

ETHICS AND SYNTHETIC GAMETES

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ABSTRACT

The recent in vitro derivation of gamete-like cells from mouse embryonic stem (mES) cells is a major breakthrough and lays down several challenges, both for the further scientific investigation and for the bioethical and biolegal discourse. We refer here to these cells as gamete-like (sperm-like or oocyte-like, respectively), because at present there is still no evidence that these cells behave fully like bona fide sperm or oocytes, lacking the fundamental proof, i.e. combination with a normally derived gamete of the opposite sex to yield a normal individual. However, the results published so far do show that these cells share some defining features of gametes. We discuss these results in the light of the bioethical and legal questions that are likely to arise would the same process become possible with human embryonic stem (hES) cells.

I INTRODUCTION¹

Three independent laboratories recently reported the derivation of gamete-like cells from mouse embryonic stem (mES) cells.² In the wider field of sex cell specification, the emphasis had been so

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² N. Geijsen, M. Horoschak, K. Kim, J. Grinau, K. Eggan & G.Q. Daley. Derivation of Embryonic Germ Cells and Male Gametes from Embryonic Stem Cells. *Nature* 2004; 427: 148–154; Y. Toyooka, N. Tsunekawa, R. Akasu & T. Noce. Embryonic stem cells can form germ cells in vitro. *Proc Natl Acad Sci USA* 2003; 100: 11457–11462. K. Hubner, G. Fuhrmann, L.K. Christenson,

far on trying to develop gametes *in vitro* starting with their precursors taken, for example, from foetal gonads (ovaries or testes). The three reports above add a completely new dimension raising the concrete possibility that gametes could be derived from ES cells. ES cells, both in mice (mES) and in humans (hES), are derived from the inner cell mass (ICM) within the blastocyst. Mouse embryonic stem cells are pluripotent in that they give rise, in the fetus, to all somatic cells as well as to the germ cells. Nearly three decades of work with mES cells have shown the tremendous versatility of these cells and their ability, *in vitro* and under the appropriate culture conditions, to differentiate into a variety of cell types, including, it now seems, also those most significant cells that are the gametes. The potential impact of human ES cells for medicine became apparent only in recent years. This was largely the result of two parallel developments: the successful cloning of a mammal (Dolly the sheep) from a somatic cell nucleus³ and the isolation of embryonic stem cells from human blastocysts.⁴ The intersection of these two lines of research sparked the vision for tailored cell therapies, achieved through cloning from a patient's own cell his/her ES cell line from which cells could be differentiated to replace damaged tissues. This procedure, commonly referred to as 'therapeutic cloning' (TC), became one pole (the other being 'reproductive cloning' (RC)) in the ethical and political debate on cloning and stem cell research.⁵

J. Kehler, R. Reinbold, R. De La Fuente, J. Wood, J.F. Strauss, M. Boiani & H.R. Scholer. Derivation of oocytes from mouse embryonic stem cells. *Science* 2003; 300: 1251–1256.

³ I. Wilmut, A.E. Schnieke, J. McWhir, A.J. Kind & K.H. Campbell. Viable offspring derived from fetal and adult mammalian cells. *Nature* 1997; 385: 810–813.

⁴ J.A. Thomson, J. Itskovitz-Eldor, S.S. Shapiro, M.A. Waknitz, J.J. Swiergiel, V.S. Marshall & J.M. Jones. Embryonic stem cell lines derived from human blastocysts. *Science* 1998; 282: 1145–1147.

⁵ That the pluripotent cells isolated from human blastocysts are commonly referred to as human ES cells implies that they are the human counterpart of mES cells. This is however not as straightforward as commonly perceived. The studies of the last few years have indeed confirmed that hES cells share many fundamental features with mES cells, foremost the ability to differentiate *in vitro* in a wide variety of cell types, and the ability to be cultured *in vitro* indefinitely without undergoing malignant transformation. But the cornerstone feature to define bona fide ES cells is their ability, when combined with a donor blastocyst, to contribute to all tissues of the ensuing chimera including the germline; since this experiment in humans is ethically untenable, it has been rightly suggested that, in the absence of this fundamental proof, these human cells be more cautiously referred to as human pluripotent stem cells (hPSC), thus underscoring pluripotency as their defining feature while avoiding to assume *tout court*

We wish to focus here on the specific implications that would arise as a result of the capacity of deriving gametes from hPSC cells (in which case they should arguably be regarded indeed as hES cells), and the ways in which these developments redefine and reshape the debate on human cloning. While awaiting for the experimental test of this possibility with hPSC, at the time of writing there remain outstanding questions on these synthetic gametes. First, can they function, in combination with natural and/or synthetic gametes of the opposite sex, in the development of normal individuals? As this requires, at the very least, the proper establishment of genomic imprinting, we will dedicate the first section to a brief introduction to the central role of genomic imprinting in gametogenesis, highlighting how this could affect safety evaluations and the comparison with other assisted reproduction technologies (ART). Second, will it be possible also to derive synthetic gametes from cloned ES cells, a decisive issue for the application of this technology to human reproduction? In

