XXXVIII
SABATO DELL'ANDROLOGIA

COLLOQUI IN PMA TRA GINECOLOGI, BIOLOGI E ANDROLOGI

17 FEBBRAIO 2018

PADERNO DUGNANO

Clinica San Carlo - Via Ospedale, 21
(Auditorium del Nuovo Ospedale)

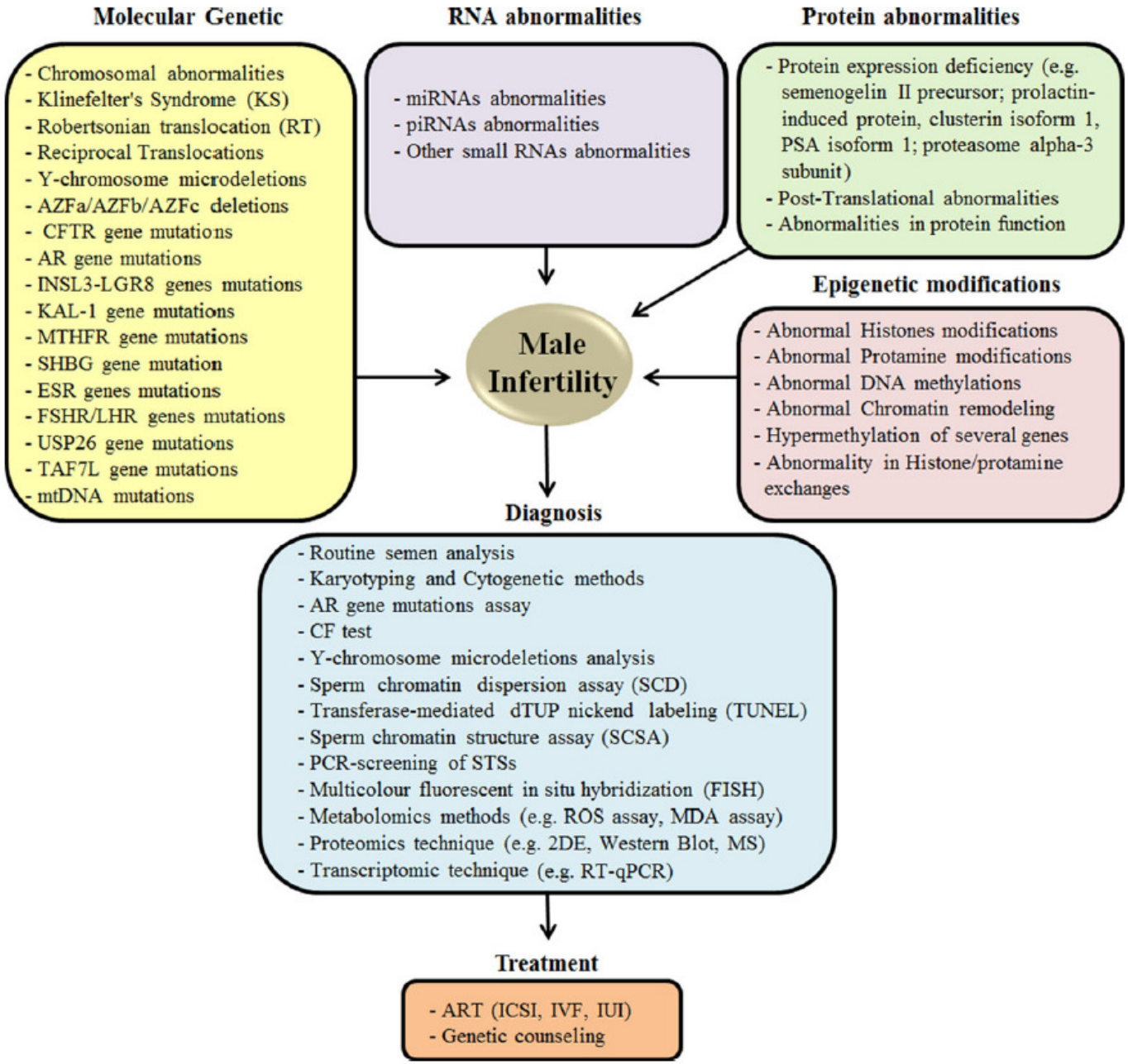
I SESSIONE - L'ANDROLOGO PARLA AL GINECOLOGO E AL BIOLOGO

MODERATORI: **G. Franco, M. Grasso**

10:00 Il ruolo del genoma maschile nella riuscita della PMA
E. Caroppo

ASLBA

Dipartimento Materno-Infantile
U.O. Fisiopatologia della Riproduzione Umana e P.M.A.
PTA «F Jaia», Conversano (BA)


Molecular Genetic
- Chromosomal abnormalities
- Klinefelter's Syndrome (KS)
- Robertsonian translocation (RT)
- Reciprocal Translocations
- Y-chromosome microdeletions
- AZFa/AZFb/AZFc deletions
- CFTR gene mutations
- AR gene mutations
- INSL3-LGR8 genes mutations
- KAL-1 gene mutations
- MTHFR gene mutations
- SHBG gene mutation
- ESR genes mutations
- FSHR/LHR genes mutations
- USP26 gene mutations
- TAF7L gene mutations
- mtDNA mutations
RNA abnormalities
- miRNAs abnormalities
- piRNAs abnormalities
- Other small RNAs abnormalities
Protein abnormalities
- Protein expression deficiency (e.g. semenogelin II precursor; prolactin-induced protein, clusterin isoform 1, PSA isoform 1; proteasome alpha-3 subunit)
- Post-Translational abnormalities
- Abnormalities in protein function
Epigenetic modifications
- Abnormal Histones modifications
- Abnormal Protamine modifications
- Abnormal DNA methylations
- Abnormal Chromatin remodeling
- Hypermethylation of several genes
- Abnormality in Histone/protamine exchanges
Male Infertility
Diagnosis
- Routine semen analysis
- Karyotyping and Cytogenetic methods
- AR gene mutations assay
- CF test
- Y-chromosome microdeletions analysis
- Sperm chromatin dispersion assay (SCD)
- Transferase-mediated dTUP nickend labeling (TUNEL)
- Sperm chromatin structure assay (SCSA)
- PCR-screening of STSs
- Multicolour fluorescent in situ hybridization (FISH)
- Metabolomics methods (e.g. ROS assay, MDA assay)
- Proteomics technique (e.g. 2DE, Western Blot, MS)
- Transcriptomic technique (e.g. RT-qPCR)
Treatment
- ART (ICSI, IVF, IUI)
- Genetic counseling

Prevalence of genetic abnormalities in infertile men

A multi-faceted approach to understanding male infertility: gene mutations, molecular defects and assisted reproductive techniques (ART)

Eisa Tahmasbpour · Dheepa Balasubramanian · Ashok Agarwal

Table 2 Prevalence and phenotype of the most common genetic anomalies associated with male infertility

Genetic abnormalities	Phenotype	Prevalence (%)	References
Chromosome aberrations	Azoospermia to normospermia	2–10 %	[1]
Numerical disorders			
Klinefelter's syndrome	Azoospermia to severe oligospermia	5–10 % azoospermia 2–5 % oligospermia	[1]
Other sex chromosomes	Azoospermia to normospermia	0.1–0.2 %	[20]
Structural disorders			
Robertsonian translocations	Azoospermia to severe oligospermia	0.5–1 %	[20]
Reciprocal translocations	Azoospermia to severe oligospermia	0.5–1 %	[32]
Y chromosome deletions and microdeletions	Azoospermia to severe oligospermia	5–10 %	[226]
AZFa	Azoospermia to SCOS	0.5–1 %	[34]
AZFb	Azoospermic to arrest of spermatogenesis	0.5–1 %	[34]
AZFc	Azoospermia to severe oligospermia	3–7 %	[20]
AZFb,c	SCOS to arrest of spermatogenesis	0.5–1 %	[34]
Partial deletions of AZFc	Azoospermia to normozoospermia	3–5 %	[34]
Genetic mutations			
CFTR	Obstructive azoospermia	4–5 %	[20]
AR	Azoospermia to oligospermia	2–3 %	[82]
KAL-1	Hypogonadism hypogonadotropic	5 %	[18]
INSL3-LGR8	Cryptorchidism	4–5 %	[227]

J Assist Reprod Genet 2014; 31: 115-37

Chromosomal abnormalities in 1663 infertile men with azoospermia: the clinical consequences
Donker RB, Vioeberghs V, Groen H, Tournaye H, Van Ravenswaaij-Arts CMA, Land JA.
Hum Reprod 2017; 32: 2574-80 (December)

- 1663 pz with azoospermia
- 14,4% with chromosomal abnormalities

	Normogonadotropic (FSH<10)	Hypergonadotropic (FSH>10)
Chromosomal abnormalities	4,9%	20.2%
Klinefelter		83%

	Number of pz needed to be screened to identify one man with chromosomal abnormalities
Increased risk of absent spermatogenesis	72
Prevent one miscarriage	370-739
Prevent one child with congenital malformations	4751-23757

Chromosomal polymorphisms and IVF/ICSI outcome

[a]Infertile couples with male chromosomal polymorphisms only.
[b]Infertile couples with female chromosomal polymorphisms only.
[c]Infertile couples without chromosomal polymorphisms.

Effect of chromosomal polymorphisms of different genders on fertilization rate of fresh IVF–ICSI embryo transfer cycles

Ji Liang, Yongsheng Zhang, Yang Yu, Wentao Sun, Jili Jing, Ruizhi Liu

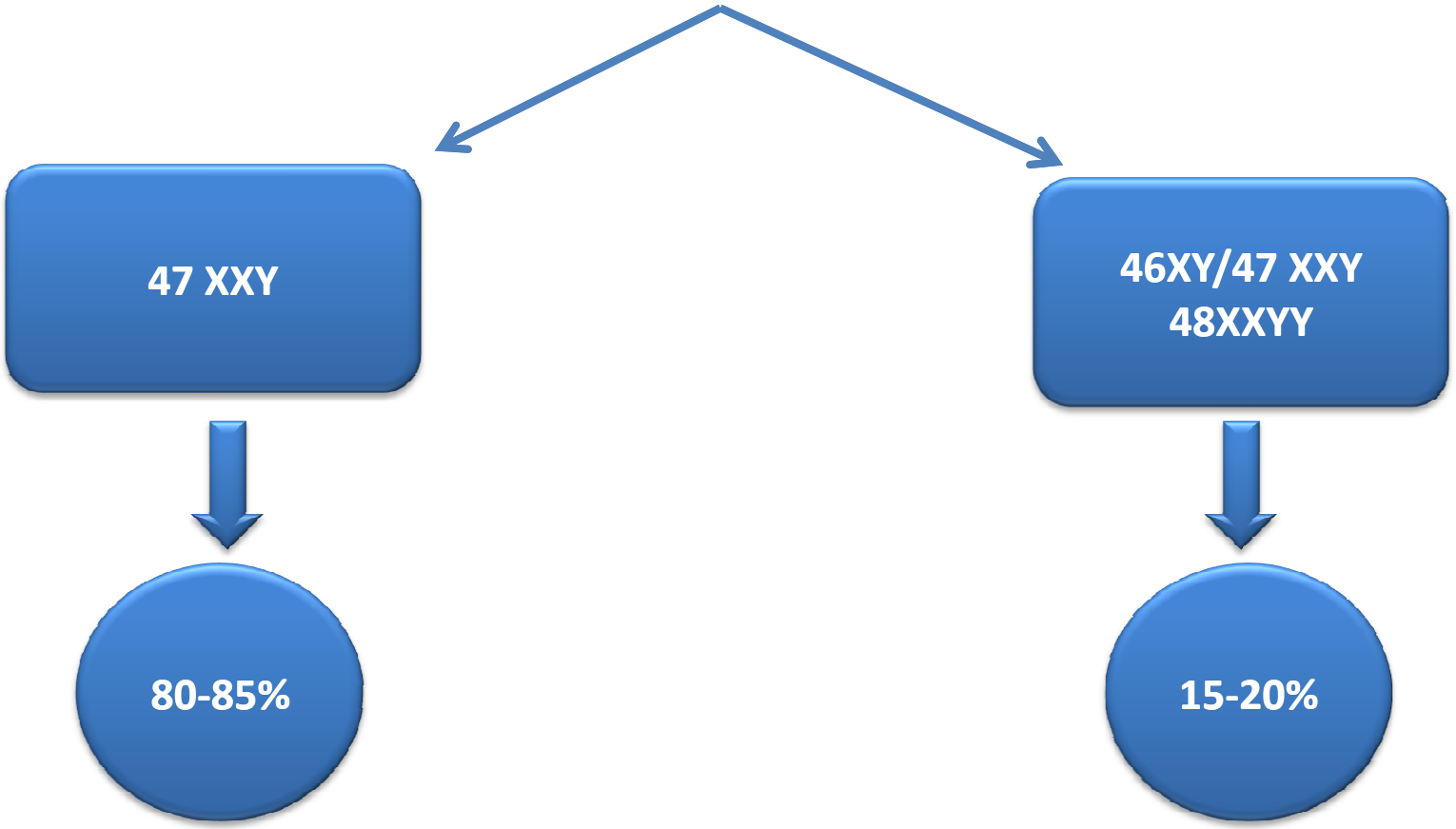
Table 3 Comparison of the outcomes of fresh IVF–ICSI-embryo transfer cycles among three groups.

Outcomes n *(%)*	*Group 1*[a]	*Group 2*[b]	*Group 3*[c]	*P-value*
Fertilization rate (fertilized oocytes/inseminated oocytes)	106/187 (56.68)[h]	142/182 (78.02)	198/276 (71.74)	<0.001[b]
Cleavage rate (cleaved zygotes/fertilized oocytes)	102/106 (96.23)	135/142 (95.07)[i]	197/198 (99.49)[i]	0.013[d]
Good quality embryo rate (good quality embryos/cleaved zygotes)	35/102 (34.31)	58/135 (42.96)	94/197 (47.72)	NS[e]
Pregnancy rate (positive beta HCG/fresh embryo transfer cycles)	8/20 (40.00)	12/17 (70.59)	10/19 (52.63)	NS[e]
Biochemical pregnancy rate (biochemical pregnancies/positive pregnancies)	1/8 (12.50)	1/12 (8.33)	3/10 (30.00)	NS[e]
Clinical pregnancy rate (clinical pregnancies/fresh embryo transfer cycles)	7/20 (35.00)	11/17 (64.71)	7/19 (36.84)	NS[e]
Early miscarriage rate (early miscarriages/clinical pregnancies)	1/7 (14.29)	1/11 (9.09)	1/7 (14.29)	NS[d]
Ongoing pregnancy rate (ongoing pregnancies/fresh embryo transfer cycles)	6/20 (30.00)[f]	10/17 (58.82)[g]	4/19 (26.32)	NS[d]
Live birth rate (live births/fresh embryo transfer cycles)	5/20 (25.00)	9/17 (52.94)	5/19 (26.32)	NS[d]

Reproductive BioMedicine Online (2014) **29**, 436–444

Klinefelter syndrome

- 152-223/100.000 soggetti di sesso maschile



Eur J Endocrinol 2014; 171: R67-77

Euploid sperm in patients with KS!

Human Reproduction, Vol.26, No.12 pp. 3486–3493, 2011
Advanced Access publication on October 20, 2011 doi:10.1093/humrep/der351

human reproduction ORIGINAL ARTICLE *Reproductive genetics*

Hidden mosaicism in patients with Klinefelter's syndrome: implications for genetic reproductive counselling†

L. Garcia-Quevedo[1], J. Blanco[1], Z. Sarrate[1], V. Català[2], L. Bassas[3], and F. Vidal[1,*]

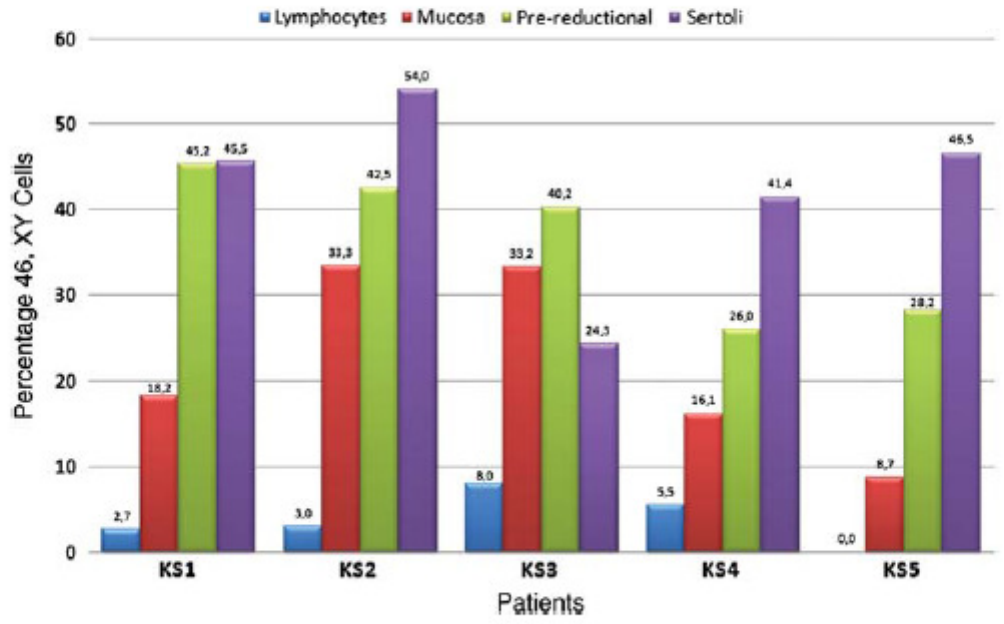
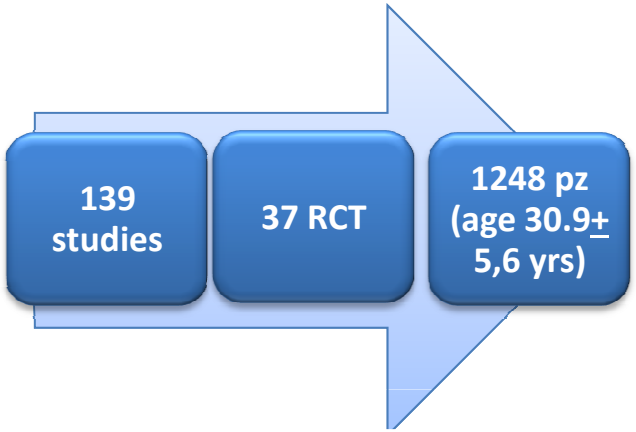


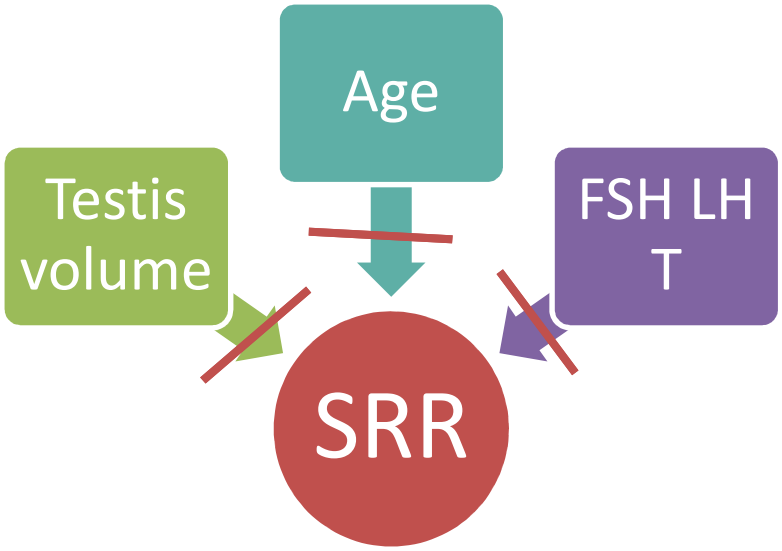
Figure 3 Percentage of 46,XY cells in five patients with KS.

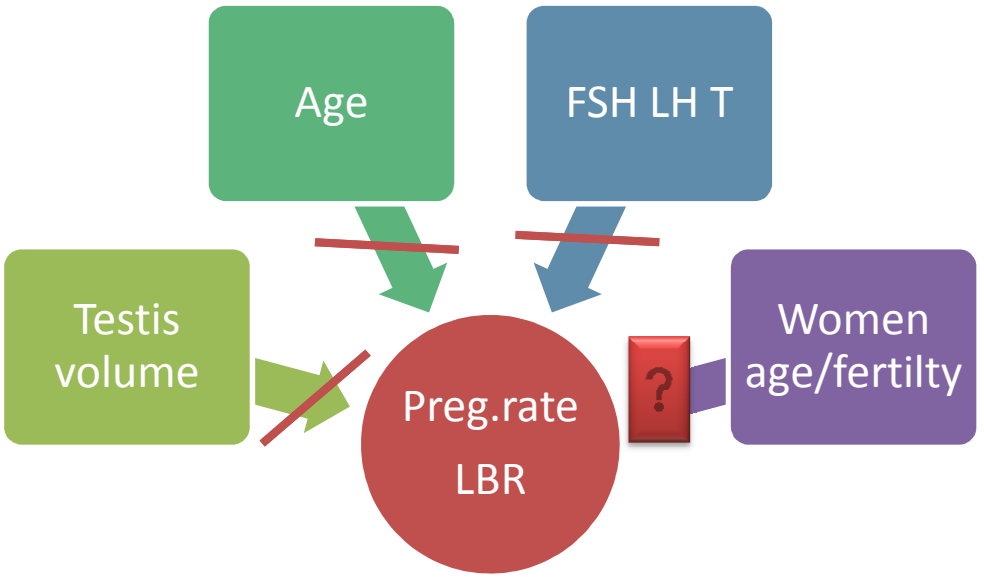
Sperm recovery and ICSI outcomes in Klinefelter syndrome: results from a systematic review and meta-analysis



	cTESE	mTESE
SRR	43%	45%

Pregnancy rate	43%
Live Birth Rate	43%





Hum Reprod Update 2017; 23: 265-75

Microtese seems to perform better than tese in KS patients

Klinefelter syndrome: an argument for early aggressive hormonal and fertility management

Akanksha Mehta, M.D., and Darius A. Paduch, M.D., Ph.D.
Department of Urology, Weill Cornell Medical College, New York, New York

Fertility and Sterility® Vol. 98, No. 2, August 2012

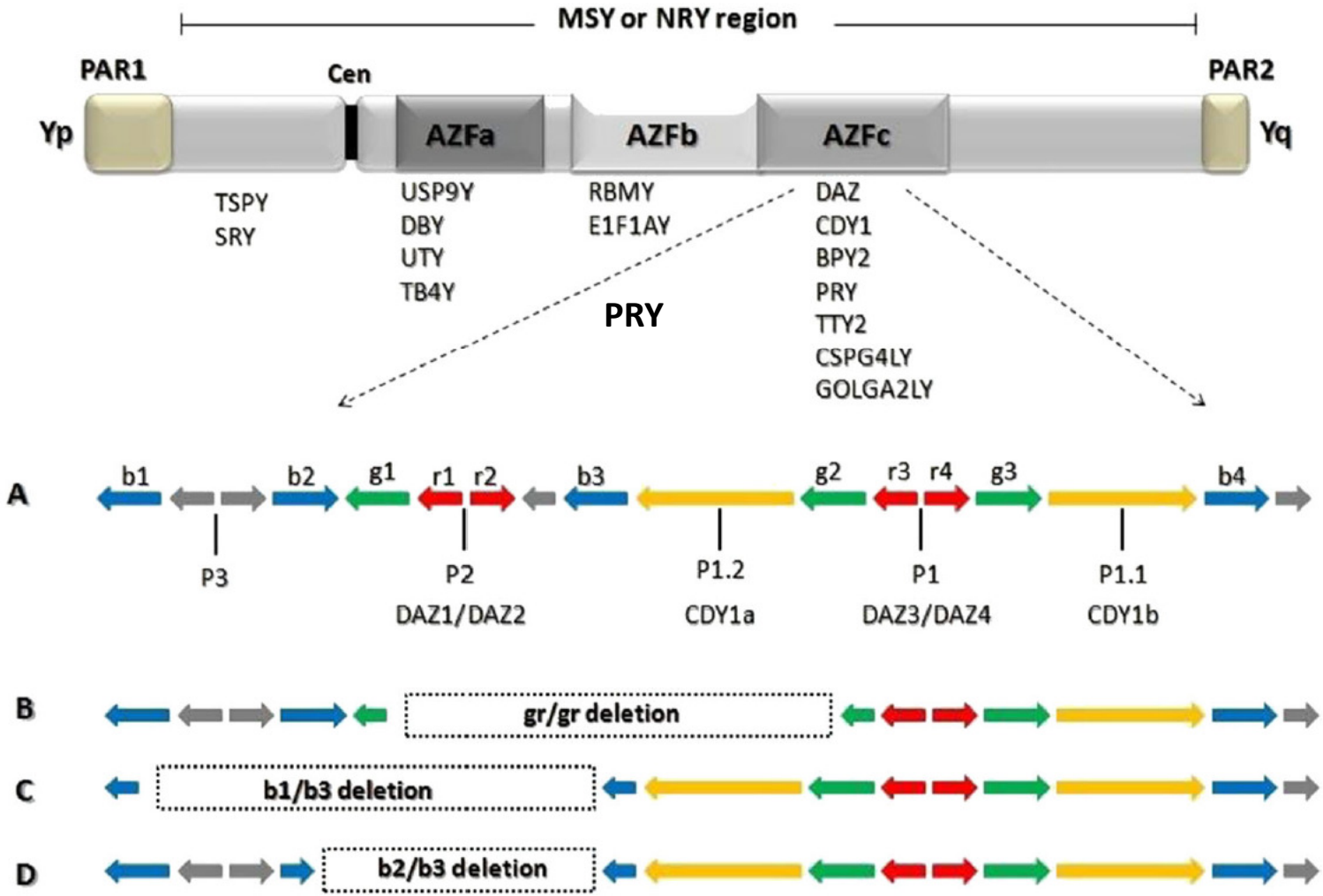
	TESE	mTESE	P
SRR	88/212 (41.5%)	150/261 (57.4%)	0.0005

