Epigenetics: Explaining Male Infertility and Beyond

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Disclosures

- Owner/ Co-Owner Founder/ Co-Founder:
 - Inherent Biosciences/ Wasatch Biolabs

- Describe the balance between epigenetics and genetics
- Discuss the mechanistic role of epigenetics
- Summarize Predictive utility vs. mechanistic utility of epigenetics
- Discuss Epigenetics in male fertility
- Discuss Epigenetics in the clinic



The Fallacy: Nature M. Nurture

The Reality: Nature <u>AND</u> Nurture

Extreme views exist

Human Genome Project (2003)

"...revolutionize the diagnosis, prevention and treatment of most, if not all, human diseases." – Bill Clinton

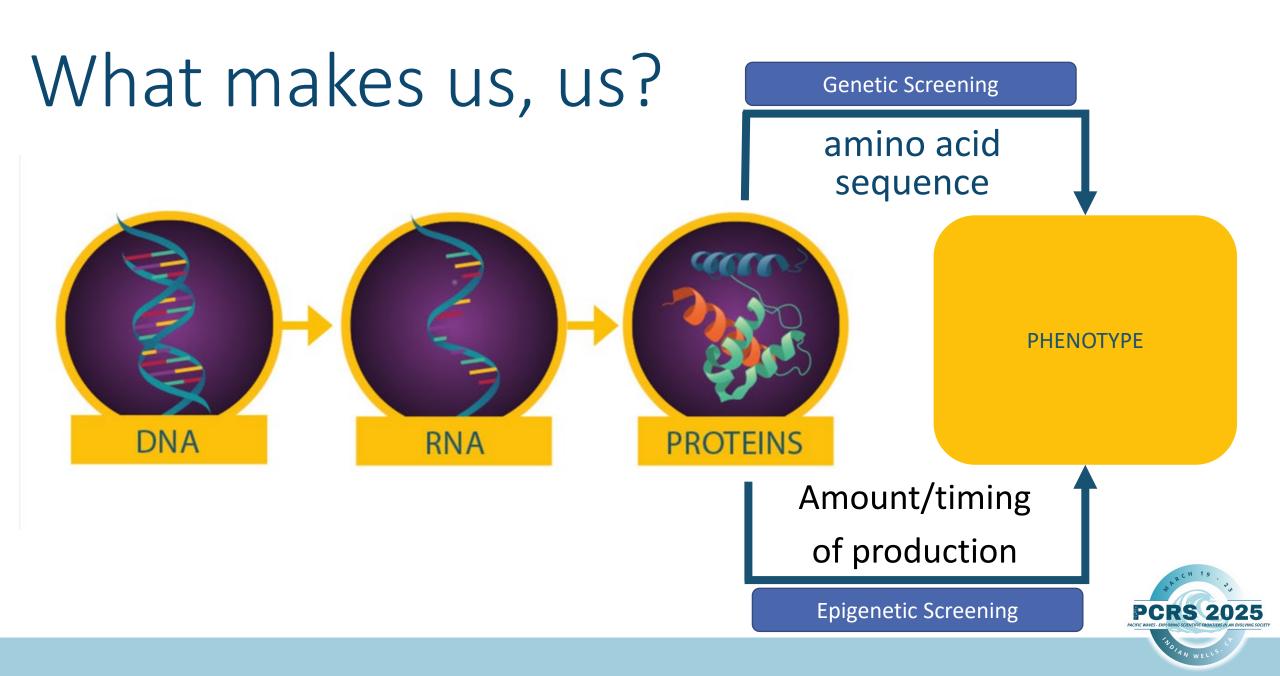
"you will see a complete transformation in therapeutic medicine." – Francis Collins

Underlying premise:

If we understand the genome, we'll understand all phenotypes and pathologies





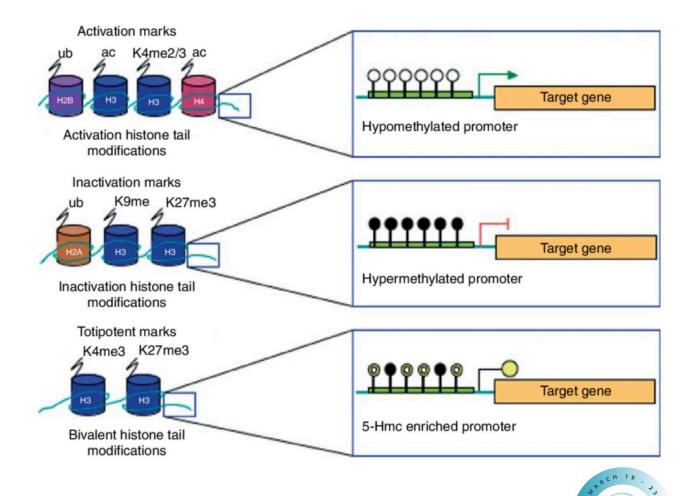


- The balance between epigenetics and genetics
- The mechanistic role of epigenetics
- Predictive utility vs. mechanistic utility of epigenetics
- Epigenetics in male fertility
- Epigenetics in the clinic



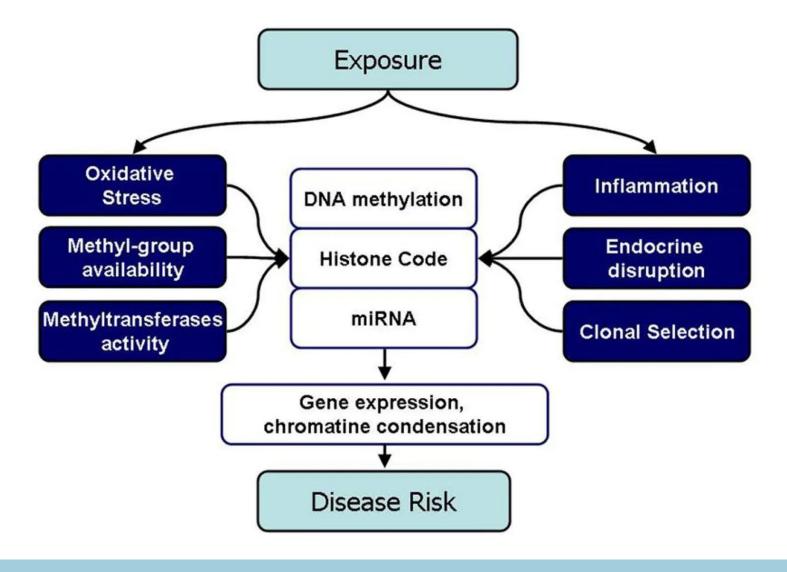
Epigenetics and gene regulation

- Histon tail modifications
 - Methylation
 - Acetylation
 - Ubiquitination
- DNA methylation
 - 5-mC and 5-hmC
 - At CpG dinucleotides
- Non-coding RNAs
 - snRNA
 - IncRNA
 - miRNA
 - Etc.



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The link between environment and gene regulation





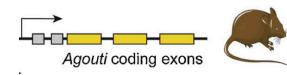
Agouti Variable Yellow Link between environment and phenotype



Genetically identical inbred mice - Agouti viable yellow allele



Methylated promoter



Agouti coding exons

Bisphenol A (BPA)

More likely if mother consumed Folate or genistein (soy)

More likely if mother consumed

certain environmental toxins:





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Agouti expression can impact metabolism:

Agouti mice (yellow) are also obese and have type II diabetes

Why difference in methylation?