identity with their murine counterpart (A.G. Smith. Embryo-derived stem cells: of mice and men. *Annu Rev Cell Dev Biol* 2001; 17: 435–462). This is relevant to the theme of this essay, because, lacking the direct *in vivo* test of hPSC cells' contribution to the germline, it should not be taken for granted that, since synthetic gametes were obtained from mES cells *in vitro*, the same will be necessarily true also for hPSC. Nonetheless, this could be a matter of technological development, as suggested by the observation that so far, despite intense effort, it has been possible to isolate authentic ES cells (defined through their ability to colonize, after extensive passages of *in vitro* culture, the germline of the chimeric animal) only from certain strains of mice. This fact offers reasons for both caution and optimism in extrapolating the findings from mES to hES cells. On the one hand it highlights the importance of the genetic background for the ability to establish ES cell lines, and if this is true for different strains of inbred mice, it could potentially be much more relevant to the possibility of therapeutic cloning within the human population, whose genetic heterogeneity might influence the relative success at obtaining customized ES lines from different individual patients. But on the other hand the failure to obtain true ES cells from other species, together with our increased understanding of the differences that exist between the ES cells *in vitro* and the epiblast from which they were derived, point us to an altogether more contingent interpretation of the data at hand; namely to the suggestion that the ES cell status, rather than being an *a priori* condition within the embryo epiblast, should be interpreted more correctly as the epigenetic adaptation (with its host of phenotypic consequences) of pluripotent cells within the epiblast to a defined set of culture conditions. And since these culture conditions were first defined for the mouse, it is perhaps not too surprising that their direct application to other species' epiblast cells failed to produce bona fide ES cells. Thus, while a historical look at ES cell research does not enable automatic extrapolations of data across species, it also hints at the possibility that failures reflect more our limited understanding and technical capabilities rather than insurmountable differences among different species.

theory, since cloned ES cells appear very similar to sexually generated ES cells in terms of their differentiation potential, the answer to this question should be 'yes', but clearly the experimental evidence is needed. Third, will synthetic oocytes be as proficient as natural ones at reprogramming somatic nuclei so as to become an endless supply of 'reprogramming force'?

The dramatic implications of synthetic gametes have so far gone largely unnoticed outside a very small scientific community. This paper will explore these implications in some detail. Following a brief introduction to gametogenesis and imprinting, we will first focus on the implications of synthetic oocytes for somatic cell nuclear transfer, both as a way to study and to potentially cure human diseases. We will then move on to the application of ES-gametes for human reproduction and then consider the impact of this technology for the alteration of the human germline. Finally, we will examine the implications of mitochondrial DNA for ES-gametes.

I Gametogenesis and imprinting: an overview

Following fertilization by sperm, the fertilized egg (zygote) starts dividing to generate all cell types of the adult organism, including the germ cells, which will pass the genetic information to the following generation. Understanding how the same pool of approximately 30,000 genes is used during development to generate and maintain all differentiated cell types of an adult organism is a central and fascinating task of modern biology. As most of the cells in our bodies retain the same genetic information as the zygote from which they originated, the profound differences in shape, function and life span observed among different cell types arise as a result of the regulation of this stable genetic information: the orchestrated choice of which genes need to be active or silent in which cell at what stage of development. This regulatory activity is commonly referred to as epigenetic to underscore the fact that it operates upon (-epi) the genetic information (DNA) without affecting the gene sequence. By and large, epigenetic regulation involves chemical modification of either DNA (methylation and demethylation) or of the histones, proteins around which DNA is wrapped in a complex structure called nucleosome, the central unit of chromosomal organization. Thus, throughout development and in adult life, the functional state of a given gene or genomic region will be ultimately determined by the combination of these epigenetic modifications. Importantly, these modifications are heritable, which

allows cells to maintain their identity through cell division, but they are at least potentially reversible. The most spectacular example of this reversibility is the possibility that the fully differentiated genome of a somatic cell can be coaxed, upon transfer into an enucleated oocyte, into recapitulating development to yield a new organism, a process commonly referred to as 'cloning'.⁶ That all cloning experiments so far have been highly inefficient shows that epigenetic marks, though potentially reversible, are indeed quite stable.

Eggs and sperm are highly specialized cells and present two main features that distinguish them from all other cells of an adult organism. First, mature oocytes and sperm contain a haploid complement of the genome, i.e. half of the genetic material, so that upon fertilization, their combination restores the full genetic complement characteristic of the species. Second, in mammals these two haploid genomes (hereafter referred to as parental genomes) are not equivalent, because certain genes are differentially marked in oocytes and sperm with molecular tags that reflect their parental origin, commonly referred to as genomic imprints.⁷ At the molecular level, these tags are differences in the methylation of specific genes or genetic regions; their effect is that, after fertilization, certain genes will be expressed only from the paternal or the maternal genome, but not from both. So, the diploid genome of a zygote can be distinguished into two functional families: on the one side the vast majority of genes, both copies (also termed alleles) of which from this point onward will be regulated during development regardless of the parent they came from, and on the other side the imprinted genes (only about 50, but crucial for normal development), only one copy of which will be expressed and this choice will depend on the maternal or paternal origin of this copy. What is the function of imprinting, why did this epigenetic asymmetry between parental genomes evolve? Various theories have tried to explain the evolutionary meaning of this phenomenon, and it is beyond the scope of this paper to examine them; what appears certain, mainly from studies in mice in which imprinted genes were disrupted, is that such genes are crucial for normal mammalian physiology, with functions in placentation, energy metabolism, lactation, and behaviour.

