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COMMENTARY

Reproductive medicine involving genome editing: clinical uncertainties and embryological needs

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Abstract Genome editing based on site-directed nucleases facilitated efficient and versatile genetic modifications in human cells. However, recent reports, demonstrating CRISPR/Cas9-mediated genome editing in human embryos have raised profound concerns worldwide. This commentary explores the clinical justification and feasibility of reproductive medicine using germline genome editing. Despite the perceived utility of reproductive medicine for treating intractable infertility, it is difficult to justify germline genome editing from the perspective of the prospective child. As suggested by the UK legalization regarding mitochondrial donation, the prevention of genetic disease in offspring by genome editing might be acceptable in limited cases of serious or life-threatening conditions, where no alternative medicine is available. Nonetheless, the mosaicism underlying human embryos as well as the off-target effect by artificial nucleases will likely hamper preimplantation genetic diagnosis prior to embryo transfer. Such considerations suggest that this type of reproductive medicine should not be developed toward a clinical application. However, the clinical uncertainties underscore the need for embryology that can address fundamental questions regarding germline aneuploidy and mosaicism using genome editing.

KEYWORDS: aneuploidy, disease prevention, embryology, genome editing, germline, mosaicism

Introduction

The genetic modification of germ cells or zygotes (germline) can impact the entire body of the progeny as well as subsequent generations via modified germ cells. For this reason, germline genetic modification has been considered to be effective against some genetic diseases. Transferring donor oocyte-derived cytoplasm (containing mitochondrial DNA (mtDNA)) to putatively non-viable oocytes or zygotes was practised in cases of unexplained infertility from the late 1990s to the early 2000s. However, such cytoplasmic transfers resulted in pregnancies affected with Turner syndrome (Barritt et al., 2001b), fetal deaths (Zhang, 2003) and the onset of pervasive developmental sisorder in progeny (Barritt et al., 2001b). Conversely, the UK has recently become the first country to allow the clinical use of karyoplast transfer to an enucleated donor oocyte or zygote (so-called mitochondrial donation) in order to prevent the inheritance of pathogenic mtDNA mutations in offspring (HFEA, 2015).

Genome editing tools, such as zinc-finger nucleases (ZFN), transcription activator-like effector nucleases (TALEN) and the clustered regularly interspaced short palindromic repeat (CRISPR)/Cas9, have facilitated the insertion of an exogenous gene, correcting a gene mutation (or copying of a variant) and disrupting an endogenous gene in human cells. The artificial, site-directed nucleases can unintentionally break DNA double strands at non-target sites (Ishii, 2015b; Kim and

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Kim, 2014), although a recent clinical trial concluded that the infusion of T cells modified by ZFN is safe in HIV-positive patients, despite no investigation of off-target mutations in the infused cells (Tebas et al., 2014). With regard to germline genome editing, two groups recently reported that the microinjection of CRISPR/Cas9 into tripronuclear zygotes can produce human embryos with an intentional genetic modification, but also indicated three technical problems: low efficiency of on-target gene modification, off-target mutations and the mosaicism of genetic modification in the embryos (Kang et al., 2016; Liang et al., 2015). More recently, a non-human primate (NHP) study demonstrated that the microinjection of optimized ZFN/TALEN into zygotes can avoid the mosaicism of genetic modification in resultant monkeys, causing them to display immune-deficiency similar to human patients (Sato et al., 2016). These reports suggest that reproductive medicine involving genome editing is theoretically feasible although there are still concerns regarding the safety and efficacy related to its clinical use. However, the two human embryo editing studies raised serious concerns over its medical use and non-medical (social) use worldwide, prompting several global discussions such as the International Summit on Human Gene Editing (NASEM, 2015).

The present commentary discusses the two objectives of reproductive medicine involving germline genome editing: infertility treatment and disease prevention. Then, the clinical feasibility of such reproductive medicine is examined in terms of risk assessment. In addition, the wider implications of the findings are discussed in scientific contexts.

