Germ-line genetic modification: what is wrong with it?

Jacques Cohen - disclosures

- ✓ Reprogenetics, consultant
- ✓ ART Institute of Washington, Director
- ✓ Althea Sciences, product developer and director
- ✓ Life Global, product developer and consultant

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PGDIS, Bologna, 2016

Genetic modification

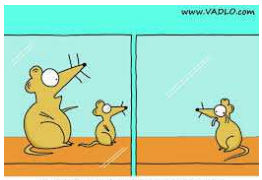
- (I) **Somatic** Gene Therapy - **Not heritable (?)**
- (II) **Germ-line** Gene Therapy - **Heritable.**

www.genome.gov – NIH
Genetics and Public Policy Center, 2005

Genomic Modification Can Occur Intentionally or Unintentionally

✓Unintentional – epigenetic change (mouse - diet, human - famine)
✓Unintentional – epigenetic change after IVF (possibly long-term)
✓Unintentional - change in mtDNA (Cytoplasmic Transfer)

✓Intentional - mtDNA sequence - mitochondrial replacement therapy (MRT)
✓Intentional - repair/change/elimination of mutation of mtDNA (mito disease)
✓Intentional - repair/change of mutation of nuclear DNA (genetic disease)
✓Intentional - repair/change of mutations of nuclear DNA (polygenic trait)



"Don't play with him, he is Wild Type."

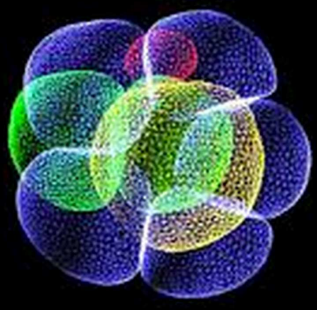
Lecture Content

1.Unintentional change - genomic expression (Bouillon et al 2016)

2.Intentional change in mtDNA sequence - mitochondrial replacement therapy (MRT)

3.Intentional elimination of mtDNA mutation (mitochondrial disease – TALENS, CRISPR)

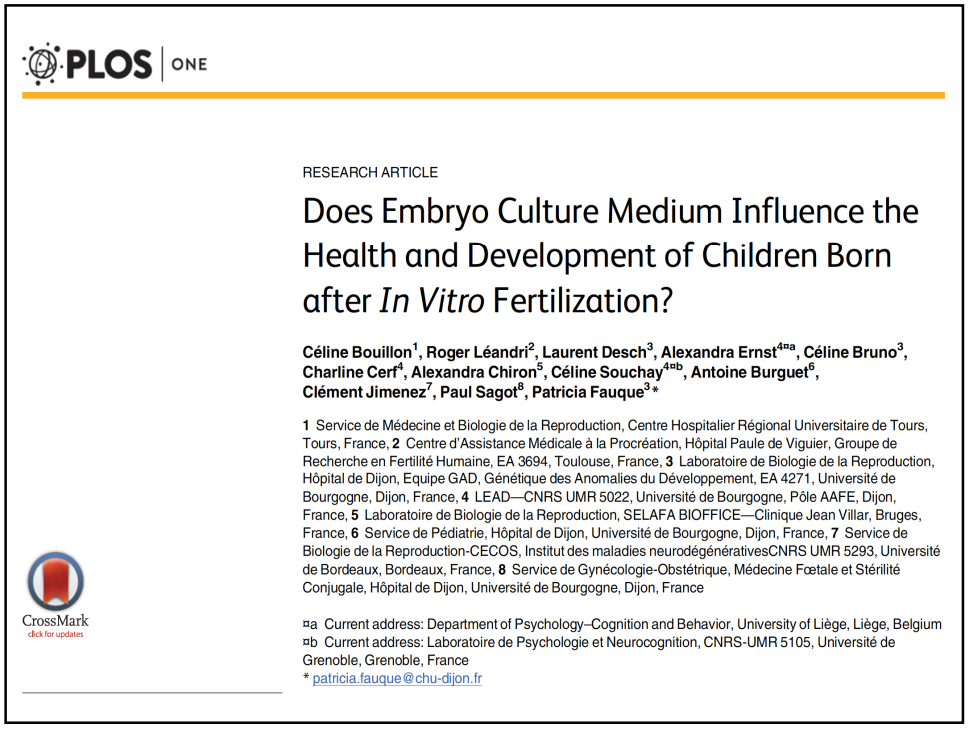
4.Intentional repair/change of mutation of nuclear DNA (genetic disease - CRISPR/cas9)