⁶ I. Wilmut et al., *op. cit.* 3.

⁷ M.A. Surani. Reprogramming of genome function through epigenetic inheritance. *Nature* 2001; 414: 122–128.

Thus looking at the cycle of reproduction, we start with two gametes carrying parental imprints for about 50 genes, on to the zygote (male or female) that retains these imprints and starts development to give origin, among all other cells and tissues, also to the new germ cells, but which now must be newly imprinted according to the appropriate sex of the germline (maternal in a female individual and paternal in a male individual). Thus, one of the defining features of defining germ cells is their capacity to erase imprints (since initially, like any other cell in the developing embryo, they obviously carry both parental imprints) and to reset them so that in mature gametes they match the sex of that germline (paternal imprints in sperm and maternal imprints in oocytes).

Imprinting is essential for normal development, as shown by the failure to obtain development to term when using, in nuclear transplant experiments, an imprint free donor nucleus (taken from primordial germ cells at the stage when they have erased all imprints). Most recently, direct *in vivo* evidence was provided that the imprinted asymmetry between parental genomes is indeed the reason why in mammals parthenogenesis (i.e. reproduction that occurs exclusively through the maternal germ cells without any male contribution) is not compatible with normal development.⁸ In this seminal work, Hogawa and colleagues reconstructed an oocyte from two maternal haploid genomes, producing a parthenogenetic embryo that normally would not be able to proceed to term. However, when the authors used standard gene engineering technology to remove from one of the two haploid maternal genomes one of the crucial imprinted loci, they managed to obtain, albeit at very low frequencies, an adult, fertile mouse. This mouse, as widely publicized in the media, was the offspring of two mothers: the asymmetry between imprinted genes had in this case been accomplished artificially through genetic manipulation, resetting for two maternal genomes the appropriate dosage of imprinted genes normally achieved through the combination of a paternal and a maternal genome. Interestingly, this work provides not only proof of the *in vivo* relevance of imprinting; it also shows that artificially tuned parthenogenesis is possible in mammals.

Somatic cell nuclear transplant (SCNT) studies have also shown that imprints can be left intact during reprogramming,

⁸ T. Kono, Y. Obata, Q. Wu, K. Niwa, Y. Ono, Y. Yamamoto, E.S. Park, J.S. Seo & H. Ogawa. Birth of parthenogenetic mice that can develop to adulthood. *Nature* 2004; 428: 860–864.

however they can be erased, and in fact aberrant imprinting patterns have been observed in most cloned animals. The observation that cloned animals often display fetal and placental abnormalities similar to the phenotypes observed in both patients and mice carrying mutation in imprinted genes strengthens the connection between faulty imprinting and cloning failures. However, normal imprinting is also affected by culture conditions associated with IVF procedures, disturbances that are probably exacerbated in cloning. And importantly the frequent detection of imprinting defects in surviving clones also shows that full development can tolerate significant imprinting defects.

II Implications for somatic cell nuclear transfer (SCNT) in the understanding of disease

If the ability to derive 'synthetic' oocytes from ES cells is confirmed also for human ES cells, this could potentially eliminate one of the main caveats of SCNT, namely the supply of human oocytes to perform nuclear reprogramming. Public debate on cloning has been so far polarized around the juxtaposition of 'reproductive' versus 'therapeutic' cloning. Relatively little attention has been dedicated to the potential usefulness of SCNT in the study and understanding of a variety of human disorders. In fact, for most diseases, the availability of tissue samples is scarce, and primary cells (i.e. cells taken directly from the patient) are usually very difficult to culture, so that only a very limited number of experiments are possible. Immortal cell lines originally derived from human cancers have been available for a long time, and although they have enabled great progress in the molecular cell biology of the various tissues from which they were derived, clearly they do not recapitulate the function of either normal or abnormal cells from a variety of diseases. In fact, most of our molecular understanding of human disorders has come in the past decade from mouse studies: by introducing in the mouse genome mutations which are found in human diseases, such studies have allowed, in several cases, to develop useful mouse models of the human conditions. However, some diseases have proven resilient to these modelling attempts. More importantly, the majority of diseases with great social impact are polygenic – i.e. they are caused by the interaction of several genetic mutations and/or polymorphisms. We still do not understand this polygenic inheritance in molecular detail, and even if we did, modelling such a disease in the mouse by introducing all relevant mutations

will remain for some time a very substantial challenge. Therefore, it would be highly useful to open an experimental window directly on patients' diseased tissues. SCNT could provide a powerful approach. In analogy to 'therapeutic cloning' the process would require transplanting a somatic nucleus from a patient into an enucleated oocyte to generate a cloned embryo which would be allowed to grow up to the blastocyst stage, at which point, ES cells would be derived. Contrary to 'therapeutic cloning' (see below), the goal here would not be to differentiate ES cells for transplantation, but rather to have them as an unlimited source from which to derive differentiated diseased cells and diseased tissue as an unprecedented opportunity to understand molecular pathogenesis. If the donor nucleus comes from patients with very well defined pathologies, such an approach would enable the study of the disease without requiring *a priori* knowledge of the underlying mutations, since they would be automatically present in the donor genome.

