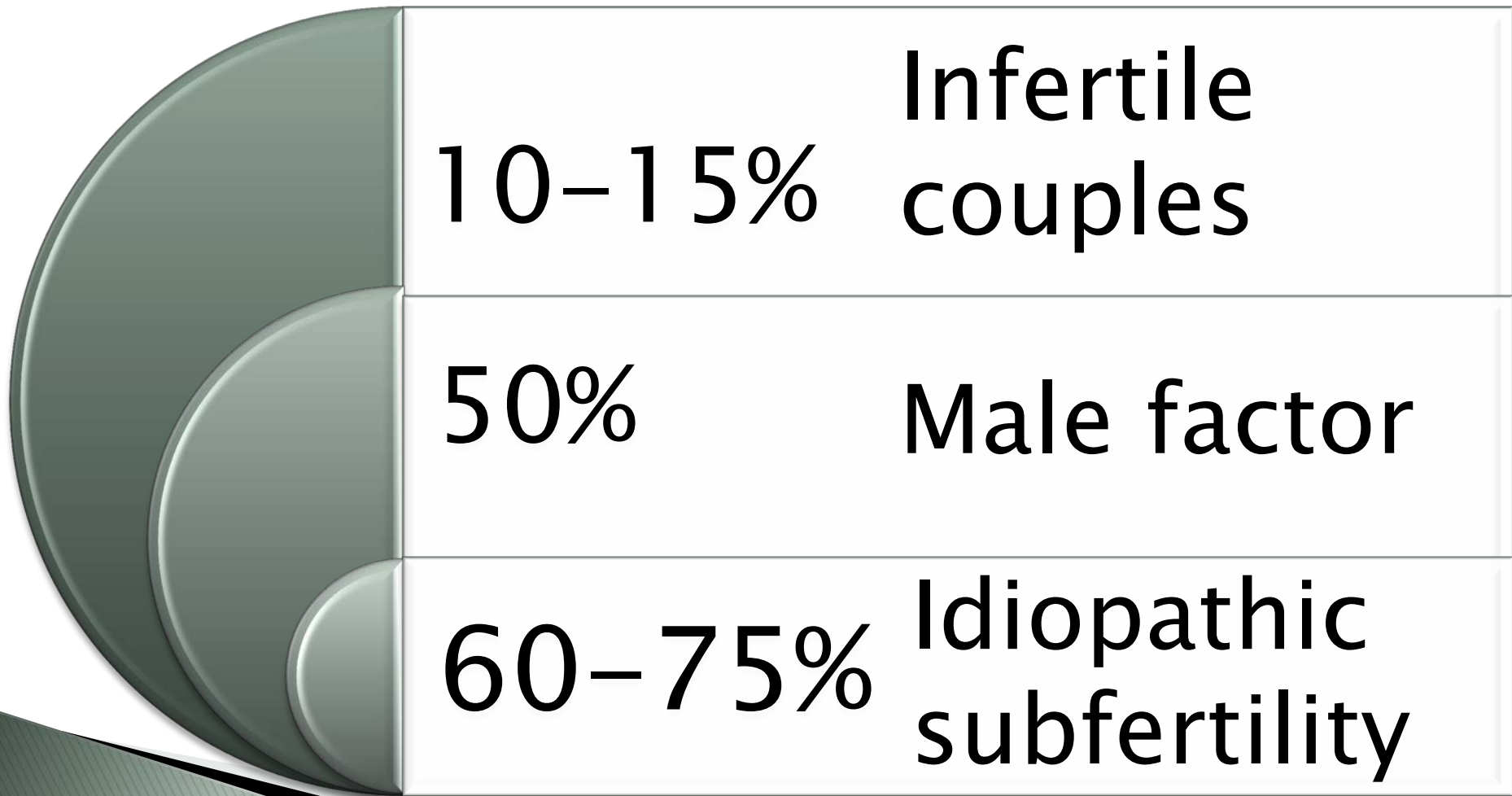


microRNAs as biomarkers of male subfertility

Dorota Trzybulska

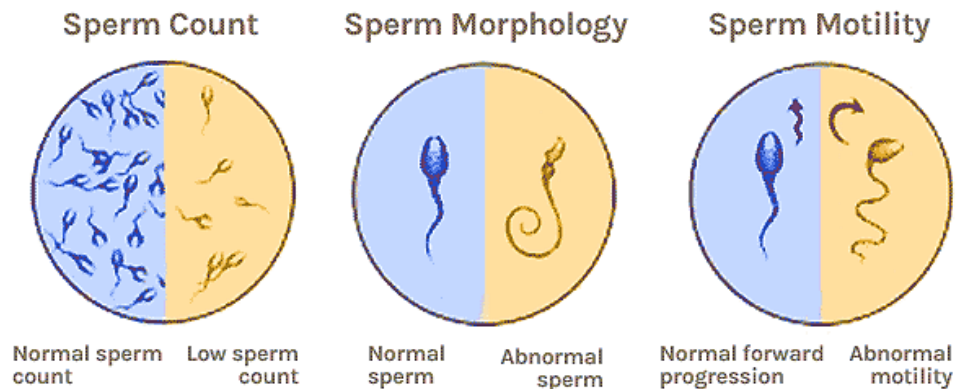
*Prof. Aleksander Giwercman's Group
Molecular Reproductive Medicine
Department of Translational Medicine, Malmö
Lund University*