Authors	Tecnique	Sample size	SSR	ICSI outcome
Tournaye 1997	TESE	15	47%	Not reported
Friedler 2001	TESE	12	42%	6 babies (2 twins)
Levron 2000	TESE	20	40%	1 triplet, 1 twins, 2 singletons
Madgar 2002	TESE	20	45%	Not reported
Westlander 2003	TESE	19	21%	2 pregnancies
Sao 2004	TESE	25	16%	Fert rate 50%
Vernaeve 2004	TESE	50	48%	Not reported
Okada 2005	TESE	51	51%	Not reported
Okada 2005	mTESE	10	60%	3 singletons
Schiff 2005	mTESE	42	69%	18 pregnancies, 21 live births
Emre Bakiricioglu 2006	mTESE	74	57%	Not reported
Kyono 2007	mTESE	17	35%	6 singletons 1 twins
Koga 2007	mTESE	26	50%	Not reported
Ramasamy 2009	mTESE	68	66%	LBR 41%
Selice 2010	mTESE	24	38%	Not reported

Role of age and testosterone level on SSR in KS

- Higher testosterone level correlates with higher sperm retrieval rates (TESE) at least in adolescent KS patients [JCEM 2015; 100: 961-67]
- When Leydig cell function is at its best (late pubertal-young adulthood KS) 50% chance of retrieving sperm with mTESE exists
- Sperm in the ejaculate are associated with T levels > 7 nmol/l in late pubertal and young adult KS [Andrology 2016; 4: 1178-86]
- Mean age was lower and T levels were higher among KS men (N=134) with SSR compared to those with failed SR [Urology 2014; 83: 107-10]
- Younger age correlated with SSR in KS men [J Urol 2014; 191: 175-8; J Urol 2013; 189: 638-42]

Y chromosome microdeletions



5-10% azoospermic
2-5% oligozoospermic men

Gr/gr deletions on Y-chromosome correlate with male infertility: an original study, meta-analyses, and trial sequential analyses

SCIENTIFIC REPORTS | 6:19798 (2016).

Sandeep Kumar Bansal[1], Deepika Jaiswal[2], Nishi Gupta[1], Kiran Singh[2], Rima Dada[3], Satya Narayan Sankhwar[4], Gopal Gupta[1] & Singh Rajender[1]

		Partial deletions distribution (%)				Complete AFZc deletion (%)
Subjects	N	gr/gr	b1/b3	b2/b3	Total (%)	b2/b4
All cases	822	48 (5.84)	1 (0.12)	2 (0.24)	51 (6.20)	8 (0.97)
Azoospermic	251	3 (1.20)	Nil	1 (0.40)	4 (1.59)	4 (1.59)
Oligozoospermic	105	7 (6.67)	Nil	Nil	7 (6.67)	Nil
Asthenozoospermic	34	Nil	Nil	Nil	Nil	Nil
Normozoospermic	203	18 (8.87)	Nil	Nil	18 (8.87)	Nil
Uncategorized idiopathic infertile	229	20 (8.73)	1 (0.44)	1 (0.44)	22 (9.61)	4 (1.75)
Fertile controls	225	2 (0.89)	Nil	1 (0.44)	3 (1.33)	Nil

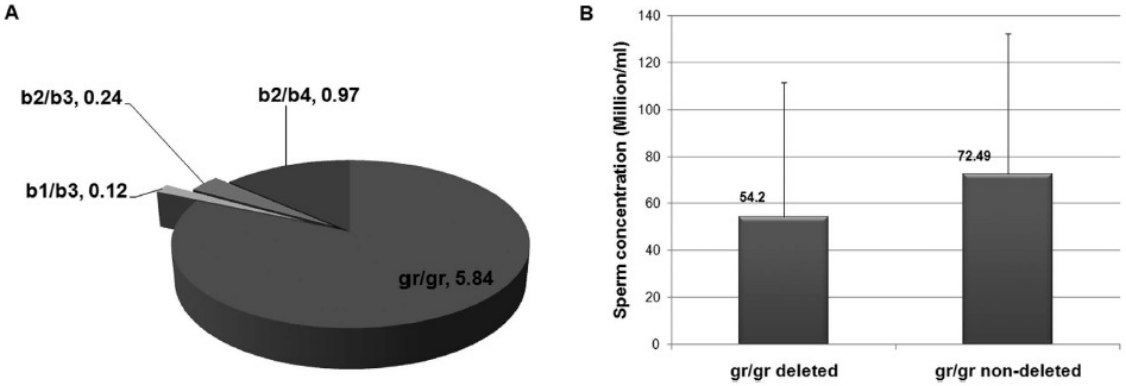


Figure 1. Distribution and the effect of Y-partial deletions: (A) Pie chart showing the frequencies (%) of the AZFc partial deletions in the cases (n = 822), (B) Cohort analysis showing sperm count between groups with and without gr/gr deletions.

Outcomes of intracytoplasmic sperm injection in oligozoospermic men with Y chromosome AZFb or AZFc microdeletions

X.-Y. Liu[1], R.-X. Wang[1], Y. Fu[2], L.-L. Luo[1], W. Guo[1] & R.-Z. Liu[1]

ANDROLOGIa

Andrologia 2017, **49**, e12602;

Table 2 AZF microdeletion and control group data

Variables	AZF microdeletion group	Control group	*t*	*P*
Female's age (year)	27.69 ± 4.64	29.85 ± 2.85	−1.872	0.069
Female's BMI	22.81 ± 3.39	22.20 ± 2.81	0.635	0.529
Male's age (year)	29.37 ± 4.06	31.35 ± 3.53	−1.659	0.105
Infertility duration (year)	4.50 ± 3.18	3.29 ± 1.84	1.566	0.125
Number of OR	12.5 ± 9.77	12.92 ± 6.79	−0.166	0.869
MII oocyte	10.19 ± 8.57	12.23 ± 6.56	−0.872	0.388

Values are mean ± SD (95% CI).
OR, oocytes retrieved; BMI, body mass index; AZF, azoospermia factor; MII, metaphase II.

Table 3 Azoospermia factor (AZF) microdeletion and control group semen parameters

Variables	AZF microdeletion group	Control group	*t*/*Z*	*P*
Semen volume	3.30 ± 1.63	2.23 ± 1.49	2.185	0.035
Sperm motility a + b (%)[a]	19.06	23.00	−1.026	0.305

Values are mean ± SD (95% CI).
[a]Mann–Whitney *U*-test was used.

Table 4 Clinical outcomes in the azoospermia factor (AZF) microdeletion and control groups

Variables	AZF microdeletion group *n* (%)	Control group *n* (%)	X^2	*P*
Fertilised oocyte rate[a]	104/200 (52.00)	278/336 (82.74)	57.850	0.000
Cleaved embryo rate[b]	95/104 (91.35)	241/278 (86.69)	1.549	0.213
High-grade embryo rate	49/95 (51.58)	129/241 (53.53)	0.104	0.747
Blastocyst formation rate	18/41 (43.90)	70/124 (56.45)	1.950	0.163
Embryo implantation rate[c]	8/24 (33.33)	20/53 (37.74)	0.138	0.710
Clinical pregnancy rate[d]	6/25 (24.00)	13/30 (43.33)	2.254	0.133
Delivery rate	6/24 (25.00)	13/53 (24.53)	0.002	0.965
Birth defect rate	0	0	–	–

Microdissection TESE is superior to conventional TESE in patients with nonobstructive azoospermia caused by Y chromosome microdeletions

SchwarzerJU et al

Andrologia 2016; 48: 402-5

- 25 male patients with AZF microdeletions

	TESE	MicroTESE
N	11	14
Sperm retrieval	25%	67%

- Overall pregnancy rate: 33%

In summary…

Review of Azoospermia

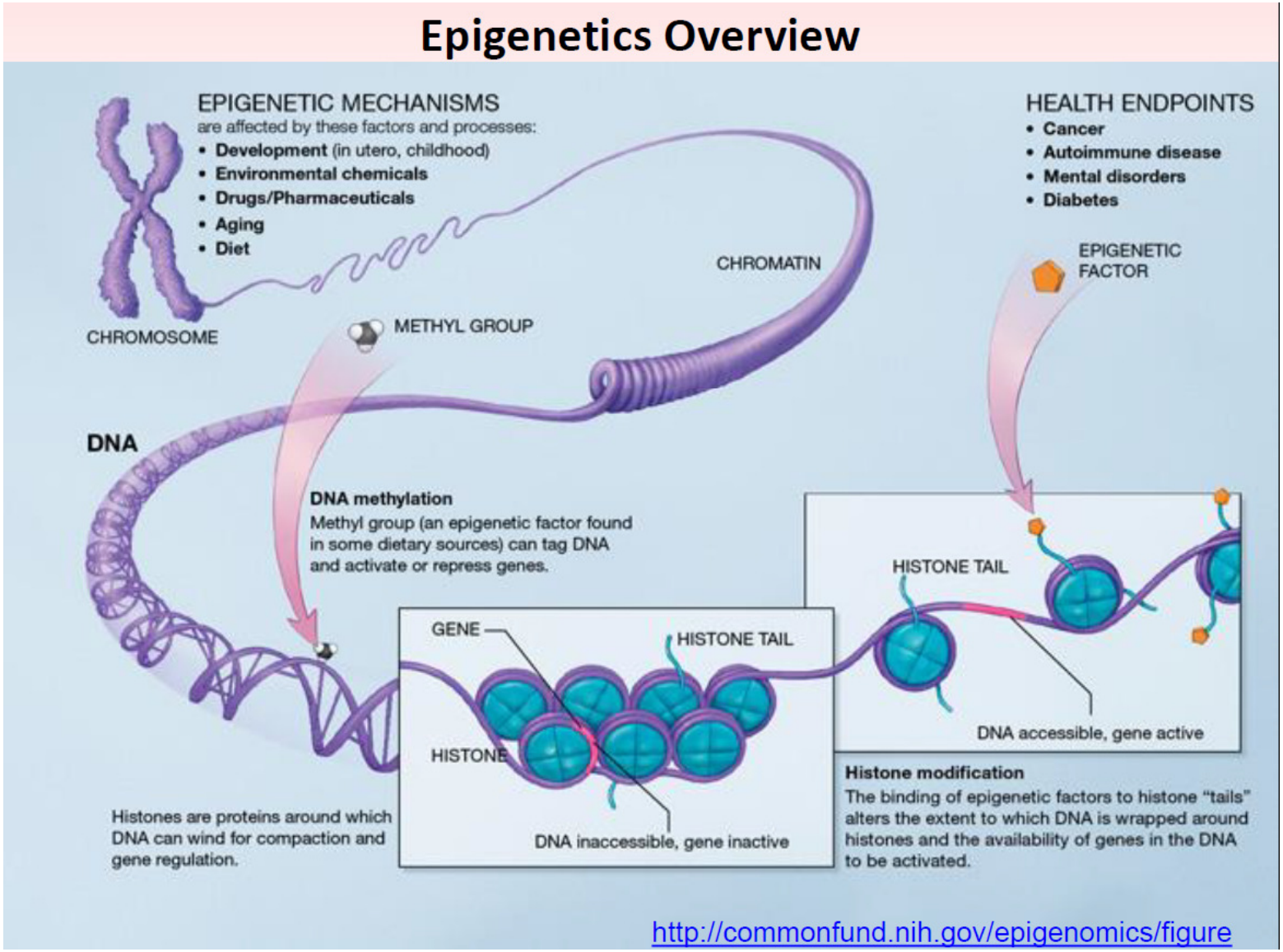
Matthew Wosnitzer[1,*], Marc Goldstein[2], and Matthew P Hardy[1,3,4]

Table 1. Microdissection TESE outcomes at Cornell

Condition	Overall sperm retrieval rate	Pregnancy rate
	per micro-TESE cycle	
Cryptorchidism	64%	50%
Post-chemotherapy azoospermia	48%	40%
KS (classic and mosaic)	65%	40%
AZFc deletion (Y chromosome microdeletion)	72%	46%
Uniform Maturation Arrest	50%	29%
Sertoli cell only	44%	46%

Spermatogenesis 4, e28218; March 2014

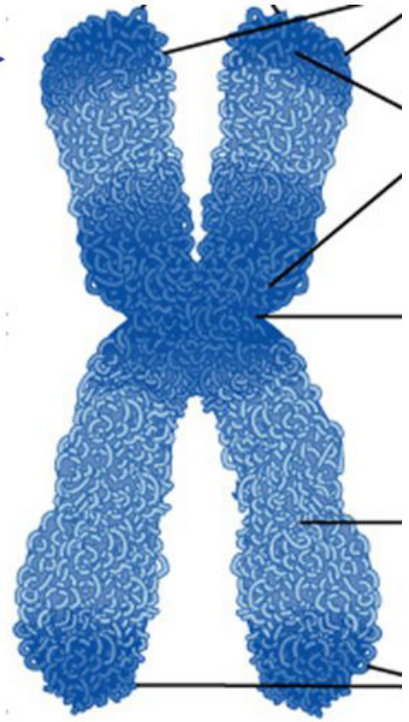
Epigenetics



Genome and epigenome

Chromosome Parts:

- Heterochromatin:
  - More condensed
  - Silenced genes (methylated)
  - Gene poor (high AT content)
  - Stains darker
- Euchromatin:
  - Less condensed
  - Gene expressing
  - Gene rich (higher GC content)
  - Stains lighter



Epigenetic marks

- **Epigenetic marks** are a fundamental component of the mechanisms that functionally interpret DNA sequence. These marks contribute to the **establishment and maintenance of specialized gene expression patterns** that determine cell identity.
- Epigenetically reinforced transcriptional states **can be propagated through cell division**, acting as a long-term marker of developmental origin during lineage specification.
- **DNA methylation** is an epigenetic mark that can be established *de novo*, maintained through cell division and be interpreted by transcription machinery and DNA-binding proteins. It is a **repressive mark** that characterizes heterochromatin in mammalian cells, and **when found at gene promoters** generally suppresses transcription

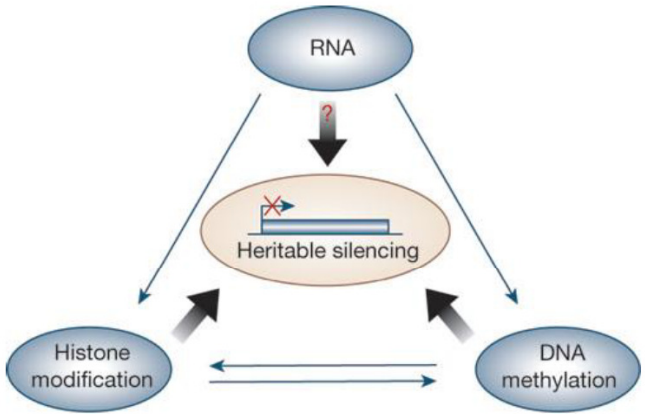
Epigenomics (2016) 8(10), 1399–1413

Epigenetic marks

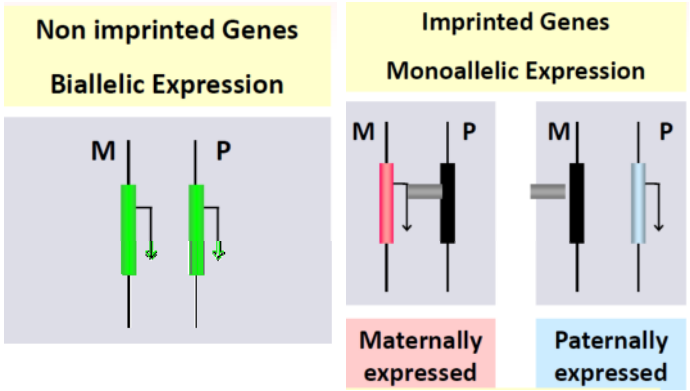
Epigenesis:

Changes in the genetic information not involving DNA sequence

→ **Modifications in gene expression**



- **DNA methylation**
- **Histone modification**
- **Chromatin remodeling**
- **RNA interference**



Epigenetic Mark/Imprint

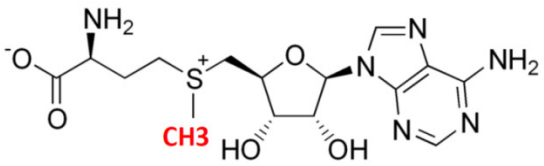
Genomic imprinting implies a differential marking of the two parental genomes

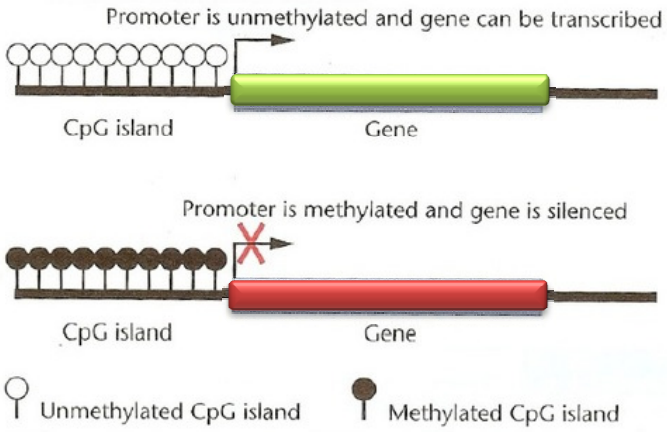
1. Apposed during gametogenesis and early embryogenesis
2. Specific
3. Stable and transmissible
4. Reversible, imprint resetting

- >80 imprinted genes (0.1%-1% of all genes)
- Key role in embryonic growth and placental function, cognition and maternal behavior
- Maternal and paternal alleles carry different epigenetic modifications ("imprints")
- Defective imprinting involved in carcinogenesis and in human genetic diseases
  - Angelman syndrome
  - Prader-Willi syndrome
  - Beckwith-Wiedemann syndrome

Methylation

- Biochemical process
- Intermediate metabolism
- Universal effector in DNA and histone methylation
  - SAM (S Adenosyl methionine)

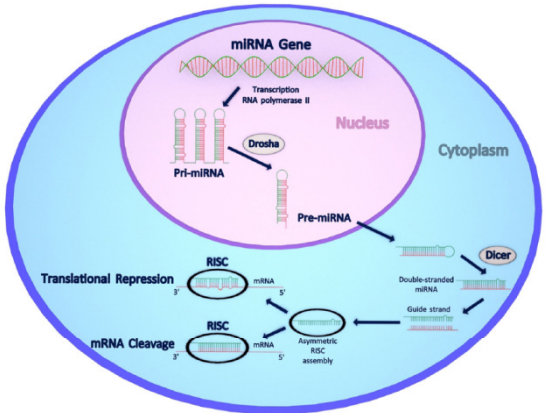




- 70% to 80% of CpG sites are methylated in humans
- 20% remaining are located in clusters: CpG islands
- CpG islands represent 1%–2% of the genome and contain 50% of unmethylated sites

- Estimated 29,000 CpG islands in the genome are frequently found in the promoter regions of human genes either tissue-specific or “housekeeping”

Short non-coding RNAs



Short non-coding RNAs are a class of functional RNA molecules that regulate gene expression at the post-transcriptional level via epigenetic mechanisms. These RNA molecules are shorter than 30 nucleotides, and they do not code for a particular protein. Short non-coding RNAs can be classified into three main groups called microRNAs (miRNAs), small-interfering RNAs (siRNAs), and piwiinteracting RNAs (piRNAs).

