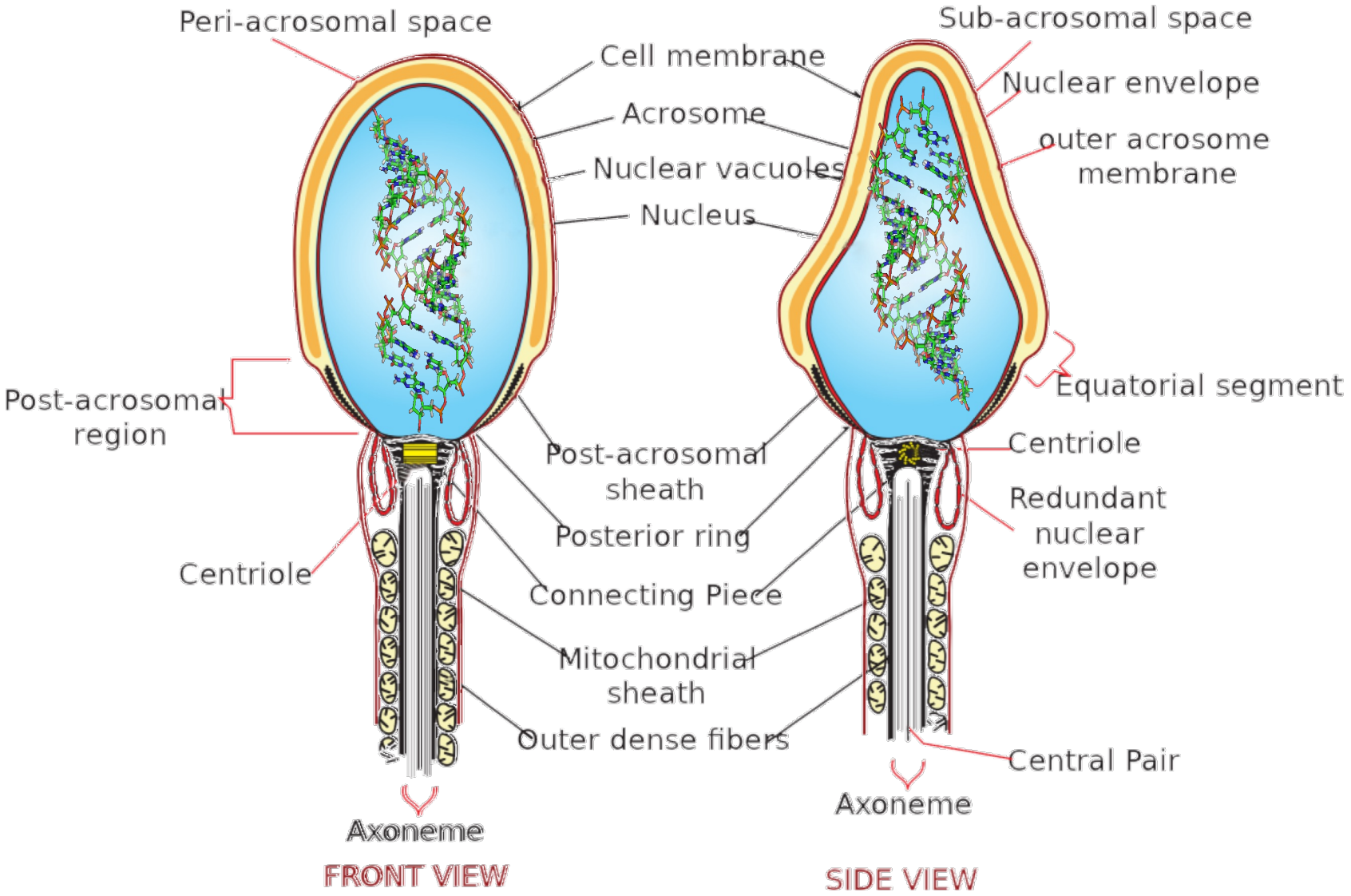
Sélection des SPZ par IA avant ICSI

Nino Guy Cassuto **ART Unit Drouot Paris**


Peri-acrosomal space
Cell membrane
Acrosome
Nuclear vacuoles
Nucleus
Post-acrosomal region
Post-acrosomal sheath
Posterior ring
Centriole
Connecting Piece
Mitochondrial sheath
Outer dense fibers
Axoneme
FRONT VIEW
Sub-acrosomal space
Nuclear envelope
outer acrosome membrane
Equatorial segment
Centriole
Redundant nuclear envelope
Central Pair
Axoneme
SIDE VIEW

Different Sperm Head Morphology

Same genetic information
Double helix DNA sequency

Different epigenetic
Chromatin Methylation
5mcytosine

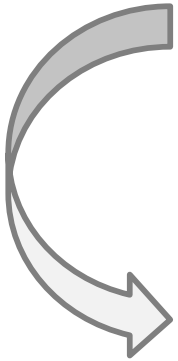
Different Outcome

Why it is Important to investigate the sperm DNA ?

In reproductive physiology many **abnormalities** may occur during **spermatogenesis** and **spermiogenesis**, resulting in **spermatozoa defects**.

These disorders may concern:

Sperm morphology
Numerical or structural chromosomes abnormalities
Abnormal chromatin
Sperm DNA defects



Resulting in oocyte activation failure,
No fertilization or zygote blocking, (Shaoqin et al 2014)
Chaotic early embryo development,
Negative blastocyst culture
Miscarriage
Birth defects
(Schagdarsurengin et al 2012; Cassuto et al 2014)

JBRA Assisted Reproduction 2021;**25**(3):466-472
doi: 10.5935/1518-0557.20210030

Original Article

Impact of Intracytoplasmic Morphologically Selected Sperm Injection (IMSI) on Birth Defects: A Systematic Review and Meta-Analysis

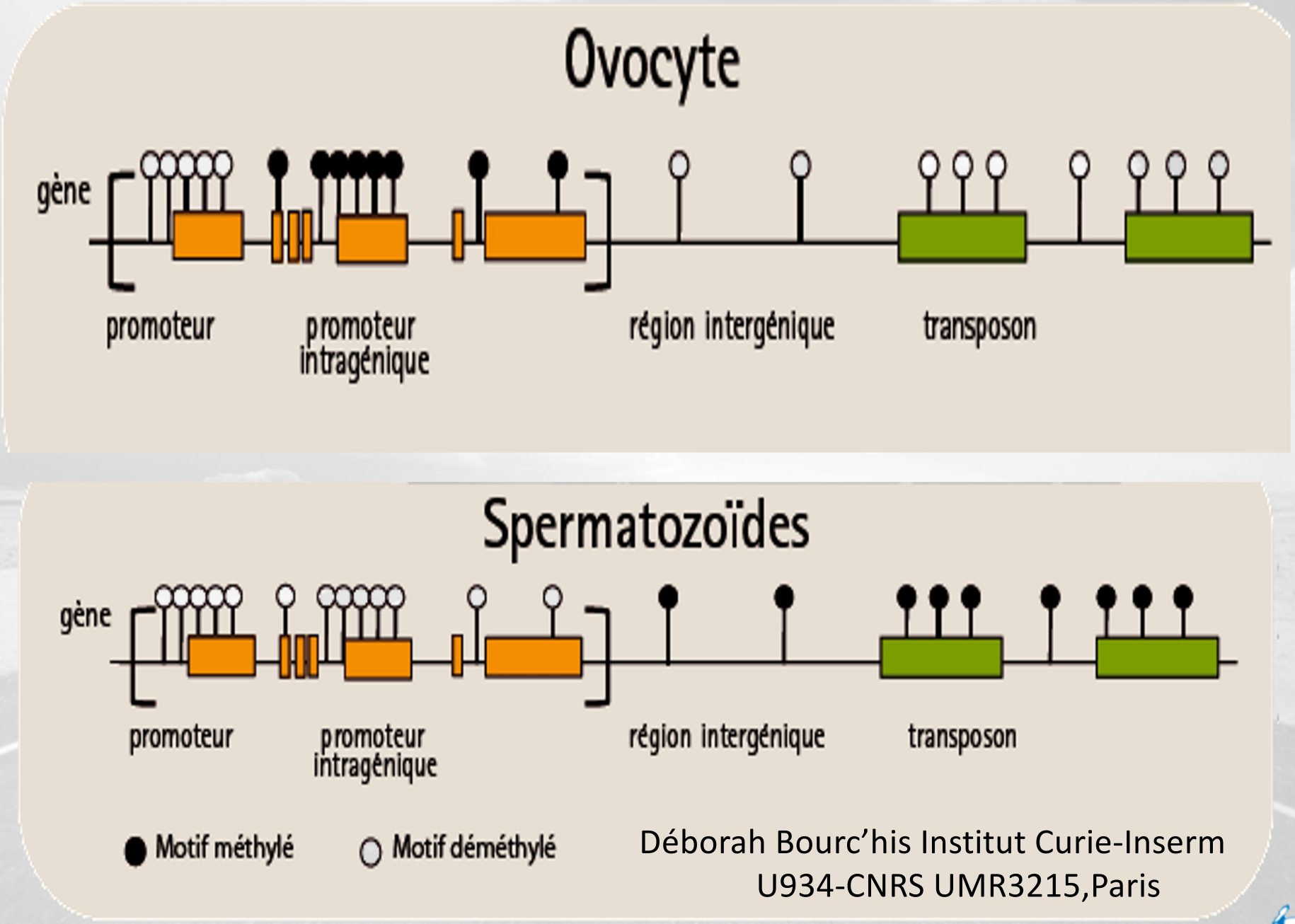
Felipe Dieamant[1,2], Claudia G Petersen[1,2], Laura D Vagnini[2], Adriana Renzi[2], Bruna Petersen[1,2], Fabiana Massaro[1], Camila Zamara[1], Andreia Nicoletti[1], Juliana Ricci[1], Antonio H Oliani[3], João Batista A. Oliveira[1,2], José G. Franco Jr[1,2]

May 2021

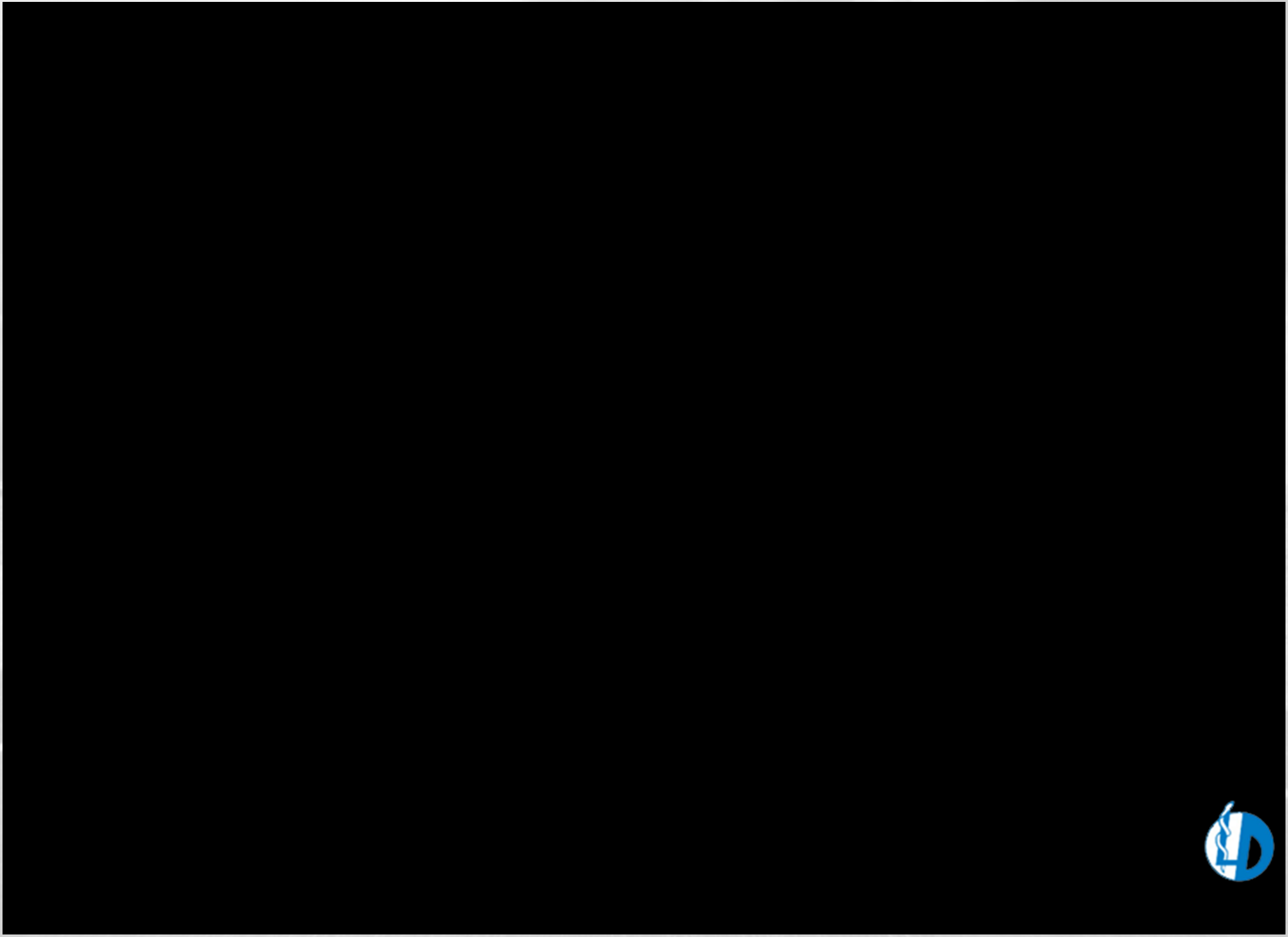
IMSI vs. ICSI: Total birth defects(structural-defects/chromosomal- abnormalities).

