Human procreation in unchartered territory: new twists in ethical discussions

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ABSTRACT: Since their validation in mammals, there have been profound ethical discussions on the possible applications of somatic cell nuclear transfer, human embryonic stem cells and induced pluripotent stem cells to reproductive medicine. This has been the case whether these technologies were considered as direct (i.e. when procreation is the ultimate goal) or indirect applications. In most countries, the majority of these approaches have been either stringently regulated, or regulation has been strongly and consensually suggested. However, this is not necessarily the case for possibilities such as same-sex chimaeras or the direct differentiation of gametes from somatic cells, skipping a pluripotent cell intermediate. The author suggests that the field of reproductive medicine should be more proactive in discussing both current and emerging developments with possible implications for human reproduction, even those reaching beyond current paradigms.

Key words: ethics / induced pluripotency / nuclear transfer / chimaera / gametogenesis

Introduction
The continuous introduction of novel technological approaches to human health has led to clear improvements in patient care, and generally raises few ethical issues in terms of the medical science itself. Rather, ethical discussions focus on the prioritization, cost and availability of treatments. Reproductive medicine seems to be a notable exception, both in terms of how its patients are viewed, and as to what it aims to accomplish. Indeed, possible interventions touch what some perceive to be the unchanging (and unchangeable) essence of human existence. The negative reactions to the 2010 Nobel Prize awarded to Prof. Robert Edwards for his pioneering efforts in establishing human IVF, while limited, stress an underlying tension, not seen in other fields.

Of course in assisted reproduction not all interventions are ethically equal, and common procedures, for example IVF, ICSI, testicular biopsies or embryo transfer, should not be compared with the more technically challenging, controversial, experimental or even theoretical applications. In particular, the use of human embryos and human embryonic stem cells (hESCs) for research and therapeutic applications, and the possible reproductive application of somatic cell nuclear transfer (SCNT, aka ‘cloning’) techniques have been hotly debated for many years (for different perspectives, see for example, Rhind et al., 2003; Lee, 2004; Hall and Stojkovic, 2006; Pearson, 2008). The recent development of human-induced pluripotent stem cells (iPSCs; Takahashi et al., 2007; Yu et al., 2007) has added to ongoing discussions (Testa et al., 2007; Devolder, 2009; Lo et al., 2010).

Direct and indirect use of cells with reproductive potential
It is very important to note that the SCNT and iPSC technologies do not relate to human procreation in exactly the same way as IVF or ICSI for example, in that involvement in this case can be either direct or indirect. In direct use, the immediate goal of a clinical intervention/research programme is of a reproductive nature, while in indirect use it is not.

For example, the putative use of SCNT to generate an individual whose nuclear genome is identical to that of the somatic donor (‘reproductive cloning’) would be a direct involvement. On the other hand, a main use of pluripotent hESC lines isolated from the inner cell mass of blastocysts is to differentiate these cells for regenerative medicine applications. The putative use of SCNT to generate patient-matched cells (‘therapeutic cloning’) joins both concepts, as it involves the reprogramming of adult somatic cells by introducing the nucleus into an enucleated oocyte, and subsequently deriving pluripotent cells from the resulting blastocysts, not using the resulting embryos for reproduction. Although complicity arguments can be raised in terms of involvement, i.e., that a need for oocytes or
blastoscyts is created indirectly (Devolder, 2010), the technology is not viewed in terms of reproduction as its ultimate goal (Rhind et al., 2003; Hall and Stojkovic, 2006). This research does, however, raise important ethical issues concerning the non-reproductive use of human oocytes and embryos, regardless of whether one considers those issues essential or exaggerated. In an ever-evolving field, the recent derivation of pluripotent cells in the adult human testis might add another indirect involvement of reproductive medicine to this type of research (Conrad et al., 2008; Kossack et al., 2009), although there is still controversy concerning reproducibility of these findings (Ko et al., 2010 and reply by Conrad et al. herein).

**Will iPSC solve all ethical problems?**

The introduction of iPSC has been greeted favourably in general because it was assumed that at least some indirect ethical issues related to the field could be mitigated, if not eliminated, in the near future (Testa et al., 2007). iPSC technology uses the forced expression of a few genes to reproducibly reprogramme adult somatic cells to a pluripotent state, similar to that of hESCs. The possibility of differentiating these iPSC into all somatic cell types would avoid the use of embryos to generate more hESC lines, and allow for the establishment of patient-specific cells for therapy and basic research into somatic cell reprogramming without the need for oocytes (Ramalho-Santos, 2009; Yamanaka, 2009). While the application of SCNT technology to reproduction was largely dismissed because of technical inefficiency at many levels, from oocyte availability, to the challenges of micromanipulation, to well-documented embryonic arrest and fetal malformations in model animals (Rhind et al., 2003; Hall and Stojkovic, 2006), the same is not necessarily the case after iPSC.

