

Remember to turn on zoom

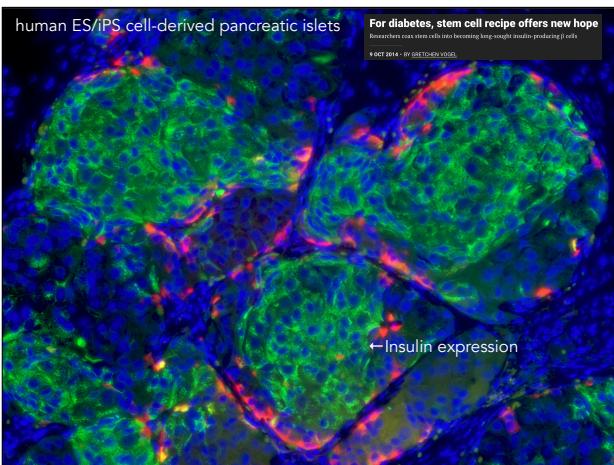
Week 14 — Tuesday, 30 November

Organoids, evolution and disease

Core course concepts and principles and their application / pre-exam 2 review

- Big topics (in class group work):
 - Communications & Cooperation (Brian)
 - Cellular responses (Zach)
 - Asymmetries and Axes (Chhavy)
- We will discuss these in detail - including how specific model systems shaped our understanding and what confusions (you may have) remain
 - study guide will be posted the DEVO website.
- Today - Review last beSocratics
- Finish organoids, human evolution & diseases and environmental effects
 - [Reintroduction of the archaic variant of NOVA1 in cortical organoids alters neurodevelopment](#)

- TEAMS devchat reminder
<https://www.microsoft.com/en-us/microsoft-teams/download-app>



The lab-generated cells should be a valuable tool for studying diabetes and, Melton hopes, could eventually be used to treat patients.

A Cure for Type 1 Diabetes? For One Man, It Seems to Have Worked.

Doug Melton et al

Limb formation

You identify a DNA sequence in the mouse that, when mutated, leads to loss of Shh expression in the limb buds. A similar sequence is identified in the coelacanth or bat. If the mouse sequence is replaced by coelacanth or bat (donor) sequences, the pattern of Shh expression in the limb bud is normal. Predict what will happen to the limb as a whole? (→) and explain your logic (!)

What does Shh expression determine?

The homologous region from the python leads to limbless mice when it is used to replace the mouse sequence. Predict how this change (python → mouse) might influence gene regulatory interactions, e.g. as visualized by "Chromosome conformation capture techniques" (!)

What will be different?

○ unaltered
○ like that of the donor sequence
○ intermediate between mouse and donor
○ no idea

The proximal-distal axis of the limb is analogous to the anterior-posterior/rostral-caudal axis of the body; predict which Hox genes would you expect to be expressed first during limb development and why?

more

WT

Hoxa13/d13 \sim /-

Hoxa11/d11 \sim /-

HoxA/D \sim /-

← Here are phenotypes associated with null mutations in various Hox genes.

Is Hox13 expression dependent on Hox11? (→)
Explain your reasoning; what if anything can you conclude as to the expression of Hox13 in a Hox11 null limb (!)?

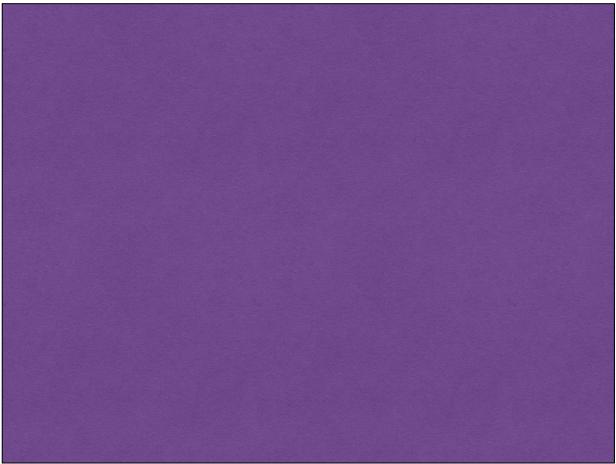
explain here (please)

