Genome editing in the germline

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Outline

Genome editing: Mechanisms, Indications

Genome editing in the germline

Human embryo genome editing

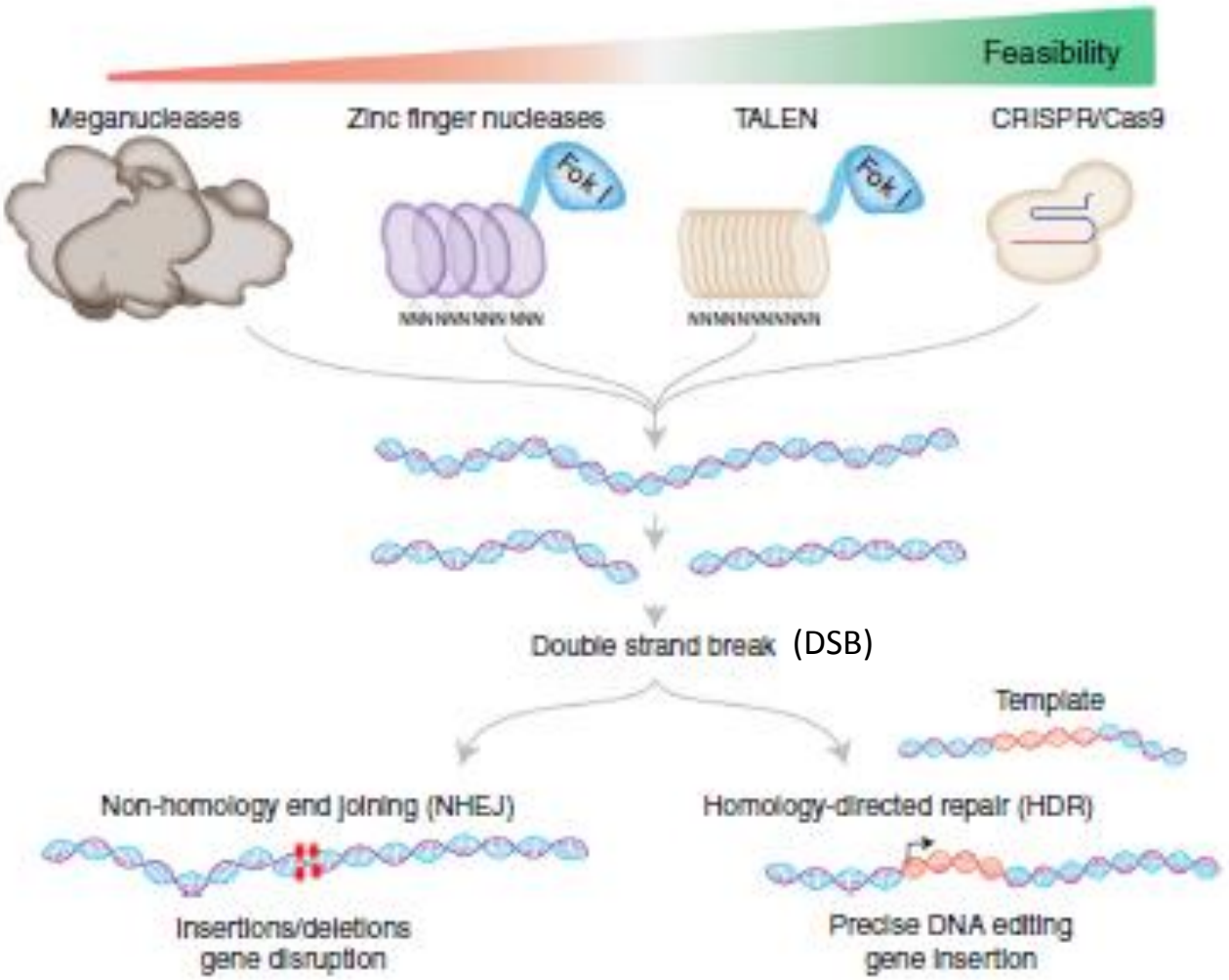
Conclusions

Genome editing
From Wikipedia, the free encyclopedia

Genome editing, or **genome engineering** is a type of genetic engineering in which DNA is inserted, deleted, modified or replaced in the genome of a living organism.

Genome editing was selected by *Nature Methods* as the 2011 Method of the Year.

The CRISPR-Cas system was selected by *Science* as 2015 Breakthrough of the Year.