- miRNAs and endo-siRNAs are abundantly expressed in male germ cells throughout spermatogenesis, whereas piRNAs are only present in spermatocytes at the pachytene stage and in round spermatids. 221 miRNAs have been found in normozoospermic men
- Germ-cell specific deletion of Dicer1 in mice models has led to complete male infertility due to alterations in meiotic progression, increased spermatocyte apoptosis, and failure of haploid male germ cell differentiation
- when compared to ones from Dicer knockouts, the testes from Drosha knockouts were more severely disrupted in terms of spermatogenesis

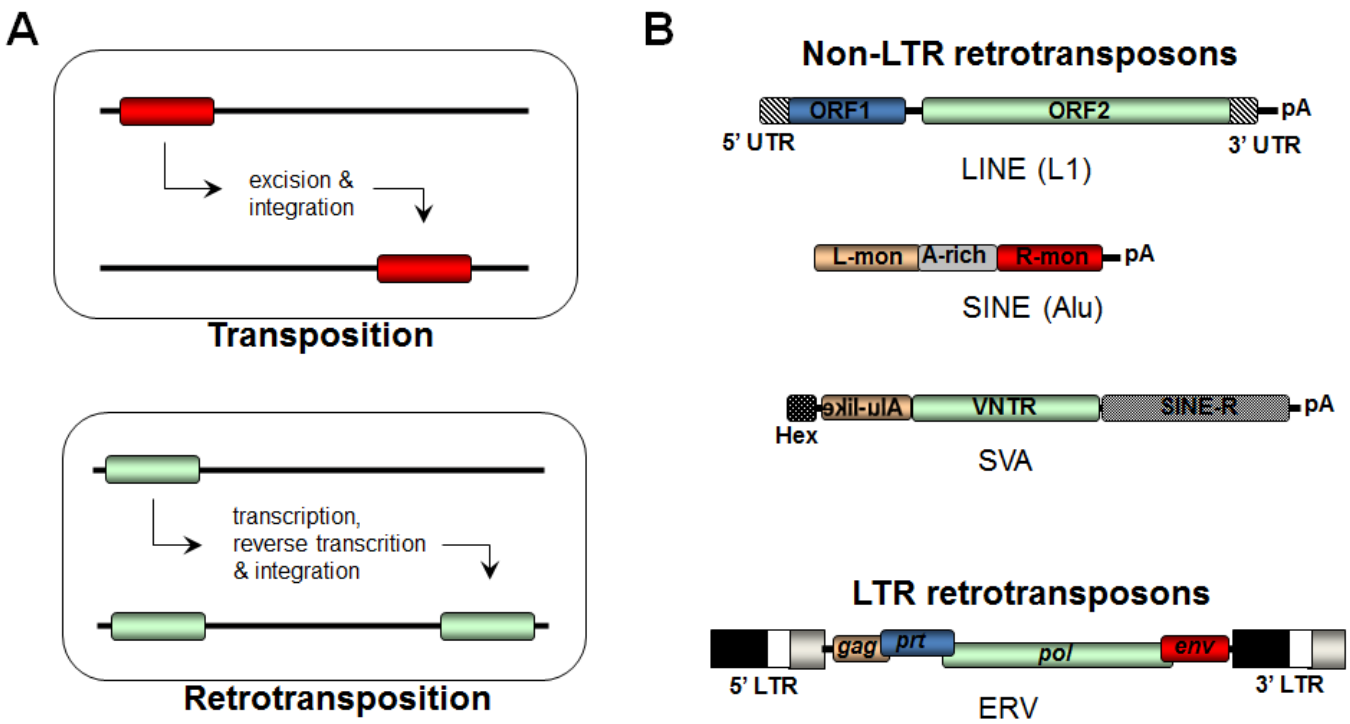
J Assist Reprod Genet (2016) 33:553–569

Sperm non-coding RNAs

- sperm cells contain a large repertoire of small non-coding RNAs.
- male germ cells express and require the activity of microRNAs *(Hayashi et al., 2008; Maatouk et al., 2008; Romero et al., 2011; Wu et al., 2012)* and many can still be detected in mature sperm *(Amanai et al., 2006; Krawetz et al., 2011; Hammoud et al., 2014)*
- inhibition, in the zygote, of sperm-delivered microRNAs leads to developmental delays *(Liu et al., 2012; Hammoud et al., 2014)*
- traumatic stress in early life of males alters the sperm microRNA (and PIWI- interacting RNA) profile and behavioral and metabolic responses in the offspring *(Gapp et al., 2014)*
- Male germ cells express PIWI-interacting RNAs (piRNAs; *Aravin et al., 2006; Girard et al., 2006; Grivna et al., 2006; Lau et al., 2006;Watanabe et al., 2006*); their most deeply conserved function is protection of the germline genome from transposons (*reviewed in O'Donnell and Boeke, 2007; Thomson and Lin, 2009; Siomi et al., 2011*). piRNAs target transposon transcripts for degradation and silencing when DNA methylation is nearly completely depleted during germcell development

Front Genet 2014; 5:330

Retrotransposons



LINE=long interspersed nuclear elements
SINE= short interspersed nuclear elements
LTR= long terminal repeats
SVA= SINE-VNTR-Alu elements

Retrotransposon expression and incorporation of cloned human and mouse retroelements in human spermatozoa

Leandros Lazaros, Ph.D.,[a,b,c] Chrysoula Kitsou, Ph.D.,[a] Charilaos Kostoulas, B.Sc.,[a] Sofia Bellou, Ph.D.,[d] Elissavet Hatzi, Ph.D.,[b] Paris Ladias, B.Sc.,[a] Theodoros Stefos, M.D., Ph.D.,[b] Sofia Markoula, Ph.D.,[a] Vasiliki Galani, Ph.D.,[e] Georgios Vartholomatos, Ph.D.,[f] Theodore Tzavaras, Ph.D.,[g] and Ioannis Georgiou, Ph.D.[a,b]

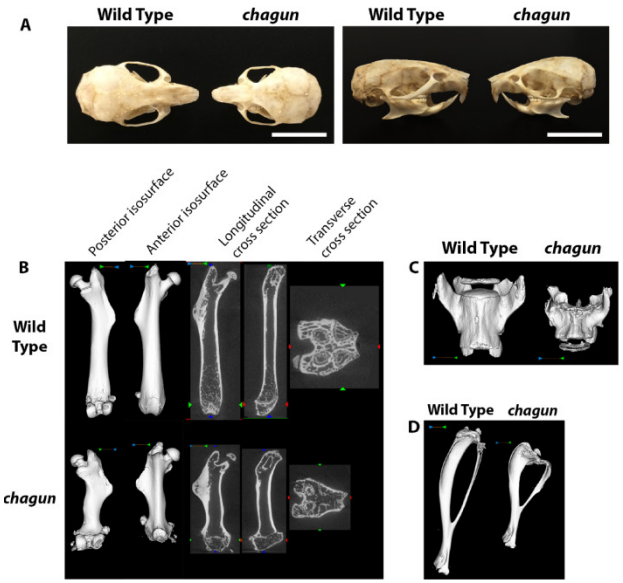
Fertility and Sterility® Vol. 107, No. 3, March 2017

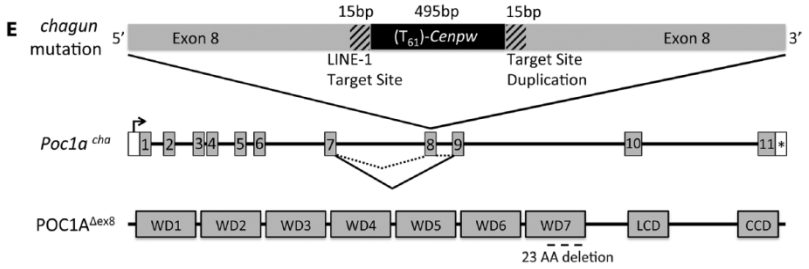
LINE-1, HERV-K10, and SVA Retrotransposons Are Transcriptionally Active in Human Spermatozoa

LINE-1 Mediated Insertion into *Poc1a* (Protein of Centriole 1 A) Causes Growth Insufficiency and Male Infertility in Mice

Krista A. Geister[1¤], Michelle L. Brinkmeier[2], Leonard Y. Cheung[2], Jennifer Wendt[3], Melissa J. Oatley[4], Daniel L. Burgess[3], Kenneth M. Kozloff[5], James D. Cavalcoli[6], Jon M. Oatley[4], Sally A. Camper[1,2]*

PLOS | GENETICS October 23, 2015

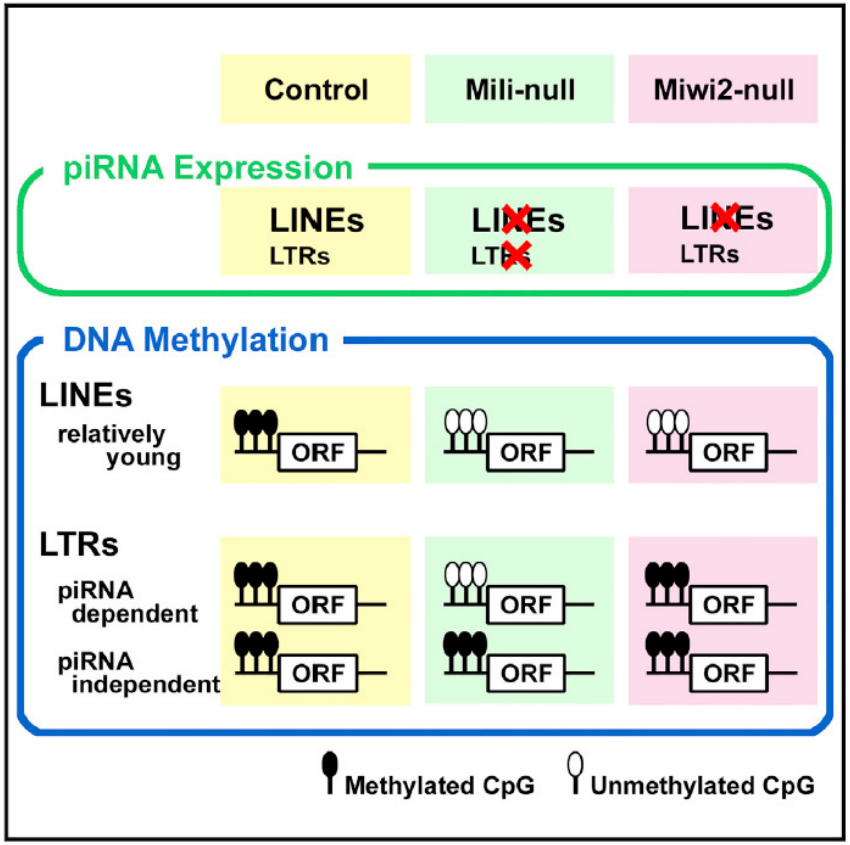


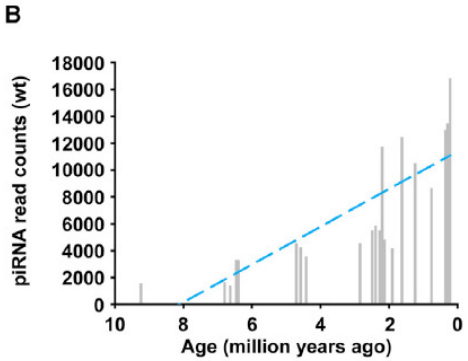


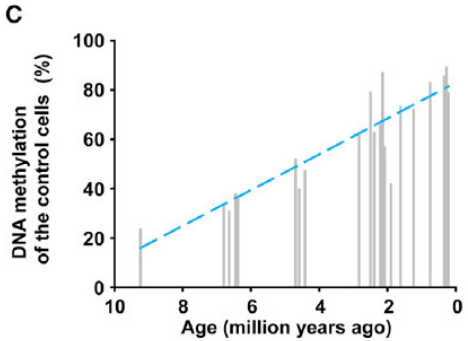
Comprehensive DNA Methylation Analysis of Retrotransposons in Male Germ Cells

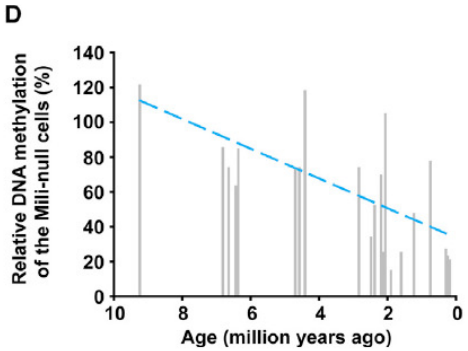
Cell Reports
2015 *12*, 1541–1547

Ippei Nagamori,[1] Hisato Kobayashi,[2] Yusuke Shiromoto,[1,5] Toru Nishimura,[3] Satomi Kuramochi-Miyagawa,[1,5] Tomohiro Kono,[4] and Toru Nakano[1,3,5,*]





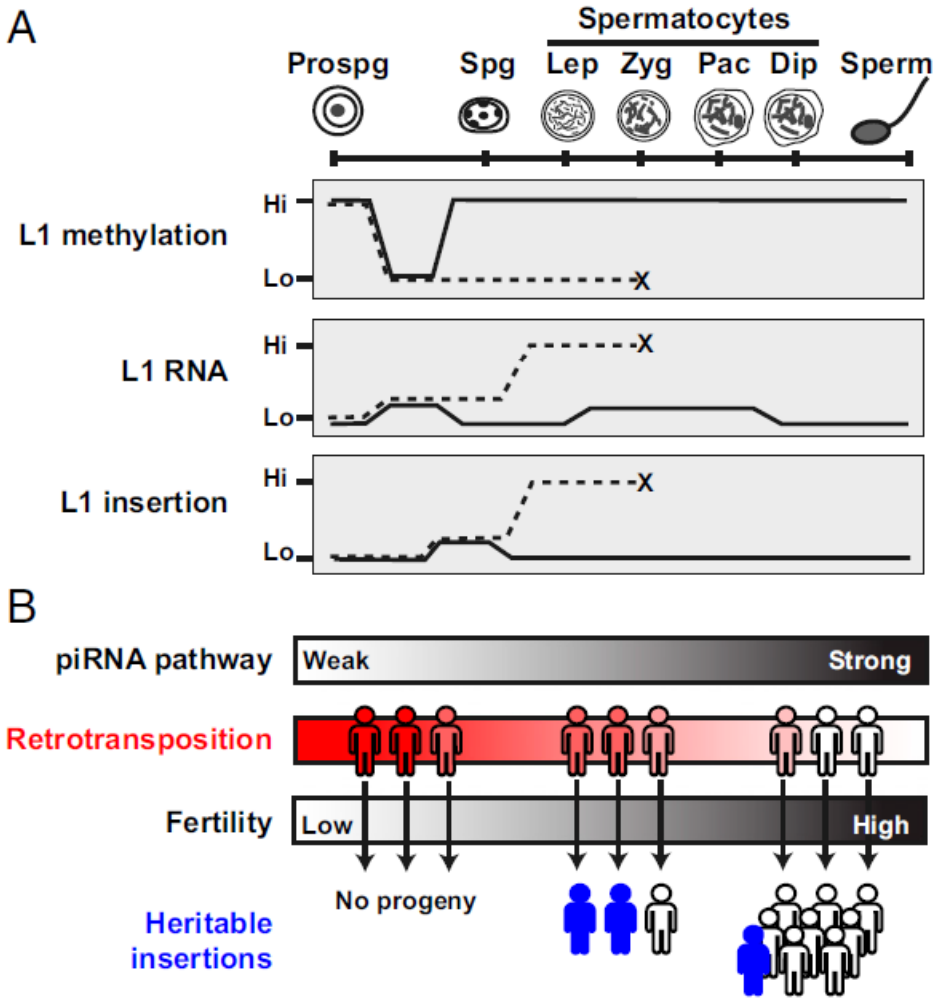




Intact piRNA pathway prevents L1 mobilization in male meiosis

PNAS | Published online June 19, 2017 | E5635–E5644

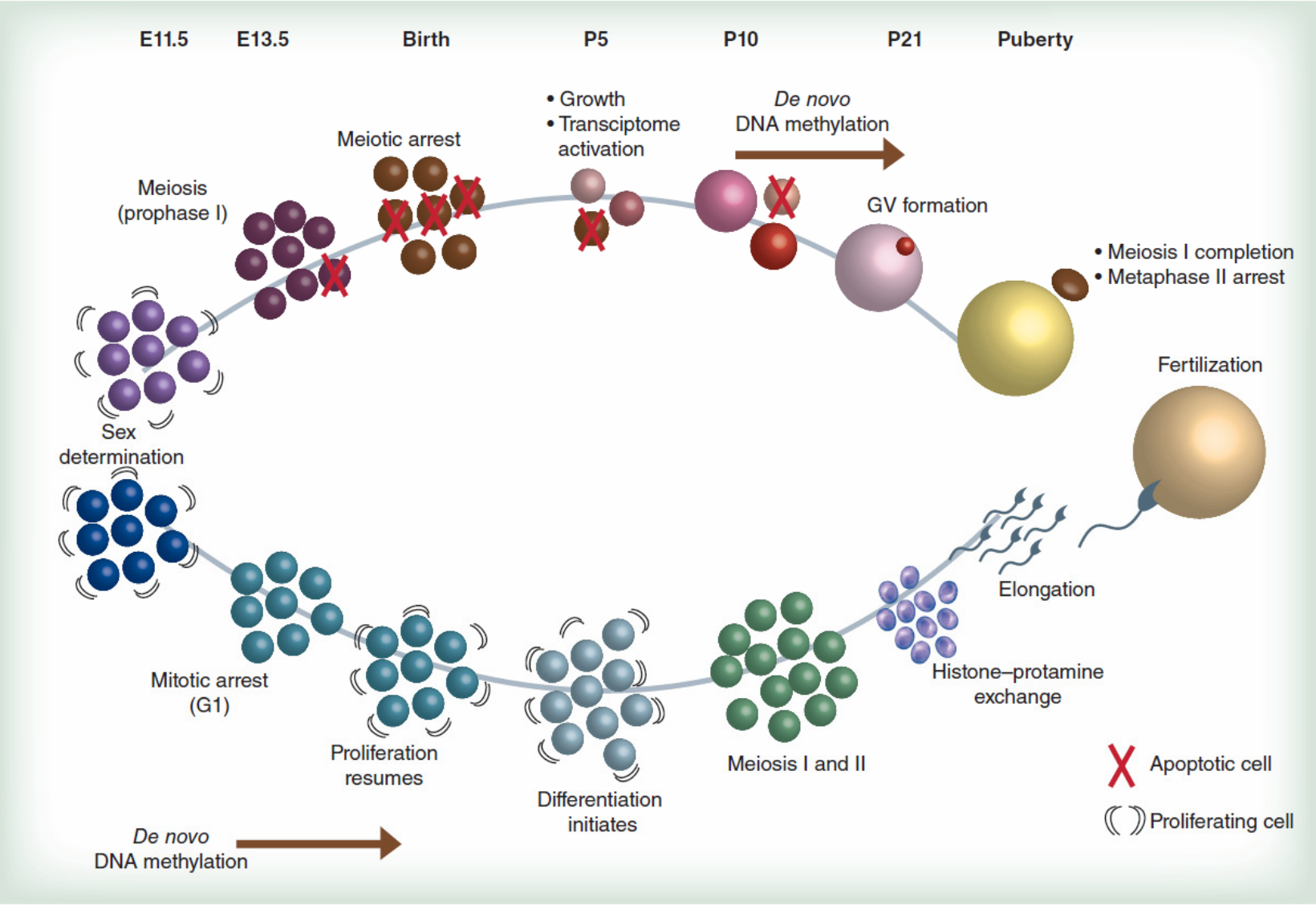
Simon J. Newkirk[a,b], Suman Lee[a], Fiorella C. Grandi[b,1], Valeriya Gaysinskaya[c], James M. Rosser[b,2], Nicole Vanden Berg[a], Cathryn A. Hogarth[b], Maria C. N. Marchetto[d], Alysson R. Muotri[d,3], Michael D. Griswold[b], Ping Ye[e,f], Alex Bortvin[c], Fred H. Gage[d,4], Jef D. Boeke[g], and Wenfeng An[a,4]



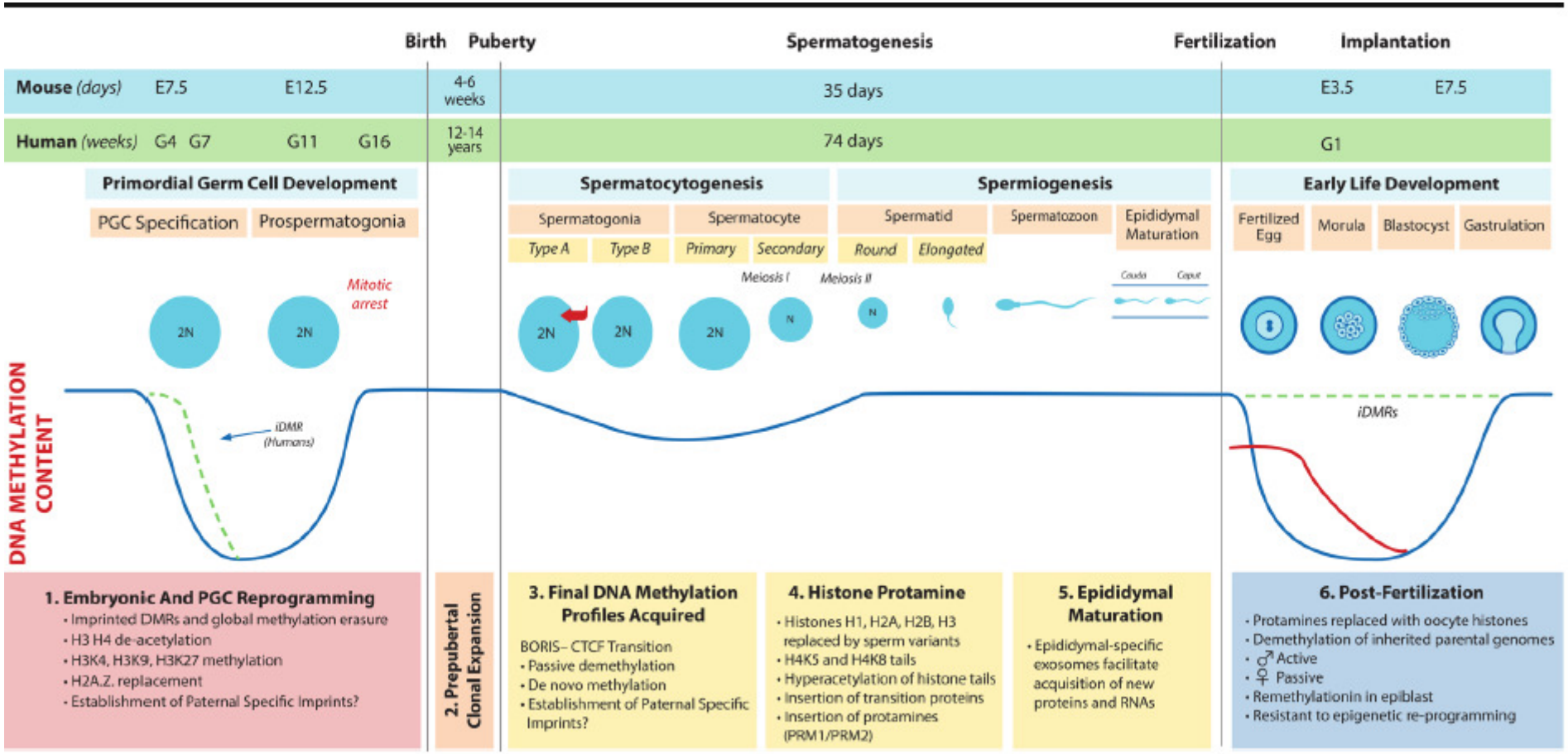
Significance

Retrotransposons make up the bulk of the human genome and, if unleashed, threaten the genomic integrity through DNA damage and insertional mutagenesis. In germ cells, an intact PIWI-interacting RNA pathway is essential for suppressing the expression of L1 retrotransposons. Deficiencies in the PIWI-interacting RNA pathway have dire consequences because mutant males are invariably sterile. To address the role of retrotransposon activation in these mutants, we developed an L1 reporter transgenic mouse. This mouse model allowed us to detect significant and stage-specific increases of new insertions in mutant germ cells, to draw attention to the importance of other L1-related activities for germ-cell health, and to predict the timing and origin of heritable L1 insertions in the human population.

DNA methylation timeline in the human male and female



Windows of epigenetic instability



Developmental origins of epigenetic transgenerational inheritance

Mark A. Hanson[1] and Michael K. Skinner[2,*]

Environmental Epigenetics, 2016, 1–9

ENVIRONMENTAL EPIGENETICS

Table 1: exposure induced epigenetic transgenerational inheritance

Toxicants	Species	Generation	References
Vinclozolin (agricultural fungicide)	Rat and mouse	F4	[5, 9, 10]
Methoxychlor (agricultural pesticide)	Rat	F4	[5, 11]
TCDD/dioxin (industrial contaminant)	Rat, mouse, fish	F3	[12, 13, 40]
Plastics (bisphenol-A, phthalate-DEHP and DBP)	Rat	F3	[6, 14, 15]
Jet fuel [JP8] (hydrocarbon mixture)	Rat	F3	[47]
Permethrin and DEET pesticide and insect repellent	Rat	F3	[48]
DDT (pesticide)	Rat	F4	[16]
Bisphenol A (BPA) (plastic toxicant)	Rat, mouse, fish	F3	[49, 50, 98]
Phthalates (plastic toxicant)	Rat	F3	[17]
Tributyltin (industrial toxicant)	Rat	F3	[18]
Nutrition			
Folate (nutrition)	Mouse		[25]
High fat diet (nutrition)	Mouse and rat	F2, F3	[23, 24]
Caloric restriction (nutrition)	Human, rat, mouse, pig, worm, flies	F2, F3	[19–22, 36, 37, 39, 42]
Other types exposures			
Temperature and drought (plant flowering and health)	Plant	F2, F3	[26–29]
Stress (behavioral)	Mouse, rat, human	F2, F3	[30, 31, 44–46]
Smoking (health)	Human	F2, F3	[32, 33]
Nicotine (health)	Rat	F3	[34]
Alcohol (health)	Rat	F3	[35]

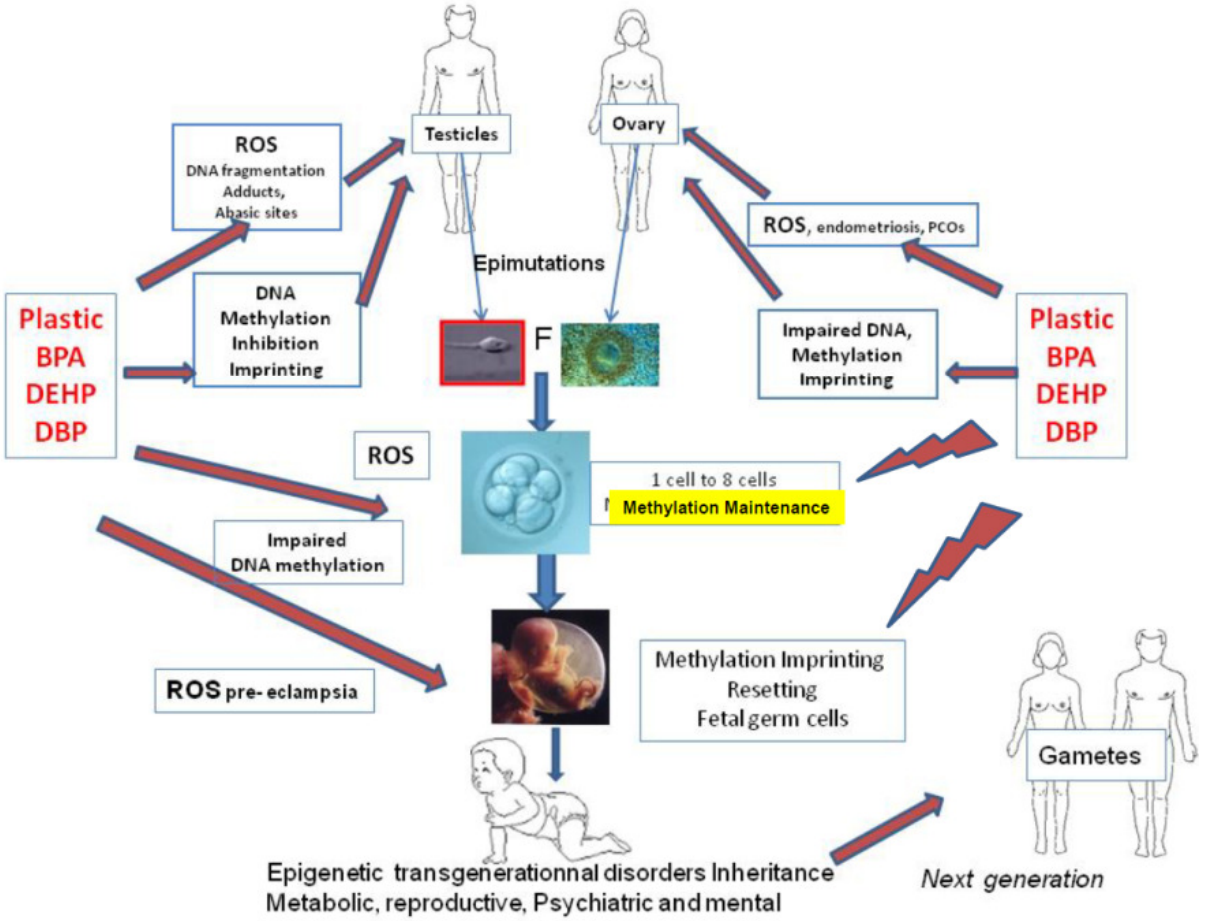
Table 2: sites of action and phenotypes of environmental factors

Site of action	Biological response and toxicology
Somatic cells	Allows tissue-specific toxicology and critical for adult onset disease in the individual exposed but not capable of transmitting a transgenerational phenotype.
Germ cells	Allows transmission between generations and in the absence of direct exposure to promote a transgenerational phenotype.