Unintended change in genomic expression after IVF

Bouillon et al, 2016

PGDIS, Bologna, 2016



PLOS ONE

RESEARCH ARTICLE

Does Embryo Culture Medium Influence the Health and Development of Children Born after *In Vitro* Fertilization?

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Bouillon et al 2016

- RCT comparing two KSOM-derived media and looking at pregnancy outcomes from one IVF laboratory – early study termination (University Hospital Dijon)
- Follow-up of developmental milestones at age 5
- Many IVF studies show immediate outcome differences
- Some studies show perinatal and long-term developmental effects of ART procedures, whereas other studies do not (Bowen et al 1998; Knoester et al, 2008; Leslie et al, 2005)

Bouillon et al 2016 study

- Comparing two manufacturers of KSOM-derived media: Global (A) and SSM (B)
- There were two composition differences (Taurine and Gly-Glu or Ala-Gln)
- Probably other differences – concentrations, QC

Table 1. Components of both culture media according to published analyses*.

Component Type			*Global medium*	*SSM medium*
Salts and Ions		Sodium Chloride	+	+
		Potassium Chloride	+	+
		Magnesium Sulfate	+	+
		Calcium Chloride	+	+
		Potassium Phosphate	+	+
Buffer		Sodium Bicarbonate	+	+
Energy Substrates		Glucose	+	+
		Sodium Pyruvate	+	+
		Sodium Lactate	+	+
Amino Acids	***Essential amino acids***	Arginine	+	+
		Cysteine	+	+
		Histidine	+	+
		Isoleucine	+	+
		Leucine	+	+
		Lysine	+	+
		Methionine	+	+
		Phenylalanine	+	+
		Threonine	+	+
		Tryptophan	+	+
		Tyrosine	+	+
		Valine	+	+
	Non-essential amino acids	Alanine	+	+
		Asparagine	+	+
		Aspartic acid	+	+
		Glutamic acid	+	+
		Glycine	+	+
		Proline	+	+
		Serine	+	+
		Taurine	-	+
Di-peptide			Glycyl-L-Glutamine	Alanyl-L-Glutamine
		EDTA	+	+
		Phenol Red	+	+
Indicator		Phenol Red	+	+
Antibiotic		Gentamicin	+	+

* Zhao et al., 2013; Morbeck et al., 2014

doi:10.1371/journal.pone.0150857.t001

Table 2. Pregnancy outcomes.

	Global group (No. 179)	SSM group (No. 192)	Rate Ratio (95% CI)	p
Implantation (% per embryo transferred[a])	82 (29.1%)	48 (16.3%)	1.78 [1.31–2.43]	0.001

Table 3. Malformations.

