Table 1 Hidden, cryptic and undisclosed variables in human nuclear transfer

Variable	Possible relevance of variable
Microinjectionist	Unidentified differences in competence produce variation in developmental outcomes
Micromanipulation	Is cell fusion preferable to microinjection in humans?
Oocyte source	Oocyte donor age and diet are among poorly understood influences on developmental potential
Hormone hyperstimulation	Unknown or possibly adverse effects on oocyte quality
Nucleus donor cell	Can adult-derived nuclei work efficiently (as in mice)? What are the cell cycle and other key culture parameters?
First polar body	More prevalent in oocytes that fail in IVF, but the reason is uncertain
Oocyte collection–micromanipulation interval	<1 h improves development, but why?
Cellular reprogramming environment	Is metaphase II essential, and how long do human oocytes take for sufficient remodeling?
Cytoskeletal integrity	Poorly characterized spindle defects cause aneuploidy, but little is known about cytoskeletal behavior apart from that of lamins
Histone acetylation	Hyperacetylation correlates with poor development, but why?
Histone methylation	Key determinant of gene expression in other systems, but few data exist for humans
DNA methylation	Essential for global and imprinted gene expression control in mice, but effects in humans are unknown
Embryo culture conditions	Effects on epigenetic regulation and potentially on development
Other environmental factors	Air quality and overlaying mineral oil may affect development
Preimplantation development	Development *in vitro* not guarantee of quality; efficient mouse blastocyst parthenogenesis may be followed by developmental failure
Blastocyst quality	For example, cell number and trophectoderm ($CDX2^+$) and inner cell mass ($OCT4^+$) lineage specification rarely recorded
Pharmacological agents (e.g., caffeine)	What are their relevant targets in human eggs and embryos? How are they affected and are the agents specific?

Some hidden developmental influences are easier to discover than others and potentially include factors contributed by the biology of human cells and the techniques used to manipulate them.

remodeled by recipient oocyte factors in a process taking hours (although remodeling could continue during embryogenesis) with no drug selection and with an overall ntES cell derivation rate of ~12%. The genesis of human iPS cells from somatic cells is initiated by overexpressing the transcription factors OCT4 and usually SOX2 with one or more other factors, includes selection, and takes 2–3 weeks, with a success rate of ~0.1% and often much lower[11]. Induced pluripotency may reflect a balanced equilibrium between competing differentiation forces[14] and is promoted by the expression of oncogenes (of which *OCT4* and *SOX2* are examples) or the removal of tumor suppressors[11]. It is not known to what extent the reprogramming pathways that lead to ntES and iPS cell generation overlap, if at all. What is more apparent is that these pathways represent black boxes whose contents are often concealed by hidden variables (**Fig. 1**, **Table 1**).

Looked at one way, the efficiencies of cloned embryo and ntES cell derivation here[1] are impressively high. This may reflect the youth of oocyte donors. The four carefully characterized ntES cell lines were all derived from the eggs of the same donor ('donor A'), but these oocytes may have been anomalously proficient nucleus recipients. Moreover, there are cases in mammals where ES cells can be derived after efficient formation of developmentally incompetent blastocysts. For example, mouse parthenogenetic blastocysts develop efficiently—and parthenogenetic ES cells can be derived from them—but developmental catastrophe ensues and mammalian parthenogenetic offspring have not been reported. This shows that neither blastocyst development nor (nt)ES cell derivation necessarily reflect developmental normality, a difficulty that is exacerbated in man because much of the fundamental biology of human oocytes and the technical aspects of their manipulation remain hidden (**Table 1**).

The availability, albeit limited, of naturally impaired human oocytes and early embryos represents a unique opportunity to delineate molecular processes that direct the initiation of embryogenesis in fertilization. These processes are likely to include key determinants of genome reprogramming in nuclear transfer, and as long as they are unknown, human oocytes will remain refractory to prescriptive manipulation. This is a call to redouble efforts, not to give up: weaknesses and strengths inherent to human ntES and iPS cells argue that each should be pursued absent an alternative to both. To succeed, such efforts will have to produce a fix that is a lot stronger than caffeine.

COMPETING FINANCIAL INTERESTS
The author declares no competing financial interests.

1. Tachibana, M. *et al. Cell* **153**, 1228–1238 (2013).
2. Stojkovic, M. *et al. Reprod. Biomed. Online* **11**, 226–231 (2005).
3. Willadsen, S.M. *Nature* **320**, 63–65 (1986).
4. Mitalipov, S.M. *et al. Hum. Reprod.* **22**, 2232–2242 (2007).
5. Egli, D. *et al. Nature* **447**, 679–685 (2007).
6. Perry, A.C.F. & Verlhac, M.-H. *EMBO Rep.* **9**, 246–251 (2008).
7. Noggle, S. *et al. Nature* **478**, 70–75 (2011).
8. Han, Z. *et al. J. Proteome Res.* **9**, 6025–6032 (2010).
9. VerMilyea, M.D. *et al. EMBO J.* **30**, 1841–1851 (2011).
10. Nazor, K.L. *et al. EMBO Rep.* **13**, 890–894 (2012).
11. Masip, M. *et al. Mol. Hum. Reprod.* **16**, 856–868 (2010).
12. Polo, J.M. *et al. Nat. Biotechnol.* **28**, 848–855 (2010).
13. Kim, K. *et al. Nature* **467**, 285–290 (2010).
14. Shu, J. *et al. Cell* **153**, 963–975 (2013).

Research Highlights

Papers from the literature selected by the Nature Biotechnology editors. (Follow us on Twitter, @NatureBiotech #nbtHighlight)

Glycan receptor binding of the influenza A virus H7N9 hemagglutinin
Tharakaraman, K. *et al. Cell* **153**, 1486–1493 (2013)

Structural determinants for naturally evolving H5N1 hemagglutinin to switch its receptor specificity
Tharakaraman, K. *et al. Cell* **153**, 1475–1485 (2013)

Vascularized and functional human liver from an iPSC-derived organ bud transplant
Takebe, T. *et al. Nature* doi:10.1038/nature12271 (3 July 2013)

Whole-genome sequence–based analysis of high-density lipoprotein cholesterol
Morrison, A.C. *et al. Nat. Gene.* doi:10.1038/ng.2671 (16 June 2013)

Nanoscopy with more than 100,000 'doughnuts'
Chmyrov, A. *et al. Nat. Methods* doi:10.1038/nmeth.2556 (7 July 2013)

Biosynthesis of antinutritional alkaloids in solanaceous crops is mediated by clustered genes
Itkin, M. *et al. Science* **341**, 175–179 (2013)