Sperm transcriptome profiling in oligozoospermia

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Abstract

Purpose Investigate in what extent sperm transcriptome of infertile men is different from that of fertile individuals.

Methods Semen samples were collected for determination of sperm parameters as well as for RNA isolation. Gene expression profile was investigated in spermatozoa of 8 infertile and 3 fertile men by microarray analysis using the Affymetrix Chip HG-U133 Plus 2.0.

Capsule Sperm transcriptome is altered in oligozoospermia.

This work has been presented at the 27th Annual Meeting of ESHRE, Rome 2010.

We analyzed and compared the whole transcriptome profile of oligoasthenoteratozoospermic sperm patients to normal fertile men to understand gene expression deregulation associated with oligoasthenoteratozoospermia.

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Result(s) We observed up to 33-fold reduction expression of genes involved in spermatogenesis and sperm motility. Furthermore, there is an important decrease in expression of genes involved in DNA repair as well as oxidative stress regulation. In this study, we also show a striking drop in expression of histone modification genes.

Conclusion(s) We found that transcription profile in germ cells of men with idiopathic infertility is different from that of fertile individuals. Interestingly, about 15% of the regulated genes (Eddy Rev Reprod 4:23–30, 1999) play a role in spermatogenesis.

Keywords Sperm · Transcripts ·
Oligoasthenoteratozoospermia · Male infertility · Microarray

Introduction

Approximately 15% of the couples worldwide have fertility problems with a male factor found in 45% of the cases. Multiple factors are known to impair spermatogenesis: exposure to environmental disruptors, genetics, testis pathologies, lifestyle [1, 2]. However, about two-thirds of the cases of non-obstructive spermatogenic defects with apparently normal semen quality remain unexplained, suggesting that other factors have to be investigated. It is now established that mature spermatozoa contain a complex panel of transcripts [3, 4]. Some studies showed that these transcripts have a crucial role to play in normal spermatogenesis [5–10]. We suspect inappropriate gene expression in developing male germ cells to have a negative effect on spermatogenesis. Here, we investigated transcripts in

sperm of oligozoospermic infertile men ($n=8$) as compared to that of normospermic fertile men ($n=3$) in order to detect any difference that could provide new insights in the molecular events happening during physiological and pathological spermatogenesis. As a recent study has identified a series of human sperm transcripts as candidate markers of male fertility [5], we compared our results to these data.

Material & methods

Patients

Human spermatozoa samples were collected from patients consulting for infertility. Semen samples parameters were assessed according to the WHO criteria (1999). Normal semen were obtained from healthy men of proven fertility (sperm counts >20 million spermatozoa/ml), $n=3$ and oligozoospermic infertile men seek for infertility counseling (sperm counts <20 million spermatozoa/ml), $n=8$. These patients were screened for known causes of infertility including chromosome anomalies, Y chromosome AZF deletions. The studied individuals had normal karyotype, clinical examination (no varicocele, infection or other reversible cause of infertility was found) and hormonal profile (FSH: 1.0–10.5 IU/L, LH: 0.7–8.0 IU/l, Testosterone: 3.0–10.0 ng/ml, Inhibin B: 80–400 pg/ml, Oestradiol: 22–49 ng/l, Prolactin: 2–15 ng/ml).

RNA purification

Semen samples were collected by masturbation after 3–4 days of sexual abstinence. After liquefaction at 37°C, spermatozoa were selected and separated from somatic cells by centrifuging 1 to 2 ml of semen through a two-layer (45% and 90%) gradient of Percoll at 400 g for 20 min. The fraction in the 90% layer was washed in 2 ml of Tyrode and the tube was centrifuged at 400 g for 10 min. The pellet was collected with extreme care to avoid any somatic cells contamination and was subjected to mini swim-up modified technique. Total RNA was extracted from spermatozoa isolated by mini swim-up using TRIzol® reagent according to the manufacturer's instructions (Life Technologies, Gaithersburg, MD, USA). The purified RNA samples were subjected to RNAase-free DNase treatment (TURBO™ DNase, Ambion Inc). The purity and integrity of the total RNA preparation was verified using both the eukaryote total RNA nano-chip (Bioanalyzer 2100, Agilent Technologies, France) and Reverse Transcription PCR using the one step RT-PCR kit (Qiagen®) according to the manufacturer's recommendations.

