2026 PCRS ANNUAL MEETING

REPRODUCTIVE FRONTIERS: BRIDGING BIOLOGY, PRACTICE, AND POSSIBILITY

MARCH 18-22 | RANCHO MIRAGE, CA

PACIFIC COAST REPRODUCTIVE SOCIETY

Can In Vitro Spermatogenes Prevent Heritable Disease: De Novo Mutations, Oligozoospermia & the $7M /10 yr graph

James M. Hotaling, MD MS FECSM

Chief Innovation Officer, UU health
Associate Vice President of Research for Innovation UofU
Prof Surgery (Urology)
Adjunct Prof OBGYN
Director of Men's Health
Director of Urologic Research
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REPRODUCTIVE FRONTIERS:
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Disclosure Slide - Sample

- Owner/Co-Owner Founder/Co-Founder
  - Paterna Bio, Firmtech,
- Consulting Fee (e.g., Advisory Board)
  - Maximus, Carrot, Repronovo, Coloplast
- Stock Shareholder (Individual stocks/Stock options; diversified mutual funds do not need to be disclosed)
  - Carrot, Maximus, Repronovo, Firmtech, Inherent (path fertility), Paterna

Needs Assessment Statement and Expected Learning Outcomes

- Discuss the impact of de novo mutations on male infertility and IVF success
- Describe the relationship between male infertility and somatic health and what mechanisms might drive this relationship
- Explain how in vitro spermatogenesis might impact our field in the future

Male Infertility is lagging 30 years behind IVF focused female treatment

8%
of all men are infertile

1%
of men have no sperm in ejaculate (azoospermia)

No FDA
approved therapeutic treatment for male infertility

For men with low or no sperm, the only option is testicular surgery to find sperm and IVF.

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What are the implications of male infertility beyond pregnancy?

One ? has driven my work over past decade

Why do infertile men and their families have higher rates of cancer and shorter lifespans?

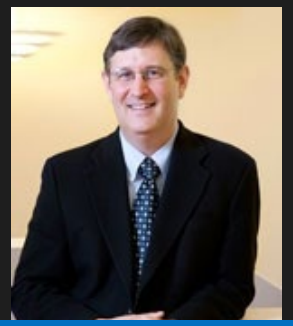


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One ? has driven my work over past decade

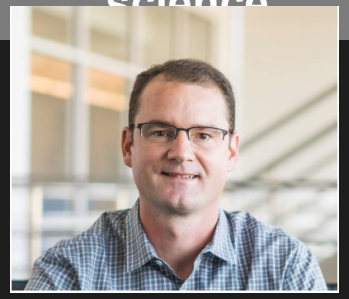
- Why do infertile men and their families have higher rates of cancer and shorter lifespans?

Collaborators

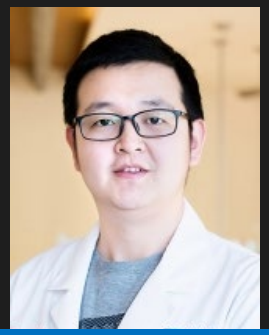


Brad Cairns PhD

The key to this research program has been team science



Aaron Quinlan, PhD



Jingtao Guo PhD



Qi Chen PhD



Ki Aston, PhD



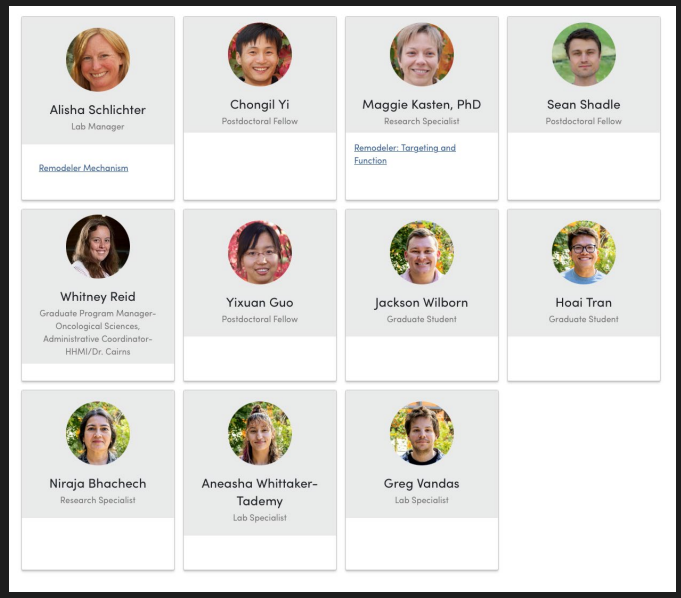
Alex Pastuszak, MD, PhD



Ben Emery, MS



Joemy Ramsay, PhD

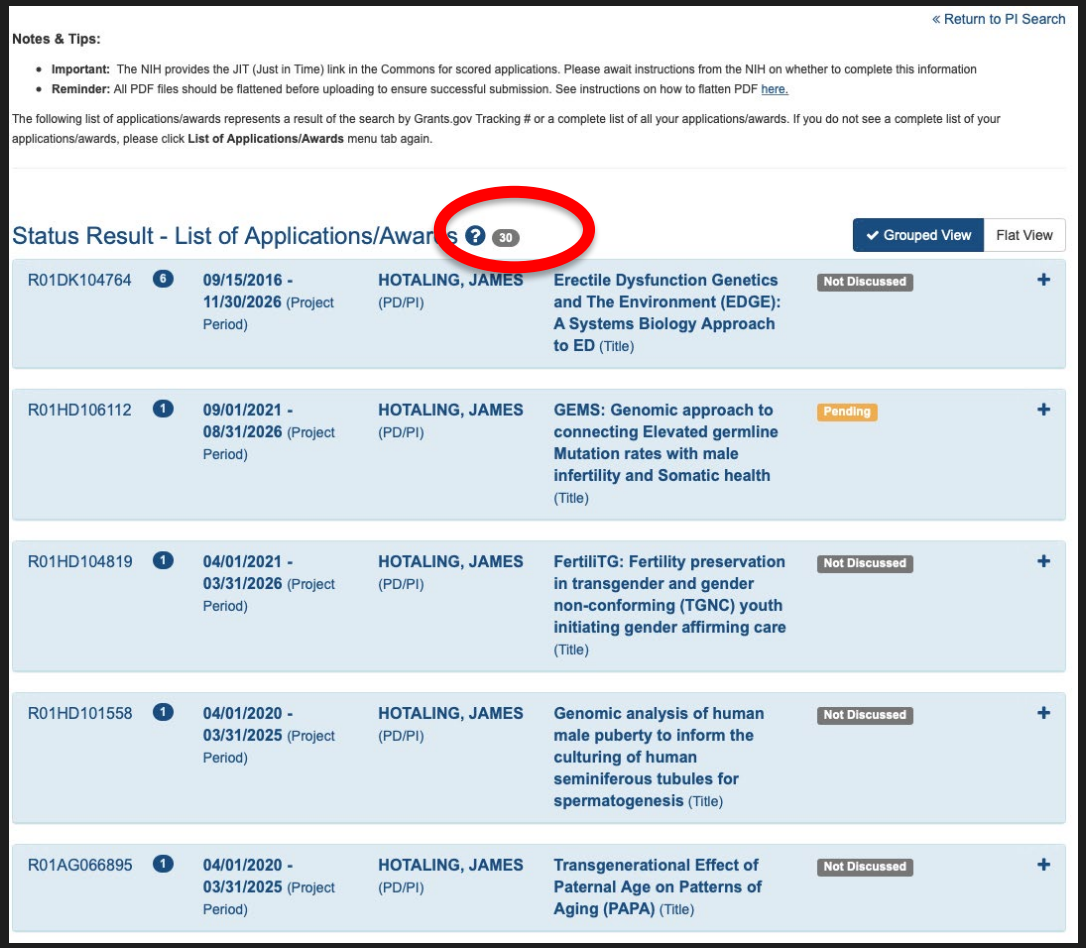




Jason Kunisaki, MD, PhD



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« Return to PI Search

Notes & Tips:

- **Important:** The NIH provides the JIT (Just in Time) link in the Commons for scored applications. Please await instructions from the NIH on whether to complete this information
- **Reminder:** All PDF files should be flattened before uploading to ensure successful submission. See instructions on how to flatten PDF here.

The following list of applications/awards represents a result of the search by Grants.gov Tracking # or a complete list of all your applications/awards. If you do not see a complete list of your applications/awards, please click **List of Applications/Awards** menu tab again.

Status Result - List of Applications/Awards 30

Grouped View | Flat View

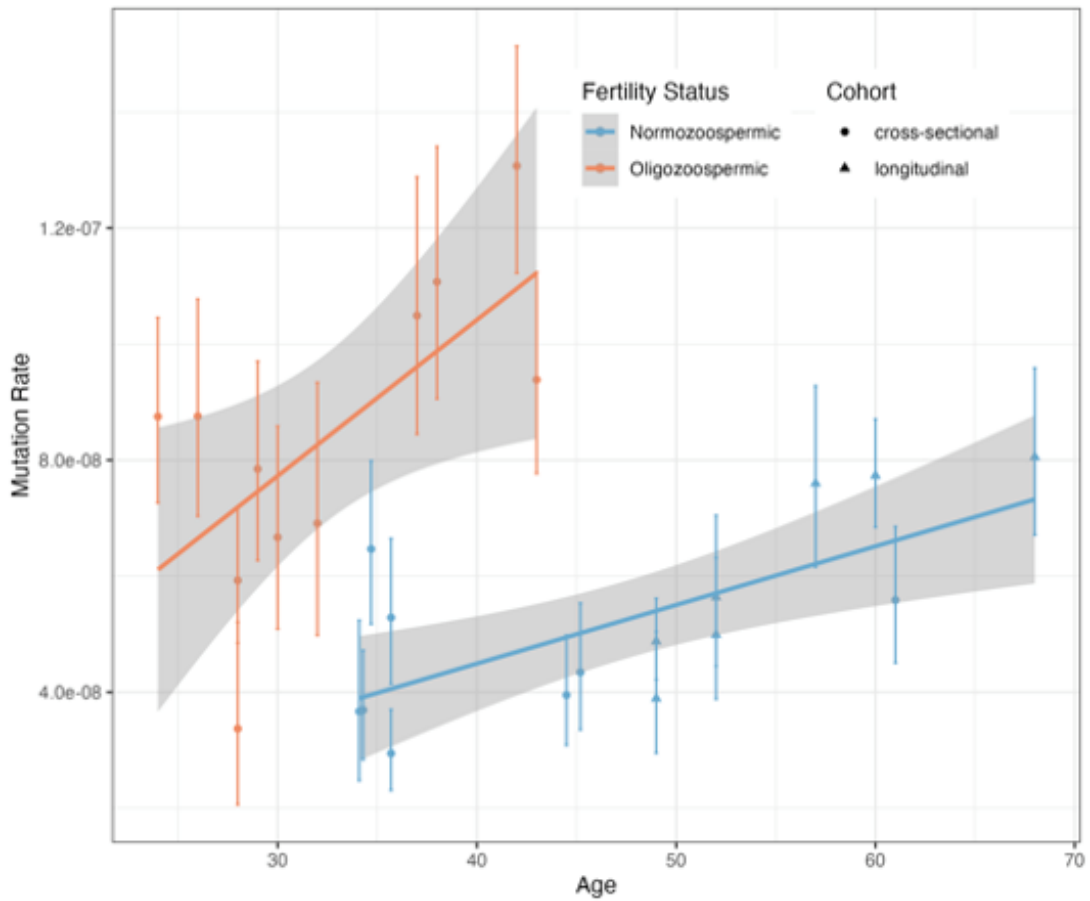
R01DK104764	6	09/15/2016 - 11/30/2026 (Project Period)	HOTALING, JAMES (PD/PI)	Erectile Dysfunction Genetics and The Environment (EDGE): A Systems Biology Approach to ED (Title)	Not Discussed
R01HD106112	1	09/01/2021 - 08/31/2026 (Project Period)	HOTALING, JAMES (PD/PI)	GEMS: Genomic approach to connecting Elevated germline Mutation rates with male infertility and Somatic health (Title)	Pending
R01HD104819	1	04/01/2021 - 03/31/2026 (Project Period)	HOTALING, JAMES (PD/PI)	FertiliTG: Fertility preservation in transgender and gender non-conforming (TGNC) youth initiating gender affirming care (Title)	Not Discussed
R01HD101558	1	04/01/2020 - 03/31/2025 (Project Period)	HOTALING, JAMES (PD/PI)	Genomic analysis of human male puberty to inform the culturing of human seminiferous tubules for spermatogenesis (Title)	Not Discussed
R01AG066895	1	04/01/2020 - 03/31/2025 (Project Period)	HOTALING, JAMES (PD/PI)	Transgenerational Effect of Paternal Age on Patterns of Aging (PAPA) (Title)	Not Discussed

31 applications

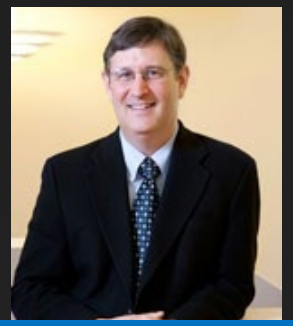
8 funded…

“The population of Utah does not reflect the population of the US as they make their own clothes, do not smoke or drink, grow their own food and have high birth rates”

10 yrs, $7M to generate one slide ($500k sequencing)

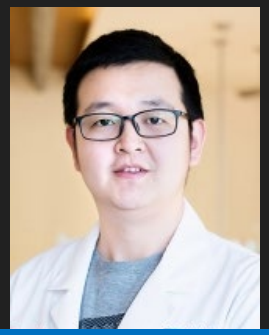


Research pipeline

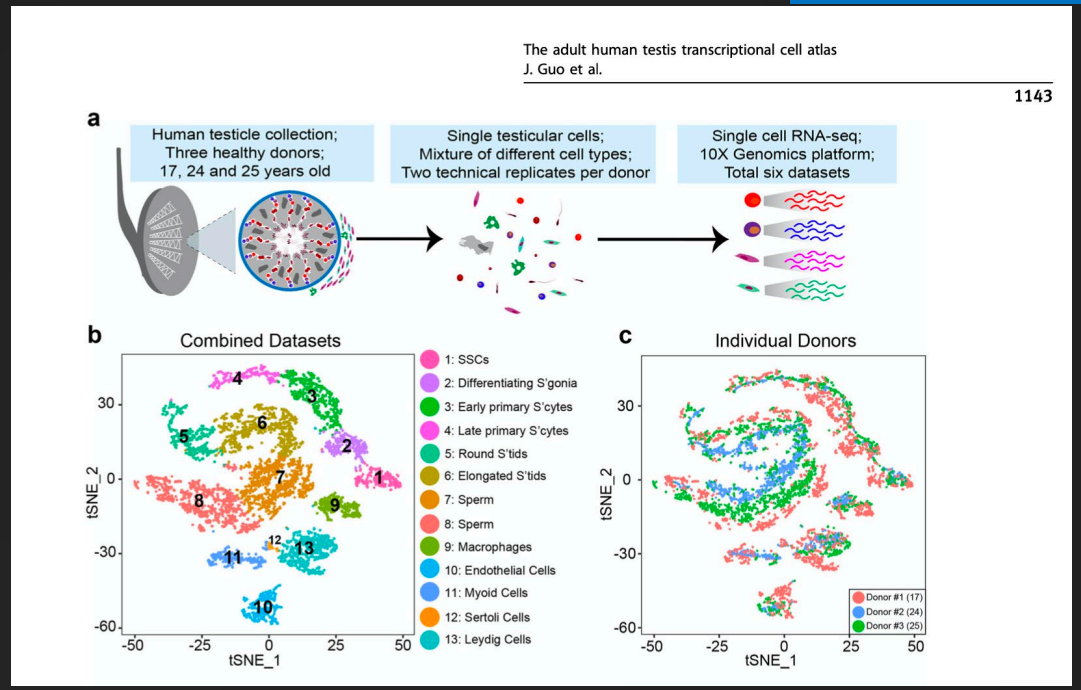


Brad Cairns PhD

The key to this research program has been team science,



Jingtao Guo PhD



Cadaveric Organ donors (Donor Connect)

