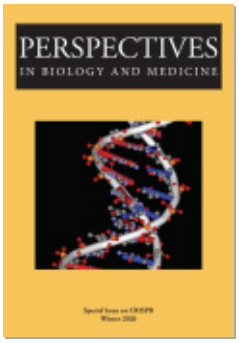
Clinical Germline Genome Editing: *When Will* Good *be* Good Enough?



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ABSTRACT Ensuring experimental outcomes are of the highest clinical caliber is crucial prior to the introduction of germline genome editing. However, if we are to police scientific progress using probability or the potential to go wrong, then we must account for the specious standards of human reproduction. With 15% of clinically recognized pregnancies estimated to end in spontaneous miscarriage within the first trimester, and 25% of all pregnancies ending in miscarriage, human reproduction has a high failure rate. These figures, coupled with the percentage of all births with congenital defects and the number of these who will die in the first year of life, paint two scenarios: one, that evolutionary checkpoints are cruel but critical, and two, that for the seemingly inevitable 3%, or 8 million babies born annually with congenital disorders, perhaps more must be done for prevention, when methods exist for prediction. Unifying progress in three coevolving technologies—assisted reproduction, genome editing, and genome sequencing—could produce revolutionary clinical changes in the harsh global statistics of hereditary disease. A historical perspective on the rocky foundations upon which IVF was built suggests that lessons should be learned from the misalignment of research and clinical practice due to funding and research restrictions. At present, it seems likely that history will repeat itself, and that progress in research will be hampered by hypocritical hesitation.

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The year 2018 was the 40th anniversary of the birth of Louise Joy Brown, marking four decades of clinical in vitro fertilization (IVF) and embryo transfer (Steptoe and Edwards 1978). Though this milestone, reached first by Steptoe and Edwards in the United Kingdom, is well acknowledged through Nobel accolade (2010), the achievement was not entirely celebrated at the time. Global contention was not just moral, but political and legislative. In the United States, the achievement led in 1978 to the freezing of federal funds by the National Institute of Health (NIH) for research on human embryos and oocytes (Biggers 2012).

A report published on May 4, 1979, by the Ethics Advisory Board of the Department of Health Education and Welfare (HEW) contained evidence from both opponents and supporters of research into infertility. The decision to ban funding led to a unique path for the field of assisted reproduction as evidenced in the US. Despite the ban on federal funding for research, a demand for infertility treatment led to the opening of the first clinic in Eastern Virginia Medical School, on the grounds of a "Certificate of Need" from the State of Virginia (the legal requirement for opening a new clinic at the time) (Thompson 2016). The first US IVF baby—the 15th IVF baby in the world—was born in 1981, over three years after Louise Brown. The legislation was, however, still unclear: procedures that were done for the "benefit to the patient" were legal in clinical laboratories, yet the same procedures were illegal in research laboratories (Biggers 2012; Biggers and Racowsky 2018).

The ethos of this misaligned first step has continued in the field of assisted reproduction, where restrictions on the use of human embryos and oocytes for research has meant that insufficient preclinical research is carried out prior to the introduction of IVF treatments to the clinical IVF laboratory globally (Cheung, Bernard, and Lam 2019; Harper et al. 2012). The commercial domination of the field allows for clinical approval without clinical trial.

The number of planned long-term ab initio studies on children born through IVF and medically assisted reproduction has been relatively scant. When investigating data through follow-up studies and meta-analyses, a further major problem is that the quality of evidence is limited by the quality of the studies and the inconsistency of data collection, stratification, and methods used (Kissin et al. 2019). These problems result, in most instances, in an inconclusive knowledge of the root of adverse events (Heijligers et al. 2018). For example, children born from IVF treatment have been potentially linked to increases in total body fat composition, increased blood pressure, and advancement of bone age, as well as to increased risk of congenital and sex chromosome abnormalities (Hart and Norman 2013; Källén et al. 2010; Kalra and Barnhart 2011). Perhaps most alarming are the links to imprinting disorders (Cortessis et al. 2018: Lazaraviciute et al. 2014), but the diversity of patients' ages, genetic backgrounds, and mixture of laboratory and clinical treatments make proof of causality nearly impossible to ascertain.

