RNA, Exosomes and Epigenetics:

How Much We Still Don't Know About Male Reproduction

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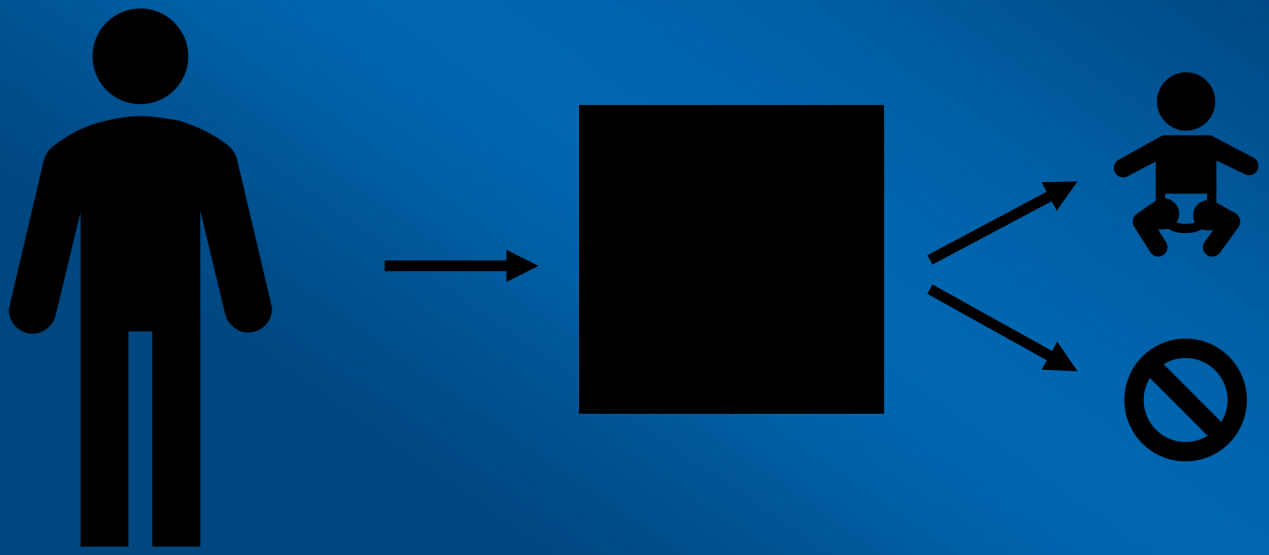
Disclosures

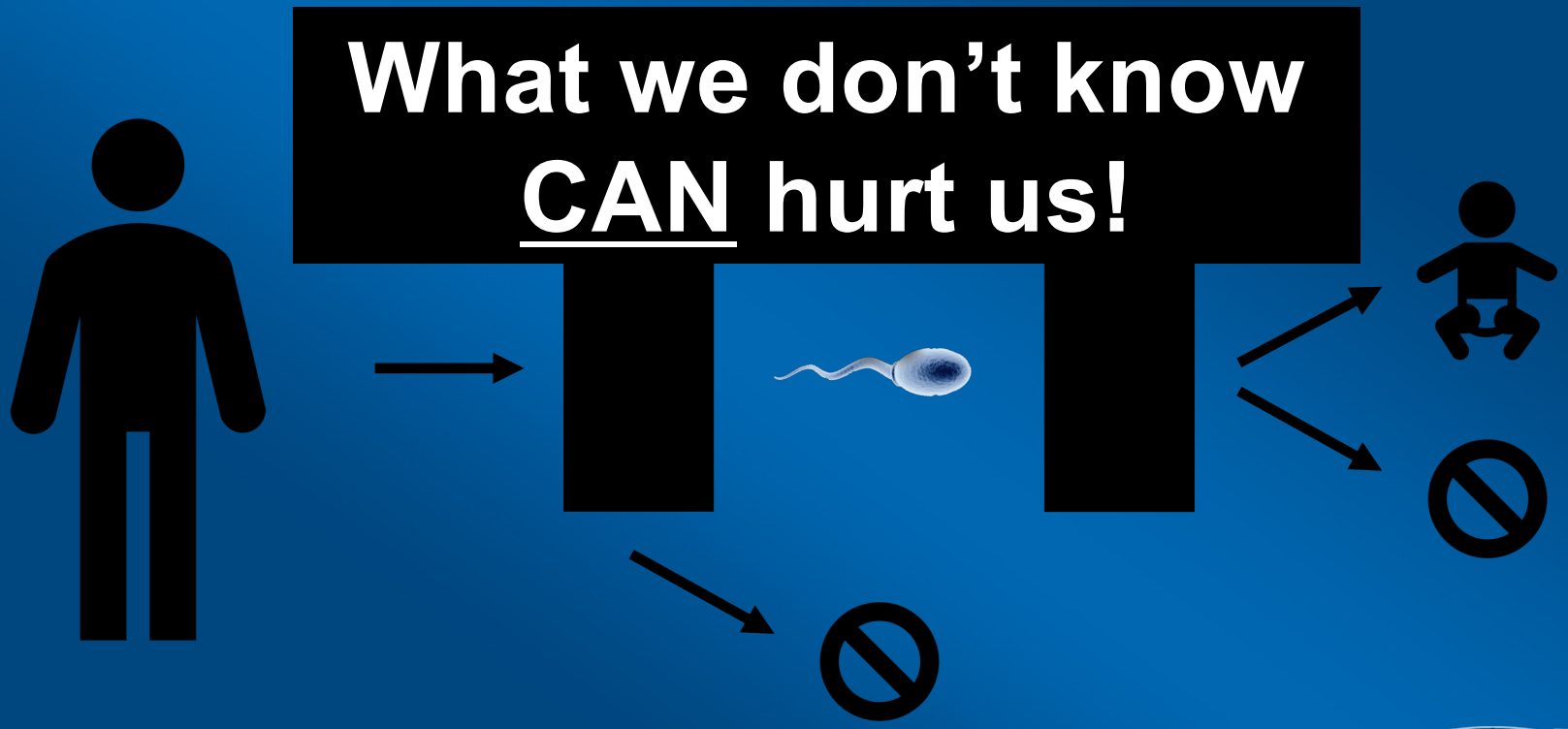
- None

I probably should have chosen to talk about what we DO know about male reproduction…

