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Human germline genome editing: update on basic and preclinical research, ethical considerations and possible candidate to substitute PGT in the clinics

La edición del genoma humano en la línea germinal: actualización de investigación básica y preclínica, consideraciones éticas y posible candidato para sustituir PGT en las clínicas

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SUMMARY

Nowadays thousands of families are affected by a genetic disease. Germline genome editing has the potential to correct mutations and prevent the transmission of such diseases. Different editing tools are currently available to edit the genome, and they have evolved in the last few years. Such tools include the CRISPR/Cas9 system and, more recently, the BE3 system. Nonetheless, these tools have technical

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and ethical problems, which are discussed here, but it seems that the scientific community is moving towards a future application of gene editing in the human germline. In this non-systematic review we provide an update on the last experiments performed in embryos, and on the gene editing tools employed, and review the main technical and ethical problems that arise from this topic. We conclude this work considering different points of view regarding the applicability of gene editing in the clinics and its relationship with the current PGT techniques. The articles reviewed reveal that human germline gene editing still comes associated with technical and ethical problems that need to be solved before it can be applied in the clinics. However, progress made in the pre-clinic research is promising, especially with the BE3 system. Furthermore, it seems that the scientific community is in favour of using gene editing in the human germline in the future once all the problems are solved and the technologies are perfectioned. The progress presented here in the preclinic research, regarding the BE3 system, and our call for more basic research, will allow in the future to overcome the technicals and ethical problems presented here, in order to be able to apply gene editing techniques in assisted reproduction clinics. The benefits would have a great impact in the life of many couples. Nonetheless, there is still a long way to go, and more basic research, as well as international debates, are urgently needed in order to discuss how to regulate, and for what applications, gene editing in the human germline once the tools are ready to be used.

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Key words: *CRISPR, Cas9, BE3, genome editing, embryo, assisted reproduction, PGT.*

RESUMEN

Actualmente miles de familias están afectadas por una enfermedad genética. La edición genética de la línea germinal tiene el potencial de corregir mutaciones y prevenir la transmisión de dichas enfermedades. Diferentes tecnologías de edición genética están actualmente disponibles para editar el genoma, las cuales han ido evolucionando en los últimos años. Estas tecnologías incluyen el sistema CRISPR/Cas9 y, más recientemente, el sistema BE3. Sin embargo, estas tecnologías vienen asociadas con problemas técnicos y éticos, los cuales son discutidos aquí, pero parece que la comunidad científica se mueve hacia una futura aplicación de la edición genética en la línea germinal humana. En esta revisión no sistemática, se muestra una actualización de los últimos experimentos realizados con embriones humanos, y de las tecnologías empleadas, y se revisan los principales problemas técnicos y éticos derivados de la edición genética en embriones humanos. Se concluye este trabajo considerando diferentes puntos de vista en cuanto a la aplicabilidad de la edición genética en las clínicas de fertilidad, y su relación con la actual técnica de PGT. Los artículos revisados revelan que la edición genética de embriones humanos aún va asociada a problemas técnicos y éticos que necesitan ser resueltos antes de que se pueda aplicar en las clínicas. Sin embargo, el progreso realizado a nivel de investigación preclínica es prometedor, especialmente usando el sistema BE3. Además, parece que la comunidad científica está a favor de usar la edición genética en la línea germinal humana en el futuro, una vez se hayan resuelto todos los problemas existentes. El progreso científico presentado aquí, junto con una llamada de atención para la realización de más investigación básica, permitirán en el futuro superar y resolver los problemas técnicos y éticos expuestos en esta revisión, con el fin de que las tecnologías de edición genética se puedan aplicar en clínicas de fertilidad. Los beneficios podrían tener un gran impacto en la vida de muchas parejas. Sin embargo, aún queda un largo camino por recorrer, y más investigación básica, así como debates internacionales, son tremendamente necesarios para discutir y decidir cómo regular, y para qué aplicaciones permitir, la edición genética en la línea germinal humana una vez que las tecnologías sean completamente seguras y eficaces.

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Palabras clave: *CRISPR, Cas9, BE3, edición genética, embrión, reproducción asistida, PGT.*

INTRODUCTION

Nowadays there are around 10,000 monogenic diseases (caused by a mutation in a single gene), which affect thousand of families worlwide. However, approved treatment is available only for less than 6% of these diseases (1). The correction of the mutations that cause these diseases in human embryos could decrease the prevalence of such diseases in fetuses and new borns and prevent the transmission to the offspring, and at the same time it could improve the success rates of the assisted reproduction techniques. The technologies to edit the DNA have a great potential to modify human and non human genomes and they have allowed for great scientific discoveries in the last decades. Such technologies include zinc-finger nucleases (ZFNs) (2), transcription activator-like effector nucleases (TALENs) (3), the clustered regularly interspaced short palindromic repeat (CRISPR)/CRISPR-associated (Cas) nuclease system (CRISPR/Cas) and different base editor systems, specifically the Base Editor 3 system (BE3) (4). The CRISPR/Cas9 system has been the gene editing tool mainly used in the last years, due to its high efficiency, the ease of design, the simplicity and the possibility to direct it to virtually anywhere in the genome (5). Nonetheless, the BE3 system has been used recently to edit human embryos as it has high efficiency as well, and seems to produce less off-target mutations and mosaicism (6).

Both systems are used to modify the genome, but there are differences between them and they have a different mode of action. The CRISPR/Cas9 system consists of a Cas9 protein and a guide RNA (gRNA) of around 100 nucleotides (nt) (7). The first 20 nt in the 5' end of the gRNA give specificity to the system, as they are complementary to the target sequency and bind to the DNA. The rest of the nucleotides of the gRNA form a scaffold, which the Cas9 uses to attach to the DNA and create a double-strand break (DSB) (8). This complex can be directed to virtually any secuence in the genome, as long as it is adjacent to a protospacer adjacent motif (PAM), consisting of the trinucleotide NGG (5, 8, 9). Following Cas9 target site cleavage, the DSB generated can be repaired either by non-homologous end joining (NHEJ), in the absence of a repair template, or by homology-directed repair (HDR), in the presence of a repair template (7, 10, 11). The NHEJ repair pathway results in indels, such as insertions or deletions that can alter the gene function and introduce unwanted mutations in the target site (11), whereas the HDR repair pathway allows for precise modifications of the target site (7). To edit human embryos, precise gene editing through HDR is required, nonetheless the HDR repair pathway occurs at a much lower frequency than the NHEJ repair pathway, which in turn results in a high rate of off-target mutations (12).

