
1. Introduction

- 1.1.** As a regulator, the HFEA oversees all research in the UK that involves using human embryos. Centres make research applications to us and these are reviewed by a Licence Committee made up of Authority members. Since October 2009, it has been possible for centres to apply for a research licence to carry out genome editing of human embryos. A licence was granted for the first time to a project studying genetically modified embryos in 2016, taking place at the Francis Crick Institute. As set out in the Human Fertilisation and Embryology Act 1990 (as amended), genetically modified embryos cannot be used in treatment, and cannot be grown in culture for more than 14 days.
- 1.2.** Recently the House of Commons Science and Technology Committee launched an inquiry into genome editing. [The committee's report](#) highlighted regulatory concerns, including how UK regulatory standards should align with international standards and also how the licensing process is fit for purpose, particularly for research involving human embryos. A new inquiry has been opened that will be looking at genomic therapies in the NHS.
- 1.3.** Genome editing was the subject of an ethical review carried out by the Nuffield Council on Bioethics. The review discussed applications of genome editing across many fields including human health, food production, wildlife and ecosystems, and military use. In applications of genome editing in human reproduction, the review comments that the safety of the genome editing technique has not been sufficiently demonstrated through research in human embryos. Along with livestock, human reproduction was considered an issue that needs to be addressed urgently in relation to ethical considerations. A new working group has been established that will produce further reports on application of genome editing in humans.
- 1.4.** There is increasing interest in the prospects of genome editing on a public level and as any research involving genome editing of human embryos in the UK needs to be licensed by us it is therefore important for SCAAC to keep abreast of ongoing research.
- 1.5.** Techniques used for genomic editing have developed considerably with nuclease driven mechanisms such as zinc finger nucleases and CRISPR-Cas9. This review highlights recent studies using genome editing techniques on both animal and human embryos.

2. Review of recent studies

CRISPR studies

- 2.1.** CRISPR stands for Clustered Regularly Interspaced Short Palindromic Repeats and is a system adapted from bacteria that is used in research on genome editing. In genome editing, CRISPR is used to target a specific gene and

together with an enzyme called Cas9 forms a complex that can cut a certain gene out of the genome and can be replaced with another gene. This means that CRISPR-Cas9 could be used to avoid the inheritance of diseases, by removing a defective gene that causes disease and replacing it with the normal functioning gene. The CRISPR-Cas9 technology is still quite new and as such has limitations. For example, sometimes other genes besides the target gene are edited. It is also possible that genes are edited in one or some cells in an embryo but not all cells, leading to a mosaic embryo. The studies below look at how CRISPR-Cas9 can edit genes, as well as investigating ways to reduce risks such as off-site targeting and mosaicism.

- 2.2.** Risks of off-target effects were analysed in a study by Hay et al. (2017) who injected guide RNA and Cas9 mRNA into mouse embryos and carried out deletions of two genes. They analysed whether certain sites that carried similar sequences to the two target genes had been affected. They found that there was no evidence of Cas9 deletions on these off-target sites, and suggested risks are exaggerated.
- 2.3.** Another study looking at off-target sites constructed a modified version of the Cas9 enzyme that is designed to reduce non-specific DNA contacts (Kleinstiver et al., 2016), that is prevent Cas9 from editing genes which are not the target gene. They found using this variant of the enzyme reduced interaction between Cas9 and the phosphate backbone of DNA which made off-target sites undetectable.
- 2.4.** One study looked at an alternative to microinjection for delivery of mRNAs (Hashimoto and Takemoto, 2015). Electroporation - a method where an electric field is used to reduced permeability of cells - was used to introduce mRNAs into fertilised mouse eggs. Embryos that were electroporated had a higher survival rate than embryos that were microinjected.
- 2.5.** In a study by Chen et al. (2016), electroporation was used to deliver Cas9/ preassembled with sgRNA - a molecule which guides Cas9 to the target gene - into mouse zygotes. This was found to be more efficient than microinjection.
- 2.6.** Hashimoto et al. (2016) used electroporation to introduce the CRISPR system into IVF mouse zygotes before the first replication. This generated embryos that were non-mosaic where all the cells carried the same mutations.

Studies on human embryos

- 2.7.** At SCAAC's June 2015 meeting the Committee discussed the recent research conducted by a Chinese group using CRISPR-Cas9 (Liang et al., 2015). The group demonstrated that CRISPR-Cas9 could effectively cleave the endogenous β -globin gene (HBB), which is the gene when mutated causes B-thalassemia. The HBB gene was removed and replaced with another version of the HBB gene, however the efficiency of the replacement process - called homologous recombination directed repair (HDR) - was low and the resulting edited embryos were mosaic. The research group highlighted that their work

demonstrated a need to further improve the fidelity and specificity of this technique.

- 2.8.** A study by Kang et al. (2016) was able to use the Cas9 system to introduce the naturally occurring CCR5 Δ 32 allele which is involved in HIV resistance into early human 3PN embryos (abnormal embryos with three nuclei, as opposed to normal embryos which contain two nuclei, one nucleus from each parent). However, insertion/deletion (indel) mutations did occur on other alleles, where off-target genes had been edited. The repairing process non-homologous end-joining (NHEJ) pathway which joins up gaps in DNA where Cas9 has cut out DNA, was more efficient than homologous directed repair (HDR) using donor DNA for introducing the CCR5 Δ 32 allele.
- 2.9.** Tang et al. (2017) looked at CRISPR gene editing in 2PN (normal embryos with two nuclei, one nucleus from each parent) human zygotes for correction of point mutations in two genes. They injected CRISPR/Cas9 protein complex with guide RNA and a donor template into one-cell human embryos and through homologous recombination were able to correct point mutations in the β -globin gene (HBB) and a gene coding for an enzyme called Glucose-6-phosphate dehydrogenase (G6PD). The authors highlighted that the correction procedure has limitations.
- 2.10.** A recent US study (Ma et al., 2017) used the CRISPR system to correct the heterozygous mutation in the MYBPC3 gene. They used the maternal wild type gene to repair double stranded breaks instead of a synthetic DNA template. The CRISPR system was injected into eggs during fertilisation, before any division into multiple cells takes place, which led to reduced mosaicism.
- 2.11.** A recent Chinese study (Liang et al., 2017) used the base editor system to correct a point mutation of the HBB gene in human embryos. The base editor system uses CRISPR/Cas9 to target point mutations without introducing double stranded breaks. They were able to correct a point mutation in the HBB gene with an efficiency of over 23%, although the embryos were still mosaic.

3. Conclusions

- 3.1.** Genome editing of human embryos and germ line cells has the potential to replace disease causing genes, as the studies outlined above demonstrate. However, there are significant risks and limitations. Research needs to continue, particularly to optimise genome editing technologies to ensure issues such as unintended editing of off-site targets and mosaicism can be avoided.
- 3.2.** Many studies of gene editing on human embryos have been carried out in China, where the regulation system is considerably different to that in the UK.
- 3.3.** Members are asked to note this update and:
 - consider whether there are aware of any further studies or developments in the area

- identify particular concerns or issues that should be highlighted
- discuss potential clinical applications of this technology.

4. References

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