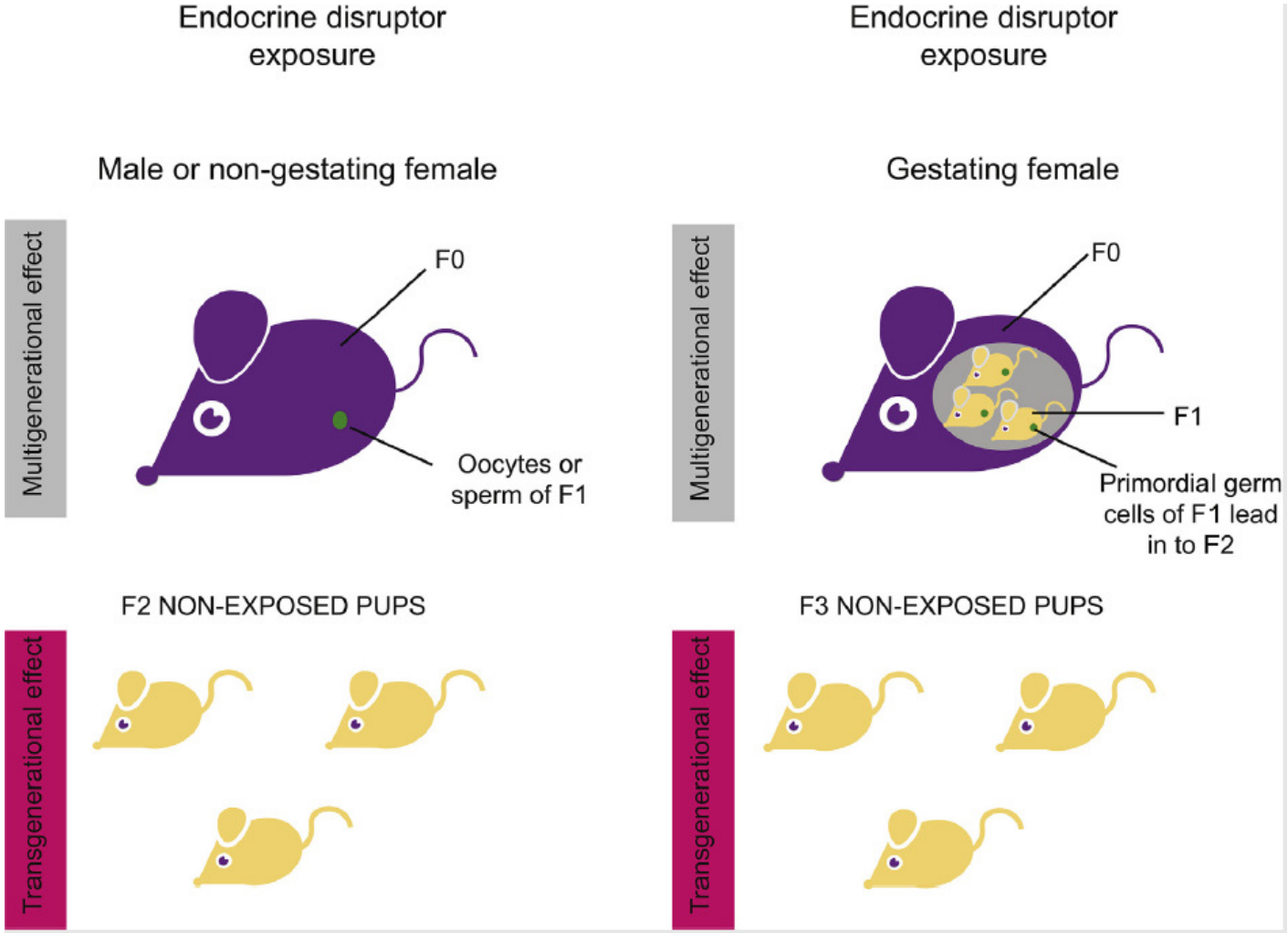
Table 3: transgenerational versus multigenerational phenotypes

Phenotype	Exposure	Definition
Multigenerational	Direct 	Coincident direct exposure of multiple generations to an environmental factor promoting alterations in the multiple generations exposed.
Transgenerational	None, except the initial generation 	After the initial exposure, the transgenerational phenotype is transmitted through the germ line in the absence of direct exposure.

Environmental-induced epigenetic alterations



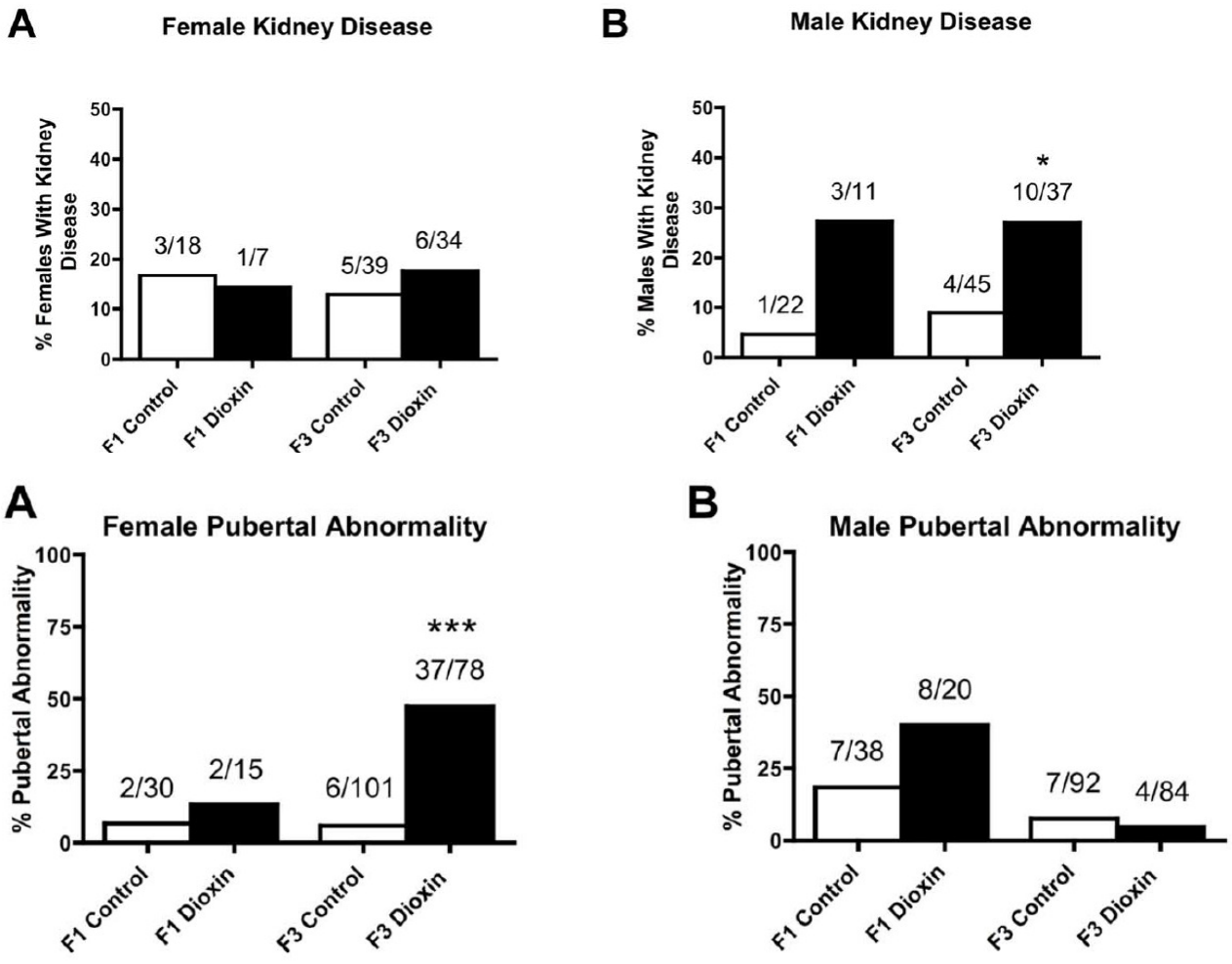
Transgenerational epigenetic inheritance

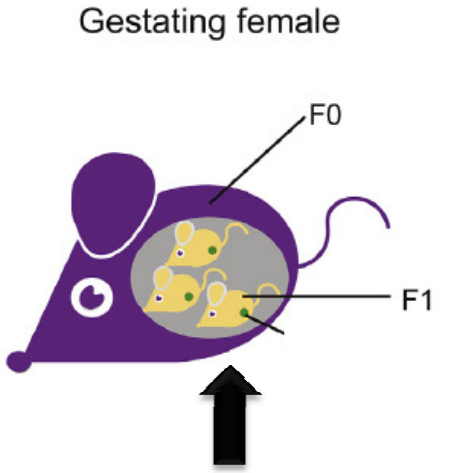


Dioxin (TCDD) Induces Epigenetic Transgenerational Inheritance of Adult Onset Disease and Sperm Epimutations

Mohan Manikkam, Rebecca Tracey, Carlos Guerrero-Bosagna, Michael K. Skinner*

PLOS ONE September 2012 | Volume 7 | Issue 9 | e46249





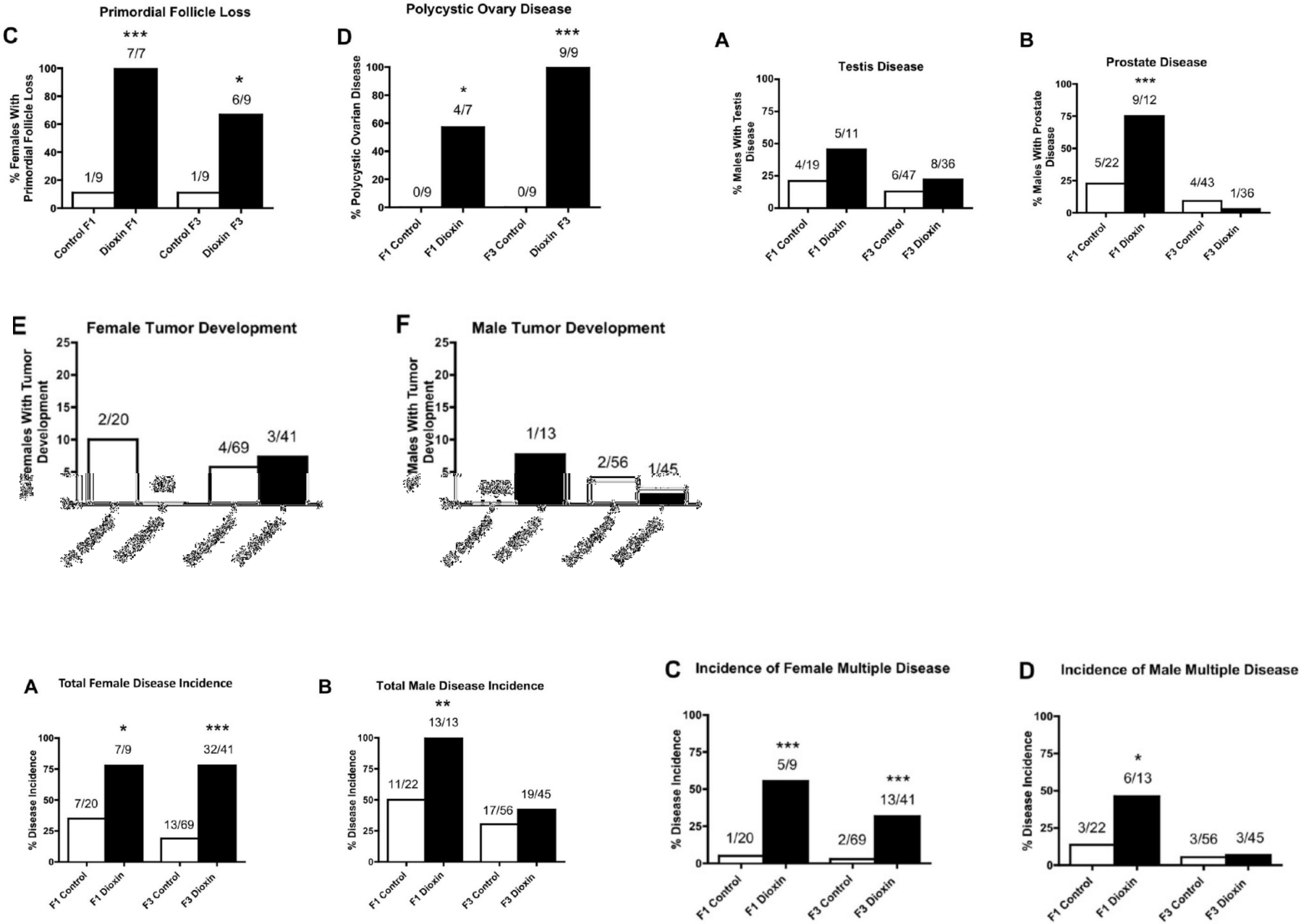


Figure 4. Dioxin and control lineage F1 and F3 generation adult-onset diseases in rats. Incidences of total female disease (panel A), total male disease (panel B), female multiple disease (panel C) and male multiple disease (panel D) and number of diseased rats/total number of rats (*P<0.05; **P<0.01; ***P<0.001).

doi:10.1371/journal.pone.0046249.g004

Genetic & epigenetic mechanisms involved in epigenetic transgenerational inheritance

RESEARCH ARTICLE

Tertiary Epimutations – A Novel Aspect of Epigenetic Transgenerational Inheritance Promoting Genome Instability

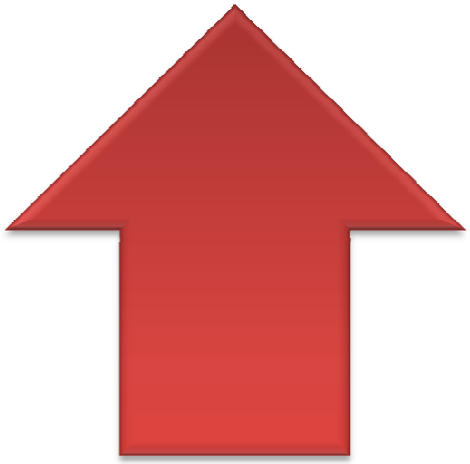
PLOS ONE December 19, 2016

John R. McCarrey[1]*, Jake D. Lehle[1], Seetha S. Raju[1], Yufeng Wang[1], Eric E. Nilsson[2], Michael K. Skinner[2]

Table 4. Transmission of Genetic and Epigenetic Defects.

Type of Defect	Initial Disruption[1]	Manifestation of Disruption[2]	Mode of Transmission
Genetic Mutation	Genome	Genome	Genetic
Primary Epimutation	Epigenome	Epigenome	Epigenetic
Secondary Epimutation	Genome	Genome & Epigenome	Genetic or Epigenetic
Tertiary Epimutation	Epigenome	Epigenome & Genome	Epigenetic or Genetic

Trends of genetic and epigenetic diseases over time



Epigenetic diseases

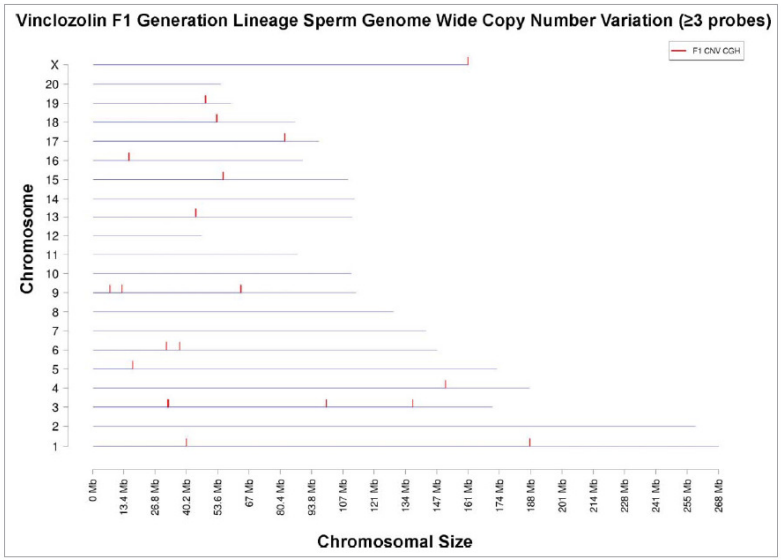
Genetic diseases

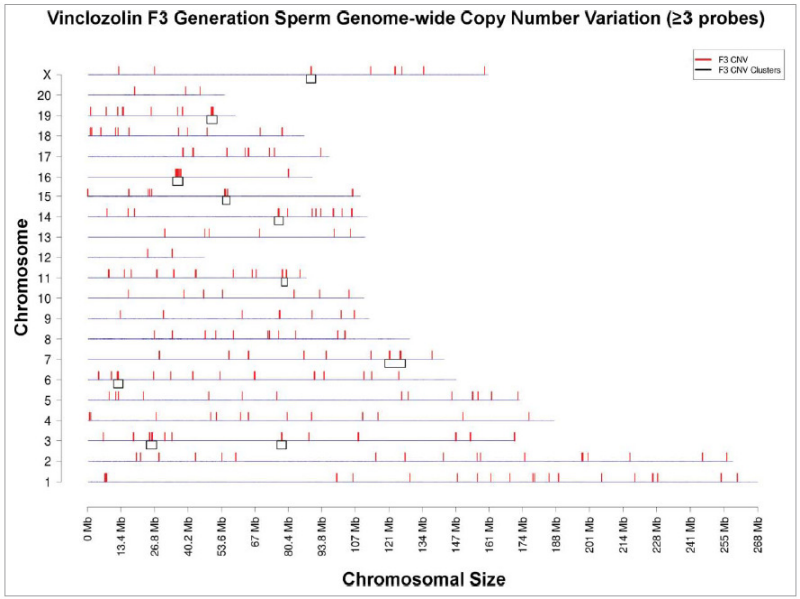
Epigenetic transgenerational inheritance and male infertility

Environmentally induced epigenetic transgenerational inheritance of sperm epimutations promote genetic mutations

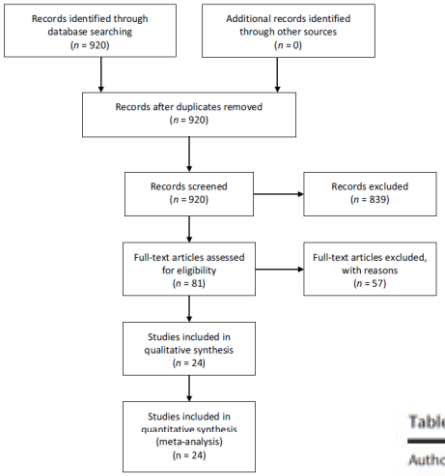
Michael K Skinner*, Carlos Guerrero-Bosagna, and M Muksitul Haque

Epigenetics 10:8, 762–771; August 2015;





Impairment of sperm DNA methylation in male infertility



"The main limitation of our study is as a result of the high heterogeneity imprinted genes"

Table 1 Population characteristic and imprinted genes evaluated in each study included in the meta-analysis

Author	Journal	Year	Imprinted genes	Inclusion criteria	Study group (n)	Age (years)	Sperm count (millions/mL)	Control group (n)	Age (years)	Sperm count (millions/mL)
Marques	Lancet	2004	*MEST, H19*	Oligozoospermia	96	NA	(range: 5.00–20.00)	27	NA	(range: >20.00)
Marques	Mol Hum Reprod	2008	*MEST, H19, LINE-1, CFTC-6*	OAT or OT	20	NA	(range: <20.00)	5	NA	(range: >20.00)
Navarro-Costa	Hum reprod	2010	*DAZL*	OAT	5	39.40 ± 7.23	6.66 ± 2.58	5	NA	NA
Boissonnass	Eur J Hum Res	2010	*H19, LINE-1, IGF2, IGF2R, PEG3*	OAT and/or teratozoospermia	22	36.60 ± 5.70	15.27 ± 2.20	17	39.2 ± 7.29	83.52 ± 23.12
Hammoud	Fertil & Steril	2010	*MEST, H19, LIT1, SNRPN, ZAC*	Oligozoospermia	13	NA	NA	7	36.60 ± 5.70	118.90 ± 28.70
Poplinsky	Int J Androl	2010	*MEST, H19*	Idiopathic infertility	69	35.50 ± 3.39	11.55 ± 8.09	33	37.00 ± 8.46	65.00 ± 14.95
Nanassy	Asian J Androl	2011	*CREM*	Oligozoospermia	20	NA	64.84 ± 17.00	10	34.10 ± 2.40	(range: >20.00)
Minor	Reproduction	2011	*MEST, H19, GTL2*	Oligozoospermia	18	37.00 ± 5.30	(range: <20.00)	9	32.81 ± 0.88	137.06 ± 7.08
Nanassy	Fertil & Steril	2011	*CREM*	Oligozoospermia	32	33.00 ± 1.28	7.32 ± 1.12	40	31.85 ± 3.88	101.99 ± 35.63
Sato	Fertil & Steril	2011	*H19, PEG3, LIT1, GTL2, ZAC*	Oligozoospermia	57	NA	NA	204	NA	NA
El Hajj	Sex Dev	2011	*H19, MEST, LINE-1, SNRPN*	Idiopathic infertility	106	NA	NA	28	31.40 ± 5.10	97.7 ± 56.5
Camprubi	Epigenetics	2012	*H19, SNRPN*	Idiopathic infertility	107	36.00 ± 5.50	NA	30	32.50 ± 6.50	113.60 ± 32.10
Ankolkar	Fertil & Steril	2012	*H19*	Idiopathic infertility	26	NA	61 ± 27.23	26	32.16 ± 3.26	63.31 ± 3.27
Kläver	Andrology	2013	*H19, MEST*	Idiopathic infertility	37	34.5 ± 7.2	NA	31	26.00 ± 6.15	NA
Li	PlosOne	2013	*H19, LINE-1, CFCTC-6, DAZL*	Oligozoospermia and/or asthenozoospermia	20	31.25 ± 5.63	5.22 ± 3.33	20	32.22 ± 3.59	115.98 ± 31.12
Montjean	Fertil & Steril	2013	*H19, MEST*	Oligozoospermia	175	NA	NA	118	38.50 ± 5.30	55.70 ± 33.00
Tian	Biol Reprod	2014	*H19, LINE-1, LIT1, BRD-T, MTHFR*	Idiopathic infertility	29	31.40 ± 5.10	41.00 ± 56.5	29	34.5 ± 7.2	NA
Botezatu	Reprod Biomed Online	2014	*SNRPN, MTHFR*	Idiopathic infertility	27	35.77 ± 19.65	14.01 ± 24.04	11	NA	132.50 ± 12.81
Richardson	Hum Mol Genet	2014	*MEST, RHOX*	Idiopathic infertility	95	36.00 ± 9.82	53.00 ± 84.43	45	NA	NA
Laurentino	Hum Mol Genet	2015	*H19, MEST, MEG3, KCNQ1OT1*	Idiopathic infertility	7	35.33 ± 1.70	9.13 ± 1.85	5	NA	NA
Montjean	Andrology	2015	*H19, MEST*	Oligozoospermia and/or asthenozoospermia	30	38.30 ± 6.00	5.80 ± 3.90	62	35.00 ± 9.96	18.30 ± 29.96
Xu	Andrologia	2016	*MEST, H19, LINE-1, GNAS; FAM50B*	Idiopathic infertility	46	31.95 ± 2.21	43.93 ± 3.37	49	33.68 ± 1.58	65.53 ± 10.49
Li	Exp & Therap Med	2016	*H19*	OAT	15	35.50 ± 8.50	11.80 ± 7.20	15	NA	75 ± 97.30
Dong	Reprod Sci	2016	*H19, LINE-1, SNRPN*	OAT	48	31.52 ± 3.58	10.90 ± 3.86	50	30.45 ± 9.60	32.22 ± 15.21

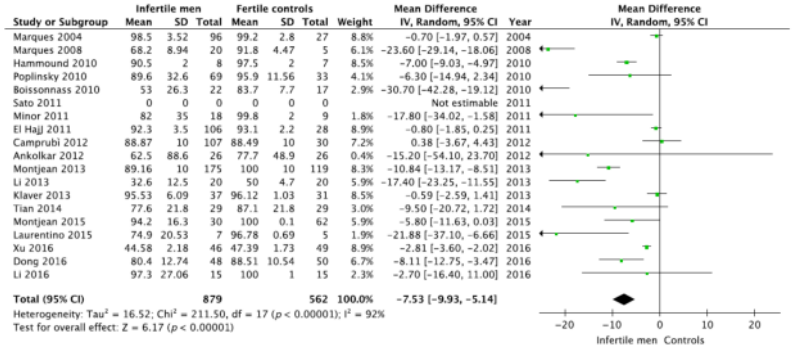
OAT, oligoasthenoteratozoospermia; OT, oligoteratozoospermia.

