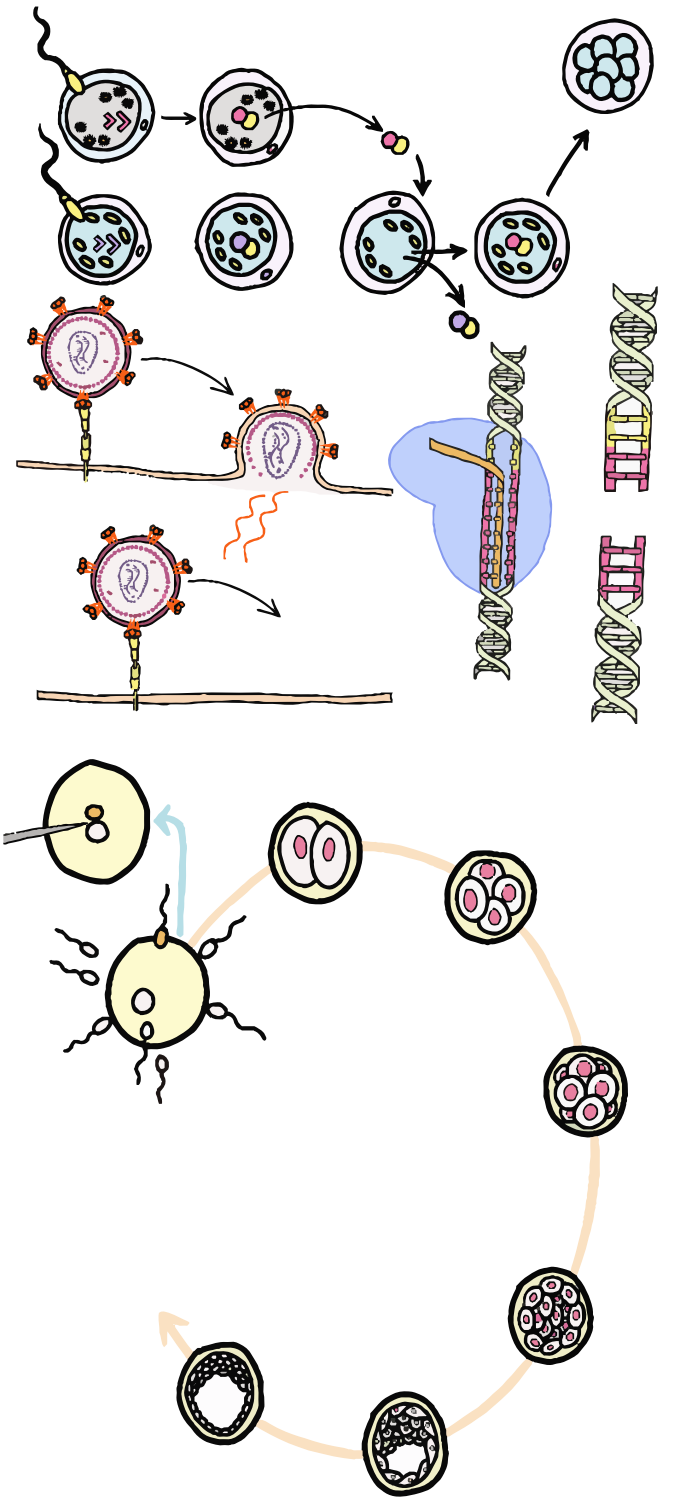
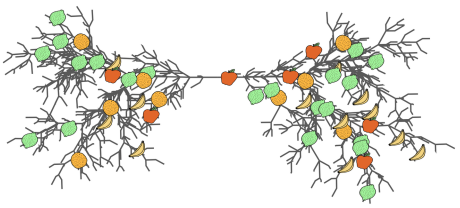
The Three Genome Problem



Top (Mitochondrial Replacement Therapy): A fertilized nucleus is inserted into a donor egg with healthy mitochondria, creating an embryo with parental nuclear DNA and donor mtDNA — preventing transmission of mitochondrial disorders.
Middle Left (CCR5 and HIV Resistance): HIV entry requires CCR5. Individuals with the CCR5-Δ32 mutation lack a functional receptor, blocking viral entry. This natural resistance underpins CCR5-targeted therapies.
Middle Right (CRISPR-Cas9 Genome Editing): Cas9 cuts DNA at a targeted site guided by RNA. This enables correction or knockout of specific genes, forming the basis of precise gene therapy.
Bottom Left (Embryonic Germline Editing): CRISPR introduced at the zygote stage edits all descendant cells, including germline — causing permanent, heritable changes. Ethically and legally restricted.
Bottom Right (Embryo Development): Post-fertilization, the embryo progresses through cleavage stages to blastocyst.