Base editors, on the contrary, do not modify the genome by cleaving the DNA, so they could be an efficient and highly specific alternative. The base editor is an RNA-complex consisting of a cytidine deaminase enzyme (rAPOBEC1), the Cas9 protein, and a uracil DNA glycosylase inhibitor (UGI) (4). This complex can deaminate cytidine (C) to uridine (U) without creating DSBs in the target sequence, and finally result in C-to-T (or G-to-A) conversion in the target sequence, so this system could be used to correct specific mutations that cause many genetic diseases, such as b-thalasemia, caused by mutations in the HBB gene (13). Kim et al. engineered different types of base editors, using Cas9 proteins containing different mutations, and the base editor 3 described in his work (BE3) (rAPOBEC1-nCas9-UGI) showed the best results (14). This is the system that has been used to edit human embryos in the papers reviewed in this work.

The CRISPR/Cas9 system has been widely used in human cells and in animals as a gene therapy, for gene function investigation and other applications (5, 8, 15-19). The same for the base editor systems, wich have been used for base-editing at single base resolution with high efficiencies in plants, yeast, human cells, mouse zygotes and human tripronuclear zygotes (4, 20-28). In viable and non viable human embryos these technologies are allowed to be used only in a few countries, but only for basic and preclinical research purposes. Nonetheless, the generation of fetuses from genome-engineered embryos is forbiden worlwide.

In this work we have reviewed all the available data on experimental works of gene editing in human embryos up to date, using both the CRISPR/Cas9 system and the BE3 system, and we have also reviewed and analysed the most important ethical aspects of human germline gene editing. To conclude, as the main application of gene editing of human embryos in the future would be to prevent the transmission of genetic diseases, we also reviewed articles about the possibility that gene editing could someday replace the PGT, and have exposed the main arguments for and against the case. In the light of the recent advances in this field, and with the recognision of the World Health Organisation (WHO), attributted by publishing a guide with recommendations for gene editing in humans (29), it seems that gene editing in the human germline will be performed sooner than expected. Thus, this review will be of great interest to help in its regulation and to identify the main obstacles that still need to be resolved before the technique can be applied in fertility clinics.

METHODS

PubMed was used to search for the literature that was then reviewed for this non-systematic review. Two different se-

arch equations were employed: "genome editing" AND "embryo" AND "human"; and "human germline" AND "gene editing". The articles selected were written in English language and focused on gene editing of human embryos, the ethical considerations it has associated and also articles that compared gene editing and PGT. All articles considered to be out of topic were discarded, and eventually, a total of 41 papers were included in this review.

GENOME EDITING OF HUMAN EMBRYOS

Compared to other cell types or animal models, there are only few papers about genome editing of human embryos at the experimental level. This can be attributted to different reasons, but mainly it is due to the lack of embryos available to be edited. Many countries do not allow the creation of embryos solely for research, and many others have strong regulations for the use of embryos in research. These countries refer to the moral concerns of creating and using embryos to be destroyed in the end (30). Because of this, most of the research presented here has been perfomed in not viable 3 pronucleus (PN) embryos, which could be seen as a burden to move forward, as results may differ if the gene editing would be performed in viable embryos instead. The papers reviewed can be divided into preclinical research or basic research.

USE OF GENOME EDITING TOOLS FOR PRECLINICAL RESEARCH

The first time gene editing was applied in human embryos was in 2015, when the CRISPR/Cas9 system was used to demonstrate that the B-globine gene (HBB), could be edited in 3 PN embryos, with efficiency rates higher than 50%. Nonetheless, the DNA was repaired mainly by the NHEJ pathway, hence introducing indels in the target sequence. Furthermore, the authors reported the presence of off-target mutations in other loci of the genome, as well as mosaic embryos (31). Another two studies also employed the CRISPR/Cas9 system in both 3 PN embryos (32, 33) and 2 PN embryos (33), in order to edit the genes CCR5 (32), RAG1, NEK1, G6PD and HBB (33), with efficiency rates in all cases between 50% and 80%. As demonstrated in the Liang et al. work (31), these two studies also detected mosaic embryos, both in 3 PN embryos and in 2 PN embryos, and again, the DNA was preferentially repaired through the NHEJ pathway. It is worth mentioning that these works didn't find off-target mutations in the sites analysed, although the authors akwnowledge the need of a more exhaustive analysis to completely rule out the presence of off-target mutations in other loci of the genome (32, 33). The last of the works that used the CRISPR/Cas9 system edited both 2 PN embryos and gametes, to correct a mutation in the MYBPC3 gene, which causes a hypertrophic cardiomiopathy (34). Efficiency rates were above 70% in both cell types. Interestingly, microinjection of the CRISPR/Cas9 system in 2 PN embryos resulted in the production of mosaic embryos, whereas the co-injection of the CRISPR/Cas9 system and the sperm in MII oocytes didn't produce mosaic embryos. Regarding off-target mutations, no differences were found between embryos and gametes, as no off-target mutations were detected in any of them. Furthermore, in both cell types the DNA was preferentially repaired using the wild-type allele as a DNA template (34).

The last human germline gene editing works used the BE3 system to edit the genome. This gene editing system was used in 2 PN embryos (1, 35) and in 3 PN embryos (23, 27) to correct different genes (Table 1). In all cases the efficiecy rates were above 40%, with most of the genes showing an efficiency rate between 80% and 100%. None of these works reported the presence of mosaic embryos, as well as only very few off-target mutations were detected. Only those works that edited 3 PN embryos reported low rates of off-target mutagenesis, and only in specific genes (23, 27).

One of the main limitations of the genome editing tools that need to be solved are the off-target mutations, both in the target site and in other loci. Based on the reviewed papers, we compared the results from the works that used the CRISPR/Cas9 system, regarding the DNA repair pathway, which as mentioned before, can be responsible for the introduction of non-desired mutations in the target site. Figure 1 shows the different genes edited in the diverse works, and the cell type used. As can be seen in the figure, when 3 PN embryos are used the DNA is preferentially repaired through NHEJ, which causes non desired indels in the target sequence. Only when 2 PN embryos were used results improved and the frequency of the HDR repair pathway increased, being even predominant in the Ma et al. work. We would like to point out here that in the Tang et al. work, when the HBB gene is edited both in 3 PN and 2 PN embryos, the DNA is mainly repaired through NHEJ in 3 PN embryos, whereas in 2 PN embryos, the DNA is repaired both by NHEJ and HDR at the same frequency. This result suggest that the cell type used for gene editing experiments can affect the outcomes, so priority should be given to 2 PN embryos or gametes for these type of works, as this would be the cell type edited if gene editing tools are finally applied in the clinics.