Microarray data analysis

As small amounts of RNA were recovered after purification (10 to 100 ng), the RNA samples had to undergo Affymetrix two-step amplification protocol (along with two universal RNA samples as amplification internal controls) before microarray analysis (Affymetrix, HG-U133 Plus 2.0). The transcription profile in sperm of infertile men was compared to that of fertile individuals.

Quality control assessment, chip normalization, and filtering

The quality of the expression distribution at the probeset level between chips was evaluated by using the Relative Log Expression (RLE) and standard quality metrics (3'/5' ratios, hybridization and labeling quality metrics). Microarray data from CEL files for all 11 chips was normalized simultaneously and expression levels were generated using RMA from Expression Console (Affymetrix, Santa Clara, CA). The normalized data was filtered for low-expressed probesets. Only transcripts with a mean signal ≥ 5.5 were retained for further analysis.

Statistical analysis of microarray data

Differentially expressed transcripts were identified by both Anova (p -value ≤ 0.05) and unpaired T -Test (p -value ≤ 0.05 and fold-change ≥ 1.5).

Clustering of microarray data and functional analysis of microarray data

Hierarchical clustering was carried out to cluster among the gene signal intensities and among the samples with Mev4.0 software from TIGR (The Institute of Genome Research). Finally, the functional analyses were generated through the use of PANTHER [11, 12].

Quantitative RT-PCR

Microarray results were confirmed by quantitative RT-PCR. A total of 2.5 ng of RNA samples were reverse transcribed using SuperScript® VILO™ Master Mix following the manufacturer's recommendations. RT-PCR results were normalized to one reference gene: *SAP130* that was found by microarray analysis to be consistently expressed in spermatozoa of both fertile and infertile men. The selected genes were among the most regulated when comparing oligozoospermic to normospermic men

Table 1 Sperm parameters of both fertile and infertile men. Highlighted in light grey, normospermic fertile patients and in dark grey oligozoospermic infertile patients

Donor	Sperm counts ($\times 10^6/\text{ml}$)	Volume (ml)	Progressive motility (%)	Abnormal morphology (%)	Vitality (%)
1	83.6	6.2	45	91	82
5	67.2	4	53	80	78
8	137.6	5.2	69	84	61
6	17.6	5.2	16	81	78
7	19.4	1.5	35	99	74
10	19.2	3.2	22	80	78
18	3.2	4	18	96	47
19	19.2	6.3	39	82	74
20	16	7	46	81	79
21	0.36	2.9	3	96	12
22	0.96	0.96	16	96	38

(*TPD52L3*, *PRM2*, *JMJD1A* and *NIPBL*). Reactions were prepared using both QuantiTect Primer Assay and QuantiTect SYBR Green PCR Kit according to the manufacturer's recommendations and were run in duplicates in ABI 7900HT fast real time PCR system machine.

Results

Patients and RNA samples

Sperm parameters of the investigated patients are summarized in Table 1. The purity of these samples was estimated

Fig. 1 Scatter plot showing transcripts level within the normospermic fertile group (**a**) and within the oligozoospermic infertile cohort (**b**). **c** Correlation coefficients for the transcripts levels detected in 3

normospermic fertile individuals and 8 oligozoospermic infertile men. *Red* characters: correlation coefficients for the transcripts level detected in spermatozoa of men with different fertility status

by RT-PCR using protamine-2 specific primers [13] that produce a 310 bp in samples containing genomic DNA (not shown). The reverse-transcribed cDNA amplicon is 148 bp long. Obviously, samples containing genomic DNA were rejected from the study. 20 samples (5 normospermic fertile and 15 oligozoospermic infertile samples) were originally selected for this study. The integrity of the RNA samples was assessed by the Agilent 2100 Bioanalyser. However, only 11

(3 normospermic fertile and 8 oligozoospermic infertile samples) have passed the RNA integrity quality control.