The Three Genome Problem

Every human inherits two distinct genomes: nuclear DNA from both parents and mitochondrial DNA almost exclusively from the oocyte. This second genome — 37 genes controlling cellular energy production — mutates 10–100 times faster than nuclear DNA, causing devastating diseases when defective. Traditional IVF cannot prevent mothers from passing faulty mitochondria to children. Enter mitochondrial replacement therapy: scientists transfer nuclear DNA from an affected mother's egg into a donor egg with healthy mitochondria. From single-base edits to chromosome transfers — many ethical questions arise to be discussed.



In Vitro Fertilization ◦ Mitochondrial DNA Inheritance ◦ Maternal mtDNA Transmission ◦ Mitochondrial Disease ◦ Mitochondrial Replacement ◦ Three-Parent Baby ◦ NUMTs Confusion ◦ Genetic Parentage Redefined ◦ He Jiankui CRISPR Case ◦ Oxidative Phosphorylation ◦ Heteroplasmy

"Who in the world am I? Ah, that's the great puzzle!"

— Alice, 1865

"It has not escaped our notice that the specific pairing we have postulated immediately suggests a possible copying mechanism for the genetic material."

— James D. Watson and Francis H. Crick, 1953

The Three Genome Problem

The effort to prevent the maternal transmission of mitochondrial DNA (mtDNA) diseases originated from the recognition, during the late twentieth century, that certain debilitating disorders such as Leigh syndrome, MELAS, and Leber's hereditary optic neuropathy were caused by mutations (Wallace et al., 1988) in the mitochondrial genome. Unlike nuclear genetic diseases, mitochondrial disorders presented a unique challenge: strict maternal inheritance, random bottleneck effects during oogenesis, and heteroplasmy complicated genetic counseling and prediction of disease severity.

Early experiments in the 1990s explored the feasibility of cytoplasmic transfer between oocytes, aiming to improve oocyte competence rather than prevent disease. These procedures, known as ooplasmic transfer, resulted in the births of children carrying a mixture of maternal and donor mitochondria, raising ethical and regulatory concerns. In 2001, the U.S. Food and Drug Administration (FDA) halted cytoplasmic transfer procedures, citing insufficient safety data and concerns regarding heritable genetic modification.

Scientific focus then shifted toward targeted nuclear transfer techniques. In 2009, Tachibana et al. demonstrated in rhesus macaques that meiotic spindle transfer (MST) could successfully prevent the transmission of maternal mtDNA mutations without compromising embryo viability. This work provided the first preclinical evidence supporting mitochondrial replacement as a viable therapeutic strategy.

Pronuclear transfer (PNT), originally demonstrated in murine models in 1983, was adapted for use in human embryos by Craven et al. in 2010. Subsequent refinements by research groups in the United Kingdom and United States established that both MST and PNT could achieve low levels of mitochondrial carry-over and support normal embryonic development to the blastocyst stage.

In 2015, the United Kingdom became the first country to formally legalize mitochondrial replacement therapies (MRTs) under strict regulation, following extensive public consultation and scientific review by the Human Fertilization and Embryology Authority (HFEA). Clinical licenses were granted on a case-by-case basis for preventing the transmission of serious mitochondrial diseases.

The first reported live birth resulting from MRT occurred in 2016 via spindle transfer, performed by a clinical team led by Dr. John Zhang. The procedure was carried out partially in the United States and partially in Mexico to circumvent regulatory barriers, marking a controversial milestone in the field.

Parallel developments occurred at the Nadiya Clinic in Kyiv, Ukraine. In 2016–2017, researchers led by Dr. Valery Zukin and Dr. Pavlo Mazur implemented pronuclear transfer protocols adapted for infertility treatments, reporting multiple pregnancies and births.

Research efforts have since expanded to include polar body transfer (PB1T and PB2T) as alternative MRT strategies, aiming to further minimize mitochondrial carry-over and ethical concerns related to zygote destruction. Long-term follow-up studies and multigenerational observations remain essential to fully assess the safety, efficacy, and societal implications of mitochondrial replacement technologies.