- Partnered with cadaveric organ donor program in 2016
- Now have 325+ pairs of human testis age prenatal - 70+ AND 500 clinical tissue samples

Extensive fertility database w/comprehensive cradle to grave info and pedigree data linked with biobank

- Testis tissue bank (n=700+)
- Banked Sperm 7k+
- >25 Transgender tissue samples
- Database of >40k Semen Analyses linked to controls and family members (n=1.5M)

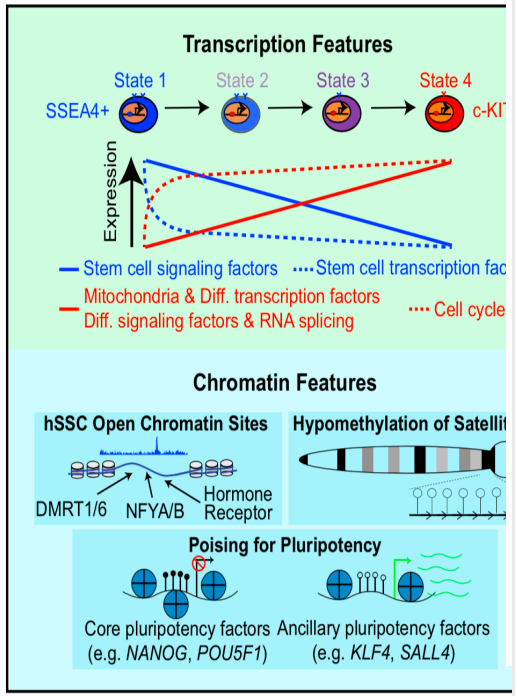
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Single Ce

Cell Stem Cell

**Chromatin and Single-Cell R
Dynamic Signaling and Meta
Human Spermatogonial Ster**

Graphical Abstract

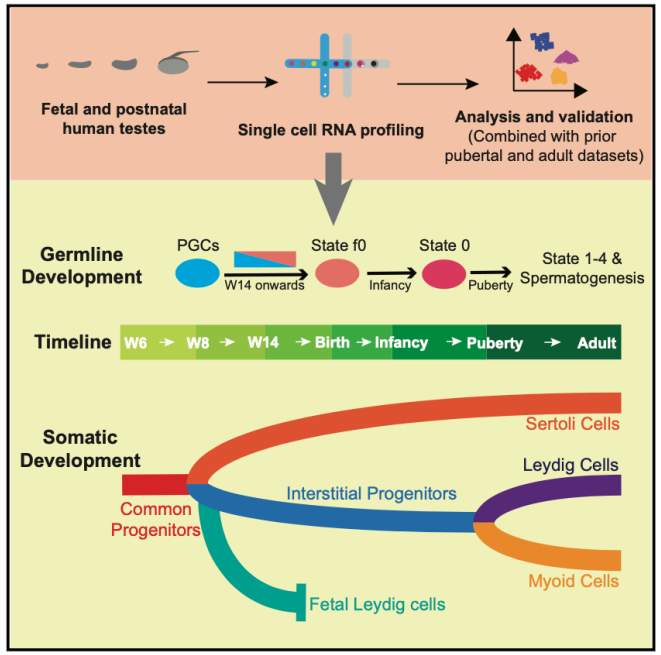


Cell Stem Cell

Resource

Single-cell analysis of the developing human testis reveals somatic niche cell specification and fetal germline stem cell establishment

Graphical Abstract



Highlights

- A transcriptional single-cell atlas of the fetal and postnatal human testes
- Somatic niche cell types derive from a common progenitor ~7 weeks after fertilization
- PGCs transition directly into fetal state 0-like cells (state f0) starting at week 14
- Fetal somatic niche cell specification precedes the PGC-to-state f0 transition

Authors

Jingtao Guo, Enrique Sosa, Tsotne Chitiashvili, ..., Jan-Bernd Stukenborg, Amander T. Clark, Bradley R. Cairns

Correspondence

jingtao.guo@hci.utah.edu (J.G.), clarka@ucla.edu (A.T.C.), brad.cairns@hci.utah.edu (B.R.C.)

In Brief

Guo et al. provide a transcriptional cell atlas of the fetal and postnatal human testes. Remarkably, starting from ~14 weeks postfertilization, fetal primordial germ cells transition to a cell state highly similar to postnatal spermatogonial stem cells. Furthermore, somatic niche specification precedes this transition, which is consistent with guiding fetal germline development.

estis tissue

Resource

**l Atlas of Testis
erty**

uthors

ingtao Guo, Xichen Nie,
laria Giebler, ..., Anne Goriely,
ames M. Hotaling, Bradley R. Cairns

orrespondence

rad.cairns@hci.utah.edu

n Brief

uo et al. provide a transcriptional cell tlas of the developing human testis uring puberty, revealing dramatic evelopmental changes in both germ and omatic niche cell lineages. Furthermore, erm cells and Sertoli cells from estosterone-suppressed transfemale estes display partial developmental eversal, revealing critical roles for estosterone in maintaining testis aturation.

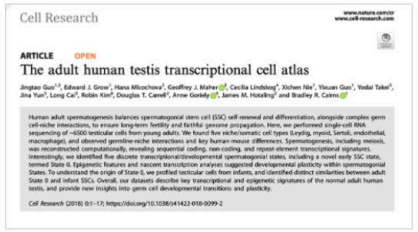
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Fundamental insights into human testis and germ cell development along entire lifespan: embryo, infancy, puberty, adulthood, aging



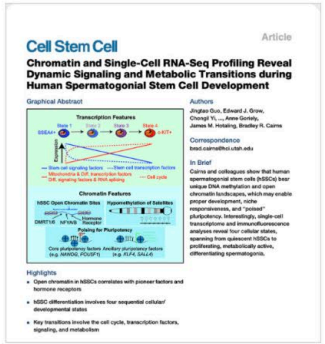
Fetal — Prepubertal — Adult — Aging →




Cell Research

ARTICLE OPEN

The adult human testis transcriptional cell atlas


Cell Stem Cell

Article

Chromatin and Single-Cell RNA-Seq Profiling Reveal Dynamic Signaling and Metabolic Transitions during Human Spermatogonial Stem Cell Development

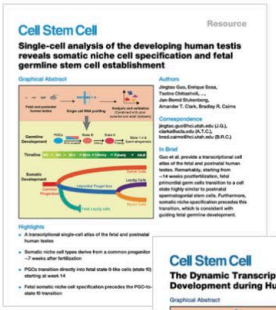
Graphical Abstract

Highlights

- Open chromatin in hSSCs correlates with pioneer factors and hormone receptors
- hSSC differentiation involves four sequential cellular/developmental states
- Key transitions involve the cell cycle, transcription factors, signaling, and metabolism

Human testis transcriptome atlas by single cell RNA-seq.

Validation of genomic findings by cellular and molecular biology approaches.

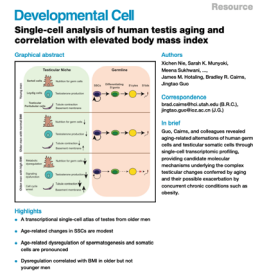

Cell Stem Cell

Resource

Single-cell analysis of the developing human testis reveals somatic niche cell specification and fetal germline stem cell establishment

Graphical Abstract

Highlights


Developmental Cell

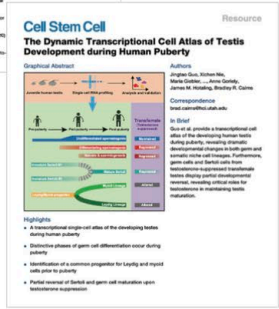
Resource

Single-cell analysis of human testis aging and correlation with elevated body mass index

Graphical abstract

Highlights

- A transcriptional single-cell atlas of testes from older men
- Age-related changes in SSCs are modest
- Age-related dysregulation of spermatogenesis and somatic cells are pronounced
- Dysregulation correlated with BMI in older but not younger men


Cell Stem Cell

Resource

The Dynamic Transcriptional Cell Atlas of Testis Development during Human Puberty

Graphical Abstract

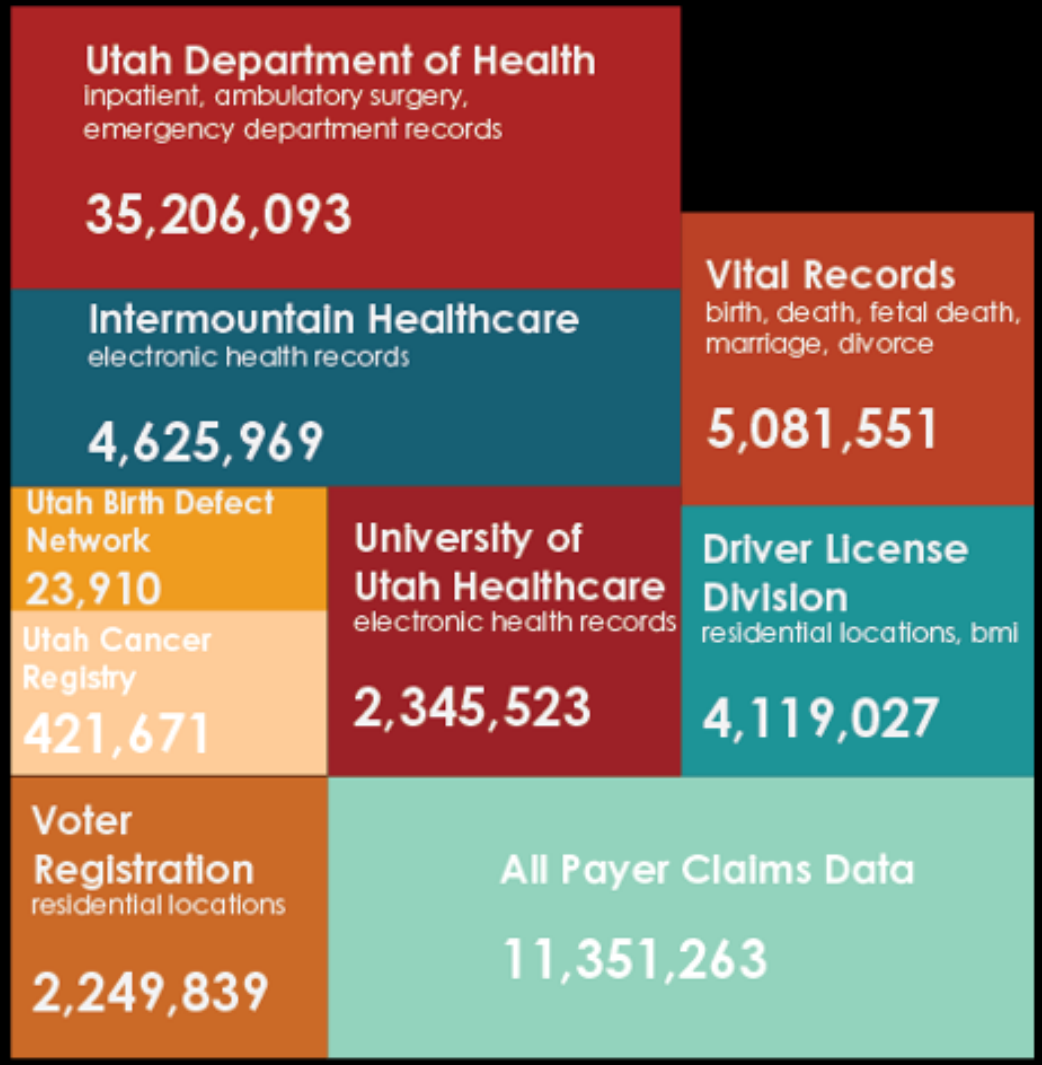
Highlights

- A transcriptional single-cell atlas of the developing testes during human puberty
- Distinctive phases of germ cell differentiation occur during puberty
- Identification of a common progenitor for Leydig and myoid cells prior to puberty
- Partial reversal of Sertoli and germ cell maturation upon testosterone suppression

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Utah Population Database (UPDB)

- linked population-based information
  - demographic, genetic, & epidemiological studies
  - multi-generational pedigrees
- data on over 11 million individuals
  - late 18th century to present



https://uofuhealth.utah.edu/huntsman/utah-population-database/data

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Subfertility, Health and Assisted Reproduction (SHARE) cohort



genealogic & family history records







children pre-, post-SA female partner records



men with SAs
~1980 – 2017
University of Utah Healthcare
Intermountain Healthcare











residential locations

medical records, biospecimens

- men with semen analyses (SAs)
- linked to UPDB
  - longitudinal follow-up
- age-matched fertile controls

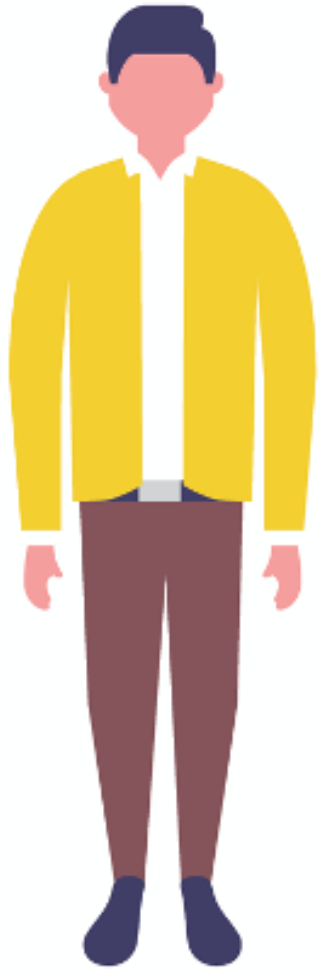
familial association between male infertility and somatic health

Male subfertility as a **biomarker** of **overall somatic health**

epigenetic, **genetic**, and **environmental** insults impact fertility and somatic health

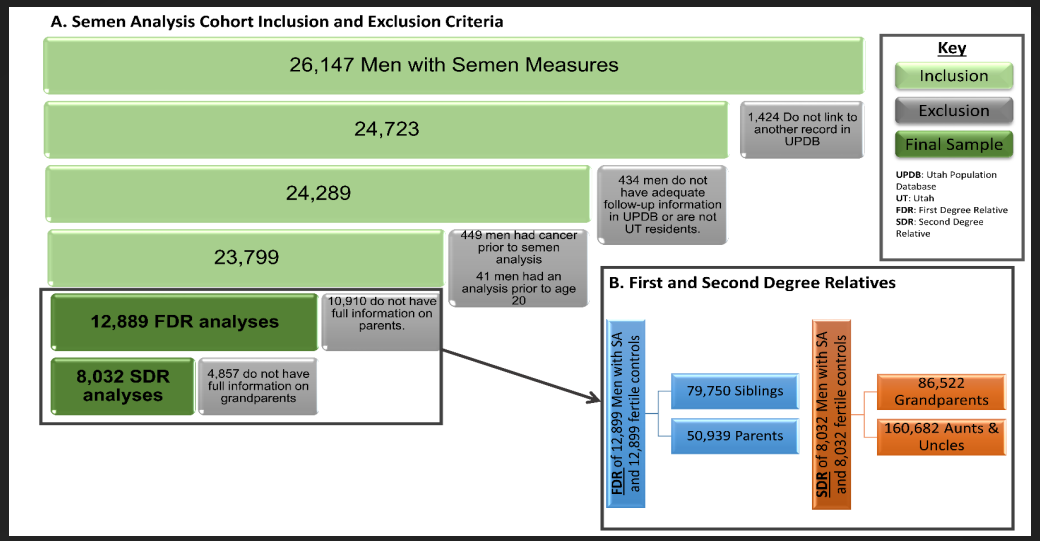
family members can have **shared exposure** to these factors

semen quality and infertility are associated with **poor health**



- cardiovascular disease
- autoimmune disease
- cancer
- increased hospitalizations from chronic conditions
- increased mortality from chronic conditions
- reduced lifespan

