THE ROLE OF THE SPERM IN FACILITATION OF FERTILIZATION

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Has Conventional IVF Reached Its Limits?

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NO CONFLICTS – RESEARCH AND MENTORING SPONSORED BY THE US NATIONAL INSTITUTES OF HEALTH
sperm nucleus

centriole

cytoplasm

membranes

mitochondria

sperm tail

RNA
PATERNAL INHERITANCE OF MITOCHONDRIAL DNA

MARIANNE SCHWARTZ, PH.D., AND JOHN VISSING, M.D., PH.D.

Malian mitochondrial DNA (mtDNA) is thought to be strictly maternally inherited. Sperm mitochondria disappear in early embryogenesis by selective destruction, inactivation, or simple dilution by the vast surplus of oocyte mitochondria. Very small amounts of paternally inherited mtDNA have been detected by the polymerase chain reaction (PCR) in mice after several generations of interspecific backcrosses. Studies of such hybrids and of mouse oocytes microinjected with sperm support the hypothesis that sperm mitochondria are targeted for destruction by nuclear-encoded proteins. We report the case of a 28-year-old man with mitochondrial myopathy due to a novel 2-bp mtDNA deletion in the ND2 gene (also known as MTND2), which encodes a subunit of the enzyme complex I of the mitochondrial respiratory chain. We determined that the mtDNA harboring this mutation was paternally inherited by PCR in muscle. The abnormal findings in muscle-biopsy specimens from both thighs and the finding of severely impaired oxygen extraction when the forearm muscles were repeatedly contracted suggested generalized muscular involvement.

METHODS

DNA was isolated from the patient's blood, muscle, hair roots, and fibroblasts (derived from a skin biopsy) by standard methods. DNA was also isolated from the blood of the patient's parents and paternal uncle, and from the blood and the quadriceps muscle of the patient's sister. The mtDNA was amplified into two products with the primers OLA (5756-5781) + D1B (5282-255) and D1A (336-363) + OLB (5745-5721), and the products were purified. We sequenced most of the mtDNA, including all transfer RNA (tRNA) genes, CYTB, and all seven genes encoding subunits of enzyme complex I, using a genetic analyzer (ABI PRISM 310, Applied Biosystems) and a terminator cycle-sequencing ready-reaction kit (ABI PRISM BigDye, Applied Biosystems). The sequences obtained were compared with the revised Cambridge reference sequence10 (AC J01415) with use of the DNAsis program (Hitachi software Engineering Europe).

Two different mtDNA haplotypes were found in the patient; presumably, one came from the father and the other from the mother. Solid-phase minisequencing5 was performed to establish the ratios of the mtDNA haplotypes in blood and muscle. The target was nucleotide position 3197, which, among others, distinguished the paternal haplotype (3197T) from the maternal one (3197C). PCR products spanning the position in question were generated by the 5'-biotinylated forward primer (3014-3034) and the reverse primer (3376-3356). PCR products were immobilized on a streptavidin-coated solid support (96-well plate) and denatured by sodium hydroxide. A sequencing primer (3220-3198) was designed to anneal adjacent to (upstream from) nucleotide 3197.

The nucleotide at position 3197 was analyzed by the primer extension reaction, in which a tritium-labeled deoxycytidine triphosphate corresponding to either the maternal nucleotide (deoxyadenosine triphosphate) or the paternal nucleotide (deoxyguanosine triphosphate) was added to two parallel reactions. After washing, the elongated primers were eluted by sodium hydroxide, and the amount of incorporated [3H]deoxycytidine monophosphate was determined with a liquid scintillation counter. The ratios of adenine to guanine incorporated into each sequencing primer were determined and compared with the values on a standard curve constructed on the basis of known proportions of cloned segments of mtDNA harboring 3197T and 3197C, respectively.

