Expression génique des spermatozoïdes : Nouveau biomarqueur dans la fertilité masculine

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Spermatozoa over the time

1951	100 Millions/ ml
1970	90 Millions/ ml
1974	75 Millions/ ml
1998	55 Millions/ ml
2005	51 Millions/ ml



50 Millions / ml decrease in 50 years

Human Reproduction Update November 2017







ASRM Denver 2018 US Geographic Trends in Semen Quality From 2007 to 2017

City	Change	P Value	
Total sperm count (millions)			
Boston	-2.35	.1069	
Los Angeles	-3.73	.0001	
New York City	+2.75 🗸	.3369	
Palo Alto	-3.97	.0003	
All four cities	-2.94	<.0001	
Sperm concentration (millions/ml)			
Boston	-1.41	.0006	
Los Angeles	-2.08	<.0001	
New York City	-0.17	.8984	
Palo Alto	-1.57	<.0001	
All four cities	-1.76	<.0001	
Total motile sperm (millions)			
Boston	-2.58	.0155	
Los Angeles	-3.11	<.0001	
New York City	+3.39 🗸	.1500	
Palo Alto	-2.96	.0004	
All four cities	-2.45	<.0001	





The Sperm

Spermatozoa survival 3 to 4 days in cervical crypts Oocyte 1 day post-ovulation then it degenerates

The smallest cell 3µ / 5µ Course 20 cm in the female genital tract 20 000 times its size to reach the oocyte in the oviduct

But is Sensitive to Oxidative Reaction, ROS Sensitive to its environment: Temperature, Infection



The Sperm

Quality Control DNA repair DNA abnormalities in sperm

But is terminated; transcription and translation stops post-spermiogenesis!!!

These spermatozoa have no mechanism to repair the damage occurred during their transit through the epididymis and post-ejaculation

Gonzàlez-Marin C, Gosàlvez J Int J Mol Sci Oct 2012





Causes of Male Infertility





Potential effects of exposure to environmental factors on declining fertility rates







Rôle du stress oxydatif : dérivés réactifs de l'oxygène

Les dérivés réactifs de l'oxygène (ROS) sont l'une des principales causes de l'infertilité masculine et causent des dommages aux spermatozoïdes





Example of Bisphenol A

- Bisphenol A (BPA) is an industrial manufacturing monomer used in the polymerization of **plastics**, specifically polycarbonate plastics, and as an additive in **epoxy resins**.
- The main route of exposure to Bisphenol A (BPA) is through food consumption. BPA can be found in serum, urine, breast milk, amniotic fluid, and other biological fluids, indicating its presence in the human body as a result of dietary intake.







- Bisphenol A (BPA) is a weak agonist of estrogen that can bind to both estrogen receptors (ERα and ERβ) and activate a response that has been widely considered as the source of its adverse effects in some animal studies.
- It can also bind to androgen receptors (in vitro).



Distribution of Sperm Anomalies

Azoospermia No Obstructive: NOA	6%
Azoospermia Obstructive: OA	3%
Tératozoospermia	10%
Oligozoospermia	2%
Asthénozoospermia	16%
OAT	28%
ACAS	2%





A new real-time morphology classification for human spermatozoa: a link for fertilization and improved embryo quality

Nino Guy Cassuto, M.D.,^a Dominique Bouret, M.D.,^a Jean Michel Plouchart, M.D.,^a Sonia Jellad, M.D.,^a Pierre Vanderzwalmen, M.S.,^a Richard Balet, M.D.,^b Lionel Larue, M.D.,^c and Yona Barak, Ph.D.^d

TABLE 1

Study 1: fertilization, rate of development, and blastocyst expansion in correlation to the classification of the injected motile spermatozoon.

Sperm classification	Class 1 21 % (46/218)	Class 2 59% (128/218)	Class 3 20% (44/218)	Total number of spermatozoa (N = 218)
Fertilization rate	84% (39/46) ^a	73% (94/128) ^a	61% (27/44) ^a	73% (160/218)
Total blastocysts and morulae	37% (17/46)	26% (33/128)	16% (7/44)	26% (57/218)
Expanded blastocysts	15% (7/46) ^b	9% (12/128) ^b	0 (0/44) ^b	33% (19/57)

Score = Head x^2 + Vacuole x^3 + Base x^1 = 6

Vanderzwalmen P. RBMO 2008 Setti AS. J A Rep Gen 2012 Greco E. F S 2013 Balaban B. RBMO 2011 Tanaka A. F S 2012 Knez K. Rep Bio Endoc 2011 and RBMO 2012 El Khattabi L. F S 2013