Needless to say, this approach could only be feasible with a virtually endless supply of oocytes; this would allow reprogramming a sufficient number of donor nuclei for the relevant diseases even if the efficiencies of reprogramming remain low.

But how good should reprogramming be in order to be useful in this setting, and therefore, how good should these synthetic oocytes be in order to perform this function?

Although a final answer can only await experimentation, it seems reasonable to assume that for this application oocytes would not need to be perfect (remembering, of course that naturally produced oocytes are of very variable quality and many are far from a standard that would be regarded as acceptable), since it would not be asked of them to reprogram the somatic nucleus so that a healthy newborn can be generated, but rather to rewind the 'developmental clock' far enough so that ES cells can be generated. At present, the first question remains of course whether such synthetic oocytes can reprogram, and if so, understand the extent and efficiency of their performance. There remains the possibility that faulty reprogramming, though still compatible with the derivation of ES cells, could impair later differentiation along certain pathways, and this could conceivably limit the use of this technology for the study of certain diseases. Equally important, appropriate control experiments should be set up, so that an impairment in differentiation observed on an ES cell line cloned from a diseased patient could be unequivocally traced to the disease and not to the consequences of faulty reprogramming.

III Implications for somatic cell nuclear transfer (SCNT) in the treatment of treat disease

In ‘therapeutic cloning’, the exactly same procedure described above would be used to generate, from each patient, autologous ES cells from which to differentiate a specific cell type in order to replace lost or damaged tissue. The three main advantages of this procedure are the following: i) the cloned cells would have the same genetic makeup (except for mitochondrial DNA) as the individual to be treated and thus would not be rejected; ii) the ES cell line would constitute an endless supply of material, so that repeated administrations of cells would be possible over time should the disease require it; and iii) the ES cells could be genetically altered in a very precise manner (using the same technology – homologous recombination – successfully used in mouse ES cells for over two decades and shown last year to also function in hES cells) prior to transplantation into the patient. In the case of diseases caused by cell-autonomous mutations – mutations that cause the disease in the cells that carry them, this would permit the repair of the mutation so that healthy cells can replace the diseased tissue. Other genetic manipulations include the introduction of genetic switches that would enable us to selectively kill cells after transplantation should adverse effects arise. It is beyond the scope of this paper to discuss exhaustively the scientific, technical and ethical challenges posed by ‘therapeutic cloning’. We limit our analysis to the impact of synthetic oocytes on this technology, and observe that, as noted above, their availability would be likely a prerequisite to make ‘therapeutic cloning’ a viable option.

The question as to exactly how good the oocytes should be for this application is of both scientific and ethical significance. It would be safe to assume that in this case the standards of safety should be high. If considering SCNT to understand diseases we could be happy with a patient derived ES cell line that is able to differentiate *in vitro* along the desired developmental pathways; in the case of therapeutic cloning, we require that the differentiated cells derived from the patient’s clone integrate into the damaged tissue, perform the relevant function *in vivo* and establish proper communication with the cellular context. Could any of these requirements be affected by faulty reprogramming from ‘imperfect’ synthetic oocytes? It is possible, but needless to say, the only way to find out is to do extensive research, first in animal models, to test the long-term efficacy of reprogramming performed by synthetic oocytes.

IV Implications for human assisted reproduction

The possibility to derive gametes from ES cells could have a very significant impact on assisted reproduction, enabling infertile individuals to have their own genetic children. In the case of an infertile couple, the procedure would involve first cloning an embryo from the infertile member of the couple by transplanting his/her somatic nucleus into an enucleated oocyte. This cloned embryo would be allowed to proceed to the blastocyst stage, at which point embryonic stem cells would be derived from the inner cell mass of the embryo and put into culture. Following the appropriate culture conditions, whose identification will be greatly aided by the methods that recently led to the first ES-derived gametes, it should be possible to generate mature gametes from these ES cells, which would finally be employed in *In vitro* Fertilization (IVF) together with the naturally generated gamete from the fertile member of the couple. It is clear that, although the procedure does involve Somatic Cell Nuclear Replacement (SCNR), this would not be an exercise in reproductive cloning, since the resulting embryo would have, as in normal reproduction and IVF, the genomic contribution of both parents. Whereas the half genome of the fertile member of the couple will have been reprogrammed ('made fit for fertilization') through the normal process of gametogenesis, in the case of the infertile member, this will have occurred through an 'assisted reprogramming effort' – i.e. exposing a somatic genome to the reprogramming activity of a donor oocyte followed by *in vitro* differentiation into the appropriate gamete. Thus, both ethically and legally, we suggest that this procedure is most appropriately framed as a therapeutic intervention (to treat infertility) that replaces *in vitro* the physiological function normally responsible for reprogramming the germline genome, in analogy to the host of medical technologies that restore other deficient functions of the human body.