Black Boxes of Male Infertility



Black Boxes of Male Infertility



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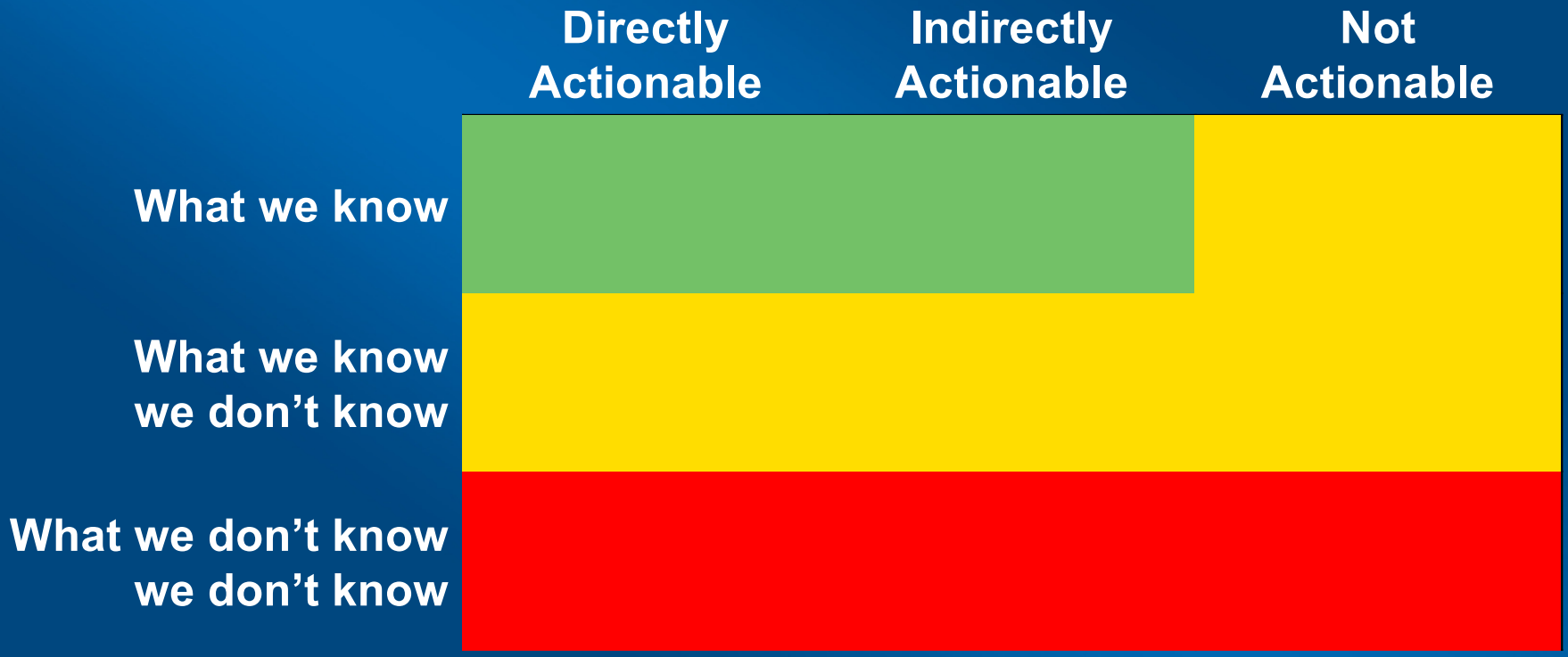
Beyond the Genome

- Epigenetics
- Exosomes

Why not talk about genetics?

- Heritability – the more deleterious the mutation the less likely it is to be passed on
- Likely many genes, partial penetrance
- Outcome measures are complex, multifactorial and delayed
- High variability in sperm counts, motility, morphology
- Controls often not phenotyped for fertility

Knowledge versus Action



Paternal Transmission Phenotypes

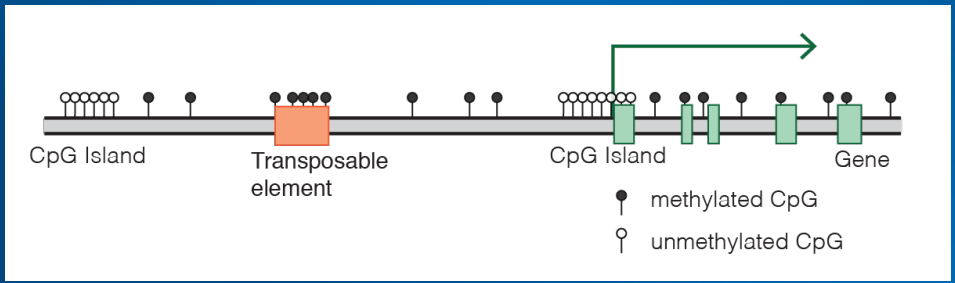
- High fat/Western Diet
- Nicotine/Ethanol
- Obesity*
- Fear conditioning
- Endocrine disruptors
- Stress/Trauma
- Temperature
- Toxin exposures (e.g., vincazolin)*

Epigenetics

- Non-DNA mediated transfer of heritable properties from one generation to the next
  - DNA Methylation
  - Post-translational histone modifications
  - Small RNAs

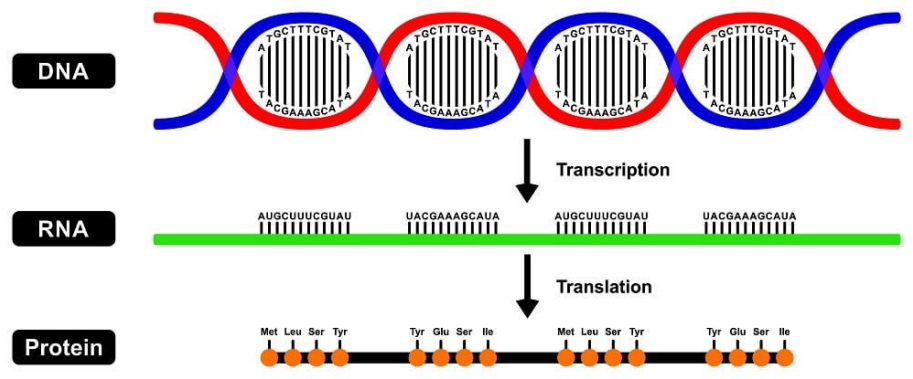
DNA Methylation

- Regulates gene expression
- Cell-type specific
- Important for genome stability
- Dynamic
  - Response to environment
  - Altered by mutation/ DNA damage



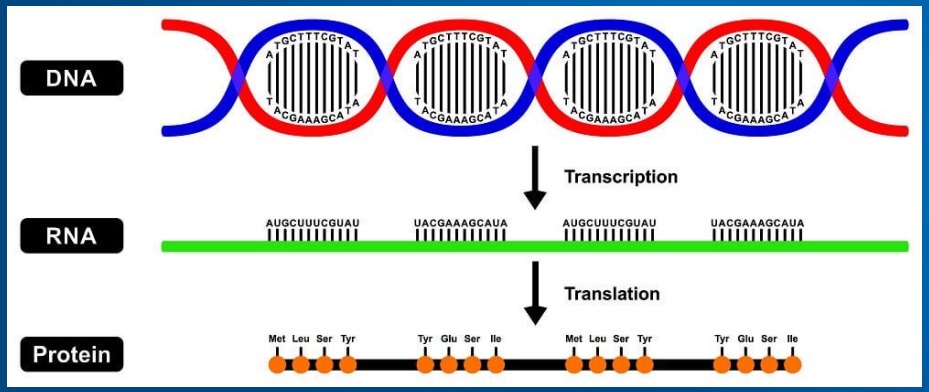
By Mariuswalter - Own work, CC BY-SA 4.0, https://commons.wikimedia.org/w/index.php?curid=54319988