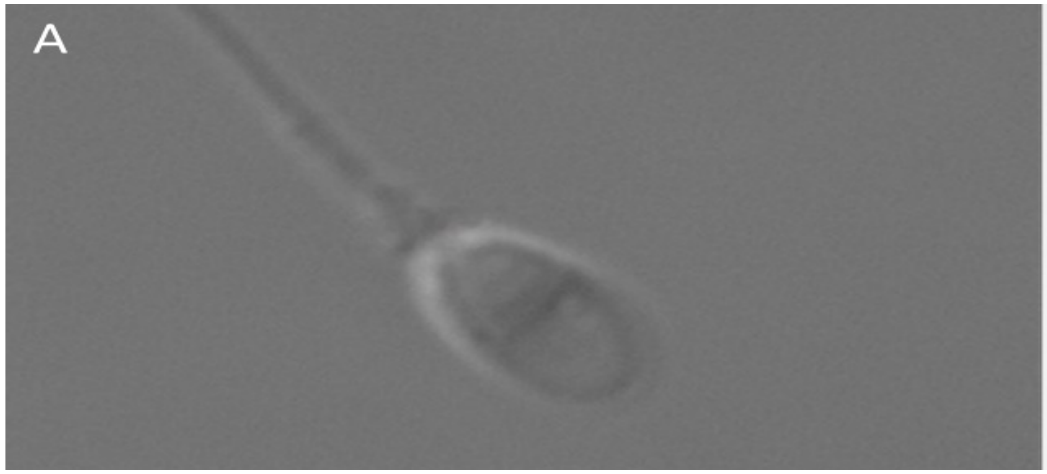
Total birth defects	**ICSI(n/N)**	**IMSI(n/N)**	**RR**	**95% CI**
Cassuto *et al.*,2014	22/578	6/450	0.35	0.15-0.83
Hershko-Klement *et al.*,2016	71/1394	18/498	0.71	0.43-1.17
Gaspard *et al.*,2018	26/655	8/332	0.61	0.28-1.30
Total	**119/2627**	**32/1280**	**0.60**	**0.41-0.88**
Chi^2=6.7;*P*<0.01				
Cochran's Q=1.8;*P*=0.4				

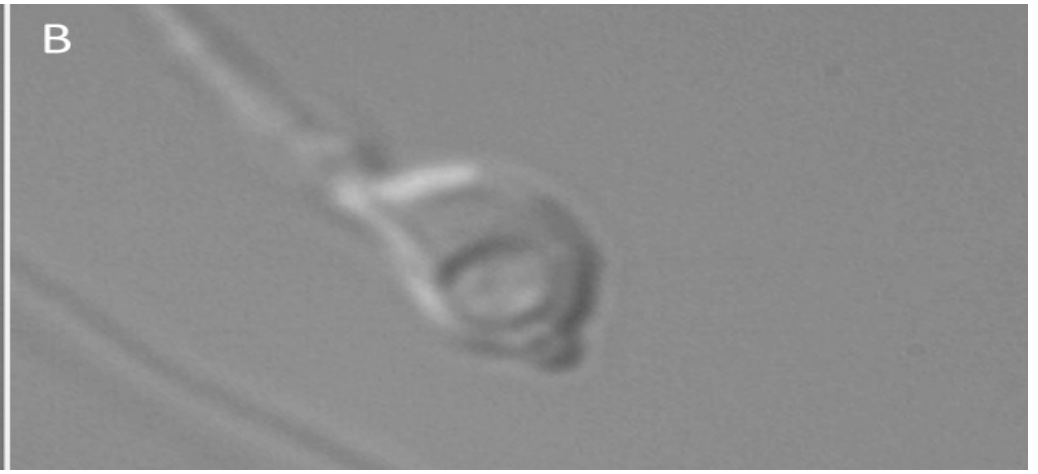
ESHRE - Vienna June 2019



Déborah Bourc'his Institut Curie-Inserm U934-CNRS UMR3215,Paris







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

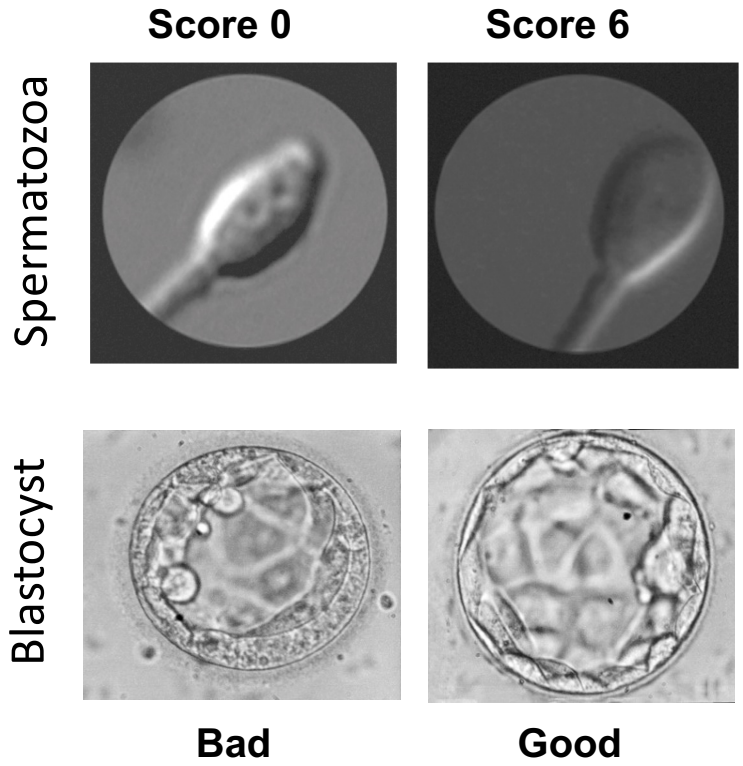
Study 1: fertilization, rate of development, and blastocyst expansion in correlation to the classification of the injected motile spermatozoon.

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 x2 + Vacuole x3 + Base x1 = 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

Strict morphological criteria for sperm head



Morphological criteria are used to score spermatozoa at high magnification (6100x) and to assess expended good blastocyst quality

Cassuto et al., 2021

Sperm fluorescence in situ hybridization study in nine men carrying a Robertsonian or a reciprocal translocation: relationship between segregation modes and high-magnification sperm morphology examination

Nino Guy Cassuto, M.D.,[a] *Nathalie Le Foll, M.D.,*[b] *Sandra Chantot-Bastaraud, M.D.,*[b] *Richard Balet, M.D.,*[c] *Dominique Bouret, M.D.,*[a] *Alexandre Rouen, M.D.,*[b] *Rakia Bhouri, M.D.,*[b] *Capucine Hyon, M.D.*[b] *and Jean Pierre Siffroi, M.D., Ph.D.*[b]

TABLE 3

Sperm fluorescence in situ hybridization (FISH) result… translocations carriers.

MSO				P6	P8
	…: 35	Class II: 50 Class III: 40	Class I: 25 Class II: 50 Class III: 25	Class I: 10 Class II: 60 Class III: 30	Class I: 5 Class II: 60 Class III: 35
Altern…					
NS	50%	44.40%	52.60%	47.80%	37.10%	44%
Class…	X	43.40%	53%	59.60%	23.40%	40%
Class II	43.30%	53.50%	64%	55%	35.50%	46%
Class III	50%	38.30%	41.20%	51.70%	39.10%	44.7

High-magnification in translocation carriers cannot be used to select sperm cells with a balanced chromosomal content

Chelli MH. J A Rep Gen 2013

Table II Evaluation of sperm DNA fragmentation in large vacuole spermatozoa, isolated using MSOME.

Perdrix A HR 2013

Studies	Studied population	Large vacuole definition	Sperm DNA fragmentation analysis	Percentage of spermatozoa with fragmented DNA (mean ± s.e.m.) (%)			P	
				MSOME normal spermatozoa (*n*)	Large vacuole spermatozoa (*n*)			
Franco *et al.* (2008)	30 Infertile patients	>50% of the sperm head area	TUNEL (fixation: methanol–acetic acid)	15.9% (410)	29.1% (382)		<0.0001	
Garolla *et al.* (2008)	10 Infertile patients oligozoospermia	Not defined	TUNEL (fixation: paraformaldehyde 4%)	9.3 ± 4.8% (100)	40.1 ± 11.6% (100)		<0.001	
Wilding *et al.* (2011)	8 Infertile patients	>4% of the sperm head area	TUNEL (fixation: paraformaldehyde 4%)	6.1 ± 7.2% (331)	14.7 ± 7.2% (529)		=0.031	
Boitrelle *et al.* (2011)	15 Infertile patients	>25% of the sperm head area	TUNEL (fixation: ethanol 95%)	0.7 ± 0.4% (450)	1.3 ± 0.4% (450)		NS (=0.25)	
Perdrix *et al.* (2011)	20 Infertile patients Teratozoospermia	>13% of the sperm head area	TUNEL (fixation: methanol)	Unselected spermatozoa from native sample 11.5 ± 1.22% (10040)	14.5 ± 3.45% (560)		NS (=0.68)	
Watanabe *et al.* (2011)	10 Infertile patients 2 Sperm donors	>1.5 µm and visible at ×400 magnification	TUNEL (fixation: paraformaldehyde 4%)	3.5% (2252) 2.3% (398)	3.3% (209) 0% (18)		NS NS	
Hammoud *et al.* (2012)	8 Infertile patients with high DNA fragmentation rates >13%	>4% of the sperm head area	TUNEL (fixation: methanol–acetic acid)	4.1 ± 1.1% (191)	Anterior vacuoles 15.9 ± 2.9% (368) (a)	Posterior vacuoles 22.5 ± 3.6% (402)(b)	(a): $P = 0.013$	(b): $P = 0.0007$
				Unselected spermatozoa from native sample 26.1 ± 1.5% (8000)			(a): $P = 0.02$	(b): $P = 0.44$
Cassuto *et al.* (2012)	26 Infertile patients, Oligoasthenoteratozoospermia, Previous IVF failures	Score 0 spermatozoa (Cassuto *et al.*, 2009)	TUNEL	Unselected spermatozoa after density gradient 3.7 ± 6.7% (2600)	Score 0 4.2 ± 5.5% (2600)		NS	

Table I Evaluation of sperm chromatin compaction in large vacuole spermatozoa, isolated using MSOME.

Perdrix A HR 2013

Studies	Studied population	Definition of large vacuoles	Sperm chromatin compaction analysis	Abnormal sperm chromatin compaction (mean ± s.e.m.) (%)		P
				MSOME normal spermatozoa (*n*)	Large vacuole spermatozoa (*n*)	
Franco *et al.* (2008)	30 Infertile patients	>50% of the sperm head area	Orange		67.9% (371)	<0.0001
Garolla *et al.* (2008)	10 Infertile patients, oligozoospermia				71.9 ± 11.1% (100)	<0.001
Boitrelle *et al.* (2011)	15 Infertile pa				36.2 ± 1.9% (450)	<0.0001
Perdrix *et al.* (2011)	20 Infertile patie Teratozoospermia			Unselected spermatozoa from native sample 26.5 ± 2.57% (9980)	50.4 ± 3.1% (460)	<0.0001
Cassuto *et al.* (2012)	26 Infertile patients oligoasthenoteratozoospermia Previous IVF failures	Score 0 spermatozoa (Cassuto *et al.*, 2009)	Aniline blue	Unselected spermatozoa after density gradient 10.1 ± 6.0% (2600)	19.5 ± 8.7% (2600)	<0.0001
Franco *et al.* (2012)	66 Infertile patients	>50% of sperm head area	CMA3	40.3% (835)	53.2% (1351)	<0.0001

Failure of Chromatin Condensation

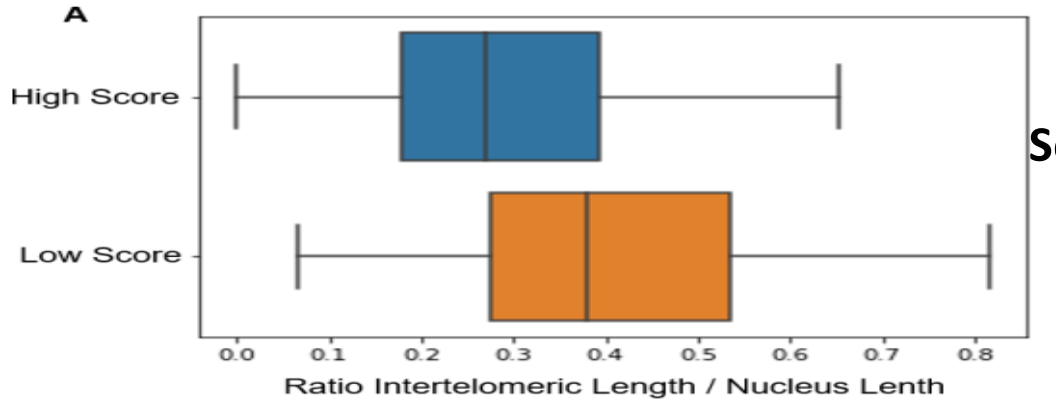
genes April 2024

MDPI

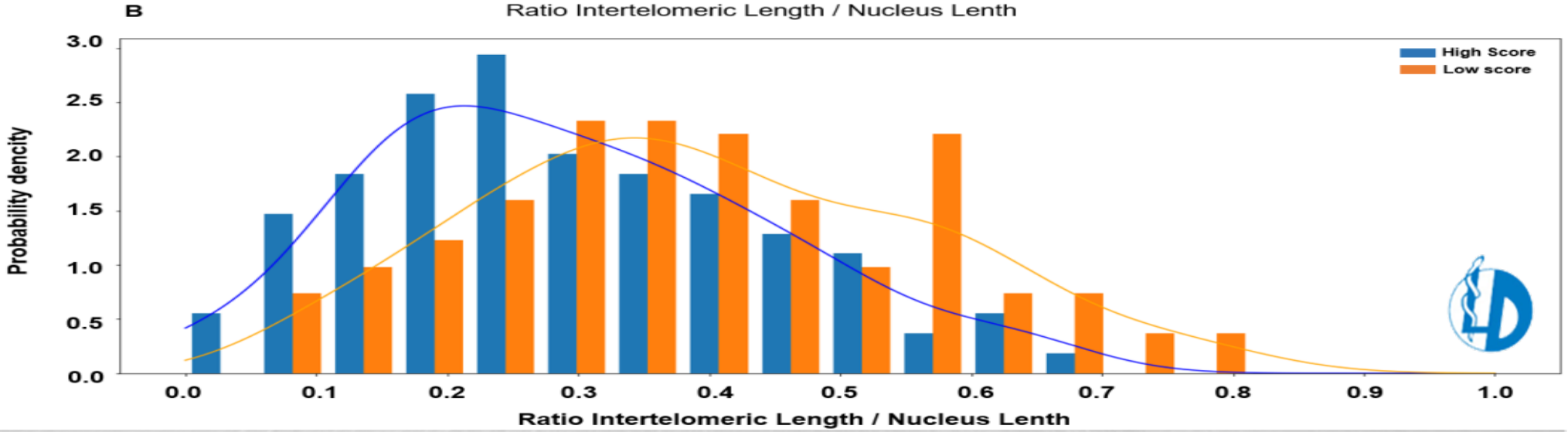
Brief Report

Different Nuclear Architecture in Human Sperm According to Their Morphology

Nino-Guy Cassuto [1,*], Nesrine Ogal [2], Said Assou [3], Lea Ruoso [1], Eli-Jonathan Rogers [2], Miguel-José Monteiro [1], Daniel Thomas [1], Jean-Pierre Siffroi [2] and Alexandre Rouen [4,5,*]



Score 0 higher inter-telomeric distances



genes April 2024

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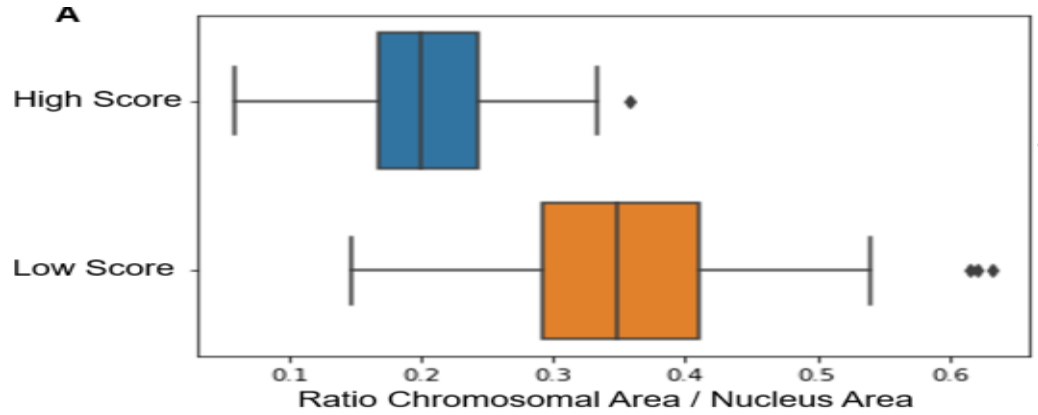
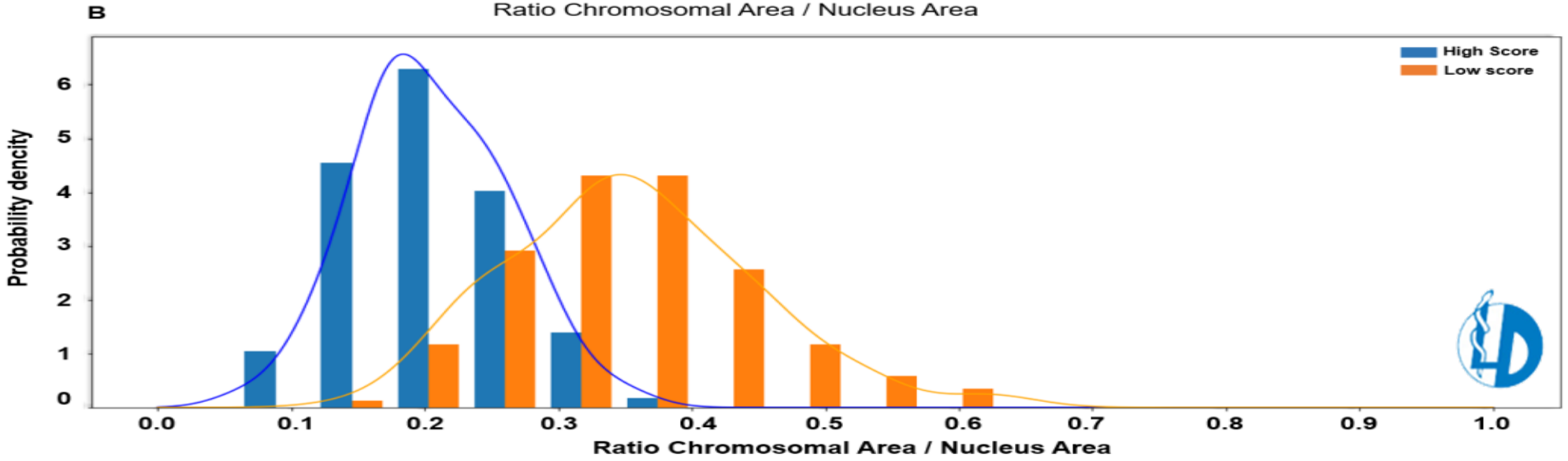