Male subfertility as a BIG health problem

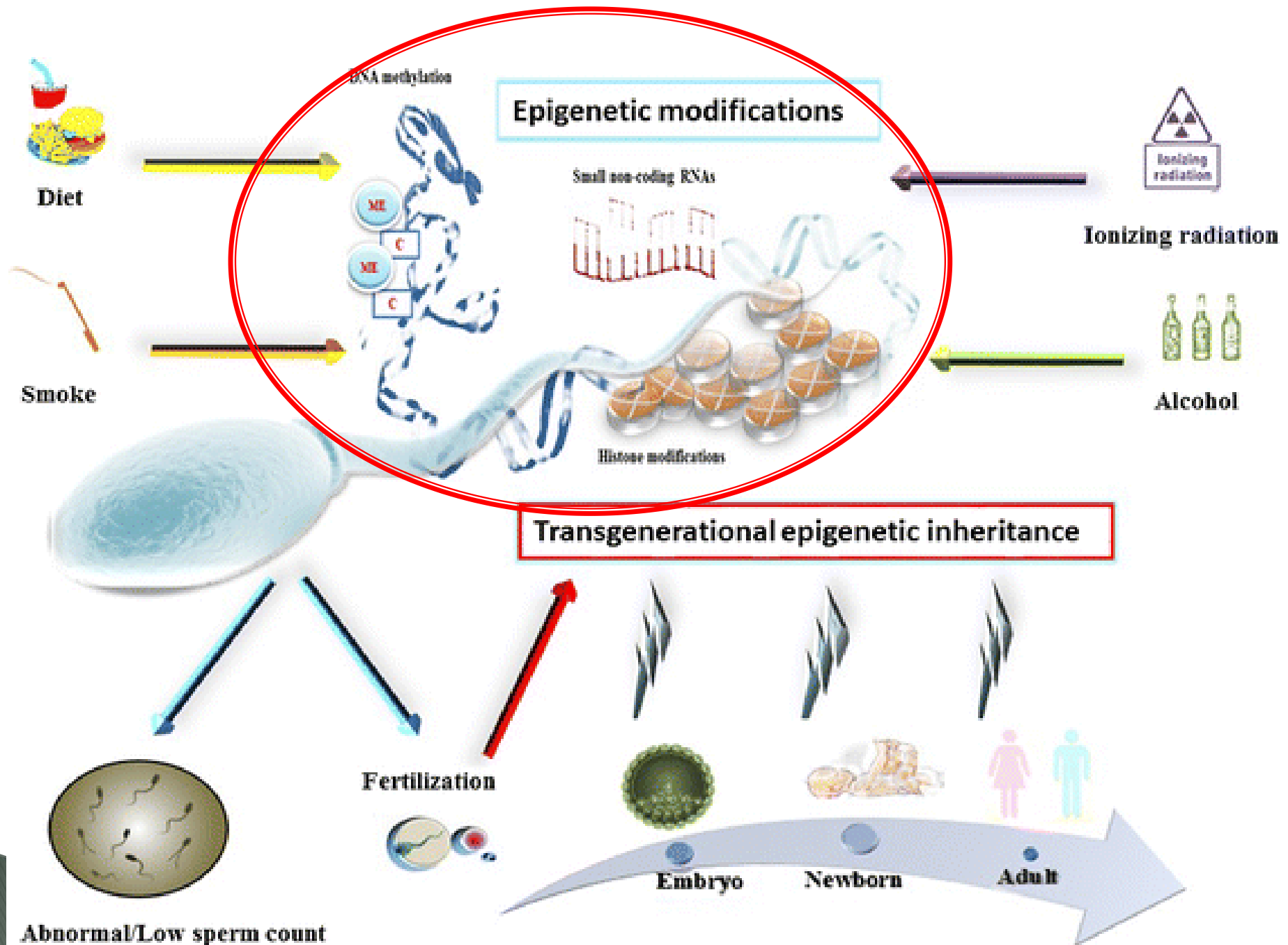


Conventional male fertility diagnostics

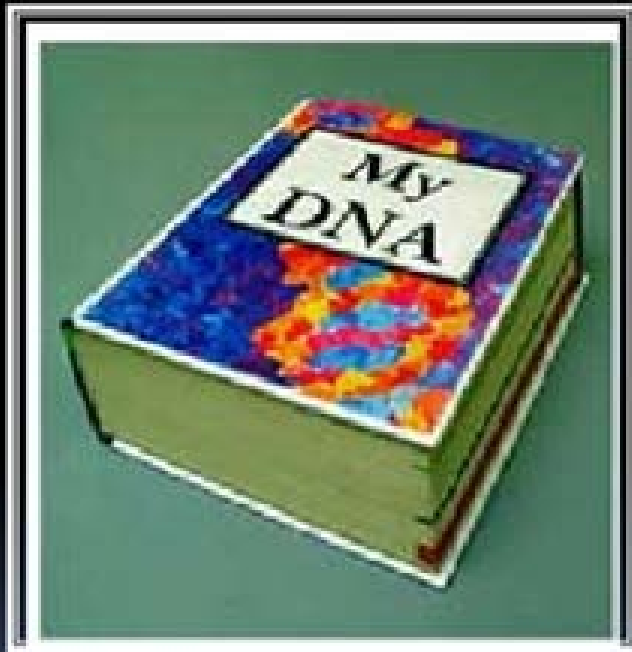
- ▶ Sperm count, morphology and motility
- ▶ Semen volume
- ▶ pH
- ▶ Fructose levels
- ▶ Liquefaction
- ▶ Contaminants