Central Dogma of Molecular Biology



https://biologydictionary.net/central-dogma/

Central Dogma of Molecular Biology



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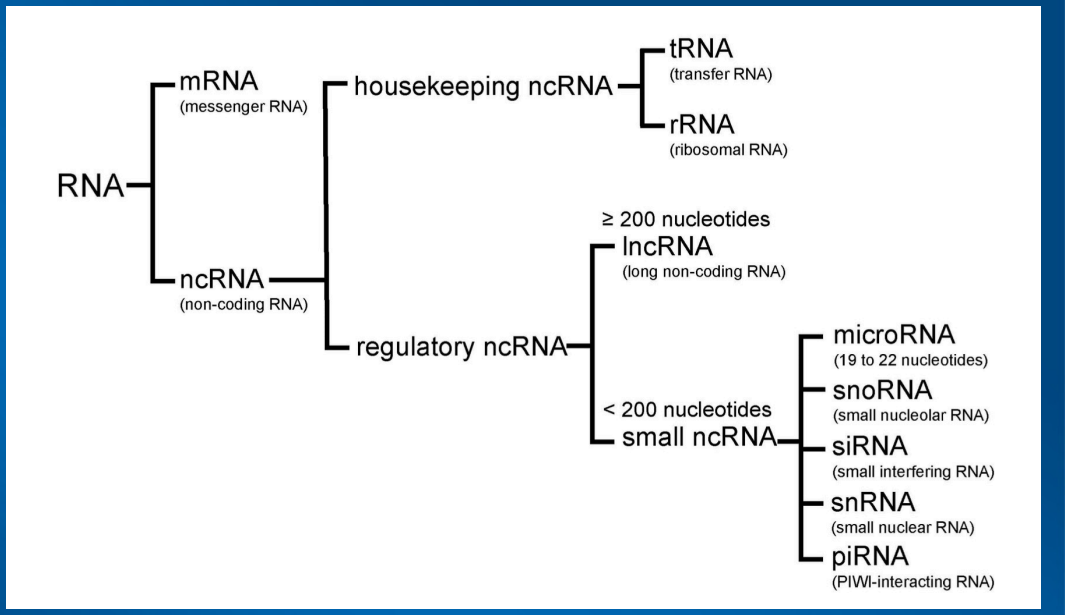
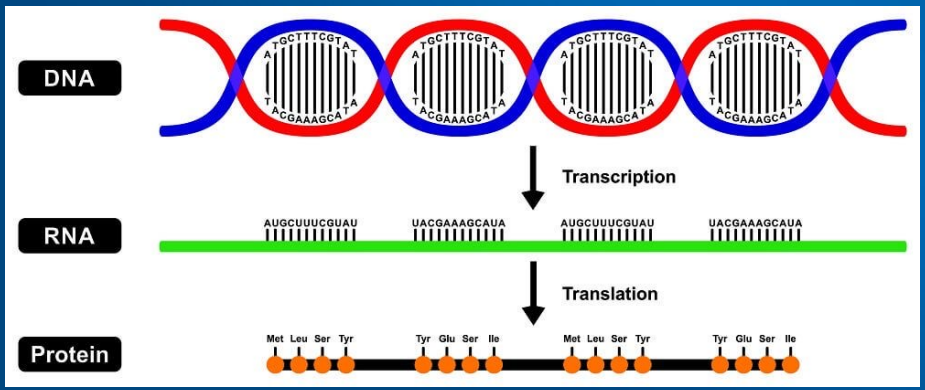


doi:10.3390/cells6020012

Central Dogma of Molecular Biology



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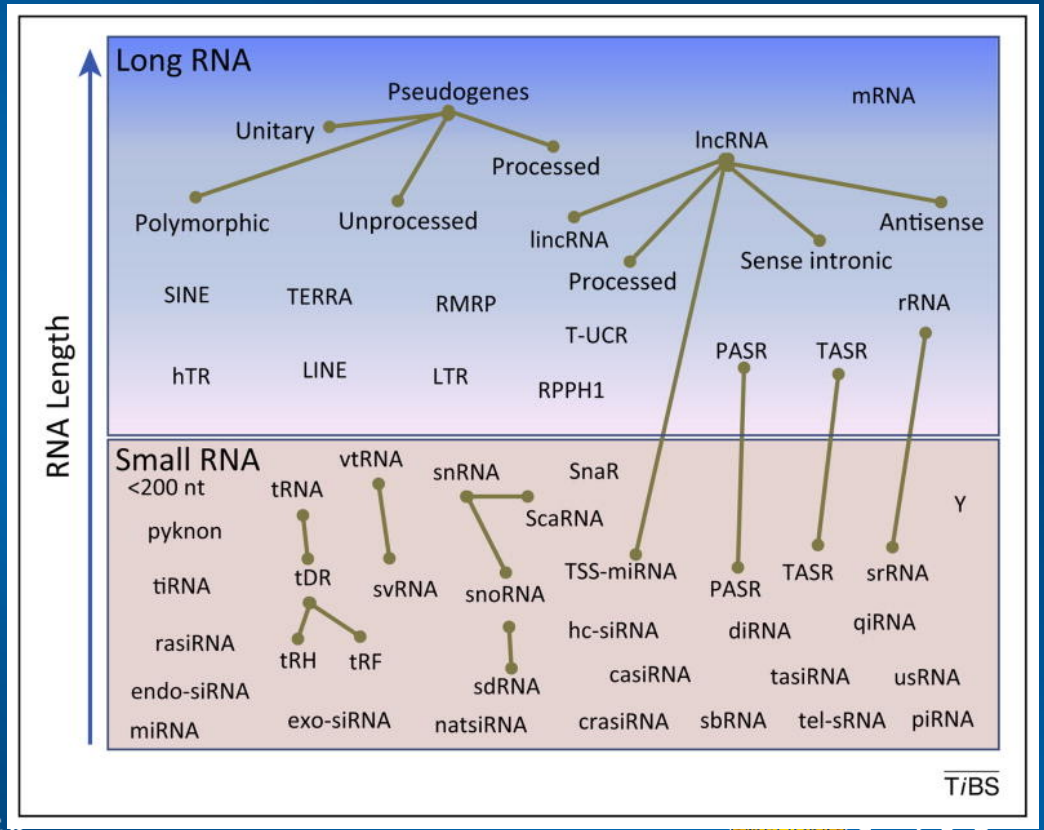
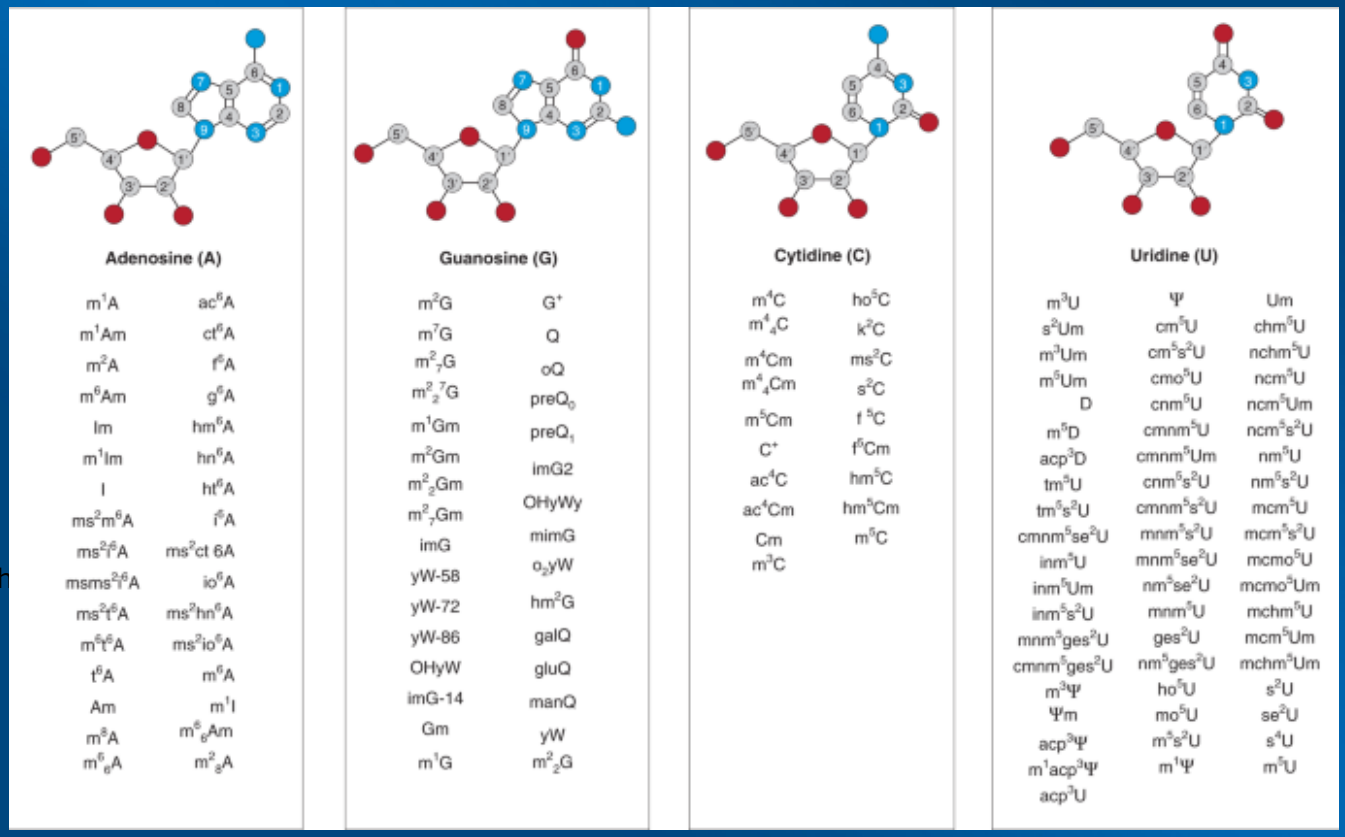