		Global group* (No. 40)	SSM group* (No. 31)
Major malformations (according to EUROCAT)	Detected during the neonatal period	2 (5.1%)	1 (3.2%)
	Detected after the neonatal period	1 (2.8%)	1 (3.3%)
Minor malformations	Detected during the neonatal period	7 (18.0%)	3 (9.7%)
	Detected after the neonatal period	9 (25.0%)	7 (23.3%)
Major malformations by organ type	Cardiac[a]	2 (5.6%)	1 (3.3%)
	Nervous system[b]	0 (0%)	1 (3.3%)
	Limbs[c]	1 (2.8%)	0 (0%)

PLOS ONE | Effects of Culture Medium on Health and Development

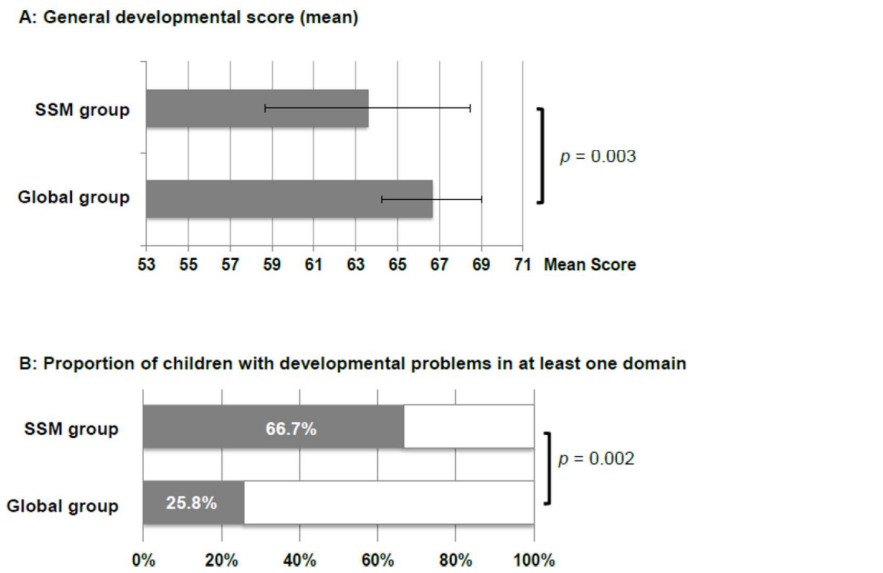
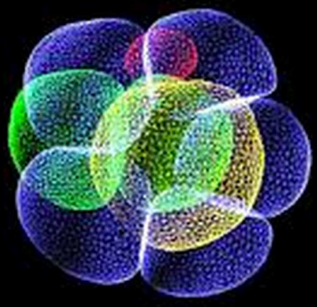


Fig 1. Development. General developmental score (A) and proportion of children with developmental problems in at least one out of eight domains according to the CDI norms (B) in Global and SSM groups.

doi:10.1371/journal.pone.0150857.g001



Treatment of Mitochondrial Disease by Laboratory-Assisted Reproduction

Mitochondrial Replacement Therapy - MRT

PGDIS, Bologna, 2016

Mitochondrial disease

- ~1/100, MERFF, LHON, MELAS, NARP
- Threshold mutant mtDNA load 25-80%
- Threshold dependent on disease type
- Wild-type mtDNA and mutant
- Heteroplasmy (malignant)
- Benign form is common in aging
- Disease is untreatable