Score 0 higher chromosomal territory areas

.



genes April 2024

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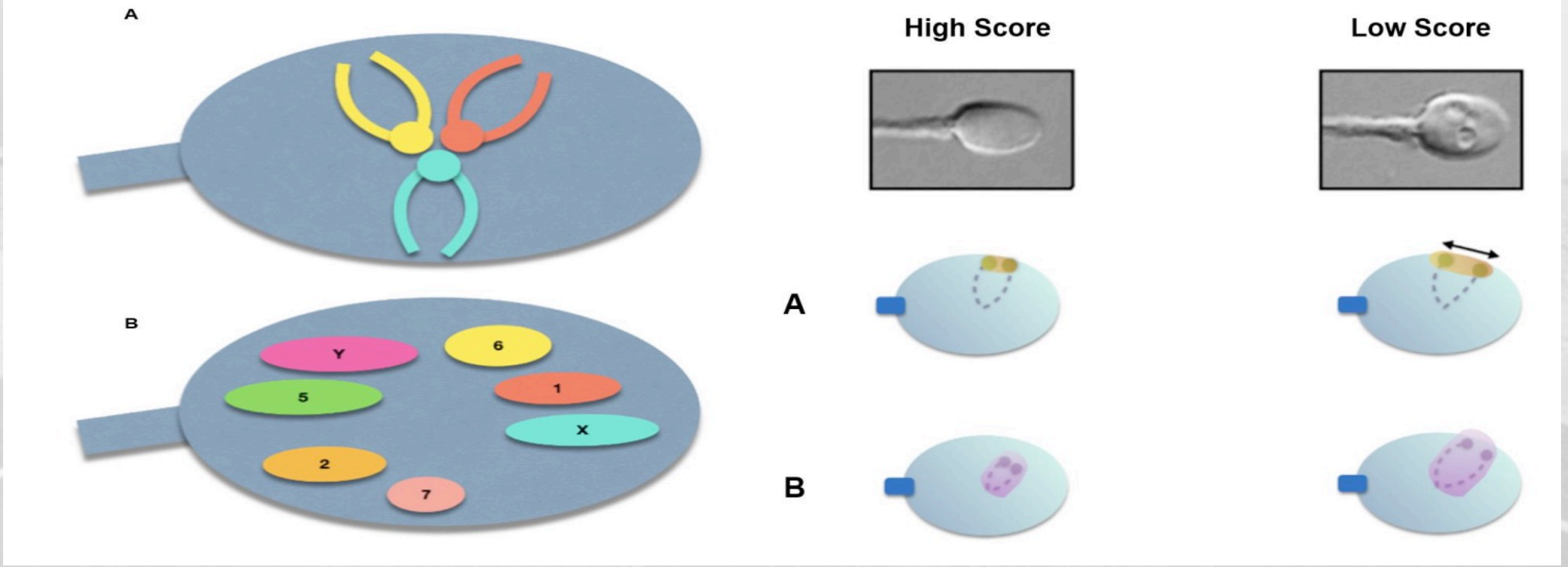


A Score 0 spermatozoa exhibit significantly higher inter-telomeric distances
B Score 0 spermatozoa exhibit significantly higher chromosomal territory areas for chromosome 1.

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 Bouret,[1] Flora Marzouk,[1] Henri Copin,[4] and M

[1]*ART Unit, Drouot La*
[2]*Reproductive Medici*
[3]*Medical Genetics and*
75012 Paris, France
[4]*Reproductive Biology*
Picardy University Ju

Hindawi

Research Article | Open Access
Volume 2016 | Article ID 6372171 | https://doi.org/10.1155/2016/6372171

Show citation

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 Bouret,[1] Flora Marzouk,[1] Henri Copin,[4] and Moncef Benkhalifa[4]

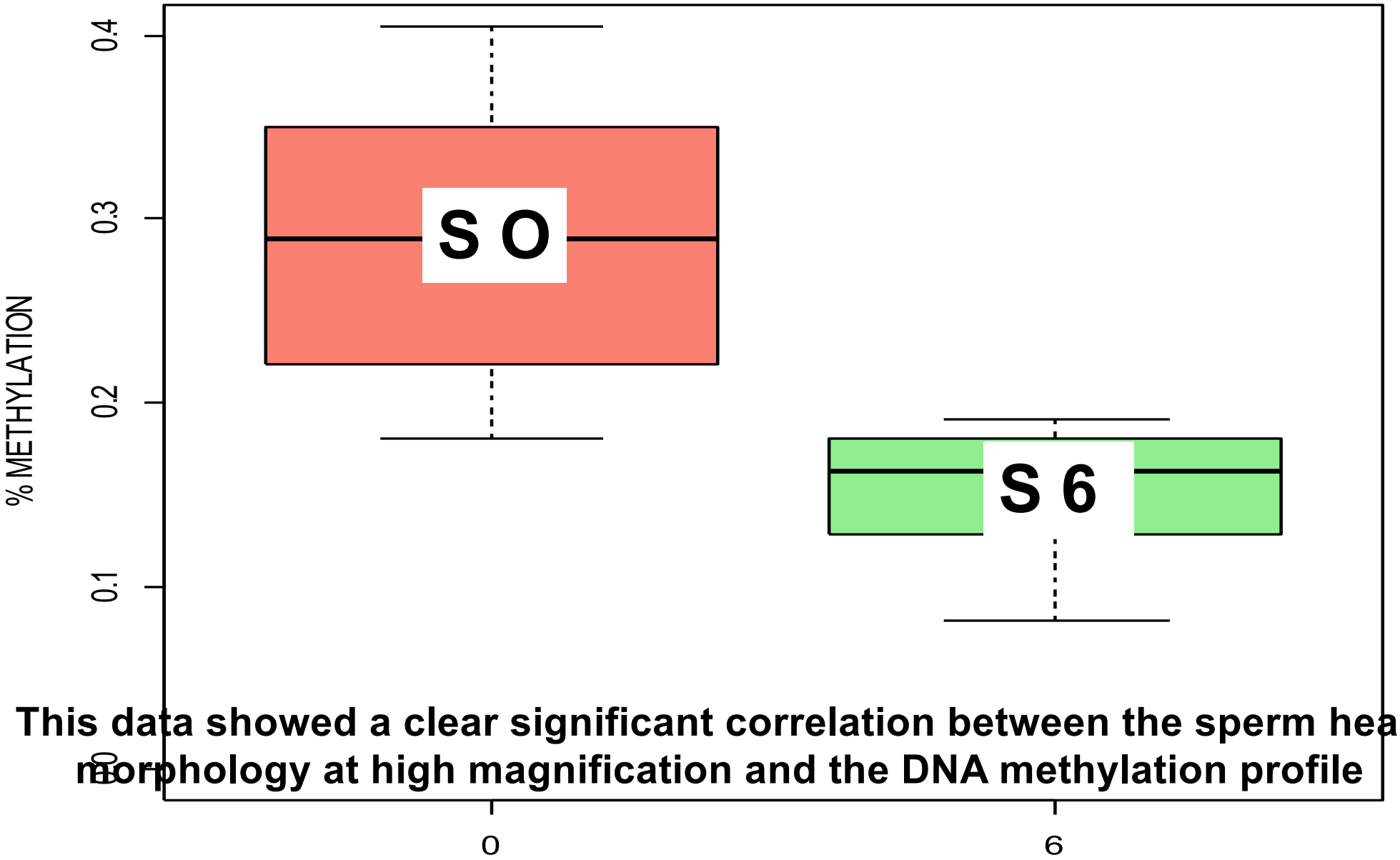
Hum Reprod. 2018 Dec 1;33(12):2256-2267. doi: 10.1093/humrep/dey319.

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

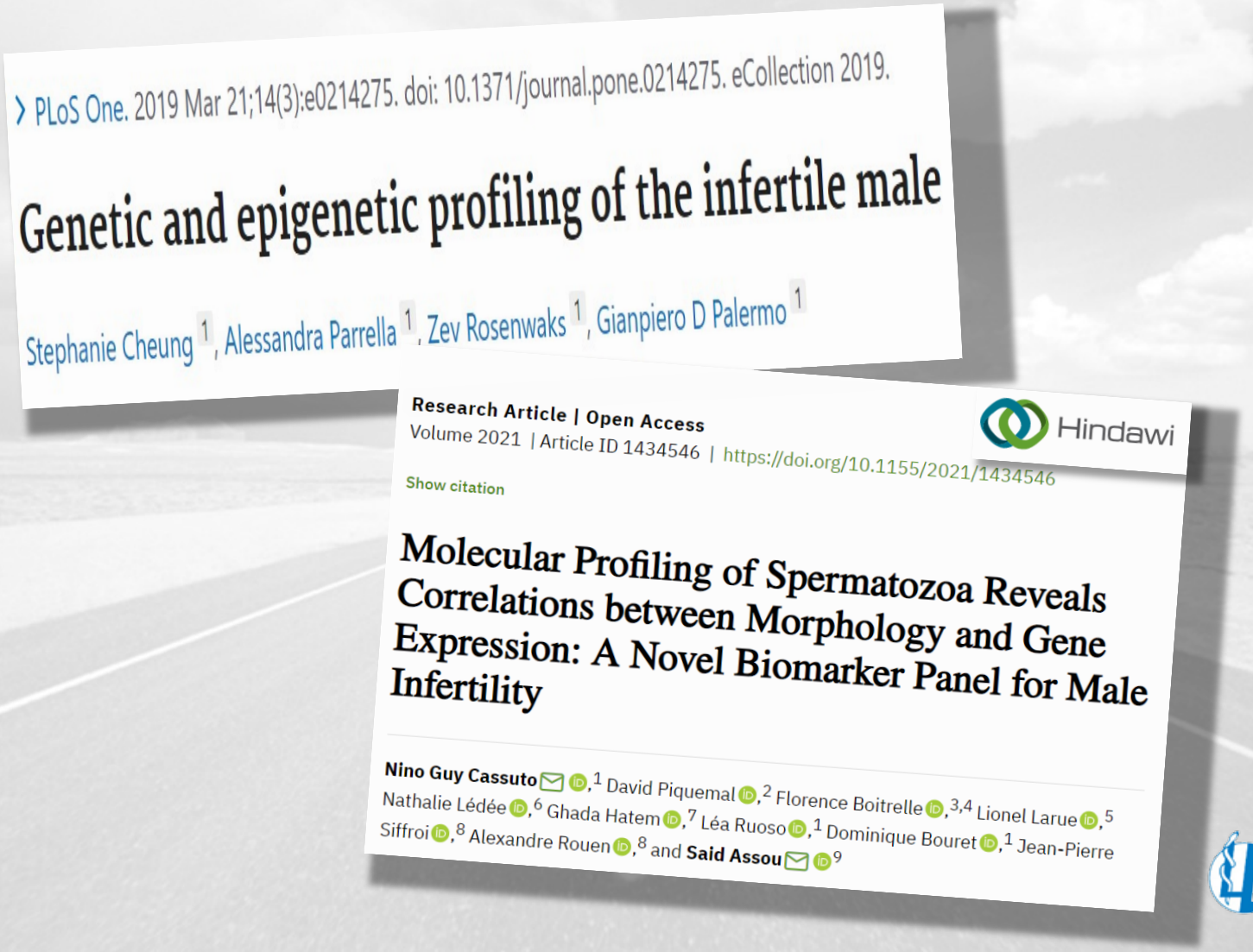
The overall sperm DNA methylation patterns from the 10 patients according to the Score 6 and the Score 0.



This data showed a clear significant correlation between the sperm head morphology at high magnification and the DNA methylation profile

***p* < 0.00001** OR S0 / S6 = 2.31

Morphology and Gene Expression

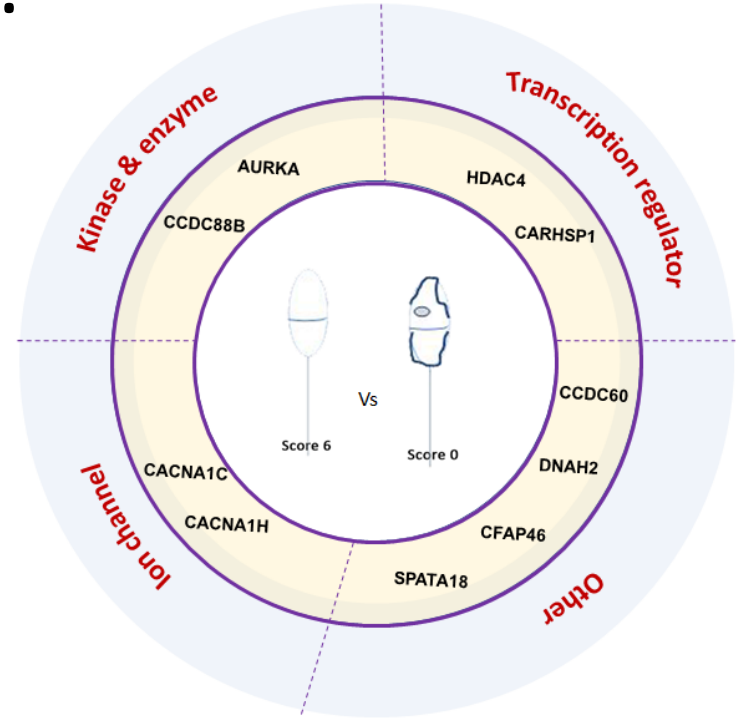


Genes selected by differential methylation level and sperm morphology with Gene Ontology

.