Research Article

Different Levels of DNA Methylation Detected in Human Sperms after Morphological Selection Using High Magnification Microscopy

Nino Guy Cassuto,¹ Debbie Montjean,² Jean-Pierre Siffroi,³ Dominique Bouret¹ Flora Marzouk,¹ Henri Copin ⁴ and M Hindawi Volume 2016 | Article ID 6372171 | https://doi.org/10.1155/2016/6372171 ¹ART Unit, Drouot Le ²Reproductive Medici Different Levels of DNA Methylation Detected ter, ³Medical Genetics and in Human Sperms after Morphological 75012 Paris, France ⁴Reproductive Biology Selection Using High Magnification Picardy University Ju Microscopy Nino Guy Cassuto 2,1 Debbie Montjean,2 Jean-Pierre Siffroi,3 Dominique Bouret,1 Flora Marzouk,¹ Henri Copin,⁴ and Moncef Benkhalifa⁴

> Hum Reprod. 2018 Dec 1;33(12):2256-2267. doi: 10.1093/humrep/dey319.

Genome-wide differential methylation analyses identifies methylation signatures of male infertility

Kumar Mohanty Sujit ¹, Saumya Sarkar ¹, Vertika Singh ², Rajesh Pandey ^{3 4}, Neeraj Kumar Agrawal ⁵, Sameer Trivedi ⁶, Kiran Singh ², Gopal Gupta ¹, Singh Rajender ¹



BioMed Research International Volume 2016, Article ID 6372171

The overall sperm DNA methylation patterns from the 10 patients according to the Score 6 and the Score 0.



Stress oxydatif : conséquences épigénétiques

Les dérivés réactifs de l'oxygène (ROS) induisent des déméthylations



Morphology and Gene expression

> PLoS One. 2019 Mar 21;14(3):e0214275. doi: 10.1371/journal.pone.0214275. eCollection 2019.

Genetic and epigenetic profiling of the infertile male

Stephanie Cheung¹, Alessandra Parrella¹ Zov Rosenwaks¹, Gianpiero D Palermo¹

Research Article | Open Access Volume 2021 | Article ID 1434546 | https://doi.org/10.1155/2021/1434546



Show citation

Molecular Profiling of Spermatozoa Reveals Correlations between Morphology and Gene Expression: A Novel Biomarker Panel for Male

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Research Article

Molecular Profiling of Spermatozoa Reveals Correlations between Morphology and Gene Expression: A Novel Biomarker Panel for Male Infertility



Genes selected by differential methylation level and sperm morphology with Gene Ontology

AURKA, HDAC4, CFAP46, SPATA18, CACNA1C, CACNA1H, CARHSP1, CCDC60, DNAH2, and CDC88B



Graphical representation of the 10 genes differentially expressed in score 0 and score 6 spermatozoa.

Functional classification of methylated genes



Expression in various human tissues



Transcript Expression (LOG2(FPKM+0.1))

Differential Gene Expression between

Low Score 0 and High Score 6



Descriptive results show a general trend for under expression for 10 genes in blue bar



Top-ranked functional protein interaction network



CFAP46 Cilia And Flagella Associated Protein 46

Epigenetic regulation Orchestrates genes expression

Because epigenetic regulation is not only on a specific-site event but also spans along stretches of chromosome regions consisting of clusters of contiguous CpG islands

Epigenetic determinants of reproductive potential augment the predictive ability of the semen analysis

F S September 2023

Ryan H. Miller, M.S.,^a Elizabeth A. DeVilbiss, Ph.D.,^b Kristin R. Brogaard, Ph.D.,^a Carter R. Norton,^c

Developments in reproductive biology and medicine

Could the sperm epigenome become a diagnostic tool for evaluation of the infertile man?

Ettore Caroppo 🝺 ^{1,*} and Michael K. Skinner 🝺 ²

H R December 2023



Surrounding Response Sperm Epigenome Signatures





Ettore Caroppo and Michael K. Skinner 2023

Study Design



Expression Génique AURKA / SCORE

Rapport qAURKA/qPRM1



La hauteur de chaque histogramme reflète le niveau d'expression du marqueur considéré.