The ongoing need for evidence-based medicine has not prevented the routine adoption of many unproven treatments in clinics. The debate surrounding the clinical benefit of many expensive additional assisted reproductive treatments, known as "IVF add-ons," has led to a consensus statement by the Human Fertilization and Embryology Authority (HFEA), the governing body for embryo use and fertility treatment in the UK. The HFEA introduced a traffic light system presented to help patients understand whether a so-called treatment can still be considered experimental and unproven (orange and red) or effective (green) (HFEA 2019). Despite this, procedures with as yet no proven evidence of increased chance of live birth, such as assisted hatching and the use of embryo glue, are still offered at high cost to patients. Indeed, 60% of all biopsied embryos are biopsied for preimplantation genetic testing for aneuploidy (PGT-A), a screening method whose benefit to improving chances of live births is still hugely contested (De Rycke et al. 2017; Mastenbroek et al. 2011; Munné et al. 2019; Vermeesch, Voet, and Devriendt 2016).

While human trial is the only method for truly assessing clinical competence, it should be carried out as a randomized controlled trial, not as a charged extra service to patients. But to date, clinical lessons have largely been learned on a trial-and-error basis. Proof or disproof of appropriate practice using embryos created and altered through medically aided means is difficult when the sources for potential errors are abundant; despite this, very few of these trials have led to catastrophic errors, with 8 million babies having been born using this technology and close to 16 million parents benefitting as well.

Many of the events that followed the birth of the first IVF baby have been echoed in the aftermath of the birth of the first genome-edited babies in Hong Kong in November 2018. When He Jiankui announced that he had not only edited human embryos but had transferred them, leading to the birth of twin girls, the news was met with a global outcry, moral abhorrence, and calls for a moratorium (Daley, Lovell-Badge, and Steffann 2019). While there is no doubt that the scenario in this instance was premature and unethical, the call for a moratorium could also be considered unethical. Interestingly, the 1979 report by the Ethics Advisory Board of HEW also included calls for a moratorium on human in vitro fertilization research by the American Medical Association, similar to the present day calls (Lander et al. 2019; Lanphier et al. 2015; Walters 1979). Esteemed scientists and tabloids alike are quoting *Brave New World* and hypothesizing about dystopian uses for new technology, making unevolved ethical arguments cloned from those used for early recombinant DNA technologies, embryonic stem cell research, and the use of cloning itself.

Can We Justify Germline Genome Editing?

Intervention that alters the course of familial heritability to prevent disease is not a new clinical practice. The intuitive pathway for clinical germline genome

editing therefore would not be that of an entirely new field, but one that mirrors the use of preimplantation genetic diagnosis (PGD), first carried out over 30 years ago (Handyside et al. 1989). Preimplantation genetic testing (PGT) is the genetic and karyotypic profiling of oocytes or embryos prior to in vitro fertilization and implantation, where only unaffected embryos are transferred for pregnancy. Germline genome editing maintains most of the clinical characteristics of PGT for monogenic disorders—endocrine stimulation protocols followed by follicular rupture to retrieve oocytes, in vitro fertilization, ex vivo culture methods, and cell biopsy for testing—but, rather than selecting against a mutation and discarding an affected embryo, CRISPR-mediated editing could be used to correct a mutation without the need to discard affected embryos.

This proposed application of genome editing invokes a number of rebuttals that aim to emphasize the adequacy of PGD as a preventative method for the transmission of hereditary disorders. The assumption that genome editing would replace PGD as a screening tool serves to divide the field (Cyranoski 2015; Greenfield 2018; Savulescu et al. 2015). Instead of replacing PGD, genome editing should be used as a companion tool, in the rare but significant instance that no embryo from a given cycle (or collection of cycles) is free from harmful mutation.