Microarray results

The Affymetrix, HG-U133 Plus 2.0 ChIP enabled us to investigate of the expression level of over 47,000 transcripts in human sperm. We detected the presence of 5,382

Fig. 2 **a** Dendrogram of the 157 regulated genes clusters differentially expressed between infertile and fertile men. Up-regulated genes are marked in *red* and down-regulated genes in *green*. Controls:1-5-8.

Infertile men: 6-7-10-18-19-20-21-22. **b-c** List of some differentially expressed transcripts, gene function and fold difference

transcripts in our sperm samples. Transcripts expression level in spermatozoa is quite homogeneous within the two populations (Normospermic fertile and oligozoospermic infertile) (Fig. 1a and b). The correlation coefficients (r^2) for the transcripts levels detected in 3 fertile and 8 infertile men are depicted in Fig. 1c. r^2 range from 0.79 to 0.82 and from 0.66 to 0.92 in the fertile and the infertile group, respectively. However, the correlation scores are lower when the infertile individuals are compared to the fertile ones ($0.38 \leq r^2 \leq 0.66$). This observation suggests that the transcripts found in spermatozoa are not equally expressed in infertile and fertile men (Fig. 1c).

157 transcripts proved to be either up- or down-regulated in sperm of oligozoospermic infertile men as compared to normospermic fertile individuals. These 157 transcripts are a part of a series of biological processes (I- Protein-lipid modification II-mRNA transcription III-Spermatogenesis and motility IV-Protein targeting and localization, Phospholipid metabolism V-Nucleoside, nucleotide and nucleic acid metabolism) and molecular functions (1-RNA helicase 2-mRNA processing factor 3-Helicase 4-Chaperone 5-mRNA splicing factor 6- Nucleic acid binding 7- Transmembrane receptor regulatory/adaptor protein), which, according to our data are seriously disturbed in infertile men. The combined expression data of all transcripts grouped by fold difference and fertility status is depicted in Fig. 2a. About 83% of the regulated genes are at least two-fold down-regulated in germ cells of infertile patients (Fig. 2a). We observe up to 42.96-fold reduction in expression of genes involved in spermatogenesis, sperm motility and germ cells anti-apoptotic process (*PRM2*, *SPZ-1*, *SPATA-4*, *MEA-1*, *CREM*). Furthermore, there is an important decrease in expression (15.12 and 16.36-fold) of

genes involved in DNA repair (*NIPBL*) as well as oxidative stress regulation (*PARK7*). We also note a striking drop (up to 29-fold) in expression of histone modification genes expression (*DDX3X*, *JMJD1A*) (Fig. 2b).

Only 17% of regulated genes are over-expressed in infertile men as compared to the controls (Fig. 2c). However the folds are considerably less important and range from 1.51 to 4.08. Some of these up-regulated genes encode for proteins with an oxido-reductase activity or involved in response to oxidative stress process and abortive spermatogenesis (*ADH4*, *HSD17B7*, *CYGB* and *NXNL1*). Surprisingly, LMNA, an important gene for spermatogenesis is 1.7 fold up-regulated in germ cells of infertile men (Fig. 2c).

Further pathway analysis revealed that transcripts encoding components of ubiquitin proteasome pathway (*PSMD8*, *UBE2E3*, *UBE2V1*: $p=1.08.10^{-2}$) and transcripts encoding purine biosynthesis pathway (*GMPS*, *NME5*: $p=1.71.10^{-2}$) are seriously perturbed.

Quantitative RT-PCR data confirming microarray results are depicted in Fig. 3. The selected genes were among the most regulated when comparing oligozoospermic to normospermic men: *TPD52L3*, *PRM2*, *JMJD1A* and *NIPBL*. qRT-PCR results were normalized to *SAP130*. Similarly to microarray analysis, qRT-PCR showed a down regulation of *TPD52L3* (Fold change: -28.4), *PRM2* (Fold change: -14.1), *JMJD1A* (Fold change: -8.0) and *NIPBL* (Fold change: -12.7), p -values <0.001 .