The basic working principle of major genome-editing technologies
Adli, 2018

Germline genome editing

DNA editing of germline can be performed in

1. Stem cells
   - Pluripotent stem cells to be differentiated into gametes to further give rise to an embryo (*in vitro* gametogenesis-IVG)
   - Spermatogonial stem cells
2. Zygotes or Embryos

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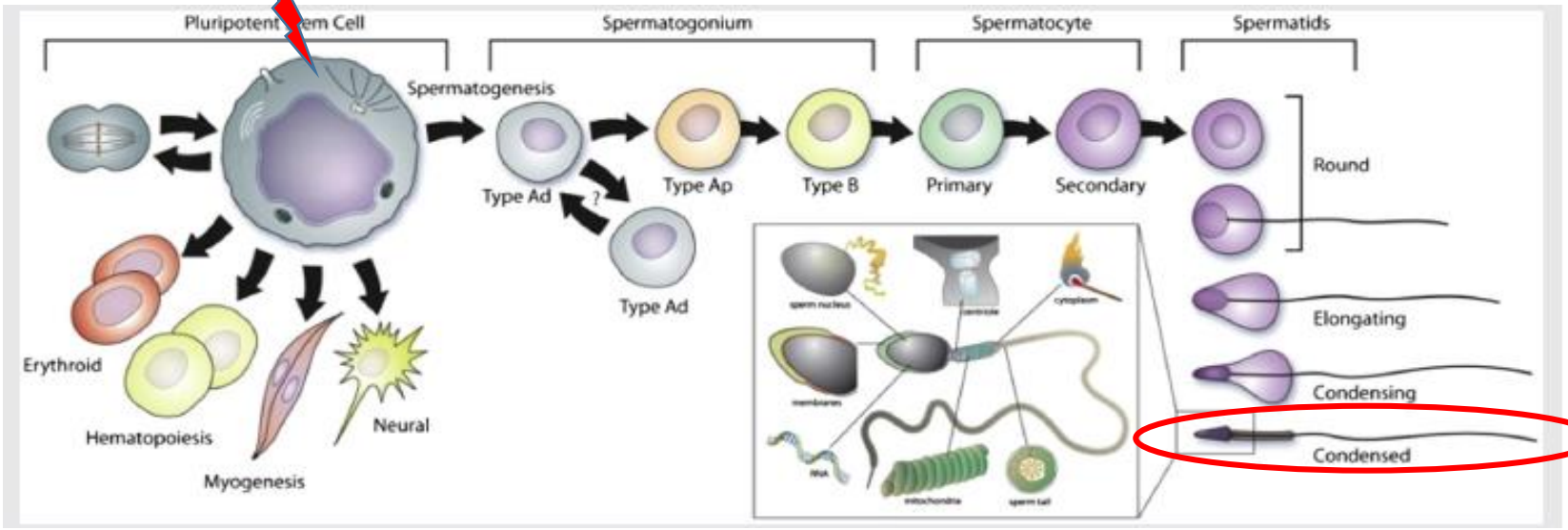
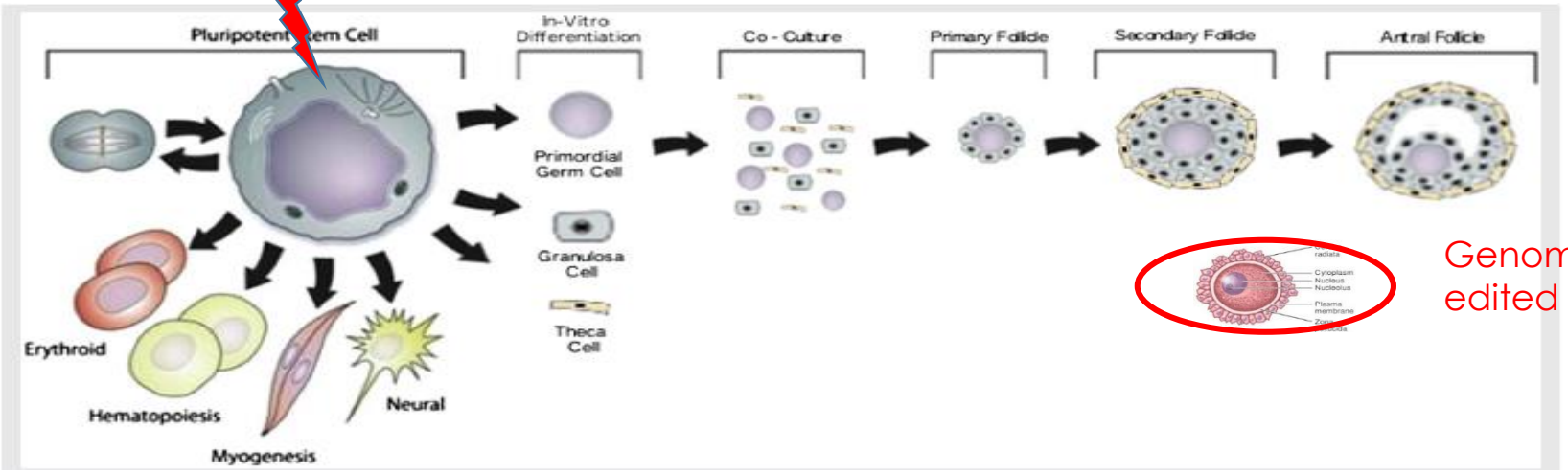
Genome editing



