

TRANSCRIPTOMIC ANALYSIS OF MALE INFERTILITY

INTRODUCTION

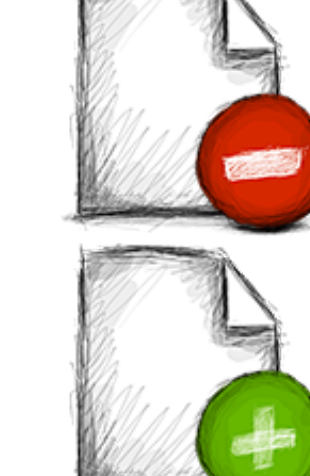
- ✓ **Infertility:** Disease defined as failure to achieve a clinical pregnancy after 12 months or more of unprotected sex (World Health Organization).
- ✓ Affects approximately 10-15% of couples of reproductive age. About **50%** of these cases can be associated with male factors.
- ✓ Causes: Epigenetic, genetic, physiological and endocrine failure, testis pathologies...
- ✓ A normal seminal evaluation **does not necessarily** indicate a fertile potential. Transcriptomics represents a promising approach for the discovery of new biomarkers able to improve the management of male factor infertility.
- ✓ **Transcriptome:** Collection of all molecules of RNA derived from genes whose biological information is required by the cell, at a specific time and under certain conditions.

OBJECTIVE

Get a list of candidate genes, with alterations in the transcriptome, as potential markers for the diagnosis of different forms of male infertility: **idiopathic** [1], **azoospermia** [2], **asthenozoospermia** [3], **oligozoospermia** [4] and **teratozoospermia** [5].

METHODOLOGY

Review of transcriptomic studies in infertility



Inclusion criteria

- Infertility classification
- N° patients (>10)
- Microarray analysis
- RT-PCR validation

Functional annotation and classification by gene ontology (GO)



IDIOPATHIC

Unexplained infertility

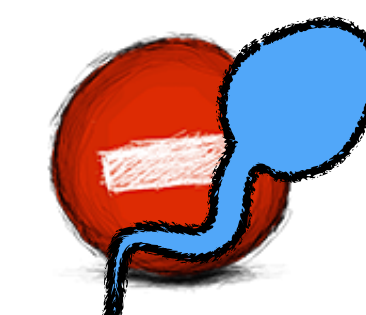


- ✓ **TRY1, CAB39L, GGT1** and ribosomal proteins (+ other 133), were found to be differentially expressed at least two times.
- ✓ If the over- or under-expressed genes are grouped by function, between 80 and 100% of them are involved in spermatozoa differentiation.

Infertility markers may not be related to sperm production in terms of sperm count, but are probably related to sperm function. [1]

AZOOSPERMIA

Complete lack of sperm in the ejaculate

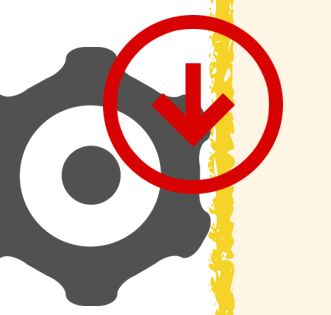


- ✓ Yq microdeletions study is useful for azoospermia diagnosis (AZF deletions).
- ✓ YBX2 network: **ODF1, TNP1** and **PRM2**.
- ✓ ARNT2 network: **CRISP, TSSK2, MDM4** and **EGR4**.

The **DAZ1** gene is the leading candidate that causes the failure of the spermatogenesis process [2].

ASTHENOZOOSPERMIA

> 40% of sperm with reduced motility



- ✓ **ANXA2, BRD2, OAZ3** and **IL6ST**: significant positive correlation between the sperm progressive motility and the relative mRNA levels.
- ✓ **PRM1, PRM2, TNP1** and **TNP2** reduction increased DNA strand breaks, which may induce the inactivation of mitochondria.

Protamine mRNAs appear to have a role in the formation of fully functional mature spermatozoa including sperm motility [3].

Function	Symbol	Name	ID
SPERMATOGENESIS (GO:0007283)	GGT1	Gamma-glutamyltranspeptidase 1	2678
	CAB39L	Calcium binding protein 39-like	81617
	TRY1	Trypsin 1	5644
	OAZ3	Ornithine decarboxylase antizyme 3	51686
	ADAM29	ADAM metallopeptidase domain 29	11086
	CABYR	Calcium-binding tyrosine phosphorylation-regulated protein	26256
TRANSCRIPTION (GO:0006351)	HMGB2	High mobility group box 2	3148
	RPS3	40S ribosomal protein S3	6188
	NKX3-1	NK3 homeobox 1	4824
CELL DEATH (GO:0008219)	RPS3	Ribosomal protein S3	6188
	RPS3A	Ribosomal protein S3A	6189
CELL PROLIFERATION (GO:0008283)	RPS15A	Ribosomal protein S15A	6210
	NKX3-1	NK3 homeobox 1	4824