What can Pedigree data do…



Cancer risk in first- and second-degree relatives of men with poor semen quality

Ross E. Anderson, M.D., M.C.R.,[a] Heidi A. Hanson, Ph.D., M.S.,[c] Darshan P. Patel, M.D.,[a] Erica Johnstone, M.D.,[b] Kenneth I. Aston, Ph.D., H.C.L.D.,[a] Douglas T. Carrell, Ph.D., H.C.L.D.,[a,d] William T. Lowrance, M.D., M.P.H.,[a] Ken R. Smith, Ph.D.,[e] and James M. Hotaling, M.D., M.S.[a]

[a] Division of Urology, Department of Surgery, University of Utah; [b] Division of Reproductive Endocrinology and Infertility, Department of Obstetrics and Gynecology, University of Utah; [c] Department of Family and Preventive Medicine and Population Sciences, Huntsman Cancer Institute, University of Utah; [d] Department of Human Genetics, University of Utah School of Medicine; and [e] Department of Family and Consumer Studies and Population Sciences, Huntsman Cancer Institute, University of Utah, Salt Lake City, Utah

Human Reproduction, Vol.32, No.1 pp. 239–247, 2017
Advanced Access publication on December 6, 2016 doi:10.1093/humrep/dew289

human reproduction ORIGINAL ARTICLE *Reproductive epidemiology*

Risk of childhood mortality in family members of men with poor semen quality

Heidi A. Hanson[1,2,*], Erik N. Mayer[3], Ross E. Anderson[3], Kenneth I. Aston[3,4,5], Douglas T. Carrell[3,5], Justin Berger[2], William T. Lowrance[3], Ken R. Smith[2,6], and James M. Hotaling[3,4]

[1]Department of Family and Preventive Medicine, University of Utah, Salt Lake City, UT, USA [2]Population Sciences, Huntsman Cancer Institute, University of Utah, 675 Arapeen Drive, 204, Salt Lake City, UT 84112, USA [3]Division of Urology, Department of Surgery, University of Utah, Salt Lake City, UT, USA [4]Center for Men's Health and Reconstructive Surgery, Salt Lake City, UT, USA [5]Department of Human Genetics, University of Utah School of Medicine, Salt Lake City, UT, USA [6]Department of Family and Consumer Studies, University of Utah, Salt Lake City, UT, USA

*Correspondence address. University of Utah, Department of Family and Preventive Medicine Population Sciences, Huntsman Cancer Institute, 675 Arapeen Drive, 204, Salt Lake City, UT 84112, USA. Tel: +1-801-585-6794; E-mail: heidi.hanson@hci.utah.edu

Submitted on March 24, 2016; resubmitted on October 14, 2016; accepted on October 27, 2016

Subfertility increases risk of testicular cancer: evidence from population-based semen samples

Heidi A. Hanson, Ph.D.,[a,b] Ross E. Anderson, M.D., M.C.R.,[d] Kenneth I. Aston, Ph.D.,[d,e,f] Douglas T. Carrell, Ph.D., H.C.L.D.,[d,e,f] Ken R. Smith, Ph.D.,[b,c] and James M. Hotaling, M.D., MS[e]

[a] Department of Family and Preventive Medicine, and [b] Population Sciences and [c] Department of Family and Consumer Studies and Population Sciences, Huntsman Cancer Institute, [d] Division of Urology, Department of Surgery, University of Utah, Salt Lake City, Utah; [e] Center for Men's Health and Reconstructive Surgery; and [f] Department of Human Genetics, University of Utah School of Medicine, Salt Lake City, Utah

Childhood Cancer Risk in the Siblings and Cousins of Men with Poor Semen Quality

Ross E. Anderson,* Heidi A. Hanson, William T. Lowrance, Jeffrey Redshaw, Siam Oottamasathien, Anthony Schaeffer, Erica Johnstone, Kenneth I. Aston, Douglas T. Carrell, Patrick Cartwright, Ken R. Smith and James M. Hotaling

From the Division of Urology, Department of Surgery (REA, WTL, JR, SO, AS, KIA, DTC, PC, JMH) and Division of Reproductive Endocrinology and Infertility, Department of Obstetrics and Gynecology (EJ, DTC), Department of Family and Preventive Medicine and Population Sciences (HAH) and Department of Family and Consumer Studies and Population Sciences (KRS), Huntsman Cancer Institute and Department of Human Genetics, School of Medicine (KIA, DTC), University of Utah, Salt Lake City, Utah

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Anderson et al, FNS, 2016; Hanson FNS 2016; Anderson J Urol 2017; Hanson Hum Reprod 2017

Male Factor: Individual vs. Familial Health

Individual

1. DM, cardiac disease, EtOH/drug abuse
2. Mortality, life expectancy
3. Testis, Prostate CA
4. Psychiatric disease?

Eisenberg FNS 2016; Eisenberg HR 2014;
Jensen Am. J Epi 2009; Jacobsen BMJ 2000;
Walsh Arch Int Med. 2009; Walsh Cancer 2010

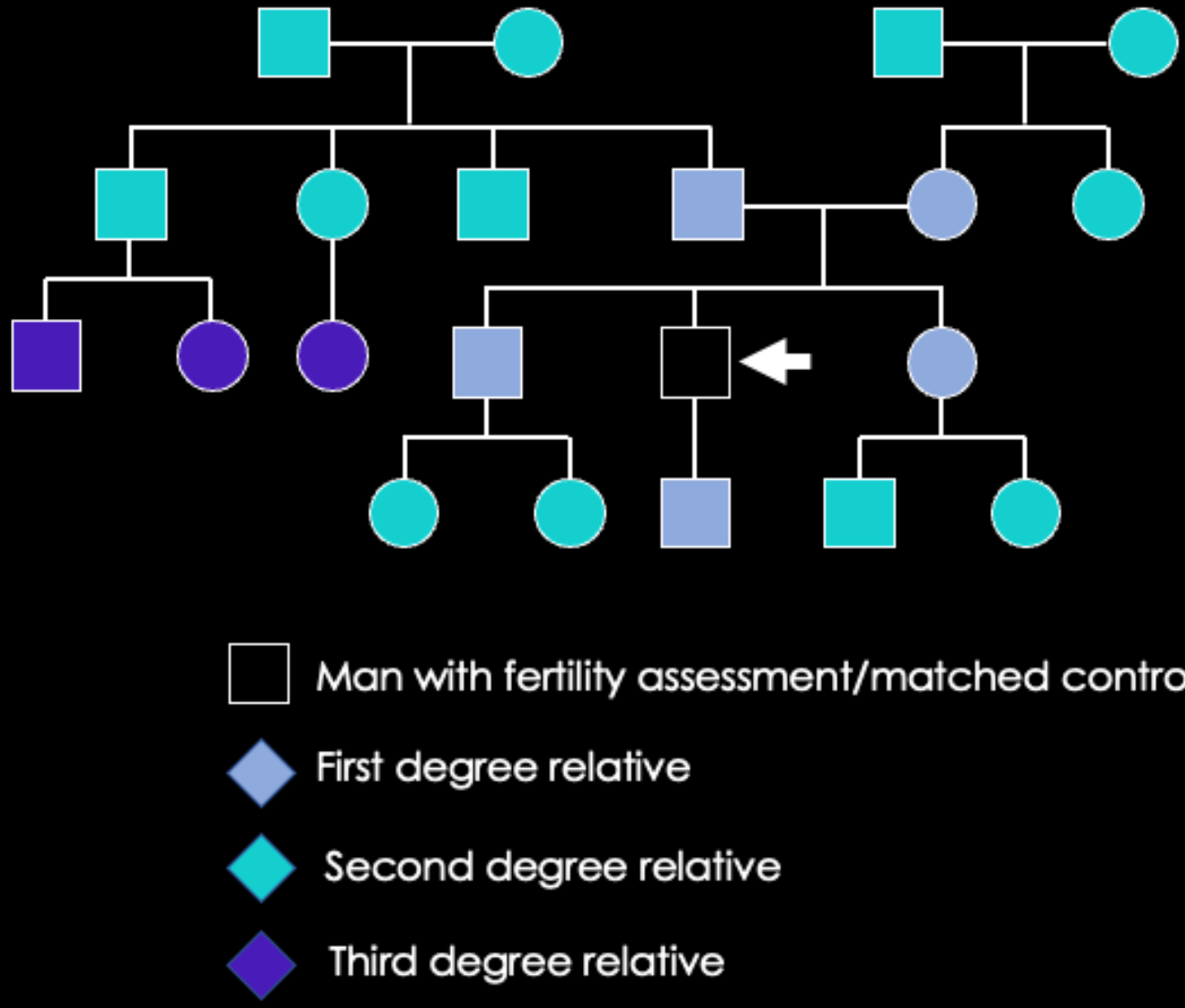
Familial

1. Testis CA, thyroid CA
2. Pediatric CA, acute lymphoblastic leukemia
3. Pediatric mortality, death from major congenital anomalies

Anderson et al, FNS, 2016; Hanson FNS 2016;
Anderson J Urol 2017; Hanson Hum Reprod 2017

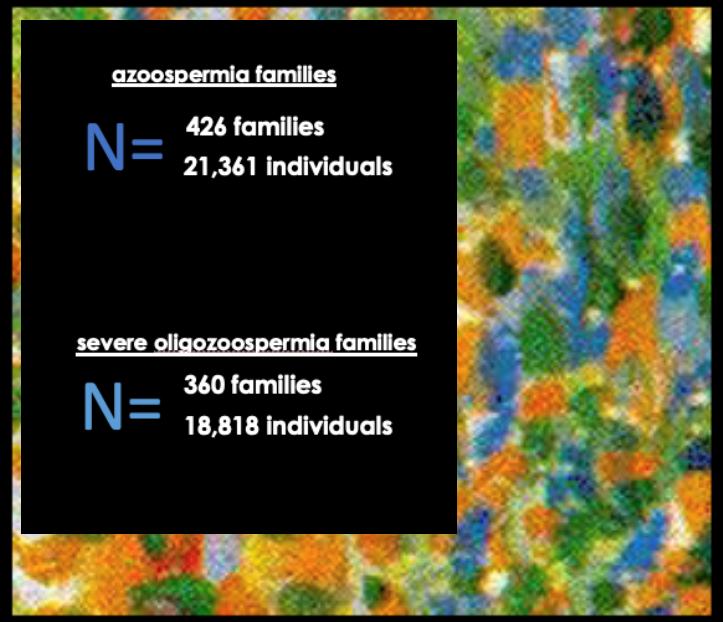
cohort design

- Men with fertility assessment in SHARE
- 5:1 fertile controls
- family to 3rd degree
  - at least 2nd degree
- ≥10 informative family members



familial multi-cancer clustering (FMC)

- identify multi-cancer patterns
- 34 cancer sites/site groups
- azoospermia and severe oligospermia families assessed separately
- famcluster R package



multi-cancer perspective allows us to see the full picture

~65% of families have NO elevated risk of cancer, 35% with unique patterns of elevated risk

summary of familial multi-cancer patterns

azoospermia families

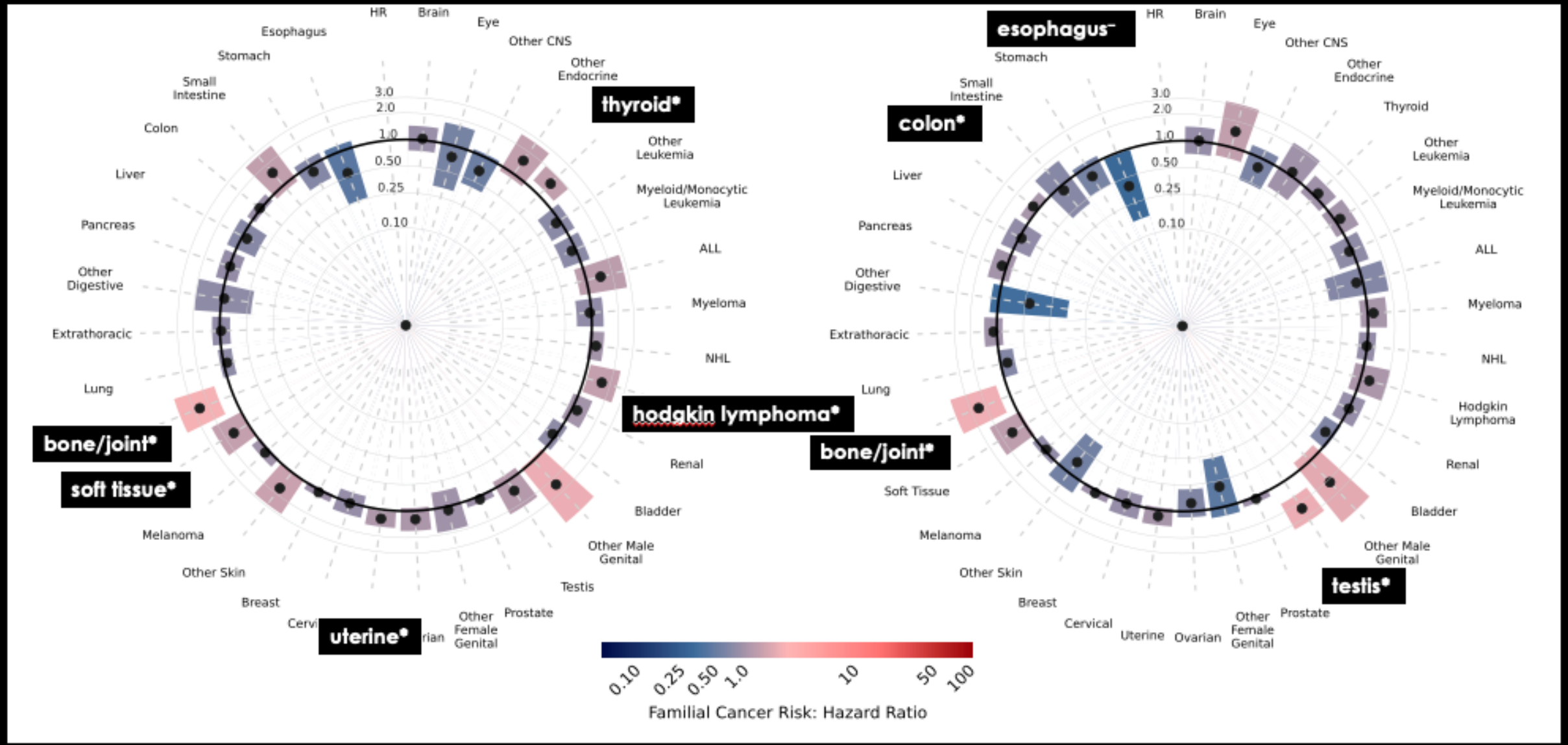
multi-cancer pattern	families	individuals	significant cancers
1	281 (66.0%)	12,707 (60.9%)	0
2	22 (5.2%)	1,138 (5.4%)	2
3	16 (3.8%)	1,057 (5.1%)	7
4	16 (3.8%)	727 (3.5%)	2
5	15 (3.5%)	760 (3.6%)	3
6	14 (3.3%)	944 (4.5%)	3
7	13 (3.1%)	566 (2.7%)	2
8	10 (2.3%)	593 (2.8%)	3
9	10 (2.3%)	600 (2.9%)	2
10	10 (2.3%)	537 (2.6%)	2
11	7 (1.6%)	399 (1.9%)	2
12	6 (1.4%)	393 (1.9%)	2
13	6 (1.4%)	461 (2.2%)	2

severe oligozoospermia families

multi-cancer pattern	families	individuals	significant cancers
1	224 (62.4%)	10,395 (56.1%)	2*
2	27 (7.5%)	1,348 (7.3%)	1
3	18 (5.0%)	999 (5.4%)	2
4	17 (4.7%)	956 (5.2%)	3
5	16 (4.5%)	847 (4.6%)	2
6	12 (3.3%)	797 (4.3%)	2
7	10 (2.8%)	580 (3.1%)	2
8	9 (2.5%)	574 (3.1%)	3
9	9 (2.5%)	638 (3.4%)	3
10	6 (1.7%)	319 (1.7%)	1
11	6 (1.7%)	666 (3.6%)	2
12	5 (1.4%)	399 (2.2%)	1