The ratio of the 2-bp deletion to wild-type mtDNA in tissues (the level of heteroplasmy) was determined by PCR fragment analysis. The mtDNA was amplified by the 5'-fluorochrome-labeled forward primer (5041-5060) and the reverse primer (5196-5177). The PCR products were analyzed on a genetic analyzer with a GeneScan standard (PE Applied Biosystems) as a size marker. The areas of the mutant (2-bp deletion) and wild-type peaks were used to calculate the percentage of mutant (paternal) mtDNA in the patient's muscle.

The nuclear genotypes of the patient, his parents, and his sister were determined for the highly polymorphic markers (microsatellites) D7S212, D7S817, D19S219, D19S559, and TNFβ. PCR primers were analyzed on a genetic analyzer with GeneScan software (Applied Biosystems) as size markers. The areas of the mutant (2-bp deletion) and wild-type peaks were used to calculate the percentage of mutant (paternal) mtDNA in the patient's muscle.

The abnormal findings in muscle-biopsy specimens from both thighs and the finding of severely impaired oxygen extraction when the forearm muscles were repeatedly contracted suggested generalized muscular involvement.

CASE REPORT

The patient was a 28-year-old man with severe, lifelong exercise intolerance. He had never been able to run more than a few steps. His cardiac and pulmonary functions were normal, and he was orthopneic at rest. Both parents and a 23-year-old sister were healthy and had normal exercise tolerance.

The myopathic symptoms were associated with severe lactic acidosis induced by minor physical exertion. His plasma lactate level after walking 100 m at a slow pace was 6 to 8 mmol per liter (the normal level is below 2.5 mmol per liter). His creatine kinase levels were marginally elevated in periods of no physical exertion. Biopsies of the right and left quadriceps muscles revealed that 15 percent of the fibers were of the ragged-red type, a result consistent with the accumulation of abnormal mtDNA with impaired respiratory function. Biochemical analysis demonstrated an isolated deficiency of the mitochondrial enzyme complex I of the respiratory chain and an isolated deficiency of the mitochondrial enzyme complex III of the respiratory chain. The abnormal findings in muscle-biopsy specimens from both thighs and the finding of severely impaired oxygen extraction when the forearm muscles were repeatedly contracted suggested generalized muscular involvement.

The myopathies of the patient, his parents, and his sister were determined for the highly polymorphic markers (microsatellites) D7S212, D7S817, D19S219, D19S559, and TNFβ. PCR primers were analyzed on a genetic analyzer with GeneScan software (Applied Biosystems) as size markers. The areas of the mutant (2-bp deletion) and wild-type peaks were used to calculate the percentage of mutant (paternal) mtDNA in the patient's muscle.
Biparental Inheritance of Mitochondrial DNA in Humans

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Although there has been considerable debate about whether paternal mitochondrial DNA (mtDNA) transmission may coexist with maternal transmission of mtDNA, it is generally believed that mitochondria and mtDNA are exclusively maternally inherited in humans. Here, we identified three unrelated multigeneration families with high-level of mtDNA heteroplasmy (ranging from 24 to 76%) in a total of 17 individuals. Heteroplasmy of mtDNA was independently examined by high-depth whole mtDNA sequencing analysis in our research laboratory and in two Clinical Laboratory Improvement Amendments and College of American Pathologists-accredited laboratories using multiple approaches. A comprehensive exploration of mtDNA segregation in these families shows biparental mtDNA transmission with an autosomal dominant inheritance mode. Our results suggest that, although the central dogma of maternal inheritance of mtDNA remains valid, there are some exceptional cases where paternal mtDNA could be passed to the offspring. Elucidating the molecular mechanism for this unusual mode of inheritance will provide new insights into how mtDNA is passed on from parent to offspring and may even lead to the development of new avenues for the therapeutic treatment for pathogenic mtDNA transmission.