Genome edited sperm

Genome editing



Genome edited oocyte

Germline genome editing

DNA editing of germline can be performed in

1. Stem cells
   - Pluripotent stem cells differentiated into gametes to further give rise to an embryo (*in vitro* gametogenesis – IVG)
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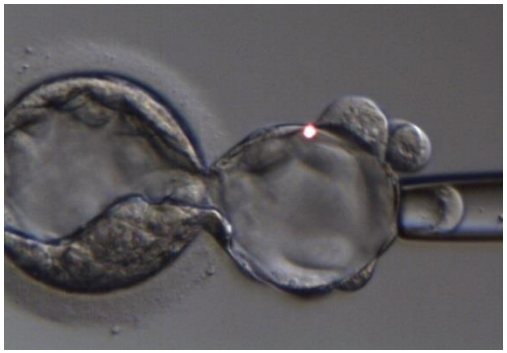
Embryo genome editing

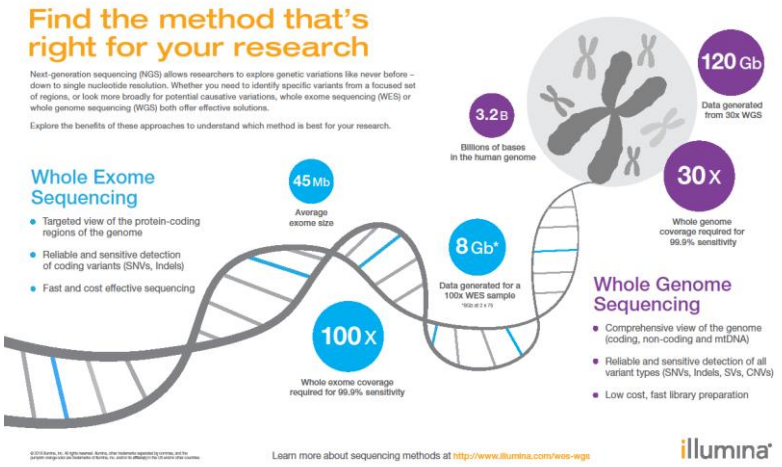
- Highest efficiency expected in **one cell stage zygotes**
- High efficiency to produce both single mutants (95%) and double-mutants (70-80%) using direct injection of Cas9 mRNA and sgRNA into zygotes

Embryo genome editing- Limitations

- Genome editing of human embryos requires the **use of PGD** in order to identify the modified embryos from the **non modified** or the one's with **off-target effects**.

- **Sequencing** of the full genome required.





Embryo genome editing- Limitations

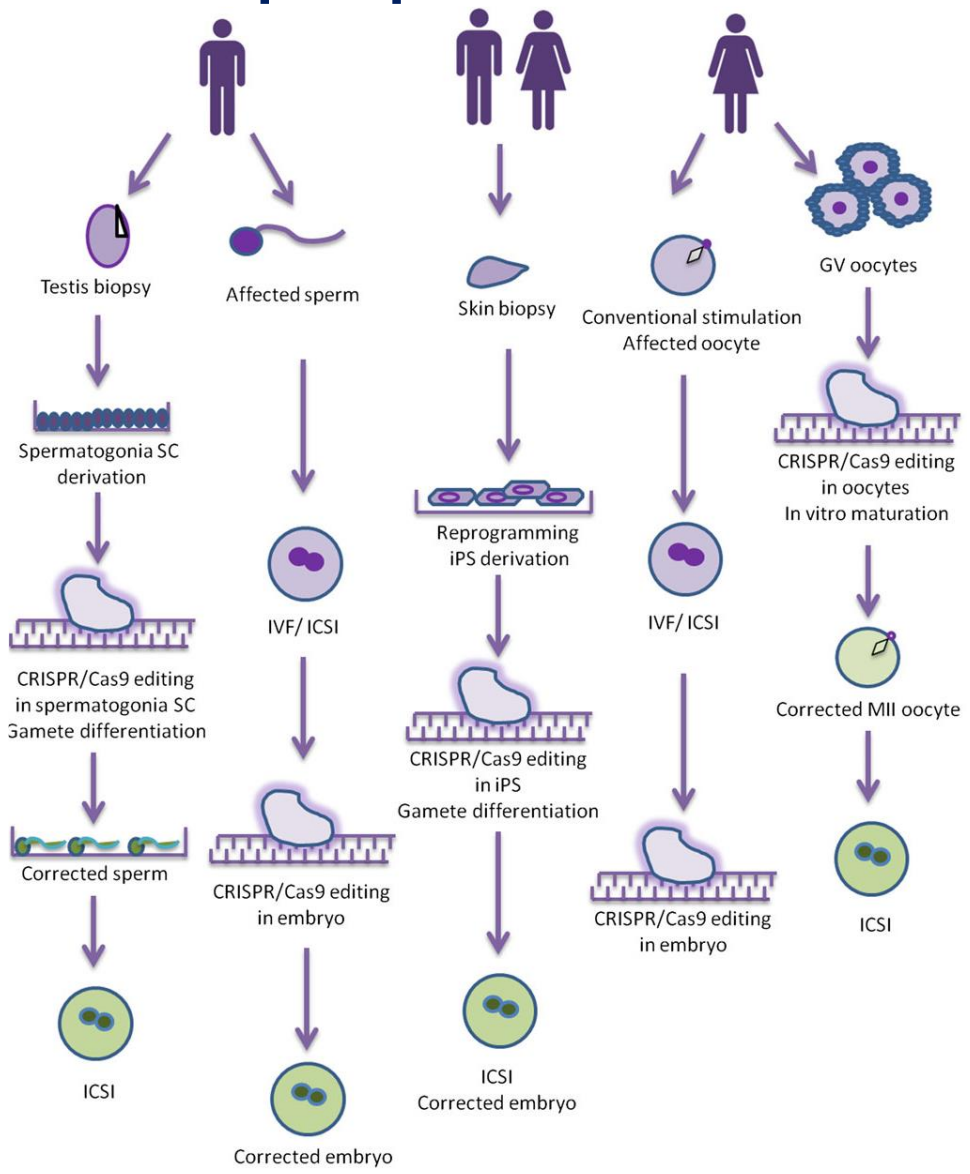
- Generation of **chimera/mosaic** embryos as a result of inefficient nuclease cutting and/or inaccurate DNA repair before the embryo undergoes cleavage will remain **undetected.**