Trends Biochem Sci. 2015 Jan; 40(1): 4–7

Central Dogma of Molecular Biology



Adenosine (A)

m^1A	ac^6A
m^1Am	ct^6A
m^2A	f^6A
m^6Am	g^6A
Im	hm^6A
m^1Im	hn^6A
I	ht^6A
ms^2m^6A	i^6A
ms^2i^6A	$ms^2ct\ 6A$
$msms^2i^6A$	io^6A
ms^2t^6A	ms^2hn^6A
m^6t^6A	ms^2io^6A
t^6A	m^6A
Am	m^1I
m^3A	$m^6{}_8Am$
$m^6{}_6A$	$m^2{}_8A$

Guanosine (G)

m^2G	G^+
m^7G	Q
$m^2{}_7G$	oQ
$m^2{}_2{}^7G$	$preQ_0$
m^1Gm	$preQ_1$
m^2Gm	imG2
$m^2{}_2Gm$	OHyWy
$m^2{}_7Gm$	mimG
imG	o_2yW
yW-58	hm^2G
yW-72	galQ
yW-86	gluQ
OHyW	manQ
imG-14	yW
Gm	$m^2{}_2G$
m^1G	

Cytidine (C)

m^4C	ho^5C
$m^4{}_4C$	k^2C
m^4Cm	ms^2C
$m^4{}_4Cm$	s^2C
m^5Cm	f^5C
C^+	f^5Cm
ac^4C	hm^5C
ac^4Cm	hm^5Cm
Cm	m^5C
m^3C	

Uridine (U)

m^3U	Ψ	Um
s^2Um	cm^5U	chm^5U
m^3Um	cm^5s^2U	$nchm^5U$
m^5Um	cmo^5U	ncm^5U
D	cnm^5U	ncm^5Um
m^5D	$cmnm^5U$	ncm^5s^2U
acp^3D	$cmnm^5Um$	nm^5U
tm^3U	cnm^5s^2U	nm^5s^2U
tm^5s^2U	$cmnm^5s^2U$	mcm^5U
$cmnm^5se^2U$	mnm^5s^2U	mcm^5s^2U
inm^5U	mnm^5se^2U	$mcmo^5U$
inm^5Um	nm^5se^2U	$mcmo^5Um$
inm^5s^2U	mnm^5U	$mchm^5U$
mnm^5ges^2U	ges^2U	mcm^5Um
$cmnm^5ges^2U$	nm^5ges^2U	$mchm^3Um$
$m^3Ψ$	ho^5U	s^2U
Ψm	mo^5U	se^2U
$acp^3Ψ$	m^5s^2U	s^4U
$m^1acp^3Ψ$	$m^1Ψ$	m^5U
acp^3U		

https://doi.org/10.1038/s41588-021-00903-1

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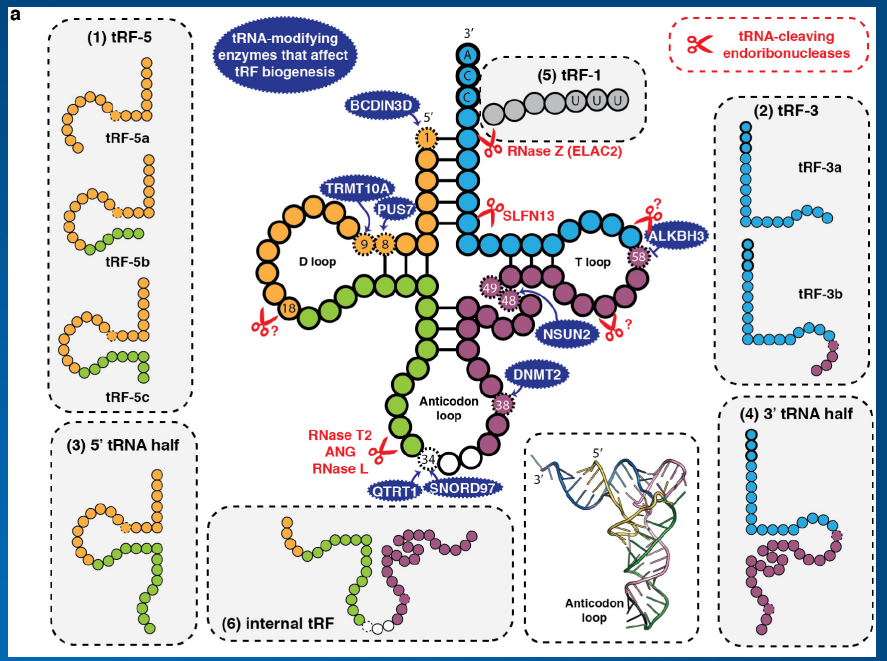
Small RNAs

- Sperm are largely transcriptionally silent, but RNA payload changes as they mature
- Most common sperm RNAs:
  - piRNAs — DNA methylation, repetitive element silencing
  - miRNAs, tDF — Early embryo development; Paternal epigenetic inheritance
  - rsRNAs — ??? There are a LOT of these

tRNA Derived Fragments

- Thought to be degradation products of tRNAs, but…
- Expression is cell type specific
- RNA nucleotide alterations affect resistance to cleavage
- Fragments bind targets RNAs *very* specifically



Annu Rev Genet. 2020 Nov 23; 54: 47–69.

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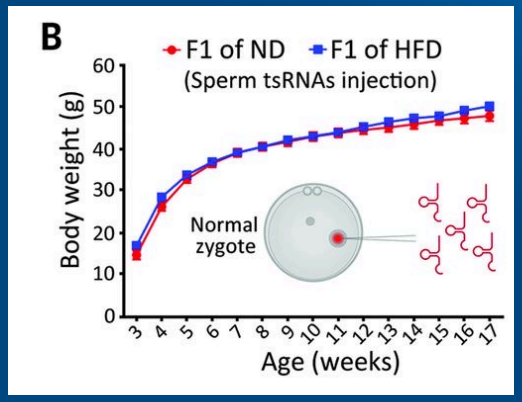
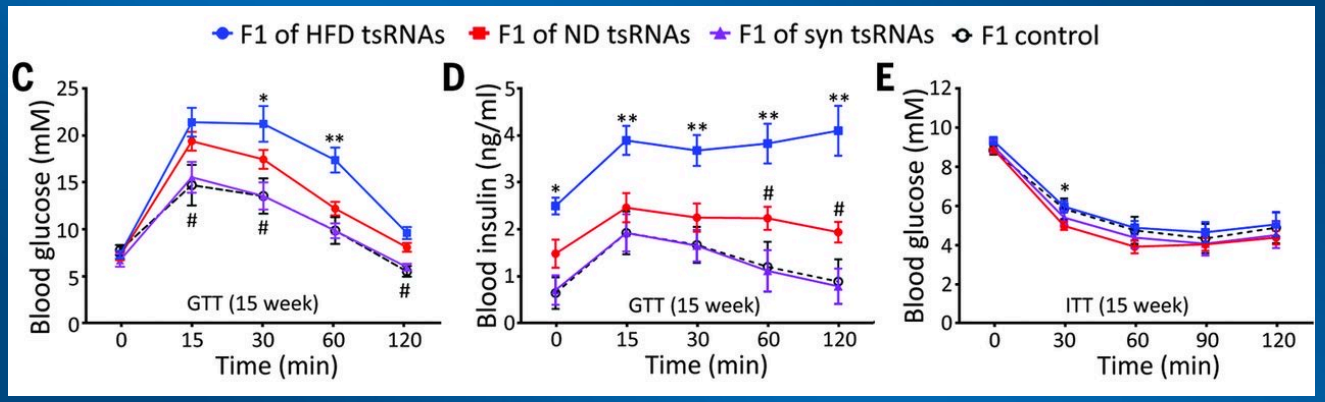
SMALL RNAS