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

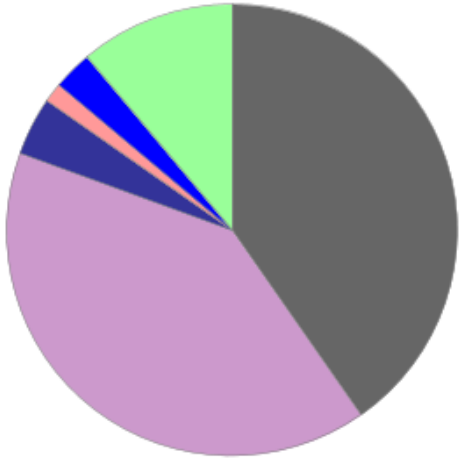
Gene_Name	Pvalue
AURKA	0.004316
CACNA1C	0.3057
CACNA1H	0.6764
CARHSP1	0.4697
CCDC60	0.09758
CCDC88B	0.1028
CFAP46	0.01651
DNAH2	0.1815
HDAC4	0.07368
HMGB4	0.3814
SPATA18	0.6764



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

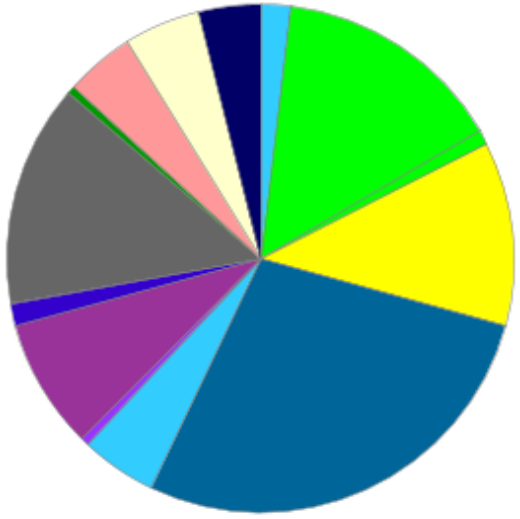
Functional classification of methylated genes

Molecular function



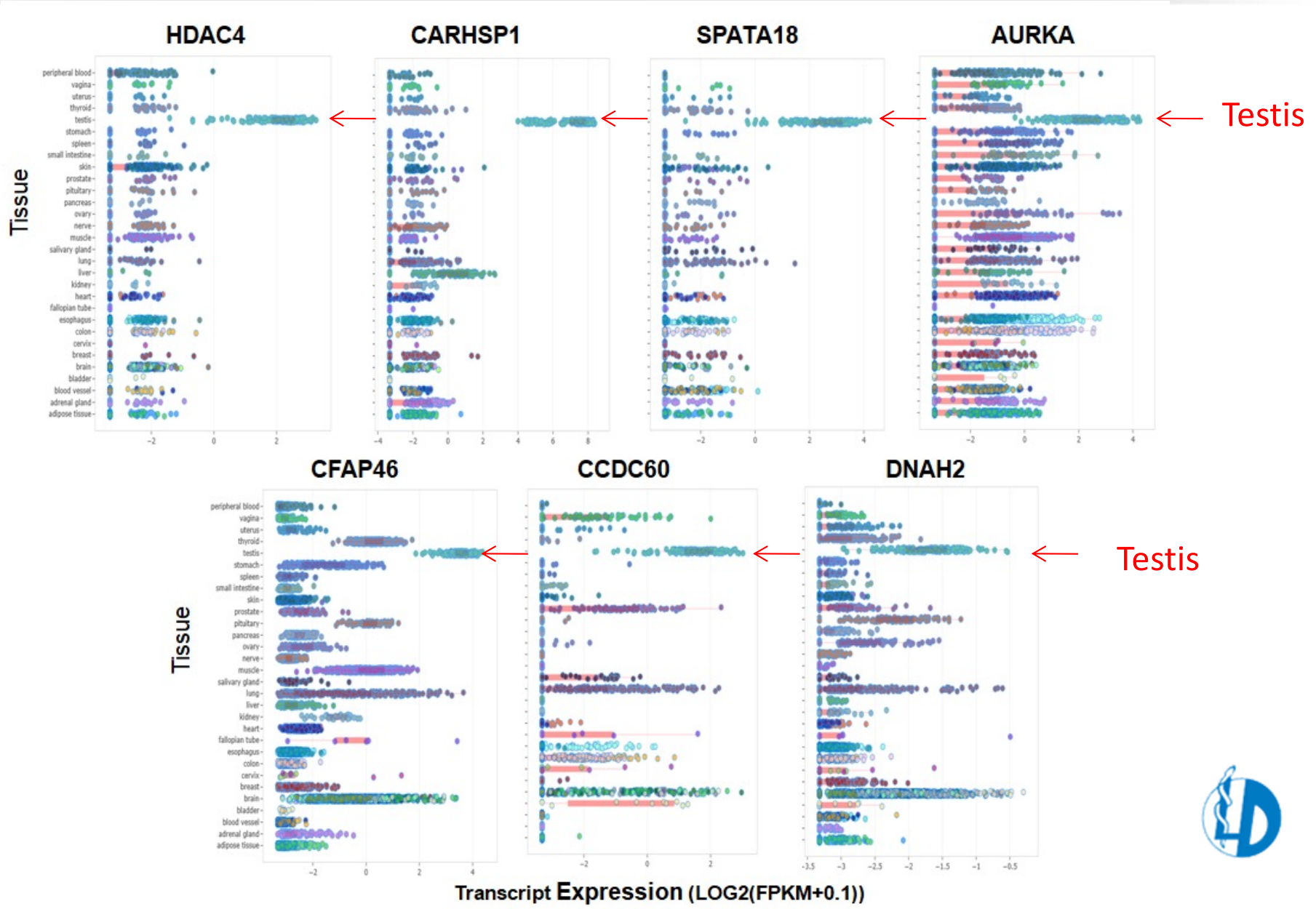
- Binding (GO:0005488)
- Catalytic activity (GO:0003824)
- Molecular function regulator (GO:0098772)
- Molecular transducer activity (GO:0060089)
- Transcription regulator activity (GO:0140110)
- Transporter activity (GO:0005215)

Biological process

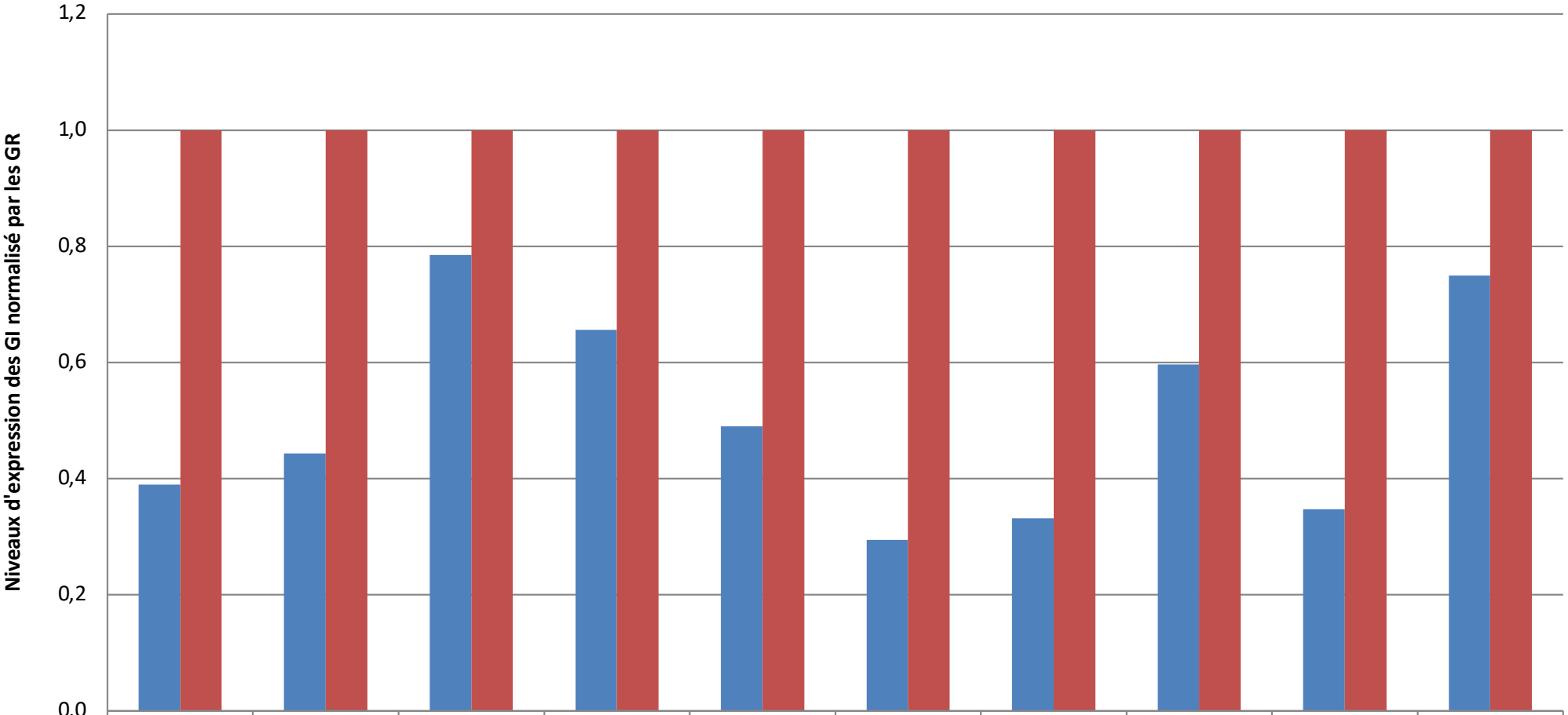


- Biological adhesion (GO:0022610)
- Biological regulation (GO:0065007)
- Cell population proliferation (GO:0008283)
- Cellular component organization (GO:0071840)
- Cellular process (GO:0009987)
- Developmental process (GO:0032502)
- Immune system process (GO:0002376)
- Localization (GO:0051179)
- Locomotion (GO:0040011)
- Metabolic process (GO:0008152)
- Multi-organism process (GO:0051704)
- Multicellular organismal process (GO:0032501)
- Response to stimulus (GO:0050896)
- Signaling (GO:0023052)

Expression in various human tissues



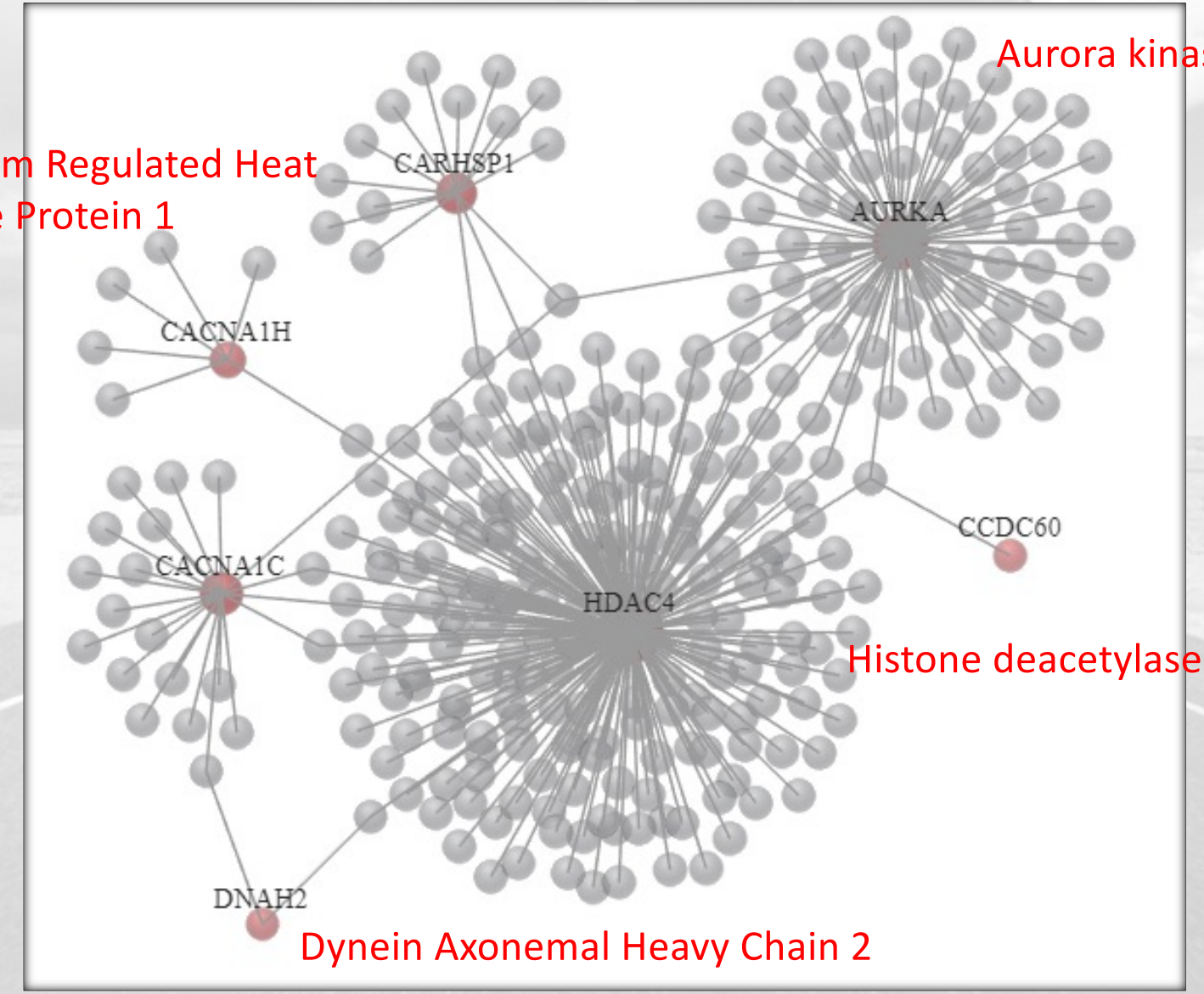
Differential Gene Expression between Low Score 0 and High Score 6



	AURKA	CACNA1C	CACNA1H	CARHSP1	CCDC60	CCDC88B	CFAP46	DNAH2	HDAC4	SPATA18
Score 0	0,4	0,4	0,8	0,7	0,5	0,3	0,3	0,6	0,3	0,7
Score 6	1,0	1,0	1,0	1,0	1,0	1,0	1,0	1,0	1,0	1,0

Descriptive results show a general trend for under expression for 10 genes in blue bar for the score 0

Top-ranked functional protein interaction network



Aurora kinase A

Calcium Regulated Heat Stable Protein 1

Histone deacetylase 4

Dynein Axonemal Heavy Chain 2

CFAP46 Cilia And Flagella Associated Protein 46

ESHRE 40th Annual Meeting 2024

P-029

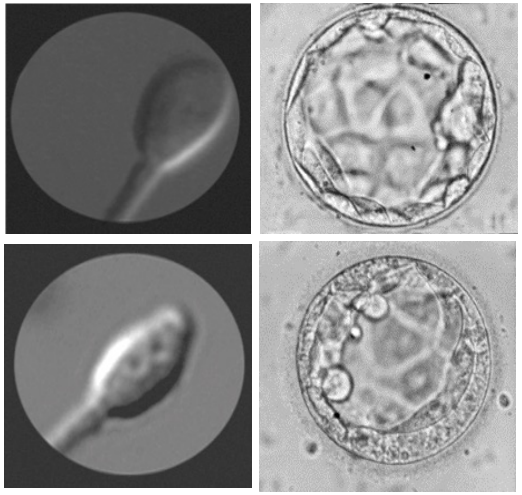
Genome-wide microRNA expression profiling in human spermatozoa and its relation to sperm quality