Discussion

Male factor infertility represents about 45% of infertility cases in northern countries. Multiple factors such as

Fig. 3 Validation of microarray data by quantitative RT-PCR. qRT-PCR assay confirmed that *SAP130* is equally expressed in oligozoospermic and normospermic samples (Fold change, oligo vs. Normo = 1.1). *PRM2*, *TPD52L3*, *JMJD1A* and *NIPBL* proved to be 14.1, 28.4, 8.0 and 12.7 fold down regulated in oligozoospermic men as compared to normospermic men, respectively, p -values <0.001 . *Oligo* Oligozoospermic samples. *Normo* Normospermic samples

Table 2 Transcripts not detected in our study, described as fertility markers by Lalancette and collaborators, gene description and function

Gene symbol	Gene title	Entrez gene ID	Molecular function
ALS2CR14	Homo sapiens amyotrophic lateral sclerosis 2 (juvenile) chromosome region, candidate 14 (ALS2CR14), mRNA.	130026	Function Unknown
ARL2	Homo sapiens ADP-ribosylation factor-like 2 (ARL2), mRNA.	402	GTPase inhibitor activity
ARL6IP4	Homo sapiens ADP-ribosylation-like factor 6 interacting protein 4 (ARL6IP4), transcript variant1, mRNA.	51329	Function Unknown
BSCL2	Homo sapiens Bernardinelli-Seip congenital lipodystrophy 2 (seipin) (BSCL2), mRNA.	26580	Function Unknown
CDC26	Homo sapiens cell division cycle 26 homolog (<i>S.cerevisiae</i>) (CDC26), mRNA.	246184	RNA binding
CLCNKA	Homo sapiens chloride channel Ka (CLCNKA), transcript variant 1, mRNA.	1187	voltage-gated chloride channel activity
DGCR6	Homo sapiens DiGeorge syndrome critical region gene 6 (DGCR6), mRNA.	8214	Function Unknown
DUSP15	Homo sapiens dual specificity phosphatase 15 (DUSP15), transcript variant 1, mRNA.	128853	hydrolase activity
FCRL6	Homo sapiens Fc receptor-like 6 (FCRL6), mRNA.	343413	Function Unknown
FLJ10081	Homo sapiens hypothetical protein FLJ10081 (FLJ10081), mRNA.	55683	glucan 1, 4-alpha-glucosidase activity
FOXD4L2	Homo sapiens forkhead box D4-like 2 (FOXD4L2), mRNA.	100036519	Function Unknown
HNRPH1	Homo sapiens heterogeneous nuclear ribonucleoprotein H1 (H) (HNRPH1), mRNA.	3187	protein binding
HNRPUL1	Homo sapiens heterogeneous nuclear ribonucleoprotein U-like 1 (HNRPUL1), transcript variant 4, mRNA	11100	protein binding
IGFL3	Homo sapiens IGF-like family member 3 (IGFL3), mRNA.	388555	RNA binding
IRX6	Homo sapiens iroquois homeobox 6 (IRX6), mRNA.	79190	sequence-specific DNA binding
KIAA1026	Homo sapiens kazrin (KIAA1026), transcript variant B, mRNA.	23254	Function Unknown
LOC349196	Homo sapiens hypothetical protein LOC349196 (LOC349196), mRNA.	653618	Hypothetical Protein
LOC374395	Homo sapiens similar to RIKEN cDNA 1810059G22 (LOC374395), mRNA.	374395	RNA binding
LOC374768	Homo sapiens hypothetical protein LOC374768 (LOC374768), mRNA.	374768	RNA binding
LOC389517	Homo sapiens Williams Beuren syndrome chromosome region 19 pseudogene (LOC389517) on chromosome 7.	389517	Function Unknown
LOC391811	PREDICTED: Homo sapiens similar to DNA polymerase delta subunit 2 (DNA polymerase delta subunit p50) (LOC391811), mRNA.	391811	Function Unknown
LOC441087	Homo sapiens hypothetical gene supported by AK125735 (LOC441087), mRNA.	441087	Function Unknown
LOC645317	PREDICTED: Homo sapiens similar to Coiled-coilhelix-coiled-coil-helix domain-containing protein 2 (HCV NS2 trans-regulated protein) (NS2TP), transcript variant 3 (LOC645317), mRNA.	645317	Function Unknown
LOC650152	PREDICTED: Homo sapiens similar to tropomyosin 3 isoform 2 (LOC650152), mRNA.	650152	Function Unknown
LOC729466	PREDICTED: Homo sapiens hypothetical protein LOC729466 (LOC729466), mRNA.	729466	Function Unknown
LOC88523	Homo sapiens CG016 (LOC88523), mRNA.	88523	Function Unknown
OKL38	Homo sapiens pregnancy-induced growth inhibitor (OKL38), transcript variant 1, mRNA.	29948	growth factor activity
OR2A42	Homo sapiens olfactory receptor, family 2, subfamily A, member 42 (OR2A42), mRNA.	403218	olfactory receptor activity
PCDHAC2	Homo sapiens protocadherin alpha subfamily C, 2 (PCDHAC2), transcript variant 2, mRNA.	56134	calcium ion binding
PIP5K2B	Homo sapiens phosphatidylinositol-4-phosphate 5-kinase, type II, beta (PIP5K2B), transcript variant 2, mRNA.	8396	transferase activity
RAXL1	Homo sapiens retina and anterior neural fold homeobox like 1 (RAXL1), mRNA.	84839	sequence-specific DNA binding
RUNDC2C	Homo sapiens RUN domain containing 2C (RUNDC2C) on chromosome 16.	440352	Function Unknown
SAC	Homo sapiens testicular soluble adenylyl cyclase (SAC), mRNA.	55811	phosphorus-oxygen lyase activity
SFTPA2B	Homo sapiens surfactant, pulmonary-associated protein A2B (SFTPA2B), mRNA. XM_001133043 XM_001133049 XM_001133054	6436	sugar binding
TMSB4X	Homo sapiens thymosin, beta 4, X-linked (TMSB4X), mRNA.	7114	protein binding
TNFRSF6B	Homo sapiens tumor necrosis factor receptor superfamily, member 6b, decoy (TNFRSF6B), transcript variant M68C, mRNA.	8771	ATP binding
TOMM70A	Homo sapiens translocase of outer mitochondrial membrane 70 homolog A (<i>S. cerevisiae</i>) (TOMM70A), nuclear gene encoding mitochondrial protein, mRNA.	9868	protein binding
TUBA3E	Homo sapiens tubulin, alpha 3e (TUBA3E), mRNA.	112714	GTP binding
VCX	Homo sapiens variable charge, X-linked (VCX), mRNA.	26609	chromatin binding
ZP3	Homo sapiens zona pellucida glycoprotein 3 (sperm receptor) (ZP3), mRNA.	7784	receptor activity