However, the argument for considering iPSC as ethically more neutral entities may not be as clear cut. In fact, the creation of a pluripotent cell state may lead to iPSC being viewed much as hESCs in terms of theoretical development potential (Lo et al., 2010). Furthermore, human iPSC could presumably, and despite the clearly complex events of imprinting, chromosome recombination and meiotic disjunction (Mathews et al., 2009), give rise to gametes. This suggests that the non-standard production of gametes from adult somatic tissue, both for assisted reproduction and for research purposes, such as the in vitro study of meiosis, might be envisioned (Mathews et al., 2009). In addition, the use of oocytes from animal models to study human cell reprogramming, given that human oocytes are not generally available for this purpose (St John et al., 2008), could also be avoided if iPSC-derived human oocytes can be obtained.

All in all, embryo-derived hESC are still needed as a ‘golden standard’ for pluripotency (Devolder, 2010), and the important epigenetic and genomic instability differences that have been found in iPSC relative to this standard may be relevant for future clinical applications (Kim et al., 2010; Bock et al., 2011; Gore et al., 2011; Hussein et al., 2011; Lister et al., 2011). Regardless, iPSCs seem more promising than putative pluripotent adult stem cells (Jiang et al., 2002), which were never developed reproducibly, and are now more realistically being proposed to treat the tissue of origin, or closely related tissues (Testa et al., 2007).

While the main conceptual notions of pluripotency and differentiation are not difficult to understand, there is a wide gulf between what is theoretically possible and what is achievable or has indeed been achieved. For example, having pluripotent stem cells (either ESC or iPSC) differentiate in culture is one thing, but having them reproducibly and exclusively differentiate into specific cells of interest is quite another (Testa et al., 2007). In fact, after the initial observations showing some differentiation of mouse ESCs into gametes (Hubner et al., 2003; Toyooka et al., 2003; Geijsbeek et al., 2004), it became clear that the process is inefficient in terms of reliably generating functional cells (Dalay, 2007). Given the similarities between ESC and iPSC, it seems reasonable to assume that generating gametes from iPSCs will be equally problematic. Thus, although regulatory measures are discussed in international research and clinical organizations as well as in local government bodies, with some aspects in common with debates concerning the management of germline banks, namely the use of material from deceased individuals (Mathews et al., 2009), it seems that technical difficulties still play the role of practical gatekeeper, much as was the case for SCNT.

**Going into unchartered territory**

Even if iPSC-related technology may lead to the in vitro production of gametes, there is another possibility for the direct use of iPSC in human procreation. The true test for cell pluripotency in the mouse is proving that the cells can contribute to the formation of a whole adult individual, with germline transmission. This is generally performed using one of two methodologies: creating chimerae via the injection of mouse ESC or iPSC into a native blastocyst (Okita et al., 2007), or by tetraploid complementation (Boland et al., 2009; Kang et al., 2009; Zhao et al., 2009). In tetraploid complementation, the first two blastomeres of a mouse embryo are fused forming a 4n structure, which at the blastocyst stage will not contribute to the inner cell mass but can develop into the trophoectoderm/trophoblast and help the development of the iPSC. In successful chimæra formation, the resulting mouse carries genetic information from both the native inner cell mass cells and the added iPSC, while in tetraploid complementation, the mouse is derived solely from iPSC, with the embryonic contribution to the placenta originating from the 4n structure. These procedures are obviously not acceptable for confirming the true nature of human pluripotent cells. In this case, in vitro differentiation or in vivo teratoma formation with production of cells from all three germ layers are the standard, although these methods are considered to be less stringent proofs of pluripotency (Ramalho-Santos et al., 2009; Varum et al., 2009).