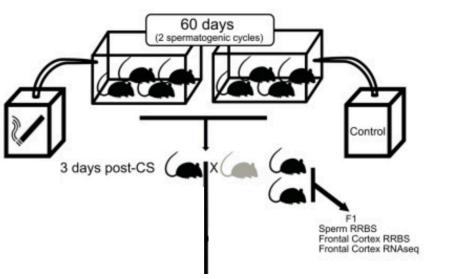
Unmethylated promoter

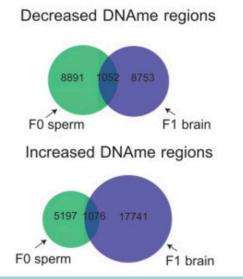
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Typical findings in sperm...

- >2,000 publications: search for "DNA Methylation Sperm"
- Differential methylation ≠ differential gene expression in every case
- Can't check the impact methylation differences directly





Despite our best efforts:

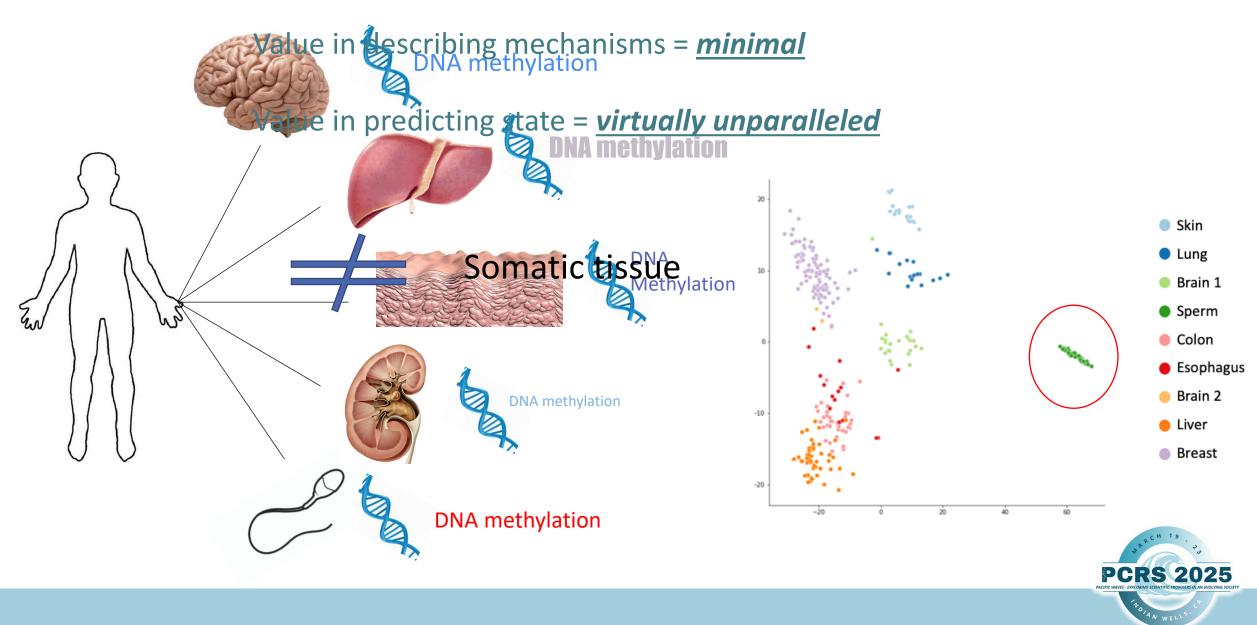
Epigenetic data = Not interpretable







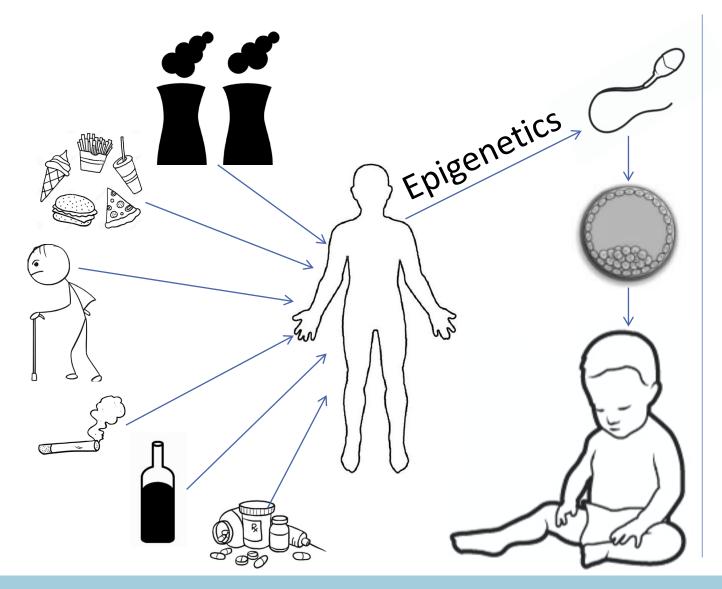
So, what is the value of Epigenetic data?



- The balance between epigenetics and genetics
- The mechanistic role of epigenetics
- Predictive utility vs. mechanistic utility of epigenetics
- Epigenetics in male fertility
- Epigenetics in the clinic



Environment / Epigenetics and male fertility



The impact of air pollution on sperm DNA methylation

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Table 1

The identified DMRs are each associated with two genes according to the GREAT analysis. Detailed DMRs include chromosome, start genomic position, stop genomic position, and region-gene associations with their corresponding distance to TSS.

Chromosome	Start	Stop	Genomic Region Size (b)	Region-gene Association (Distance to TSS)
chr10	131686103	131686886	783	EBF3 (+75,610), MGMT (+421,047)