A

The role of Hox genes during vertebrate limb development
Zimmer, Lavery, and Fallon (2008)

How is this classic experiment in the chick limb (!) similar to or different from the Xenopus organizer transplant experiments we took about early in the course (!).

explain



When making iPS cells from mouse embryo fibroblasts, using the doxycycline-regulated expression of the four transgenic Yamanaka factors (Oct4, Klf4, Sox2 & Myc - see [reference paper](#)). Why don't all of the cells become iPS cells? And do expect all somatic cell types to behave the same (why or why not).

answer here please

In the graph, where would cells make "decisions" about that type of cell they will eventually become (↑);
What is likely to occur at these decision points? (↓)

answer here please

In their reprogramming method, Schiebinger et al used cells from a transgenic mouse in which the four Yamanaka factors are expressed from a DOX-regulated gene cassette. The expression of this gene cassette is turned off after 8 days. Why (↓) don't the cells revert back to embryo fibroblasts?

Media
Doxycycline expression
Days
Collections

or do they?

Blast from the Past 3:

Part 1: Draw a schematic (\rightarrow) of a gene that contains multiple promoters and enhancers that combine to control its expression at specific times and in specific cell types.

Part 2: Indicate how the geometry of the gene changes (\rightarrow) in a specific cell type in which it is expressed.

Part 3: What methods might you use to identify (experimentally) the specific enhancer and promoter sequences involved in cell-type specific expression of a gene, and the accessory factors (proteins) involved (1).

what methods would be useful and how?

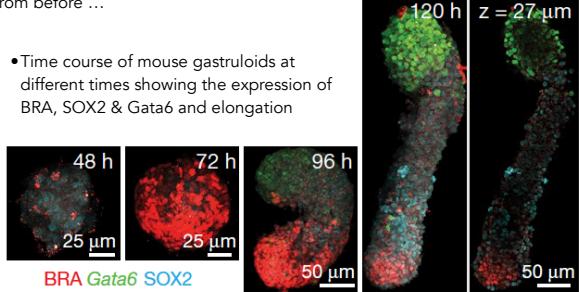


Organoids (using ES and iPS cells) can be used to model human developmental and pathogenic processes.

Multi-axial self-organization properties of mouse embryonic stem cells into gastruloids

Leonardo Beccari^{1,6}, Naomi Morris^{2,6}, Mehmet Gingin^{3,5}, David A. Turner², Peter Murchison², Luisa P. Lanza^{2,7}, Donie Dabholkar^{4,2,7*} & Alfonso Martinez-Arrieta^{2,7*}

- Time course of mouse gastruloids at different times showing the expression of BRA, SOX2 & Gata6 and elongation



Emergent asymmetries (stochastic decisions)

Multi-axial self-organization properties of mouse embryonic stem cells into gastruloids

Chondrocytes from bone marrow mesenchymal stem cells into gastr

In a gastruloid generated from embryonic stem cells, the originally spherical aggregate of 200-300 cells was first treated with a Wnt agonist (Chi) while in suspension culture & then placed in a shaking culture. During this period asymmetric gene expression appears.

Propose & explain a model (considering class materials) for how the initial asymmetry of the gastruloid might appear and be maintained? (1)

answer here please

a Elongation of gastruloids.

b

Based on the [Beccari et al paper](#) (and your class notes) as in vitro gastruloid development proceeds, describe the expression of the Hoxd genes, and explain why their expression is similar to or different from the expression in the developing embryo. (→)

answer here please

Organoids (using ES and iPS cells) can be used to model human developmental and pathogenic processes.

In situ hybridization of 168 h AA gastruloids using probes for various Hoxd genes.