NG. CASSUTO , L. RUOSO , G. KEROMNES , L. PRAT-ELLENBERG , N. LEDEE , C. CHAO , H. MOUIK , S. ASSOU

The relevance of sperm morphology in male infertility

Score 6

Score 0



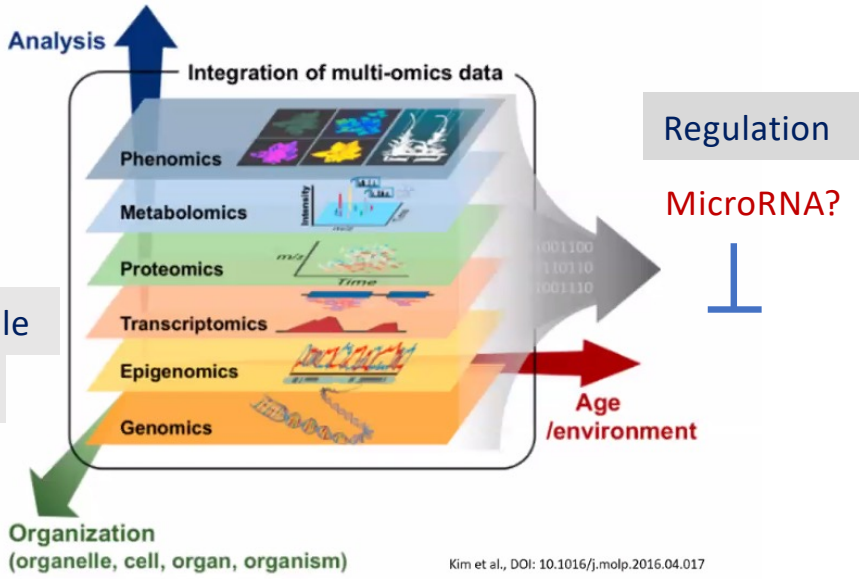
Integration with Omics approaches

Gene expression profile

Methylation portrait

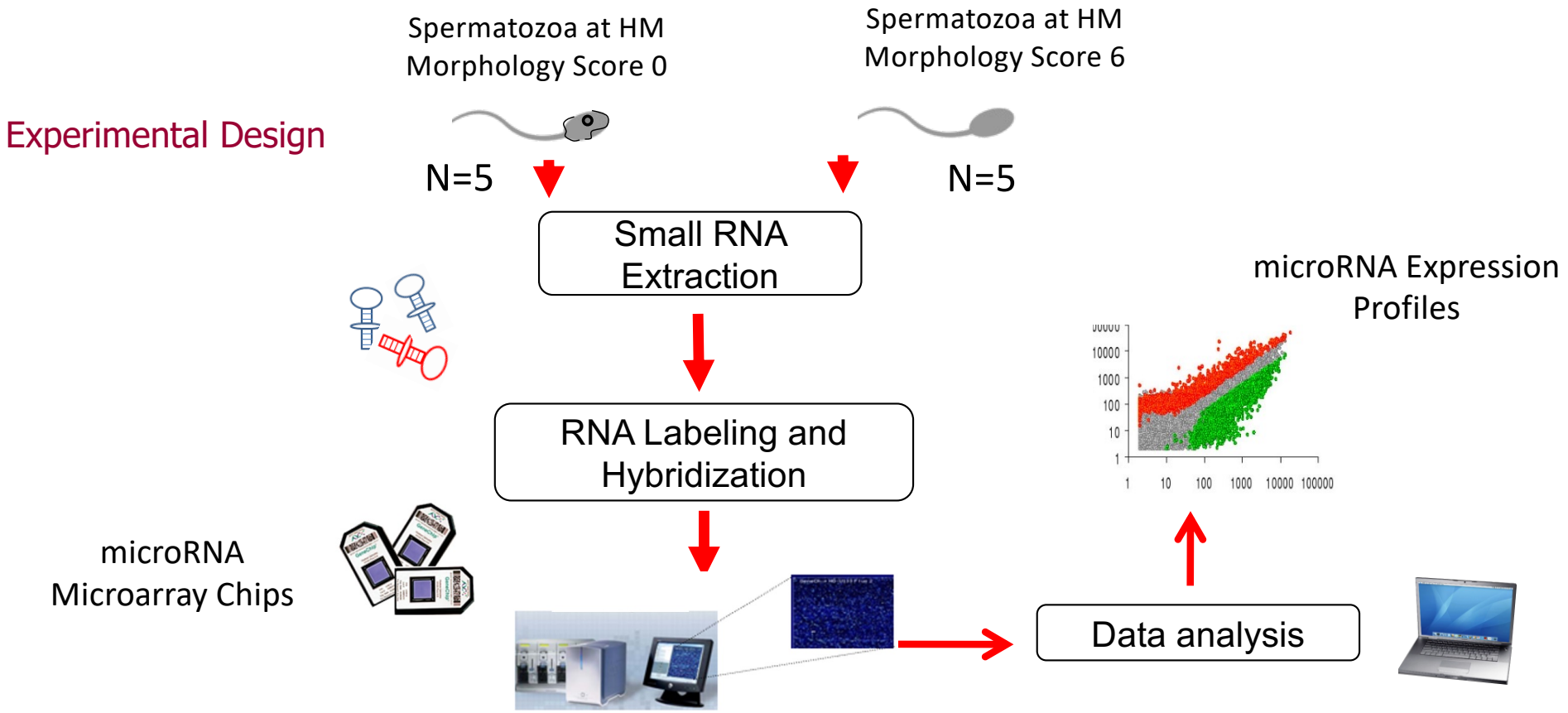
Epigentic pattern

DNA analysis

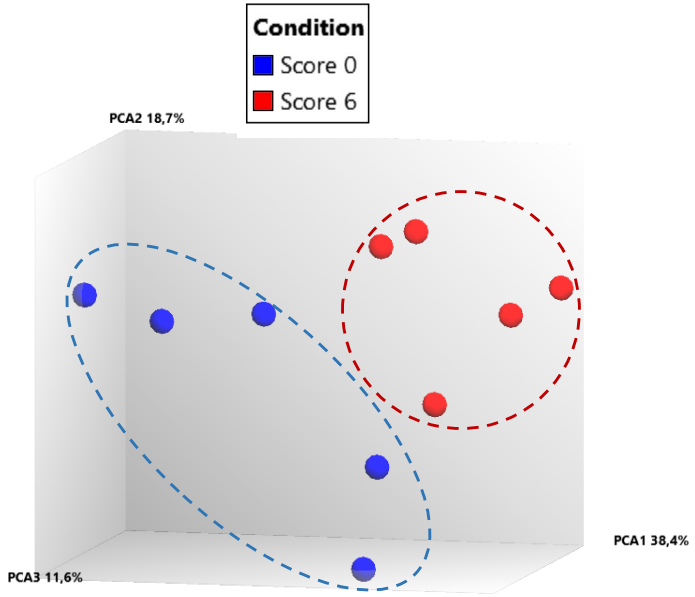


Microarray analysis of microRNA expression patterns in the spermatozoa with good and poor morphology

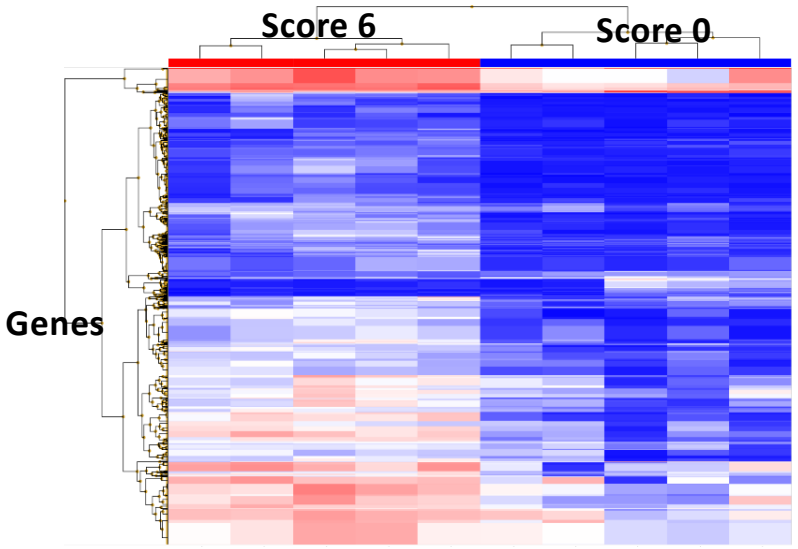
Experimental Design



Differences in the global miRNA expression profiles of sperm samples with good and poor morphology



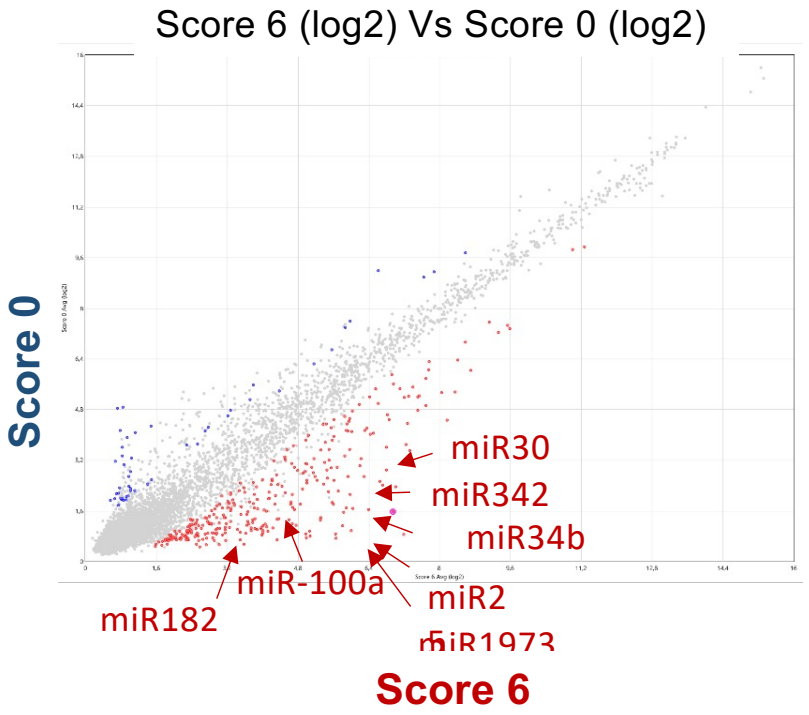
Principal Component Analysis (PCA) in 3-dimensional plots represents the different sample's gene expression patterns (Score 0 and Score 6)
Each dot represents a sample.
10 samples can be divided into 2 distinct groups based on their gene expression profiles



Heat map. Dendrogram

The 5 samples Score 6 clustered in 1 Red branch
The 5 samples Score 0 samples in 1 Blue branch
The molecular signatures of Score 6 and Score 0 were visualized by a hierarchical clustering diagram by the algorithm based on the differential expression genes.
The 10 samples are arranged in columns
Genes are arranged in rows

Differential microRNA expression in Score 6 and Score 0



Two-dimensional Scatter plots representing the top miRNAs that are differentially expressed in Score 6 and Score 0
Each dot represents a sample: Red=Score 6 and Blue= Score 0.

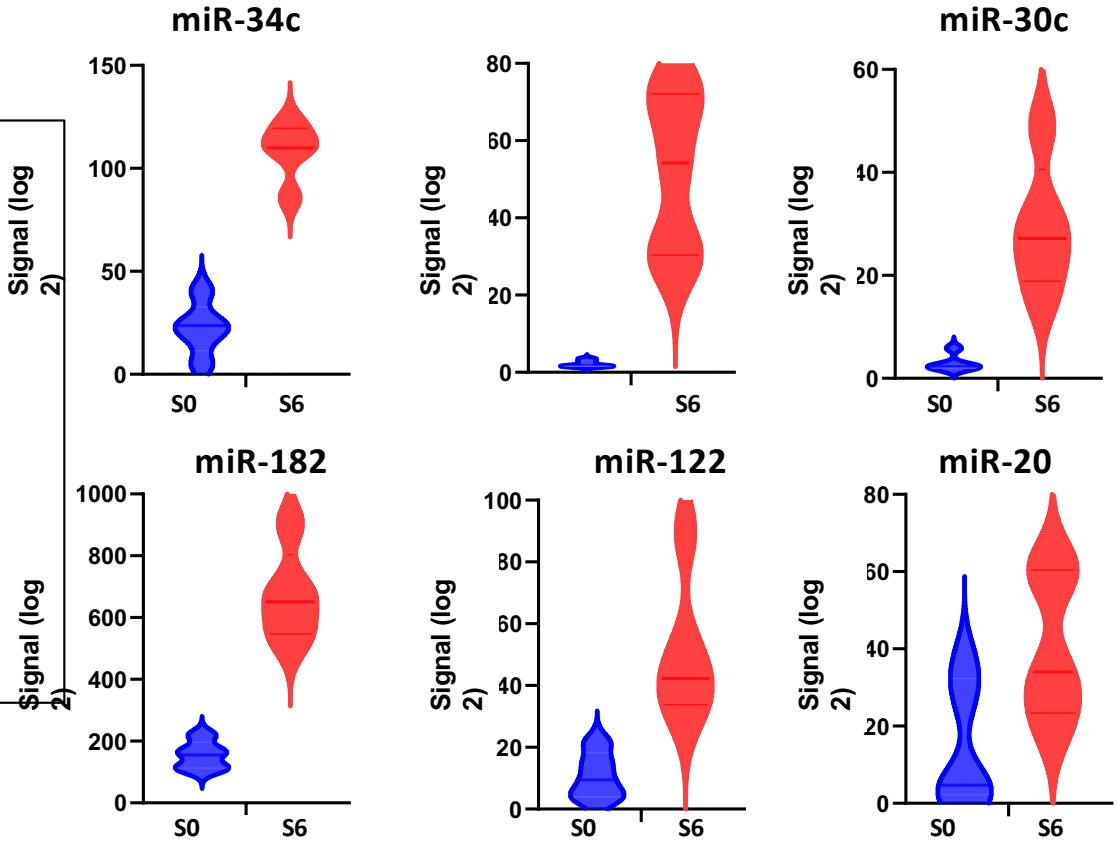
Differential microRNA expression in Score 6 and Score 0

Filter criteria:
Fold Change: > 2 or < -2
P-value: < 0.05

Genes passed filter criteria: 107

Up-Regulated in Score 6:
86 miRNAs

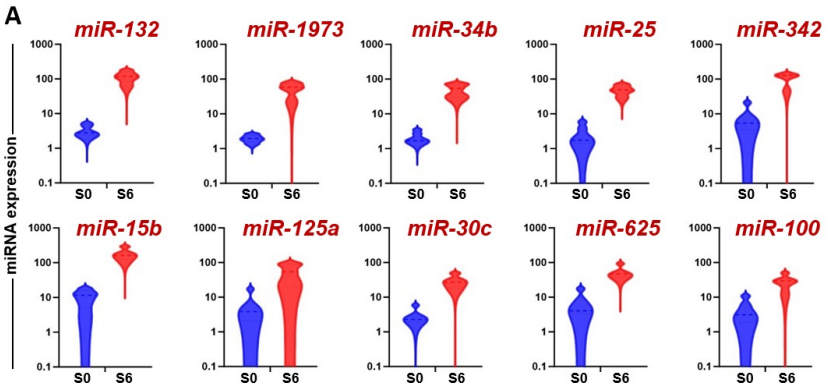
Up-Regulated in Score 0:
21 miRNAs



Violin plot - Box-and-whisker plots – comparing microRNAs that are differently expressed in Score 6 and Score 0
(FC >2 and p-value ≤0.05)

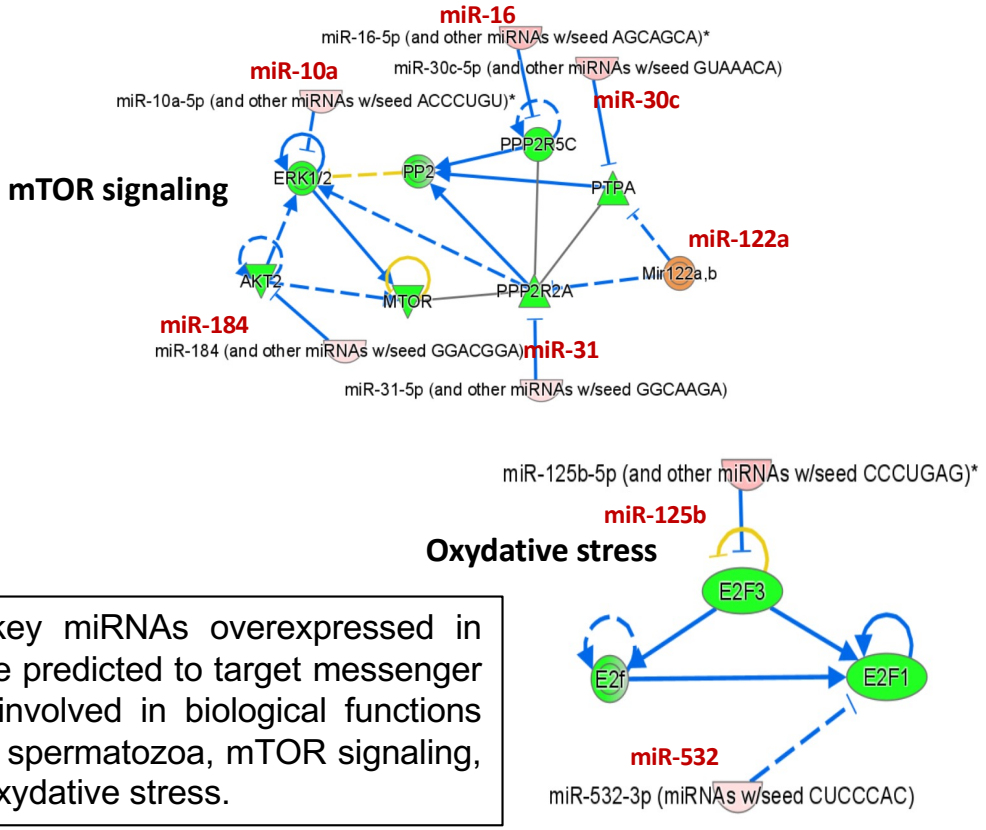
Regulatory roles of the identified miRNAs in S6

Top ten miRNA overexpressed in S6 Vs. S0



Violin plot comparing the top ten microRNAs that are differently expressed in Score 6 (n = 5) and Score 0 samples (n = 5).