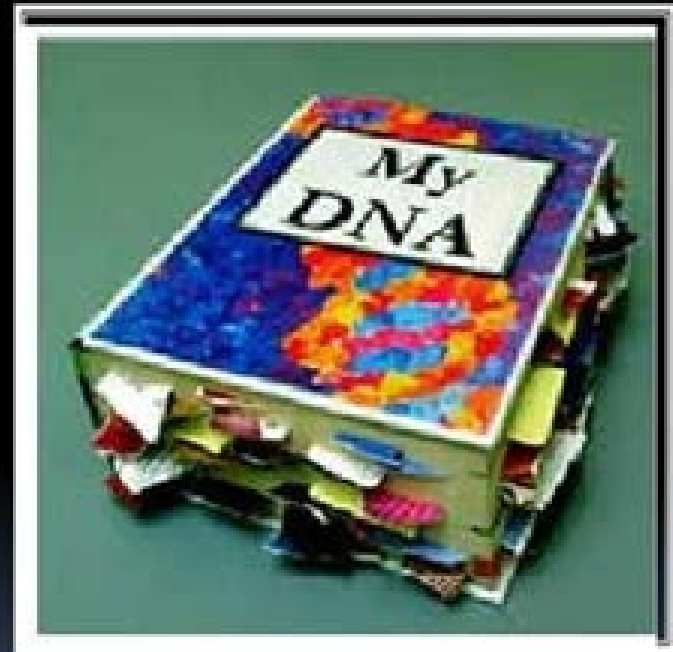
<http://low-sperm-count.com/>



Genetics vs epigenetics

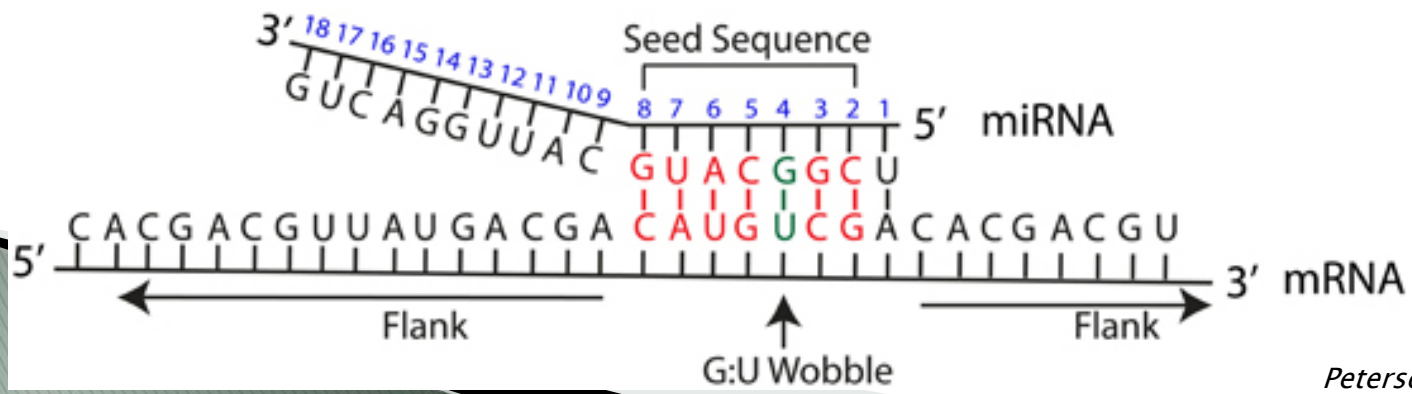
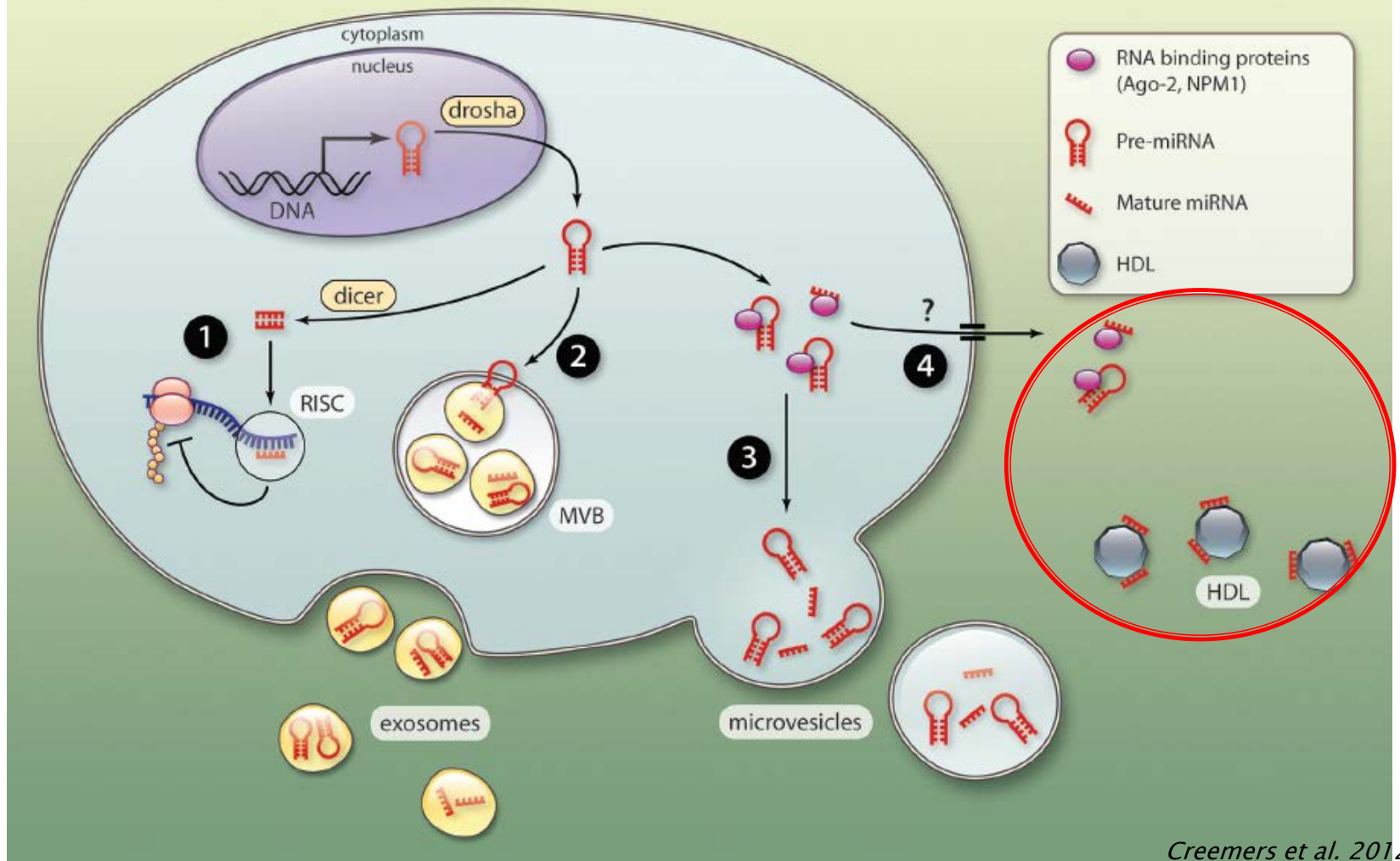


