

microRNAs as biomarkers of male subfertility

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Male subfertility as a BIG health problem

Infertile 10-15% couples

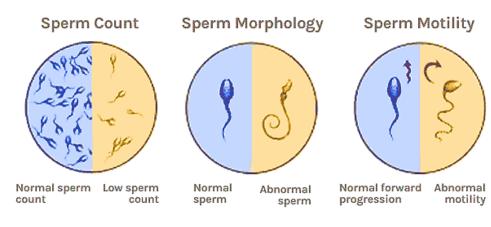
50%

Male factor

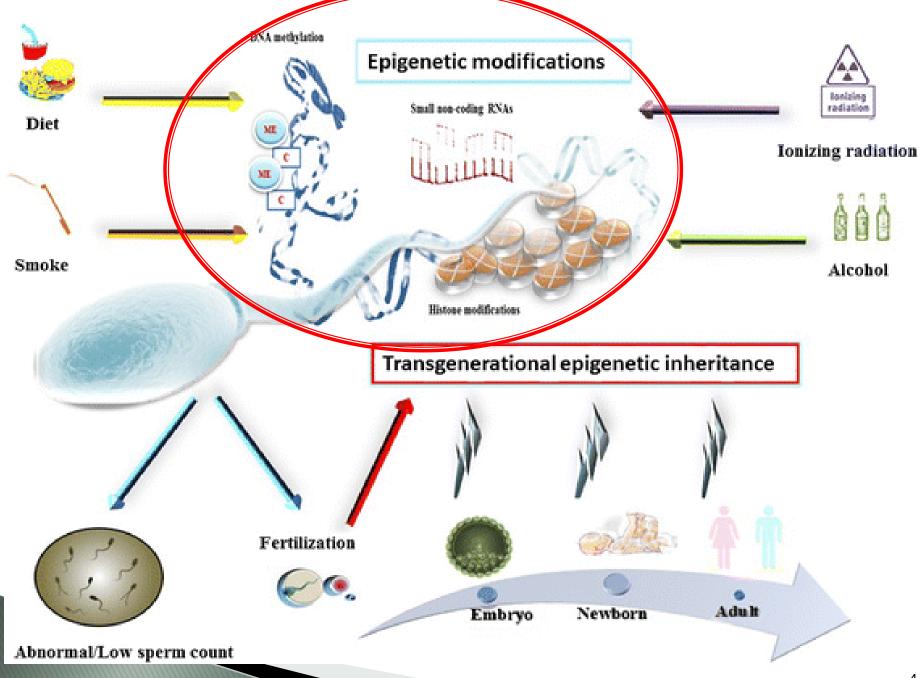
60-75% Idiopathic subfertility

Conventional male fertility diagnostics

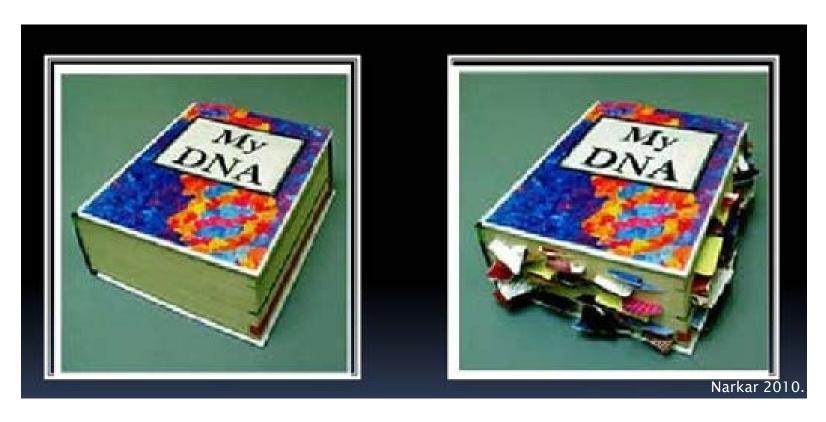
- Sperm count, morphology and motility
- Semen volume
- ▶ pH
- Fructose levels
- Liquefation
- Contaminants