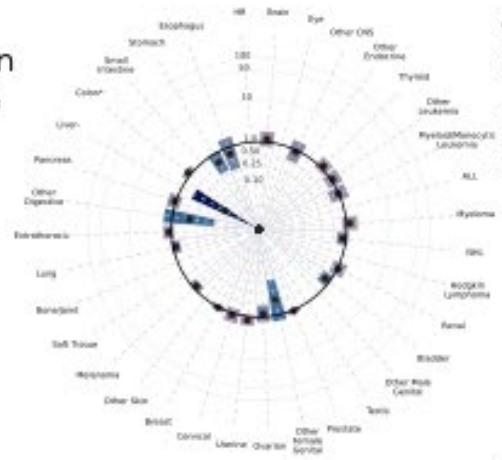
* 1 increased risk, 1 decreased risk

multi-cancer risk in subfertile families vs fertile controls

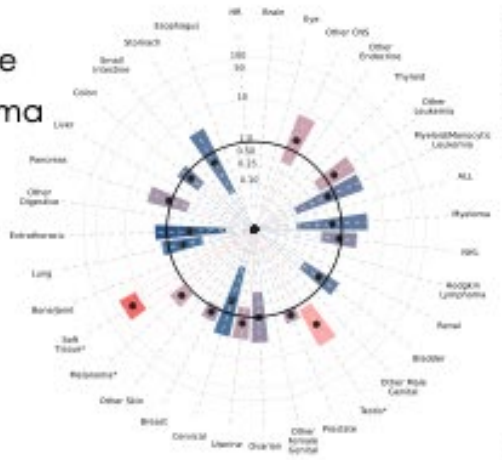


oligozoospermia families

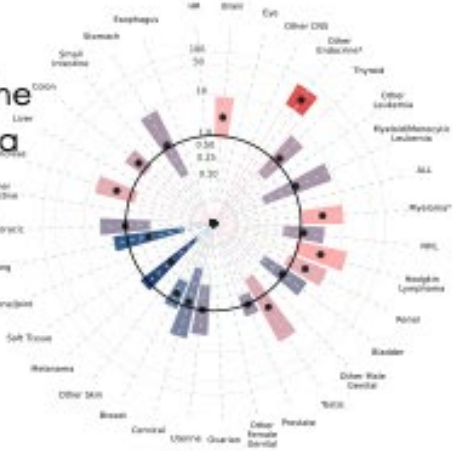
1
colon
liver*



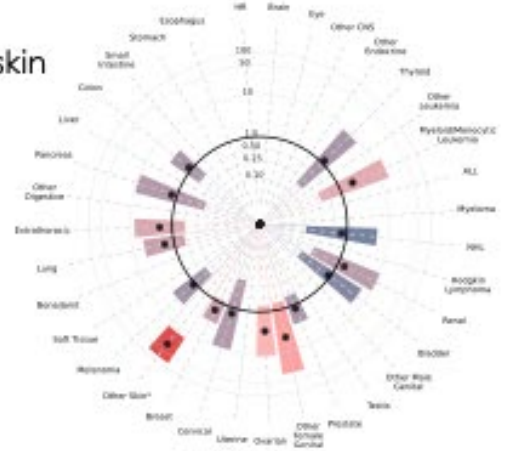
4
soft tissue
melanoma
testis



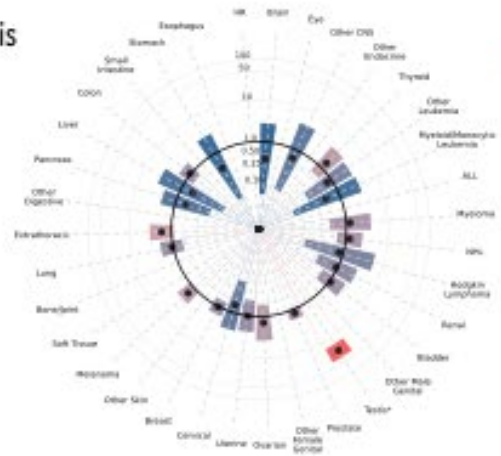
7
other endocrine
myeloma



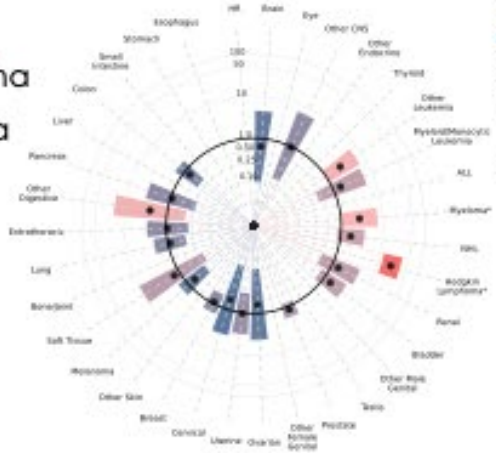
10
other skin



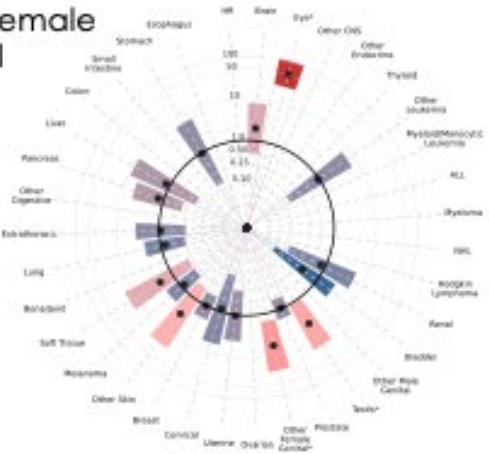
2
testis



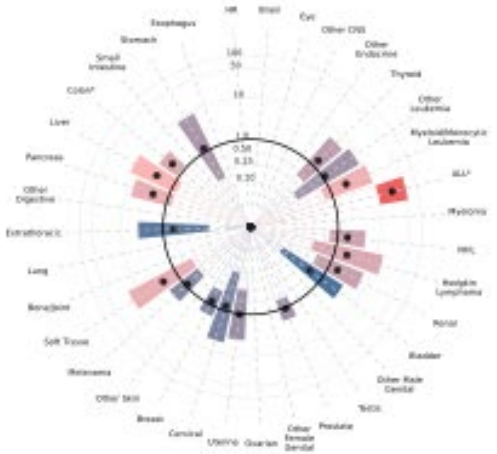
5
hodgkin lymphoma
myeloma



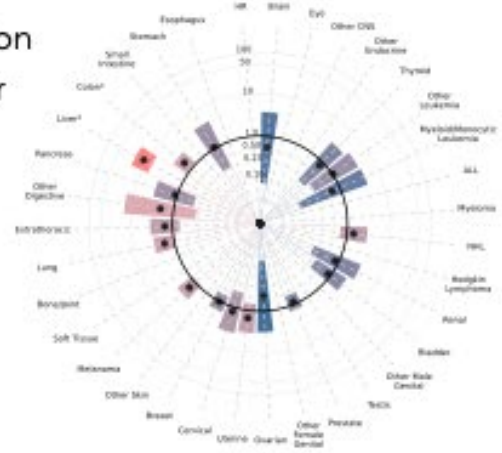
8
other female genital
testis
eye



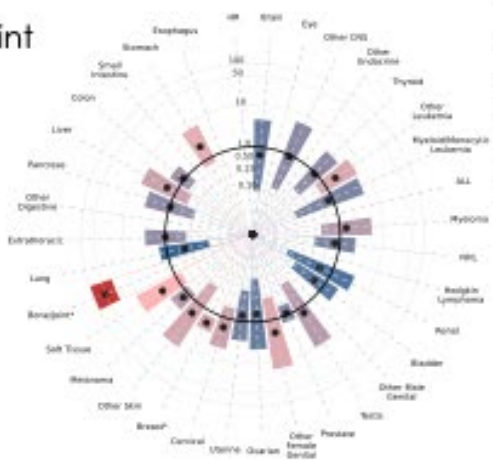
11
colon
ALL



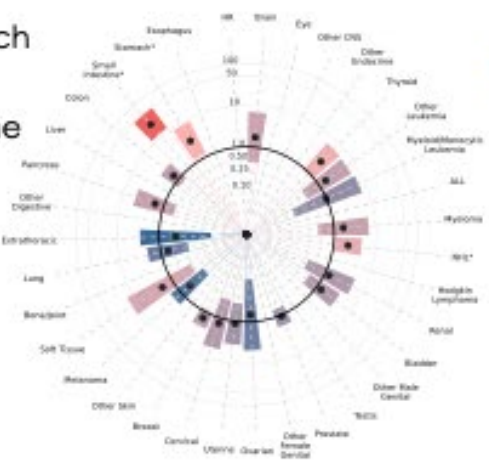
3
colon
liver



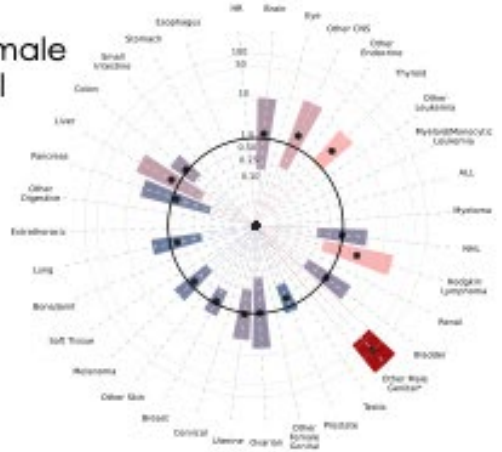
6
bone/joint
breast



9
stomach
small intestine
NHL



12
other male genital



Is TMC 50-100M the new 20M? Major improvements in conception rate if TMC>50M

Human Reproduction, Vol.00, No.0, pp. 1–13, 2021
doi:10.1093/humrep/deab133

human reproduction ORIGINAL ARTICLE *Infertility*

Semen parameter thresholds and time-to-conception in subfertile couples: how high is high enough?

Sorena Keihani [1,*], Lauren E. Verrilli[2], Chong Zhang[3], Angela P. Presson[3], Heidi A. Hanson [1,4], Alexander W. Pastuszak[1], Erica B. Johnstone[2], and James M. Hotaling[1]

[1]Division of Urology, Department of Surgery, University of Utah, Salt Lake City, UT, USA [2]Division of Reproductive Endocrinology and Infertility, Department of Obstetrics and Gynecology, University of Utah, Salt Lake City, UT, USA [3]Division of Epidemiology, Department of Internal Medicine, University of Utah, Salt Lake City, UT, USA [4]Huntsman Cancer Institute, Salt Lake City, UT, USA

*Correspondence address. Division of Urology, Department of Surgery, University of Utah School of Medicine, 30 North 1900 East, Salt Lake City, UT 84132, USA. Tel: +1-801-213-2700; Fax: +1-801-213-2700; E-mail: sorena.keihani@hsc.utah.edu https://orcid.org/0000-0001-9006-5809

Submitted on June 24, 2020; resubmitted on April 25, 2021; editorial decision on May 4, 2021

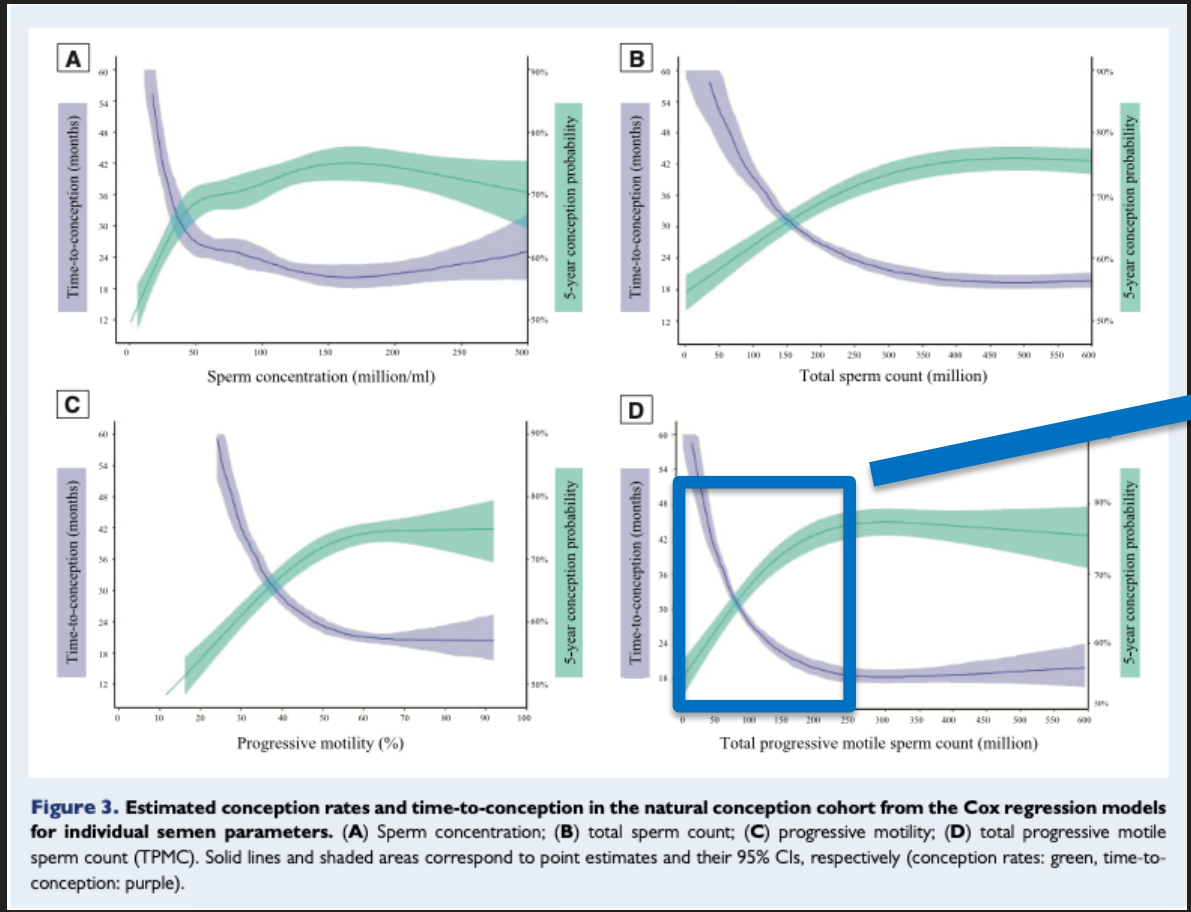


Figure 3. Estimated conception rates and time-to-conception in the natural conception cohort from the Cox regression models for individual semen parameters. (**A**) Sperm concentration; (**B**) total sperm count; (**C**) progressive motility; (**D**) total progressive motile sperm count (TPMC). Solid lines and shaded areas correspond to point estimates and their 95% CIs, respectively (conception rates: green, time-to-conception: purple).

What if this whole range of TMC was passing on deleterious de novo mutations (DNM) to offspring?

What if low TMC was a biomarker of familial health mediated through DNA mismatch repair mutations?

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Skinny Pedigree: A Marker of Familial Health?