Andrology, 2017, 5, 695–703

Methylation levels

Altered methylation

Figure 2 Comparison between fertile and infertile men considering the methylation levels at *H19* gene. [Colour figure can be viewed at wileyonlinelibrary.com].

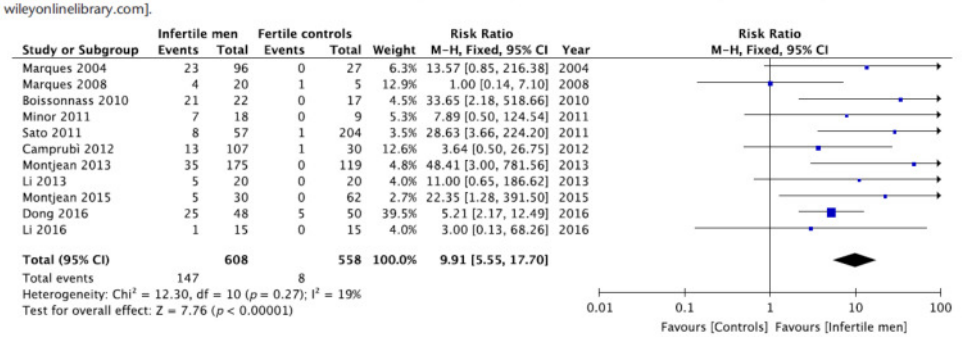


Study or Subgroup	Infertile men Mean	SD	Total	Fertile controls Mean	SD	Total	Weight	Mean Difference IV, Random, 95% CI	Year
Marques 2004	98.5	3.52	96	99.2	2.8	27	8.8%	−0.70 [−1.97, 0.57]	2004
Marques 2008	68.2	8.94	20	91.8	4.47	5	6.1%	−23.60 [−29.14, −18.06]	2008
Hammound 2010	90.5	2	8	97.5	2	7	8.5%	−7.00 [−9.03, −4.97]	2010
Poplinsky 2010	89.6	32.6	69	95.9	11.56	33	4.1%	−6.30 [−14.94, 2.34]	2010
Boissonnass 2010	53	26.3	22	83.7	7.7	17	2.9%	−30.70 [−42.28, −19.12]	2010
Sato 2011	0	0	0	0	0	0		Not estimable	2011
Minor 2011	82	35	18	99.8	2	9	1.8%	−17.80 [−34.02, −1.58]	2011
El Hajj 2011	92.3	3.5	106	93.1	2.2	28	8.9%	−0.80 [−1.85, 0.25]	2011
Camprubi 2012	88.87	10	107	88.49	10	30	7.2%	0.38 [−3.67, 4.43]	2012
Ankolkar 2012	62.5	88.6	26	77.7	48.9	26	0.4%	−15.20 [−54.10, 23.70]	2012
Montjean 2013	89.16	10	175	100	10	119	8.3%	−10.84 [−13.17, −8.51]	2013
Li 2013	32.6	12.5	20	50	4.7	20	5.9%	−17.40 [−23.25, −11.55]	2013
Klaver 2013	95.53	6.09	37	96.12	1.03	31	8.5%	−0.59 [−2.59, 1.41]	2013
Tian 2014	77.6	21.8	29	87.1	21.8	29	3.0%	−9.50 [−20.72, 1.72]	2014
Montjean 2015	94.2	16.3	30	100	0.1	62	5.9%	−5.80 [−11.63, 0.03]	2015
Laurentino 2015	74.9	20.53	7	96.78	0.69	5	1.9%	−21.88 [−37.10, −6.66]	2015
Xu 2016	44.58	2.18	46	47.39	1.73	49	8.9%	−2.81 [−3.60, −2.02]	2016
Dong 2016	80.4	12.74	48	88.51	10.54	50	6.7%	−8.11 [−12.75, −3.47]	2016
Li 2016	97.3	27.06	15	100	1	15	2.3%	−2.70 [−16.40, 11.00]	2016
Total (95% CI)			**879**			**562**	**100.0%**	**−7.53 [−9.93, −5.14]**	

Heterogeneity: Tau² = 16.52; Chi² = 211.50, df = 17 ($p < 0.00001$); I² = 92%
Test for overall effect: Z = 6.17 ($p < 0.00001$)

Mean Difference IV, Random, 95% CI
−20 −10 0 10 20
Infertile men Controls

Figure 3 Comparison between fertile and infertile men considering the percentage of altered methylation at *H19* gene. [Colour figure can be viewed at wileyonlinelibrary.com].

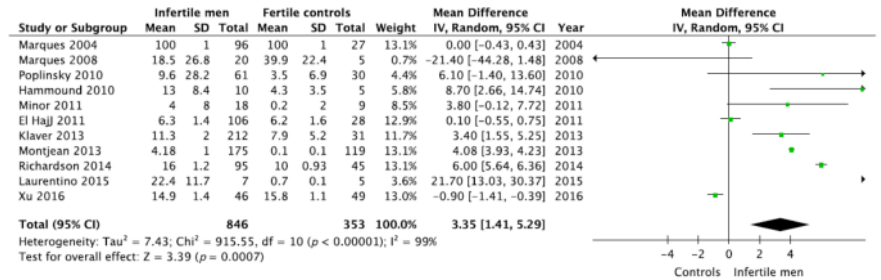


Study or Subgroup	Infertile men Events	Total	Fertile controls Events	Total	Weight	Risk Ratio M-H, Fixed, 95% CI	Year
Marques 2004	23	96	0	27	6.3%	13.57 [0.85, 216.38]	2004
Marques 2008	4	20	1	5	12.9%	1.00 [0.14, 7.10]	2008
Boissonnass 2010	21	22	0	17	4.5%	33.65 [2.18, 518.66]	2010
Minor 2011	7	18	0	9	5.3%	7.89 [0.50, 124.54]	2011
Sato 2011	8	57	1	204	3.5%	28.63 [3.66, 224.20]	2011
Camprubi 2012	13	107	1	30	12.6%	3.64 [0.50, 26.75]	2012
Montjean 2013	35	175	0	119	4.8%	48.41 [3.00, 781.56]	2013
Li 2013	5	20	0	20	4.0%	11.00 [0.65, 186.62]	2013
Montjean 2015	5	30	0	62	2.7%	22.35 [1.28, 391.50]	2015
Dong 2016	25	48	5	50	39.5%	5.21 [2.17, 12.49]	2016
Li 2016	1	15	0	15	4.0%	3.00 [0.13, 68.26]	2016
Total (95% CI)		**608**		**558**	**100.0%**	**9.91 [5.55, 17.70]**	
Total events	147		8				

Heterogeneity: Chi² = 12.30, df = 10 ($p = 0.27$); I² = 19%
Test for overall effect: Z = 7.76 ($p < 0.00001$)

Risk Ratio M-H, Fixed, 95% CI
0.01 0.1 1 10 100
Favours [Controls] Favours [Infertile men]

Figure 4 Comparison between fertile and infertile men considering the methylation levels at *MEST* gene. [Colour figure can be viewed at wileyonlinelibrary.com].

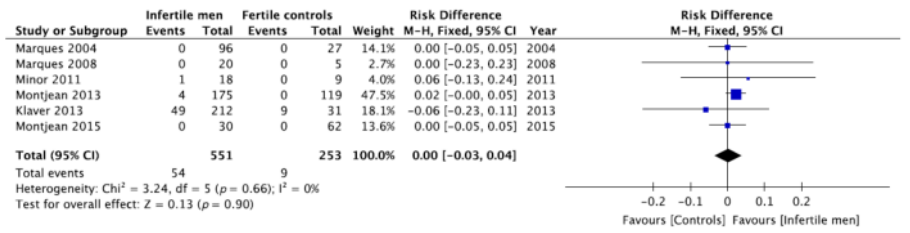


Study or Subgroup	Infertile men Mean	SD	Total	Fertile controls Mean	SD	Total	Weight	Mean Difference IV, Random, 95% CI	Year
Marques 2004	100	1	96	100	1	27	13.1%	0.00 [−0.43, 0.43]	2004
Marques 2008	18.5	26.8	20	39.9	22.4	5	0.7%	−21.40 [−44.28, 1.48]	2008
Poplinsky 2010	9.6	28.2	61	3.5	6.9	30	4.4%	6.10 [−1.40, 13.60]	2010
Hammound 2010	13	8.4	10	4.3	3.5	5	5.8%	8.70 [2.66, 14.74]	2010
Minor 2011	4	8	18	0.2	2	9	8.5%	3.80 [−0.12, 7.72]	2011
El Hajj 2011	6.3	1.4	106	6.2	1.6	28	12.9%	0.10 [−0.55, 0.75]	2011
Klaver 2013	11.3	2	212	7.9	5.2	31	11.7%	3.40 [1.55, 5.25]	2013
Montjean 2013	4.18	1	175	0.1	0.1	119	13.1%	4.08 [3.93, 4.23]	2013
Richardson 2014	16	1.2	95	10	0.93	45	13.1%	6.00 [5.64, 6.36]	2014
Laurentino 2015	22.4	11.7	7	0.7	0.1	5	3.6%	21.70 [13.03, 30.37]	2015
Xu 2016	14.9	1.4	46	15.8	1.1	49	13.0%	−0.90 [−1.41, −0.39]	2016
Total (95% CI)			**846**			**353**	**100.0%**	**3.35 [1.41, 5.29]**	

Heterogeneity: Tau² = 7.43; Chi² = 915.55, df = 10 ($p < 0.00001$); I² = 99%
Test for overall effect: Z = 3.39 ($p = 0.0007$)

Mean Difference IV, Random, 95% CI
−4 −2 0 2 4
Controls Infertile men

Figure 5 Comparison between fertile and infertile men considering the percentage of altered methylation at *MEST* gene. [Colour figure can be viewed at wileyonlinelibrary.com].

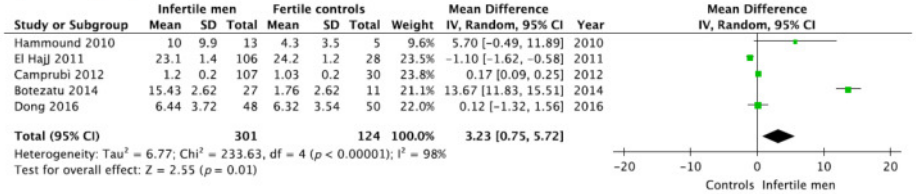


Study or Subgroup	Infertile men Events	Total	Fertile controls Events	Total	Weight	Risk Difference M-H, Fixed, 95% CI	Year
Marques 2004	0	96	0	27	14.1%	0.00 [−0.05, 0.05]	2004
Marques 2008	0	20	0	5	2.7%	0.00 [−0.23, 0.23]	2008
Minor 2011	1	18	0	9	4.0%	0.06 [−0.13, 0.24]	2011
Montjean 2013	4	175	0	119	47.5%	0.02 [−0.00, 0.05]	2013
Klaver 2013	49	212	9	31	18.1%	−0.06 [−0.23, 0.11]	2013
Montjean 2015	0	30	0	62	13.6%	0.00 [−0.05, 0.05]	2015
Total (95% CI)		**551**		**253**	**100.0%**	**0.00 [−0.03, 0.04]**	
Total events	54		9				

Heterogeneity: Chi² = 3.24, df = 5 ($p = 0.66$); I² = 0%
Test for overall effect: Z = 0.13 ($p = 0.90$)

Risk Difference M-H, Fixed, 95% CI
−0.2 −0.1 0 0.1 0.2
Favours [Controls] Favours [Infertile men]

Figure 6 Comparison between fertile and infertile men considering the methylation levels at *SNRPN* gene. [Colour figure can be viewed at wileyonlinelibrary.com].



Study or Subgroup	Infertile men Mean	SD	Total	Fertile controls Mean	SD	Total	Weight	Mean Difference IV, Random, 95% CI	Year
Hammound 2010	10	9.9	13	4.3	3.5	5	9.6%	5.70 [−0.49, 11.89]	2010
El Hajj 2011	23.1	1.4	106	24.2	1.2	28	23.5%	−1.10 [−1.62, −0.58]	2011
Camprubi 2012	1.2	0.2	107	1.03	0.2	30	23.8%	0.17 [0.09, 0.25]	2012
Botezatu 2014	15.43	2.62	27	1.76	2.62	11	21.1%	13.67 [11.83, 15.51]	2014
Dong 2016	6.44	3.72	48	6.32	3.54	50	22.0%	0.12 [−1.32, 1.56]	2016
Total (95% CI)			**301**			**124**	**100.0%**	**3.23 [0.75, 5.72]**	

Heterogeneity: Tau² = 6.77; Chi² = 233.63, df = 4 ($p < 0.00001$); I² = 98%
Test for overall effect: Z = 2.55 ($p = 0.01$)

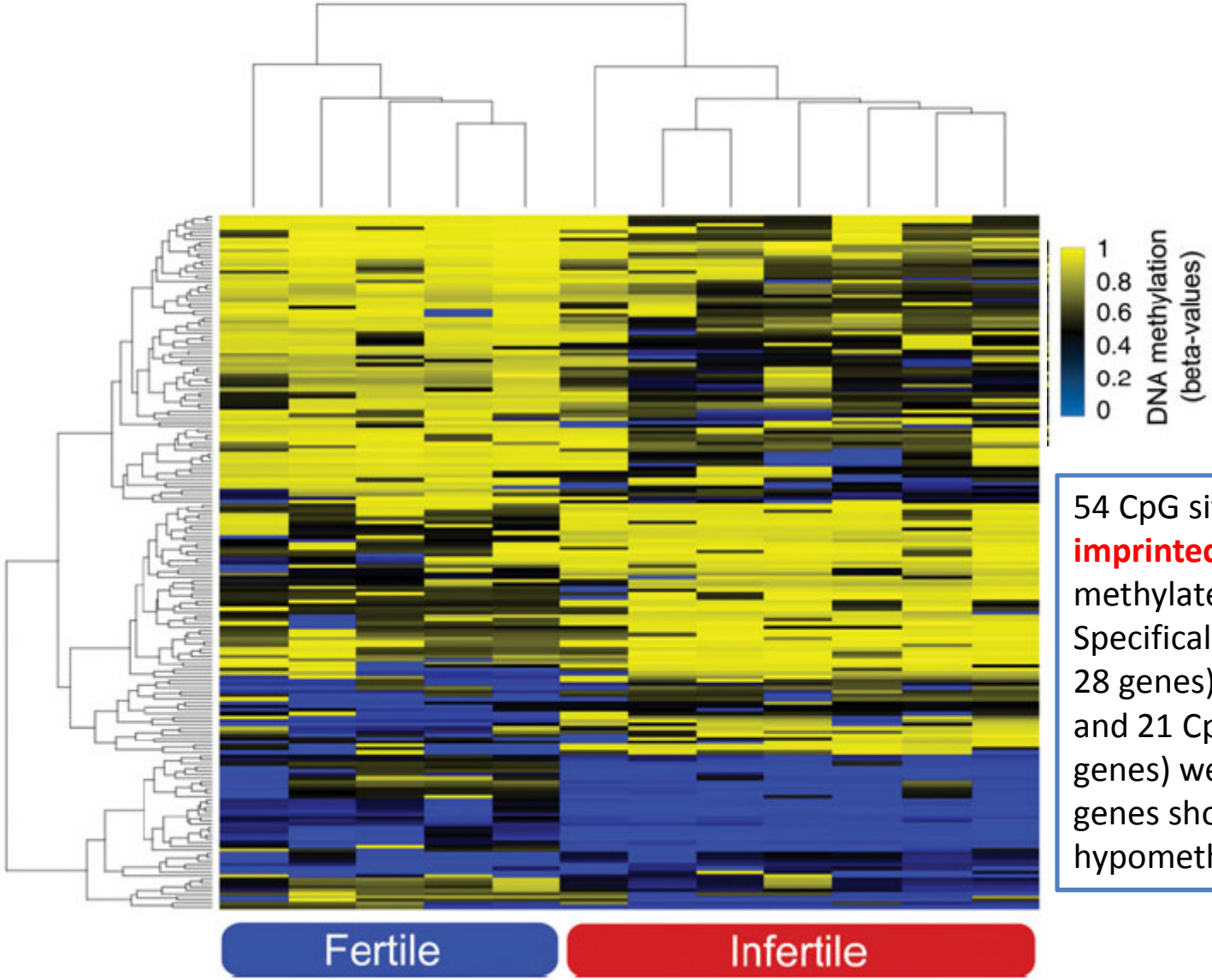
Mean Difference IV, Random, 95% CI
−20 −10 0 10 20
Controls Infertile men

Aberrant DNA methylation patterns of spermatozoa in men with unexplained infertility

Human Reproduction, Vol.30, No.5 pp. 1014–1028, 2015

Rocío G. Urdinguio[1,†], Gustavo F. Bayón[1,†], Marija Dmitrijeva[1], Estela G. Toraño[1], Cristina Bravo[1], Mario F. Fraga[1,2], Lluís Bassas[3], Sara Larriba[4,*], and Agustín F. Fernández[1,*]

human reproduction



54 CpG sites associated with **48 imprinted genes** were aberrantly methylated in infertile patients. Specifically, 33 CpG sites (related to 28 genes) were hypermethylated and 21 CpG sites (related to 28 genes) were hypomethylated; 8 genes showed both hyper- and hypomethylation

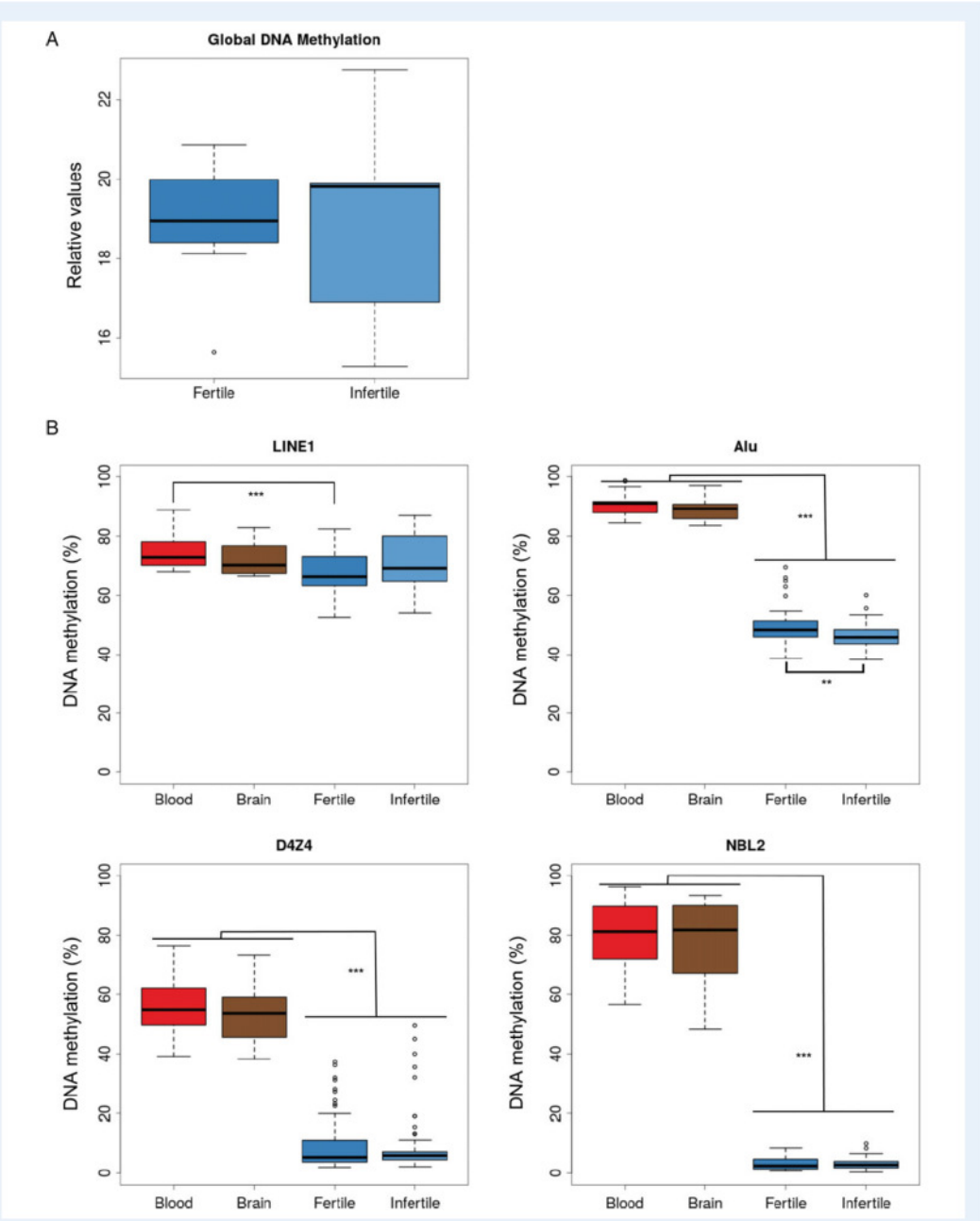


Figure 3 Global DNA methylation patterns in sperm. (**A**) Global DNA methylation levels of sperm from fertile individuals and normospermic infertile patients obtained in a colorimetric assay. (**B**) DNA methylation values of several repetitive regions (LINE-1, Alu Yb8, NBL-2 and D4Z4) measured by bisulfite pyrosequencing in sperm (controls and patients) and somatic cells (blood and brain). ***$P < 0.001$; **$P < 0.01$.