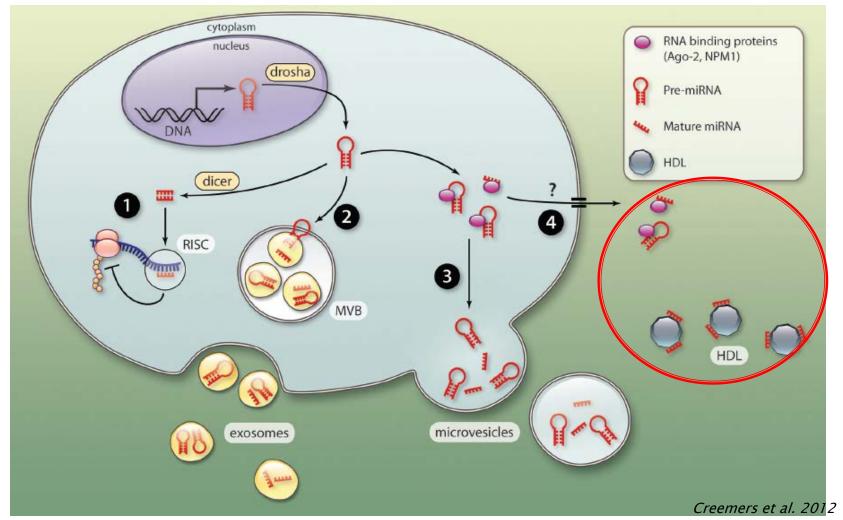
http://low-sperm-count.com/

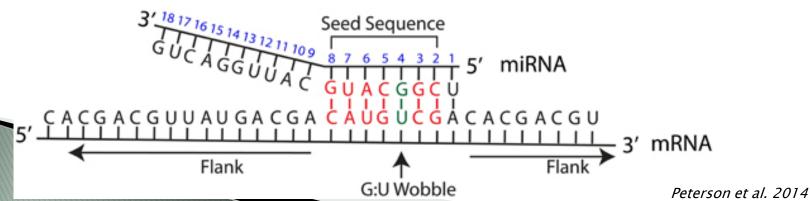


Genetics vs epigenetics



Mutations Polymorphisms Chromatin remodelling DNA methylation sncRNA - miRNA

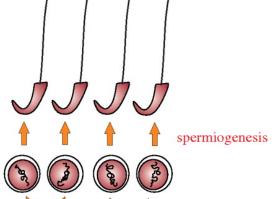




Function of miRNA during male germ cells differentiation

sperm cells (haploid)

spermatids



miR-34c miR-449

miR-221/222

miR-20

miR-21

miR-106a

miR-34c

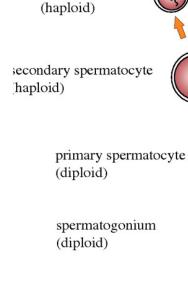
miR-21

miR-34c

miR-182

miR-183

miR-146a

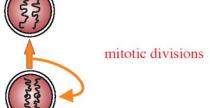


meiosis I

mitotic divisions

meiosis II

spermatogonial stem cell (diploid)



primordial germ cell

mitotic divisions

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

miR-942 miR-324-3p miR-1255b-5p miR-212-3p miR-616-3p miR-520h miR-342-3p miR-27a-5p miR-591 miR-604 miR-629-3p miR-432-3p miR-770-5p miR-143-3p miR-491-5p miR-19b-1-5p miR-370 miR-615-5p miR-34b-3p miR-184 miR-605 miR-939 Chromatin miR-1275 modification hsa-miR-1303 hsa-miR-1254 hsa-miR-636

miR-942 miR-629-3p miR-615-5p miR-143-3p miR-605 miR-770-5p miR-342-3p miR-491-5p miR-1303 miR-212-3p miR-939 miR-520h miR-1275 miR-34b-3p miR-636 miR-616-3p miR-1254 miR-604 miR-370 miR-19b-1-5p miR-324-3p miR-432-3p Chordate miR-591 embryonic miR-1255b-5p development miR-184 miR-27a-5p

miR-942 miR-143-3p Embryonic miR-342-3p development miR-212-3p ending in birth or miR-605 egg hatching miR-629-3p miR-1303 miR-491-5p miR-1275 miR-520h miR-939 miR-34b-3p miR-636 miR-616-3p miR-1254 miR-324-3p miR-591 miR-370 miR-27a-5p miR-604 miR-615-5p miR-19b-1-5p miR-184 miR-770-5p miR-432-3p miR-1255b-5p

miR-34b-3p miR-1303 miR-370 Cell motion miR-432-3p miR-636 miR-1255b-5p miR-629-3p miR-27a-5p miR-1275 miR-520h miR-939 miR-615-5p miR-143-3p miR-19b-1-5p miR-942 miR-770-5p miR-324-3p miR-212-3p miR-342-3p miR-184 miR-605 miR-616-3p miR-604 miR-1254 miR-491-5p miR-591

Teratozoospermia

miR-1305 miR-101-5p miR-32-3p miR-616-3p miR-320b miR-935 miR-195-5p miR-380-5p miR-34a-5p miR-198 miR-605 miR-191-3p miR-770-5p miR-125a-3p miR-130b-5p miR-509-5p miR-16-1-3p

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Negative regulation of cell differentiation

Cell cycle

morphogenesis

Cellular component morphogenesis

Embryonic morphogenesis

Cell projection

morphogenesis

Cell part morphogenesis

Cell

miR-1305 miR-16-1-3p miR-32-3p miR-616-3p miR-320b miR-935 miR-195-5p miR-101-5p miR-34a-5p miR-125a-3p miR-509-5p miR-380-5p miR-191-3p miR-130b-5p miR-770-5p miR-198 miR-605