Mutations
Polymorphisms



Narkar 2010.

Chromatin remodelling
DNA methylation
sncRNA – miRNA



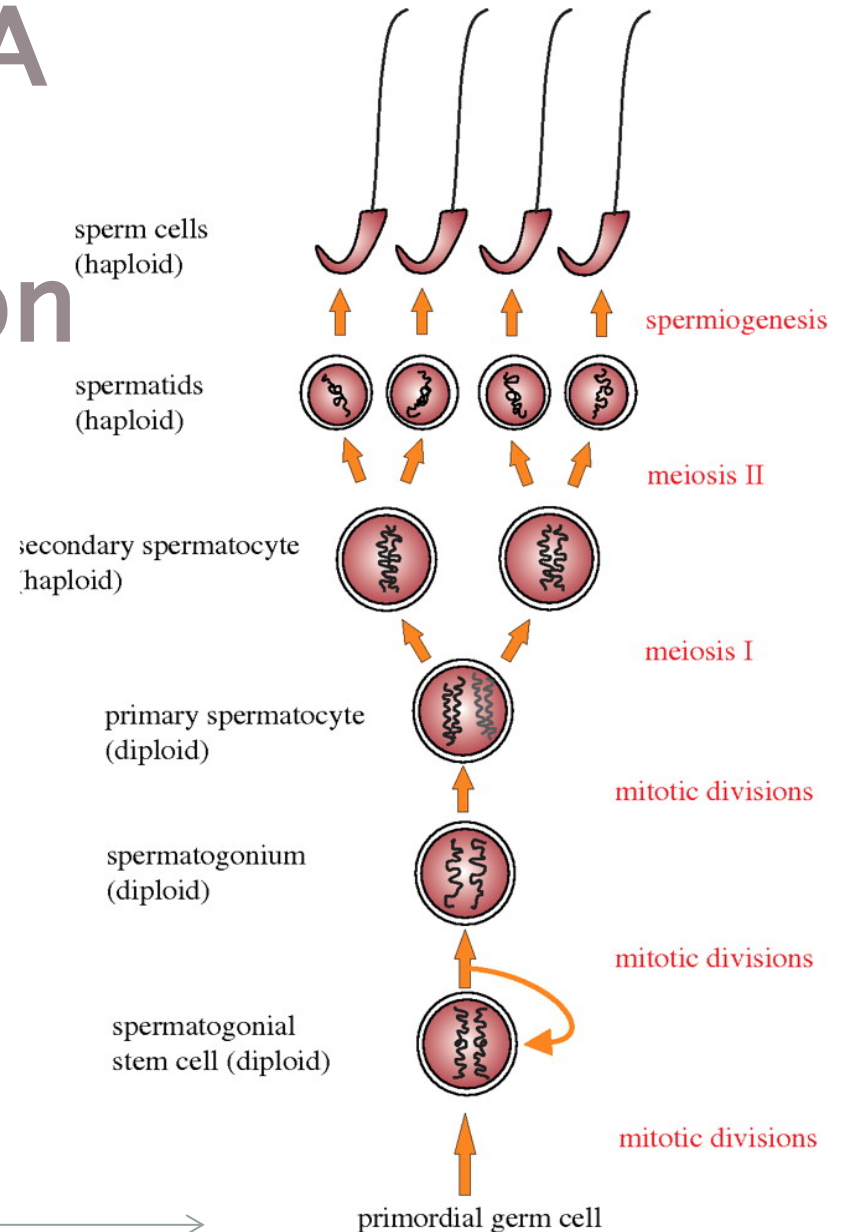
Function of miRNA during male germ cells differentiation