A sad reality in PGD cycles is the often unacceptably low number of embryos available for transfer following oocyte retrieval, fertilization, biopsy, and testing (Steffann et al. 2018). All of these factors are heavily dependent on the age of the parents, the quality of the embryos, and the penetrance of disease transmission (whether a single or double mutation copy is causative). While it is argued that successive "freeze-all" stimulation cycles will yield sufficient oocyte quantities for fertilization and testing, it is not just the risk to the embryo that must be accounted for, but the risk to the mother-to-be also. Risk of death from ovarian hyperstimulation syndrome (OHSS) is a rare but not insignificant risk of stimulation protocols for oocyte retrieval, and while frequencies are declining, severe hyperstimulation affected 10% of patients (Mourad, Brown, and Farquhar 2017; Nelson 2017; Petrenko et al. 2019).

Transgenerational Inheritance

There are those who argue that altering the germline has untold consequences for future generations, but in certain family lines, preserving the germline has incontestable consequences for the future generation and represents a growing health burden in terms of mortality, disability, and health-care costs. In these families, nature is a trial that will lead to certain error.

Well over a billion people live in countries with high rates of customary consanguinity. Many Middle Eastern, North African, and West Asian communities have consanguinity rates ranging from 40–55% between first-cousin marriages (Bener and Mohammad 2017). Even those who do not practice consanguinity suffer from shared inherited bottleneck transmission within communities. They

are not just carriers of one allele, but of multiple dominant and recessive alleles as well.

Since the early 1970s, there have been successful mandatory (and elective) screening programs for couples prior to marriage to reduce the genetic burden of common heritable disorders (Goonasekera, Paththinige, and Dissanayake 2018). These have been most successful for autosomal recessive conditions such as the hemoglobinopathies, which are the most common single gene disorders in the world, and for which screening programs exist on all continents of the globe (Cousens et al. 2010). A significant decrease in the incidence of β-thalassemia, for example has been seen after the introduction of screening programs. These screening programs initially involved prenatal diagnosis and the termination of affected fetuses, but this practice has largely been supplanted (though not in all instances) by preimplantation genetic diagnosis, where affected embryos are discarded.

Certainly the course of evolution has been altered, but so too has the health cost burden, with a 95–100% decrease in carrier frequencies in certain regions, some of which have not had any affected births for many years (Mitchell et al. 1996). This has led to increased access to blood supplies for affected individuals, who previously suffered from lack of access due to high demand (Bate 1975).

High use of PGD and carrier screening may prevent transmission of one disorder, but when many conditions are being screened for at once, including de novo family-specific mutations, the probabilities of generating or selecting an unaffected embryo are hugely diminished. These are the families and individuals for whom germline genome editing would be suitable.

WHEN WE MATE, WE MUTATE

Genetic bottlenecks and consanguinity aside, consideration must also be given to the natural mutation rate per generation (Keightley 2012). The occurrence of mutations is an essential phenomenon for diversity, with every new generation inheriting new mutations. It has been shown that age is the biggest catalyst for this mutation rate: the older we get the more mutations we accrue and pass on. The rate of de novo mutations per generation is between $1.20 - 2.3 \times 10^{-8}$ per nucleotide (Kong et al. 2012; Sun et al. 2012). Most strikingly, this single nucleotide polymorphism rate is predominantly attributed to the paternal line. The older a father is at a child's conception, the higher the number of mutations passed on, with an increase of two mutations per year with an increase in the age of the father. This rate doubles every 16.5 years in men, and while studies have found no association with maternal age and this increased prevalence for mutagenic alleles, it is well established that advances in maternal age lead to an increase in chromosomal abnormalities or aneuploidy (Hassold and Chiu 1985).

Aneuploidy is a common phenomenon in human embryos. It is responsible for over 50% of all miscarriages and missed abortions, as well as being the leading

cause of congenital birth defects (Hassold and Hunt 2001). Meiotic errors during oocyte generation are largely to blame for embryo aneuploidy, and these become increasingly common with maternal age, with the rate rising predictably after age 26 (Chiang, Schultz, and Lampson 2012; Franasiak et al. 2014; Gruhn et al. 2019; Hassold, Hall, and Hunt 2007; Treff 2012). Only 2% of clinically recognized pregnancies of women in their mid-20s are trisomic, compared to 35% in women over 40 (Hassold and Hunt 2001).