Mitochondrial disease

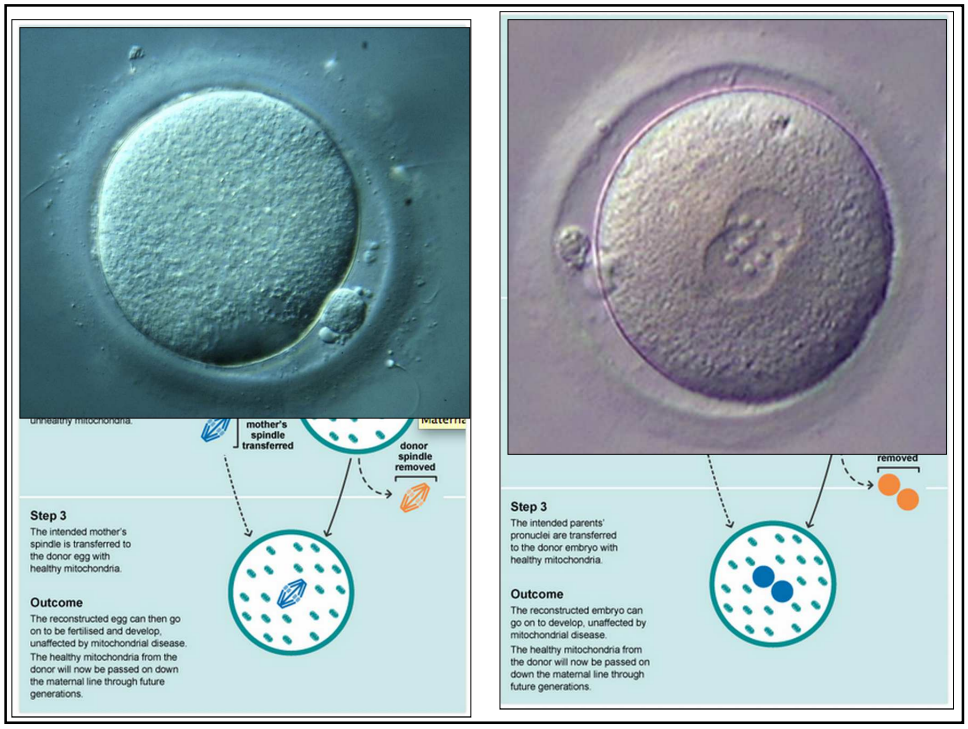
- 15% of disease mutations on mtDNA
- 85% dysfunction of nuclear genes of OXPHOS
- Symptoms: neurological, respiratory, heart, liver, kidney, gastro-intestinal, muscular, visual, learning disabilities
- Patient-specific

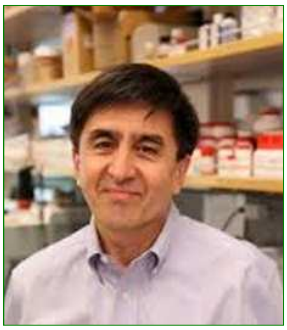
Therapeutic embryology-based approaches:

I. PGD (Preimplantation Genetic Diagnosis)

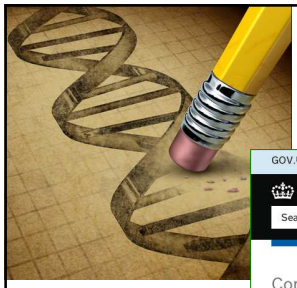
II. MRT (Mitochondrial Replacement Therapy)

III. Genome Editing





Tachibana et al, 2009, Nature



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Consultation outcome

Serious mitochondrial disease: new techniques to prevent transmission

From: Department of Health
History: Updated 22 July 2014, see all updates

This consultation has concluded

Download the full outcome

Mitochondrial Donation: government response to the consultation on draft regulations to permit the use of new treatment techniques to prevent the transmission of a serious mitochondrial disease from mother to child

Published: 22 July 2014
PDF, 253KB, 47 pages

INSIGHTS

Hurdles for biosimilars p. 188
Seeing clearly with chaotic lasers p. 189

PERSPECTIVES

MEDICINE

Transatlantic lessons in regulation of mitochondrial replacement therapy

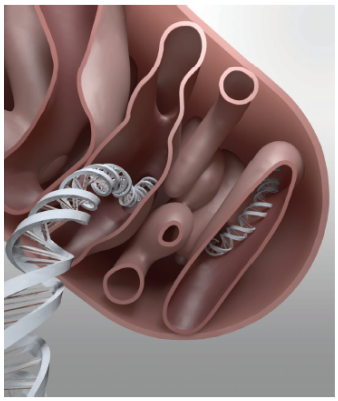
The UK has approved MRT for clinical use, but the discussion has just begun in the U.S.