The crucial issue is again safety. Here the main problem is constituted by the fact that as we proceed in life, our genome progressively accumulates genetic damage (for example UV induced mutations etc.); thus, it has been rightly emphasized that a somatic cell nucleus will have most likely low (or at least, lower) quality DNA. Some evidence suggests that germ cells might have enhanced capacity to repair and/or limit DNA damage, which would be broadly consistent with the evolutionary vision of the germline as some sort of protected 'genetic sanctuary'. Of course also in the case of DNA, quality is a relative matter, and defining the extent to which somatic cell DNA is damaged will require a

systematic comparison of DNAs from a variety of tissues (it is conceivable, for example, that DNA of tissues which are more directly exposed to environmental mutagens may be more easily damaged, or that non dividing cellular compartments will accumulate relatively more mutations than rapidly cycling ones). More importantly, if DNA quality constitutes the main criterion upon which to assess the technique's safety, it will be crucial to compare the genome integrity of somatic cells against germ cells of comparable age. This is especially true for the female germline, in which all germ cells are already present at birth, and thus potentially exposed to environmental and/or spontaneous DNA damage throughout life. But also in the case of the male germline, it would be interesting to assess the extent to which the stem cells of the testis, which originate mature sperm suffer DNA damage throughout life. For it is clear that if sperm from relatively older men or oocytes from relatively older women present levels of DNA damage similar to at least some of the somatic lineages, an ethical argument based on DNA quality would not be tenable. For, as with IVF and all other assisted reproduction technologies, safety must be evaluated in comparison with the safety of the natural process rather than of ideal absolutes.

V Implications for assisted reproduction for same-sex couples

Synthetic oocytes were derived from both male (XY) and female (XX) cells.⁹ Though this may appear at first as the most startling feature of the technology, this result is not particularly surprising: in gonads which lack expression of key regulators of male sexual development (like the SRY gene), male primordial germ cells develop down the female germline differentiation pathway and undergo the first step of oogenesis. In the cell culture aggregates described by Hubner et al. for the isolation of oocytes, Sry was apparently not expressed in the areas from which female germ cells originated. This implies that what happened fortuitously in this case (the microarchitecture of the cell colonies in which differentiation took place and the patterns of gene expression within them are a largely stochastic processes) could be in the future intentionally manipulated. In fact, if the past is any guide for future developments, the existence of *in vitro* differentiation systems has always heralded the rapid characterization of the main signals and molecules involved in such pathways. Thus, it is conceivable that culture conditions will be set up to direct in

⁹ Hubner et al., *op. cit.* note 1.

vitro ES-gametogenesis towards the female or the male germline with relative reliability; and the process does not even need to be highly efficient, provided that methods exist to isolate the few cells which have successfully differentiated. Assuming that the process could be successfully completed – i.e. derivation of a mature oocyte from a male ES cell line, the social implications could not be more obvious. It has not escaped our attention that a gay couple could potentially generate for the first time a child to which both fathers contribute their genomes, one through the natural process of spermatogenesis, the other through the assisted process of genome reprogramming down the female germline. Clearly, a woman would need to carry this embryo to term, but this would not be different from current arrangements in which two gays and one woman collaborate in a pregnancy. The fundamental difference however would be that while so far only one man in a gay couple could genetically father the offspring, synthetic gametogenesis could allow both fathers to do so simultaneously, just like fathers and mothers in heterosexual couples.

Could this be relevant also for female gay couples? This seems at present a more distant possibility, since the production of mature sperm requires the genetic information stored on the Y chromosome. And while it is conceivable that technologies of gene and/or chromosome transfer may at some point overcome this problem, we shall not consider here this possibility in detail largely because, contrary to the ES-derived gametes, in this case the necessary proof of principle has yet to be provided. However, as described in section 1, the recent generation of a viable mouse from two mothers through an artificial resetting of genomic imprinting does offer at least the conceptual framework within which two women could simultaneously contribute to the genetic makeup of their offspring. However, the low frequencies obtained, lower in fact than in reproductive cloning, make it highly premature to envision the practical application of this technology to humans. Especially because in the above cited study even the apparently normal mouse born from two mothers showed a wide pattern of aberrant gene expression, warranting a detailed characterization of the molecular consequences of artificially resetting imprinting mechanisms. Furthermore, this ‘two-mothers’ scenario would result, by definition, in a genetically modified offspring (the genetic change being in fact necessary to overcome the imprinting requirement), and thus we suggest that it be more properly framed within the context of human germline alteration.

Clearly, monumental challenges still lie ahead. The most pressing question in this context is whether male ES cells could not only differentiate into oocytes, but do so showing the pattern of erasure and resetting of imprints appropriate and characteristic of the maternal germline. For, as noted above, the combination of a correctly imprinted sperm and a correctly imprinted oocyte is a prerequisite for a healthy offspring.