Sperm tsRNAs contribute to intergenerational inheritance of an acquired metabolic disorder

Qi Chen,[1,3*†] Menghong Yan,[2†] Zhonghong Cao,[1,5†] Xin Li,[1†] Yunfang Zhang,[1,5†] Junchao Shi,[1,5†] Gui-hai Feng,[1] Hongying Peng,[1,4] Xudong Zhang,[1,5] Ying Zhang,[1] Jingjing Qian,[1,5] Enkui Duan,[1*] Qiwei Zhai,[2*] Qi Zhou[1*]





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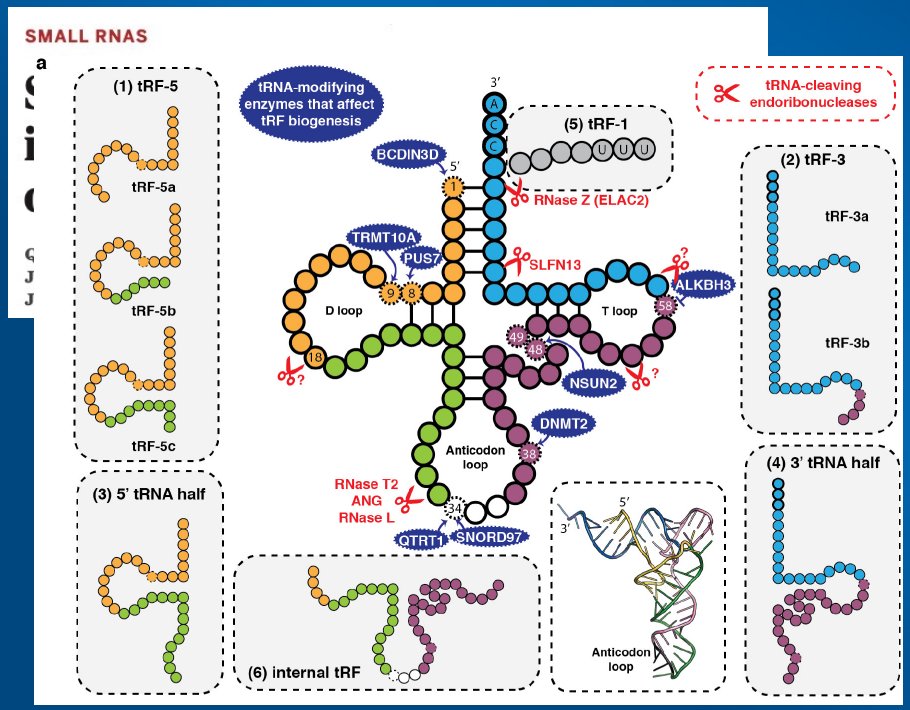
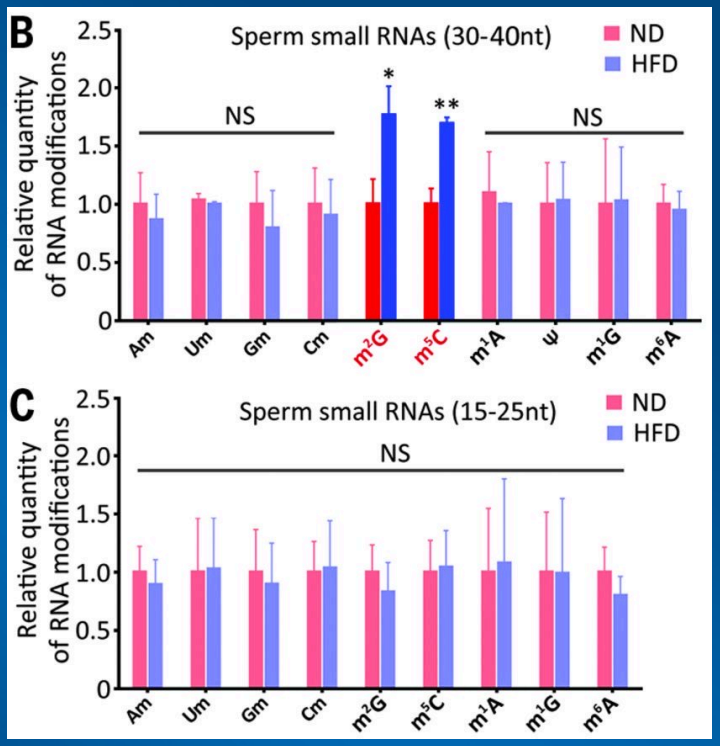

SMALL RNAS
a
(1) tRF-5
tRF-5a
tRF-5b
tRF-5c
tRNA-modifying enzymes that affect tRF biogenesis
tRNA-cleaving endoribonucleases
(5) tRF-1
BCDIN3D
RNase Z (ELAC2)
TRMT10A
PUS7
SLFN13
ALKBH3
D loop
T loop
NSUN2
DNMT2
Anticodon loop
RNase T2
ANG
RNase L
QTRT1
SNORD97
(2) tRF-3
tRF-3a
tRF-3b
(3) 5' tRNA half
(4) 3' tRNA half
(6) internal tRF
Anticodon loop


B
Sperm small RNAs (30-40nt)
ND
HFD
Relative quantity of RNA modifications
NS
NS
*
**
Am
Um
Gm
Cm
m2G
m5C
m1A
ψ
m1G
m6A
C
Sperm small RNAs (15-25nt)
ND
HFD
Relative quantity of RNA modifications
NS
Am
Um
Gm
Cm
m2G
m5C
m1A
m1G
m6A

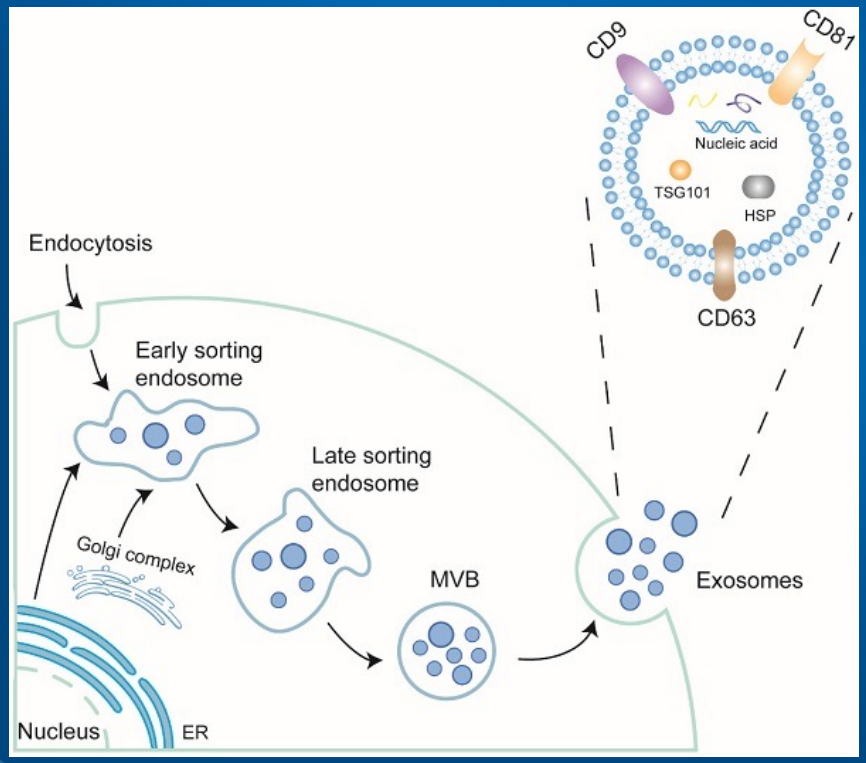
Paternal Transmission Phenotypes

- High fat/Western Diet
- Nicotine/Ethanol
- Obesity*
- Fear conditioning
- Endocrine disruptors
- Stress/Trauma
- Temperature
- Toxin exposures (e.g., vincazolin)*