By I. Glenn Cohen,[1]* Julian Savulescu,[2] Eli Y. Adashi[3]

Mutant mitochondrial DNA (mtDNA) gives rise to a broad range of heritable clinical syndromes (*1*). A cure for those affected remains out of reach (*1*). However, recently developed mitochondrial replacement therapy (MRT) has raised the prospect of disease-free progeny for women carriers (*2*–*4*). Moreover, the feasibility of replacing mutant oocytic or zygotic mtDNA with a donated wild-type counterpart in humans has now been firmly established (*2*–*4*). In the United Kingdom, legislation regulating the clinical application of MRT, now 10 years in the making, has recently been approved by the House of Commons (*5*) and the House of Lords (*6*). The regulatory vetting of MRT in the United States, under way for a year, remains a work in progress (*7*). Here, we compare and contrast the regulatory history of MRT in the United Kingdom and the United States and examine potential lessons learned.

POLICY

THE UK REGULATORY EXPERIENCE. In the United Kingdom, matters relating to reproductive technologies are wholly governed by the Human Fertilisation and Embryology Authority (HFEA), an independent regulatory agency established by Parliament through the Human Fertilisation and Embryology Act of 1990 (HFE Act). Among its responsibilities, the HFEA licenses and monitors human embryo research. In 2005, the HFEA issued a research license to the Newcastle Centre for Mitochondrial Re-

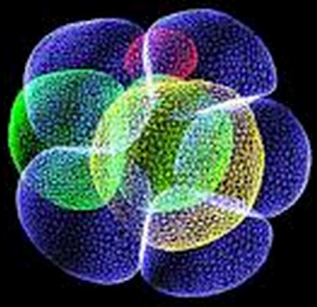


178 10 APRIL 2015 • VOL 348 ISSUE 6231 sciencemag.org SCIENCE

Regulatory Path in UK & USA

- UK HFEA
- HFEA Act 1990
- 2005 research license
- 2011, 2012, 2014 HFEA reports
- 2012 - Public consultation
- 2015 - Parliament gives green light

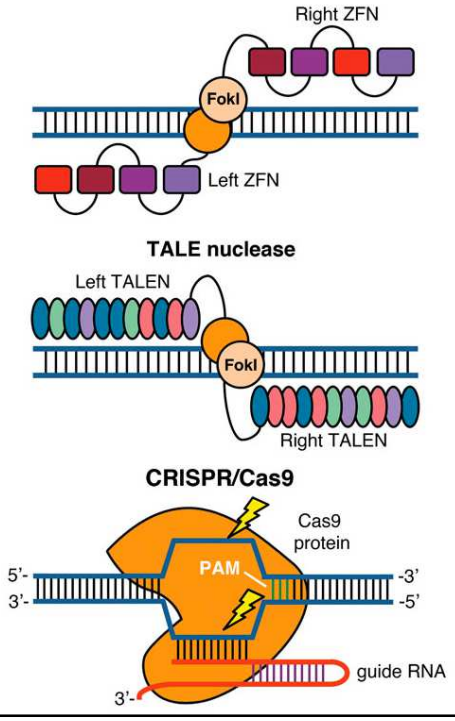
- USA - FDA argued jurisdiction
- 2001 - IND protocol
- 2015 - Committee of IOM
- 2016 - Positive report



Treatment of Mitochondrial Disease

DNA Editing - Talens

PGDIS, Bologna, 2016



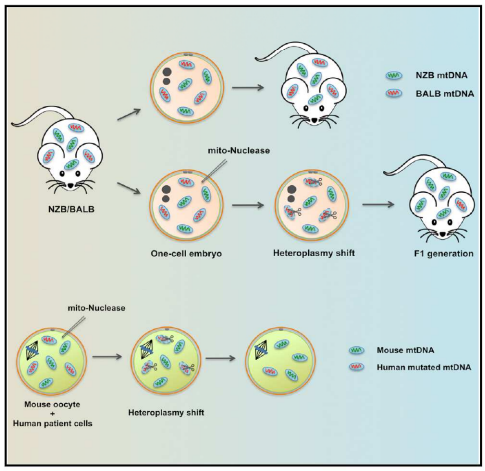
Year	Complexity	Precision
1991	+	-
2009 2011	+	+/-
2012	-	+