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Fertilization in humans begins when a sperm cell successfully penetrates the outer membrane of the oocyte. Upon entry, the sperm delivers its haploid set of 23 chromosomes into the oocyte cytoplasm. The oocyte, already arrested in metaphase II of meiosis, completes its meiotic division and expels the second polar body. The fusion of the male and female pronuclei forms a single diploid nucleus, establishing the genomic foundation of the zygote.

Following fertilization, the zygote undergoes a series of rapid mitotic divisions known as cleavage. These divisions increase cell number without increasing the overall size of the embryo, partitioning the cytoplasm into progressively smaller blastomeres. Around the 16- to 32-cell stage, the embryo compacts to form a morula, and by the fifth to sixth day post-fertilization, a fluid-filled cavity called the blastocoel develops, creating a blastocyst. The blastocyst consists of an inner cell mass, destined to form the embryo, and an outer trophoblast layer that facilitates implantation into the uterine endometrium.

The nuclear DNA of the zygote comprises 46 chromosomes, organized into 23 homologous pairs. One chromosome of each pair is inherited from the oocyte, and the other from the sperm. These chromosomes encode the genetic information required for human development, regulating processes from cell cycle control to tissue differentiation. The nuclear genome is distributed across the nucleus of each embryonic cell, and its faithful replication is critical for maintaining genomic integrity throughout embryogenesis.

In addition to nuclear DNA, each human cell contains mitochondria in the cytoplasm that produce cellular energy. Within each mitochondrion exists a small, circular DNA molecule known as mitochondrial DNA (mtDNA). Unlike nuclear DNA, which is packaged into chromosomes within the nucleus, mtDNA is physically separate and exists in multiple copies per mitochondrion. The mitochondria are inherited maternally through the oocyte's cytoplasm.

Mitochondrial DNA encodes components for oxidative phosphorylation, the biochemical pathway that generates adenosine triphosphate (ATP) through electron transport and proton gradient-driven synthesis (Mitchell, 1961). Specifically, mtDNA contains 37 genes: 13 encoding protein subunits of the respiratory chain complexes, 22 encoding transfer RNAs, and 2 encoding ribosomal RNAs necessary for mitochondrial protein synthesis. These elements are indispensable for cellular metabolism.

Unlike nuclear DNA, mtDNA is highly susceptible to mutations. It lacks protective histone proteins, possesses limited DNA repair mechanisms, and sits near the electron transport chain, a major source of reactive oxygen species. These conditions result in a mutation rate for mtDNA that is approximately 10 to 100 times higher than that of nuclear DNA. Mutations in mtDNA accumulate over time and can disrupt the efficiency of oxidative phosphorylation, impairing cellular energy production.

Mutations in mitochondrial DNA cause diseases characterized by impaired energy metabolism. Because mitochondria are responsible for supplying the majority of cellular ATP, defects in oxidative phosphorylation have the greatest impact on tissues with high metabolic demands. Clinical manifestations include neurological disorders such as encephalopathy and seizures, muscular disorders such as myopathy and exercise intolerance, cardiomyopathies, and sensory deficits including optic neuropathy and hearing loss. The severity of these diseases

often correlates with the proportion of mutated mtDNA within affected cells, a condition known as heteroplasmy, and with the energy thresholds required by different tissues.

In vitro fertilization (IVF) combines retrieved oocytes and prepared sperm outside the human body under controlled laboratory conditions. The process begins with controlled ovarian hyperstimulation, during which exogenous gonadotropins are administered to stimulate the development of multiple follicles. Once sufficient follicular maturation is confirmed by ultrasound and hormone measurements, oocyte retrieval is performed transvaginally under ultrasound guidance. Retrieved oocytes are assessed for maturity and subsequently exposed to motile sperm, either by conventional insemination or by intracytoplasmic sperm injection (ICSI) (Palermo et al., 1992), in which a single sperm cell is mechanically introduced into the oocyte cytoplasm.

Fertilized embryos are cultured in specialized media supporting preimplantation development. Embryos are monitored for cleavage patterns, morphology, and progression to the blastocyst stage, typically over a period of five to six days. Selection criteria based on morphological quality and developmental timing guide the choice of embryos for transfer. One or more embryos are transferred into the uterine cavity using a catheter, aiming to establish implantation and initiate a clinical pregnancy. Remaining viable embryos may be cryopreserved for future use.

In standard IVF procedures, the oocyte's cytoplasm, including its mitochondrial content, is transmitted unchanged to the resulting embryo. Because mitochondria and mtDNA are maternally inherited, IVF does not prevent the transmission of pathogenic mtDNA mutations. Someone with defective mtDNA can pass these mutations to offspring through the oocyte cytoplasm in both natural conception and IVF.