VI Implications for the alteration of the human germline

Perhaps the most profound implications of synthetic gametogenesis concern the genetic alteration of the human germline. The topic has figured prominently in the bioethical debate, however, in this case, technological development has been lagging behind. For so far, the most widely proposed methods to introduce genetic changes in the human germline rested upon the inefficiencies of 'reproductive cloning'. The scheme here (referred to as A) would be that two parents, for example carriers of a genetic mutation, conceive an embryo by standard IVF. This embryo is then allowed to proceed to the blastocyst stage to obtain ES cells, which can then be genetically engineered *in vitro*. Once introduction of the desired genetic change has been confirmed, the nucleus of this altered ES cell is transferred to an enucleated oocyte to initiate development and then transferred to the uterus of the mother. As noted above, also in this case cloning would not produce a genetic copy of either parent, since the resulting child would carry genomes of both parents, except that the disease-causing mutations would have been repaired. The main pitfall of this approach however is that, assuming cloning remains highly inefficient, the chances of obtaining healthy offspring would be very low; and this would pose possibly insurmountable ethical quandaries from the viewpoint of the ethics of experimenting with human subjects. It is true that when cloning was done using ES cell nuclei as donors, the efficiencies were significantly higher than with any other somatic cell nucleus (evidence that the ES cell genome is more easily reprogrammed), but they would probably still remain below an acceptable threshold of risk. What changes then with the possibility of generating gametes from ES cells? The process of gametogenesis has evolved specifically to reprogram genomes, in eggs and sperm, and fit them for fertilization. Thus, if the process of gametogenesis *in vitro* would be sufficiently similar to the natural one, as hinted at by the recent results, the 'reprogramming power' of gametogenesis could be conveniently exploited.

In this scenario (referred to as B), the above considered couple would first produce two independent embryos, one cloned from a somatic cell of the father and the other from a somatic cell of the mother (in both cases of course, an external supply of oocytes would be needed). After development to the blastocyst stage, ES cells would be derived from both embryos and altered genetically to repair the mutation. At this point, these corrected ES cell clones, one from the father and one from the mother, would be allowed to differentiate down the respective germline (female and male for the mother and the father respectively) to generate genetically altered egg and sperm, which would then be combined through standard IVF. Also in this case, the offspring would not be a genetic copy of either parent, rather an individual with unique genetic composition in which disease causing mutations have been repaired. Would procedure B be better than A? Likely yes. In A we are asking the oocyte to reprogram the nucleus from the repaired ES cell directly into a state compatible with healthy development to term and beyond; there is no intermediate step, it's a win or lose bet. In B we are introducing a potentially critical selection step: the reprogramming oocytes would be required to be just as good as to let us derive ES cells. Then, upon ES cell differentiation (which is likely to improve considerably over the currently available and largely stochastic methods) we would be able to select the possibly few ones that have properly undergone gametogenesis and use them for standard IVF. Being able to select out the few cells, which have undergone a process that specifically evolved to reprogram genomes (gametogenesis) could be the key improvement to make germline gene modification a feasible reality.

We have intentionally outlined above one of the most difficult cases, in which both prospective parents carry a disease mutation. Other variations are clearly possible, first of all the possibility that only one parent would need to undergo the procedure (in the case of dominant mutations), while the other could contribute his/her natural gamete.

VII Implications of mitochondrial DNA

In SCNT the donor nucleus is reprogrammed by an enucleated oocyte, which is the only source of mitochondria. As a result, the product of SCNT (embryo, ES cells and ES derived gametes) all carry the mitochondrial DNA inherited from the donor oocyte. Though mitochondria host only a tiny fraction of the whole genome, ample evidence suggests that mitochondrial DNA (mtDNA) influences neuronal development and brain

activity. This is by no means surprising, especially considering that most mtDNA encodes proteins required for the mitochondrial function of energy factories within the cell. And in fact, the majority of deleterious mitochondrial mutations in humans are associated with brain disorders. Recent results in mice point to a significant role of mtDNA in cognition, a role played out in combination with the nuclear genome and, importantly, with age.¹⁰

In the cloning debate, the issue of mtDNA has been usually either neglected or presented as one more reason why a cloned individual would not be a perfect genetic copy of the donor. In fact, nuclear transfer has been advocated as a potential means to allow women carriers of mtDNA mutations to still pass their nuclear genome to the offspring, which would then have three genetic parents, a constellation arguably more profound than the three-person relationship involved in maternal surrogacy.

With respect to ES-gametes, the issue of mtDNA appears relevant to the reproductive possibilities. For, in the context of SCNT to either understand or treat diseases (2 and 3), the origin of mtDNA of the reprogramming oocyte will have in most cases no relevance. On the contrary, if cloned embryos are to be used to generate gametes (4, 5 or 6), should consideration be given to the effect of mtDNA on such complex traits as behavior and cognition, especially if, as appears from the studies cited above, some of these effects are only uncovered during ageing? Clearly, we chart here a largely unknown territory. For how polymorphic is mtDNA in the human species? And which ES cells (i.e. from which person) should we choose as the source of our reprogramming oocytes, with their non-neutral 'baggage' of mitochondria? As noted previously, also in this case, evaluation of risk and safety needs to confront the imponderables inherent to natural reproduction and other, already accepted, assisted reproduction technologies. For example, it was recently noted that mice born from ARTs behave differently when adult, appearing to be on the whole bolder.¹¹ Extrapolating behavioral studies from mice to men is always a tricky business, but on the other hand, as noted above,

¹⁰ P.L. Roubertoux, F. Sluyter, M. Carlier, B. Marcet, F. Maarouf-Veray, C. Cherif, C. Marican, P. Arrechi, F. Godin & M. Jamon. Mitochondrial DNA modifies cognition in interaction with the nuclear genome and age in mice. *Nat Genet* 2003; 35: 65–69.