miR-34c
miR-449 →

miR-221/222
miR-20
miR-21
miR-106a →
miR-34c

miR-21 →
miR-34c
miR-182
miR-183
miR-146a

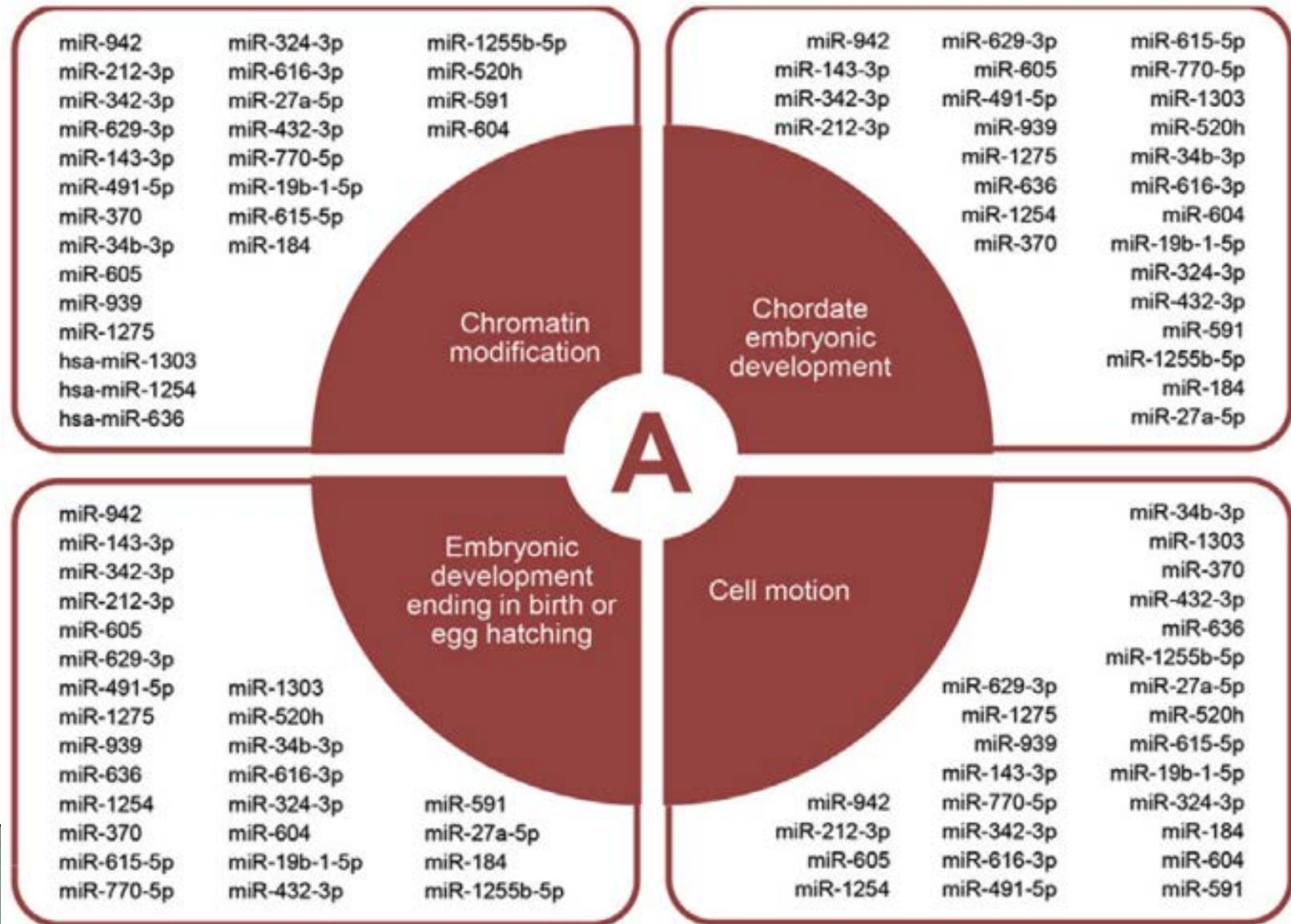
miR-17-92 →



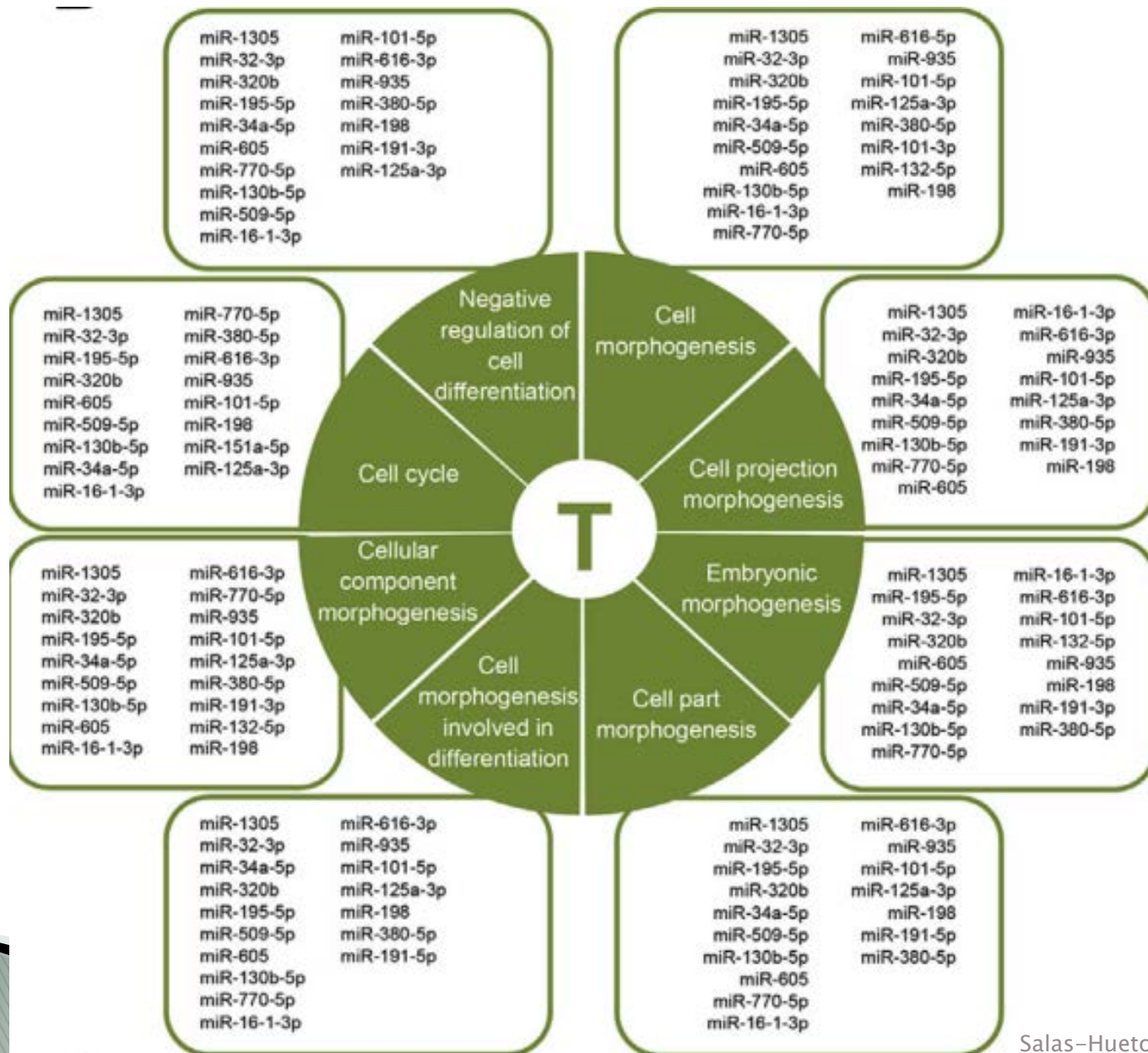
Why circulating miRNAs are promising biomarkers