Excess of Rare Variants in Genes that are Key Epigenetic Regulators of Spermatogenesis in the Patients with Non-Obstructive Azoospermia

Zesong Li[1,2,3*], Yi Huang[2,3*], Honggang Li[4*], Jingchu Hu[5*], Xiao Liu[5*], Tao Jiang[5], Guangqing Sun[5], Aifa Tang[2,3], Xiaojuan Sun[2,3], Weiping Qian[6], Yong Zeng[7], Jun Xie[1], Wei Zhao[5], Yu Xu[5], Tingting He[5], Chengliang Dong[5], Qunlong Liu[6], Lisha Mou[1,2,3], Jingxiao Lu[2,3], Zheguang Lin[1], Song Wu[2,3], Shengjie Gao[5], Guangwu Guo[5], Qiang Feng[5], Yingrui Li[5], Xiuqing Zhang[5], Jun Wang[5], Huanming Yang[5], Jian Wang[5], Chengliang Xiong[4], Zhiming Cai[2,3] & Yaoting Gui[1]

SCIENTIFIC REPORTS | 5 : 8785 | 2015

Table 1 | The top genes that showed an excess of rare, non-silent variants in the NOA patients

Gene	BURDEN	FRQWGT	UNIQ	VT	Carrier frequency (%) Cases	Carrier frequency (%) Controls	OR	Fisher *P*
BRWD1[a]	7.4×10^{-4}	9.9×10^{-4}	2.7×10^{-3}	1.5×10^{-3}	7.27	4.09	1.84	0.01
PDGFC	3.6×10^{-3}	1.6×10^{-3}	–	3.8×10^{-3}	2.51	0.71	3.62	0.007
ATF4	4.1×10^{-3}	9.1×10^{-3}	–	7.9×10^{-3}	2.64	0.85	3.18	0.01
LIMK2[a]	6.7×10^{-3}	8.1×10^{-3}	4.1×10^{-2}	9.5×10^{-3}	3.43	1.13	3.11	0.005
ADORA1[a]	7.5×10^{-3}	7.0×10^{-3}	1.5×10^{-2}	2.2×10^{-2}	1.72	0.42	4.11	0.02
CDA	1.3×10^{-2}	2.6×10^{-2}	9.4×10^{-3}	2.6×10^{-2}	1.85	0.56	3.32	0.03
TSSK2[a]	1.4×10^{-2}	1.4×10^{-2}	1.5×10^{-2}	2.0×10^{-2}	2.38	0.85	2.85	0.02
UBR2[a]	1.9×10^{-2}	3.0×10^{-2}	–	–	5.68	3.39	1.97	0.04
USP26	2.0×10^{-2}	3.2×10^{-2}	1.5×10^{-2}	2.0×10^{-2}	1.19	0.28	4.25	0.07
TCEB3B	2.1×10^{-2}	5.4×10^{-3}	–	3.4×10^{-2}	7.00	4.37	1.65	0.03
RAD23B[a]	2.6×10^{-2}	2.3×10^{-2}	–	–	1.19	0.28	4.25	0.07
SOX9	2.8×10^{-2}	1.9×10^{-2}	–	3.1×10^{-2}	1.32	0.42	3.15	0.09
SLC19A2[a]	3.3×10^{-2}	2.6×10^{-2}	1.9×10^{-2}	4.7×10^{-2}	1.32	0.42	3.15	0.09
VDR[a]	4.5×10^{-2}	2.1×10^{-2}	2.9×10^{-2}	1.1×10^{-2}	1.45	0.56	2.60	0.12
SLC19A1[a]	–	–	1.6×10^{-2}	–	1.85	0.99	1.89	0.19
CDKN1B[a]	–	–	4.9×10^{-3}	–	1.32	0.56	2.36	0.18
ETV5[a]	–	–	2.3×10^{-2}	–	0.66	0.14	4.70	0.22
RNF17[a]	–	4.5×10^{-2}	1.4×10^{-2}	2.2×10^{-2}	3.70	2.54	1.47	0.23
DNMT3B	–	–	2.5×10^{-2}	–	3.96	2.82	1.42	0.25
SULT1E1[a]	–	–	–	2.0×10^{-2}	1.19	0.56	2.12	0.27
DNMT1	–	3.9×10^{-2}	–	1.3×10^{-2}	1.72	0.99	1.75	0.27

[a]genes proven to cause infertility in mouse mutants when deleted. Genes shown in bold are epigenetic regulators of spermatogenesis.

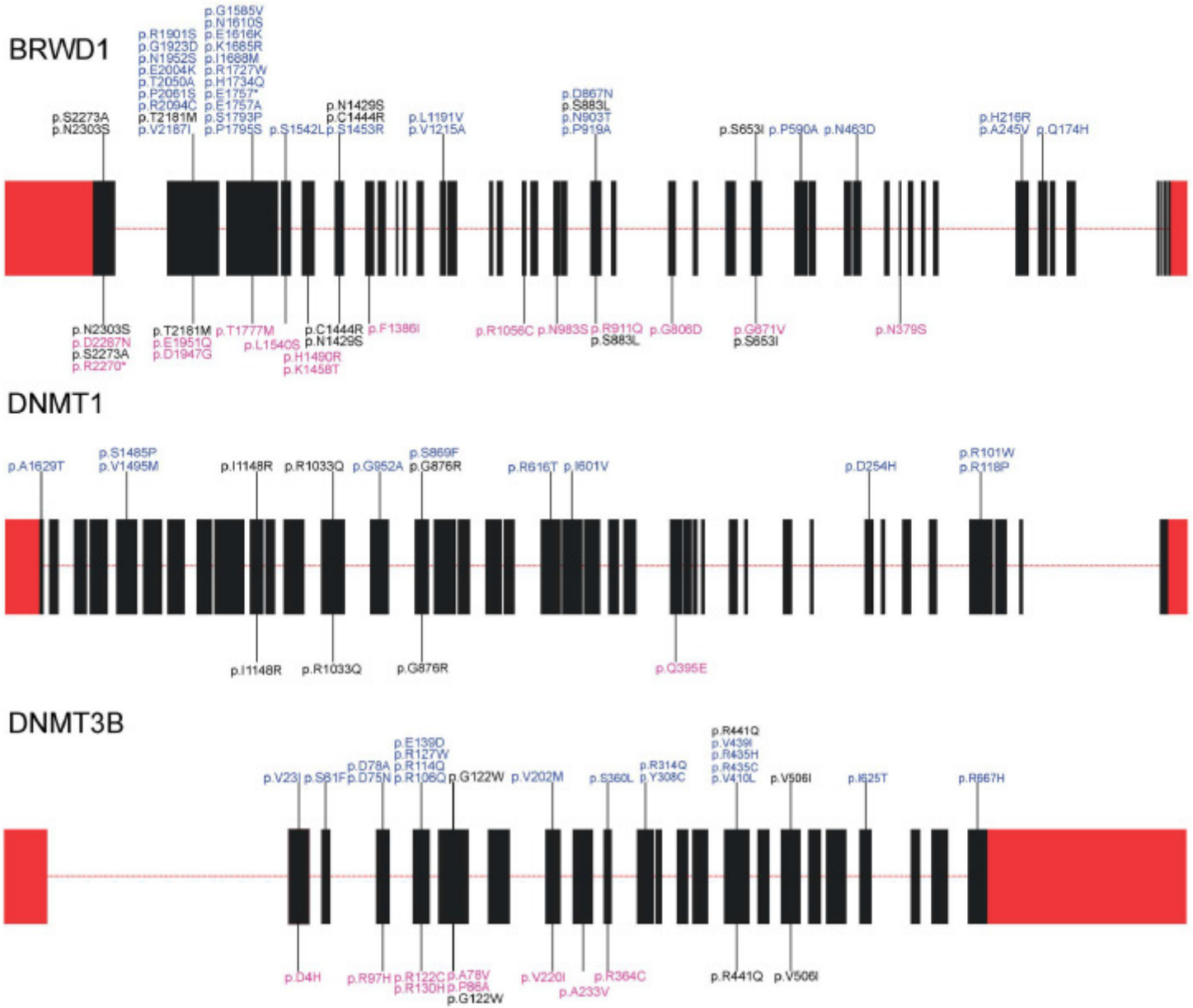


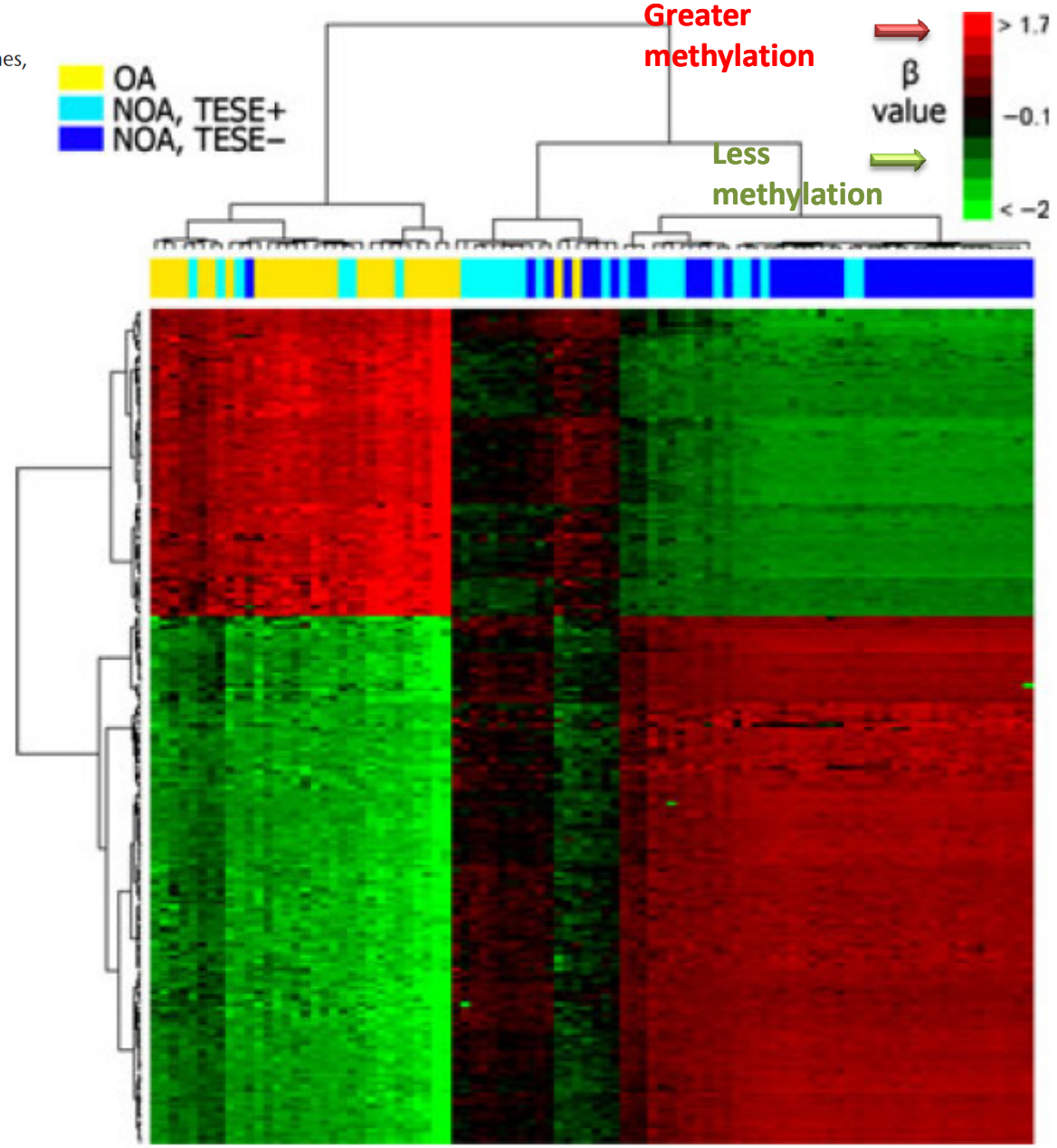
Figure 2 | Rare non-silent variants identified in genes that are key epigenetic regulators of spermatogenesis. Variants shown above the indicated gene maps were detected in NOA patients, and variants shown below the indicated gene maps were detected in controls. Rare variants that were identified in both the patient and control groups are colored black, and rare variants that were exclusive to the NOA patients and normal controls are colored blue and pink, respectively. Boxes labeled in red represent the UTRs and boxes labeled in black represent the exons.

A genome-wide DNA methylation study in azoospermia

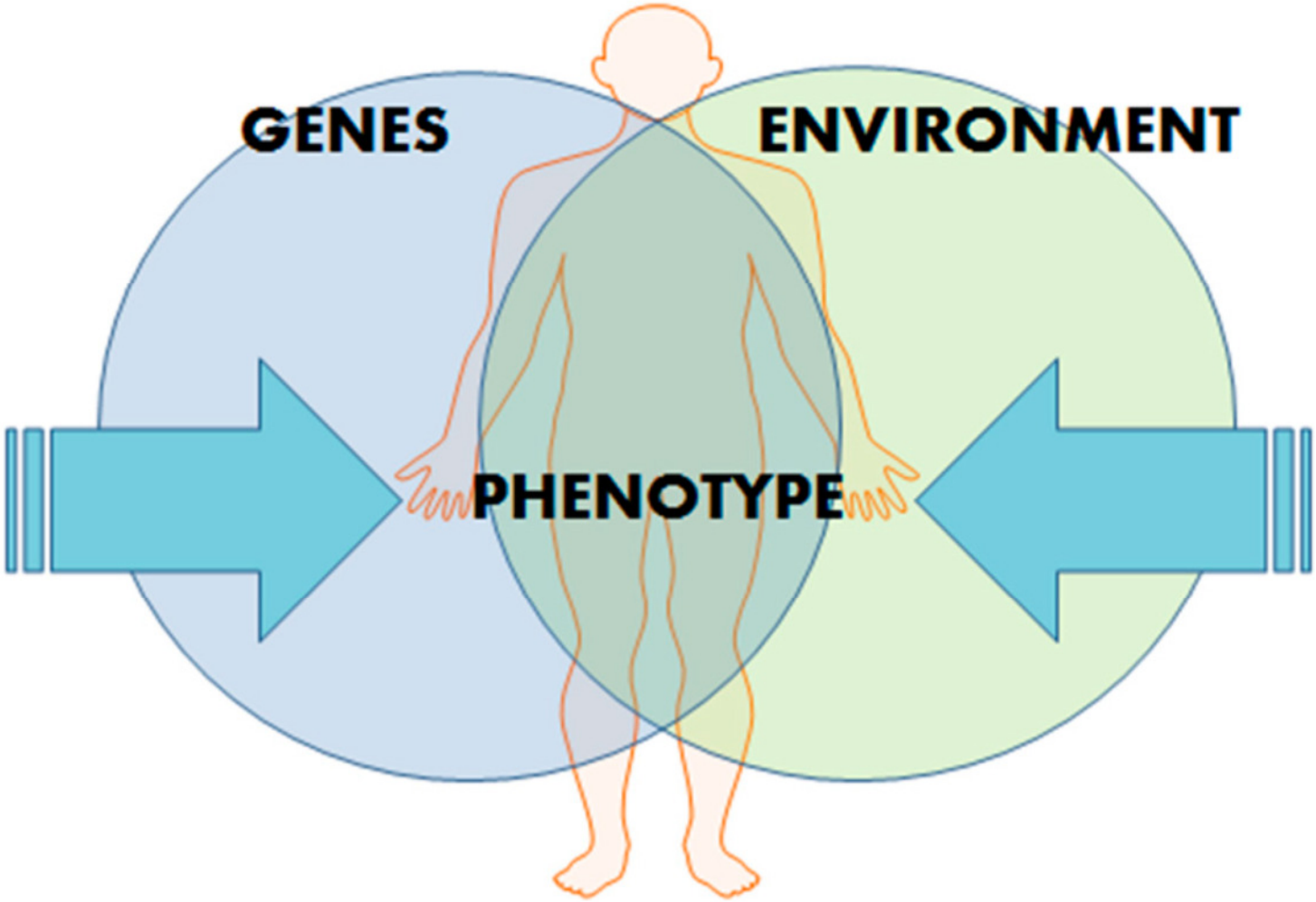
[1,2]F. Ferfouri, [1,2]F. Boitrelle, [3]I. Ghout, [1,2]M. Albert, [1,2]D. Molina Gomes, [1,2]R. Wainer, [1,2]M. Bailly, [1,2]J. Selva and [1,2]F. Vialard

ANDROLOGY

Andrology, 2013, 1, 815–821



Epigenetic transgenerational inheritance: phenotypes

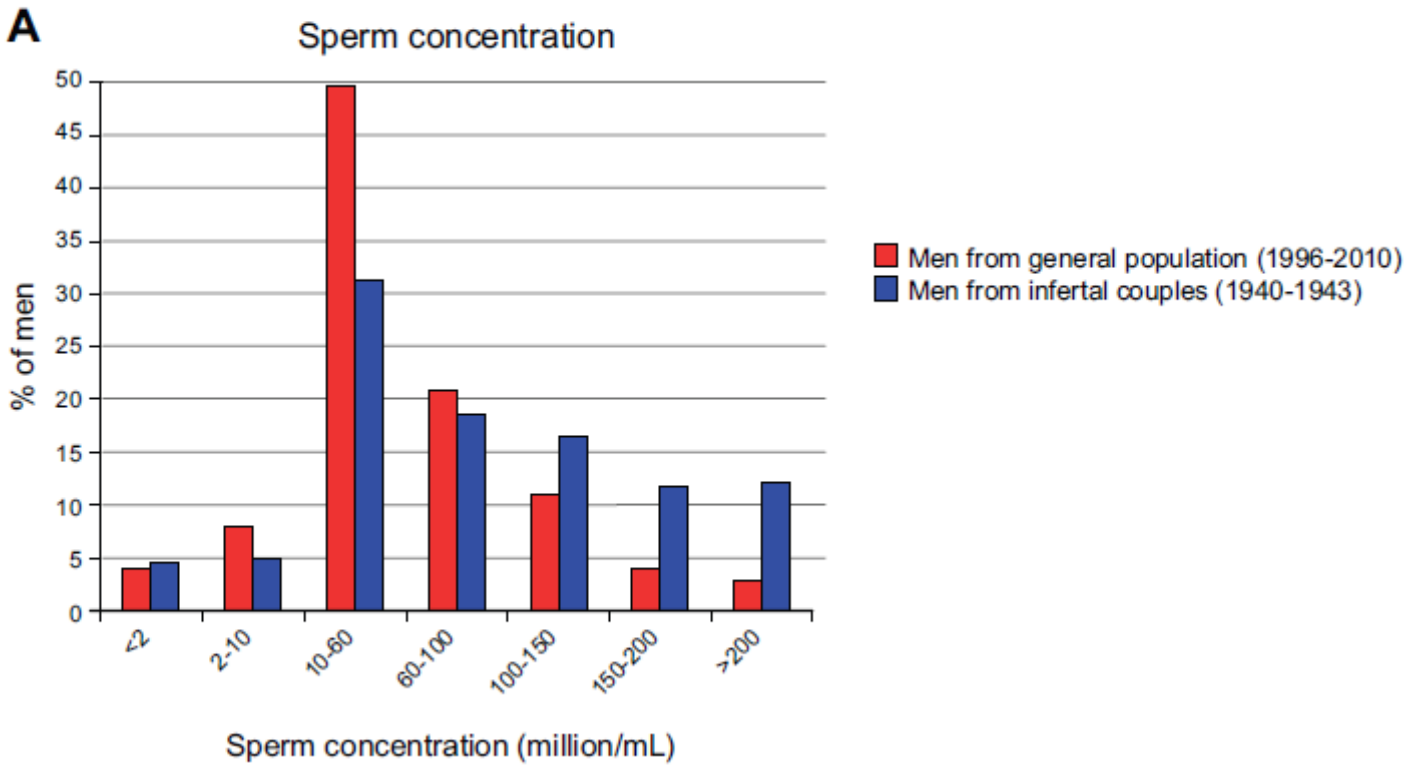


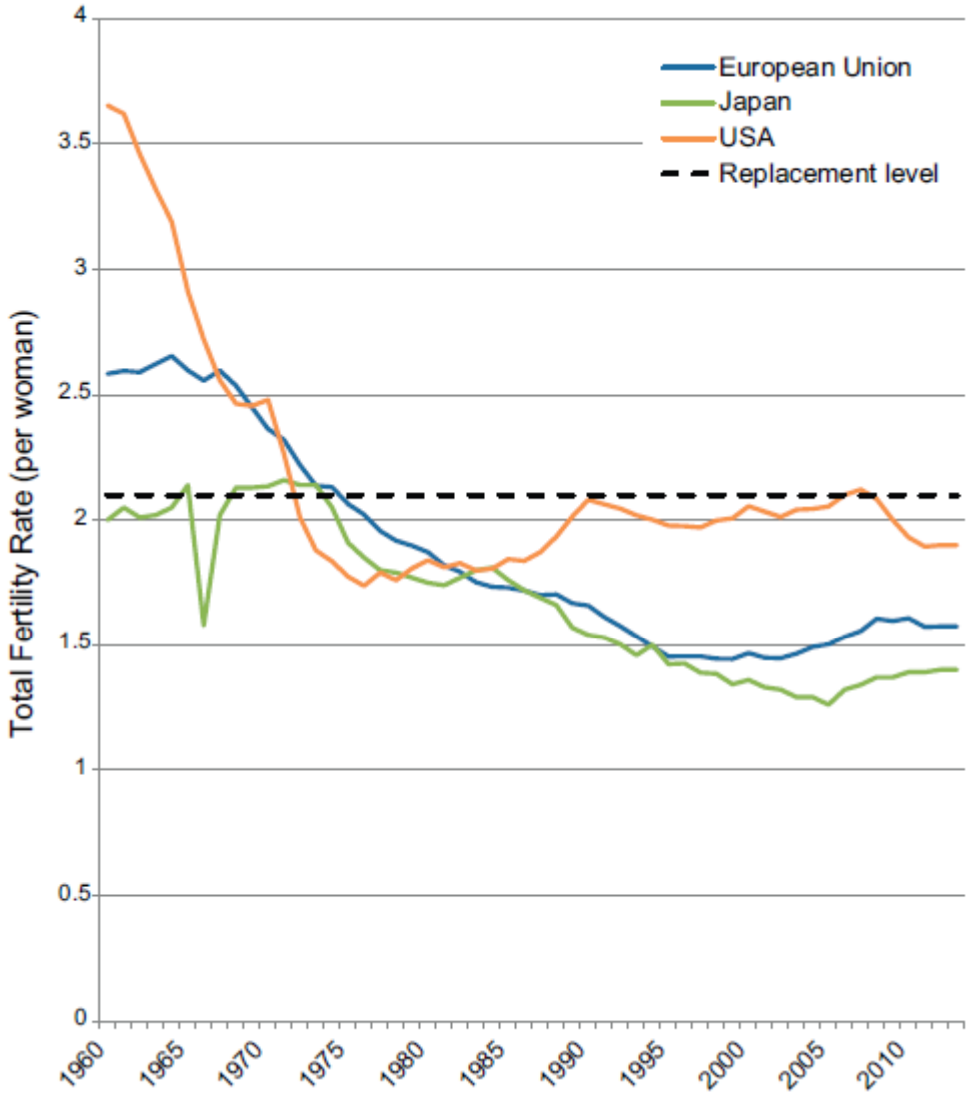
MALE REPRODUCTIVE DISORDERS AND FERTILITY TRENDS: INFLUENCES OF ENVIRONMENT AND GENETIC SUSCEPTIBILITY

Niels E. Skakkebaek, Ewa Rajpert-De Meyts, Germaine M. Buck Louis, Jorma Toppari, Anna-Maria Andersson, Michael L. Eisenberg, Tina Kold Jensen, Niels Jørgensen, Shanna H. Swan, Katherine J. Sapra, Søren Ziebe, Lærke Priskorn, and Anders Juul

Department of Growth & Reproduction and EDMaRC, Rigshospitalet, University of Copenhagen, Copenhagen, Denmark; Division of Epidemiology, Statistics and Prevention Research, Eunice Kennedy Shriver National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, Maryland; Department of Physiology & Pediatrics, University of Turku and Turku University Hospital, Turku, Finland; Male Reproductive Medicine & Surgery Program, Stanford University, Stanford, California; Icahn School of Medicine at Mount Sinai, New York, New York; and The Fertility Clinic, Rigshospitalet, Copenhagen, Denmark

Physiol Rev 96: 55–97, 2016





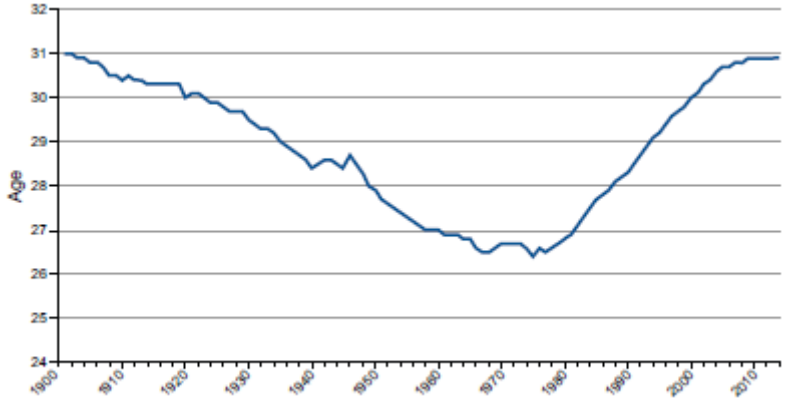
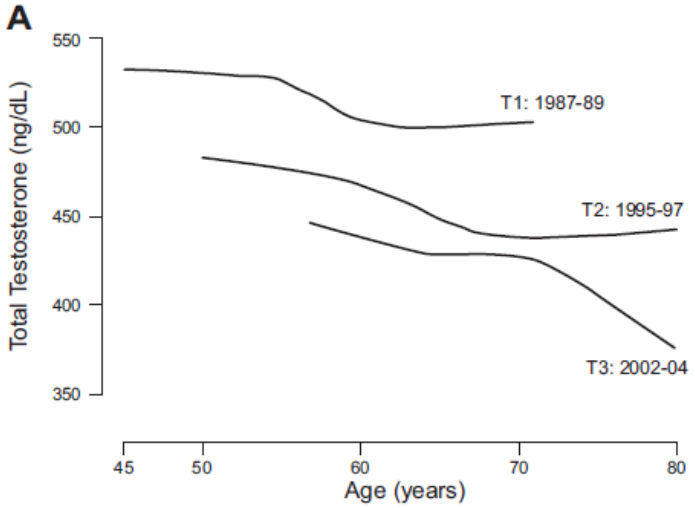


FIGURE 19. Mean ages of Danish women delivering from 1901–2014. From Statistics Denmark: http://www.statistikbanken.dk/statbank5a/default.asp?w=1600.