chr16	86714536	86715555	1019	FOXL1 (+102,931), FBXO31 (+702,317)
chr17	113233	114320	1087	DOC2B (-82,350), RPH3AL (+88,821)

Decreased sperm motility is associated with air pollution in Salt Lake City

Ahmad Hammoud, M.D., M.P.H.,^a Douglass T. Carrell, Ph.D., H.C.L.D.,^b Mark Gibson, M.D.,^a Matt Sanderson, B.S.,^b Kirtly Parker-Jones, M.D.,^a and C. Matthew Peterson, M.D.^a

^a University of Utah, Division of Reproductive Endocrinology and Infertility; and ^b University of Utah, Andrology and IVF laboratories, Department of Surgery (Urology) and Physiology, Salt Lake City, Utah



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Future of DNA methylation testing: two flavors

Methylation Risk Scores (MRS)

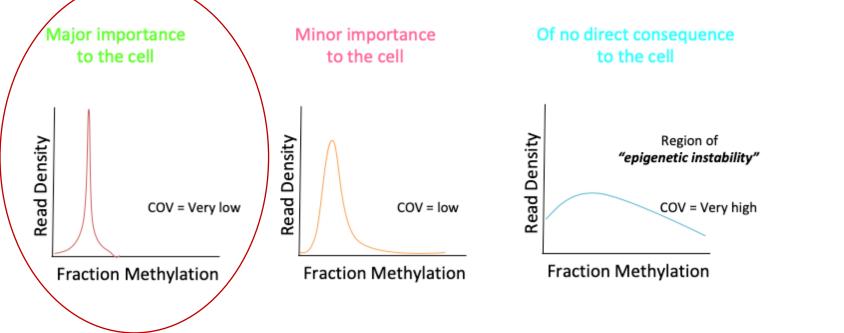
- Based on machine learning
 - Too big for us to interpret
- Cumulative assessment of every tree to understand the forest
- Black box

Tissue of origin assessment

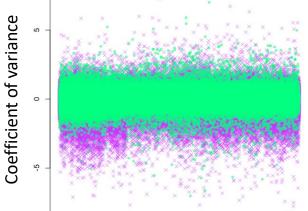
- ID genomic regions whose methylation patterns are predictive of tissue type
- Generation of a ratio
 - Ex. % of unknown DNA that was sperm derived

MRS for IUI fertility success: Sperm Epigenetic Instability





At genes important to sperm



- Infertile patient
- Fertile patient



SpermQT: IUI or straight to IVF?