Sperm epigenome becomes a diagnostic tool for the evaluation of male infertility













Results obtained with the quadruplex final kit: EBX 100 Sperm Messenger 4 Fertility kit





Methylation profiles evaluated by mRNA augment the predictive ability of semen analysis and outcome in term of pregnancy



Combined expression of the 3 target genes



Index Expression < 290 Under-expression Index=290 < Expression < 320 Intermediate expression Index Expression > 320 Normal expression



Spécificité - Sensibilité : 89%



Résultats - Biostatistiques

Sperm Messenger 4 Fertility Analyse

Seuils déterminés:

"Sous expression des gènes cibles" si "index" < 290 "Expression normale des gènes cibles" si "index" > 320 "Expression intermédiaire des gènes cibles" si 290 < "index" < 320

2									
4	Nom de l'échantillon	nb de spermatozoïdes mobiles dans 0,3 mL	Volume échantillon extrait	Résultat quantitatif Gène de ménage (CY5)	Résultat quantitatif CARHSP1 (Texas Red)	Résultat quantitatif Aurka (FAM)	Résultat quantitatif HDAC4 (HEX)	Index	Interprétation
5	XXX303	0.3	120	3.39E+03	2.20E+02	1.20E+01	1.21E+01	34.47	sous expression des gènes cibles
6	XXX116	6.72	300	1.22E+04	3.46E+02	2.76E+01	1.39E+01	813.89	expression normale des gènes cibles
7	XXX021	0.018	300	3.82E+03	1.77E+02	2.87E+01	1.05E+01	4.16	sous expression des gènes cibles
8	XXX020	3.78	160	9.36E+03	1.10E+03	1.81E+01	3.81E+01	914.45	expression normale des gènes cibles

Index : > 320 : Expression normale des gènes

eurobio

SCIENTIFIC

Index : > 290 < 320 : Expression intermédiaire des gènes

Index : < 290 Sous expression des gènes



Gene expression in human sperm as biomarkers and tools for the assessment of male infertility

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> What is known already?

Diagnostic male infertility is largely based on semen parameters according to the World Health Organization (WHO) reference values. While these parameters assess sperm quality, they do not provide insights into sperm quality and have a limited predictive value on natural fecundity, fertilization rates, and assisted reproductive technology (ART) outcomes



We proposed a scoring scale for sperm morphology at high magnification, useful for ICSI protocol, with a score of 6 points for a good spermatozoon to assess expended good blastocyst and a score of 0 for a bad spermatozoon with an altered nucleus. The sperm were ranked according to their scoring and ability to reach blastocyst stage.

> Top network of selected biomarkers

The selected genes involved in mitosis, epigenetic regulation, and early embryo development interact with each other



> AURKA, HDAC4, and CARHSP1 are used for the diagnosis of sperm quality exploring male infertility



Analyzing the mRNA expression levels of the three biomarkers between Score 6 and Score 0



The thresholds for the spermatozoa function index were established by assessing the combined expression of these target genes, considering the morphology and the capability to reach a blastocyst stage:

- > 320 for an "over-index value" spermatozoa with a normal capacity
- < 290 for an "under-index value"</p>
- 290 < index < 320 for an "intermediate-index value".

> Evaluation of potential sperm quality markers

Receiver operating characteristic (ROC) curve analysis was conducted to assess the quality of spermatozoa for three biomarkers by analyzing their mRNA expression levels. The AUC score (0.891) was achieved by the combination of the 3 biomarkers.



Among the 277, we find 112 (40.4%) have an overexpression index; 161 (58.1%) have an under-expressed index and 4 (1.4%) have an intermediate-index.



Among the 129 samples with the three normal WHO parameters, we show that 82 (63.5%) have an over-index value, 44 (34.1%) have an under-index value, and 3 (2.3%) display an intermediate-index value

Conclusion

This study's results highlight understanding a chaotic early embryo development in ART and used to explore male infertility; particularly in unexplained infertility. Furthermore, even in cases with normal sperm parameters, there may be an under-expressed index, indicating lower functionality in terms of fertilization and early embryo development.

Study Results All population



Population with 3 normal WHO parameters





INTRODUCTION^{1,2}

Fertility rates have declined dramatically worldwide since 1950.

The total fertility rate (TFR) has fallen below replacement levels (minimum accepted is 2-1)¹

Male infertility affects approximately 7% of the male population; contributing to 50% of all infertility cases.²

Infertility causes can be identified or not

50% are Identified causes

Testistucal dysfunction
Postinfectious

- Mechanical
- Endcrinopathies...

• At least 2,000 genes are involved in spermatogenesis, lifestyle factors such as tobacco, drugs, stress nutrition, obesity, and surrounding factors such as toxic

exposures, endocrine, disruptors, and aging.³



Environmental factors modify methylation profiles of sperm. Theses signals do not affect the DNA sequence.

Epigenetic diagnostics with gene expression levels open new avenues of exploring male infertility.