exposure to environmental disruptors, genetics, testis pathologies, lifestyle [1, 2] can alter male germ cells development at various levels, leading to abortive spermatogenesis. It has recently been shown that transcripts levels in spermatozoa are altered in defective spermatogen-

esis cases [8–10]. An association was found between gene expression in spermatozoa and teratozoospermia [6]. The current study represents an attempt to associate gene expression deregulation with oligozoospermia. Microarray results, validated by quantitative RT-PCR, showed that

infertile oligozoospermic men display a major alteration of gene expression profile when compared to fertile individuals. Interestingly, a large proportion of down regulated genes in infertile men are involved in germ cells development: *SPZ1*, *CREM*, *MEAI*, *SPATA4* [14–16]. During mammalian spermatogenesis, the chromatin structure undergoes substantial condensation. The key role in this process is played by protamines 1 and 2. It is now known that a reduction in levels of *PRM2* leads to asthenozoospermia [17] and probably to spermatogenic failure [18], which is in line with our results. Indeed, we observe a 25-fold down-expression of *PRM2* in infertile men who also have a reduction in sperm motility. *JMJD1A*, involved in histone modification and essential for spermatogenesis [19], turned out to be 17 fold down-regulated in spermatozoa of infertile men. Furthermore, A-type lamin (*LMNA*) that is an important determinant of male fertility in mice [20] is slightly (1.7-fold) up-regulated in sperm of infertile men. However, the fold is very small and calls into question the significance of this observation.