Human genetics | mitochondrial inheritance | paternal transmission | mtDNA transmission

Mitochondria are energy-generating organelles that play a critical role in numerous cellular functions, including ATP production, cellular homeostasis, and apoptosis (1). Unlike nuclear DNA, in which there are only two copies of each gene per cell, thousands of copies of mitochondrial DNA (mtDNA) are present in every nucleated cell. Typically, each individual harbors only one mtDNA genotype (that of the mother), and all mitochondrial genotypes are approximately genetically identical (homozygous). In many mitochondrial diseases, however, wild-type and mutant maternal alleles coexist, and this is known as heteroplasmy (2). The extent of heteroplasmy may vary among tissues and contribute to mitochondrial disease severity. In this paper, we present work that shows that there are rare exceptions to the strict maternal inheritance pattern and that paternal contributions can be made to the mtDNA of the offspring.

In humans, since mitochondria (and thus mtDNA) are typically transmitted to subsequent generations exclusively through the maternal lineage (3), a clinically asymptomatic woman with low-levels of a deleterious heteroplasmic mtDNA mutation may pass it to all of her offspring, resulting in mtDNA dysfunction and disease. The severity of clinical symptoms in affected children is often associated with the level of mtDNA heteroplasmy (i.e., the percentage of the deleterious mutation) (4). For example, the heteroplasmic mtDNA m.8993T>G mutation causes Leber hereditary optic neuropathy (5, 6), a condition that is associated with regression in mental and motor skills, disability, and death due to seizures and respiratory failure (4, 5). When the mtDNA m.8993T>G mutation load is less than 10%, it is not expected to be symptomatic. The probability of having an asymptomatic woman with the m.8993T>G load reaching 60–70% for the m.8993T>G mutation (6). Given their strict maternal inheritance, the options for treating pathogenic mtDNA remain limited. Transmission of mtDNA mutations can potentially be avoided by using technologies, such as oocyte or sperm transfer to reconstitute a carrier embryo in the cytoplasm of enucleated donor oocytes that do not carry any mtDNA mutations. Once reconstituted, such embryos could be implanted in vitro fertilized and used to establish in vitro fertilization procedures, resulting in a so-called “three-parent baby.” This process has already been successfully used to treat a m.8993T>G carrier with an extensive history of miscarriages and early death of offspring, resulting in the birth of a healthy child in early 2016 (7). However, most countries do not currently permit carrying embryos created through mitochondrial replacement therapy to term due to ethical controversies over mixing genetic material from different individuals. In addition, the procedure

Significance

The energy-producing organelle mitochondrion contains its own compact genome, which is separate from the nuclear genome. In nearly all mammals, this mitochondrial genome is inherited exclusively from the mother, and transmission of paternal mitochondrial or mitochondrial DNA (mtDNA) has not been convincingly demonstrated in humans. In this paper, we have uncovered multiple instances of biparental inheritance of mtDNA spanning three unrelated multigeneration families, a result confirmed by independent sequencing across multiple laboratories with different methodologies. Surprisingly, this pattern of inheritance appears to be determined in an autosomal dominant manner. This paper profoundly alters a widespread belief of mitochondrial inheritance and potentially opens a novel field in mitochondrial medicine.


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Biparental mtDNA inheritance pattern in Family A. (A) Pedigree of Family A. The black filled symbols indicate the four family members (II-1, II-3, II-4, and III-6) showing biparental mtDNA transmission, and the diagonal filled symbols indicate the...
The safety of intracytoplasmic sperm injection and long-term outcomes

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This paper is part of an Anniversary Issue celebrating 25 Years of intracytoplasmic sperm injection. The Guest Editor for this section was Professor Gianpiero Palermo. Professor Gianpiero Palermo was not involved in the peer review of this paper, on which he is listed as an author.

