

Key references for human germline genetic editing

v3

Narrative outline of our project: [Human Germline Genetic Modification](#)

Reviews

[Nucleic acids delivery methods for genome editing in zygotes and embryos](#) Recent (2016) review of the methods available - see Table 1 and Figure 1 for an overview of methods. Limited consideration of CRISPR and/or sperm selection to enhance efficiency. Refs 67-74 in that paper are also relevant for our project.

[In vivo Gene Transfer into Testis and Sperm: Developments and Future Application](#) Another good review, it references e.g. [In vivo transfection of testicular germ cells](#) and [testis-mediated gene transfer](#) which succeeded in generating transgenic sperm.

[Gene transfer to sperm and testis](#) also does not consider the impact of CRISPR or cell sorting and only deems methods viable if the mice can produce transgenic progeny from natural mating (no IVF), but is otherwise a good review.

Cell Sorting

[Single Cell Isolation and Analysis](#) Summary of cell sorting techniques (FACS/MACS/microfluidics), specific example here: [A method for high purity sorting of rare cell subsets applied to TDC](#). Allows us to extract transgenic sperm even if they are a tiny fraction of the total. [Sperm selection using magnetic activated cell sorting](#) has been investigated as well.

CRISPR

[Efficient precise knockin with a double cut HDR donor after CRISPR/Cas9-mediated double-stranded DNA cleavage](#) Much-improved method for CRISPR-based gene knock-in, another related technique is [MMEJ-assisted gene knock-in](#). Both allow the insertion of transgenes at specific locations in the genome.

Base Editing

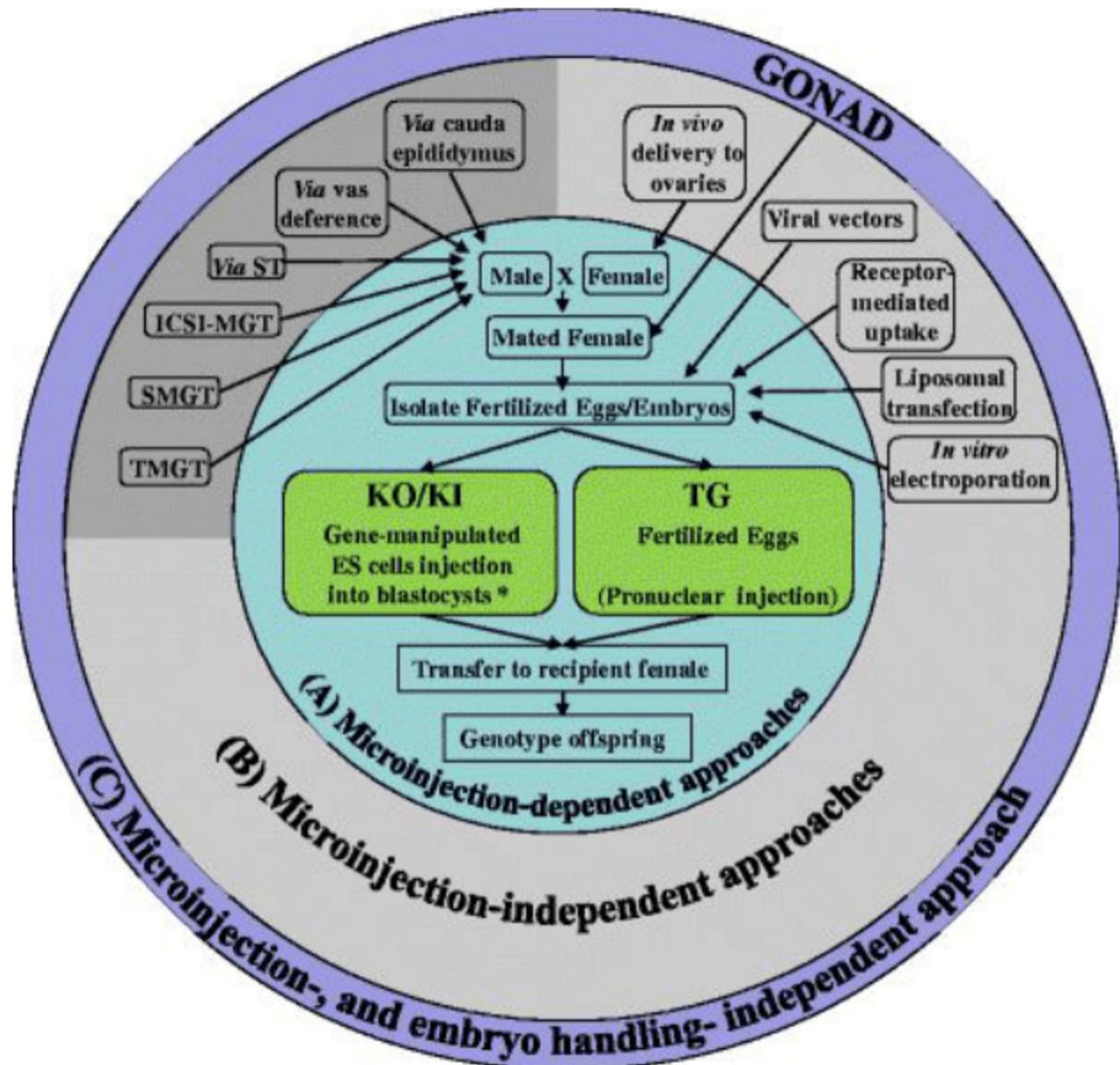
[Correction of the Marfan Syndrome Pathogenic FBN1 Mutation by Base Editing in Human Cells and Heterozygous Embryos](#) Base editing in human embryos; has limited utility for now but it's a good option for single point mutations.