The other main problem of genome editing in the human germline is the mosaicism. The BE3 system appears to improve the results (Figure 2), and in all cases the survival

TABLA 1

Summary of the results presented in the papers reviewed that use genome editing tools to edit human embryos. For each author, there is information about the technology employed, the cell type edited, the gene or genes edited, survival rates, efficiency rates and if mosaicism or off-target mutations were identified.

Author	Technology	Cell type	Gene	% Survival	% Efficiency	Mosaicism?	Off-target mutations?
Liang et al. (31)	CRISPR/Cas9	3PN embryo	HBB	82.6	51.9	Yes	Yes
Kang et al. (32)	CRISPR/Cas9	3PN embryo	CCR5	64	> 50	Yes	No (in the studied sites)
Tang et al. (33)	CRISPR/Cas9	3PN embryo	RAG1 NEK 1 G6PD	Not specified	60 70 75-80	Yes	Not determined
		2PN Embryo	HBB	Not specified	50	Yes	No
Ma et al. (34)	CRISPR/Cas9	2PN embryo Gamete	MYBPC3 MYBPC3	97,1 97,1	72,2 100	Yes No	No No
Liang et al. (35)	BE3	2PN embryo	HBB	86.6	45.4	No	No
Li et al. (23)BE3	BE3	3PN embryo	HEK293 RNF2	86-100	87,5 87.5	No No	Yes (very low%) No
Zhou et al. (27)	BE3 y SaKKH-BE3	3PN embryo	HBB FANCF DNMT3B	Not specified	42 100 67	No No No	No Yes (very low%) No
Zeng et al. (1)	BE3	2PN embryo	FBN1	Not specified	89-100	No	No

rates were elevated, suggesting that these gene editing tools are not toxic for the cells, and when optimized, they could be used to edit the genome with high efficiency rates. In general, all the reviewed works succeeded in editing the target site with high efficiencies. However, more basic research in the appropriate cell types is needed in order to increase our knowledge abour genome editing tools and DNA repair pathways.

USE OF GENOME EDITING TOOLS FOR BASIC RESEARCH

Basic research is crucial to gain new insights about genome editing in the guman germline and finally be able to optimise the genome editing tools, in views of a future aplication in ferility clinics. The CRISPR/Cas9 system was used to investigate the function of a gene involved in embryo development (36), but mainly to study the DNA repair pathways in human embryos edited (37-39) (Table 2). These works allowed us to understand a bit better how genome editing works and what changes are produced in the DNA after the cleavage of the Cas9 protein, which could help in the optimization of the technology. Nonetheless, all the reviewed papers employed the CRISPR/Cas9 system for basic research, but none of them used the BE3 system. More experimental works for basic research are needed to fully understand how genome editing is performed and how the DNA is repaired, and to increase our knowledge about the gene editing tools, and specially more research should be performed using the BE3 system, as it holds promising results as previously shown.

PROBLEMS ASSOCIATED TO HUMAN GERMLINE GENOME EDITING

Since the development of the recombinant DNA technology more than 40 years ago, many ethical debates about gene editing have been performed. After the publication of Liang et al. work back in 2015, in which they genetically modified 3PN embryos successfully, gene editing in the human germline has been considered an option for the close future. This work prompted the realization of many meetings gathering scientifics, ethics, politicians, society representants and other people in order to debate whether to allow or to ban

FIGURA 1

Comparison of the DNA repair pathways after cleavage by the Cas9 protein. The authors of the different papers are represented on the X axis, and the percentage of embryos that repair its DNA through the different repair pathways is represented on the Y axis. Above the bars it can be found the gene mutated (in black) and the cell type edited (in green).

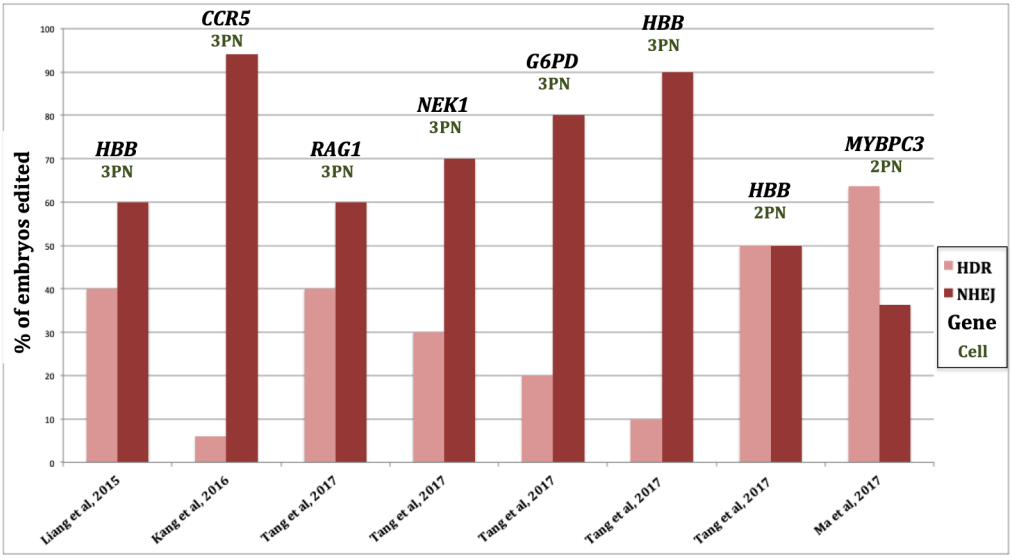


FIGURA 2

Mosaicism rates found with the CRISPR/Cas9 system or the BE3 system in the papers reviewed. Mosaicism rates were calculated according to the number of genes from the papers reviewed in which the authors found mosaicism. The CRISPR/Cas9 system caused mosaicism in most of the genes edited, whereas no mosaicism was found in any gene when embryos were edited with the BE3 system.