Abstract

The pioneering of intracytoplasmic sperm injection (ICSI) approximately 25 years ago revolutionized the treatment of infertile couples. Today, ICSI remains an indispensable part of assisted reproductive treatments (ART) and has resulted in the birth of millions of babies. The 25th anniversary of ICSI marks a chronologic landmark in its evolving history. This landmark also serves as an opportunity to thoroughly appraise the safety of ICSI and analyze the long-term outcomes of ICSI-conceived children. In this review, we collate and analyze salient data accrued over the past 25 years pertaining to the long-term safety of ICSI and ICSI conceptions. We also evaluate the effects of ICSI on the perinatal outcomes, congenital malformation rates, cognitive development and reproductive health of ICSI-conceived neonates, children, adolescents and adults, respectively. In doing so, we also highlight the existence of potential confounders and biases that frequently obscure the interpretation of clinical follow-up studies.

Reproduction (2017) 154 F61–F70

Introduction

The birth of the first babies using intracytoplasmic sperm injection (ICSI) in 1992 marked a new era of assisted reproductive technologies (ART). Although successful clinical and laboratory protocols for in vitro fertilization (IVF) were established prior to the inception of ICSI, almost 40% of all IVF cycles were inundated by poor fertilization or complete fertilization failure even in the presence of an adequate number of oocytes (Cohen et al. 1984). This was especially evident in couples undergoing IVF for severe male factor infertility (Cohen et al. 1989). As highlighted by Rosenwaks and Pereira in this special edition, micromanipulation techniques such as zona drilling, partial zona dissection and sub-zonal insemination were historically utilized to circumvent the zona pellucida and increase fertilization rates in IVF cycles. However, each of these techniques had their share of limitations, which in turn served as the basis for the development of ICSI (Palermo et al. 1992). ICSI not only bypassed the zona pellucida with high precision, but it also increased the fertilizing potential of a single spermatozoon by injecting it directly into the cytoplasm of an oocyte (Palermo et al. 1992). The technique was adopted by several clinics throughout the world, and it soon became apparent that ICSI was capable of fertilizing almost every mature oocyte that was injected, irrespective of the male partner’s semen parameters (Palermo et al. 1995). Thus, ICSI has become quintessential to modern ART. Global data from the International Committee for Monitoring Assisted Reproductive Technologies (ICMART) estimate that almost 66% of >4,461,309 ART cycles between 2008 and 2010 utilized ICSI (Dyer et al. 2016). In the United States, ICSI utilization has increased from 76.3% in 1996 to 93.3% in 2012 for male factor infertility and 15.4% in 1996 to 66.9% in 2012 for non-male factor indications (Boulet et al. 2015).

In this special edition, Palermo and coworkers have collated almost 25 years’ worth of clinical and research data pertaining to ICSI. They have also highlighted several adjuncts that have not only standardized ICSI globally, but also improved the results obtained with ICSI. However, despite the standardization of ICSI over the past 25 years, concerns about the technique have arisen, even though many are theoretical. These concerns stem from the perception that the injected spermatozoon is selected arbitrarily and that the physiologic steps of zona pellucida binding and oolemma fusion are completely bypassed (Palermo et al. 2008, 2014). Furthermore, there is still concern whether the use of...
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Semen quality of young adult ICSI offspring: the first results
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MAIN RESULTS AND THE ROLE OF CHANCE: Young ICSI adults had a lower median sperm concentration (17.7 million/ml), lower median total sperm count (31.9 million) and lower median total motile sperm count (12.7 million) in comparison to spontaneously conceived peers (37.0 million/ml; 86.8 million; 38.6 million, respectively). The median percentage progressive and total motility, median percentage normal morphology and median semen volume were not significantly different between these groups.

After adjustment for confounders (age, BMI, genital malformations, time from ejaculation to analysis, abstinence period), the statistically significant differences between ICSI men and spontaneously conceived peers remained: an almost doubled sperm concentration in spontaneously conceived peers in comparison to ICSI men (ratio 1.9, 95% CI 1.1–3.2) and a two-fold lower total sperm count (ratio 2.3, 95% CI 1.3–4.1) and total motile count (ratio 2.1, 95% CI 1.2–3.6) in ICSI men compared to controls were found.