Journal of Assisted Reproduction and Genetics (2021) 38:1207–1213
https://doi.org/10.1007/s10815-021-02115-2

REPRODUCTIVE PHYSIOLOGY AND DISEASE

Lower total motile count is associated with smaller historic intergenerational family size: a pedigree analysis from the Utah Population Database

Darshan P. Patel[1] · Huong T. Meeks[2] · Heidi A. Hanson[1,2] · Alexander W. Pastuszak[1] · James M. Hotaling[1] · Ken R. Smith[2,3]

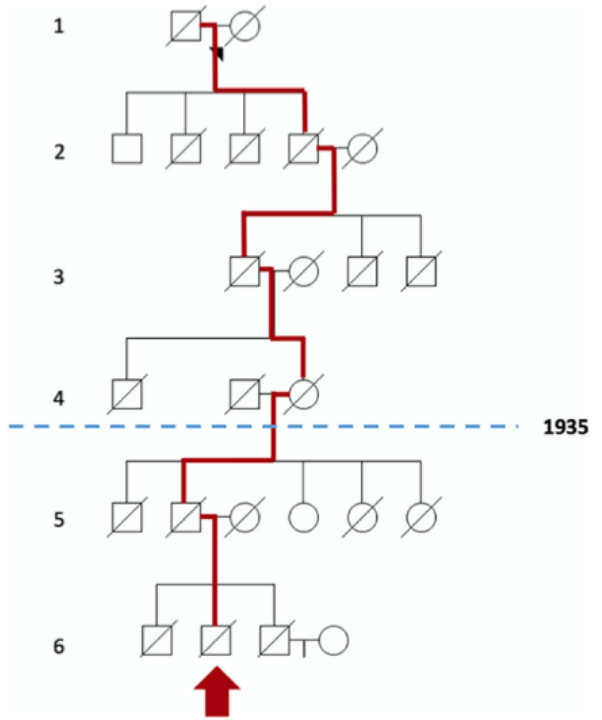


Fig. 2 Pedigree terminology (see Fig. 2). Descendant (dark arrow): male descendant with at least single measure of total motile count and complete pedigree data. Pedigree branch (dark line): pedigree path from a founder to a person

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Results:

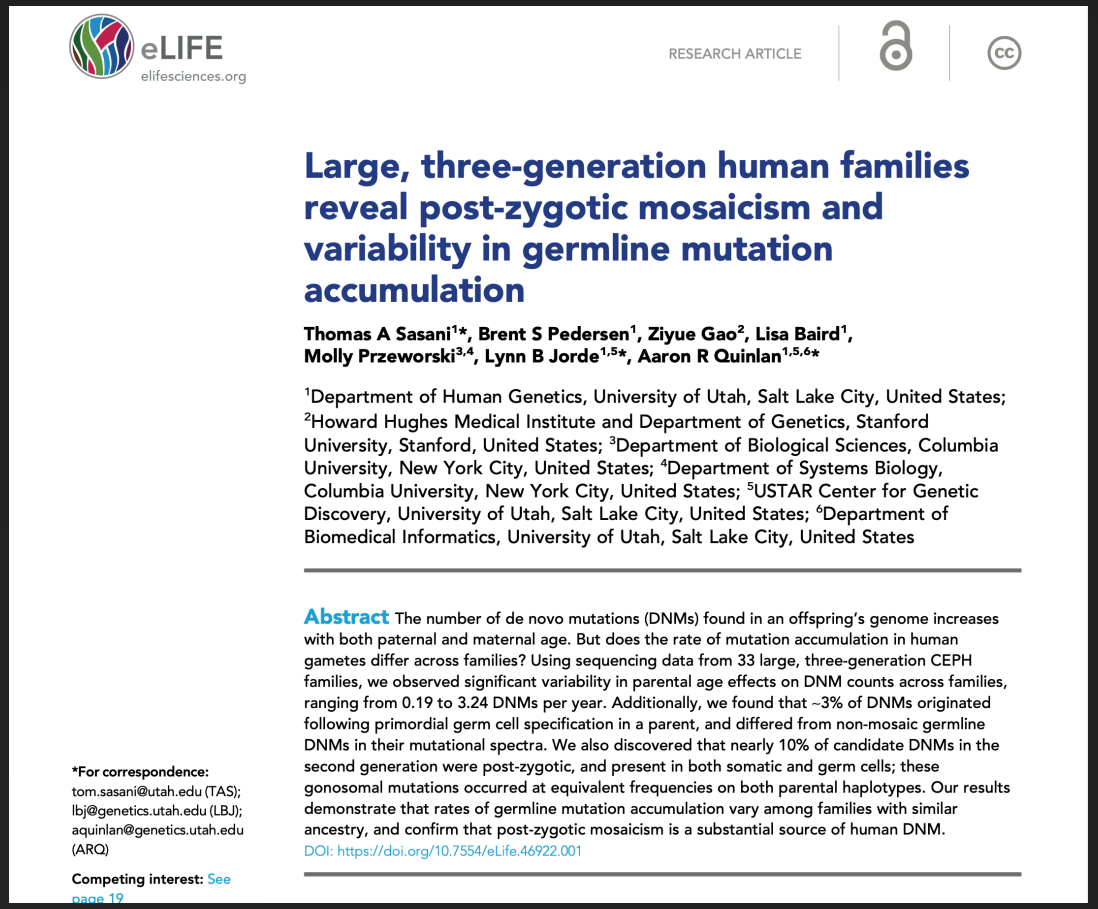
TMC as a continuous variable:

- For **every additional child** in their historical pedigree back to 9 generations, we saw an **increase in TMC of 1.88 million** ($p = 0.031$).

Lower TMC  Smaller intergenerational family size

Men in the top quartile intergenerational family size had a TMC that was 48 million higher than men in the bottom quartile

Mechanism



eLIFE elifesciences.org RESEARCH ARTICLE

Large, three-generation human families reveal post-zygotic mosaicism and variability in germline mutation accumulation

Thomas A Sasani[1]*, Brent S Pedersen[1], Ziyue Gao[2], Lisa Baird[1], Molly Przeworski[3,4], Lynn B Jorde[1,5]*, Aaron R Quinlan[1,5,6]*

[1]Department of Human Genetics, University of Utah, Salt Lake City, United States; [2]Howard Hughes Medical Institute and Department of Genetics, Stanford University, Stanford, United States; [3]Department of Biological Sciences, Columbia University, New York City, United States; [4]Department of Systems Biology, Columbia University, New York City, United States; [5]USTAR Center for Genetic Discovery, University of Utah, Salt Lake City, United States; [6]Department of Biomedical Informatics, University of Utah, Salt Lake City, United States

Abstract The number of de novo mutations (DNMs) found in an offspring's genome increases with both paternal and maternal age. But does the rate of mutation accumulation in human gametes differ across families? Using sequencing data from 33 large, three-generation CEPH families, we observed significant variability in parental age effects on DNM counts across families, ranging from 0.19 to 3.24 DNMs per year. Additionally, we found that ~3% of DNMs originated following primordial germ cell specification in a parent, and differed from non-mosaic germline DNMs in their mutational spectra. We also discovered that nearly 10% of candidate DNMs in the second generation were post-zygotic, and present in both somatic and germ cells; these gonosomal mutations occurred at equivalent frequencies on both parental haplotypes. Our results demonstrate that rates of germline mutation accumulation vary among families with similar ancestry, and confirm that post-zygotic mosaicism is a substantial source of human DNM.

DOI: https://doi.org/10.7554/eLife.46922.001

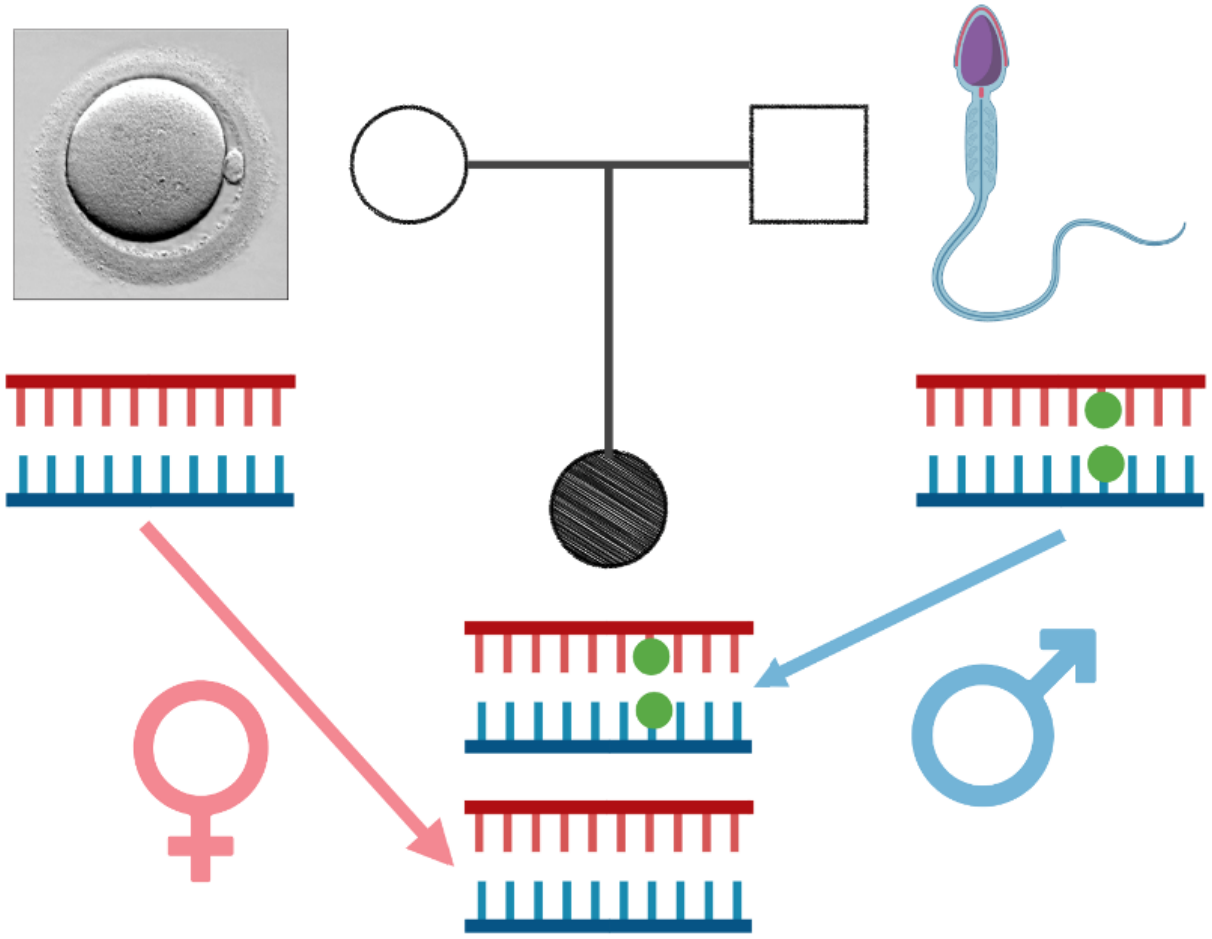
***For correspondence:** tom.sasani@utah.edu (TAS); lbj@genetics.utah.edu (LBJ); aquinlan@genetics.utah.edu (ARQ)

Competing interest: See page 19

1. 33 large Utah multigeneration families w/ average of 8 kids per generation
2. De novo mutation (DNM) rate varies from 0.19 to 3.24 DNM across the different families
3. Most DNM is driven by the male
4. Families with higher DNM have much higher rates of cancer and die on avg. 8 yrs earlier

Align Left

What are Germline *De Novo* Mutations (DNMs)?



- **Germline** = Arise in maternal or paternal gamete
- ***De novo*** = "from the new"
- Can be transmitted to offspring
- Provides substrate for evolution
- Involved in disease processes

Transmission of Germline DNMs have Important Health Implications

Spontaneous Pregnancy

De novo mutations in congenital heart disease with neurodevelopmental and other congenital anomalies

JASON HOMSY, SAMIR ZAIDI, YUFENG SHEN, JAMES S. WARE, KAITLIN E. SAMOCHA, KONRAD J. KARCZEWSKI, STEVEN R. DEPALMA, DAVID MCKEAN, HIROKO WAKIMOTO, [...] WENDY K. CHUNG +32 authors Authors Info & Affiliations

SCIENCE • 4 Dec 2015 • Vol 350, Issue 6265 • pp. 1262-1266 • DOI: 10.1126/science.aac9396

The contribution of *de novo* coding mutations to autism spectrum disorder

Ivan Iossifov, Brian J. O'Roak, ... Michael Wigler + Show authors

Nature **515**, 216–221 (2014) | Cite this article

51k Accesses | **1310** Citations | **316** Altmetric | Metrics

Prevalence and architecture of *de novo* mutations in developmental disorders

Deciphering Developmental Disorders Study

Nature **542**, 433–438 (2017) | Cite this article

38k Accesses | **666** Citations | **392** Altmetric | Metrics

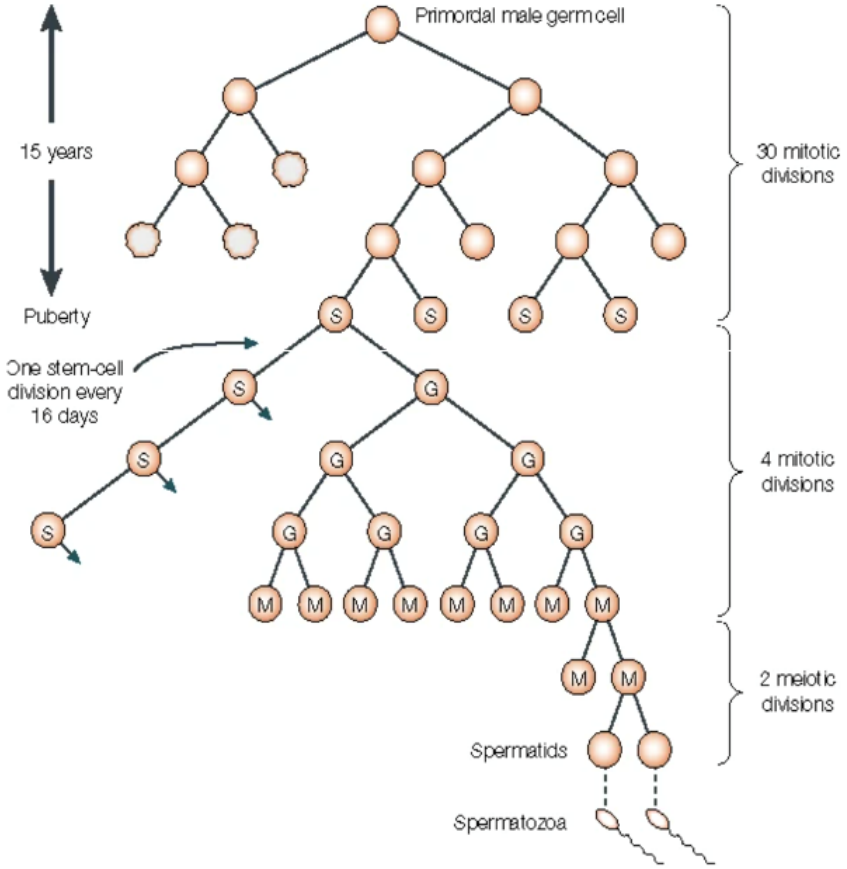
Association of assisted reproductive technology, germline de novo mutations and congenital heart defects in a prospective birth cohort study