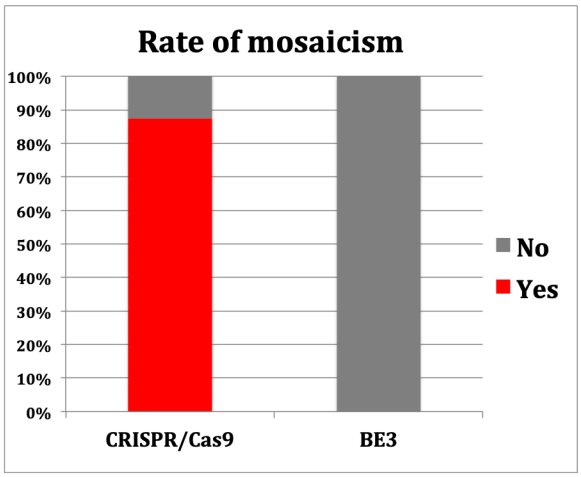


TABLA 2

Summary of the results found in the papers reviewed that employ genome editing technologies for basic research. For each author there is information about the technology employed, the objective of the study and the results found.

Author	Technology	Objective	Results
Fogarty et al. (36)	CRISPR/Cas9	Study of the function of OCT4 in embryo development	OCT4 is required in early stages of embryo development so that embryos can develop properly to bastocyst stage.
Mitalipov et al. (37)	CRISPR/Cas9	Study of the DNA repair pathways	In homocygous embryos for a mutation, the DNA is repaired mainly using the homologue wild type or by NHEJ. In heterozygous embryos, the DNA is repaired using the homologue wild type.
Alanis-Lobato et al. (38)	CRISPR/Cas9	Study of the DNA repair pathways	CRISPR/Cas9 increases the cromosomic instability in human embryos. Cromosomic gain or losses are observed adjacent to the target site of the Cas9 protein.
Zuccaro et al. (39)	CRISPR/Cas9	Study of the DNA repair pathways	In MII eggs inyected with the CRISPR/Cas9 systen, the DNA is repaired before the first mitosis. In 2-cell embryos inyected with the CRISPR/Cas9 system, the DNA is not repaired before the first mitosis, which leads to the loss of a parental cromosome.

the application of gene editing in human embryos in the clinics. Furthermore, their objective was to raise awareness about the main concerns regarding the gene editing tools and the consequences of the human germline genome editing.

ETHICAL AND TECHNICAL PROBLEMS

One of the first groups in reacting to Liang et al. work in 2015 was the Hinxton Group (40), followed later on by other three statements also published in 2015 from different organisations. These were the International Summit on Human Gene Editing, which gathered members of the National Academy of Sciences (NAS), the National Academy of Medicine (NAM) and the Royal Society of London (41); the jointed statement from the American Society for Gene and Cell Therapy (ASGCT) and the Japan Society of Gene Therapy (JSGT) (42); and the statement from the International Society for Stem Cell Research (ISSCR) (43). All of them recognised the great potential of the gene editing tools for basic research and somatic use, to prevent diseases, to create disease models or to correct gene defects, but they also stated that the technology employed was not ready to be used in human embryos aiming to become a new born, as it comes associated with technical problems, mainly mosaicism and off-target mutations, which could harm in an unexpected way the offspring of the edited embryos and the future generations. Hence, all these problems need to be sol-

ved before the technology can be applied in fertility clinics. However, these societies are in favour of continuing the basic and preclinical research to gain insights about the gene editing techonologies and solve all the problems they present, in views of a future application of the technology in the clinics (41-43).

In general, all the statements reviewed here from the different societies agree about the great potential of the gene editing technologies. However, some of them are more permisives than others. For example, members from the European Group on Ethics in Science (EGE) and the American College of Medical Genetics and Genomics (ACMG) consider that the off-target mutations and the mosaicism are problems that come with greater risks than the potential benefits for the embryos. Hence, they do not make a clear statement about basic and preclinical research, but they demand the prohibition of the clinical application of the gene editing tools in human embryos, at least until the ethical and technical problems are all resolved. Furthermore, they emphasize on the need of public debates and the need of a moratorium on genome editing of human embryos (44, 45).

In a more open view of the matter, we find the European Society of Human Reproduction and Embryology (ESHRE) (46) and the American Society of Human Genetics (ASHG) (47), which are in favour of continuing the basic and preclinical research, even with public funds, but they demand a strict regulation of the research and transparency in the publication of the results. They also detail their main ethical concerns, among which we find the risk to which the patients (the embryos) and the offspring are exposed to; the lack of regulation; the need of good quality research to increase the knowledge about gene editing tools; the impact on society, in terms of justice and equity of access to avoid discriminations (47); the preservation of the human dignity and the genetic identity as a common heritage; the use of the technology for non therapeutical uses; respect of the reproductive autonomy; or the discrimination of persons with some sort of disability (46). The ESHRE even adds certain recommendations to its statement (Table 3).

These recommendations were reinforced by a new meeting of the members of the NAS, the NAM, and the Royal Academy of London, which concluded in the Second International Summit on Human Genome Editing that the genome editing technologies had greatly evolved, especially because of the development of the base editor system, but they still had technical problems that needed to be solved. Furthermore, there isn't a proper regulation or a system that allows to do long term follow-ups of the effects caused in the children born from modified embryos. Hence, the basic and preclinical research are needed, but it was still considered irresponsible the application of genome editing in fertility clinics (48).

The last of the organisations statements has been the one published very recently by the WHO, as genome editing is a rapidly emerging science and needs to be properly regulated (29). This statement comes from a consultation that lasted more than two years, and involved hundreds of participants, such as scientists and researchers, ethics, patient groups, faith leaders and others. They recognise the enourmous potential of the genome editing technologies, especially for somatic use, but they also have great concerns when the modification would be performed in the germline, as the changes would affect future generations. The recommendations published will help the different countries to ensure that human genome editing is used safely, effectivelly and ethically, and opens the door for a future application of these technologies in the human germline once it is properly regulated and the problems associated are resolved (29). The main problems and concerns discussed over the years by all these societies are summed up in Table 4.

In addition to these societies, many people, mainly resear-

TABLA 3

Recommendations stated by ESHRE about genome editing in human embryos.