Furthermore, compared to men born after spontaneous conception, ICSI men were nearly three times more likely to have sperm concentrations below the WHO reference value of 15 million/ml (adjusted odds ratio (AOR) 2.7; 95% CI 1.1–6.7) and four times more likely to have total sperm counts below 39 million (AOR 4.3; 95% CI 1.7–11.3). In this small group of 54 father–son pairs, a weak negative correlation between total sperm count in fathers and their sons was found.
Application of a ready-to-use calcium ionophore increases rates of fertilization and pregnancy in severe male factor infertility

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Objective: To analyze whether a ready-to-use calcium ionophore improves outcomes, from fertilization to live birth, in patients with severe male factor infertility.

Design: Artificial oocyte activation offered to applicable patients over a 20-month period.

Setting: Specialized in vitro fertilization (IVF) centers in Austria and Germany.

Patient(s): Twenty-nine azoospermic and 37 cryptozoospermic men.

Intervention(s): Mature oocytes treated with a ready-to-use Ca²⁺-ionophore (GM508 Cult-Active) immediately after intracytoplasmic sperm injection (ICSI).

Main Outcome Measure(s): Rates of fertilization, implantation, clinical pregnancy, and live birth.

Result(s): Patients had had 88 previous cycles without artificial activation that resulted in a fertilization rate of 34.7%, 79 transfers (89.8%), and 5 pregnancies, which all spontaneously aborted except one. After artificial oocyte activation, the fertilization rate was 56.9%. In terms of fertilization rate, both azoospermic (64.4%) and cryptozoospermic (48.4%) men statistically significantly benefited from use of the ionophore. In 73 transfer cycles, positive h-human chorionic gonadotropin levels were observed in 34 cases (46.6%) and 29 cycles (39.7%) that ended with a clinical pregnancy. The corresponding implantation rate was 33.3%. Four spontaneous abortions occurred (11.8%) and 32 healthy children were born.

Conclusion(s): This is the first prospective multicenter study on artificial oocyte activation in severe male factor infertility. Present data indicate that a ready-to-use calcium ionophore can yield high fertilization and pregnancy rates for this particular subgroup. In addition to fertilization failure after ICSI, severe male factor infertility is an additional area for application of artificial oocyte activation.

Keywords: Azoospermia, Ca²⁺-ionophore, cryptozoospermia, TESE

Discuss: You can discuss this article with its authors and with other ASRM members at http://fertstertforum.com/ebner-calcium-ionophore-severe-male-factor-infertility/

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In vitro or in conventional in vitro fertilization (IVF), the fertilization process may be subdivided into six major events: [1] cumulus cell penetration, [2] sperm-oocyte binding and penetration, [3] sperm-oocyte fusion, [4] oocyte activation, [5] sperm processing, and [6] pronucleus formation. [4] The pre-sperm-penetration phase (events 1 to 3) may be bypassed using common micromanipulation techniques such as intracytoplasmic sperm injection (ICSI). Consequently, the number of fertilization failures is reduced in ICSI patients as compared with IVF patients. The complexity of the fertilization process may explain its susceptibility to disturbances potentially leading to impaired fertilization rates in spite of the presence of a presumably normal...
Génétique de la Fertilité

Masculine
Nuclear expansion during interphase

- Annulate Lamellae (AL)
- Nuclear Envelope (NE)

**AL-Nuclear Pore Complex**
**NE-Nuclear Pore Complex**

Transcription

Maternal control
Zygotic control

Drosophila embryogenesis

Laterally mobile Nuclear Pores
Clustered, immobile Nuclear Pores
Centrosome Structure

From: Anderhub, SJ, Kramer, A, and Maier, B. Cancer Letters 322(2012) 8-17
Sperm Centrosomes: Kiss Your Asterless Goodbye, for Fertility’s Sake

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Centrosomes are reduced to their cores in sperm. Emerging molecular explanations for centrosome construction have now helped elucidate the mechanism of their destruction in sperm. Since centrosome inaccuracies cause aneuploidy, responsible for cancers, birth defects and infertility, this new insight into centrosome behavior has broad implications.