Cheng Wang, Hong Lv, ... Zhibin Hu + Show authors

Cell Research **31**, 919–928 (2021) | Cite this article

2314 Accesses | **4** Citations | **82** Altmetric | Metrics

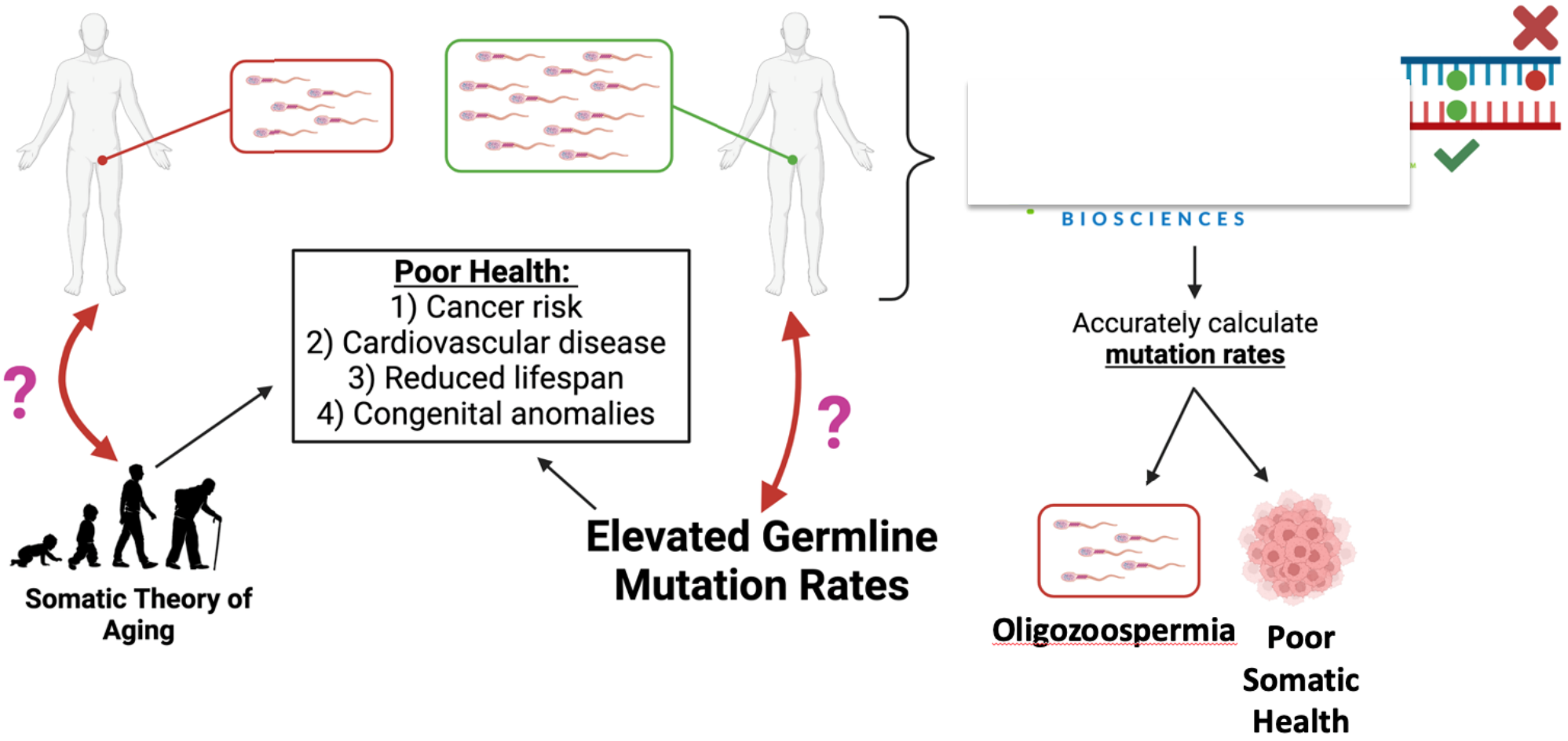
Germline DNMs Increase with Paternal Age



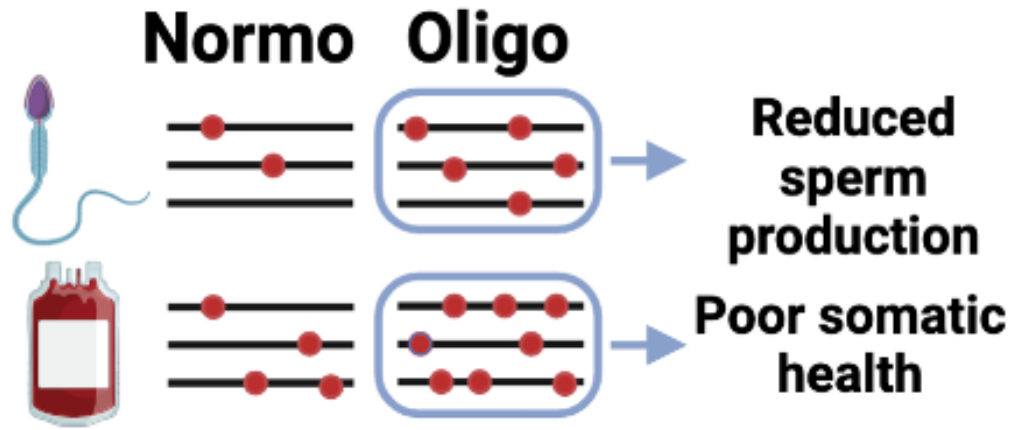
- SSCs undergo 30 rounds of replication before puberty
- SSCs divide every 16 days after puberty
- **20 y/o**: 150 chr replications
- **40 y/o**: 610 chr replications
- **Sperm quality deteriorates with age**
- Pedigrees used to study germline mutation rates

Crow, 2000

A study of mutation rates in fertile and subfertile/infertile men



Hypothesis: infertile men have elevated elevated germline and somatic mutation rates



Analysis #1: Establish “baseline” mutation rates in the sperm and blood of fertile men?

Analysis #2: Compare mutation rates and patterns between fertile and infertile men.

Significance: establish germline and somatic mutagenesis as a hallmark genetic feature of male infertility

We are using duplex sequencing to deeply profile mutations in sperm from fertile and infertile men

Normozoospermic Men

17 Fertile Patients

9 Sperm Donors

Δ 8 - 23 years

Oligozoospermic Men

15 Donors

Step 1: Somatic cell lysis and DNA extraction



Step 2: TwinStrand library prep and sequencing





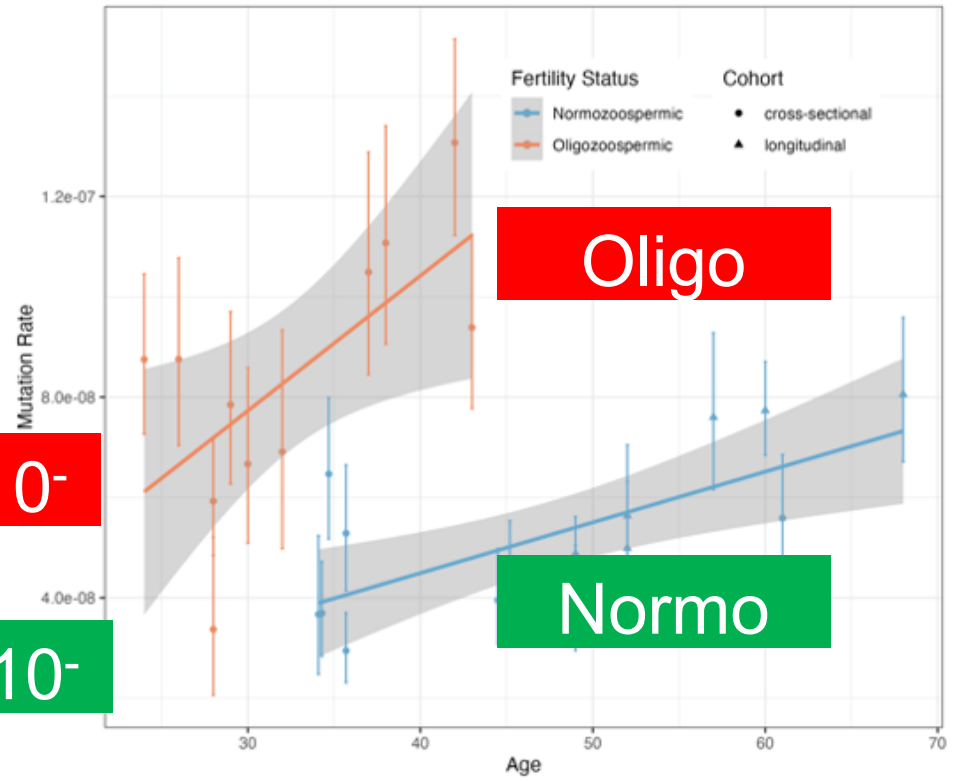
Step 3: Mutation calling and filtering





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10 yrs, $7M to generate one slide ($500k sequencing)



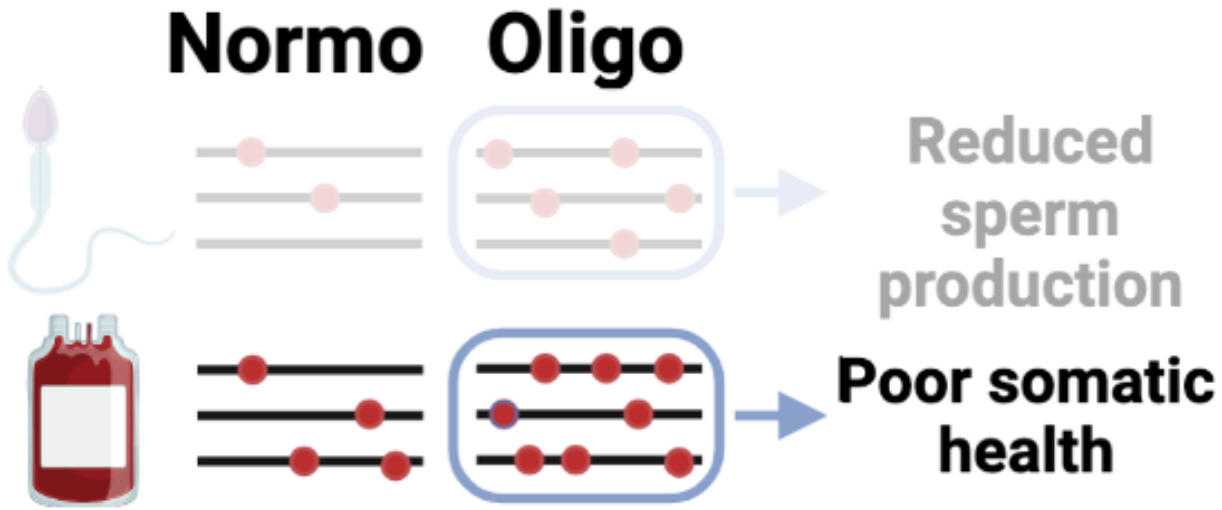
8.39×10^{-8}

3.25×10^{-8}

- Additive regression model:
  - Age
  - Fertility status
  - Dead sperm %
  - Sequencing Depth
- Significant difference in age-adjusted mutation rates (y-intercept)
- No significant difference in slopes
- Elevated C>T and T>A DNM rates

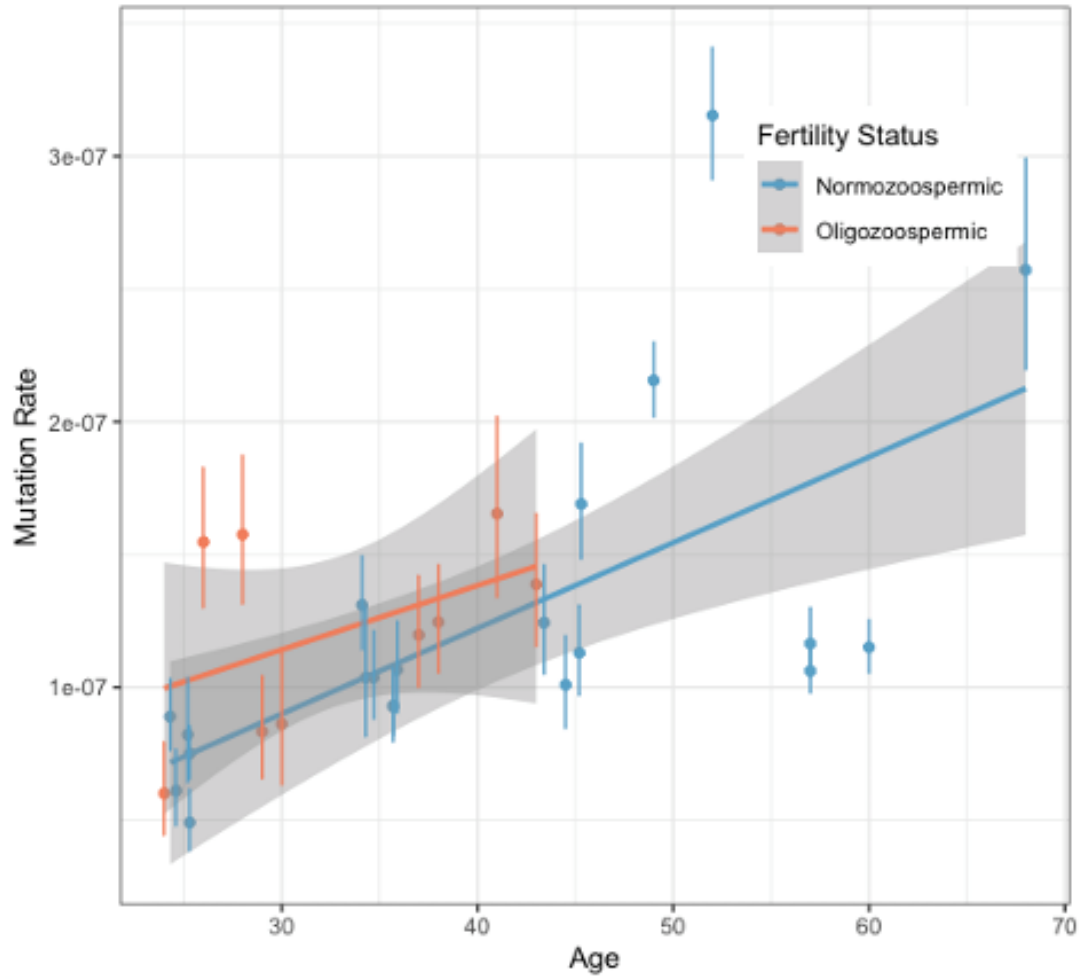
Oligo men have a 60% higher baseline DNM rate in sperm, if we adjust for age is 225%

What about the blood?