Mosaicism type	Possible TE biopsy	Diagnoses accuracy
Total Mosaic 	Euploid	Misdiagnosis
	Mosaic	Accurate
	Aneuploid	Misdiagnosis
ICM Mosaic 	Euploid	Misdiagnosis (Mosaicism never detectable)
TE Mosaic 	Euploid	Misdiagnosis
	Mosaic	Accurate
	Aneuploid	Misdiagnosis
ICM/TE Mosaic Type I 	Euploid	Misdiagnosis (Mosaicism never detectable)
ICM/TE Mosaic Type II 	Aneuploid	Misdiagnosis (Mosaicism never detectable)

Vera-Rodriguez and Rubio, 2017

Genome engenineering through CRISP/Cas 9 technology in the human germline and pluripotent stem cells. Vassena,2016

- Review of critical technical and ethical issues that should deter from employing CRISPR/Cas9 based technologies in human reproduction until clarified.



Possible uses of genome editing in human reproduction

- Both members of the couple carriers of an **Autosomal Recessive disease** (e.g. metabolic disorders, Tay Sachs disease…).

- One member affected of an **Autosomal Dominant disease** (e.g. Familial hypercholesterolemia, Von Willebrand disease…).

Genome editing would require making **IVF embryos**, using preimplantation genetic diagnosis (**PGD**) to identify those that would have the disease, **repairing the gene, and implanting the embryo.**

Easier and safer to use PGD to identify and implant the embryos that are not at risk (a parent heterozygous for a dominant disease (50%) or two parents who are carriers for a recessive disease (75%))

PGD is the method of choice in such cases

PGD for monogenic disease and Embryo genome editing

- Potentialy **viable embryos are discarded in PGD** cycles as being affected from monogenic genetic diseases
- Genome editing could be used to **repair such embryos.**

Hôpital Antoine Béclère. Paris. France.
PGD programme 2011-2016
358 couples
95 couples obtained a pregnancy with **214 cycles with no embryo transfer (73% failure rate)**
959 affected embryos were discarded.

Genome editing should be considered as a potential tool to enable the rescue of affected embryos and increase the number of available embryos for transfer in PGD couples.

Could failure in PGD justify editing the human embryo genome? Steffann et al, 2018

Possible uses of genome editing in human reproduction

- Both members affected with the **same monogenic disease** (homozygous for recessive disease)(E.g: Cystic Fibrosis)

Correction of the affected gene in the germline of one of the prospective parents or in embryos

- One member **homozygous for an Autosomal Dominant disease** () e.g Huntington disease. Achondroplasia, Marfan syndrome, policystic kidney disease)

Correction of the affected gene in the germline of the affected member or in embryos

- One member affected by a **chromosomal structural aberration** (e.g: 21;21 translocation)

Separation of the 2 chromosomes and restoration of the centromere and missing p-arms in the germline or in embryos

Gene repair. Human 3PN Model

- CRISPR/Cas9 can cleave the **endogenous β-globin gene (HBB)**
- The efficiency of homologous recombination directed repair (HDR) of HBB is **low and the edited embryos were mosaic.**
- **Off-target cleavage** was also apparent in these 3PN zygotes.
- Repair of the HBB locus occurs preferentially through the non-crossover HDR pathway.
- **Need to further improve the fidelity and specificity of the CRISPR/Cas9 platform, a prerequisite for any clinical applications of CRSIPR/Cas9-mediated editing.**

Targeted editing of the HBB gene in human 3 PN zygotes by intracytoplasmic injection