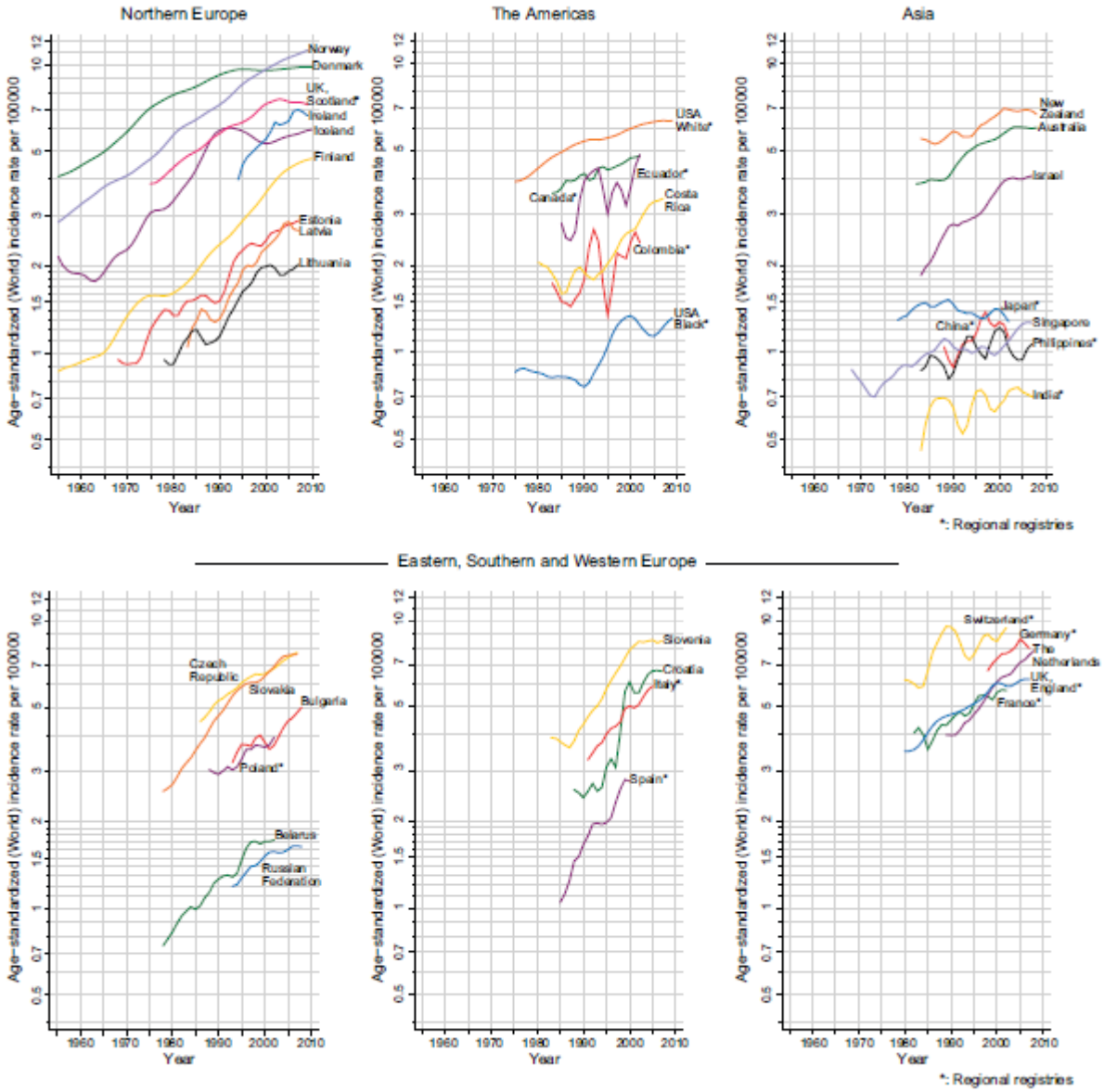


FIGURE 4. Trends in testicular cancer; age-standardized (world) incidence (regional or national), all ages.

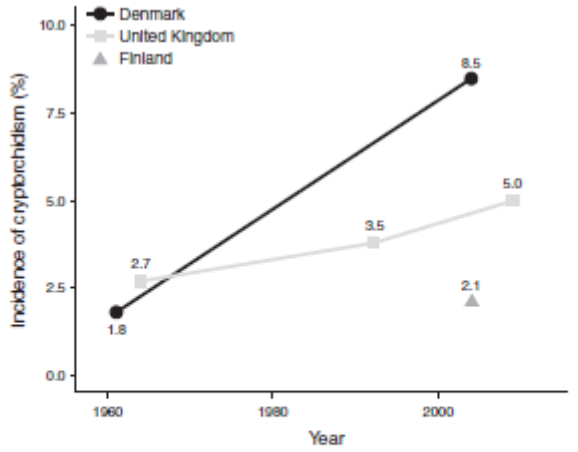


FIGURE 7. Incidence of cryptorchidism at birth on the basis of prospective clinical studies from the 1950s to the 2000s in Denmark, Finland, and United Kingdom. The data points are marked on the year of the publication of the study which represents the preceding incidence rate [3, 47, 61, 184, 377].

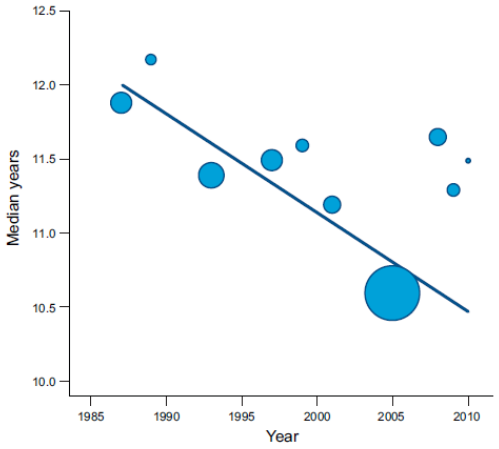
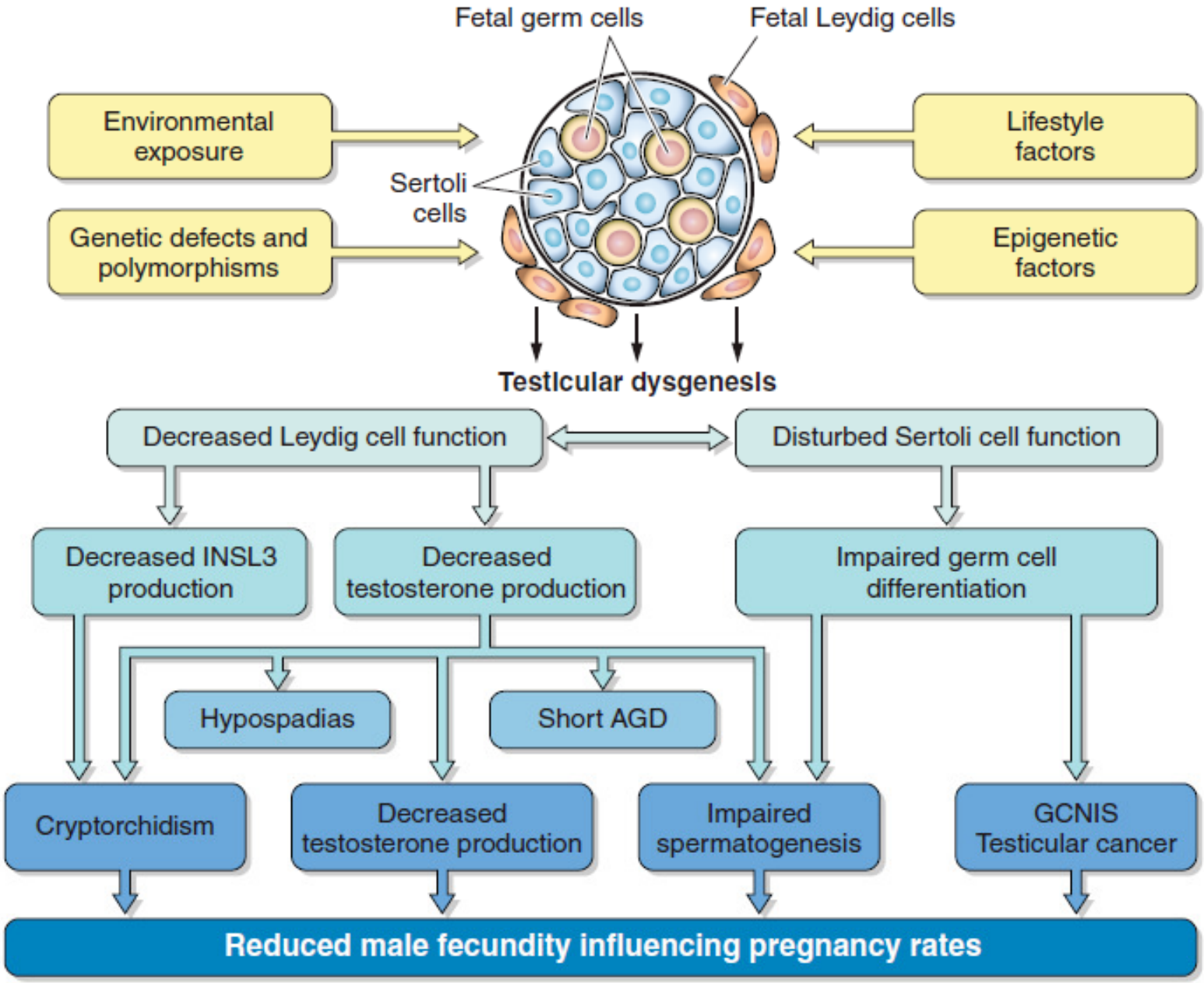


FIGURE 8. Recent changes in male pubertal timing. Testicular volume was >3 ml. [From Mouritsen et al. (293).]


Fetal germ cells
Fetal Leydig cells
Environmental exposure
Lifestyle factors
Sertoli cells
Genetic defects and polymorphisms
Epigenetic factors
Testicular dysgenesis
Decreased Leydig cell function
Disturbed Sertoli cell function
Decreased INSL3 production
Decreased testosterone production
Impaired germ cell differentiation
Hypospadias
Short AGD
Cryptorchidism
Decreased testosterone production
Impaired spermatogenesis
GCNIS Testicular cancer
Reduced male fecundity influencing pregnancy rates

Epigenetic transgenerational inheritance in F2 generation

Alterations in the sperm histone-retained epigenome are associated with unexplained male factor infertility and poor blastocyst development in donor oocyte IVF cycles

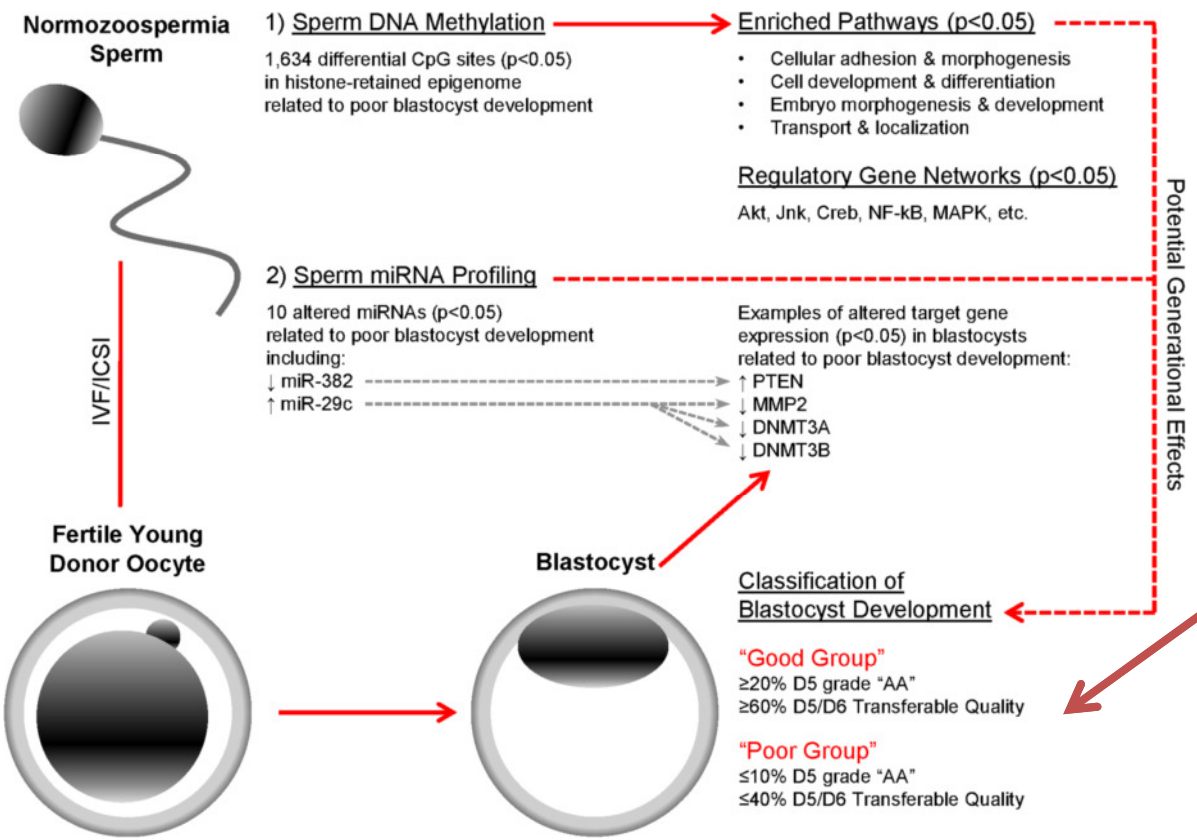
Michelle M. Denomme[1], Blair R. McCallie[1], Jason C. Parks[1], William B. Schoolcraft[2], and Mandy G. Katz-Jaffe[1,2,*]

Human Reproduction, Vol.32, No.12 pp. 2443–2455, 2017

Table I Summary of patient samples.

	Paternal age (years)	Semen parameters			
		Volume (mL)	Motility (%)	Count (million/mL)	Total motile count (million/mL)
Good Group	40.3 ± 1.3	2.9 ± 0.3	60.3% ± 3.1%	126.9 ± 14.1	221.7 ± 32.2
Poor Group	42.0 ± 1.1	3.4 ± 0.3	61.1% ± 2.6%	134.5 ± 18.8	290.4 ± 48.5
Significance (*t*-test); $P < 0.05$	0.311	0.306	0.851	0.753	0.255
	Maternal Age (years)	**Embryo Quality**			
		Oocytes Retrieved (#)	**Oocytes Fertilized (% of retrieved)**	**Grade D5 'AA' Blastocysts (% of fertilized)**	**Transferable D5&D6 Blastocysts (% of fertilized)**
Good Group	<33; donor oocyte	25.6 ± 1.7	93.3% ± 2.3%	36.5% ± 3.0%	73.0% ± 2.4%
Poor Group	<33; donor oocyte	20.8 ± 2.3	93.2% ± 2.2%	0.8% ± 0.4%	20.9% ± 2.3%
Significance (*t*-test); $P < 0.05$		0.101	0.965	**<0.001**	**<0.001**

Value after the ± reflect standard error of the mean. Bold values indicate statistically significant differences between groups.
Good Group: *n* = 25 couples; 20 sperm samples, 24 blastocyst samples.
Poor Group: *n* = 28 couples; 20 sperm samples, 24 blastocyst samples



Sperm samples (n = 40) and blastocysts (n = 48) were obtained during fertile donor oocyte IVF cycles with **normozoospermic parameters**, thereby **excluding known female and male infertility factors**. Samples were divided into two groups based on blastocyst development

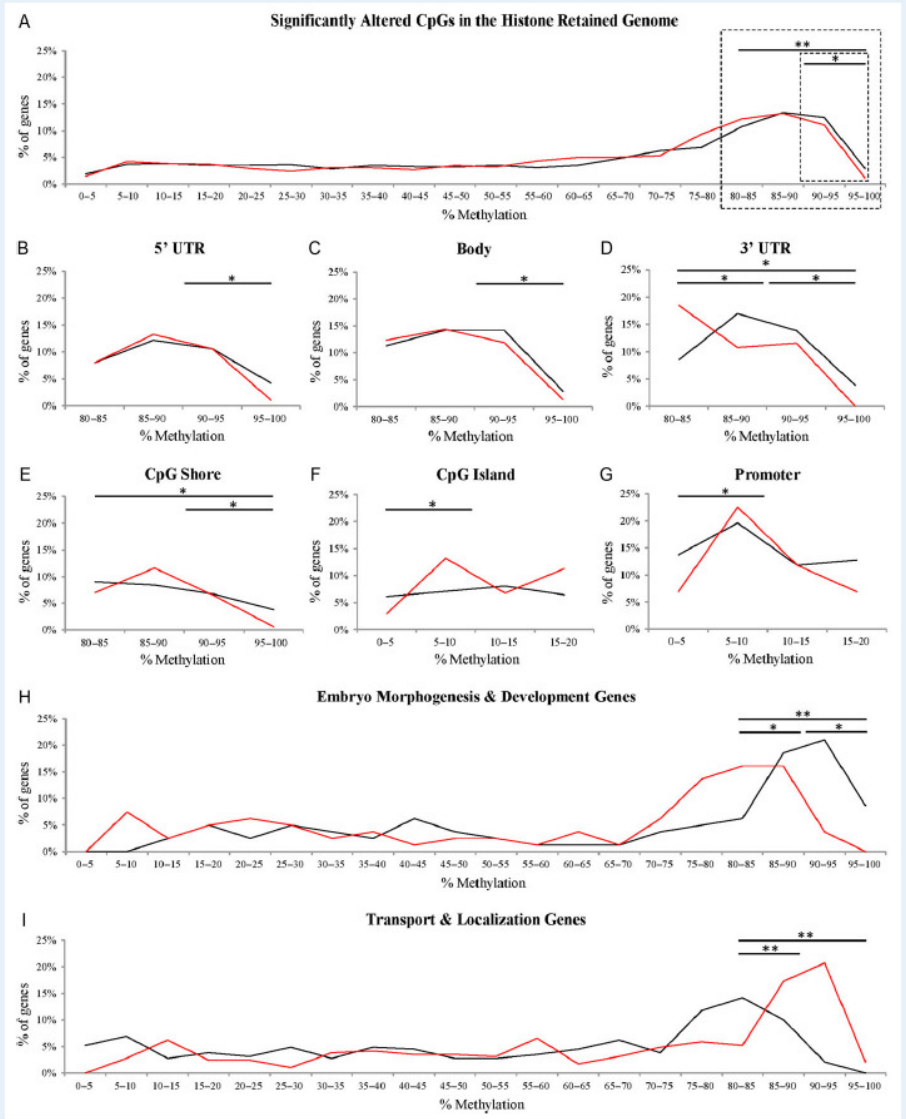


Figure 2 Sperm DNA methylation profiles of statistically significant genes for the histone-retained genome and across defined genomic regions. Mean methylation (β-values) for all significantly altered genes between the Good Group (black lines) and the Poor Group (red lines) distributed into 5% methylation intervals for (**A**) the histone-retained genome (1634 CpGs), (**B–E**) hypermethylated genomic regions: 5′UTR (189 CpGs), body (1185

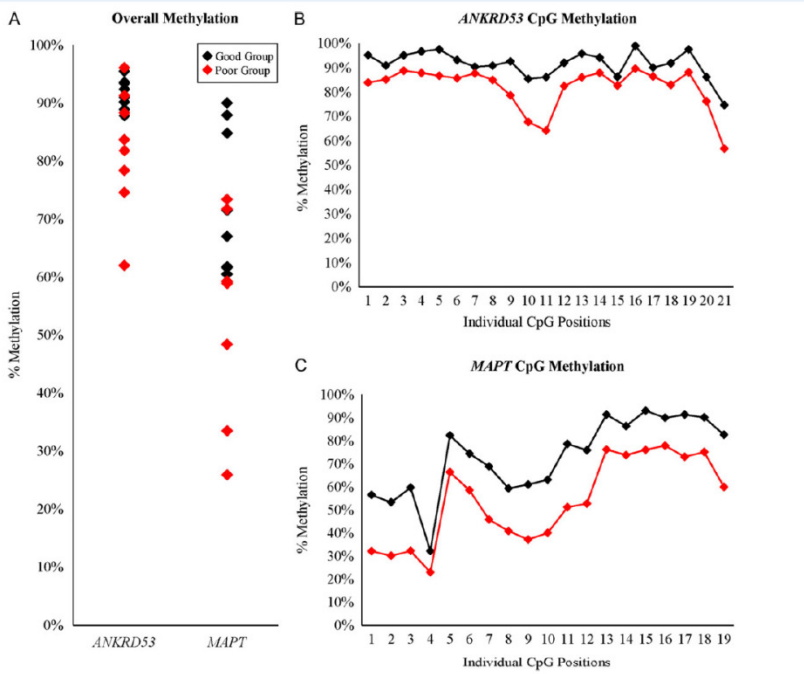
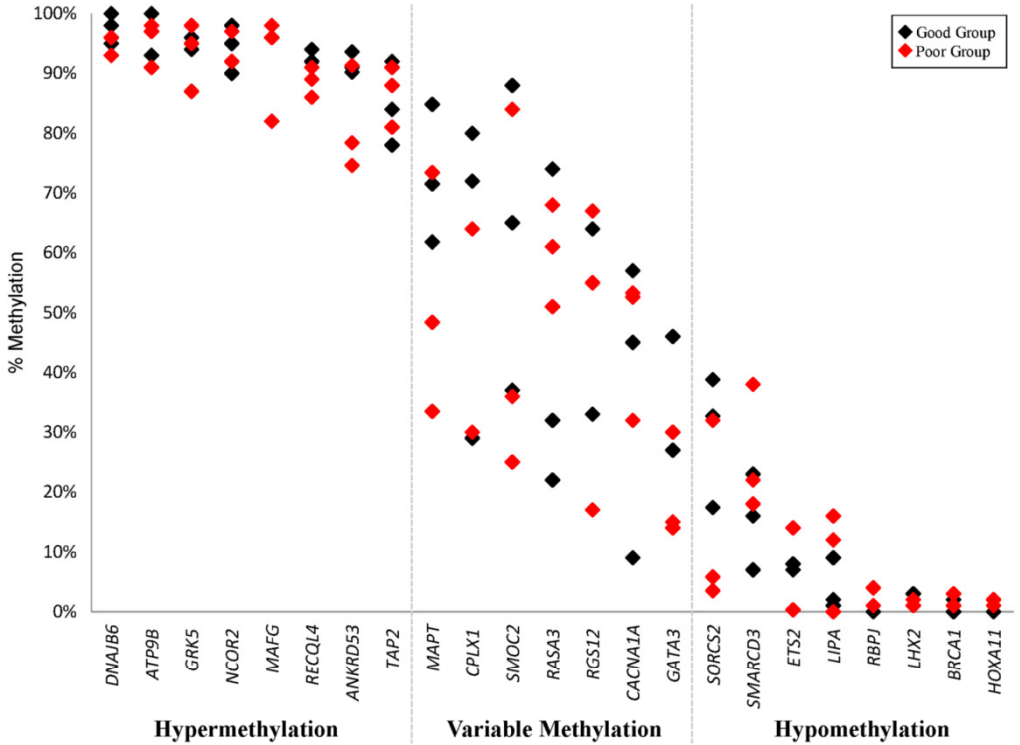
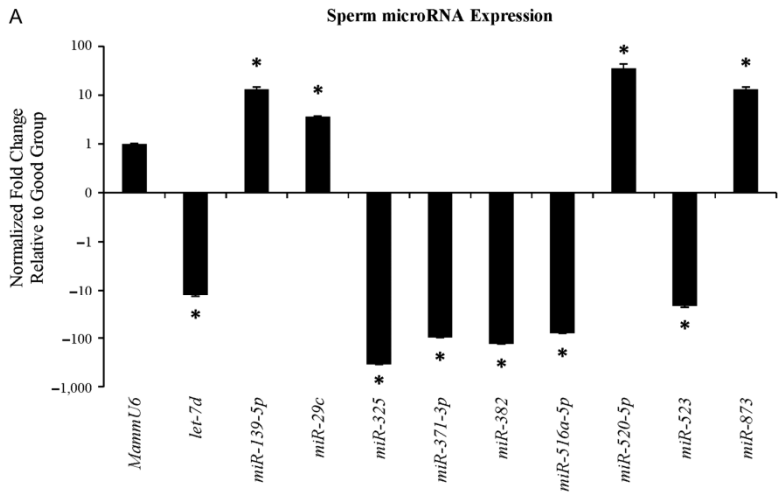
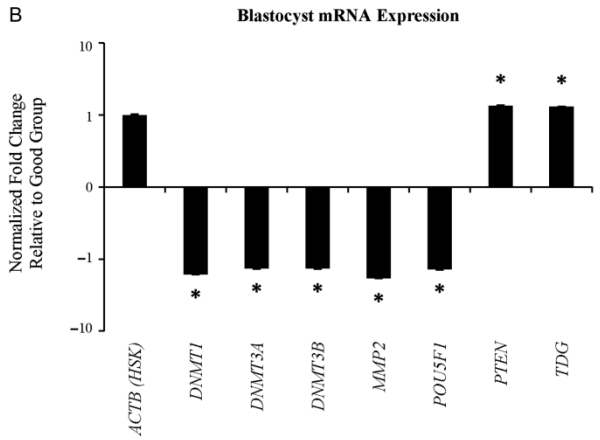