- The use of the technology must be controlled and properly regulated. Clinicians who perform it should hold a license and there should be an organism responsible to register such licenses, do quality controls and write periodical reports.
- Priority must be given to genes which cause more severe diseases.
- The difference between "therapy" and "eugenics" must be clearly defined, to decide for what applications would genome editing be permited.
- A follow-up through the years of the children born from edited embryos must be performed.
- Nowadays it would be irresponsible to apply genome editing in human embryos in the clinics and we must use other available techniques, such as the PGT.

TABLA 4

Summary of the main problems found by different societies regarding the use of genome editing technologies in human embryos. The concerns are classified into technical, ethical and social problems.

Type of problem	Concerns
Technical	• Off-target mutations
•	Mosaicism
•	Epigenetic changes
•	Unexpected harms to the children and future generations
•	Poor knowledge about gene editing technologies
Ethical	• Preservation of the genetic identity
•	Protection of the human dignity
•	Use of embryos as a tool to achieve a mean
•	Lack of informed consent from the embryo and the future generations
•	Harm in future generations due to unexpected effects from late appearance
Social	• Disrespect of the reproductive autonomy
•	Lack of justice and equity in the access to the technology
•	Use for non-therapeutic purposes, such as genetic enhancement
•	Discrimination of people with disabilities
•	Discrepancy on whether to invest public funds or not

chers and ethics, have published their opinions on this topic. The majority of them recognise the problems associated with the genome editing technologies and agree that these must be solved before the technology can be applied in the clinics. However, some of them have a more positive attitude towards the possibility of editing human embryos in the future (49-52), amongst which we find Jennifer Doudna, winner of the Nobel prize in Chemistry in 2020 for her work with the CRISPR/Cas9 system (49). All of these recognise the great benefits the genome editing could have for some families, even outstanding the risks. On the contrary, other personalities think that the risks would be greater than the benefits and the technology could be used for non-therapeutical uses, so they are in favour of banning the technology and should not be employed in the future even if it is completely safe (53-56).

LEGAL CONCERNS AND REGULATION

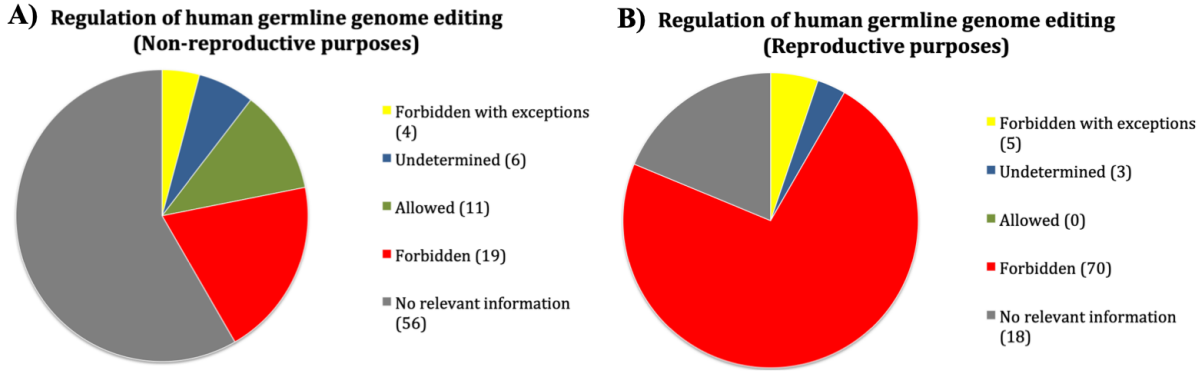
In order to be able to apply genome editing in fertility clinics in the future, this technology needs to be properly regulated. Nowadays this mentioned regulation has not been created, and let alone unified between the different countries in the world, so we can find a huge diversity of policies and guides on whether to allow or ban genome editing in the human germline based on which country we are at.

The first reaction of the society was to ban genome editing of human embryos. Back in 2015, a review of the state of the regulations for genome editing in human embryos in different countries revealed that out of 39 countries reviewed, 29 of them completely banned genome editing in human embryos through different guides and laws. Among these countries we could find Canada, Spain, France, United Kingdom or Australia (57). However, later on it was recommended to allow genome editing in the human germline for basic and preclinical research, and today, we can find a wide range of laws, normative documents and policies in the different countries which state the degree of permisivity regarding the use of genome editing for basic and preclinical research and for clinical purposes (58).

One of the most recently published papers about this topic analysed the different laws, guides and rules available in 96 different countries regarding genome editing in the human germline. For non-reproductive purposes (basic and preclinical research), most of the countries do not have proper laws or guides to regulate the research, whilst other 11 of them completely allow it and other 19 of them prohibit any kind of genome editing of embryos for research (Figure 3 A)). Regarding reproductive purposes (clinical application), genome editing of human embryos is not permited in any country. Most of these countries have firm laws to ban it, whereas only a few of them prohibit it with excepcions or do not have a proper regulation for the banning (Figure 3 B)) (59).

FIGURA 3

Regulations from 96 countries about genome editing of human embryos. **A)** Regulations regarding genome editing of human embryos for non-reproductive purposes (basic and preclinical research). **B)** Regulations regarding genome editing of human embryos for reproductive purposes.



We mentioned before that international debates are needed to decide whether to allow or not genome editing in the human germline. These debates are also required to proper regulate genome editing, and furthermore, each country has to work individually to prepare laws for this technology. This will be an important step and should be ready for when the technology is ready to be used, so that its use is not delayed because of administrative and legal issues, if it is finally approved for use in clinics.

WILL GENE EDITING SUBSTITUTE PGT IN THE FUTURE?

The main application of genome editing in the human germline at a clinical level would be the prevention of the transmission of diseases to the offspring. This would be achieved by modifiyng embryos from affected or carrier couples, in order to correct the mutation and give birth to a healthy child. Nowadays there is a technology that allows us to prevent the transmision of certain diseases, the PGT. Thus, could genome editing end up substituting PGT in the future?

Different specialists in the field have expressed their point of views, and some of them are in favour and others are against genome editing substituting PGT. We have classified in the next sections the arguments exposed, in favour or against the case. As can be seen, the arguments against are weaker than those in favour of gene editing, and allowing genome editing to be used in the clinics rather than PGT could greatly benefit certain couples unable to have healthy offspring solely with PGT (i.e. homoygous carriers for a recesive disease, mitochondrial disease in the mother, parent homozygous for a dominant disease).