We compared our data to the population transcripts that were previously identified as candidate markers of male fertility [5]. We found 90% (389) of these transcripts consistently expressed in our sperm samples. The 40 transcripts that were not detected in our study are summarized in Table 2. 2% [9] of the transcripts consistently detected above background in both 24 control men and in our cohort proved to be either up or down-represented in our infertile subjects. Gene identification, regulation, fold changes, and the involvement of these 9 transcripts in spermatogenesis are summarized in Table 3. *TPD52L3* (Tumor ProteinD52-like 3) was found to be 32-fold down regulated in infertile men. This gene is thought to play a role in testis development and spermatogenesis [21]. Moreover, a 14-fold down-expression is observed in *AKAP14* (A kinase [PRKA] Anchor Protein 14), which encodes a protein that plays a role in human ciliary axoneme beat frequency. *AKAP14* is expressed in human

sperm and is likely involved in sperm motility [22]. This observation fits with the fact that sperm motility is reduced in infertile men. Moreover, the expression of *DNAJB8* (DnaJ [Hsp40] homolog, subfamily B, member 8) is 12-fold reduced in infertile men. *DNAJB8* is a heat shock binding protein that regulates the ATPase activity of Heat Shock Protein 70 (*HSP70*). The latter protects male fertility status thanks to its crucial role in both germ cells differentiation and completion of spermatogenesis [23, 24].

Further pathway analysis showed that ubiquitin proteasome pathway is seriously perturbed in sperm of our infertile group. This observation fits with a previous study showing similar difference in teratozoospermic men [6]. *De novo* purine synthesis pathway also proved to be disturbed in infertile men. We hypothesize that purine synthesis defect may alter DNA synthesis and thus mitosis in spermatogonia leading to abnormal spermatogenesis.

Due to the cost of the technology we had to limit the number of samples analyzed. However, we observed that male infertility associated with low sperm counts is accompanied by a massive down-regulation of a series of transcripts involved in spermatogenesis. During spermatogenesis, inappropriate expression of genes involved in germ cells development could lead to an alteration of semen characteristics (sperm counts, motility, morphology, vitality) and in a larger extent fertilization, oocyte activation and embryogenesis [25–27]. However, one question remains unanswered: what is the reason for this difference in gene expression in germ cells of infertile men as compared to fertile ones? As transcriptional disorder is common within infertile men, we exclude the possibility of a genetic cause and we hypothesize that environment, though epigenetic marks could play a key role in this problem. Recent studies have shown that infertile men, with low sperm counts, are more likely to bear methylation abnormalities in their germ cells than fertile men [28–32]. It is possible that DNA methylation, which is a key regulator of transcription, could

Table 3 Genes either down- or overexpressed in spermatozoa of our oligozoospermic infertile men cohort among the 430 fertility markers published by Lalancette and collaborators, gene functions and fold difference

T-test P-value	Fold-change	Regulation	Unigene	Gene title	Gene symbol	Entrez gene ID	Role in spermatogenesis
1.09E-08	52.69	down	Hs.493739	ubiquitin associated protein 2	UBAP2	55833	Unknown
1.16E-04	32.06	down	Hs.351815	tumor protein D52-like 3	TPD52L3	89882	Possible role in testis development and spermatogenesis
1.31E-04	24.86	down	Hs.2324	protamine 2	PRM2	5620	Nuclear compaction during spermiogenesis
1.63E-04	14.64	down	Hs.592245	A kinase (PRKA) anchor protein 14	AKAP14	158798	Possible role in axoneme beat: sperm motility
3.12E-04	12.83	down	Hs.632731	myosin, light chain 6B, alkali, smooth muscle and non-muscle	MYL6B	140465	Unknown
9.86E-04	12.74	down	Hs.518241	DnaJ (Hsp40) homolog, subfamily B, member 8	DNAJB8	165721	Regulates activity of HSP70 that have a crucial role in spermatogenesis
3.46E-04	5.02	down	Hs.408236	thioredoxin domain containing 17	TXNDC17	84817	Unknown
1.67E-05	3.67	down	Hs.98910	raftlin, lipid raft linker 1	RFTN1	23180	Unknown
6.52E-04	1.70	up	Hs.594444	lamin A/C	LMNA	4000	Important role during meiosis

contribute to gene expression discrepancy in developing spermatozoa leading to abnormal semen parameters (low sperm counts, low motility, poor morphology and vitality). Analyzing the methylation profile of oligozoospermic men as compared to normospermic individuals in regards to our data may help in the understanding of the transcriptional perturbations seen in spermatozoa of infertile men.