- Non-invasive biomarkers—actively secreted extracellular miRNAs can be easily accessible and detectable
- Highly stable in extreme conditions
- Expression profile is often tissue or pathology specific
- Relatively inexpensive to measure

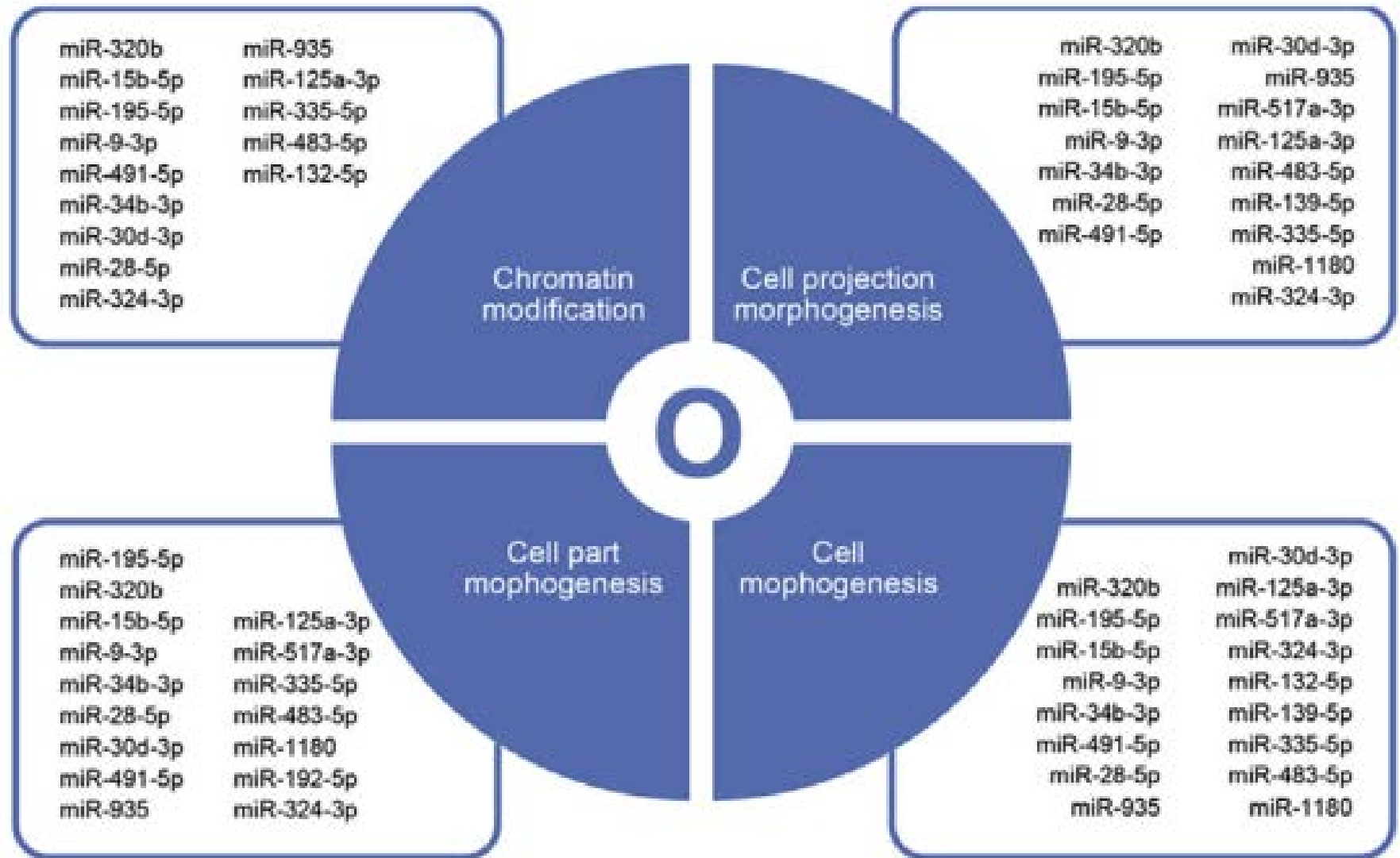
Asthenozoospermia



Teratozoospermia



Oligozoospermia



Aims of miRNA project

1. To find miRNAs-based biomarkers for male subfertility and underlying conditions
2. To find potential new targets for therapeutic intervention for male subfertility