Multi-axis self-organization properties of mouse embryonic stem cells into gastruloids

Lennardo Beccari¹, Nanni Melega², Stefano Giorgi¹, Ariadna Llorente¹, Paola Balbo¹, Alonso¹, Anne-Catherine Gossy¹, Martínez P. Llorente¹, Denis Dubrulle^{1,2*} & Alfonso Martinez Arias^{1,2*}

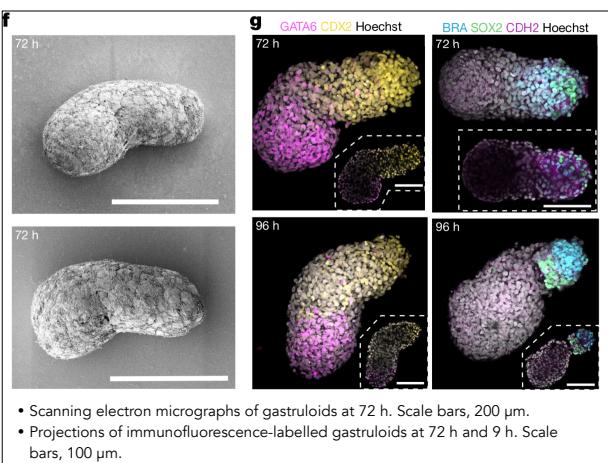
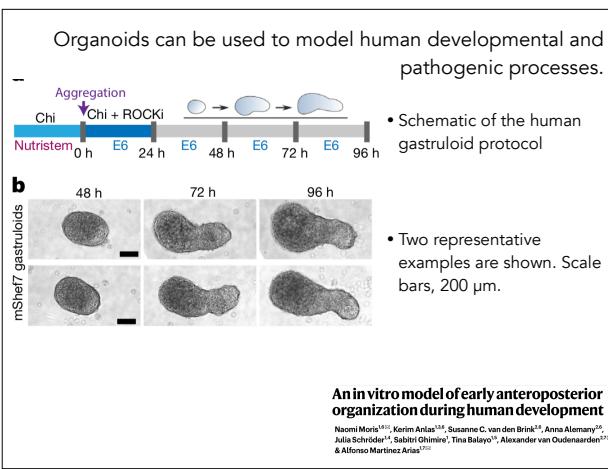
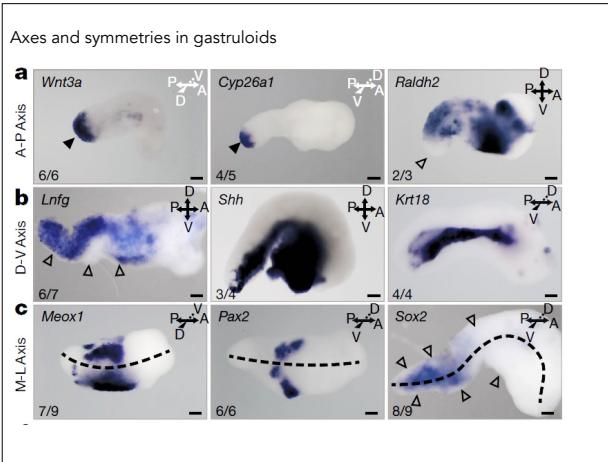
Transcript profiles over the HoxA cluster, time-sequenced pooled gastruloids.

b

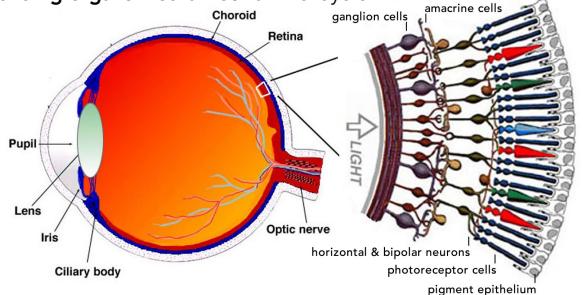
A progressive wave of transcription through Hoxa genes is observed between the 72 h and 168 h after aggregation time points.