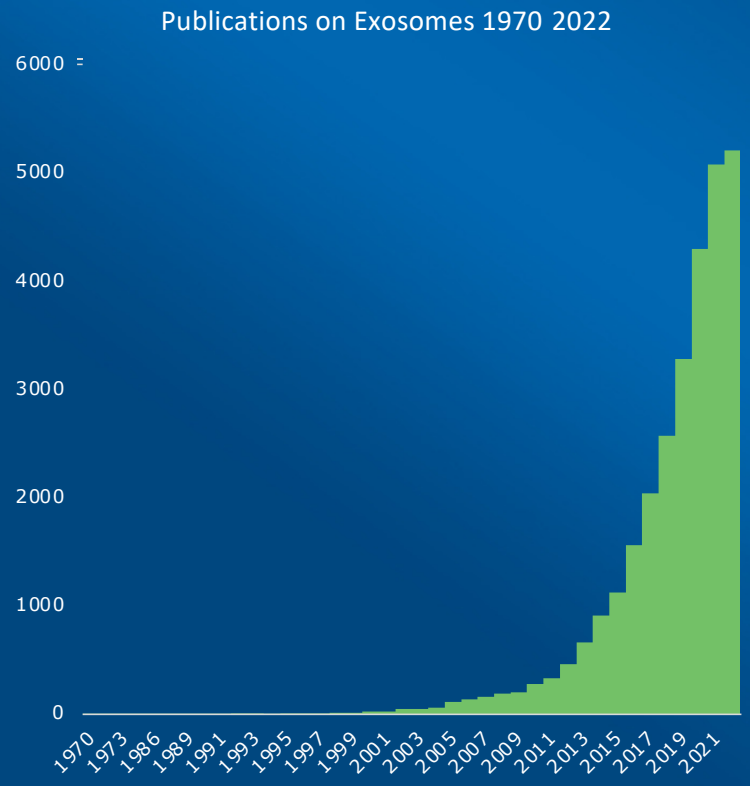
Many have been linked to alterations in sperm sRNA content

What about rsRNAs???

Exosomes



- Produced from multivesicular bodies (MVBs)
- Classified based on size and surface proteins
- Cell-to-cell communication
  - RNAs, proteins, lipids, DNA



- Produced by all cells
- Most studied in male reproduction:
  - Prostasomes
  - Epididymosomes

Promotive effect on human sperm progressive motility by prostasomes

B Stegmayr, G Ronquist

PMID: 6219486 DOI: 10.1007/BF00255932

Abstract

Seminal plasma constituents were separated on Sephadex G200 gel
was analysed with regard to protein content, ATPase activity and pro
progressive motility. Two different chromatographic fractions were al
microscopy after sedimentation by preparative ultracentrifugation. A

1982

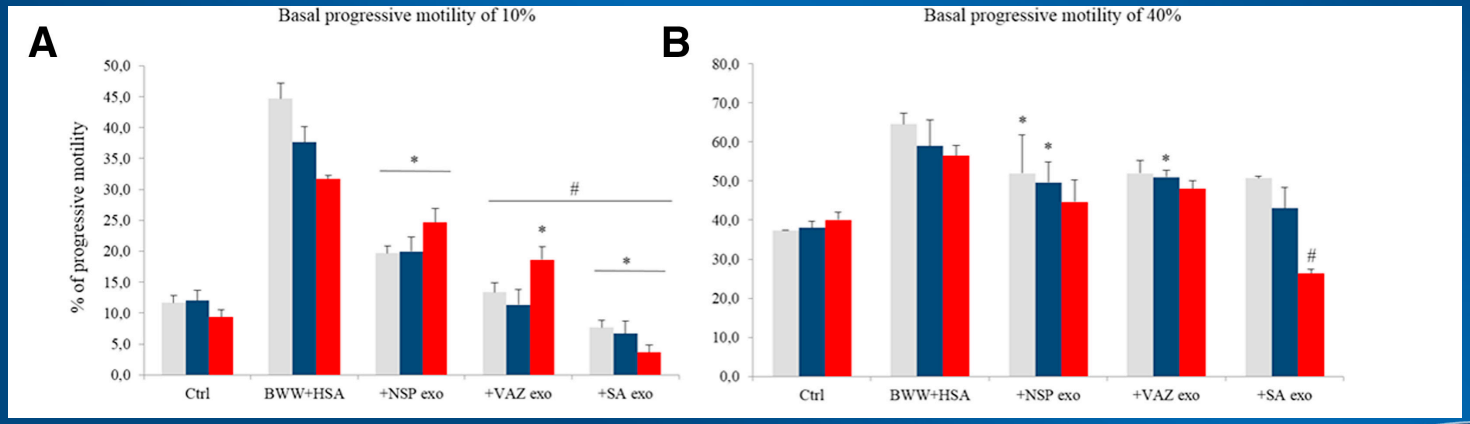
ORIGINAL ARTICLES: ANDROLOGY

Check for updates

Seminal plasma of men with severe asthenozoospermia contain exosomes that affect spermatozoa motility and capacitation

Valentina Murdica, Ph.D.,[a] Elisa Giacomini, Ph.D.,[b] Alessandra Alteri, Ph.D.,[c] Al
Greta Chiara Cermisoni, M.Sc.,[c] Natasa Zarovni, Ph.D.,[d] Enrico Papaleo, M.D.,[b] F
Andrea Salonia, M.D.,[a,e] Paola Viganò, Ph.D.,[b] and Riccardo Vago, Ph.D.[a,e]