Article

Cell

Selective Elimination of Mitochondrial Mutations in the Germline by Genome Editing

Graphical Abstract



Authors

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Carlos T. Moraes,
Juan Carlos Izpisua Belmonte

Correspondence

belmonte@salk.edu

In Brief

Using mitochondria-targeted nucleases, mtDNA mutations are specifically eliminated in the germline to prevent their transgenerational transmission. This strategy represents a potential therapeutic avenue for preventing the transmission of human mitochondrial diseases.

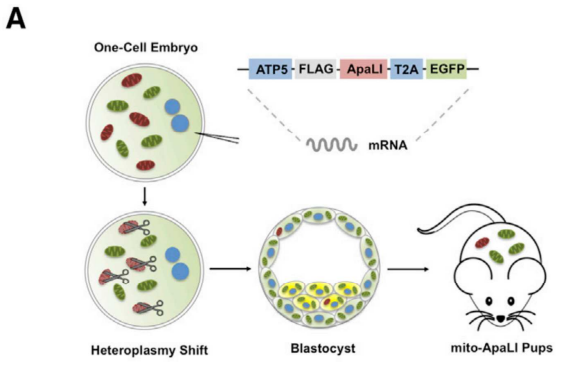
Highlights

- Mitochondria-targeted nucleases selectively reduce mtDNA

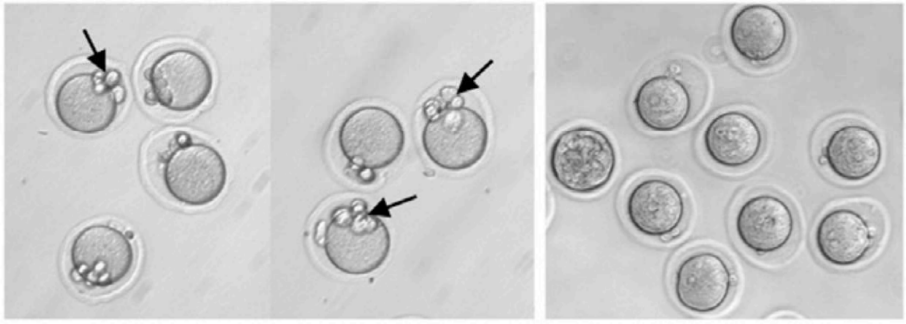
Accession Numbers

GSE67371

2015



Cell Fusion of Mouse Eggs/Zygotes and Human Carrier Cells



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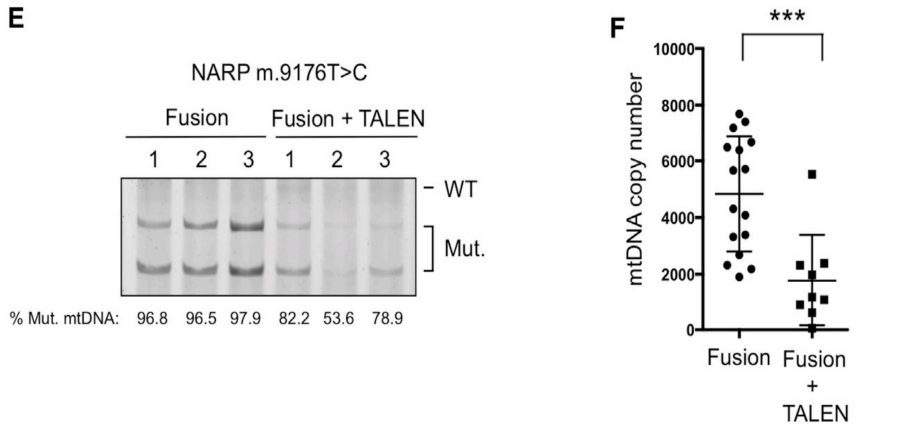
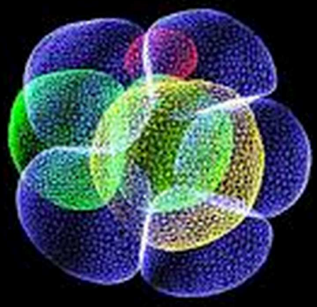