Multisite Retrospective Analysis, N=544

Result	%Pregnancy	% Live Birth	Avg. IUI Cycles	% Total Motile Count ≥20M
Normal	46.45%	34.08%	2.5	89%
Abnormal	25.49%	17.6%	2.5	<mark>80%</mark>
All Results	44.49%	32.54%	2.5	87%
P Values	0.004	0.006		· · · · · · · · · · · · · · · · · · ·

Miller RH, De Vilbiss EA, Brogaard KR, et al. Epigenetic determinants of reproductive potential augment the predictive ability of the semen analysis. F S Sci. 2023;4(4):279-285.

Real World, Multi-site, Analysis N=202

Result	%Pregnancy	Avg. IUI Cycles	% Total Motile Count ≥20M
Normal	22%	2.8	86.8%
Abnormal	0%	2.4	72.2%
All Results	19.3%	2.8	85%

P Values 0.009

Publication pending



Tissue of origin assessment in NOA patients

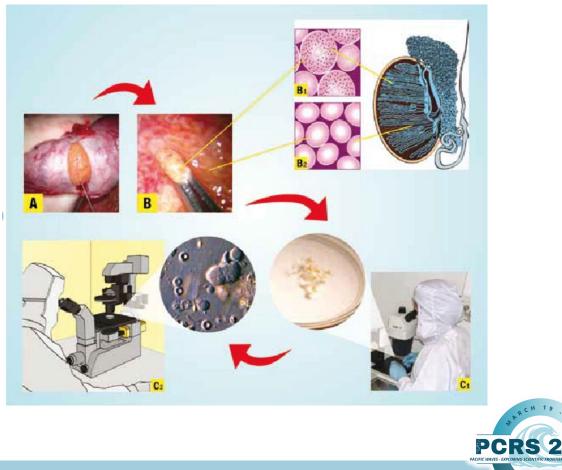
Non-obstructive azoospermia

- No sperm in the ejaculate and no known obstruction in the male reproductive tract
- 1% of all men (10-15% of all infertile men)

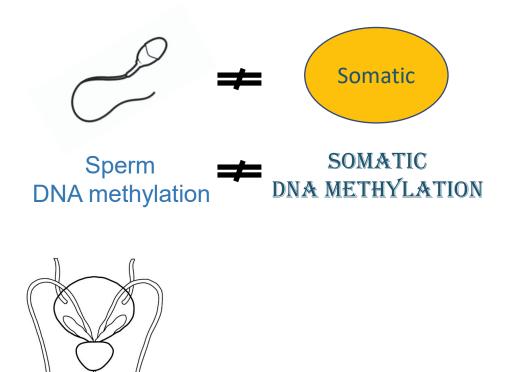




Microsurgical Testicular Sperm Extraction (microTESE)



Can DNA methylation help?



Seminal cfDNA

cfDNA in semen sample

Sperm Sperm DNA methylation DNA methylation DNA METHYLATION

> Sperm DNA methylation

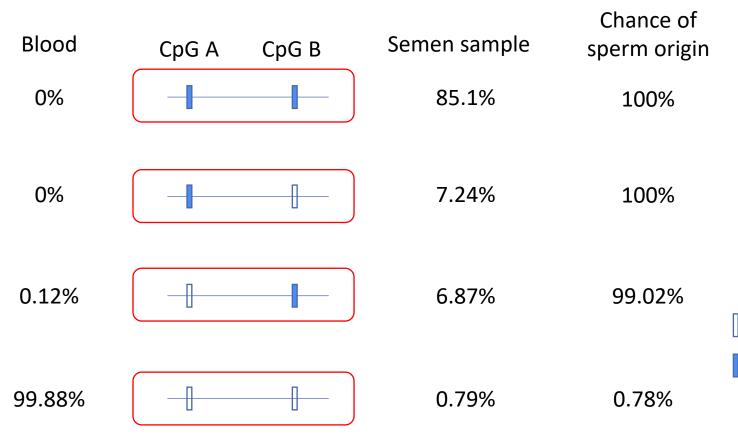
SOMATIC DNA METHYLATION SomATIC Sperm DNA METHYLATION DNA methylation

