):53-4.; Epigenetic instability in ES cells and cloned mice. Humpherys D, Eggan K, Akutsu H, Hochedlinger K, Rideout WM 3rd, Biniszkiewicz D, Yanagimachi R,

Jaenisch R. Science. 2001 Jul 6;293(5527):95-7.; Genomic alterations in cultured human embryonic stem cells. Maitra A, Arking DE, Shivapurkar N, Ikeda M, Stastny V, Kassauei K, Sui G, Cutler DJ, Liu Y, Brimble SN, Noaksson K, Hyllner J, Schulz TC, Zeng X, Freed WJ, Crook J, Abraham S, Colman A, Sartipy P, Matsui S, Carpenter M, Gazdar AF, Rao M, Chakravarti A. Nat Genet. 2005 Oct;37(10):1099-103.; Human ESCs predisposition to karyotypic instability: Is a matter of culture adaptation or differential vulnerability among hESC lines due to inherent properties? Catalina P, Montes R, Ligero G, Sanchez L, de la Cueva T, Bueno C, Leone PE, Menendez P. Mol Cancer. 2008 Oct 3;7:76.; Restriction landmark genome scanning identifies culture-induced DNA methylation instability in the human embryonic stem cell epigenome. Allegrucci C, Wu YZ, Thurston A, Denning CN, Priddle H, Mummery CL, Ward-van Oostwaard D, Andrews PW, Stojkovic M, Smith N, Parkin T, Jones ME, Warren G, Yu L, Brena RM, Plass C, Young LE. Hum Mol Genet. 2007 May 15;16(10):1253-68.

[vii] Dynamic changes in the copy number of pluripotency and cell proliferation genes in human ESCs and iPSCs during reprogramming and time in culture. Laurent LC, Ulitsky I, Slavin I, Tran H, Schork A, Morey R, Lynch C, Harness JV, Lee S, Barrero MJ, Ku S, Martynova M, Semechkin R, Galat V, Gottesfeld J, Izpisua Belmonte JC, Murry C, Keirstead HS, Park HS, Schmidt U, Laslett AL, Muller FJ, Nievergelt CM, Shamir R, Loring JF. Cell Stem Cell. 2011 Jan 7;8(1):106-18.; Identification and classification of chromosomal aberrations in human induced pluripotent stem cells. Mayshar Y, Ben-David U, Lavon N, Biancotti JC, Yakir B, Clark AT, Plath K, Lowry WE, Benvenisty N. Cell Stem Cell. 2010 Oct 8;7(4):521-31.

[viii] Aberrant gene expression patterns in placentomes are associated with phenotypically normal and abnormal cattle cloned by somatic cell nuclear transfer. Everts RE, Chavatte-Palmer P, Razzak A, Hue I, Green CA, Oliveira R, Vignon X, Rodriguez-Zas SL, Tian XC, Yang X, Renard JP, Lewin HA. Physiol Genomics. 2008 Mar 14;33(1):65-77.; Epigenetic instability in ES cells and cloned mice. Humpherys D, Eggan K, Akutsu H, Hochedlinger K, Rideout WM 3rd, Biniszkiewicz D, Yanagimachi R, Jaenisch R. Science. 2001 Jul 6;293(5527):95-7.; Aberrant gene expression in organs of bovine clones that die within two days after birth. Li S, Li Y, Du W, Zhang L, Yu S, Dai Y, Zhao C, Li N. Biol Reprod. 2005 Feb;72(2):258-65.; Aberrant epigenetic reprogramming of imprinted microRNA-127 and Rtl1 in cloned mouse embryos. Cui XS, Zhang DX, Ko YG, Kim NH. Biochem Biophys Res Commun. 2009 Feb 6;379(2):390-4.; Defective chromatin structure in somatic cell cloned mouse embryos. Zhang M, Wang F, Kou Z, Zhang Y, Gao S. J Biol Chem. 2009 Sep 11;284(37):24981-7.