¹¹ D.J. Ecker, P. Stein, Z. Xu, C.J. Williams, G.S. Kopf, W.B. Bilker, T. Abel & R.M. Schultz. Long-term effects of culture of preimplantation mouse embryos on behavior. *Proc Natl Acad Sci USA* 2004; 101: 1595–1600.

imprinted genes (which affect behaviour) are known to be exquisitely sensitive to embryo culture conditions and we already know, for example, that IVF increases the risk of certain conditions, including Beckwith-Wiedemann syndrome, which results precisely from faulty imprinting.

REMAINING ETHICAL ISSUES

Many will find the prospect of the creation of 'synthetic' gametes and the doors opened by this prospect disturbing, and the idea of two gay men being 'father and mother' to their own child, both genetically and socially, beyond the limits of toleration. We will examine the ethical issues as they arise.

a. Natural versus synthetic

People often claim that the natural is superior to the artificial or synthetic, they know that butter is natural and margarine artificial and that butter is better. People disposed to think in this way might claim that the fact that sexual reproduction is natural is morally relevant and makes it superior to artificial methods of reproduction and that we should give priority to the natural over the artificial. But this is simply absurd. A child born by assisted reproduction or one that would be born as a result of 'synthetic' gametes is the same genetically, having the same moral status as a child born by natural reproduction. Some people prefer naturally occurring foods but this distinction, so real in people's minds is extraordinarily vague and elusive. Thousands of years of farming practice and selective breeding, including of course systematic and widespread cloning of plants, which has proceeded for centuries, has all but obliterated any meaningful distinction between the natural and the artificial or synthetic. How many people are conscious that the roses they buy or grow and the tomatoes they eat have probably been cloned? In so far as the distinction can sensibly be drawn there is no reason to prefer the natural *per se*. If, for example 'natural' foods are safer or healthier, there is a reason to prefer them. But in many cases, synthetic preparations are healthier and safer and indeed are preferred for good reasons. The drug digoxin is used for heart conditions and is always given in a highly purified pharmaceutical form even though it occurs naturally as digitalis in the foxglove plant. Natural reproduction may be more enjoyable than synthetic reproduction, or cheaper, or safer or more private, these are reasons to prefer it. But there is no reason to prefer a natural process simply because it is natural.

The natural *per se* is morally neutral and although some people are naturally healthy and happy and that is good for them, it is equally natural to be unhealthy and unhappy. Moreover, the natural *per se* is often very harmful; disease, mutations, pestilence, floods, hurricanes, fire, landslides and the like can cause massive loss of human life and do terrible damage to the environment. One might characterize the practice of medicine as the comprehensive attempt to frustrate the course of nature, because people naturally fall ill, are invaded by natural organisms like viruses and bacteria, and naturally die prematurely, often as babies. If we always preferred the natural we would have to abjure the practice of medicine and the discoveries of medical science including vaccines and antibiotics.

b. Safety

Safety is always important and we want all processes, natural or synthetic, to be both as safe as possible, at least safe enough to make their use justifiable in all the circumstances of the case. If the so-called precautionary principle had held sway in the Garden of Eden it is doubtful if any of us would be here now. The tree of knowledge aside, the process of natural reproduction, which started with the first couple/s whether Eden was in Africa between 5 and seven million years ago, or in the Middle East 6008 years ago, is highly dangerous to both mother and baby.

One of the major objections to cloning a human being has been that for example, in the case of Dolly, only one clone was successfully produced after 277 attempts, showing that so far cloning is inefficient and involves very high embryo wastage. But embryo wastage *per se* cannot be an objection to reproductive cloning for those who accept natural reproduction. For we must remember that somewhere around 80% of embryos perish in natural reproduction!¹² Not only is natural reproduction inefficient, it is also unsafe. Around 3–5% of babies born have some

¹² Robert Winston gave the figure of 5 embryos for every live birth some years ago in a personal communication and also gave this figure in a television programme. Anecdotal evidence to me from a number of sources confirms this high figure but the literature is rather more conservative making more probable a figure of three embryos lost for every live birth. See Charles E. Boklage 'Survival Probability of Human Conceptions from Fertilization to Term' in *International Journal of Fertility* Vol 35. No. 2. 1990: 75–94. Also Henri Leridon *Human Fertility: The Basic Components* University of Chicago Press, Chicago 1977. Again, in a recent personal communication Henri Leridon confirmed that a figure of three lost embryos for every live birth is a reasonable conservative figure. The point however does not depend upon a precise figure.

abnormality. Natural reproduction not only involves the foreseeable and unavoidable creation of some embryos that will die but also some embryos that will go on to become very disabled human beings. Many embryos created are so genetically abnormal that they die, and some survive only to die as grossly deformed babies. Natural reproduction is of course also dangerous for the mother. It is well established that carrying a child to term is more dangerous for the mother than early abortion and much more dangerous than not having a child at all. It is salutary to remember that while we wish to improve the success rate of natural reproduction we do not find its dangers, and profligate waste of embryos unacceptable. While we might not have accepted the success rate from natural reproduction in the presence of better alternatives the fact that we have accepted it, and not obviously reluctantly, or with moral reservations, shows that while maybe wishing and working for better success, we accept the current success rate as morally acceptable. Thus, the reason why the rare success with cloning currently matters is not its absolute inefficiency in terms of embryo loss. Much more significant is our only very primitive understanding of what might actually go wrong, and how frequently, in those clones who actually survive to adulthood.