Figure 6 Sperm DNA methylation percentages at *ANKRD53* and *MAPT*. Targeted DNA methylation analysis in individual normozoospermic samples ($n = 16$) at *ANKRD53* and *MAPT*. Black diamonds represent sperm from the Good Group, and red diamonds represent sperm from the Poor Group. (**A**) Each diamond is positioned at the average percent methylation for the individual sperm sample. (**B**) Average percent methylation is distributed by the 21 CpG positions at *ANKRD53* for both groups (Good Group: 91.6%; Poor Group: 82.0%; $P < 0.05$). (**C**) Average percent methylation is distributed by the 19 CpG positions at *MAPT* for both groups (Good Group: 73.1%; Poor Group: 53.8%; $P < 0.05$).



Ten significantly altered sperm miRNAs ($P < 0.05$), correlated with altered target gene mRNA expression in the blastocysts from the Poor Group ($P < 0.05$).





DNA methylation differences between *in vitro*- and *in vivo*-conceived children are associated with ART procedures rather than infertility

Sisi Song[1], Jayashri Ghosh[1], Monica Mainigi[2], Nahid Turan[1], Rachel Weinerman[2], May Truongcao[1], Christos Coutifaris[2*] and Carmen Sapienza[1,3*]

Clinical Epigenetics (2015) 7:41

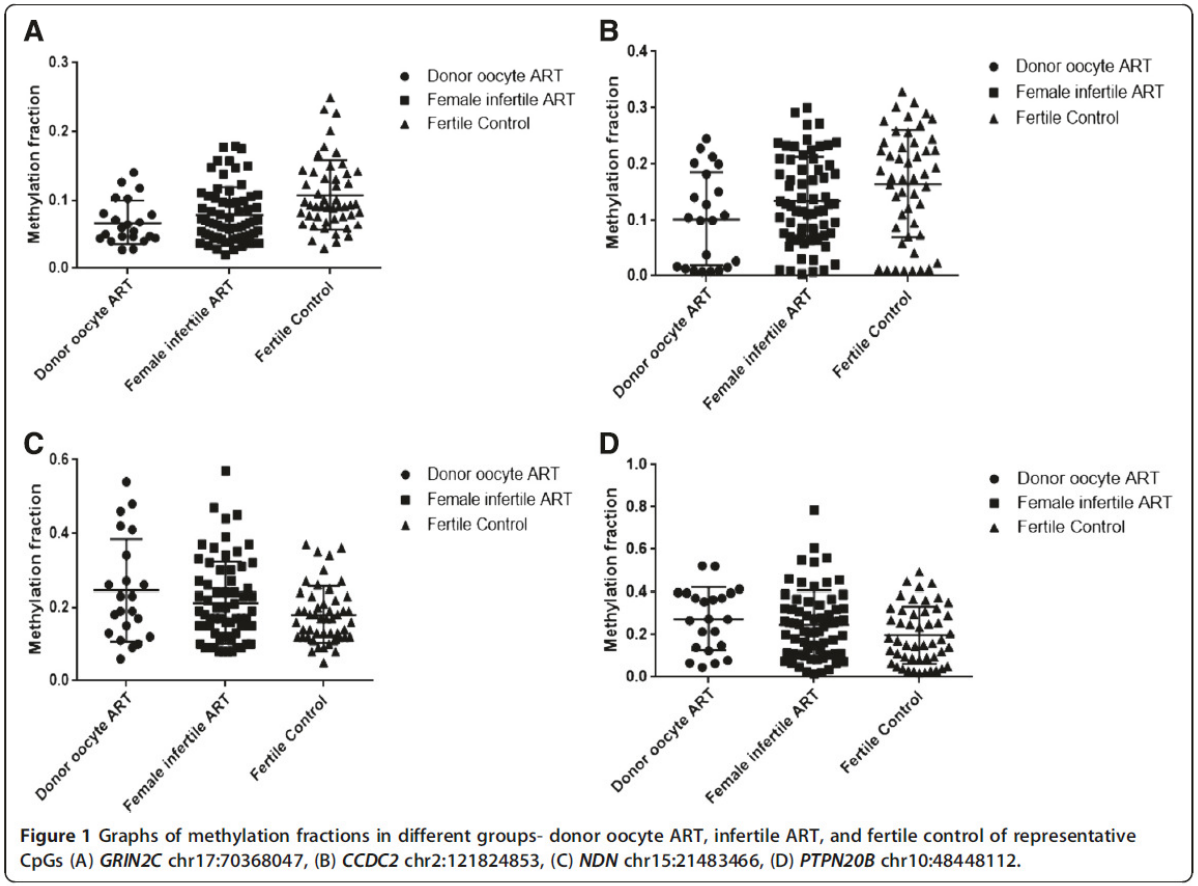


Figure 1 Graphs of methylation fractions in different groups- donor oocyte ART, infertile ART, and fertile control of representative CpGs (A) *GRIN2C* chr17:70368047, (B) *CCDC2* chr2:121824853, (C) *NDN* chr15:21483466, (D) *PTPN20B* chr10:48448112.

Placental DNA methylation of genes previously identified as being differentially methylated between in vitro- and in vivo-conceived children

The epigenetic control of transposable elements and imprinted genes in newborns is affected by the mode of conception: ART versus spontaneous conception without underlying infertility

C Choux C Binquet V Carmignac C Bruno C Chapusot J Barberet M Lamotte P SagotD Bourc'his P Fauque

Human Reproduction – epub ahead of print **December 11, 2017**

A total of 51 IVF/ICSI (15 conventional and 36 ICSI) singleton pregnancies were prospectively included from January 2013 to April 2015 and compared to 48 spontaneously conceived singleton pregnancies.

The DNA methylation and transcription of three imprinted loci (*H19/IGF2, KCNQ1OT1* and *SNURF* DMRs) and four transposon families (LINE-1, ERVFRD, AluYa5 and ERVW) in cord blood and placenta obtained at birth were assessed by pyrosequencing and quantitative RT-PCR, respectively. All data were adjusted for gestational age at delivery, sex of the newborn, parity and maternal age.

MAIN RESULTS AND THE ROLE OF CHANCE

DNA methylation levels of *H19/IGF2*, *KCNQ1OT1*, LINE-1Hs and ERVFRD-1 **were significantly lower in IVF/ICSI placentas than in control placentas**, while there was no difference for cord blood. Moreover, the expression of ERVFRD-1 and LINE-1 ORF2 in cord blood and ERVFRD-1 in placenta was lower in the IVF/ICSI group than in controls. **The expression of ERVFRD-1 in placenta correlated positively with birth weight and placenta weight**, but only in the control group, thus pointing to the potential deregulation of syncytin function after ART.

WIDER IMPLICATIONS OF THE FINDINGS

These results should encourage us to analyze the exact causes and consequences of epigenetic changes and strive to minimize these variations in the interests of epigenetic safety after ART

Inter- and Intra-Individual Variation in Allele-Specific DNA Methylation and Gene Expression in Children Conceived using Assisted Reproductive Technology

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A, B, C cord blood
D, E, F placenta

P=0,0019

P<0,0001

P=0,0039

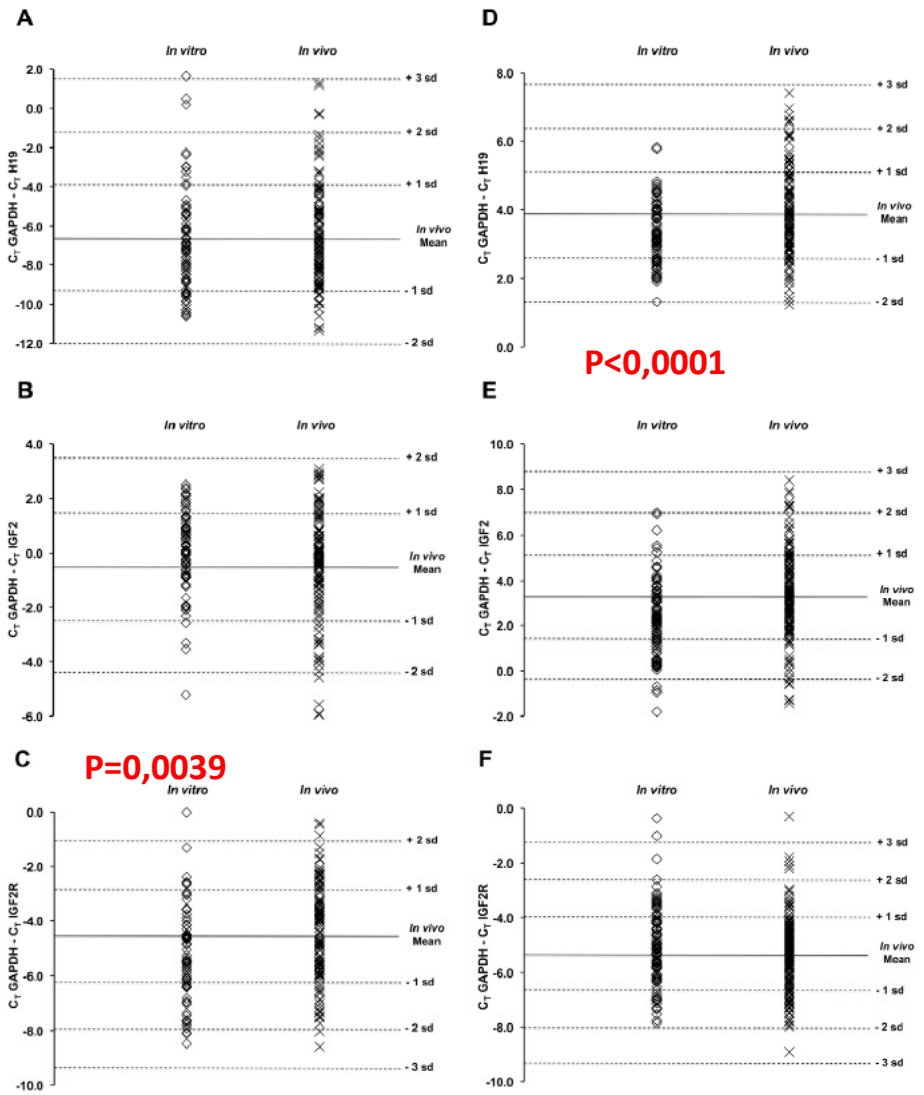

Figure 4. Scatter plots showing mRNA transcript levels in the *in vitro* and *in vivo* populations. Each symbol represents the mRNA level in one individual. (A) H19 in cord blood (*in vitro* n = 73, *in vivo* n = 118, fold change 0.88, $P = 0.8774$), (B) IGF2 in cord blood (*in vitro* n = 77, *in vivo* n = 116, fold change 1.03, $P = 0.5551$), (C) IGF2R in cord blood (*in vitro* n = 75, *in vivo* n = 121, fold change 0.61, $P = 0.0039$), (D) H19 in placenta (*in vitro* n = 84, *in vivo* n = 135, fold change 0.72, $P = 0.0193$), (E) IGF2 in placenta (*in vitro* n = 98, *in vivo* n = 160, fold change 0.52, $P < 0.0001$), and (F) IGF2R in placenta (*in vitro* n = 97, *in vivo* n = 148, fold change 1.18, $P = 0.2227$).
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A systematic review and meta-analysis of DNA methylation levels and imprinting disorders in children conceived by IVF/ICSI compared with children conceived spontaneously

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human reproduction update

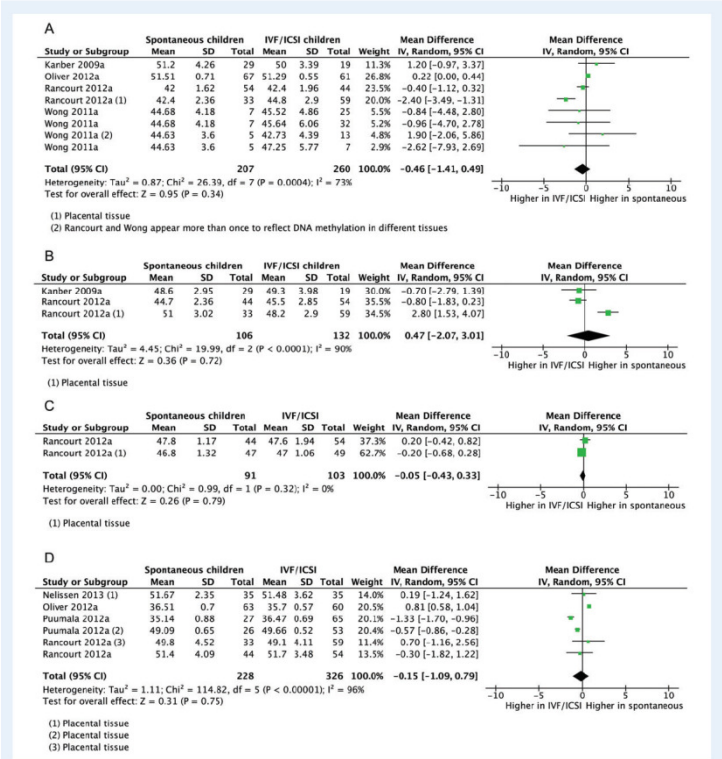


Figure 3 Forest plot analyses for weighted mean difference (95% confidence intervals) in methylation percent between IVF/ICSI versus spontaneously conceived children for (**A**) H19, (**B**) PEG1/MEST, (**C**) GRB10, (**D**) IGF2, (**E**) SNRPN, (**F**) KvDMR/KCNQ10T1 and (**G**) PEG3.

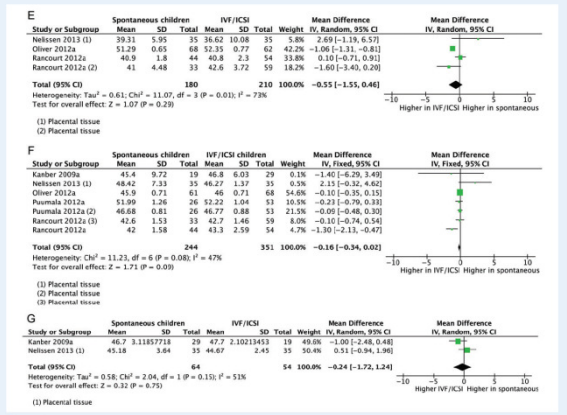


Figure 3 Continued.

Specific regions

Risk of any imprinting disorder

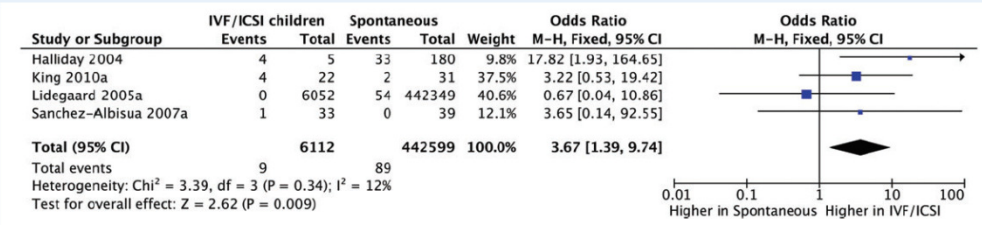
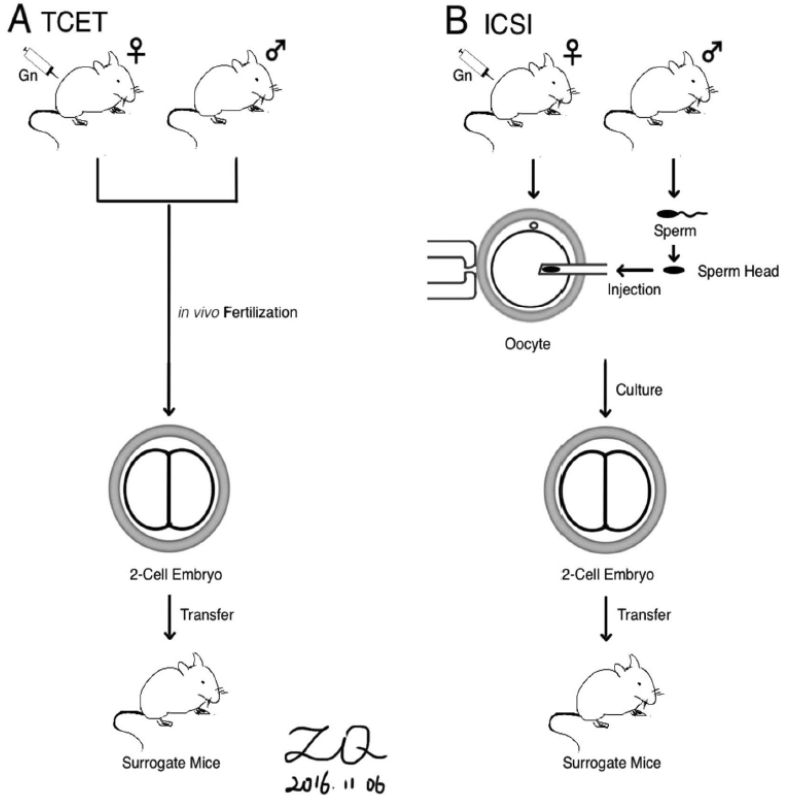


Figure 4 Forest plot analyses for risk of any imprinting disorder between IVF/ICSI versus spontaneously conceived children.

Altered methylations of H19, Snrpn, Mest and Peg3 are reversible by developmental reprogramming in kidney tissue of ICSI-derived mice



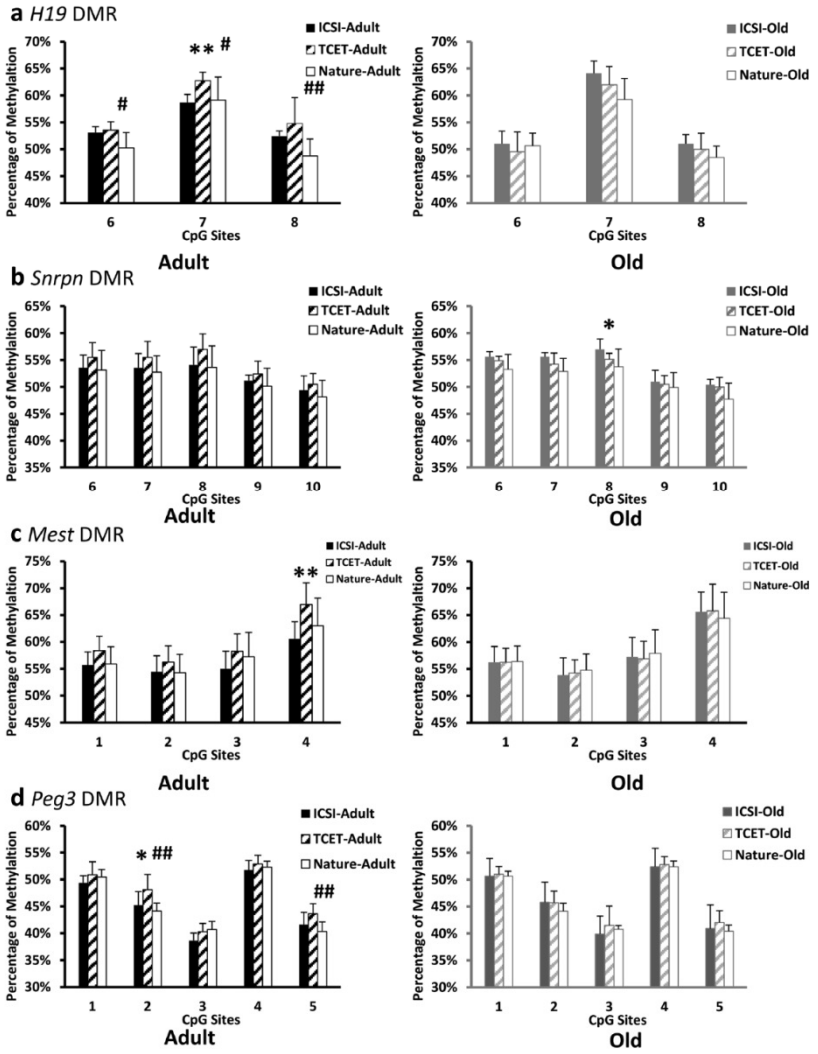


Figure 3. Pyrosequencing analyses of the methylation profiles from ICSI-derived, TCET conceived and natural mating mice of adult and old age. There are 3 CpG sites for the *H19* DMR (**a**), 5 CpG sites for the *Snrpn* DMR (**b**), 4 CpG sites for the *Mest* DMR (**c**) and 5 CpG sites for the *Peg3* DMR (**d**) (t-test was used for the analysis. ICSI versus TCET: $^{**}p < 0.01$; $^{*}p < 0.05$. TCET versus Nature: $^{\#\#}p < 0.01$; $^{\#}p < 0.05$). The pyrogram of pyrosequencing of one sample in each gene and each group was shown in Supplementary Figure S1.

Conclusioni

- Le patologie cromosomiche e genetiche sono causa di infertilità in non più del 5% dei casi
- Le alterazioni epigenetiche sembrano, invece, interessare una fascia più ampia di pazienti
- Le modificazioni epigenetiche indotte da fattori ambientali sono trasmissibili per via transgenerazionale
- L'esposizione attuale a fattori di rischio potrebbe compromettere la salute delle generazioni successive
- **IVF outcome: LBR or Healthy babies?**
- Non è ancora chiaro se le alterazioni epigenetiche transgenerazionali possano essere reversibili