Study design



deduction

- Previous knowledge: miRNA potentially associated with the cause of male subfertility based on the results found in literature

confirmation

- Assessment of miRNA levels (qPCR) in serum and seminal plasma (40 eugonadal + 39 hypogonadal + 38 healthy controls)

validation

- Between-group comparisons
- Correlation analysis
- Functional analyses

Screening (PubMed)

miRNA present in blood plasma and in seminal fluid
(Weber et al. 2010)

VS.

miRNAs in seminal plasma
(Wang et al. 2011)

VS.

miRNAs specific for testis and epididymis
(after comparison of healthy normozoospermic
individuals and vasectomized men)
(Hu et al. 2014)

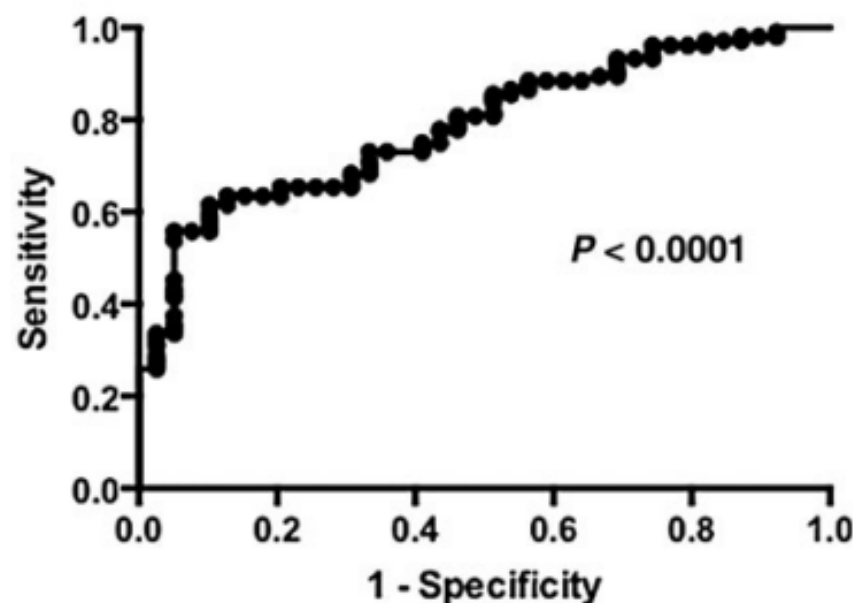
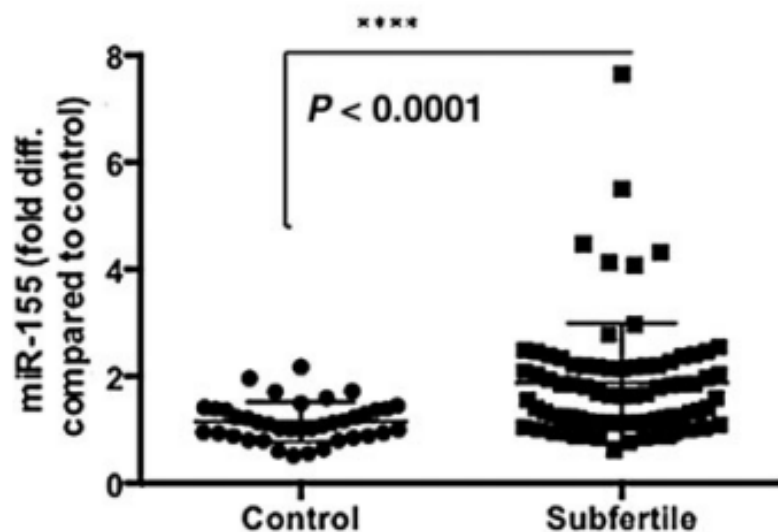
Screening (PubMed)

	miR-34c-5p	miR-122-5p	miR-200a-3p	miR-200c-3p
Present in serum	✓	✓	✓	✓
Present in seminal plasma (detection level >2000 copies)	✓	✓	✓	✓
Disregulated in azoospermia	✓	✓		
Disregulated in asthenozoospermia	✓	✓	✓	✓
Related to metabolic syndrome	✓	✓	✓	✓
Specific for testis	✓	✓		
Specific for epididymis	✓			✓

Serum miR-155 as a potential biomarker of male fertility

Christos Tsatsanis^{1,2,*}, Johannes Bobjer^{1,3}, Hamideh Rastkhani⁴,
Erini Dermitzaki², Marianna Katrinaki², Andrew N. Margioris²,
Yvonne Lundberg Giwerzman⁴, and Aleksander Giwerzman^{1,3}