Multi-axis self-organization properties of mouse embryonic stem cells into gastruloids

Lennardo Beccari¹, Nanni Melega², Stefano Giorgi¹, Ariadna Llorente¹, Paola Balbo¹, Alonso¹, Anne-Catherine Gossy¹, Martínez P. Llorente¹, Denis Dubrulle^{1,2*} & Alfonso Martinez Arias^{1,2*}



Building organs: neural retina: five layers



- Conserved across vertebrates
 - the five-layered neural retina
 - covered on the photoreceptor side by pigmented epithelium and the choroid, which contains endothelial cells, pericytes, fibroblasts, and melanocytes
- Embedded in this structure are glia and the retinal vasculature

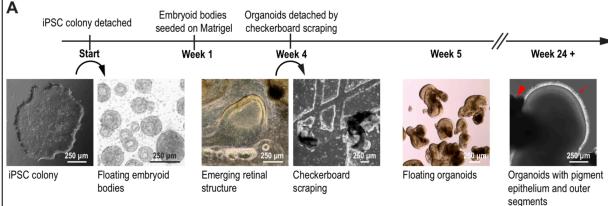
Retinal organoids from human iPSCs:

- screened 21 iPS cell lines
- 8 formed retinal organoids
- 6 had layered appearance (> 100 days in culture)

Q: what does this tell you about iPS cell lines?

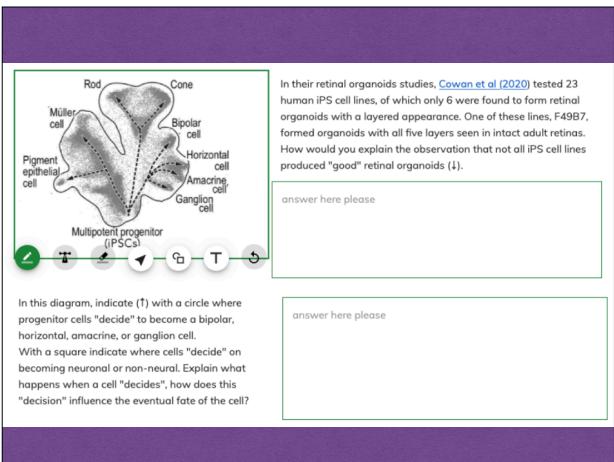
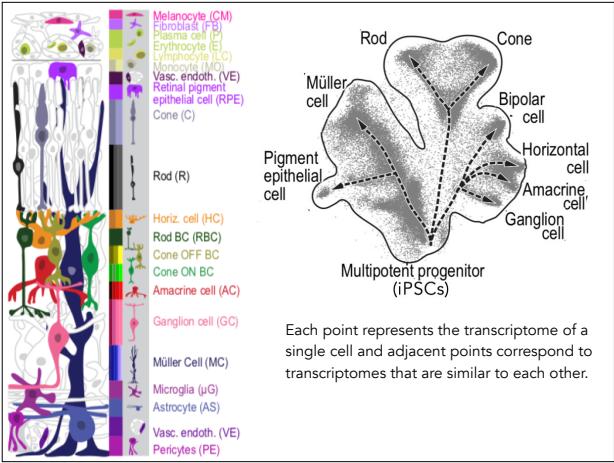
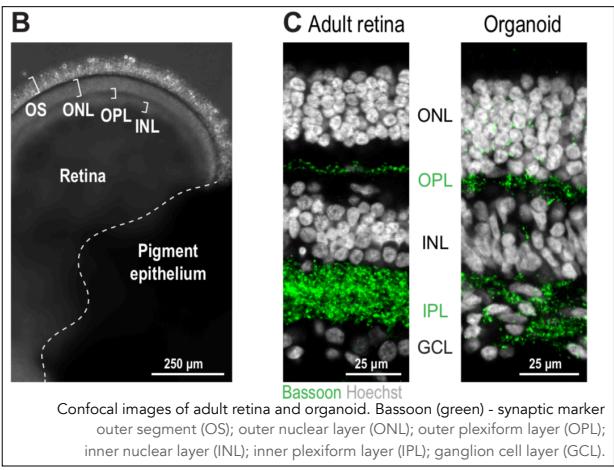
Cell types of the human retina and its organoids at single-cell resolution: developmental convergence, transcriptomic identity, and disease map Cowan et al 2019

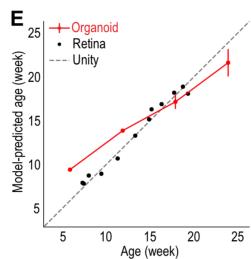
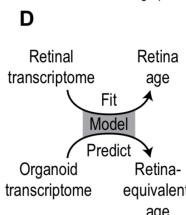
A