It is known that sperm transcripts are transmitted to the embryo and may have a role to play in early embryogenesis [5–7, 23, 24, 33]. Could alteration of gene expression in spermatozoa of infertile men also have an effect on embryo development? Further investigations are needed to clarify this issue.

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Conflict of interest None

References

- Hauser R, Sokol R. Science linking environmental contaminant exposures with fertility and reproductive health impacts in the adult male. *Fertil Steril*. 2008;89:e59–65.
- O'Flynn O'Brien KL, Varghese AC, Agarwal A. The genetic causes of male factor infertility: a review. *Fertil Steril*. 2009;93:1–12.
- Miller D, Briggs D, Snowden H, Hamlington J, Rollinson S, Lilford R, et al. A complex population of RNAs exists in human ejaculate spermatozoa: implications for understanding molecular aspects of spermiogenesis. *Gene*. 1999;237:385–92.
- Ostermeier GC, Dix DJ, Miller D, Khatri P, Krawetz SA. Spermatozoal RNA profiles of normal fertile men. *Lancet*. 2002;360:772–7.
- Lalancette C, Platts AE, Johnson GD, Emery BR, Carrell DT, Krawetz SA. Identification of human sperm transcripts as candidate markers of male fertility. *J Mol Med*. 2009;87:735–48.
- Platts AE, Dix DJ, Chemes HE, Thompson KE, Goodrich R, Rockett JC, et al. Success and failure in human spermatogenesis as revealed by teratozoospermic RNAs. *Hum Mol Genet*. 2007;16:763–73.
- Rousseaux S, Gaucher J, Thevenon J, Caron C, Vitte AL, Curtet S, et al. Spermiogenesis: histone acetylation triggers male genome reprogramming. *Gynecol Obstet Fertil*. 2009;37:519–22.
- Altmae S, Salumets A. A novel genomic diagnostic tool for sperm quality? *Reprod Biomed Online*.
- Feugang JM, Rodriguez-Osorio N, Kaya A, Wang H, Page G, Ostermeier GC, et al. Transcriptome analysis of bull spermatozoa: implications for male fertility. *Reprod Biomed Online*. 2011;21:312–24.
- Hamatani T. Spermatozoal RNA profiling towards a clinical evaluation of sperm quality. *Reprod Biomed Online*. 2011;22:103–5.
- Thomas PD, Campbell MJ, Kejariwal A, Mi H, Karlak B, Daverman R, et al. PANTHER: a library of protein families and subfamilies indexed by function. *Genome Res*. 2003;13:2129–41.
- Thomas PD, Kejariwal A, Campbell MJ, Mi H, Diemer K, Guo N, et al. PANTHER: a browsable database of gene products organized by biological function, using curated protein family and subfamily classification. *Nucleic Acids Res*. 2003;31:334–41.
- Miller D, Tang PZ, Skinner C, Lilford R. Differential RNA fingerprinting as a tool in the analysis of spermatozoal gene expression. *Hum Reprod*. 1994;9:864–9.
- Horowitz E, Zhang Z, Jones BH, Moss SB, Ho C, Wood JR, et al. Patterns of expression of sperm flagellar genes: early expression of genes encoding axonemal proteins during the spermatogenic cycle and shared features of promoters of genes encoding central apparatus proteins. *Mol Hum Reprod*. 2005;11:307–17.
- Liu SF, Lu GX, Liu G, Xing XW, Li LY, Wang Z. Cloning of a full-length cDNA of human testis-specific spermatogenic cell apoptosis inhibitor TSARG2 as a candidate oncogene. *Biochem Biophys Res Commun*. 2004;319:32–40.