Treatment of Mitochondrial and Genetic Disease

CRISPR Cas9

PGDIS, Bologna, 2016

nature
International weekly journal of science

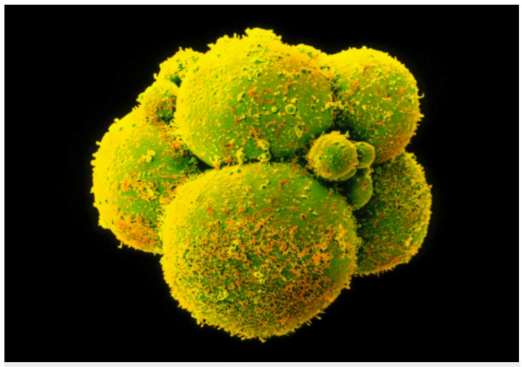
NATURE | NEWS

Chinese scientists genetically modify human embryos

Rumours of germline modification prove true — and look set to reignite an ethical debate.

David Cyranoski & Sara Reardon

22 April 2015



Dr. Yorgos Nikas/SPL

Protein Cell 2015, 6(5):363–372
DOI 10.1007/s13238-015-0153-5

CrossMark Protein & Cell

RESEARCH ARTICLE

CRISPR/Cas9-mediated gene editing in human tripronuclear zygotes

Puping Liang, Yanwen Xu, Xiya Zhang, Chenhui Ding, Rui Huang, Zhen Zhang, Jie Lv, Xiaowei Xie, Yuxi Chen, Yujing Li, Ying Sun, Yaofu Bai, Zhou Songyang, Wenbin Ma, Canquan Zhou✉, Junjiu Huang✉

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ABSTRACT

Genome editing tools such as the clustered regularly interspaced short palindromic repeat (CRISPR)-associated system (Cas) have been widely used to modify genes in model systems including animal zygotes and human cells, and hold tremendous promise for both basic research and clinical applications. To date, a serious knowledge gap remains in our understanding of DNA repair mechanisms in human early embryos, and in the efficiency and potential off-target effects of using technologies such as CRISPR/Cas9 in human pre-implantation embryos. In this report, we used tripronuclear (3PN) zygotes to further investigate CRISPR/Cas9-mediated gene editing in human cells. We found that pressing need to further improve the fidelity and specificity of the CRISPR/Cas9 platform, a prerequisite for any clinical applications of CRSIPR/Cas9-mediated editing.

KEYWORDS CRISPR/Cas9, β-thalassemia, human tripronuclear zygotes, gene editing, homologous recombination, whole-exome sequencing

INTRODUCTION

The CRISPR/Cas9 RNA-endonuclease complex, consisting of the Cas9 protein and the guide RNA (gRNA) (~99 nt), is based on the adaptive immune system of *streptococcus*

Protein & Cell

nature International weekly journal of science

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NATURE | NEWS

Second Chinese team reports gene editing in human embryos

Study used CRISPR technology to introduce HIV-resistance mutation into embryos.