ARGUMENTS IN FAVOUR OF HUMAN GERMLINE GENOME EDITING

Genome editing of human embryos could have some advantages over PGT. For instance, it could be considered that genome editing would have less moral problems associated, as it could allow the correction of the embryos with mutations for their transfer into the uterus. On the contrary, PGT selects only those embryos without mutations and discards those with mutations, which for some couples could not be morally acceptable (60-65). Furthermore, by selecting embryos by PGT, one can only prevent the transmision of a disease, but if genome editing were to be used, familiar diseases could be completely eliminated (60). And in the case all embryos were affected by a mutation, which happens at a frequency between 16% and 20% (62), the PGT would not allow the consecution of a healthy child, whereas the mutation could be corrected by genome editing and embryos could be transfered and this would increase the reproductive autonomy of the couple (60). Similarly, in the case of polygenic diseases or certain cancers, the PGT is very limited, whereas genome editing is more versatile and offers the possibility to edit different loci at the same time, which could cure polygenic diseases or reduce the sensibility to certain multifactorial diseases (61, 64). In other cases that do not involve mutations, such as for example in cases where a couple has a child with a disease which requires a

transplant, the PGT can be used to search for compatible embryos. Nonetheless, in the case no compatible embryos are found, the couple would have to start the fertility cycle again. Genome editing, on the contrary, could allow the "creation" of a completelly compatible embryo for the sick sibling, easing the obtention of a compatible child (61). All of these are strong arguments in favour of genome editing, and all of them contribute to achieve great benefits from using this technology in fertility clinics once the technology is ready.

ARGUMENTS AGAINST HUMAN GERMLINE GENOME EDITING

In the oposite side we find those who think that genome editing could never substitute PGT in the clinics. One of the main arguments for this statement is the concern about the technical and ethical problems associated with genome editing technologies. This is a totally valid argument, but for nowadays. It is assumed that if these technologies are some day used in fertility clinics, all the problems they present will have been resolved. Thus, this argument would not be valid in the future, at the time of use of the technologies (60).

Regarding the concerns about the inequity in the access to the technology, this could be solved by simply properly regulating the technology and with a good health plan. The same applies for those who think that it will be difficult to decide for what uses employ genome editing technologies, as if this is not clear, the technology could be used for genetic enhancement of the human race, which would not be morally acceptable, and could alter the integrity of the human genome (62, 66). Again, proper regulations are needed for when this technology is applied in the clinics, however, the genome editing technologies would only correct mutations, not introduce new DNA sequences, and the genetic enhancement would be very difficult to acomplish and it can be seen as science fiction rather than a possible option in the future, because the traits to improve are multifactorial (67). Hence, these are not strong enough arguments to ban genome editing technologies (62).

There are those who think that genome editing technologies do not respect the autonomy of the child to be born from edited embryo, as it is impossible to obtain the informed consent from the embryos that will be edited, and also from the future generations (62). This is considered by some as an attack to the physical integrity of these children (66), and it can be considered unethical to put these children through risks that are greater than the potential benefits (62). Nonetheless, it is important to state here that embryos do not provide their informed consent to be selected by PGT either and this didn't stop this technology to be used. It was estimated that the demand of genome editing would be low in the clinics (60-62, 65) and it would not be worth the investment of money. Nonetheless, in the cases that PGT cannot select a healthy embryo to be transferred, genome editing could modify the embryos and increase the reproductive autonomy of the couples (60-62). Do these people have less rights to have healthi children than the rest of people? Doctors should do everything in their power to help these kind of couples, and especially if they have the means to do so.

It could be considered that the only strong argument against using genome editing in the human germline is the fact that in order to determine which embryos need to be edited, these have to be analysed by PGT, so that the mutations are discovered (61). However, the best moment to edit embryos in order to avoid mosaicism is at the zygote stage. This would mean that the embryos would have to be edited "blindly", before PGT could be carried out (61). An exception would be in the case all embryos are affected by a mutation, becuase both parents present such mutation in homozygosity, but these cases are rare.

In conclusion, genome editing technologies, if allowed in the future, may not completely substitute PGT in the clinics, but both techniques could be used in a complementary way, especially in those cases where PGT would not be very useful. Most of the scientific community seems to be in favour of this situation if genome editing technologies are perfectioned and are 100% safe (61-64, 66).

CONCLUSIONS

The scientific community is moving towards a future application of genome editing in fertility clinics. Experimental works performed up to date show promising results of the genome editing technologies, even though there is still much information we need to unravel with basic science. This and the realization of proper debates will allow societies to reach a decision on whether to allow genome editing of human embryos, and this should also include the creation of proper reguation of the technology. The information exposed in this work suggests that the technology could be ready to be used in the clinics in the next few years, so it will be important to focus on the problems presented here in order to be able to overcome them for when the technology is ready. The scientific community has to work properly and toghether to reduce as much as posible the potential risks of editing human embryos, as the benefits of these technologies appear to be huge, especially for some couples that do not have other alternatives to have healthy offspring.