Group No.	Cas9/gRNA/ssDNA (ng/μL)	Survived /injected	GFP+	PCR-amplified
1	100/20/2	10/11	6	6
2	100/20/20	22/29	17	17
3	200/40/200	12/14	12	10*
4	200/40/200	27/32	24	21*
Total	-	71/86 (82.6%)	59	54

Cas9-cleaved	Edited with ssDNA	Recombined with HBD
4	0	1
7	1	0
6	2	2
11	1	4
28 (51.9%)	4 (14.3%)	7 (25.0%)

CRISPR/Cas9-mediated gene editing in human tripronuclear zygotes Protein Cell, Liang,2016

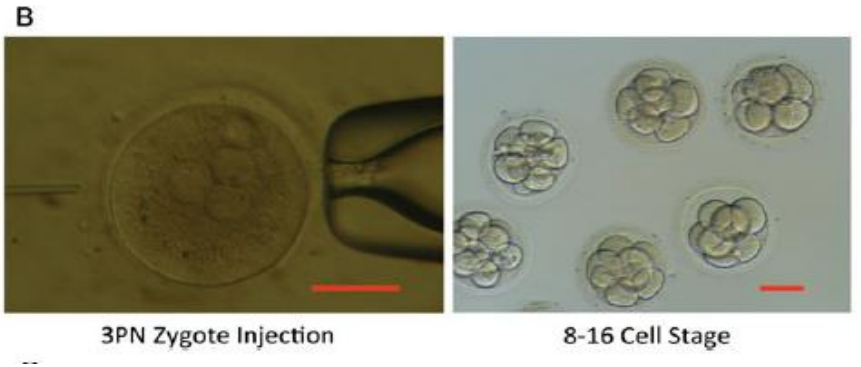
Gene insertion. Human 3PN Model

- Results By co-injecting Cas9mRNA, gRNAs, and donorDNA, **successful introduction of the naturally occurring CCR5Δ32 allele** into early human 3PN embryos.

Groups[a]	Injected 3PN zygotes	Development (%)		Genetic modification	
		Cleavage	8–16 cell	NHEJ mutations (percent)	Δ32 allele (%)
Control water	18	15 (83)	13 (72)	0	0
Cas9 + gRNA1	11	9 (82)	7 (64)	4 (57)	0
Cas9 + gRNA2	13	10 (77)	8 (62)	5 (63)	0
Cas9 + gRNA1 + ssODN1 (PN injection)	23	14 (61)	11 (48)	4 (36)	0
Cas9 + gRNA1 + ssODN1	32	28 (88)	20 (63)	10 (50)	1 (5)
Cas9 + gRNA2 + ssODN2	46	39 (85)	27 (59)	13 (48)	0
Cas9 + gRNA1 + 1 kb dsDonor	25	21 (84)	15 (60)	7 (47)	1 (7)
Cas9 + gRNA1 + gRNA2	45	37 (82)	26 (58)	13 (50)	4 (15)

[a] CRISPR/Cas system was delivered to 3PN zygotes by cytoplasmic injection in all groups, except the group labeled with PN (pronuclear) injection

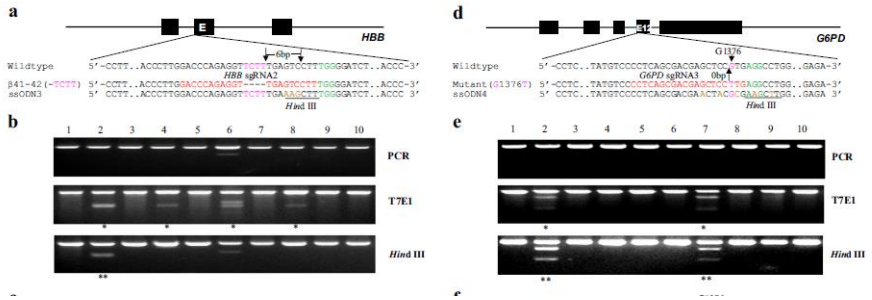
- In the embryos containing the engineered CCR5Δ32 allele, the other alleles at the same locus could not be fully controlled because they either remained wild type or contained indel mutations.



Introducing precise genetic modifications into human 3PN embryos by CRISPR/Cas 9-mediated genome editing JARG, Kang, 2016

Gene correction. Human 2PN model

- CRISPR/Cas9 is effective as a **gene-editing tool in human 2PN zygotes.**
- By injection of Cas9 protein complexed with the appropriate sgRNAs and homology donors into one-cell human embryos, efficient homologous recombination-mediated **correction of point mutations in HBB and G6PD** was demonstrated.