- Timeline of the organoid protocol
- Of the organoids produced by the checkerboard method:
 - ~76% were retinal organoids,
 - ~8.5% were pigment epithelium spheroids
 - or undefined structures (15.5%).
- Of the retinal organoids, 97% contained isolated patches of pigment epithelium at week 38. Gene expression patterns of organoids stabilize by week 30

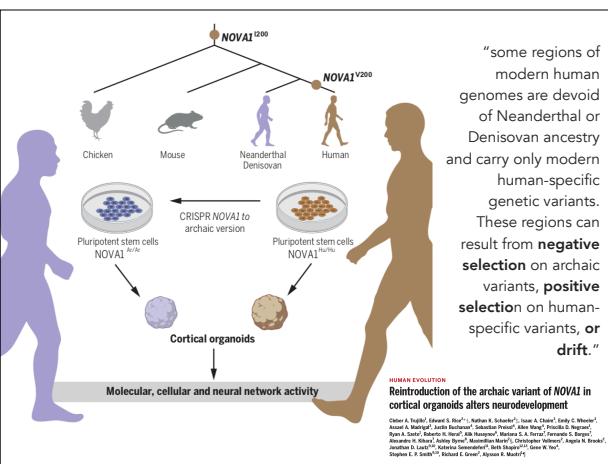
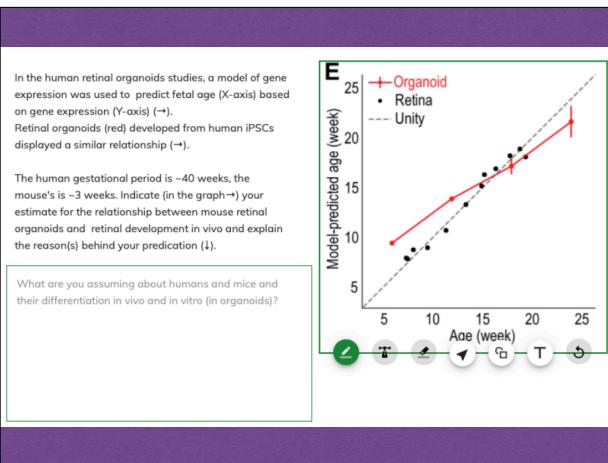
Cell types of the human retina and its organoids at single-cell resolution: developmental convergence, transcriptomic identity, and disease map Cowan et al 2019





A model, trained to predict retina age based on retinal transcriptome data, is applied to predict retina-equivalent age of organoid samples.

- Q: Would you have predicted this result (and what does it imply)?
- Q: What would you expect for mouse retina and iPSCs



Q: what did authors do? And why did they do it?

- a comprehensive analysis of genetic variation available from the 1000 Genomes Project: reveal only 61 non-synonymous, derived coding variants are both **fixed or nearly fixed in extant humans** and are **human-specific** (not found in other primates or humanoids)
 - experimentally tractable candidates for genetic variation that might underlie human- specific phenotypes.
 - Focus on **NOVA1** – regulates alternative splicing in the developing nervous system.
 - Altered NOVA1 splicing activity is associated with human neurological disorders
 - **Q:** What is “purifying selection”?



Q: what did authors do? And why did they do it?

- introduce the archaic variant of NOVA1 into iPSCs derived from two neurotypical human individuals with distinct genetic backgrounds
 - identified **277 differentially expressed genes** between NOVA1Ar/Ar and NOVA1Hu/Hu organoids at different stages of maturation
 - changes in alternative splicing in genes involved in neurodevelopment, proliferation, and synaptic connectivity
 - three genes with the largest differences:
 - mRNA-binding ribosomal protein gene RPS4Y1
 - NNAT, which codes for a protein involved in neural differentiation through calcium signaling
 - TDGF1, which codes for a membrane signaling protein involved in cell proliferation and migration during neurodevelopment