Mitochondrial replacement therapies (MRT) prevent the transmission of mutated mtDNA. These techniques involve transferring the nuclear genetic material from an oocyte or zygote carrying pathogenic mtDNA into a donor cytoplasm containing healthy mitochondria. Two methods exist: maternal spindle transfer, performed before fertilization, and pronuclear transfer, performed after fertilization but before pronuclear fusion. Both methods aim to preserve the intended parents' nuclear genome while replacing the defective mitochondrial population with functional donor-derived mitochondria.

The strict maternal inheritance of mtDNA involves active mechanisms to eliminate paternal mitochondria, including degradation during spermatogenesis and post-fertilization mitophagy.

Rare reports have described cases of paternal mtDNA transmission. High-throughput sequencing technologies occasionally detect mtDNA sequences in offspring that do not match the maternal lineage, suggesting a potential contribution from the sperm. Initial interpretations proposed that paternal mitochondria might occasionally evade elimination mechanisms and be transmitted to the offspring at detectable levels.

After some excitement in the field about this alternative inheritance mechanism, it was realized that these observations often result from nuclear mitochondrial DNA segments (NUMTs) (Lopez et al., 1994) — fragments of mtDNA incorporated into the nuclear genome over evolutionary time that closely resemble true mitochondrial sequences. NUMTs are

inherited in a Mendelian fashion and can be misinterpreted as paternal mtDNA when standard sequencing techniques co-amplify nuclear and mitochondrial DNA. Indeed, multiple studies have shown that many apparent cases of biparental mtDNA inheritance are artifacts caused by the presence of large, recently inserted NUMTs in the nuclear genome rather than true transmission of paternal mitochondria.

On the Semantics of Genetic Parentage

In laboratory settings, mitochondrial DNA replacement and more complex genomic interventions — ranging from whole chromosome transfers to single-base CRISPR edits — are becoming routine. Popular discourse often responds with labels such as "three-parent baby," referring to cases where nuclear DNA comes from two individuals and mtDNA from a third. As such procedures proliferate and more nuanced manipulations emerge, questions like "who are the parents?" or "how many parents are there if 10% of the genome is replaced?" become ill-posed.

This is not unlike the conceptual shift that occurred in physics a century ago. Questions such as "what is light?" or "what is an electron?" gave way to rigorously framed operational questions: "what signal will appear on a detector under specified experimental conditions?" Biology is undergoing a similar transition. Rather than asking "who are the parents?", the relevant question becomes "what proportion of the genome is shared with each contributor?". Genetic parentage, in this view, becomes a quantitative map of biological contribution. This technical definition intentionally separates the biological facts from the equally valid concepts of social and emotional parentage, which are defined orthogonally by nurture, commitment, and care.

A parallel imprecision pervades discussions of "biological sex." The term conflates multiple distinct but correlated traits — chromosomal karyotype, gonadal structure, hormonal profiles, anatomical morphology, and secondary sexual characteristics — that do not always align. No single definition encompasses all cases. Scientific precision requires specifying which measurable trait is under discussion: XX/XY karyotype, circulating testosterone levels, presence of particular anatomical structures, or expression of specific gene regulatory networks. The phrase "biological sex" functions as convenient shorthand but lacks the rigor of a well-defined biological variable.

This is not a debate about politics, gender identity, or social inclusion — it is a matter of definitional rigor. Science advances by defining terms precisely and specifying what is being measured. When researchers invoke "biological sex" without clarifying whether they mean chromosomal composition, hormonal milieu, or anatomical phenotype, they introduce ambiguity that weakens scientific reasoning. Sloppy terminology generates confusion across medicine, developmental biology, and public policy, much as the now-discredited concept of "biological race" once did. Scientific language should describe measurable phenomena rather than compress multidimensional biological variation into a single inherited categorical label for convenience.

CRISPR Babies

In late 2018, a Chinese biophysicist named He Jiankui announced the birth of twin girls whose genomes had been edited at the embryonic stage. Using the CRISPR-Cas9 system (Jinek et al., 2012), He targeted the CCR5 gene, seeking to introduce a mutation associated with resistance to HIV infection. The announcement, made through YouTube videos and public statements rather than peer-reviewed scientific channels, bypassed established academic and regulatory norms. This triggered international condemnation from scientists, ethicists, and government bodies.