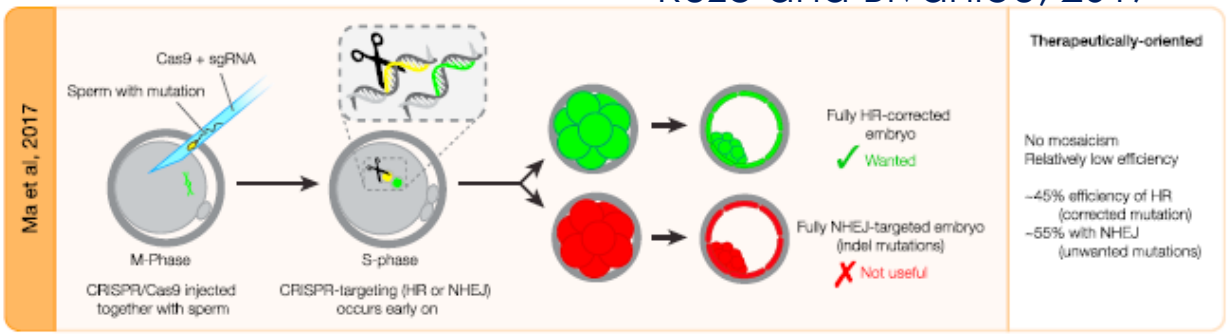


CRISPR/Cas9-mediated gene editing in human zygotes using Cas9 protein
Mol Genet. Genomics, Tang, 2017

Gene correction. Human 2 PN model

- **Correction of the heterozygous *MYBPC3* mutation** in human preimplantation embryos with precise CRISPR–Cas9-based targeting accuracy and high homology-directed repair efficiency.
- Endogenous, germline-specific DNA repair response.
- Induced double-strand breaks (DSBs) at the mutant paternal allele were predominantly **repaired using the homologous wild-type maternal gene** instead of a synthetic DNA template.
- By modulating the cell cycle stage at which the DSB was induced, **mosaicism was avoided** in cleaving embryos.

Ruzo and Bivanlou, 2017



Injection of Cas9 protein and **sgRNA together with the sperm, at the time ICSI. Correction of 45%** of the mutant embryos completely, with no mosaicism and **no detectable off-targets.**

Correction of a pathogenic gene mutation in human embryos Nature, Ma et al, 2017

Gene correction. Human 2 PN model. Ma 2017 concerns

- Genome editing occurred essentially exclusively using the normal maternal chromosome as a template? This is unlikely because **the male and female pronuclei are entirely physically separated in the 1-cell embryo**.

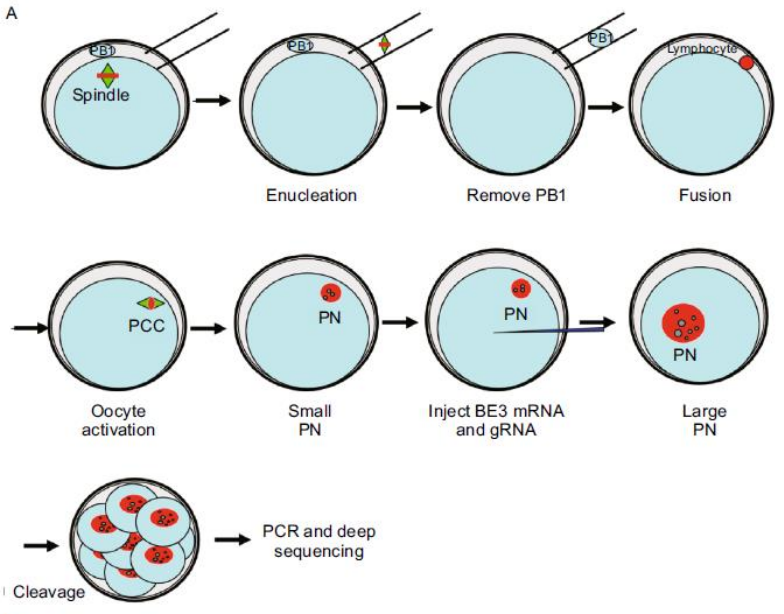
- Apparent absence of a detectable mutant allele? The altered (not repaired) mutant alleles are there but **just not detectable**.

- Zygotes with a single pronucleus are not uncommon after intracytoplasmic sperm injection, occurring in ~10% of fertilization attempts, and are mostly of **parthenogenetic origin**, containing only the maternal genome

Inter-homologue repair in fertilized human eggs? BioRXiv Egli et al, 2017

Gene correction. Human 2 PN model

- First study using *base editor (BE)* system to **correct disease mutant** in human embryos.
- Base editor could precisely correct HBB −28 (A>G) mutation in the patient's primary cells.
- To model homozygous mutation disease embryos, **nuclear transfer embryos** by fusing lymphocyte or skin fibroblast cells with enucleated in vitro matured (IVM) oocytes were constructed.
- The **gene correction efficiency was over 23.0%**
- Although these embryos were still **mosaic**, the percentage of **repaired blastomeres was over 20.0%.**