Organoids and studying the environmental effects on embryonic development

- viral infections
 - chemical (teratogens)
 - ethanol

Congenital Rubella Syndrome

- **Rubella** is an eruptive, highly contagious, and generally mild viral disease that is unnoticed and without consequences in most cases.
- Primary infection usually occurs during childhood and provides long-term immunity.
- Up to half of people infected with rubella don't have any symptoms but still can spread it to others.
- Completely avoidable through vaccination
 - Like smallpox and polio

Live Science > Health

Flight Attendant from Israel in Coma After Getting Measles Virus

By Sara G. Miller, Health Editor | April 17, 2019 10:50am ET

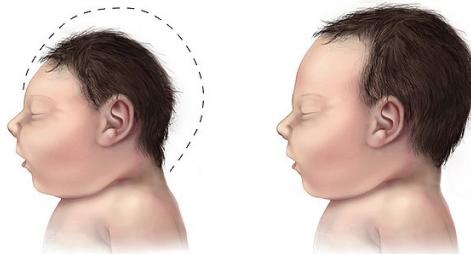
Infection during the first trimester of pregnancy can cause a fetal malformation syndrome known as congenital rubella syndrome (CRS)

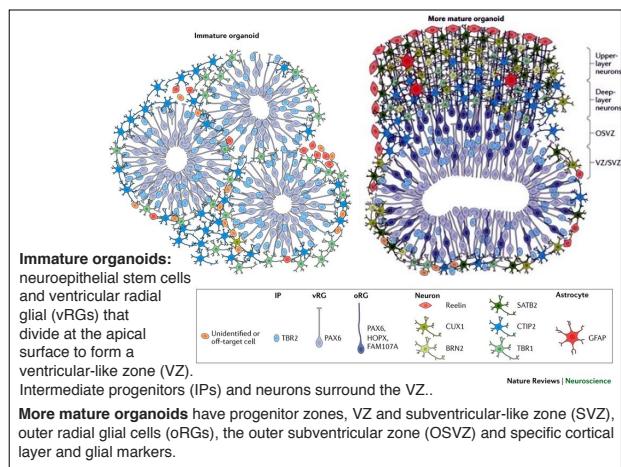
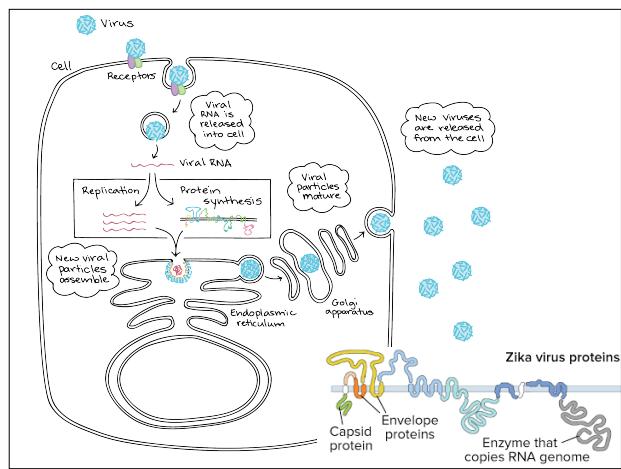
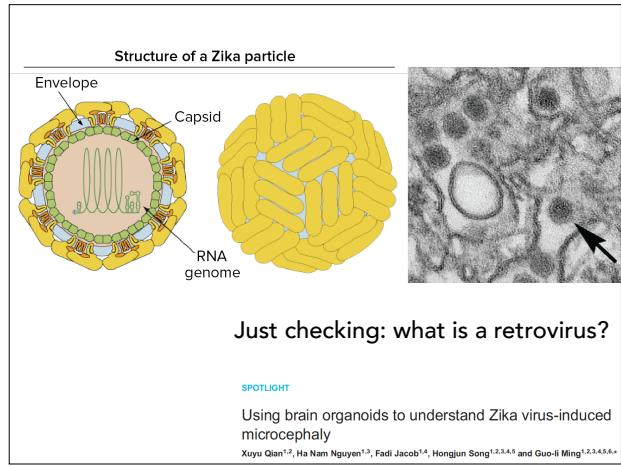
- **Ophthalmological abnormalities:** cataracts, microphthalmia, Retinopathy, chorioretinitis, & glaucoma
- **Cardiac abnormalities:** pulmonary artery stenosis, septal defects, tetralogy of Fallot, aortic stenosis, aortic coarctation, transposition of the great vessels, Ebstein's anomaly & pulmonary artery coarctation.
- **Brain abnormalities:** microcephalias, hydrocephalus, cerebral calcifications, anencephalias, cerebellar vermis agenesis, corpus callosum hypoplasia, & hydranencephaly.
- **Genitourinary disorders:** vesicoureteral reflux, renal agenesis, hydronephrosis & hypospadias.
- **Other:** low birth weight, hepatosplenomegaly, purpura, thrombocytopenia, long-bone anomalies, micrognathia, syndactyly, umbilical vein dilation, duodenal stenosis, meconium peritonitis, cleft palate & diaphragmatic hernia.