The joining of sperm and egg is the beginning of development for most animals. Over 125 years ago, the great cell biologist Theodor Boveri recognized the unique contributions of each gamete, writing:

"The ripe egg possesses all of the elements necessary for development save an active division center. The sperm, on the other hand, possesses such a center but lacks the protoplasmic substratum in which to operate. In this respect the egg and sperm are complementary structures; their union in syngamy thus restores to each the missing element necessary to further development. Accepting this it follows that the nuclei of the embryo are derived equally from the two parents; the central bodies [centrosomes] are purely of paternal origin and to this it might be added that the general cytoplasm of the embryo seems to be almost wholly of maternal origin." [1]

Today, we understand that mitochondrial DNA inheritance), and, often, essential determinants of polarity. Centrosome number is also critical, and, like chromosomes, centrosomes are reduced during gametogenesis, such that the proper number is restored in the zygote. While Boveri’s model regarding paternal centrosome inheritance has largely been validated, just how the egg discards its centrosome and exactly what the sperm contributes to the zygote, while Boveri’s model regarding paternal centrosome inheritance has largely been validated, how the egg discards its centrosome and exactly what the sperm contributes to the zygote, remains largely mysterious, with few functional insights. Now, Khire et al. [2], in this issue of Current Biology, add significantly to our understanding of the critically important event of sperm centrosome reduction.

The centrosome, with its embedded pair of centrioles, is the primary microtubule-organizing center of most cells [3,4] (Figure 1). Microtubules are polymers of α- and β-tubulin and are important for intracellular motility and cytoplasmic organization during interphase and for chromosome separation during mitosis and meiosis. Microtubules are polar polymers, with their dynamics characterized in part by nucleation occurring at the centrosome and disassembly taking place at the microtubule end that is anchored in the centrosome, as described by Boveri. The centrosome [5,9] (Figure 1) is an organelle made up of a few dozen core proteins surrounding a pair of centrioles — thus the term ‘pericentriolar material’ for the non-centriole components. Centrioles, the most spectacular intracellular structures in eukaryotes (in the unabashed opinion of the authors), are triplet-microtubule cylinders with nine-fold radial symmetry. Abnormalities in centrosome function or number are responsible for a variety of diseases and disorders, ranging from neurocognitive deficits to birth defects to cancers.

Centrosome replication is remarkable in that the daughter centriole grows orthogonally from the base of the mother centriole (Figure 1). It occurs synchronously with chromosome replication and is governed by cell-cycle licensing factors that prevent reduplication [10]. In addition to the triplet microtubules that define its structure, the centriole consists of perhaps a few dozen essential structural and regulatory proteins [3,6], including Polo-like kinase 4 (Plk4) and Astarless (Asl) — two key proteins identified in the new study to be important for sperm centriole reduction [2]. Plk4 is recognized as the...
“What I investigate is only what, without sinfully defiling myself, remains as a residue after conjugal coitus”

1677 Leeuwenhoek was the first to observe spermatozoa in humans...
**C. elegans**

- **Receptor**: SPD-2
- **Kinase**: ZYG-1
- **Inner tube**: SAS-5 ↔ SAS-6
- **Outer tube**: SAS-4
- **MT wall**: MT

**Other organisms**

- Asterless/Cep152, SPD-2/Cep192
- Plk4
- SAS-6 and Ana2/STIL
- SAS-4/CPAP
- Bld10/Cep135
- MT
LEGOs® and legacies of centrioles and centrosomes

Gerald Schatten & Calvin Simlerly

Centriole construction, now revealed by crystallography, proteomics, and imaging to be a sophisticated assembly of interlocking bricks, resembles LEGO®-albeit centrioles have remarkable dynamic capabilities, including self-assembly and disassembly, kinases and post-translational modifications, self-replication, and still mysterious mechanisms for transmission through each cell cycle and via the gametes during development. Centrioles are created by core proteins that aggregate to form unique ninefold-symmetrical paracrystalline cylinders. The centrosome then coalesces as a cloud of pericentriolar material (PCM) around the centriole. Together they comprise the cell’s microtubule organizing center (MTOC), which governs the shape, functions, and dynamics and differentiation. Yet, the 20th century provided unequivocal proof that DNA molecules are carriers of inheritable information and the constituents of mitochondrial persistence during cell cycles and over generations. Imaging and sequencing technology to analyze chromosomal and mitochondrial genomes yielded a huge bonanza of information for chromosome researchers and geneticists.