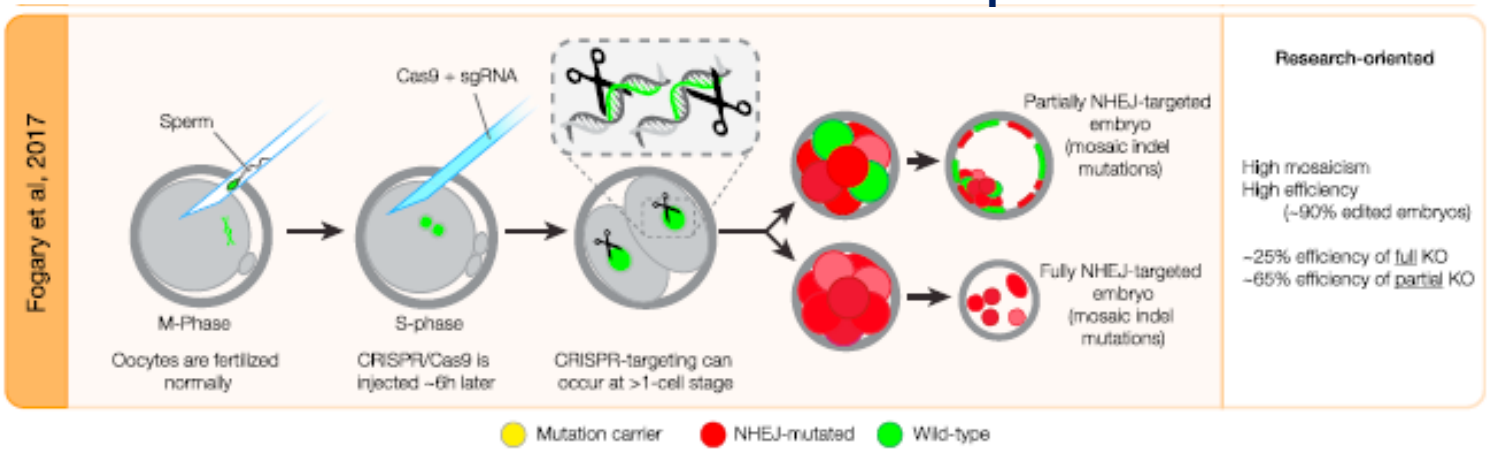
B

Injected embryo No.	Survived embryo No.	PCR-amplified embryo No.	G>A embryo No. (%)	G>C embryo No. (%)
30	26	22	9 (40.9)	1 (4.5)*

Correction of β-thalassemia mutant by base editor in human embryos
Protein Cell , Liang et al, 2017

Gene disruption. Human 2PN model for development.

- The gene encoding **OCT4 (POU5F1) was targeted** in diploid human zygotes and found that **blastocyst development was compromised**
- Transcriptomics analysis revealed that, in POU5F1-null cells, **gene expression was downregulated** not only for extra-embryonic trophectoderm genes, such as CDX2, but also for regulators of the pluripotent epiblast, including NANOG.
- CRISPR–Cas9-mediated genome editing is a powerful method for investigating **gene function in the context of human development**