2019



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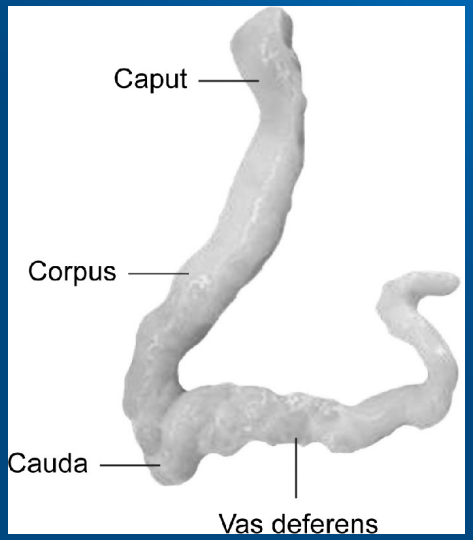
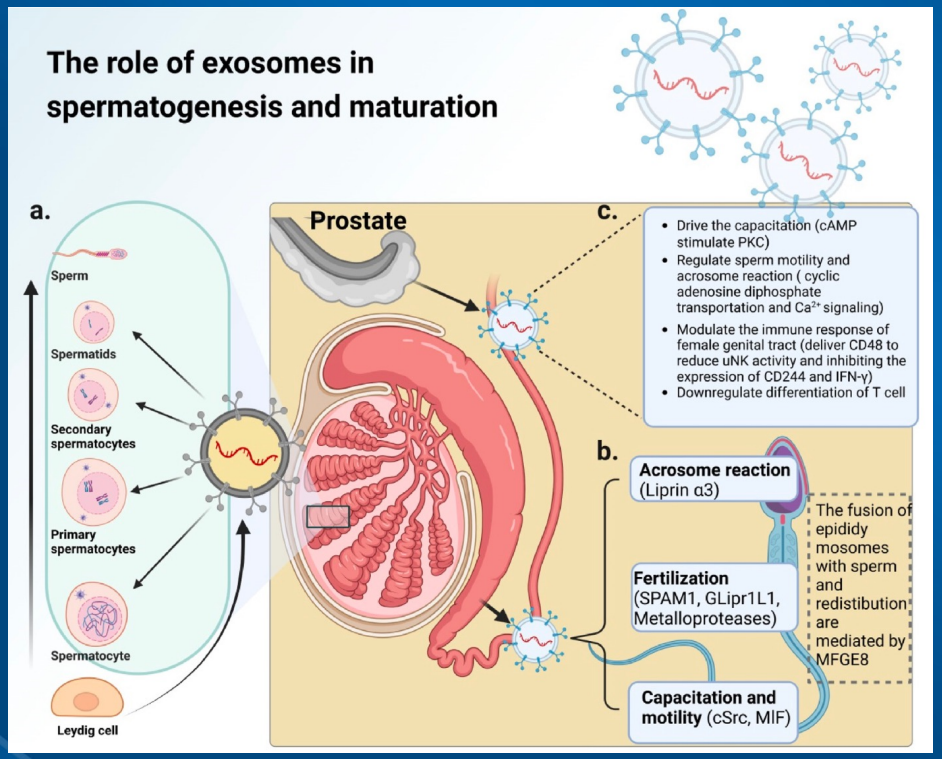
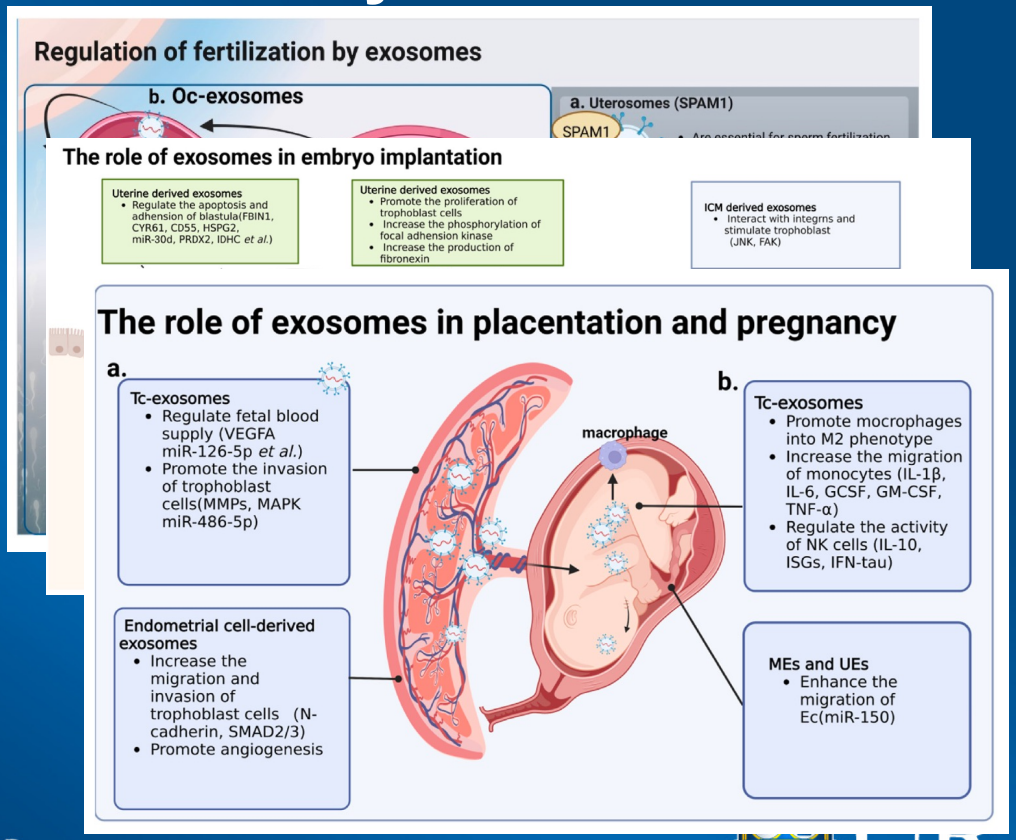
Epididymosomes



- Different cargos/roles depending on location
  - Caput — Acrosome development, motility
  - Cauda — sRNAs, tDF

Exosomes in Spermatogenesis and Beyond





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C. Chen et al. Materials Today Bio 19 (2023) 100608

MELIORA

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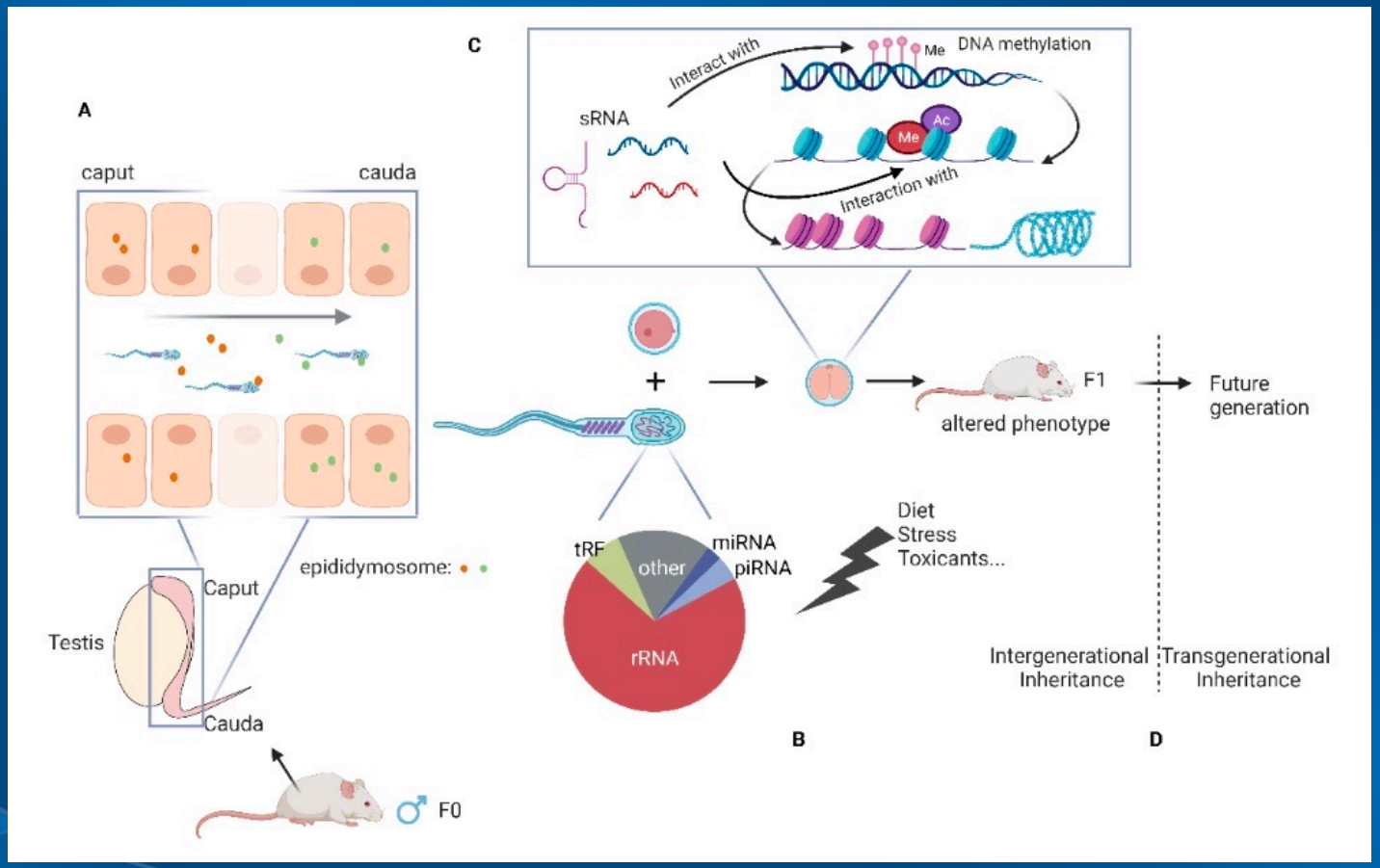
Epigenetics, RNA and Exosomes