[ix] The transcriptomic architecture of mouse Sertoli cell clone embryos reveals temporal-spatial-specific reprogramming. Cao F, Fukuda A, Watanabe H, Kono T. Reproduction. 2013 Mar 13;145(3):277-88.; Identification of inappropriately reprogrammed genes by large-scale transcriptome analysis of individual cloned mouse blastocysts. Fukuda A, Cao F, Morita S, Yamada K, Jincho Y, Tane S, Sotomaru Y, Kono T. PLoS One. 2010 Jun 30;5(6):e11274.; Aberrant profile of gene expression in cloned mouse embryos derived from donor cumulus nuclei. Tong GQ, Heng BC, Tan LG, Ng SC. Cell Tissue Res. 2006 Aug;325(2):231-43.; Irreversible barrier to the reprogramming of donor cells in cloning with mouse embryos and embryonic stem cells. Ono Y, Kono T. Biol Reprod. 2006 Aug;75(2):210-6.

[x] Nuclear transfer-derived epiblast stem cells are transcriptionally and epigenetically distinguishable from their fertilized-derived counterparts. Maruotti J, Dai XP, Brochard V, Jouneau L, Liu J, Bonnet-Garnier A, Jammes H, Vallier L, Brons IG, Pedersen R, Renard JP, Zhou Q, Jouneau A. Stem Cells. 2010 Apr;28(4):743-52.; Comparative analysis of nuclear transfer embryo-derived mouse embryonic stem cells. Part II: gene regulation. Kobolak J, Horsch M, Geissler S, Mamo S, Beckers J, Dinnyes A. Cell Reprogram. 2012 Feb;14(1):68-78.; Comparative analysis of nuclear transfer embryo-derived mouse embryonic stem cells. Part I: cellular characterization. Kobolak J, Mamo S, Rungsiwiwut R, Ujhelly O, Csonka E, Hadlaczky G, Dinnyes A. Cell Reprogram. 2012 Feb;14(1):56-67.; Differential methylation status of imprinted genes in nuclear transfer derived ES (NT-ES) cells. Chang G, Liu S, Wang F, Zhang Y, Kou Z, Chen D, Gao S. Genomics. 2009 Feb;93(2):112-9.; Nuclear transfer alters the DNA methylation status of specific genes in fertilized and parthenogenetically activated mouse embryonic stem cells. Hikichi T, Kohda T, Wakayama S, Ishino F, Wakayama T. Stem Cells. 2008 Mar;26(3):783-8.

[xi] Reprogramming efficiency following somatic cell nuclear transfer is influenced by the differentiation and methylation state of the donor nucleus. Blelloch R, Wang Z, Meissner A, Pollard S, Smith A, Jaenisch R. Stem Cells. 2006 Sep;24(9):2007-13.; Inefficient reprogramming of the hematopoietic stem cell genome following nuclear transfer. Inoue K, Ogonuki N, Miki H, Hirose M, Noda S, Kim JM, Aoki F, Miyoshi H, Ogura A. J Cell Sci. 2006 May 15;119 (Pt 10):1985-91.; Developmental ability of cloned embryos from neural stem cells. Mizutani E, Ohta H, Kishigami S, Van Thuan N, Hikichi T, Wakayama S, Kosaka M, Sato E, Wakayama T. Reproduction. 2006 Dec;132(6):849-57.

[xii] Incomplete DNA methylation underlies a transcriptional memory of somatic cells in human iPS cells. Ohi Y, Qin H, Hong C, Blouin L, Polo JM, Guo T, Qi Z, Downey SL, Manos PD, Rossi DJ, Yu J, Hebrok M, Hochedlinger K, Costello JF, Song JS, Ramalho-Santos M. Nat Cell Biol. 2011 May;13(5):541-9. Induced pluripotent stem cells expressing elevated levels of sox-2, oct-4, and klf-4 are severely reduced in their differentiation from mesodermal to hematopoietic progenitor cells. Seiler K, Soroush Noghabi M, Karjalainen K, Hummel M, Melchers F, Tsuneto M. Stem Cells Dev. 2011 Jul;20(7):1131-42.; Memory in induced pluripotent stem cells: reprogrammed human retinal-pigmented epithelial cells show tendency for spontaneous redifferentiation. Hu Q, Friedrich AM, Johnson LV, Clegg DO. Stem Cells. 2010 Nov;28(11):1981-91.