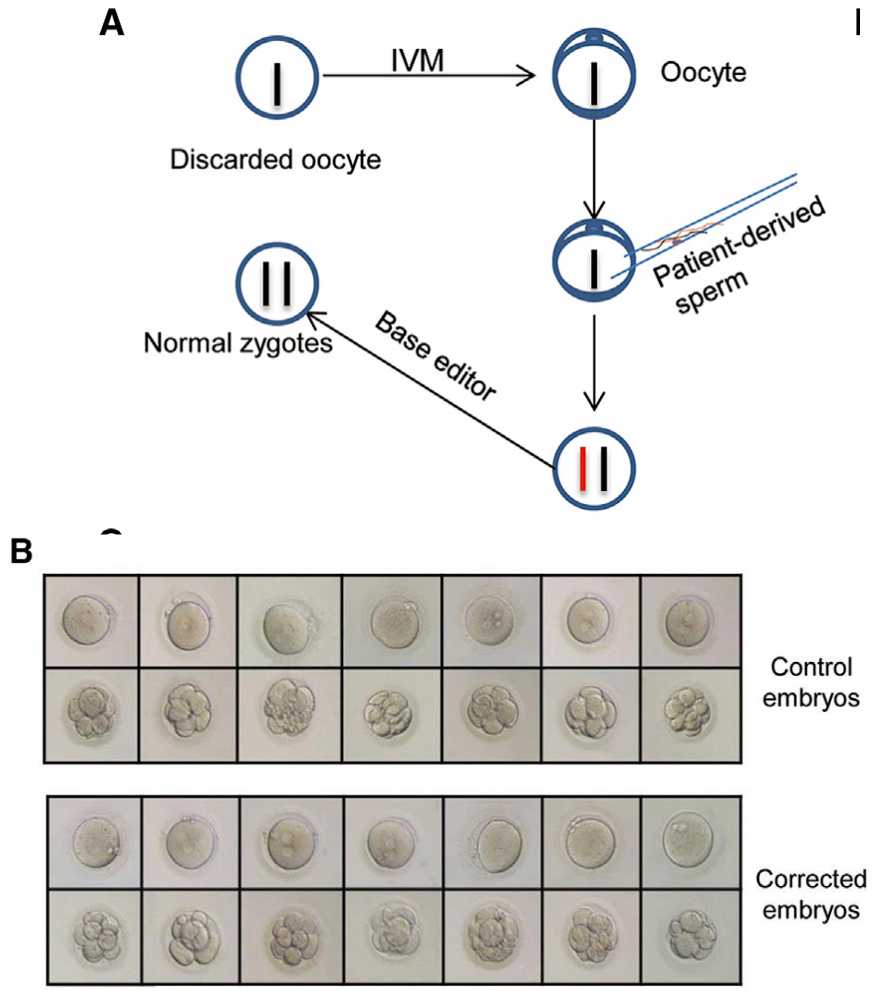
Ruzo and Bivanlou, 2017

Highly efficient **NHEJ mechanism to ablate the OCT4 gene in 90% of the injected embryos** and demonstrated that **OCT4 has different functions in human embryogenesis compared with other animal models.**

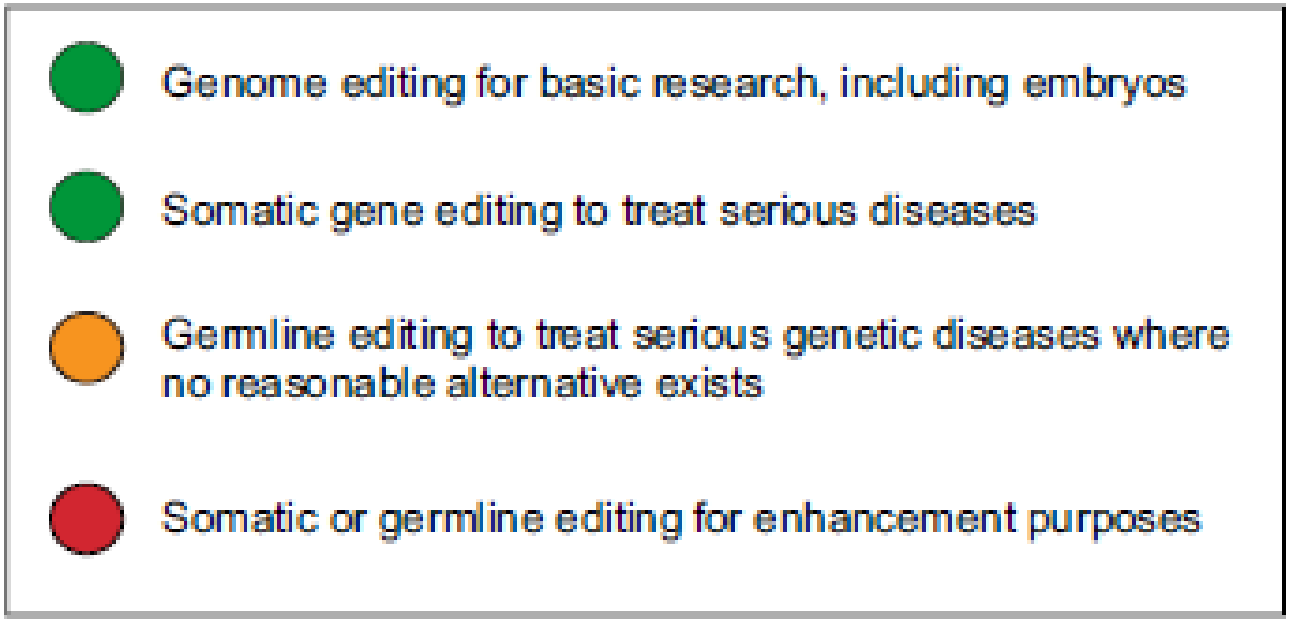
Genome editing reveals a role for OCT4 in human embryogenesis Nature, Fogarthy et al ,2017

Base editing. 2PN embryos from IVM oocytes

- Genetic correction of the **pathogenic mutation FBN1 in heterozygous human embryos by base editing.**
- BE3 mediated **perfect correction at the efficiency of 89%.**
- **No off-target and indels detected** in any tested sites in samples by high-throughput deep sequencing combined with whole-genome sequencing analysis.
- **High efficiency and genetic safety of correcting a Marfan syndrome** (MFS) pathogenic mutation in embryos by base editing.



Correction of the Marfan Syndrome Pathogenic FBN1 Mutation by Base Editing in Human Cells and Heterozygous Embryos. Zeng,2018



Major recommendations of the National Academy of Sciences report on human genome editing.

The committee did not recommend an outright ban on human germline editing but set out guidelines around when to (1) proceed under existing regulatory processes (green), (2) proceed with caution under stringent oversight and public input (orange) or (3) not proceed at this time (red).

- Genome editing is an excellent tool for the **generation of disease models** as well as for the study of genes involved in **pre** and **post implantation development**
- Genome editing cannot be actually performed with sufficient precision to allow scientists to responsibly contemplate creating genetically modified babies (**inaccurate editing, and off-target mutations**).
- **Research** in the human germline (gametes and embryos) **should be promoted** to determine the usefulness and safety of the technique

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