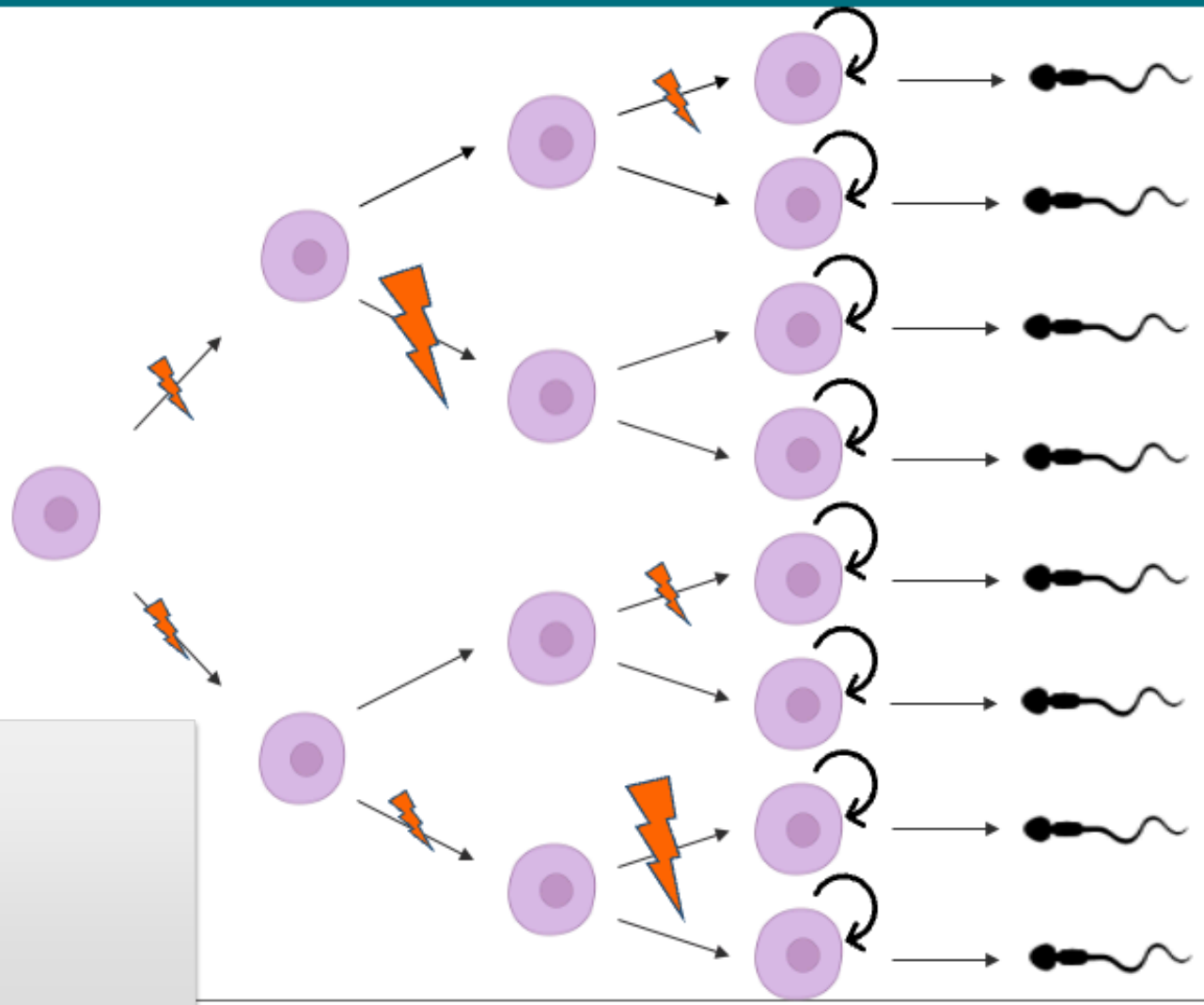
- Infertile men and their families have elevated risks for somatic health conditions (cancer, cardiovascular disease, etc.)
- Fertile men in the top quartile of age-adjusted germline mutation rates have shorter lifespans compared to the lowest quartile

Blood mutation rates are similar between fertile and infertile men



- Focusing on DNMs unique to blood samples (not in sperm)
- No significant difference between fertile and infertile men
- Selected infertile men without familial history of somatic disease

germline genomic instability is frequently observed in subfertile men



Model 1: Mutagenesis during germline development

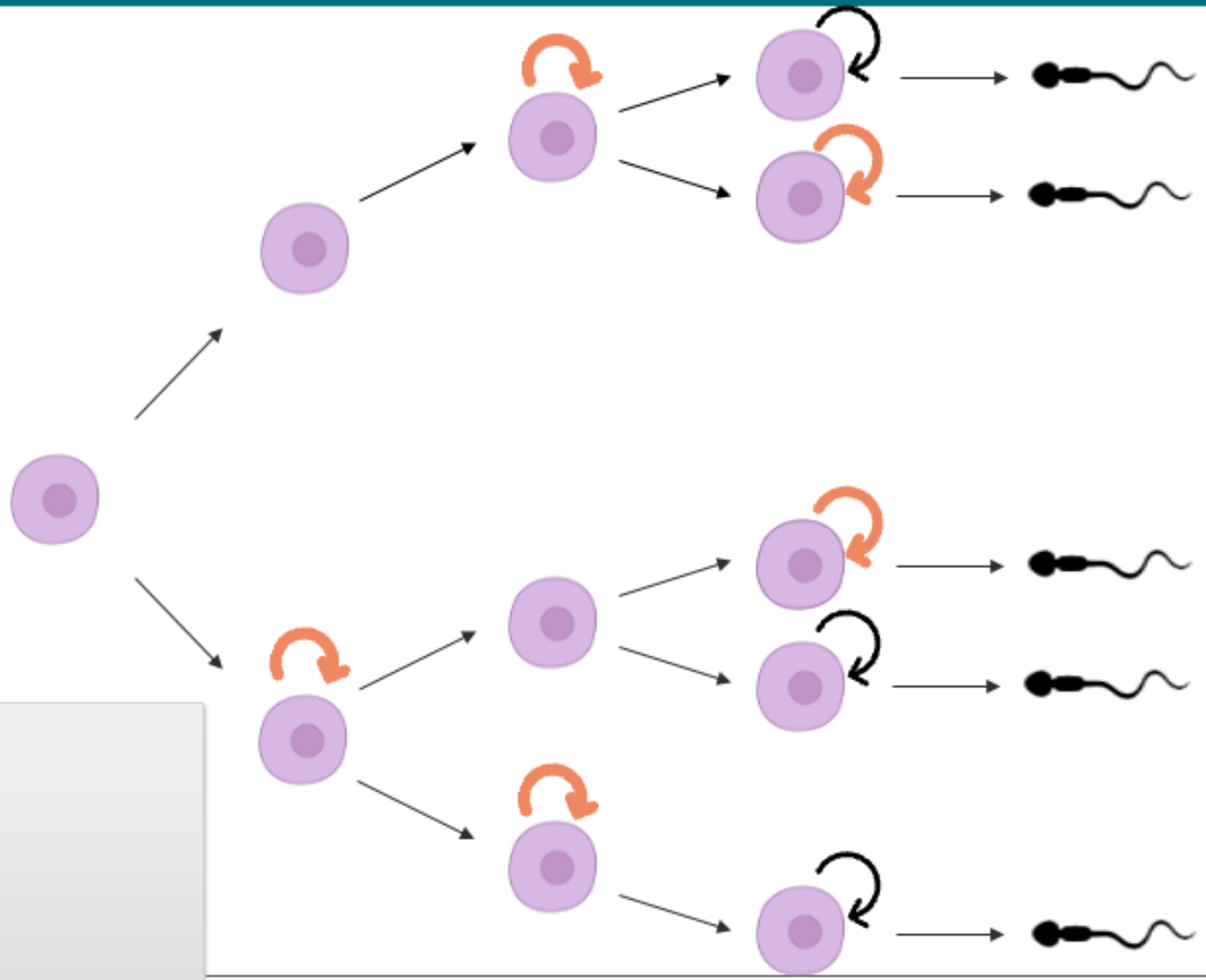
DNA Damage > DNA Repair

Fewer SSC lineages at puberty

Reduced sperm production

More mutations in sperm

germline genomic instability is frequently observed in subfertile men



Model 2: Mutagenesis during SSC replication

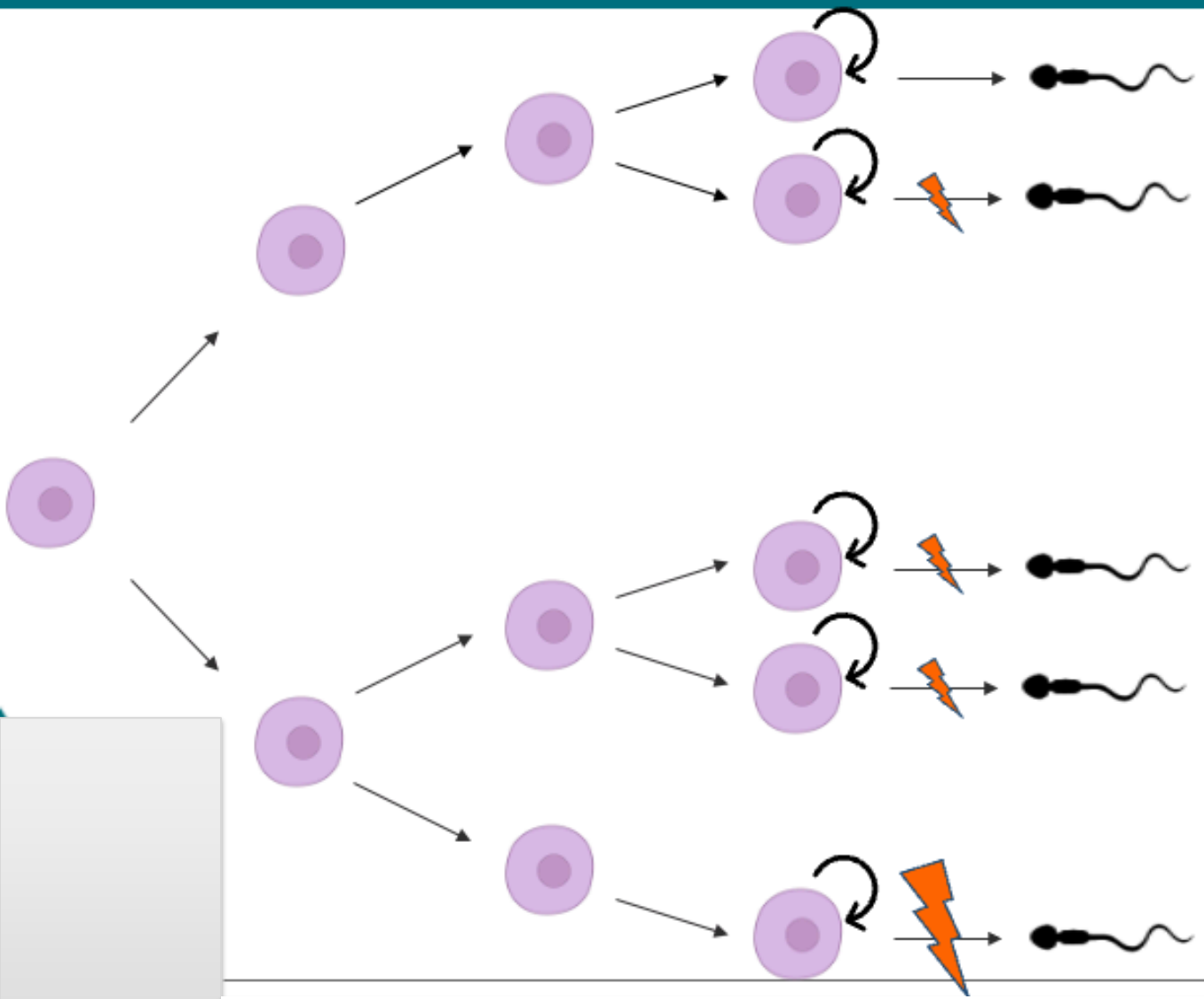
Increased cell division rate

Fewer functional SSC lineages

Reduced sperm production

More mutations in sperm

germline genomic instability is frequently observed in subfertile men



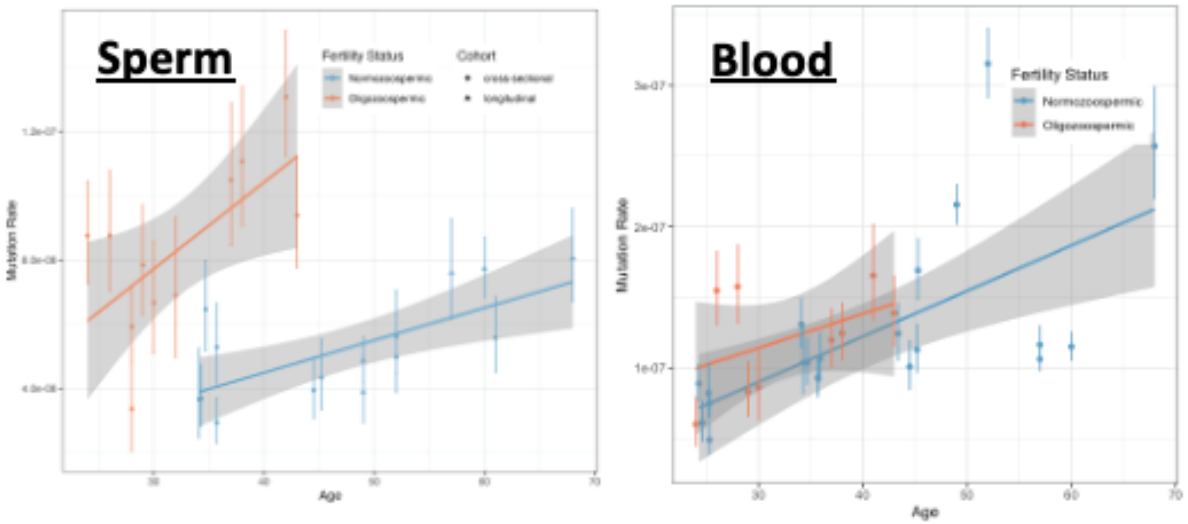
Model 3: Mutagenesis during meiosis

Errors during SSC differentiation

Reduced sperm production

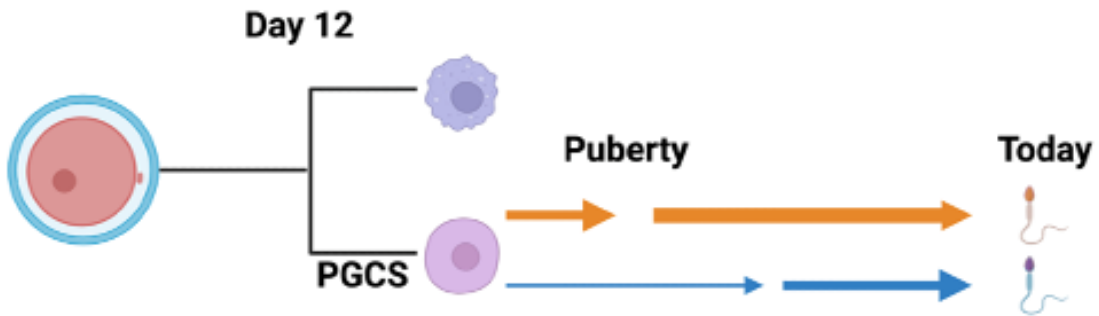
More mutations in sperm

Implications and future directions



Sperm: significant y-intercept

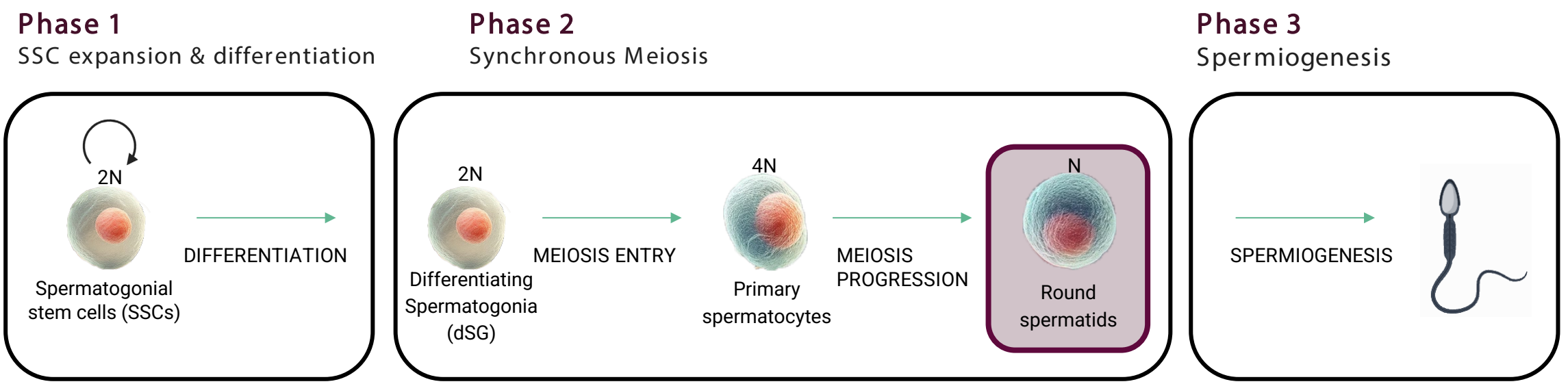
Blood: nonsignificant y-intercept and slope