The CCR5 gene, while involved in HIV susceptibility, also participates in brain development, immune response regulation, and other physiological processes. Editing this gene without comprehensive knowledge of its systemic effects introduced unknown biological risks. Established medical procedures such as sperm washing already enabled HIV-positive parents to conceive healthy children, making the intervention medically unnecessary.

The consent forms misled participating families about the experimental nature and unknown risks of the intervention. Off-target edits — unintended mutations elsewhere in the genome — were not assessed before embryo implantation. The CRISPR modifications were heritable, meaning any unintended effects would be transmitted to future generations without their consent.

In December 2019, Chinese authorities sentenced He Jiankui to three years in prison for "illegal medical practices," alongside financial penalties and professional bans. Two of his collaborators received lesser sentences. The incident marked the first criminal prosecution for human germline genome editing and catalyzed global discussions about regulatory frameworks, ethical standards, and the governance of emerging biotechnologies.

After his release from prison in 2022, He Jiankui publicly stated that the three children born from the experiment — the twin girls and a third child — are "healthy and living normal lives," though no independent medical verification or peer-reviewed follow-up data has been released. He has since attempted to re-enter research, claiming to focus on non-reproductive gene therapies, while China has enacted stricter bioethics and genetic-research regulations that explicitly ban germline editing in clinical settings. As of 2025, the health, developmental outcomes, and genetic integrity of the edited children remain unknown.

Micromanipulation and Mitochondrial Heteroplasmy

Technical Challenges in Nuclear Transfer

Maternal spindle transfer requires extracting the metaphase II spindle-chromosome complex without disrupting chromosome alignment. The spindle, a 15–20-μm birefringent structure visible under polarized light (Oosight™), must be aspirated with minimal surrounding cytoplasm. Tachibana et al. (2009) demonstrated this technique in rhesus macaques, achieving no detectable donor mtDNA carry-over and producing healthy, fertile offspring.

Critical technical parameters:

- **Timing**: Within 2 hours of oocyte retrieval before spindle depolymerization.
- **Pipette diameter**: 20 μm beveled, approaching at 30° to minimize membrane deformation.
- **Cytoplasmic volume**: <5 picoliters co-aspirated to keep mitochondrial carry-over below 2%.
- **Fusion method**: Electrofusion (1.0 kV/cm, 50 μs pulses) or HVJ-E (Sendai virus extract).

Pronuclear transfer exploits the 8–10 hour window post-fertilization when pronuclei are visible but unfused. Both pronuclei must be extracted together within a karyoplast — a membrane-bound cytoplasmic package containing ∼5% of oocyte volume. This preserves their relative positioning, which carries epigenetic information essential for proper development.

Mitochondrial Carry-over and Detection

Even stringent micromanipulation cannot eliminate all donor mitochondria. Reported carry-over levels in embryo reconstruction studies include:

- PNT: typically <2%, occasionally higher (Hyslop et al., 2016).
- MST: approximately 1–2% on average (Tachibana et al., 2013).
- PB1T: often <0.5% when optimized (reported in polar body transfer studies).

Deep sequencing can achieve ∼0.1% sensitivity, with digital droplet PCR detecting heteroplasmy as low as ∼0.01% in optimized assays.

Heteroplasmy Dynamics in Development

Low-level heteroplasmy behaves unpredictably during development due to:

The Mitochondrial Bottleneck: During oogenesis, mtDNA copy number drops from ∼100,000 in mature oocytes to ∼200 in primordial germ cells before clonal expansion. This bottleneck allows random genetic drift to dramatically shift heteroplasmy levels between generations.

Tissue-Specific Segregation: Post-mitotic tissues show divergent heteroplasmy patterns:

- **Muscle**: Can amplify from 1% to 50% by adulthood.
- **Blood**: Typically maintains stable levels.
- **Brain**: Shows regional variation, with high-energy regions (substantia nigra) potentially enriching for wild-type mtDNA.

Nuclear-Mitochondrial Compatibility

The mitochondrial proteome comprises ∼1,500 proteins: 13 encoded by mtDNA, the remainder nuclear-encoded and imported. OXPHOS complexes require precise stoichiometry between nuclear and mitochondrial subunits.

Potential incompatibilities arise where mtDNA-encoded subunits must interact with nuclear-encoded partners (e.g., Complex I: 7 mitochondrial and 38 nuclear subunits). However, the Tachibana primate studies (2009) showed reassuring intraspecies compatibility.

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