BIBLIOGRAPHY

1. **Zeng Y, Li J, Li G, Huang S, Yu W, Zhang Y, et al.** Correction of the Marfan Syndrome Pathogenic FBN1 Mutation by Base Editing in Human Cells and Heterozygous Embryos. Mol Ther. 2018;26(11):2631–7.
2. Urnov FD, Rebar EJ, Holmes MC, Zhang HS, Gregory PD. Genome editing with engineered zinc finger nucleases. Nat Rev Genet. 2010;11(9):636–46.
3. **Bogdanove AJ, Voytas DF.** TAL effectors: customizable proteins for DNA targeting. Science. 2011;333(6051):1843–6.
4. **Komor AC, Kim YB, Packer MS, Zuris JA, Liu DR.** Programmable editing of a target base in genomic DNA without double-stranded DNA cleavage. Nature. 2016 Apr 20;533:420–4.
5. **Cong L, Ran FA, Cox D, Lin S, Barretto R, Habib N, et al.** Multiplex genome engineering using CRISPR/Cas systems. Science. 2013;339(6121):819–23.
6. **Komor AC, Zhao KT, Packer MS, Gaudelli NM, Waterbury AL, Koblan LW, et al.** Improved base excision repair inhibition and bacteriophage Mu Gam protein yields C:G-to-T:A base editors with higher efficiency and product purity. Sci Adv. 2017;3(8).
7. **Jinek M, Chylinski K, Fonfara I, Hauer M, Doudna JA, Charpentier E.** A programmable dual-RNA-guided DNA endonuclease in adaptive bacterial immunity. Science. 2012;337(6096):816–21.
8. **Jinek M, East A, Cheng A, Lin S, Ma E, Doudna J.** RNA-programmed genome editing in human cells. Elife. 2013;2013(2).
9. **Cho SW, Kim S, Kim JM, Kim JS.** Targeted genome engineering in human cells with the Cas9 RNA-guided endonuclease. Nat Biotechnol. 2013;31(3):230–2.
10. **Chapman JR, Taylor MRG,** Boulton SJ. Playing the End Game: DNA Double-Strand Break Repair Pathway Choice. Mol Cell. 2012;47:497–510.
11. **Perez EE, Wang J, Miller JC, Jouvenot Y, Kim KA, Liu O, et al.** Establishment of HIV-1 resistance in CD4+ T cells by genome editing using zinc-finger nucleases. Nat Biotechnol. 2008 Jul;26(7):808–16.
12. **Mao Z, Bozzella M, Seluanov A, Gorbunova V.** Comparison of nonhomologous end joining and homologous recombination in human cells. DNA Repair (Amst). 2008;7(10):1765–71.
13. **Cao A,** Galanello R. Beta-thalassemia. Genet Med. 2010;12:61–76.
14. **Kim D, Lim K, Kim ST, Yoon SH, Kim K, Ryu SM, Kim JS.** Genome-wide target specificities of CRISPR RNA-guided programmable deaminases. Nat Biotechnol. 2017;35:475–480.
15. **Chang N, Sun C, Gao L, Zhu D, Xu X, Zhu X, Xiong JW, Xi JJ.** Genome editing with RNA-guided Cas9 nuclease in Zebrafish embryos. Cell Res. 2013;23:465–472.
16. **Cho SW, Kim S, Kim JM, Kim JS.** Targeted genome engineering in human cells with the Cas9 RNA-guided endonuclease. Nat Biotechnol. 2013;31:230–232.
17. **Long C, McAnally JR, Shelton JM, Mireault AA, Bassel-Duby R, Olson EN.** Prevention of muscular dystrophy in mice by CRISPR/Cas9-mediated editing of germline DNA. Science. 2014;345:1184–1188.
18. **Ma Y, Zhang X, Shen B, Lu Y, Chen W, Ma J, Bai L, Huang X, Zhang L.** Generating rats with conditional alleles using CRISPR/Cas9. Cell Res. 2014;24:122–125.
19. **Wu Y, Liang D, Wang Y, Bai M, Tang W, Bao S, Yan Z, Li D, Li J.** Correction of a genetic disease in mouse via use of CRISPR-Cas9. Cell Stem Cell. 2013;13:659–662.
20. **Chen Y, Wang Z, Ni H, Xu Y, Chen Q, Jiang L**. CRISPR/Cas9- mediated base-editing system efficiently generates gain-of-function mutations in Arabidopsis. Sci China Life Sci. 2017.
21. **Kim K, Ryu SM, Kim ST, Baek G, Kim D, Lim K, Chung E, Kim S, Kim JS.** Highly efficient RNA-guided base editing in mouse embryos. Nat Biotechnol. 2017;35(5):435–437
22. **Li J, Sun Y, Du J, Zhao Y, Xia L.** Generation of targeted point mutations in rice by a modified CRISPR/Cas9 system. Mol Plant. 2016;10(3):526–529.
23. **Li G, Liu Y, Zeng Y, Li J, Wang L, Yang G, Chen D, Shang X, Chen J, Huang X et al.** Highly efficient and precise base editing in discarded human tripronuclear embryos. Protein Cell. 2017a.
24. **Li J, Sun Y, Du J, Zhao Y, Xia L**. Generation of targeted point mutations in rice by a modified CRISPR/Cas9 system. Mol Plant. 2017b;10:526–529.
25. **Liang P, Sun H, Sun Y, Zhang X, Xie X, Zhang J, Zhang Z, Chen Y, Ding C, Xiong Y et al**. Effective gene editing by high-fidelity base editor 2 in mouse zygotes. Protein Cell. 2017;8(8):601–611.
26. **Ren B, Yan F, Kuang Y, Li N, Zhang D, Lin H, Zhou H. A** CRISPR/Cas9 toolkit for efficient targeted base editing to induce genetic variations in rice. Sci China Life Sci. 2017;60(5):516–519.
27. **Zhou C, Zhang M, Wei Y, Sun Y, Sun Y, Pan H, Yao N, Zhong W, Li Y, Li W, Yang H.** Highly efficient base editing in human tripronuclear zygotes. Protein Cell. 2017.
28. **Zong Y, Wang Y, Li C, Zhang R, Chen K, Ran Y, Qiu JL, Wang D, Gao C.** Precise base editing in rice, wheat and maize with a Cas9-cytidine deaminase fusion. Nat Biotechnol. 2017;35(5):438– 440.
29. **WHO** Expert Advisory Committee on Developing Global Standards for Governance and Oversight of Human Genome Editing. Human Genome Editing: recommendations. Geneva: World Health Organization; 2021. Licence: CC BY-NC-SA 3.0 IGO.
30. **Matthews, K. R., & Moralí, D.** National human embryo and embryoid research policies: a survey of 22 top research-intensive countries. Regenerative Medicine. 2020;15(7):1905-1917.
31. **Liang P, Xu Y, Zhang X, Ding C, Huang R, Zhang Z, et al.** CRISPR/Cas9-mediated gene editing in human tripronuclear zygotes. Prot Cell. 2015;6(5):363–72.
32. **Kang X, He W, Huang Y, Yu Q, Chen Y, Gao X, et al.** Introducing precise genetic modifications into human 3PN embryos by CRISPR/Cas-mediated genome editing. J Assist Reprod Genet. 2016;33(5):581–8.
33. **Tang L, Zeng Y, Du H, Gong M, Peng J, Zhang B, et al.** CRISPR/Cas9-mediated gene editing in human zygotes using Cas9 protein. Mol Genet Genomics. 2017;292(3):525–33.
34. **Ma H, Marti-Gutierrez N, Park SW, Wu J, Lee Y, Suzuki K, et al.** Correction of a pathogenic gene mutation in human embryos. Nature. 2017;548(7668):413–9.
35. **Liang P, Ding C, Sun H, Xie X, Xu Y, Zhang X, et al.** Correction of β-thalassemia mutant by base editor in human embryos. Prot Cell. 2017;8(11):811–22.
36. **Fogarty NME, McCarthy A, Snijders KE, Powell BE, Kubikova N, Blakeley P, et al.** Genome editing reveals a role for OCT4 in human embryogenesis. Nature. 2017;550(7674):67–73.
37. **Mitalipov S.** Frequent gene conversion in human embryos induced by double strand breaks. Cell. 2020.
38. **Alanis-lobato G, Zohren J, Mccarthy A, Fogarty NME, Kubikova N, Greco M, et al.** Frequent loss-of-heterozygosity in CRISPR-Cas9-edited early human embryos. 2020;1–16.
39. **Zuccaro M V., Xu J, Mitchell C, Marin D, Zimmerman R, Rana B, et al.** Allele-Specific Chromosome Removal after Cas9 Cleavage in Human Embryos. Cell. 2020;183(6):1650-1664.e15.
40. **Chan S, Donovan PJ, Douglas T, Gyngell C, Harris J, Lovell-Badge R, et al.** Genome Editing Technologies and Human Germline Genetic Modification: The Hinxton Group Consensus Statement. AJOB. Am J Bioeth. 2015;15:42–7.
41. **LaBarbera AR.** Proceedings of the International Summit on Human Gene Editing: a global discussion—Washington, D.C., December 1–3, 2015. J Assist Reprod Genet. 2016;33(9):1123–7.
42. **Friedmann T, Jonlin EC,** King NMP, Torbett BE, Wivel NA, Kaneda Y, et al. ASGCT and JSGT Joint Position Statement on Human Genomic Editing. Mol Ther. 2015;23:1282.