We do not police human reproduction with age limits in light of these figures. Rather, we have attempted to aid patients in overcoming these evolutionary barriers through screening of IVF embryos prior to transfer. The screening of embryos for aneuploidy is now routinely offered as part of clinical IVF treatment, and not just in the case of advanced maternal age. Despite improvements in IVF culture conditions and laboratory techniques, only about 50% of human embryos reach the blastocyst stage of development in vitro (McCollin et al. 2019). While many factors influence embryo development, stagnant success rates may not be due to lack of progress scientifically but potentially that we have reached our limits in bending biology to allow for reproduction.

The More We Look, the More We Find

The increased uptake of screening and advanced embryo analysis, coupled with more sophisticated methods for testing, have led to a major clinical conundrum: the more we look, the more we find. Previously, embryos were only assessed on their morphology and developmental potential (ability to survive to blastocyst stage). Only the best-looking embryos were transferred for pregnancy, with failure easily imputed to uterine insufficiency or maternal age. Early methods of chromosome analysis, such as fluorescent in situ hybridization (FISH), only looked for specific chromosomes (21, 13, 18, X, and Y) in surplus or deficiency (Griffin et al. 1991). Successive technologies coupled with alternative biopsy techniques have revealed more and more about the dynamics of DNA and chromosomes, making clinical decisions harder. Arrays, karyomapping, and whole genome sequencing now lend far more insight into the embryo in its entirety, not just portions of it (Natesan et al. 2014; Thornhill et al. 2015; Wells et al. 2014).

The evolution of testing capabilities has revealed that not all cells of an embryo are born equal. Many embryos are mosaic in their assembly, having a variety of chromosomal complements in each cell. It is now widely debated whether the transfer of a mosaic aneuploid embryo should be accommodated clinically when no other embryos are without abnormal cells (Grati et al. 2018). Until recently, it was beyond the bounds of consideration to transfer an embryo carrying any aneuploid cells for a pregnancy, even for syndromes consistent with life, such as Downs syndrome or Turners syndrome (Greco, Minasi, and Fiorentino 2015). We are more imperfect than we assumed, and we currently have no idea of what

an acceptable level of mosaicism is for survival, but we are constantly learning valuable insights into human biology. Although these insights should, more preferably, be coming from research laboratories before being used in clinics, legal restrictions preventing the creation of human embryos for research mean that basic research is largely restricted to the use of surplus embryos donated from IVF cycles. As a result, we are a long way off from true control, comparison, or comprehension of human embryogenesis.

Very little work has ever been done to look at genomic alterations, rearrangements, or unwanted effects caused by the routine procedures carried out clinically on embryos. We know that different cells respond differently to the same developmental signals (Gentsch et al. 2019). Culture conditions, biopsy methods (including the use of chemicals and lasers), assisted hatching, and in vitro maturation undoubtedly generate genomic "off-target" effects that are hard to analyze when each embryo is different, and they were previously impossible to analyze when the tools for looking were not available at the time of their clinical adoption (Swain 2019).

CRISPR-mediated editing is rightly being scrutinized for its ability to alter DNA in unintended ways, but natural cellular events such as innate recombination and increased age can result in similar alterations. We now have the tools to investigate the genome with interminable ability and increasing accuracy, but consideration must be given to the inherent dynamic nature of DNA. While the uses and applications of CRISPR and genome editing are endless, they are limited by nature's laws and nature's flaws. Work on human embryos has shown that not only are embryos distinct and elusive, but they are also robust and resilient to human interventions.

No technology is perfect—not IVF nor genome editing—but when combining these and applying them to the most flawed of systems, human biology, we may ask ourselves "When will good ever be good enough?"

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