> Sperm DNA methylation



Translation to clinical viability

- Long read sequencing (one molecule at a time)
- Simple test: one molecule, two CpGs
- Assessed ~10,000 reads



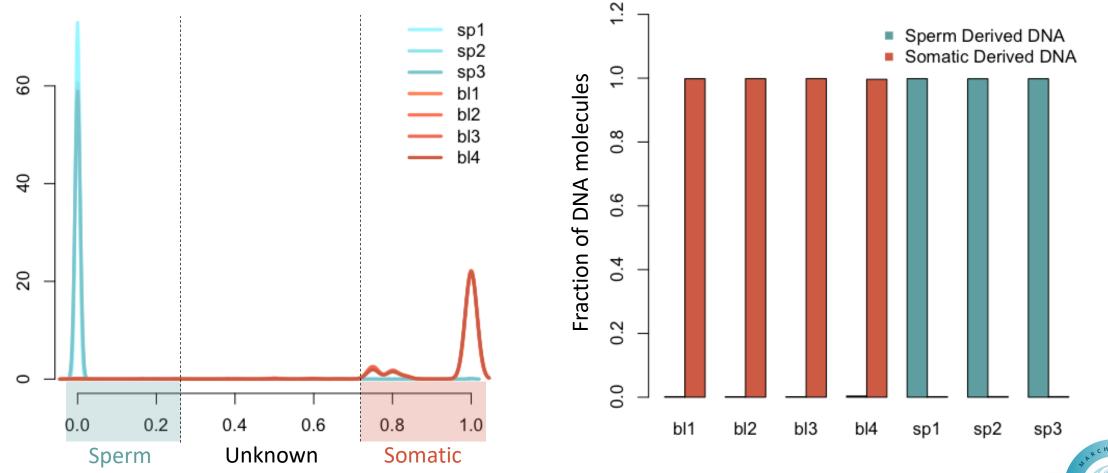
Methylation @ CpGs					
	CpG A	CpG B			
PPV	100%	99.87%			
NPV	92.89%	92.56%			
Sensitivity	92.35%	91.97%			
Specificity	100%	99.88%			

Unmethylated CpG

Methylated CpG



Single region of interest: 400bps | 9 CpGs Blood and sperm assessment



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Can we predict biopsy success?

0

% Sperm cfDNA vs. Extraction Results 0.07 Negative Sperm Extraction Positive Sperm Extraction 0.06 VOC 0.04 0.03 0.02 0.01

10834 898 150 003 053 084 068 092 011 011 0548 0934 122 026 084 0832 898 010 042 023 050 100 105 8959



Combining genetics and epigenetics

Sequencing data to screen for XXY.

Fraction of reads aligning to chrX in XX, XY, and XXY

XX (n = 12) XY (n = 51)XXY (n = 1)150 100 Density 50 0 0.00 0.01 0.02 0.03 0.04 0.05 0.06 fraction of reads aligning to X

and Y-micro deletions

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Why it matters

- Epigenetics bridges the gap between nature and nurture
- Key component to gene regulation that is responsive to the environment
- Epigenetic data will be used in the clinic in the future
- Not ideal for mechanistic studies
- Well-suited for predictive models
- Can be used for the assessment of male infertility and the prediction of ART success



Thank You!

BYU

- Ryan Barney
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- Carter Norton
- Ryan Miller
- Jonathon Hill

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- Andy Olson

Baylor College of Medicine

- Larry Lipshultz
- Jordan Kassab





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