43. THE ISSCR STATEMENT ON HUMAN GERMLINE GENOME MODIFICATION. Available from: https://scholar.google.es/scholar?hl=es&as_sdt=0%2C5&q=THE+ISSCR+STATEMENT+ON+HUMAN+GERMLINE+GENOME+MODIFICATION&btnG

44. European Group on Ethics in Science and New Technologies. Availablefrom: https://scholar.google.es/scholar?hl=es&as_sdt=0%2C5q=European+Group+on+Ethics+in+Science+and+New+Technologies+%282016%29.+Statement+on+Gene+Editing&btnG

45. Board A. ACMG Statement Genome editing in clinical genetics : points to consider — a statement of the American College of Medical Genetics and Genomics. 2017;19(7):2016–7.

46. Wert G De, Pennings G, Clarke A, Carla UE, Forzano F, Goddijn M, et al. Human germline gene editing: Recommendations of ESHG and ESHRE. Eur J Hum Genet. 2018;445–9.

47. Ormond KE, Mortlock DP, Scholes DT, Bombard Y, Brody LC, Faucett WA, et al. ASHG POSITION STATEMENT. Human Germline Genome Editing. Am J Hum Genet. 2017;101(2):167–76.

48. National Academies of Sciences, Engineering, and Medicine. Second International Summit on Human Genome Editing. The National Academies. 2018.

49. Doudna J. Perspective: Embryo editing needs scrutiny. Nature. 2015;528:S6.

50. Sugarman J. Ethics and germline gene editing. EMBO Rep. 2015;16(8):879–80.

51. Lantos JD. Hopes, Fears, and Deja Vu Regarding Germline Gene Editing. JAMA Pediatrics. 2019;173.

52. Savulescu J, Pugh J, Douglas T, Gyngell C. The moral imperative to continue gene editing research on human embryos. Protein Cell. 2015;6(7):476–9.

53. Baltimore D, Berg P, Botchan M, Carroll D, Charo RA, Church G, et al. A prudent path forward for genomic engineering and germline gene modification. Science. 2015;348:36–8.

54. Lanphier E, Urnov F, Haecker SE, Werner M, Smolenski J. Don't edit the human germ line. Nature. 2015;519:410–1.

55. Baylis F, Ikemoto L. The Council of Europe and the prohibition on human germline genome editing. 2017;18(12):2084–5.

56. Flotte TR. Senior Gene Therapy Scientists Take a Stand Against Human Embryo Gene Editing. Human Gene Therapy. 2019;30:793.

57. Ishii T. Germline genome-editing research and its socioethical implications. Trends in Molecular Medicine. 2015;21:473–81.

58. Isasi R, Kleiderman E, Knoppers BM. Editing policy to fit the genome? Framing genome editing policy requires setting thresholds of acceptability. Science. American Association for the Advancement of Science; 2016;352:337–9.

59. Baylis F, Darnovsky M, Hasson K, Krahn TM. Human Germline and Heritable Genome Editing : The Global Policy Landscape. 2020;3(5):365–77.

60. Cavaliere G. Genome editing and assisted reproduction: curing embryos, society or prospective parents? Med Heal Care Philos. 2018;21(2):215–25.

61. Ranisch R. Germline genome editing versus preimplantation genetic diagnosis: Is there a case in favour of germline interventions? Bioethics. 2020;34(1):60–9.

62. Wells D, Vermeesch JR, Simpson JL. Current Controversies in Prenatal Diagnosis 3: Gene editing should replace embryo selection following PGD. Prenat Diagn. 2019;39(5):344–50.

63. Steffann J, Jouannet P, Bonnefont JP, Chneiweiss H, Frydman N. Could Failure in Preimplantation Genetic Diagnosis Justify Editing the Human Embryo Genome?. Cell Stem Cell. 2018;22: 481–2.

64. de Miguel Beriain I, Ishii T. Comment on "Should gene editing replace embryo selection following PGD? Some comments on the debate held by the International Society for Prenatal Diagnosis". Prenat Diagn. 2019;39: 1170–2.

65. Viotti M, Victor AR, Griffin DK, Groob JS, Brake AJ, Zouves CG, et al. Estimating Demand for Germline Genome Editing: An In Vitro Fertilization Clinic Perspective. 2019;2(5):304–15.

66. Hammerstein AL V., Eggel M, Biller-Andorno N. Is selecting better than modifying? An investigation of arguments against germline gene editing as compared to preimplantation genetic diagnosis. BMC Medical Ethics. 2019;20

67. Janssens ACJW. Designing babies through gene editing : science or science fiction?. 2016:10–1.

Conflict of interest

The authors declare no conflicts of interest.