miR-16-1-3p

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miR-1305

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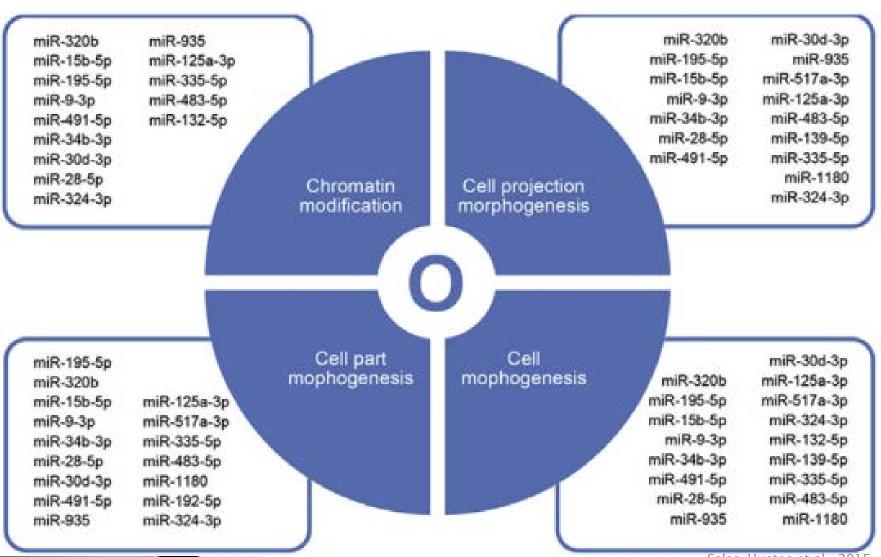
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Cell morphogenesis involved in differentiation

miR-1305 miR-616-3p miR-32-3p miR-935 miR-34a-5p miR-101-5p miR-320b miR-125a-3p miR-195-5p miR-198 miR-509-5p miR-380-5p miR-605 miR-191-5p miR-130b-5p miR-770-5p miR-16-1-3p

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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)

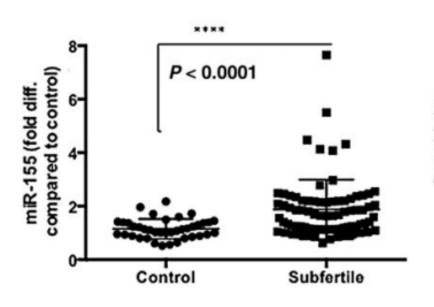
	miR- 34c-5p	miR- 122-5p	miR- 200a-3p	miR- 200c-3p
Present in serum	✓	✓	✓	✓
Present in seminal plasma (detection level >2000 copies)	✓	✓	✓	✓
Disregulated in azoospermia	✓	✓		
Disregulated in asthenozoospermia	✓	✓	✓	✓
Related to metabolic syndrome	✓	✓	✓	✓
Specific for testis	✓	✓		
Specific for epididymis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

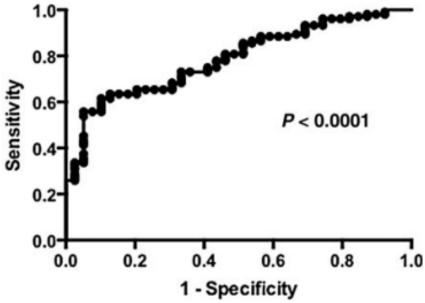
human reproduction **ORIGINAL ARTICLE Infertility**

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 Giwercman⁴, and Aleksander Giwercman^{1,3}

ROC analysis miR-155 for subfertility

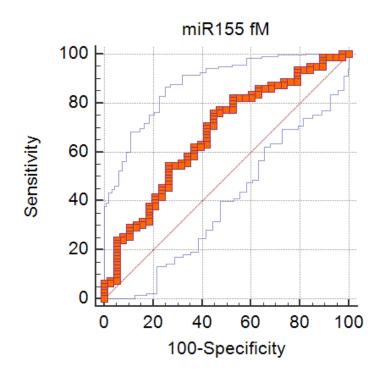




Main results

	Subfertile (fM) N=79	Healthy controls (fM) N=38	Р
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



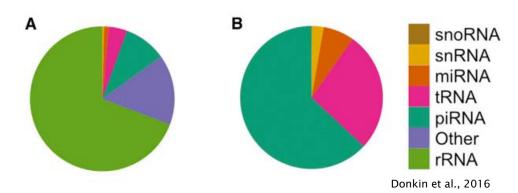
miR-122-5p associations

	miR-122-5p		
	r_{s}	р	
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.

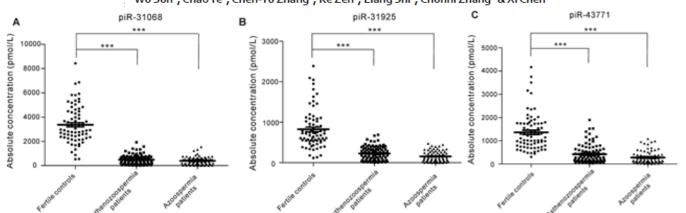
piRNAa new class of biomarkers

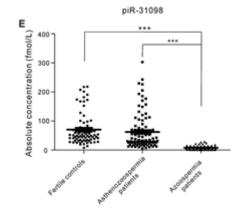


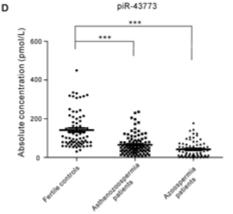
OPEN Systematic characterization of seminal plasma piRNAs as molecular biomarkers for male infertility

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