- Ohinata Y, Sutou S, Kondo M, Takahashi T, Mitsui Y. Male-enhanced antigen-1 gene flanked by two overlapping genes is expressed in late spermatogenesis. *Biol Reprod*. 2002;67:1824–31.
- Kempisty B, Depa-Martynow M, Lianeri M, Jedrzejczak P, Darul-Wasowicz A, Jagodzinski PP. Evaluation of protamines 1 and 2 transcript contents in spermatozoa from asthenozoospermic men. *Folia Histochem Cytobiol*. 2007;45 Suppl 1:S109–13.
- Imken L, Rouba H, El Houate B, Louanjli N, Barakat A, Chafik A, et al. Mutations in the protamine locus: association with spermatogenic failure? *Mol Hum Reprod*. 2009;15:733–8.
- Liu Z, Zhou S, Liao L, Chen X, Meistrich M, Xu J. Jmjd1a demethylase-regulated histone modification is essential for cAMP-response element modulator-regulated gene expression and spermatogenesis. *J Biol Chem*. 2005;280:2758–70.
- Alzheimer M, Liebe B, Sewell L, Stewart CL, Scherthan H, Benavente R. Disruption of spermatogenesis in mice lacking A-type lamins. *J Cell Sci*. 2004;117:1173–8.
- Cao Q, Chen J, Zhu L, Liu Y, Zhou Z, Sha J, et al. A testis-specific and testis developmentally regulated tumor protein D52 (TPD52)-like protein TPD52L3/hD55 interacts with TPD52 family proteins. *Biochem Biophys Res Commun*. 2006;344:798–806.
- Kultgen PL, Byrd SK, Ostrowski LE, Milgram SL. Characterization of an A-kinase anchoring protein in human ciliary axonemes. *Mol Biol Cell*. 2002;13:4156–66.
- Christians ES, Zhou Q, Renard J, Benjamin IJ. Heat shock proteins in mammalian development. *Semin Cell Dev Biol*. 2003;14:283–90.
- Eddy EM. Role of heat shock protein HSP70-2 in spermatogenesis. *Rev Reprod*. 1999;4:23–30.
- Carrell DT. Contributions of spermatozoa to embryogenesis: assays to evaluate their genetic and epigenetic fitness. *Reprod Biomed Online*. 2008;16:474–84.
- Garcia-Herrero S, Garrido N, Martinez-Conejero JA, Remohi J, Pellicer A, Meseguer M. Differential transcriptomic profile in spermatozoa achieving pregnancy or not via ICSI. *Reprod Biomed Online*. 2011;22:25–36.
- Lalancette C, Miller D, Li Y, Krawetz SA. Paternal contributions: new functional insights for spermatozoal RNA. *J Cell Biochem*. 2008;104:1570–9.
- Kobayashi H, Hiura H, John RM, Sato A, Otsu E, Kobayashi N, et al. DNA methylation errors at imprinted loci after assisted conception originate in the parental sperm. *Eur J Hum Genet*. 2009;17:1582–91.
- Kobayashi H, Sato A, Otsu E, Hiura H, Tomatsu C, Utsunomiya T, et al. Aberrant DNA methylation of imprinted loci in sperm from oligospermic patients. *Hum Mol Genet*. 2007;16:2542–51.
- Marques CJ, Carvalho F, Sousa M, Barros A. Genomic imprinting in disruptive spermatogenesis. *Lancet*. 2004;363:1700–2.
- Marques CJ, Costa P, Vaz B, Carvalho F, Fernandes S, Barros A, et al. Abnormal methylation of imprinted genes in human sperm is associated with oligozoospermia. *Mol Hum Reprod*. 2008;14:67–74.
- Poplinski A, Tuttelmann F, Kanber D, Horsthemke B, Gromoll J. Idiopathic male infertility is strongly associated with aberrant methylation of MEST and IGF2/H19 ICR1. *Int J Androl*. 2003;33:642–9.
- Ostermeier GC, Miller D, Huntriss JD, Diamond MP, Krawetz SA. Reproductive biology: delivering spermatozoan RNA to the oocyte. *Nature*. 2004;429:154.