Ewen Callaway

08 April 2016

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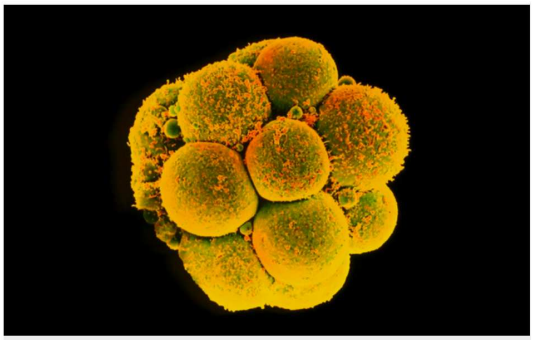

Yorgos Nikas/SPL

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Muzzled

Nine years of censorship

Canadian scientists are now allowed to speak out about their work — and the government policy that had restricted communications.

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A

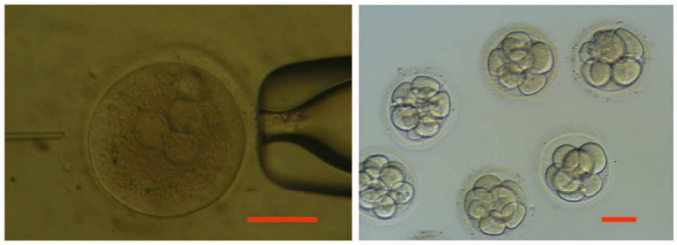
CCR5 exon 4 Δ32

5' acctgcagctctcattttccataca**GTCAGTATCAATTCTGGAAGAATTTCCAGACA**ttaaagatagtcatcttggggctgg 3'

3' tggacgtcgagagtaaaaggt**atgtCAGTCATAGTTAAGAC**CTTCTTAAA**GGTCTGTaatttctatcagtaga**accccgacc 5'

gRNA1 gRNA2

B



3PN Zygote Injection 8-16 Cell Stage

C

gRNA1 E2

5' tctcattttccataca**GTCAGTATCAATTCTGGAAGAATTTCCAGACA**ttaaagatagtcatcttggg 3' WT

tctcattttccatac-**GTCAGTATCAATTCTGGAAGAATTTCCAGACA**ttaaagatagtcatcttggg M

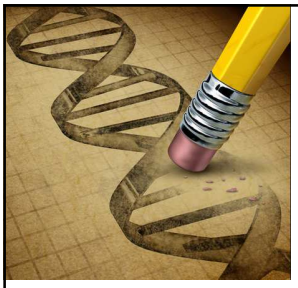
Genome Editing Services

- HSCI - Harvard
- Genscript
- Genetic Services
- Genecopoeia
- Origene
- Best-Gene
- Sigma-Aldrich
- Thermo-Fisher

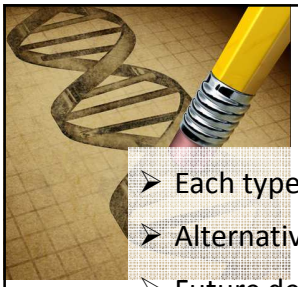
The Council of Europe's Convention on Human Rights and Biomedicine (Oviedo Convention) indicates in Article 13 that:

"*an intervention seeking to modify the human genome may only be undertaken for preventive, diagnostic or therapeutic purposes and only if its aim is not to introduce any modification in the genome of any descendants.*"

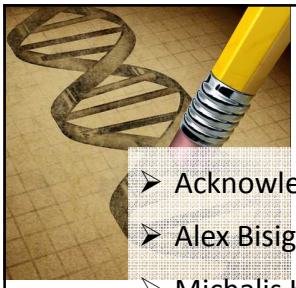
•1999
•The aim was to secure the dignity of human beings within the field of biomedicine.
•Signed 35 states and ratified by 29
•The UK and Germany have neither signed nor ratified the convention.
•The UK considered the convention too restrictive
•Germany thought it too permissive



Conclusions



- Each type of modification must be considered separately
- Alternative treatments must be considered first
- Future development will be affected by:
- (I) International Agreements – Law and Ethics
- (II) 'Smart' Politicians & Public Debate
- (III) Safety studies
- (IV) Bio-informatics
- (V) Inventions of bio-engineering / increased efficiency



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