Infertility treatment

According to the latest report on the treatments involving assisted reproductive technology by the European Society of Human Reproduction and Embryology, pregnancy rates in 2011, while the overall number of assisted reproductive technology cycles has continued to increase, decreased slightly to those reported in 2010. For all IVF cycles, the clinical pregnancy rates per aspiration and per transfer were stable with 29.1 and 33.2%, respectively. Moreover, for intracytoplasmic sperm injection (ICSI), the corresponding rates were stable with 27.9 and 31.8%, respectively (Calhaz-Jorge et al., 2016). To enhance the assisted reproductive technology success rate, personalization is one of future directions (Simon, 2013). Since half of idiopathic infertility cases are considered to have a genetic basis (Singh and Schimenti, 2015), there is a tremendous need for personalized reproductive medicine, which may be achieved by correcting a mutation responsible for infertility through genome editing (Ishii, 2015a). For instance, human oocytes with a missense mutation in the *TUBB8* undergo developmental arrest after fertilization (Feng et al., 2016b). Currently, two relevant reports are available. In 2016, the first case report identified seven *TUBB8* mutations that were responsible for oocyte meiosis I arrest in seven of the 24 families, using exome sequencing (Feng et al., 2016a). The second report discovered nine new *TUBB8* mutations in 10 patients from nine families, displaying phenotypic variability (Feng et al., 2016b). Among them, oocytes having any of three missense mutations (I210V, T238M and N348S) are of particular note. Such oocytes could extrude the first polar body and could be fertilized, despite subsequent developmental arrest. Genome editing-mediated *TUBB8* correction in premature oocytes, such as GV stage oocytes, could recover their developmental potential, although the remaining transcripts from mutated *TUBB8* could disturb the formation of microtubule via de-novo synthesis of the functional protein. It should also be noted that genome editing-mediated gene correction has not been demonstrated in mammalian oocytes.

Moreover, *TEX11* mutations cause meiotic arrest and azoospermia in infertile males (Yatsenko et al., 2015). If spermatogonial stem cells (SSC) can be retrieved from the patient's testis, viable spermatozoa could be generated from genetically corrected SSC *in vitro* in the near future (Ishii, 2015a; Ishii and Pera, 2016). Of note, a recent report demonstrated that rat offspring were born using spermatozoa regenerated following the transplantation of CRISPR/Cas9-modified SSC (Chapman et al., 2015), although SSC transplantation is still experimental in humans.

However, its use is currently unjustifiable. First, genetic modification in humans is still in the early stages. Although at least 2356 clinical trials of somatic gene therapy have been conducted worldwide, fewer than 10 products have gone on to be approved (JGM, 2016). Second, very few cases of the clinical use of human germline genetic modification have been reported. Only ooplasmic transfer and pronuclear transfer were practised for treating intractable infertility by transferring donor oocyte-cytoplasm to a patient's oocyte, or by transferring karyoplast including pronuclei to an enucleated donor zygote (Barritt et al., 2001a; Ishii, 2015b; Zhang, 2003). Third, such cytoplasmic transfers are suspected to have imposed congenital anomalies upon resultant children in some cases. Ooplasmic transfer resulted in pregnancies affected with Turner syndrome, and the onset of Pervasive Developmental Disorder after birth, pronuclear transfer led to fetal deaths (Barritt et al., 2001b; Zhang, 2003). With regard to ooplasmic transfer, the Food and Drug Administration discussions in 2002 suggested that the cytoplasmic transfer caused inappropriate mitochondrial distribution in oocytes that led to such congenital anomalies. Given that germline genome editing can potentially affect progeny with the substantial risk of off-target effects, its development for infertility treatment, which will likely promote its widespread use, should be avoided from the perspective of the prospective child's welfare.

Disease prevention

Assisted reproductive techniques are practised with prior consent by parents. However, widely accepted assisted reproductive techniques such as IVF involve no intentional genetic intervention. Therefore, under what conditions does parental consent justify the germline genetic intervention from the viewpoint of a child's welfare?

Consider the UK regulatory framework on mitochondrial transfer (HFEA, 2015). Such intervention is deemed legal provided the germline modification focuses mtDNA (not nuclear DNA) and intends to prevent the maternal transmission of 'serious' mitochondrial disease to offspring. This is employed when a mother carries the risk of transmitting the disease to the child. Its practice is limited to serious conditions among various forms of mitochondrial disease. Similarly, disease prevention via germline genome editing might be accepted in some countries. Notably, in the Interna-

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