- Infertile men may experience heightened germline mutagenesis between PGCS and puberty ⍰ why??
  - Early puberty onset
  - Mutagenic SSC niche
- Elevated germline mutation rates may be a hallmark of male infertility
- Next steps:
  - Finalize analyses (PAE genes, clonal DNMs, etc.)
  - Examine DNMs in older infertile men
  - Mutation rates ~ **motility** + fertility status

In Vitro Spermatogenesis

There are three Phases in growing mature sperm *in vitro*



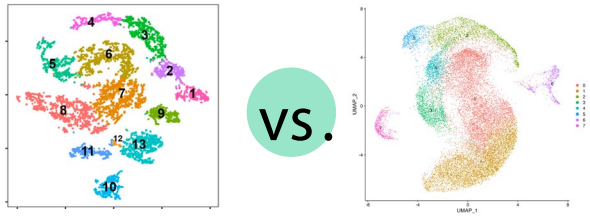
Healthy stem cells growing and replicating

Stem cells progress to become early stage gametes

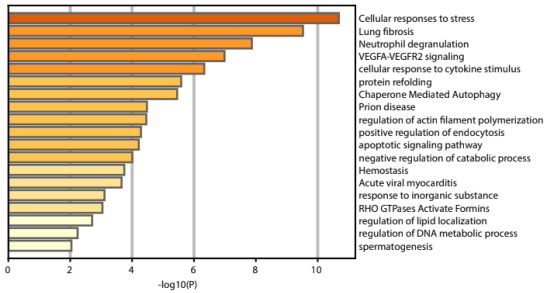
Early stage gametes complete the transition to mature sperm

Molecular-level understanding of spermatogenesis is the *foundation of ligand selection*

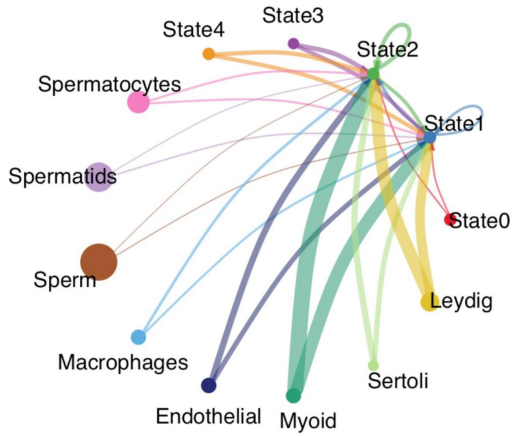
Bioinformatics workflow



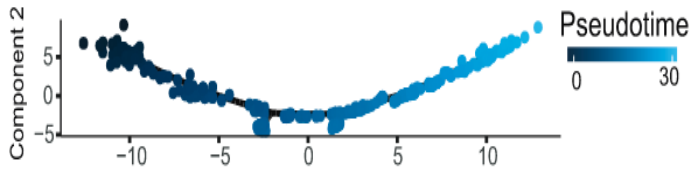
Transcriptional atlas of endogenous cells compared to cultured cells



DEG/GSEA analysis for regulated genes and signaling pathways

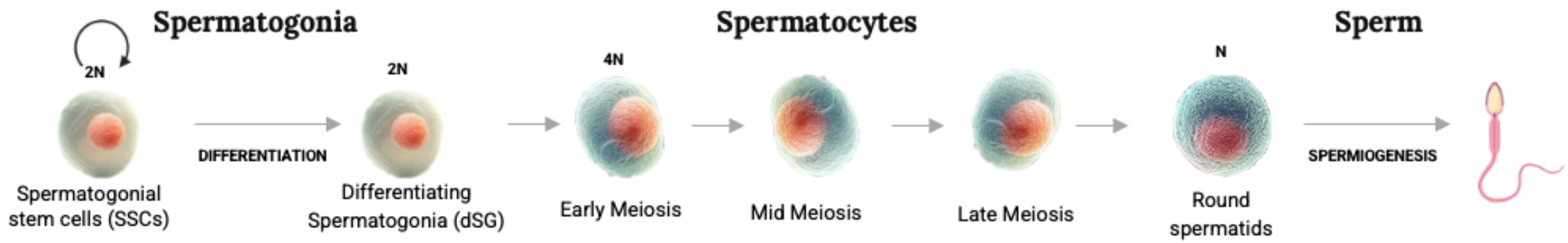


CellChat/CellPhoneDB analysis for cell-cell communications

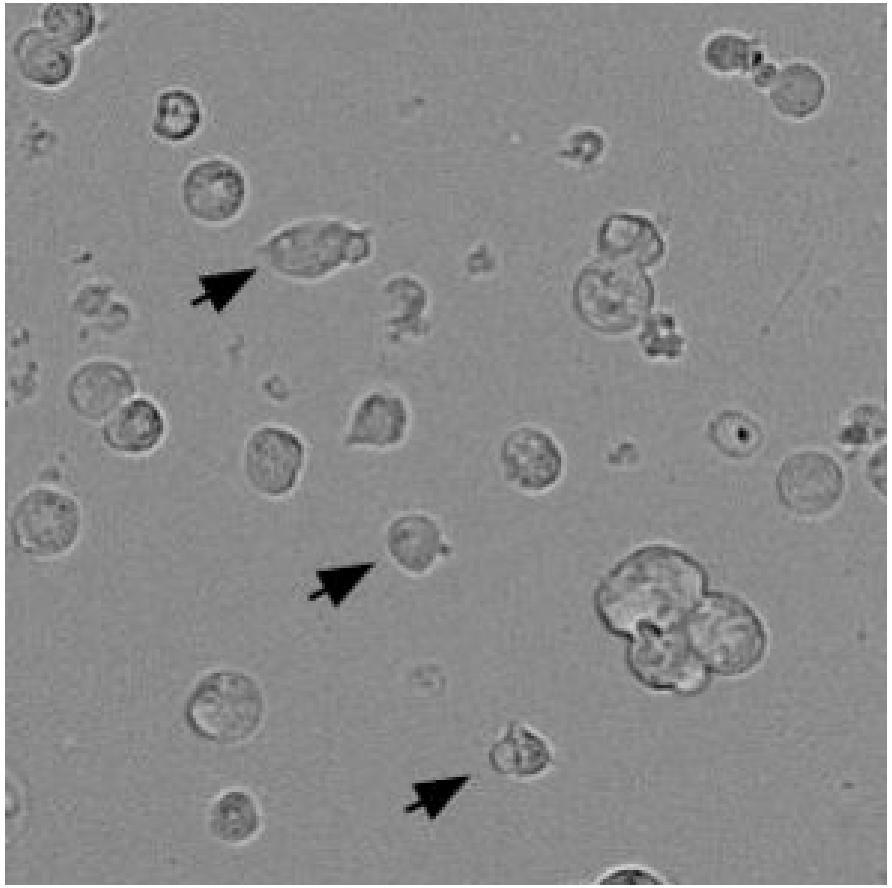


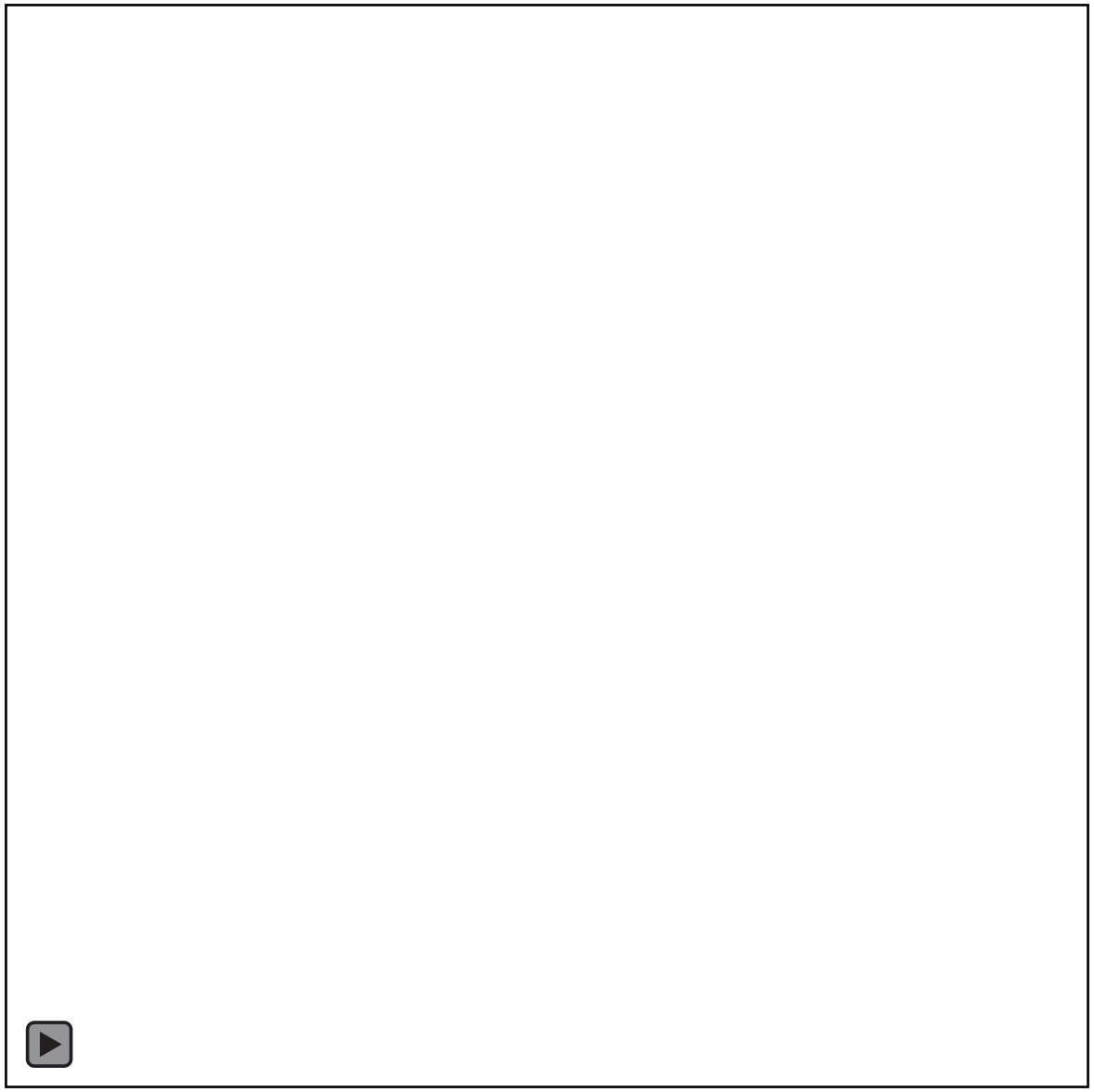
Pseudotime/Velocity analysis for cell developmental trajectory

For the first time in history - *human elongated sperm grown in vitro*



Timelapse images of spermatids growing tails *in vitro*



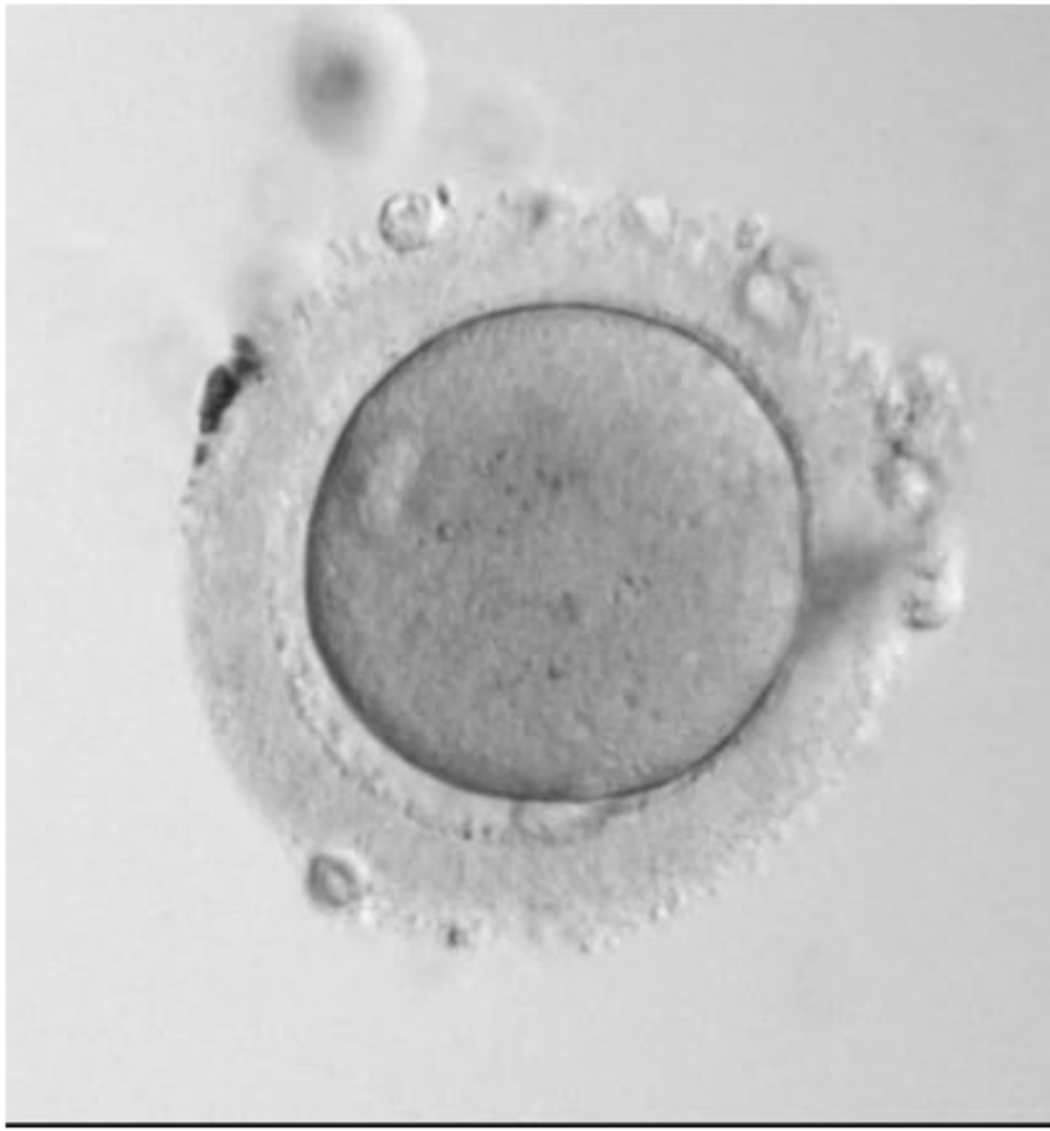


In vitro-derived sperm fertilize human eggs

Spermatid Selection & Injection



Fertilization / Embryo Formation







Targeted sequencing panel is comprised of 93 genes + “control” regions

DNA Repair*		Clonal expansion of sperm*	Infertility*	“Random Regions”
APC	LIG4	AKT3	APOA1	• GC rich/poor • Genic/non-genic • Coding/noncoding • Ostensibly under no selection
ATM	MLH1	BRAF	AR	
BRCA1	MLH3	CBL	AURKC	
BRCA2	MPG	FGFR2	DAZL	
ERCC1	MRE11	FGFR3	DCAF12L	
EXO1	MSH2	HRAS	M1AP	
FANCA	MSH3	KRAS	RB1	
FANCI	MSH4	MAP2K1	TEX11	
FANCM	MSH5	PTPN11	WT1	

*all genes not