However, these techniques in pluripotent stem cell biology can be thought of from a totally different perspective, in that tetraploid complementation in the mouse could be viewed as an animal model for related attempts in human, carried out for reproductive purposes. If iPSC from a human subject are truly pluripotent, then they should be able to develop into a genetically identical copy of the initial cell donor if allowed to become a putative blastocyst inner cell mass. In fact, the process would conceivably even duplicate mitochondrial DNA, which is not necessarily true when using SCNT and donor oocytes (St John et al., 2004). In theory, what is missing is therefore a trophoblast, which would not contribute to the offspring genome. Obtaining such a structure could be accomplished with a 4n
embryo, a trophoblast obtained from a human blastocyst ironically in a reverse procedure to the one originally employed to obtain hESCs, or differentiating a cell line, possibly the iPSC themselves, into a trophoblast phenotype. Unlike the other possibilities, in this last alternative no human embryos would be required to complete the procedure. The higher reproducibility of iPSC technology when compared with SCNT, in terms of generating patient-specific pluripotent cells in humans and viable individuals in mice, might explain the quick move to suggest banning this type of iPSC reproduction (Lo et al., 2010).

Interestingly, one recent advance that is not discussed at all in this context is direct differentiation, by which a somatic cell fate is transformed into another skipping a pluripotent cell intermediate (see for example, Zhou et al., 2008; Vierbuchen et al., 2010). This could well be the road that iPSC helped to pave, by suggesting somatic cell fates are not final but open to reprogramming provided that the correct programme can be found. This possibility might resolve some ethical challenges related to pluripotent cells. If a pluripotent embryo-like cell has, in theory, unlimited developmental potential, the same cannot be stated for a differentiated cell type that is being specifically changed into another. Or might this issue shift if instead of a fibroblast-to-neuron conversion (Vierbuchen et al., 2010) a fibroblast-to-gamete reprogramming is considered? In fact, ablation of a single gene has been shown to modify adult mouse ovaries into expressing some testicular characteristics, suggesting both that the maintenance of the adult gonad phenotype is dynamic and that some plasticity remains (Uhlenhaut et al., 2009). It is also worthwhile to note that oocyte-like structures were initially described by differentiation of male mouse ESCs (Hubner et al., 2003).

Provided that the appropriate genetic and epigenetic tools are available, it might even be conceivable to have functional sperm or oocytes produced from iPSC (or even somatic cells) derived from same-sex partners. The direct use of same-sex reproduction with iPSC has always been suggested as involving sperm-oocyte fertilization, which implies the production of either sperm from a XX individual or oocytes from a XY individual, clearly a difficult endeavour, if indeed at all possible (Mathews et al., 2009). The recent news of mice ‘with two fathers’ (Deng et al., 2010) involved the generation of an iPSC line from the first male, which spontaneously lost the Y chromosome. Given that XX mice are fertile, these X0 ‘male’ cells were then used to generate a female mouse, successfully mated with the second male. The complete impracticability of this procedure in humans, even disregarding species-specific differences, also highlights the need for more careful reporting from both scientists and the media.

However, gamete production may be circumvented altogether if iPSC from different donors could be placed together in the same reconstructed blastocyst as discussed previously, thus creating a chimeraic offspring. The individual would be a genetic mosaic (both in terms of nuclear and mitochondrial DNA) and in this case, these would have necessarily to be same-sex chimaeras, in order to avoid issues of sex determination.

Conclusions

To be clear: the author is not advocating the use of these possibilities for reproductive purposes, nor implying that any of the above is technically trivial. But if the complex differentiation of gametes from iPSC is discussed as a possibility, why are the conceptually much simpler alternatives of direct differentiation and chimaeras mentioned less often, or noted indirectly in the embryonic versus iPSC, or in the SCNT, debates (Devolder, 2009; Lo et al., 2010)? At the very least, it seems clear that the same arguments used for the discussions and preemptive regulations regarding reproductive SCNT/cloning should apply. In the mouse, both SCNT and iPSC procedures yield viable fertile animals at variable rates. Furthermore, unlike the case of SCNT offspring, where clear health concerns have been well documented (Tamashiro et al., 2007), there are no reports of any adverse effect of being a chimaera or the result of tetraploid complementation in mice, although this could be related to the fact that unlike SCNT/cloning, these procedures have been viewed as functional assays for cells, not as goals in themselves.

It is perhaps unsurprising that the focus of reproductive issues continues to be the production of gametes, even if by unconventional methods. But reproductive SCNT/cloning did introduce novel concepts to the discussion. The fact that this has not fully carried through to the discussion regarding the reproductive use of iPSC, chimaeras or direct differentiation, suggests that even the advent of radical concepts has a hard time entering into the mainstream debates concerning human procreation. All the more reason for those in this field to be especially vigilant and proactive in terms of scientific, regulatory and ethical discussions, and expand these discussions to cover all foreseeable possibilities.

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References