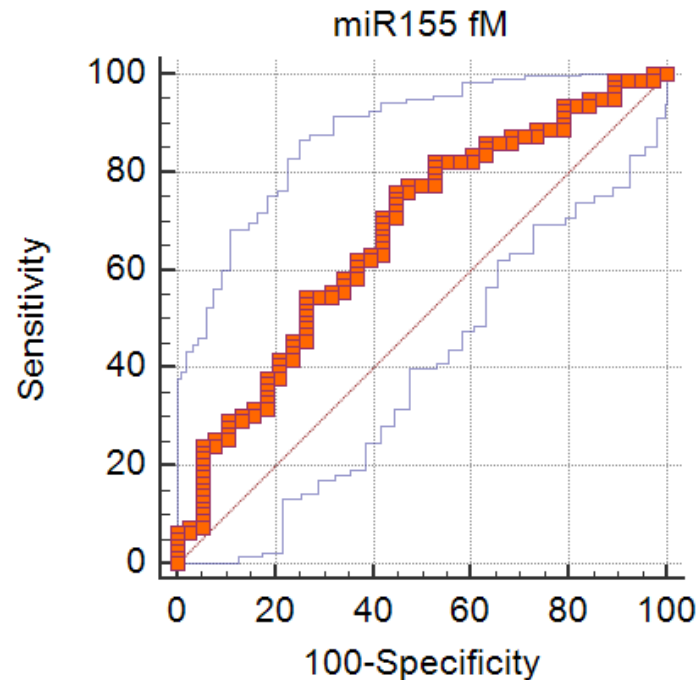
ROC analysis miR-155 for subfertility



Main results

	Subfertile (fM) N=79	Healthy controls (fM) N=38	P
miR-200c-3p	0.067 (0.047)	0.059 (0.037)	0.054
miR-200a-3p	0.015 (0.019)	0.012 (0.024)	0.970
miR-122-5p	12.5 (25.3)	10.637 (22.545)	0.219
miR-155-5p	0.118 (0.08)	0.074 (0.076)	0.003

AUC=0.673
 Sensitivity 75.95%
 Specificity 55.26%
p=0.0013



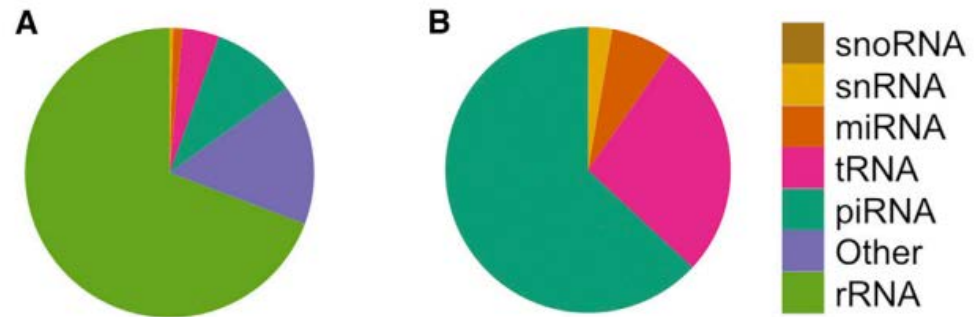
miR-122-5p associations

	miR-122-5p	
	r_s	p
Cholesterol	0.221	0.018
Fasting triglicerydes	0.281	0.002
LDL/HDL ratio	0.232	0.013
Free T3	0.253	0.007
TSH	0.198	0.034
SHBG	-.201	0.031
Fasting insulin	0.394	0.000
HOMA-IR	0.359	0.000
HbA1c	0.248	0.008
fC peptide	0.428	0.000
Adiponectin	-.360	0.000
Leptin	0.299	0.003

Functional studies

1. Identification of target mRNAs cooperating in relevant molecular processes–in silico analysis
2. Transfection of miRNA and assessment of genes expression important for Sertoli (?) cells function
3. Analysis of specificity of miRNA binding to 3'UTR in selected genes.

piRNA– a new class of biomarkers



Donkin et al., 2016

OPEN

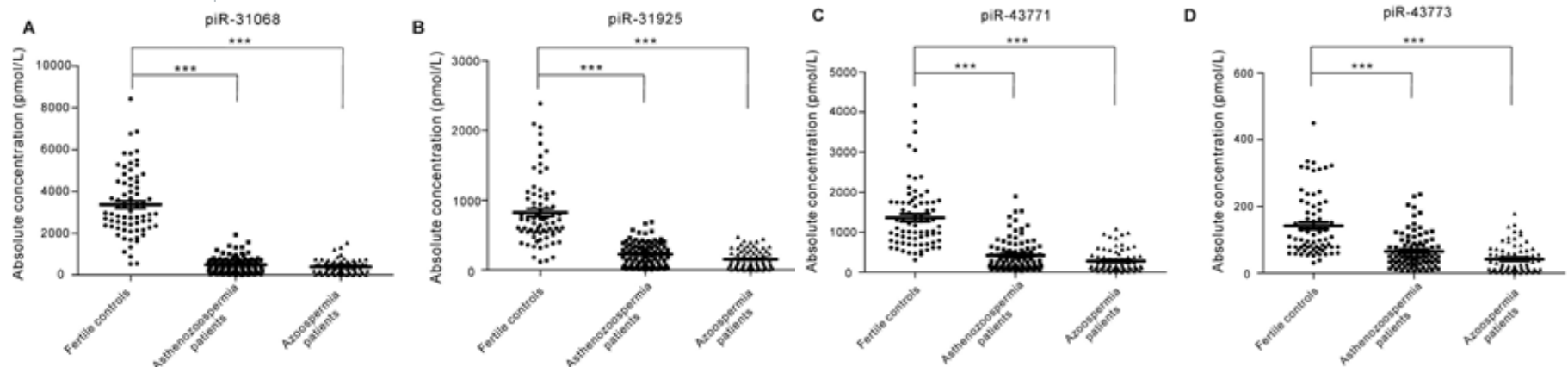
Systematic characterization of seminal plasma piRNAs as molecular biomarkers for male infertility

Received: 02 December 2015

Accepted: 22 March 2016

Published: 12 April 2016

Yeting Hong^{1,*}, Cheng Wang^{1,2,*}, Zheng Fu^{1,*}, Hongwei Liang¹, Suyang Zhang¹, Meiling Lu²,
Wu Sun⁴, Chao Ye¹, Chen-Yu Zhang¹, Ke Zen¹, Liang Shi³, Chunni Zhang² & Xi Chen¹



miRNA

piRNA

snoRNA

How close to the era
of body fluid-based
epigenetic biomarkers
we are
?



ReproUnion

To become the world leader
in overcoming infertility