Potential S6-miRNA targets involved in sperm quality



The key miRNAs overexpressed in S6 are predicted to target messenger RNA involved in biological functions of the spermatozoa, mTOR signaling, and oxydative stress.

ASRM 2023
The Past, The Present, and The Pipeline
New Orleans, Louisiana
October 14-18, 2023

Human sperm microRNA profiling correlated with head morphology

NG. CASSUTO [1], **L. RUOSO** [1], **D. BOURET** [1], **C. CHAO** [1], **N. LEDEE** [2], **L. PRAT-ELLENBERG** [1], **S. ASSOU** [3]
1 ART Unit Drouot Laboratory, Paris, France
2 Bluets Hospital, Paris, France
3 IRMB, University f Montpellier, INSERM, Montpellier, France

New players and new links who interfere in the sperm quality

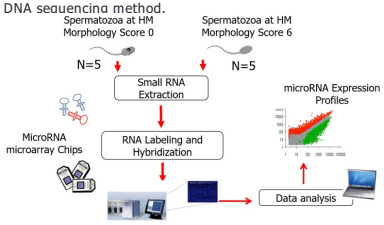
PURPOSE & OBJECTIVES

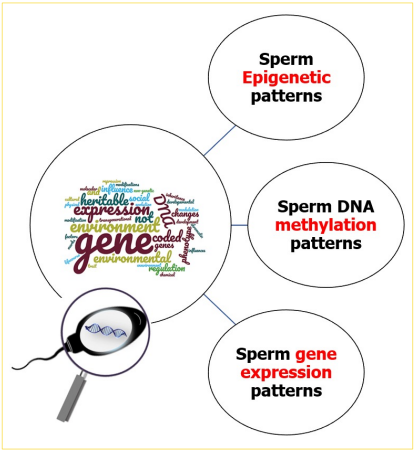
Male infertility research to understand the physiology of sperm has improved and more sophisticated techniques to identify high quality sperm function. We previously published a scoring scale where the spermatozoa with a total score of 6 points (Good spermatozoon morphology) displays normal head shape with symmetrical nuclear no extrusion and/or no invagination of the nuclear membrane, without any vacuole and normal base. The spermatozoa with a score 0 displays a nuclear-shape disorder with an abnormal base and at least one large vacuole (Bad spermatozoon). The objective of this study is to analyze microRNA (miRNA) profiles according to different morphological scoring (score 6 versus score 0) and to identify the keys regulators involved in sperm quality.

MATERIAL & METHODS

Using the GeneChip miRNA array technology, we dissected the miRNAome of ten semen samples scored according to their high-magnification morphology (score 6 versus score 0). The correlation between spermatozoa-miRNAs and their corresponding mRNA targets was analyzed using in silico prediction algorithms. The spermatozoa methylome was studied in score 6 samples using DNA sequencing method.

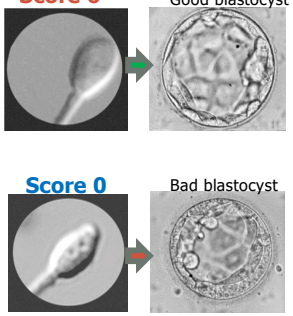
➢ Experimental design:





Morphology at High Magnification

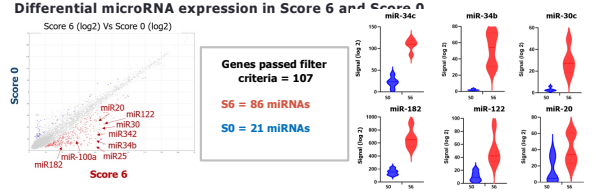
MiRNAomes



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**

RESULTS

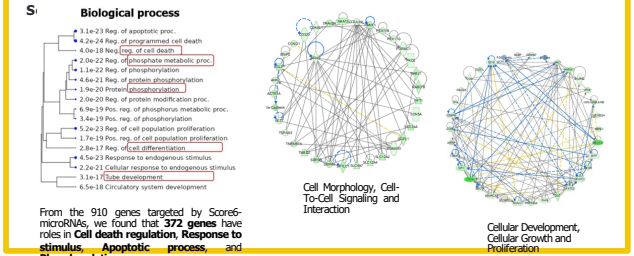
➢ **Differential microRNA expression in Score 6 and Score 0**



Two-dimensional Scatter plots representing the top miRNAs that are differentially expressed in Score 6 and Score 0. Each dot represents a sample: Red=Score 6 and Blue= Score 0

Violin plot - Box-and-whisker plots – comparing microRNAs that are differently expressed (FC >2 and p-value ≤0.05) in Score 6 (n = 5) and Score 0 samples (n = 5)

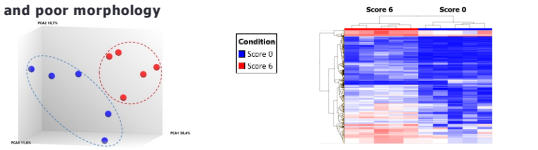
➢ **Target prediction and functional annotations of overexpressed miRNAs in S**



From the 910 genes targeted by Score6-microRNAs, we found that **372 genes** have roles in **Cell death regulation, Response to stimulus, Apoptotic process,** and **Phosphorylation**

RESULTS

➢ **Differences in the global miRNA expression profiles of sperm samples with good and poor morphology**



Principal Component Analysis (PCA) in 3-dimensional plots represents the different sample's gene expression patterns (Score 0 and Score 6). Each dot represents a sample. 10 samples can be divided into two distinct groups based on their gene expression profiles

The molecular signatures of Score 6 and Score 0 were visualized by a hierarchical clustering diagram based on the differential expression genes.
- The 5 samples Score 6 clustered in 1 Red branch
- The 5 samples Score 0 samples in 1 Blue branch

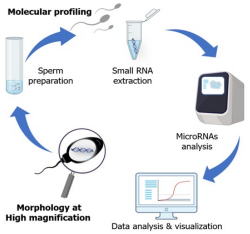
CONCLUSIONS

This study confirms the links between different **OMICS patterns**; like **epigenome**, **methylome**, **transcriptome**, **miRNAome**, and sperm head morphology at high magnification.

Today only one tool; the morphology permit in real-time to avoid the wrong epigenetic spermatozoa profile before injection in ICSI.

Reveals that microRNA profiling of good spermatozoa morphology may provide **biomarkers reflecting sperm function** to investigate its quality.

This opens new perspectives to explore male infertility; in addition to basic semen analysis information with **new diagnostic tests** particularly during ART procedures



REFERENCES

*Cassuto N.G et al. Biomed Res Int. 2021 Sep 18;2021:1434546 (PMID: 34604380). *Cassuto N.G et al. Biomed Res Int. 2016;2016:6372171 (PMID: 27148551). *Cassuto N.G et al. Reprod Biomed Online. 2012 Feb;24(2):211-8 (PMID: 22227364). *Cassuto N.G et al. Fertil Steril. 2009 Nov;92(5):1616-25 (PMID: 18990

ACKNOWLEDGEMENTS

We want to thank the patients for participating in this study. We would like to thank the ART team for their assistance during this study.

CONTACT INFORMATION

ART Unit, Drouot Laboratory, Paris, France
Mail: guycassuto@labodrouot.com

ESHRE 40th Annual Meeting 2024

Genome-wide microRNA expression profiling in human spermatozoa and its relation to sperm quality

NG. CASSUTO [1], L. RUOSO [1], G. KEROMNES [2], L. PRAT-ELLENBERG [3], N. LEDEE [4], C. CHAO [5], H. MOUIK [6], S. ASSOU [7]

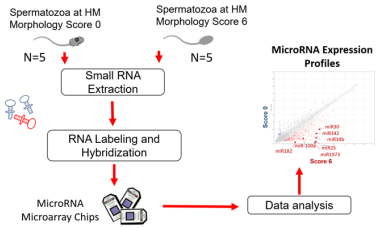
[1] ART Unit Drouot Laboratory, Paris, France, [2] Diaconesses Hospital, Île-de-France, Paris, France, [3] ART Bluets Hospital, Île-de-France, Paris, France, [4] Matricelab innove, Île-de-France, Paris, France, [5] Centre hospitalier de Saint-Denis, Saint Denis, Paris France, [6] University of Hassan II, Casablanca, Morocco, [7] IRMB, University of Montpellier, INSERM, Montpellier, France

➢ Background

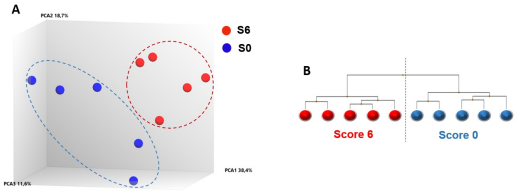
We previously introduced a strict scoring scale for the morphology of spermatozoa, where a total score of 6 points indicates good morphology. This is characterized by a normal head shape with symmetrical nuclei, no extrusion or invagination of the nuclear membrane, no vacuoles, and a normal base. Such spermatozoa are capable of reaching an expanded blastocyst stage. Conversely, spermatozoa scoring 0 exhibit nuclear shape abnormalities, an abnormal base, and at least one large vacuole, categorizing them as poor quality. This study aims to analyze microRNA (miRNA) profiles according to different morphological scoring (score 6 versus score 0) and identify the key regulators involved in sperm quality.

➢ Experimental design:

We used a miRNA microarray chip to analyze the miRNA profile (Score 6 Vs Score 0). We only consider miRNA profiles that have a Fold change>2 and p-value ≤ 0.05.

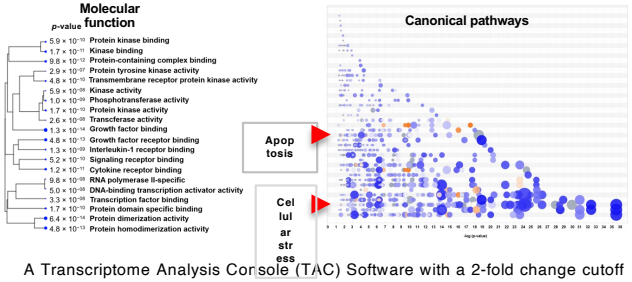


➢ Differences in the global miRNA expression profiles of sperm samples on S6 Vs. S0



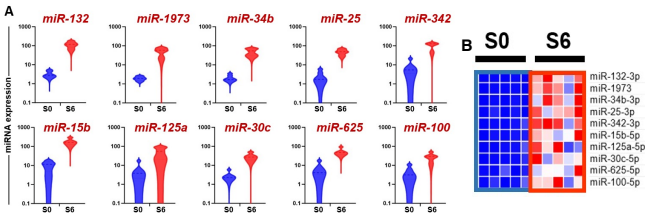
(A) Principal Component Analysis (PCA) in 3-dimensional plots represents the different miRNA expression patterns from the 10 samples. Two distinct groups were obtained based on their miRNA expression profiles. (B) The dendrogram shows that the 5 samples S6 clustered in 1 Red branch. The 5 samples S0 samples in 1 Blue branch.

➢ Functional properties of the 86 miRNAs significantly over-expressed in the S6



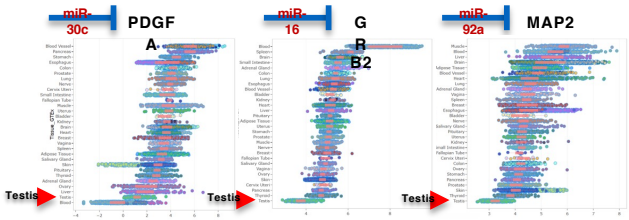
A Transcriptome Analysis Console (TAC) Software with a 2-fold change cutoff and FDR <5% identified **107** differentially expressed miRNA that significantly distinguished S6 from S0 samples. Among which only **21** miRNAs were upregulated in the S0 samples, whereas the number of known miRNAs increased to **86** in the S6 samples. The miRNAs overexpressed in Score 6 are predicted to target **910** messenger RNA involved in biological functions of the spermatozoa and several signaling pathways, such as MAPK signaling pathways, as well as cell apoptosis and cellular stress. Whereas genes targeted by score 0-miRNAs were implicated in the regulation of cellular growth and proliferation.

➢ Top ten miRNA overexpressed in S6 Vs. S0



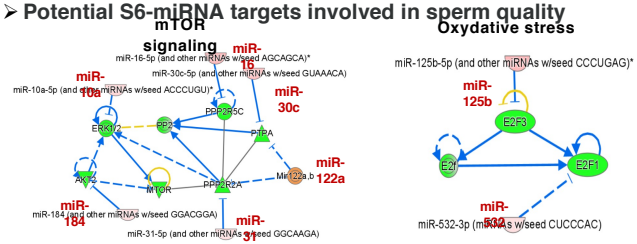
(A) Violin plot comparing the top ten microRNAs that are differently expressed in Score 6 (n=5) and Score 0 samples (n=5). (B) The heat map showed a molecular signature based on the 10 selected microRNAs upregulated in S6 Vs. S0.

➢ Target-miRNAs acquired by score 6 are downregulated in testis



Pathway analysis for the targets common to the S6-miRNAs revealed that key genes in the PDGF, EGF and MAPK signaling pathways, such as *PDGFA* and *GRB2* and *MAP2K* are regulated by miR-30c, miR-16 and miR-92a. The analysis of the expression of these target in normal tissues using RNA-seq data for 30 tissue types from the Genotype-Tissue Expression repository showed that these genes are strongly under-expressed in testis tissue compared to other tissues.

➢ Potential S6-miRNA targets involved in sperm quality

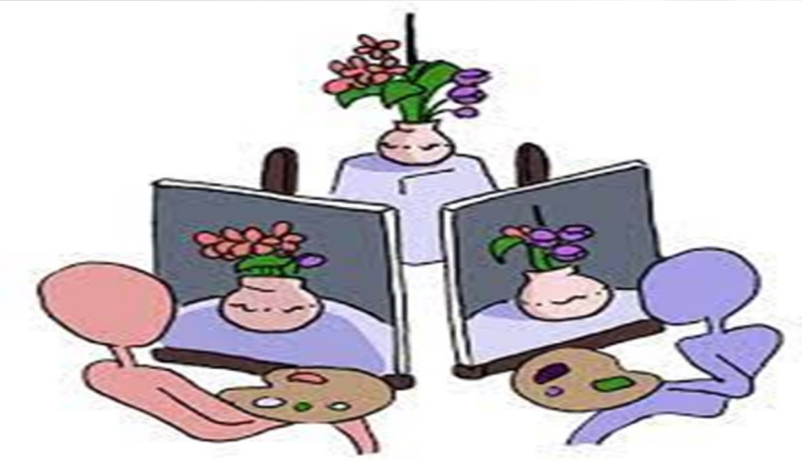


The key miRNAs overexpressed in Score 6 are predicted to target messenger RNA involved in biological functions of the spermatozoa, mTOR signaling, and oxydative stress.

➢ Conclusions

The profiling of miRNAs repertoire in scored spermatozoa morphology opens new perspectives to explore male fertility status and provides a biomarker panel for sperm analysis during the ART procedure.

Artificial Intelligence and Subjectivity



WHAT INFLUENCES SUBJECTIVITY ???

All data we generate remains stored and used for a decision
no operator-dependent

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Human Sperm Selection

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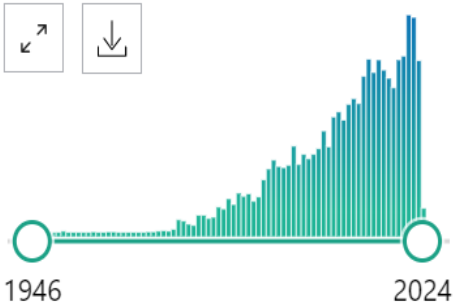
RESULTS BY YEAR



1 **Sperm Selection** for ICSI: Do We Have a Winner?