Int. J. Mol. Sci. **2023**, *24*, 5889

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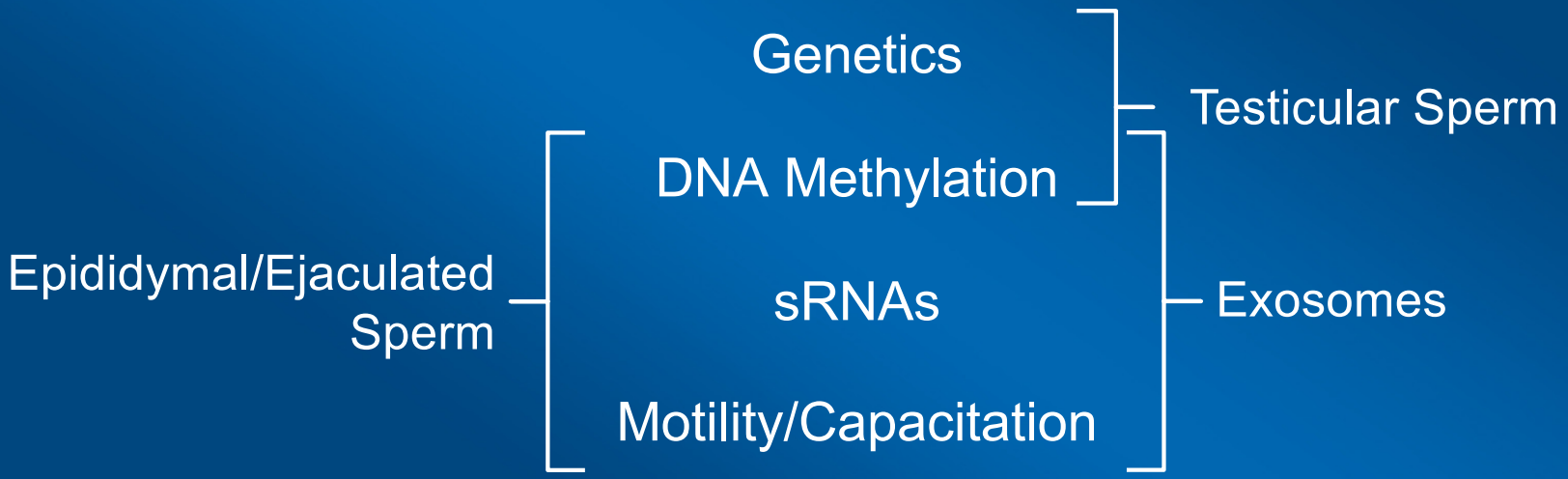
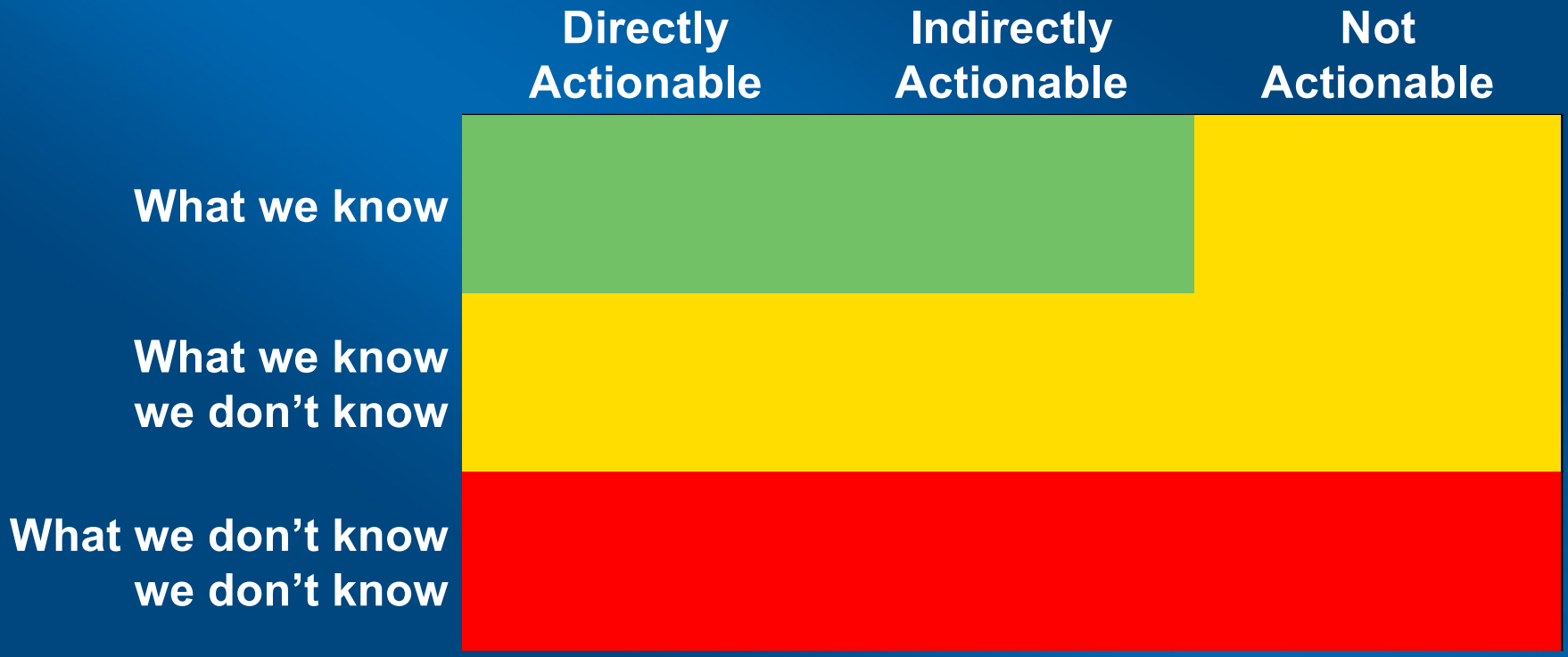
Epigenetics, RNA and Exosomes



Knowledge versus Action



A few bigger questions…

What role does DNA methylation play in spermatogenesis, fertilization, development of the embryo, health of offspring?

- Sperm DNA methylation is erased during spermatogenesis and after fertilization
- Sperm DNA methylation is strongly associated with paternal transmission phenotypes

- piRNAs are important for silencing repetitive elements and genome stability
- Other sRNAs have been shown to be associated with changes in sperm DNA methylation

- No consistent, specific changes in sperm DNA methylation have been reported on systematic reviews and metaanalyses

A few bigger questions…

Are there a finite number of sRNA-based responses for environmental conditions? Non-environmental conditions?

- Epididymosomal tDF are derived from specific tRNAs
- Can we intentionally alter sperm RNA content by adjusting environmental conditions?

- How important are tDF and other small RNAs in sperm development and fertilization?
- What are we missing by doing ICSI using testicular sperm?

- Most sperm sRNAs have been shown to degrade target RNAs and are inhibitory.
- Would there be situations in which using testicular sperm may be *better*?

A few bigger questions…

What are the role of exosomes in the testis and in other parts of the male and female reproductive tracts?

Can we alter exosome content to supplement defects in sperm development/quality?

Could synthetic exosomes be generated to improve fertility in men?

Summary (… or just the introduction?)

Male fertility is incredibly complex

There are still large gaps in our understanding that have significant impacts in diagnosis and treating male infertility.

There are also significant potential impacts for the health of the offspring as well

When you think of the trillions of things that have to go right, how is it that we can reproduce at all?

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