FOR WHICH PATIENTS ?

 (\black)

LOGY

With unexplained infertility (50% of male cases)

PATIENTS WITHOUT

- Hormonal, Chromosomal problems
- Testicular or mechanical dysfunction

NORMAL SEMEN

- Concentration
- Morphology
- ・ Motility

 $^{\mathfrak{B}}$ Sperm Messenger Prescription ! \leftarrow

EXAMPLE



OF PRESCRIPTION
+ Spermogram
+ Migration testing
+ Sperm Messenger™

1 : GBD 2021 Fertility and collaborators. Global fertility in 204 countries and territories, 1950–2021, with forecasts to 2100. The Lancet 2024.

- 2 :A unique view on male infertility around the globe. A. Agarwal et al Reproductive biology and endocrinology 2015.
- 3 : Male infertility. ML Eisenberg et al. Nat Rev Dis Primers 2023.
- 4 : Genetics of male infertility. Č. Krausz et al. Nature Reviews Urology 2018. 5 :The presence, role and clinical use of spermatozoa RNAs. Meritxell Jodar et al. Human Reproduction Update 2013.
- 6 :Molecular Profiling of Spermatozoa Reveals Correlations between Morphology and Gene Expression: A Novel Biomarker Panel for Male Infertility. Cassuto Nino Guy et al. BioMed Research International 2021.

7 : Gene expression in human sperm as biomarkers and tools for the assessment of male infertility. Cassuto Nino Guy et al. ESHRE 2024

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EXPLORE MALE INFERTILITY

Biomarker for Unexplained Male Infertility

Sperm Messenger [™] for Fertility





DIAGNOSTIC MALE INFERTILITY

Up to 50% of cases of male factor infertility are currently diagnosed as unknown and unexplained.

Discovery of novel genetic factors in idiopathic infertility is a major challenge for the field of androgenetic.⁴

10	51
	m
10	

50%

The paternal contribution has long been underestimated.

Studies shows that a spermatozoon carries approximately 3,000 mRNA transcripts; delivered to the oocyte at fertilization.



That influences fertilization, embryo development, the phenotype of the offspring, and possibly future generations.

The use of spermatozoa RNA profiles as phenotypic markers of male factor status could be used as clinical diagnostics.⁵



Semen analysis is the first step in exploring male infertility, based on routine sperm parameters such as volume, vitality, concentration, motility, and morphology evaluation.

This assessment of sperm quality does not indicate any sperm functionality.



Epigenetic diagnostics with the gene expression levels reflect sperm functionality opening new avenues for male infertility exploration.

RNA TOOL : SPERM MESSENGER™ FOR FERTILITY





EXPRESSION OF 3 TARGET GENES

AURKA (AURORA KINASE A)



It is a key regulator of cell division and is involved in mitosis.
Essential for the first mitotic spindle and chromosome segregation.
Is involved in the early development of the embryo.

HDAC4 (HISTONE DEACETYLASE 4)

- Involved in the epigenetic regulation of gene expression through methylation profile.
- Playing a role on early embryonic development, regulating gene expression.
- Involved in the regulation of motility and acrosome reaction (critical processes in fertilization and embryo development)



- · Highly expressed in the early stages of embryonic development.
- Under-expression of CARHSP1 leads to chaotic cell division and arrested embryonic development.
- It expression is significantly higher in blastocyst-developed embryos, associated with higher pregnancy rates.

The Sperm Messenger[™] test is an amplification assay for the messenger RNAs (mRNAs) of three target genes involved in fertilization and early embryo development.

A method to explore male infertility by highlighting dysfunction and quantifying the expression profile of target genes, using real-time qPCR.

This molecular biology test provides a validated biomarker signature for assessing sperm functions to determine the prevalence and distribution of 3 target gene expression levels, AURKA, HDAC4, and CARHSP1.⁶

Sperm messenger™, why is it for?

To explore male infertility, in cases of

- Unexplained infertility
- Chaotic embryonic development
- Or a low blastocyst rate in ART

A study has shown that even in cases with normal sperm parameters, there may be under-expressed genes, indicating lower functionality in terms of fertilization and early embryo development.



Conclusions

In one ejaculate all the spermatozoa have the same DNA sequence, but different epigenetics profiles and different genes expression!!!

1

Methylation Genes profiles evaluated by mRNA augment the predictive ability of outcome in term of fertilization and pregnancy

Epigenetic disorders are linked with changes in methylation profiles, controlling gene expression, as well the offspring malformations

Some of these disorders are impacted by ROS:

Treatment and lifestyle or ICSI program