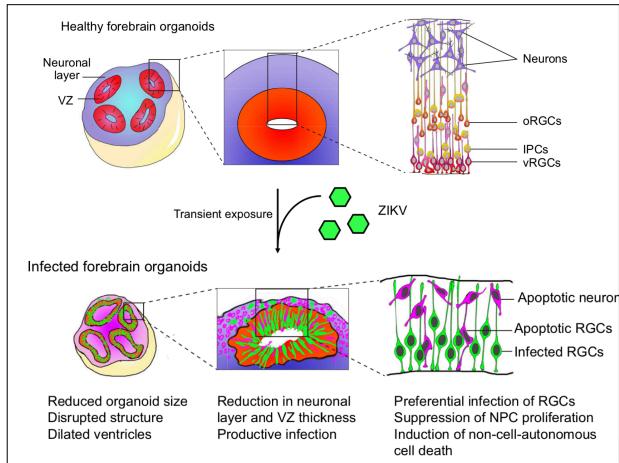
SPOTLIGHT

Using brain organoids to understand Zika virus-induced microcephaly

Xuyu Qian^{1,2}, Ha Nam Nguyen^{1,3}, Fadi Jacob^{1,4}, Hongjun Song^{1,2,3,4,5} and Guo-li Ming^{1,2,3,4,5,6,*}







Anti-Scientific & anti-vax propaganda (1926 and today)

Bioliteracy blog

- do citizens have a “right” to avoid vaccination (or decide for their children, or to place others at risk?)
- Is avoiding vaccination an example of social cheating?

Fetal alcohol syndrome

~3–5% of women drink heavily throughout pregnancy

Can result in

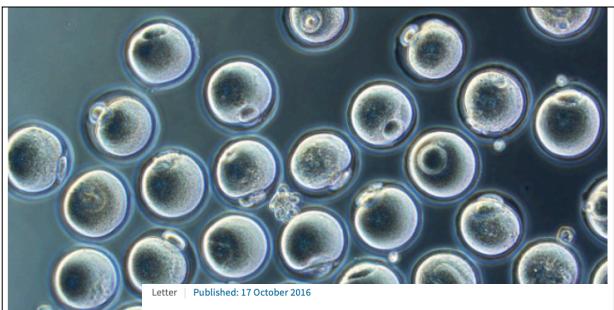
- reduced brain volume
- malformations of the corpus callosum.
- less common - effects to vasculature development.
- language problems and attention deficits were most common.
- influence activating the calcium/ calmodulin-dependent protein kinase II (CaMKII) enzyme which phosphorylates and **destabilizes active beta-catenin**.

Why is it difficult to study FAS?

- strictly limited to self-report and data collection
- unethical to host a study in that includes knowledge of pregnant women consuming (high levels) of alcohol.
- conflict of protecting embryonic life, while retaining the privacy and dignity of the patient contributing to the study.

Types of germ line “defects” to correct ...