Cite

Baldini D, Ferri D, Baldini GM, Lot D, Catino A, Vizziello D, Vizziello G.

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Cells. 2021 Dec 17;10(12):3566. doi: 10.3390/cells10123566.

PMID: 34944074 Free PMC article. Review.

In assisted reproductive technology (ART), the aim of **sperm** cells' preparation is to **select** competent **spermatozoa** with the highest fertilization potential and in this context, the intracytoplasmic **sperm** injection (ICSI) represents the most applied tech ...

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Human sperm selection; artificial intelligence

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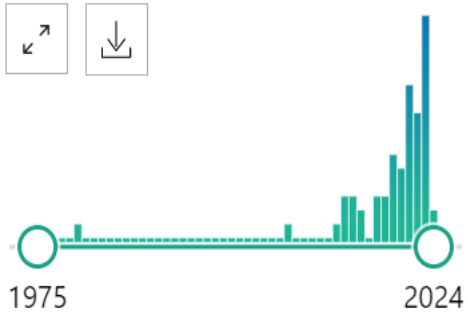
RESULTS BY YEAR



1

Cite

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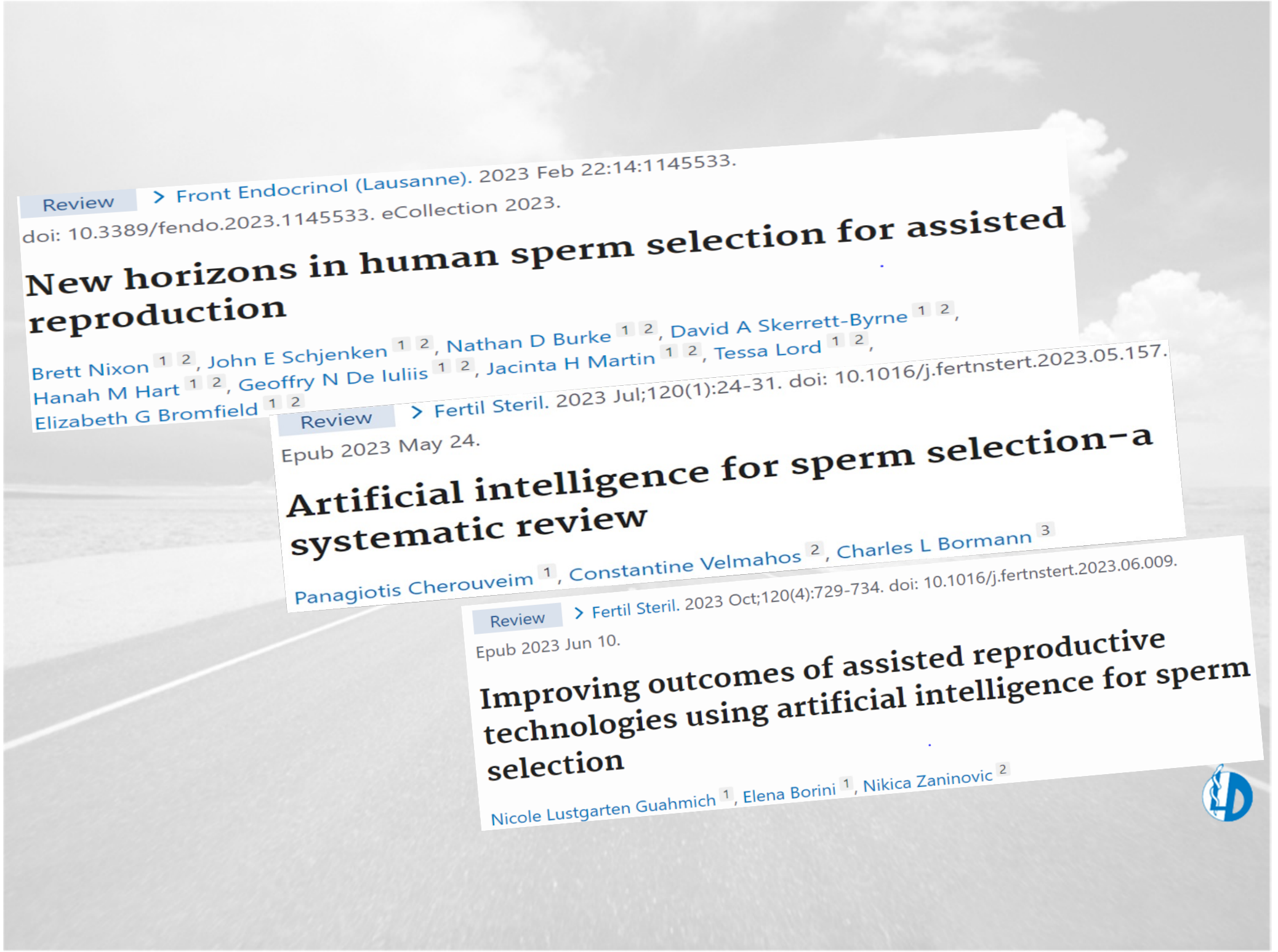
Artificial intelligence for **sperm selection**-a systematic review.

Cherouveim P, Velmahos C, Bormann CL.

Fertil Steril. 2023 Jul;120(1):24-31. doi: 10.1016/j.fertnstert.2023.05.157. Epub 2023 May 24.

PMID: 37236418 Review.

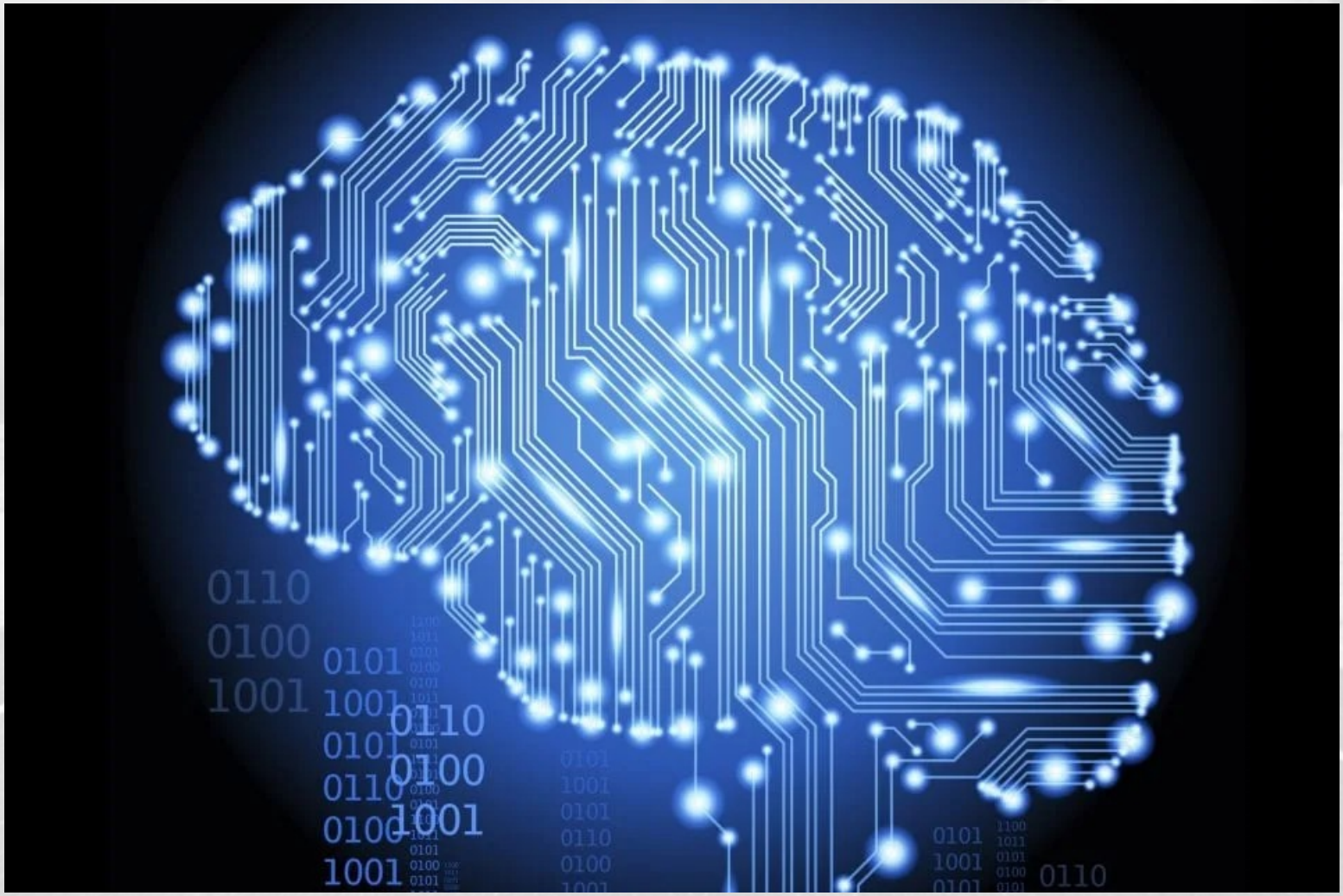
Artificial intelligence algorithms have revolutionized the field of medicine, particularly in image processing, because of their discerning abilities, efficacy, and reproducibility. **Artificial intelligence** algorithms have the potential to address the c ...

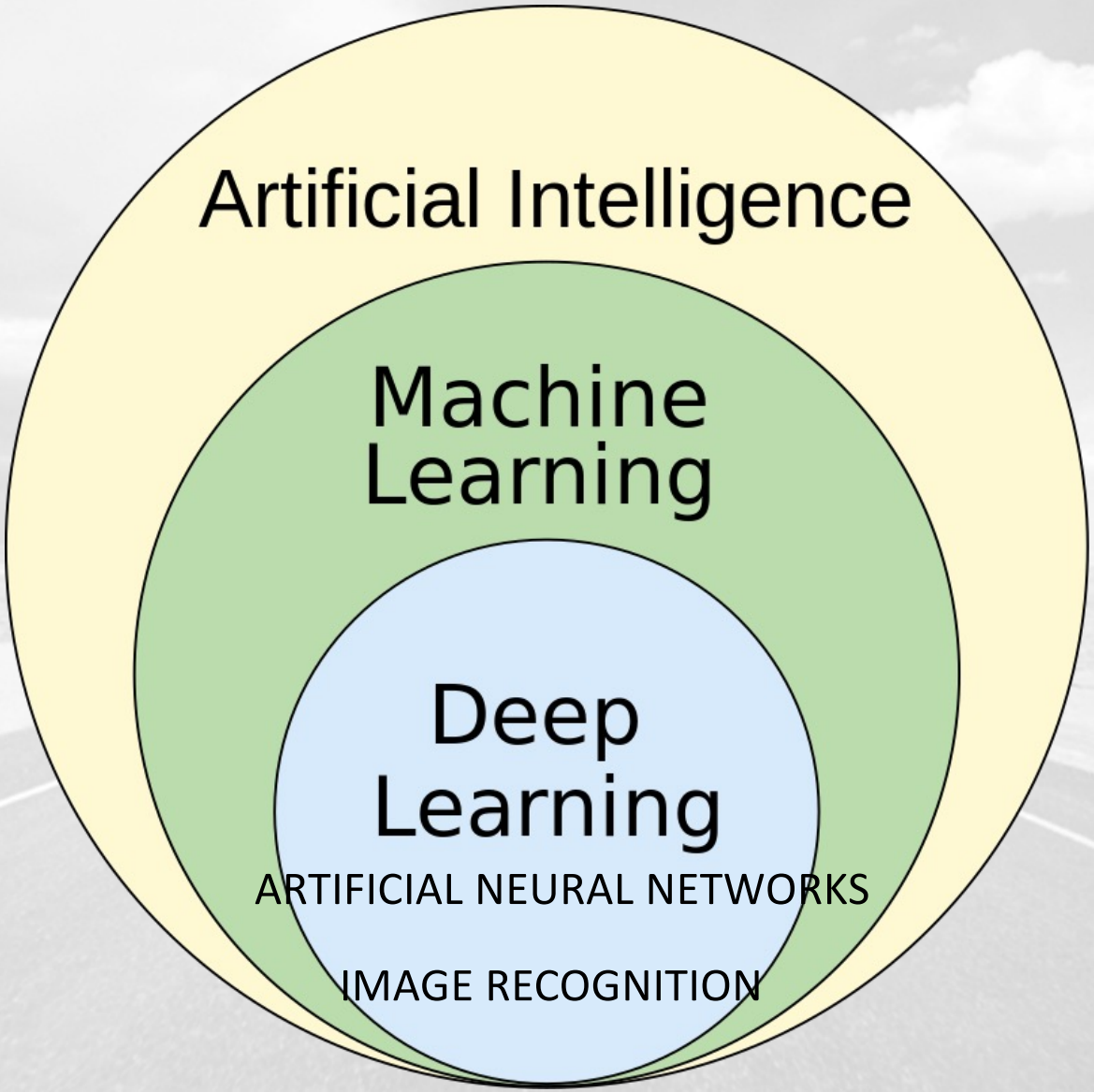

Review
Front Endocrinol (Lausanne). 2023 Feb 22:14:1145533.
doi: 10.3389/fendo.2023.1145533. eCollection 2023.
New horizons in human sperm selection for assisted reproduction
Brett Nixon [1 2], John E Schjenken [1 2], Nathan D Burke [1 2], David A Skerrett-Byrne [1 2], Hanah M Hart [1 2], Geoffry N De Iuliis [1 2], Jacinta H Martin [1 2], Tessa Lord [1 2], Elizabeth G Bromfield [1 2]
Review
Fertil Steril. 2023 Jul;120(1):24-31. doi: 10.1016/j.fertnstert.2023.05.157.
Epub 2023 May 24.
Artificial intelligence for sperm selection–a systematic review
Panagiotis Cherouveim [1], Constantine Velmahos [2], Charles L Bormann [3]
Review
Fertil Steril. 2023 Oct;120(4):729-734. doi: 10.1016/j.fertnstert.2023.06.009.
Epub 2023 Jun 10.
Improving outcomes of assisted reproductive technologies using artificial intelligence for sperm selection
Nicole Lustgarten Guahmich [1], Elena Borini [1], Nikica Zaninovic [2]

All these papers confirm the presence of different sperm epigenetics profiles in the same ejaculate.

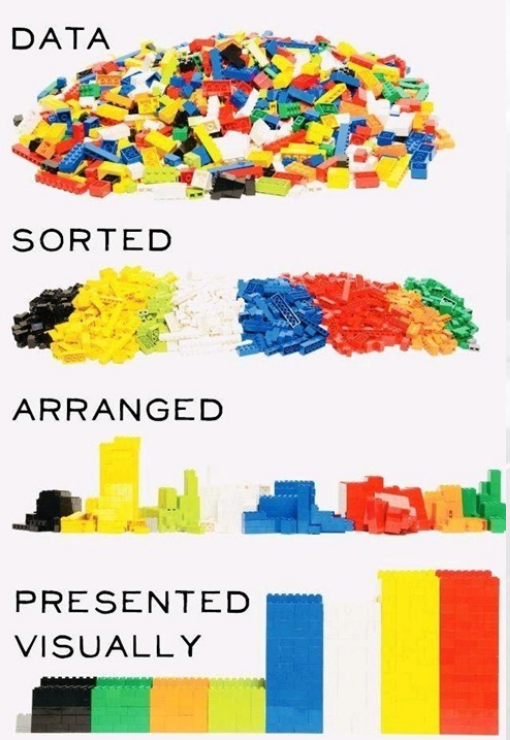
Which tool is today more appropriate, and more accurate than AI to sort, identify, and select them between millions?

What’s AI Technology ?




Artificial Intelligence
Machine Learning
Deep Learning
ARTIFICIAL NEURAL NETWORKS
IMAGE RECOGNITION

AI needs huge data! It's a hungry giant!!!