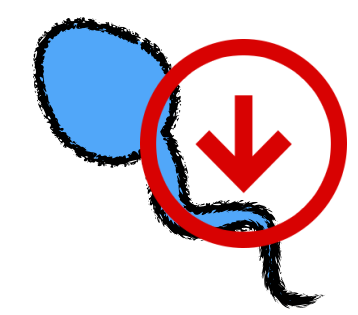
Function	Symbol	Name	ID
SPERMATOGENESIS (GO:0007283)	CREM	cAMP-responsive element modulator	1390
	DDX4	DEAD (Asp-Glu-Ala-Asp) box polypeptide 4	54514
	PRM2	Protamine-2	5620
	LMNA	Lamin A/C	4000
	MEA1	Male-enhanced antigen 1	17256
	SPATA4	Spermatogenesis-associated protein 24	202051
TRANSCRIPTION (GO:0006351)	CREM	cAMP-responsive element modulator	1390
	SAP130	Sin3A-associated protein, 130kDa	79595
	SPZ1	Spermatogenic leucine zipper 1	84654
CELL DEATH (GO:0008219)	LMNA	Lamin A/C	4000
	SPATA4	Spermatogenesis-associated protein 24	202051

Protamine mRNAs appear to have a role in the formation of fully functional mature spermatozoa including sperm motility [4].

- ✓ Aberrant **PRM1/PRM2** protein ratio is responsible for an unstable chromatin condensation.
- ✓ **DDX4** gene expression significantly decreased in the sperm of oligozoospermic men.

OLIGOZOOSPERMIA

<10 million spermatozoa per one ml of ejaculate



CONCLUSIONS



- ✓ **Omics** technologies have provided a powerful tool for transcriptomic profiling of male factor infertility and the identification of the potential diagnosis biomarkers [6].
- ✓ Despite the evidences to support these applications, there are several **deep uncertainties** to be established. From basic functional significance of the mRNAs in mature sperm, to the molecular mechanisms and the determination the mRNAs of male factor infertility.
- ✓ What is the reason for this difference in gene expression?
As transcriptional disorder is common within infertile men, the possibility of a genetic cause decreases, thereby increasing the interest of the hypothesis that the environment, through **epigenetic** marks, could play a key role in this problem.

Function	Symbol	Name	ID
SPERM MOTILITY (GO:0030317) & CELL MOTILITY (GO:0048870)	IL6ST	Interleukin 6 signal transducer	3572
	OAZ3	Ornithine decarboxylase antizyme 3	51686
	TNP1	Nuclear transition protein 1	7141
	TNP2	Nuclear transition protein 2	7142
	ANXA2	Annexin A2	302
	PRM1	Protamine-1	5619
	PRM2	Protamine-2	5620
	CRISP2	Cysteine-rich secretory protein 2	7180
	LDHC	Lactate dehydrogenase C	3948
	HILS1	Spermatid-specific linker histone H1-like protein	54388
SPERMATOGENESIS (GO:0007283)	NOS3	Nitric oxide synthase 3	4846
	BRD2	Bromodomain-containing protein 2	6046
	PRM1	Protamine-1	5619
	PRM2	Protamine-2	5620
	TNP1	Nuclear transition protein 1	7141
	TNP2	Nuclear transition protein 2	7142

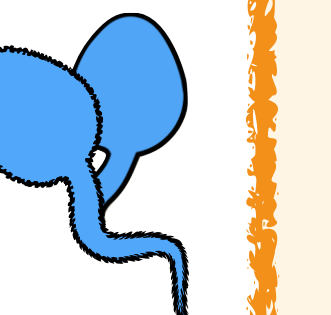
Function	Symbol	Name	ID
SPERMATOGENESIS (GO:0007283)	HSPA2	Heat shock 70kDa protein 2	3306
	ODF1	Outer dense fiber of sperm tails 1	4956
	ODF2	Outer dense fiber of sperm tails 2	4957
	ODF3	Outer dense fiber of sperm tails 3	113746
	ODF4	Outer dense fiber of sperm tails 4	146852
FERTILIZATION (GO:0009566)	PLCZ1	Phospholipase C, zeta 1	89869
	SPAM1	Sperm adhesion molecule 1 (PH-20 hyaluronidase, zona pellucida binding)	6677
CELL PROLIFERATION (GO:0008283)	CDKN3	Cyclin-dependent kinase inhibitor 3	1033

The lack of certain transcripts is indicative of a failure in the late phase of spermatogenesis [5].

- ✓ Ubiquitin - proteasomal pathway (UPP) completely suppressed. Involved in morphological progression of spermiogenesis.
- ✓ Reduction in **HSPA2** mRNA was resonant with depletion of **ODF 1-4** mRNAs and acrosomal proteins **ACRV1** and **SPAM1**.

TERATOZOOSPERMIA

>40% spermatozoa with abnormal morphology in sperm



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