c. Supply of oocytes

As noted above, a limiting factor in the potential for hES cells in the study of human disease and in therapy has been the supply of oocytes. Synthetic gametes potentially solve this problem and the perfection of this technology would potentially remove a major obstacle to a vast range of beneficial research and therapy. This fact alone would surely constitute an adequate ethical justification of work on synthetic human gametes even if none of the other ethical problems we have identified could be successfully resolved.

d. Same sex parents

The sensational possibility of an all male or female, presumably (although not necessarily) gay or lesbian couple being able to manufacture male and female gametes from either partner to enable them to have a child which shares the genetic make-up of the parents in the same proportion, and virtually in the same way as for heterosexual couples, will undoubtedly grab and indeed make the headlines. There will undoubtedly be strong objections

but can we say clearly whether these objections will have any substance or credibility? We think we can.

When synthetic gametes become available, a heterosexual couple, of whom neither can produce usable gametes, will be able to father and mother their own children for the first time. All decent people will celebrate this possibility. The same development will open the way for same sex couples to do likewise. Given that same sex couples are, in many societies, already able to use ART to help them have children to whom they will be genetically related and indeed are also able to adopt children, it is difficult to see what objections there could be to this that would be immune to charges of homophobia or of discrimination on the basis of gender or sexual orientation.

It is likely that those who feel uncomfortable about the prospect of same sex couples sharing equally the genetic contribution to their children will still object. The most plausible objections to the use of reproductive technologies in such cases would have to centre on some suggestion that the interests of the child that will result from the process will be prejudiced by the nature, novelty or 'strangeness' of the process itself, rather than to some criticism of the fitness of same sex couples to be parents, since objections to same sex couples as parents *per se* have already been made and found wanting in the case of sperm and egg donation, surrogacy, other ARTs and adoption. And of course this form of objection will not be available to those who do not object to heterosexual couples availing themselves of the technology.

A detailed dismissal of all the objections that might be made here would be beyond the purview of this paper. Suffice it to say that there is no evidence that children produced by ART so far fare significantly worse than other 'normal' children. Moreover since the children produced by ART are almost always not the same children that would have been born had ART not been used, it is always in those children's interest to have been produced in this way because unless their existence proves unbearable 'their' only alternative would have been never to have existed at all.¹³

Since it is now generally acknowledged that discrimination on the basis of sexual orientation or gender is not only unacceptable, but more importantly, profoundly immoral the most intriguing and dramatic possible use of synthetic gametes should neither alarm us nor constitute reasons to reject the immense potential

¹³ J. Burley & J. Harris. 'Human cloning and Child Welfare' in *The Journal of Medical Ethics* Vol 25. February 1999.

of these developments. It could be seen in fact as a technologically mediated way to further democratize human reproduction by overcoming natural barriers in societies that have long acknowledged, in theory and increasingly in practice (adoption still being the most conspicuous example), the distinction between genomic and social parenthood.

e. Implications for the alteration of the human germline

Germline alterations have historically proven highly controversial, for no very obvious reasons. Although the alterations will be 'permanent' and hence any mistakes made will be 'permanent' if not corrected, the fears and the objections they provoke assume the impossibility of subsequent correction or inbuilt safety 'switches' should things go wrong and they also assume that the dangers of the mistakes outweigh the ongoing dangers of not making therapeutic germline changes. All of these factors require careful assessment but there are no reasons to suppose there are any defensible objections to germline interventions in principle.¹⁴⁻¹⁵

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¹⁴ John Harris. *Wonderwoman & Superman: Ethics & Human Biotechnology*. Oxford University Press. Oxford, 1992.

¹⁵ John Harris & Søren Holm. 'Extended lifespan and the paradox of precaution' in *The Journal of Medicine and Philosophy* Vol 27. No. 3. 2002; 355-369.

GLOSSARY

Autologous: In analogy to the concept of autologous transplantation, an ES cell line cloned from a patient is autologous because it contains his same genomic DNA, except for the mitochondrial DNA.

Blastocyst: The early stage of embryonal development, at which the embryo consists of a sphere of about 150 cells, arranged into an outer layer of cells (the trophoctoderm), a fluid-filled cavity (the blastocoel), and a cluster of cells on the interior (the inner cell mass), from which the epiblast.

Epiblast: the part of the inner cell mass of the blastocyst from which embryonic stem cells are derived. It consists of a transient pool of pluripotent cells, which will quickly differentiate into the various tissues of the embryo.

Epigenetic: Epigenetic regulation refers to those features of gene function that are inherited through cell division, but which are not encoded through the nucleotide sequence of the DNA, but rather through the chemical modification of either DNA or its associated proteins, without that these modifications change the DNA sequence.

Methylation: Addition of a methyl group. This can occur both at the level of the DNA, in which the cytosine nucleotide is methylated, or at the level of proteins, in which two aminoacids, lysine and arginine, can be methylated. In the case of the histones, the proteins around which the DNA is wrapped, lysine methylation has emerged recently as a crucial mechanism to regulate gene activity.