Data: 800 hours of videos:
More than 3 months in continued vision

Sorted:15 000 frames were randomly captured

Arranged: by embryologist experts labeling and classified

Presented visually on the screen
Machine learning / Deep learning
Teach the machine to do the job

How It Works – AI Classification

AI classifies sperm cells' morphology at high magnification

- **Proprietary algorithm processes** the video stream of "live" sperm in real time
- **Autonomously classifies sperm** cells based on their morphology & motility at high-magnification (×6,100) **30 frames /Seconds**
- **Proprietary BAIBYS' algorithm controls** the motorized X-Y stage in real time to maintain the selected sperm cell in the middle of the field of view to allow reviewing the cell from all sides

BAIBYS™

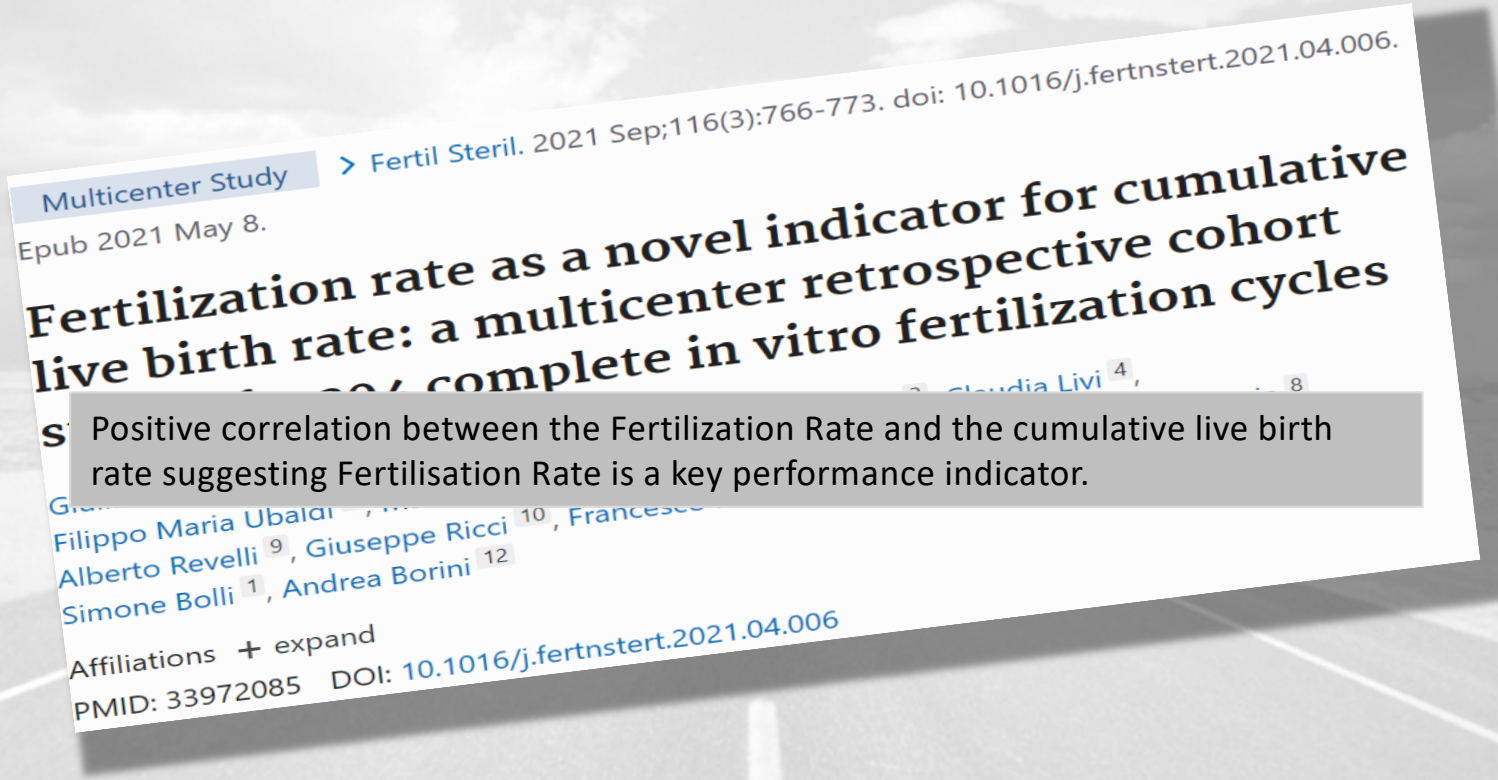
BAIBYS™

HOW IT’S WORK

Control Panel of the system and Codes for all the system generated by AI


Gradient Focus: 29.26
Contrast Focus: 311.05
Current Z: 0.00
Side
Lock ID: 3086
Side
Blue
cell

Fertilization Rate: Novel Indicator for Cumulative Live Birth Rate



agence de la biomédecine

2019

Tableau 5. Indicateurs complémentaires pour votre centre et au niveau national, des tentatives avec embryons frais en FIV et ICSI intraconjugale et avec don de sperme, hors culture prolongée de première intention, DPI, et centres ayant réalisé moins de 50 ponctions hors CP

		National					Funnel plot 1*(Moyenne)		
	Centre	Moyenne	IC 95% de la moyenne	Médiane	Minimum	Maximum	Centres au-dessous du FP	Centres dans le FP	Centres au-dessus du FP
% de ponctions avec congélation de toute la cohorte embryonnaire	17.5%	10.0%	[8.6% ; 11.4%]	8.6%	0.0%	35.8%	10.2%	10.1%	9.0%
% de transfert par ponction, hors congélation de toute la cohorte embryonnaire et ovocytaire	82.6%	77.2%	[74.4% ; 80.1%]	82.5%	0.0%	92.9%	77.1%	77.0%	79.1%
Nombre moyen d'ovocytes recueillis par ponction	8.7	8.1	[7.8 ; 8.4]	8.3	4.2	12.4	8.2	8.0	8.7
Nombre moyen d'embryons obtenus par ponction	4.9	3.8	[3.6 ; 4.0]	4.0	0.3	6.4	3.8	3.7	4.5
% d'ovocytes injectés par ovocyte recueilli en ICSI	70.8%	69.7%	[68.4% ; 71.1%]	70.2%	47.5%	87.9%	70.0%	69.6%	70.6%
% d'embryons obtenus par ovocyte recueilli	57.0%	45.8%	[44.1% ; 47.5%]	46.6%	8.0%	64.9%	46.0%	45.2%	50.3%
% d'embryons obtenus par ovocyte injecté en ICSI	81.8%	61.7%	[59.4% ; 63.9%]	64.1%	7.1%	83.8%	61.0%	61.0%	70.0%
% d'embryons transférés ou congelés par embryon obtenu	52.5%	54.8%	[53.2% ; 56.5%]	54.8%	0.0%	76.2%	53.2%	56.0%	49.1%
% d'embryons congelés par embryon obtenu	25.3%	24.4%	[22.5% ; 26.3%]	24.5%	0.0%	72.1%	25.3%	24.4%	21.9%
% d'embryons transférés par embryon obtenu	27.2%	30.5%	[28.5% ; 32.5%]	27.6%	0.0%	65.5%	27.9%	31.6%	27.2%
Nombre moyen d'embryons transférés par transfert	2.0	1.6	[1.55 ; 1.61]	1.6	1.1	2.1	1.5	1.6	1.7
% de sacs embryonnaires avec activité cardiaque par embryon transféré	21.5%	17.6%	[16.6% ; 18.5%]	17.3%	7.4%	35.3%	15.1%	17.5%	24.3%
% de grossesse échographique par transfert	35.9%	24.4%	[23.1% ; 25.7%]	25.0%	0.0%	44.2%	19.2%	24.6%	36.0%
% de grossesse évolutive par transfert	30.8%	20.6%	[19.5% ; 21.7%]	20.5%	0.0%	36.7%	16.5%	20.8%	29.6%
% de grossesse évolutive par grossesse échographique	85.8%	84.4%	[82.7% ; 86.1%]	85.7%	28.6%	100.0%	86.2%	84.1%	82.9%
% d'accouchements par grossesse évolutive	96.7%	96.2%	[94.4% ; 98.0%]	98.0%	0.0%	100.0%	97.3%	95.9%	96.0%
% d'accouchements par ovocyte recueilli, hors congélation de toute la cohorte embryonnaire et ovocytaire	3.4%	2.1%	[1.9% ; 2.2%]	2.1%	0.0%	4.2%	1.7%	2.1%	2.9%
% d'accouchements par embryon transféré	14.9%	12.7%	[11.9% ; 13.4%]	12.7%	0.0%	26.2%	10.7%	12.7%	17.4%

agence de la biomédecine

Tableau 5. Indicateurs complémentaires pour votre centre et au niveau national, des tentatives 2019 avec embryons frais en FIV et ICSI intraconjugale et avec don de sperme, hors culture prolongée de première intention, DPI, et centres ayant réalisé moins de [illegible] ponctions hors CP

		National					Funnel plot 1*(Moyenne)		
	Centre	Moyenne	IC 95% de la moyenne	Médiane	Minimum	Maximum	Centres au-dessous du FP	Centres dans le FP	Centres au-dessus du FP
% de ponctions avec congélation de toute la cohorte embryonnaire	10.8%	10.0%	[8.6% ; 11.4%]	8.6%	0.0%	35.8%	10.2%	10.1%	9.0%
% de transfert par ponction, hors congélation de toute la cohorte embryonnaire et ovocytaire	76.7%	77.2%	[74.4% ; 80.1%]	82.5%	0.0%	92.9%	77.1%	77.0%	79.1%
Nombre moyen d'ovocytes recueillis par ponction	7.8	8.1	[7.8 ; 8.4]	8.3	4.2	12.4	8.2	8.0	8.7
Nombre moyen d'embryons obtenus par ponction	4.1	3.8	[3.6 ; 4.0]	4.0	0.3	6.4	3.8	3.7	4.5
% d'ovocytes injectés par ovocyte recueilli en ICSI	63.9%	69.7%	[68.4% ; 71.1%]	70.2%	47.5%	87.9%	70.0%	69.6%	70.6%
% d'embryons obtenus par ovocyte recueilli	53.2%	45.8%	[44.1% ; 47.5%]	46.6%	8.0%	64.9%	46.0%	45.2%	50.3%
% d'embryons obtenus par ovocyte injecté en ICSI	83.8%	61.7%	[59.4% ; 63.9%]	64.1%	7.1%	83.8%	61.0%	61.0%	70.0%
% d'embryons transférés ou congelés par embryon obtenu	53.4%	54.8%	[53.2% ; 56.5%]	54.8%	0.0%	76.2%	53.2%	56.0%	49.1%
% d'embryons congelés par embryon obtenu	19.6%	24.4%	[22.5% ; 26.3%]	24.5%	0.0%	72.1%	25.3%	24.4%	21.9%
% d'embryons transférés par embryon obtenu	33.8%	30.5%	[28.5% ; 32.5%]	27.6%	0.0%	65.5%	27.9%	31.6%	27.2%
Nombre moyen d'embryons transférés par transfert	2.1	1.6	[1.55 ; 1.61]	1.6	1.1	2.1	1.5	1.6	1.7
% de sacs embryonnaires avec activité cardiaque par embryon transféré	19.4%	17.6%	[16.6% ; 18.5%]	17.3%	7.4%	35.3%	15.1%	17.5%	24.3%
% de grossesse échographique par transfert	35.4%	24.4%	[23.1% ; 25.7%]	25.0%	0.0%	44.2%	19.2%	24.6%	36.0%
% de grossesse évolutive par transfert	30.0%	20.6%	[19.5% ; 21.7%]	20.5%	0.0%	36.7%	16.5%	20.8%	29.6%
% de grossesse évolutive par grossesse échographique	84.7%	84.4%	[82.7% ; 86.1%]	85.7%	28.6%	100.0%	86.2%	84.1%	82.9%
% d'accouchements par grossesse évolutive	96.4%	96.2%	[94.4% ; 98.0%]	98.0%	0.0%	100.0%	97.3%	95.9%	96.0%
% d'accouchements par ovocyte recueilli, hors congélation de toute la cohorte embryonnaire et ovocytaire	3.1%	2.1%	[1.9% ; 2.2%]	2.1%	0.0%	4.2%	1.7%	2.1%	2.9%
% d'accouchements par embryon transféré	14.0%	12.7%	[11.9% ; 13.4%]	12.7%	0.0%	26.2%	10.7%	12.7%	17.4%

Tableau 5. Indicateurs complémentaires pour votre centre et au niveau national, des tentatives 2020 avec embryons frais en FIV et ICSI intraconjugale et avec don de sperme, hors culture prolongée de première intention, DPI, et centres ayant réalisé moins de 10 ponctions hors CP

		National					Funnel plot 1*(Moyenne)		
	Centre	Moyenne	IC 95% de la moyenne	Médiane	Minimum	Maximum	Centres au-dessous du FP	Centres dans le FP	Centres au-dessus du FP
% de ponctions avec congélation de toute la cohorte embryonnaire	16.3%	12.0%	[10.4% ; 13.7%]	10.9%	0.0%	36.9%	15.5%	11.7%	9.0%
% de transfert par ponction, hors congélation de toute la cohorte embryonnaire et ovocytaire	73.0%	74.7%	[71.6% ; 77.8%]	80.7%	0.0%	94.8%	74.5%	74.4%	79.0%
Nombre moyen d'ovocytes recueillis par ponction	7.19	7.85	[7.5 ; 8.2]	7.84	3.40	13.29	7.94	7.79	8.40
Nombre moyen d'embryons obtenus par ponction	3.65	3.66	[3.4 ; 3.9]	3.61	0.06	6.59	3.70	3.61	4.13
% d'ovocytes injectés par ovocyte recueilli en ICSI	63.8%	68.2%	[66.6% ; 69.8%]	69.8%	27.3%	100.0%	67.5%	68.0%	66.3%
% d'embryons obtenus par ovocyte recueilli	50.8%	45.2%	[43.2% ; 47.2%]	47.9%	1.4%	65.0%	45.7%	44.9%	47.0%
% d'embryons obtenus par ovocyte injecté en ICSI	83.7%	62.1%	[59.7% ; 64.5%]	64.8%	0.0%	83.7%	65.7%	61.2%	65.8%
% d'embryons transférés ou congelés par embryon obtenu	57.5%	55.7%	[54.0% ; 57.4%]	55.9%	0.0%	75.0%	57.2%	55.3%	57.2%
% d'embryons congelés par embryon obtenu	22.1%	25.5%	[23.6% ; 27.5%]	26.5%	0.0%	50.9%	29.0%	25.1%	23.4%
% d'embryons transférés par embryon obtenu	35.5%	30.1%	[28.0% ; 32.3%]	27.7%	0.0%	68.2%	28.3%	30.2%	33.8%
Nombre moyen d'embryons transférés par transfert	2.18	1.55	[1.51 ; 1.59]	1.57	1.00	2.18	1.53	1.54	1.67
% de sacs embryonnaires avec activité cardiaque par embryon transféré	15.0%	17.3%	[16.1% ; 18.5%]	16.9%	0.0%	37.2%	12.2%	17.9%	21.5%
% de grossesse échographique par transfert	27.4%	23.6%	[22.1% ; 25.1%]	23.9%	0.0%	44.1%	16.9%	24.2%	31.4%
% de grossesse évolutive par transfert	24.4%	19.8%	[18.5% ; 21.0%]	20.1%	0.0%	38.4%	13.7%	20.3%	26.3%
% de grossesse évolutive par grossesse échographique	89.2%	84.3%	[82.4% ; 86.3%]	85.8%	33.3%	100.0%	83.3%	84.4%	85.8%
% d'accouchements par grossesse évolutive	100.0%	97.3%	[96.2% ; 98.4%]	100.0%	55.6%	100.0%	91.8%	98.2%	99.0%

Conclusions

Morphology is correlated with the DNA quality;

- ☑ Sperm selection before ICSI is a crucial step;

  because we are at the beginning of the process.

- ☑ Autonomous sperm selection system;

  based on DNA quality and epigenetic profiles.

- ☑ Using AI combined with Micro Robotic.