Not so for centrosomes and their photogenic sidekicks, the centrioles [1]. Centrosomes, which help to organize the mitotic spindle poles, behave capriciously during interphase, and, worse still, the eerie appearances and spooky vanishings of centrioles, together with their bizarrely orthogonal propagation, have led scholars to describe them, appropriately, as “adver- and depleting centrioles. Remarkably, removal of the drug restored the cancer cell’s centriole amplification. These findings raise questions about the regulators and licensing factors involved in centriole biogenesis [1] and whether it might be possible to prevent or treat cancer by even more precise centriole targeting.

Centriole’s core proteins are somewhat species specific (Fig 1). In humans, it appears that PLK4 triggers centriole formation, together with SPD2/CEP192, PLK1, pericentrin, CDK5/RAP2 among others, recruiting PCM to the centriole. SAS6 and BLD10/CEP135 are important for the car- wheel’s ninefold symmetry. SAS4/CPAP tethers and/or stabilizes centriolar microtubules with CP110 at the centriole’s distal region [2]. Other proteins build the centriole...
Direct Differentiation of Human Pluripotent Stem Cells into Haploid Spermatogenic Cells
Haploid Cells are Generated from hPSCs Cultured in SSC Conditions
Haploid Spermatids from Pluripotent Stem Cells Show Similar Imprint Patterns to Human Sperm
Diagnostic of infertility

- Derivation of iPSCs
- IPSCs
- SSCs
  - Pre-meiotic germ cell
  - Post-meiotic germ cell
  - Round spermatid
- ICSI
- Sperm

in vivo

in vitro

NOT YET!
Researchers Miihon Saitou and Katsuhiko Hayashi have learned how to mimic the intricate stages of natural germ-cell development and to produce sperm and eggs in vitro that can be used to create offspring.

Pluripotent stem cells are extracted from early embryos or induced from somatic cells. These cells are then converted to germ-cell precursors using key growth factors and other signalling molecules.

The cells then develop into mature gametes in vitro.

Female cells are transplanted into ovaries.

Eggs are fertilized with a donor sperm.

The gametes are harvested and used for in vitro fertilization.

Male cells are transplanted into testes.

Sperm are used to fertilize a donor egg.

A fertilized egg is then transplanted into a surrogate mother.

Viable embryos
Adaptive light-sheet microscopy for samples with dynamic size/shape

Live imaging of mouse embryos at single-cell level from gastrulation to organogenesis

Whole-embryo analysis of cell movements and divisions

High-resolution mapping of tissue morphogenesis

Registration of embryos and assessment of developmental variability

Dynamic, cellular atlas of development

Average mouse embryo

Whole-embryo maps of tissue morphodynamics

Cellular-resolution dynamic fate maps
THE ROLE OF THE SPERM IN FACILITATION OF FERTILIZATION

MIRROR NATURE - CAPACITATION & THE ACROSOME REACTION

AVOID CAPRICIOUS SPERM SELECTION – DEVELOP RIGOROUS BIOLOGICAL CRITERIA

USE EJACULATED, OR IF UNAVAILABLE CAUDA EPIDIDYMAL – NOT CAPUT -- SPERM, OR COUNTERINTUITIVELY, TESTICULAR SPERM

AVOID ROUND SPERMATIDS

DISCOVER IMPRINTING DETAILS

EVALUATE MULTIGENERATIONAL CONSEQUENCE