Preimplantation Genetic Diagnosis (PGD)

[Technical Update: Preimplantation Genetic Diagnosis and Screening](#) Recent review of PGD technology, used in our case to confirm transgene integration in the embryo.

Graphical overview of main approaches, from literature (1st reference above, Figure 1)

Note: Our approach is testis-mediated gene transfer (transfection of sperm stem cells).
 Additional techniques can be explored but our focus is on testis-mediated:



Key: TG = transgenic, ICSI-MGT = intracytoplasmic sperm injection-mediated gene transfer, ST = seminiferous tubules, TMGT = testis-mediated gene transfer, KO/KI = knock-out/knock-in, ES = embryonic stem [cells]

Future Directions: *In vitro* gametogenesis

[Generation of human oogonia from induced pluripotent stem cells *in vitro*](#) for egg cells.

[Robust *in vitro* induction of human germ cell fate from pluripotent stem cells](#) for sperm.

In both cases, deriving germ cells from induced pluripotent stem cells (iPSCs) allows us to make far larger modifications to the genome since the iPSCs can be grown and expanded in a dish. After modifications are added and the genome is sequenced, they can be differentiated into germ stem cells and transplanted into their respective tissues.

Primary risks are pre-existing genomic damage in the iPSCs (can be mitigated with sequencing) and aberrant differentiation once in the body (will require non-human research to assess). Transplanting into the body, rather than *in vitro* culture, is likely to allow more natural differentiation into germ-cell-producing cells, but is not being actively researched due to potential risks involved.

Human cloning

[Cloning Mice and Men: Prohibiting the Use of iPS Cells for Human Reproductive Cloning](#) Paper calling for tetraploid complementation to be banned in humans, because it can be used for cloning, e.g. [iPS cells produce viable mice through tetraploid complementation](#). This is a method we are interested in using.

[A highly efficient method for generation of therapeutic quality human pluripotent stem cells by using naive induced pluripotent stem cells nucleus for nuclear transfer](#) Theoretical approach using iPSCs for nuclear transfer, sadly not researched further since there's no true "medical" need to do it. But we will try it out.

[Cloning of Macaque Monkeys by Somatic Cell Nuclear Transfer](#) Monkey cloning using SCNT, however they had to use fetal cells for the nuclear donor.

[Human Embryonic Stem Cells Derived by Somatic Cell Nuclear Transfer](#) SCNT with human cells, again needing fetal tissue for the donor.

In vitro gametogenesis (progress towards)

[Efficient generation of functional haploid spermatids from human germline stem cells by three-dimensional-induced system](#)

[Metaphase II oocytes from human unilaminar follicles grown in a multi-step culture system](#)

Primary research questions

- 1) Is it possible to reduce potential toxicity of testis-mediated gene transfer, while maintaining efficiency?
- 2) Can a small quantity of transformed sperm cells be sorted out of millions of cells?
- 3) Is it more practical to target sperm stem cells in the testis, or better to invest in R&D on in vitro spermatogenesis or in vitro oocyte genesis (which has not yet been achieved for human)?