- general infertility (failure to form functional gametes)
- maternal (mitochondrial) defects
- zygotic defects directly linked to disease
- insert alleles associated with “desirable traits”
- “repair” alleles associated “undesirable traits”
- avoid future disease (immunity to HIV infection)



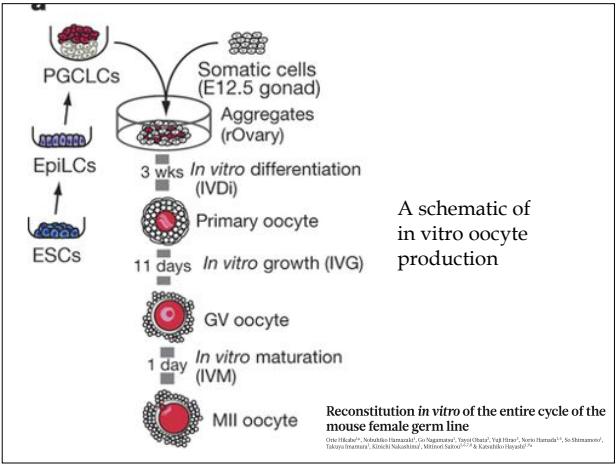
Letter | Published: 17 October 2016

Meiosis II murine oocytes dev
KATSUHIKO HAYASHI, KYUSHU

Reconstitution *in vitro* of the entire cycle
of the mouse female germ line

Orie Hikabe, Nobuhiko Hamazaki, Go Nagamatsu, Yayoi Obata, Yuji Hiroa, Norio Hamada, So
Shimamoto, Takuya Imamura, Kinichi Nakashima, Mitinori Saitou & Katsuhiko Hayashi

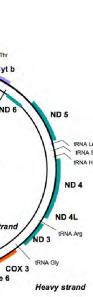
Nature 539, 299–303 (10 November 2016) | Download Citation



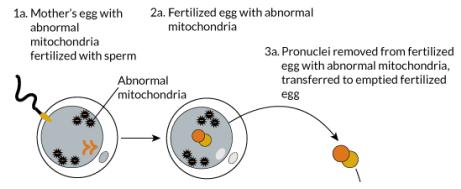
Maternal fertility effects due to dysfunctional mitochondria.

Q: why do mitochondria have a genome?

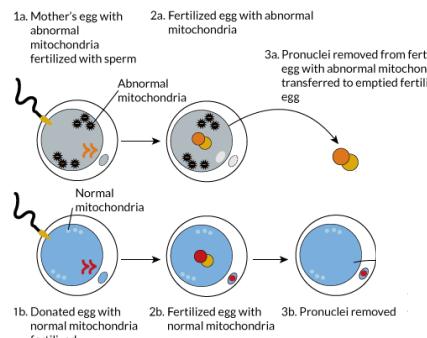
- mitochondrial genome is hyper-mutable compared with nuclear DNA
- high levels of (mutagenic) reactive oxygen species (ROS)
- mtDNA is replicated more frequently
- little non-coding mtDNA (compared to nuclearDNA)
- SO mutations are more likely to have a pathological impact.



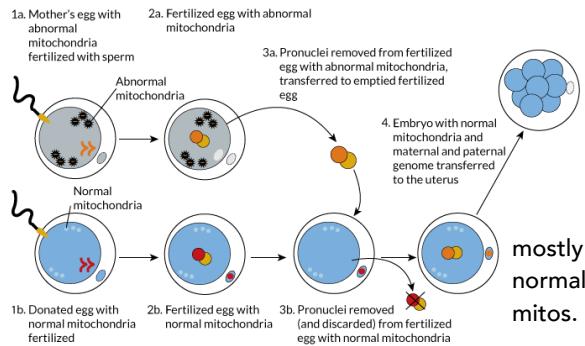
like the Gurdon experiment in frog



like the Gurdon experiment in frog



like the Gurdon experiment in frog



Opinion: Three-Parent Embryos—A Slippery Slope?

The use of pronuclear transfer to treat infertility must first be backed by evidence it can work in cases where parents seek to avoid mitochondrial mutations.

Jun 14, 2018
JOHN D. LOIKE, ALAN KADISH



Who are the parents of the child born from three DNA donors?

- do parents have a “right” to re-engineering their children?
- **To ponder:** what might be the long term socio-political and economic implications of the “unregulated” genetic engineering of babies?