Could germline genome editing be a treatment of male infertility?

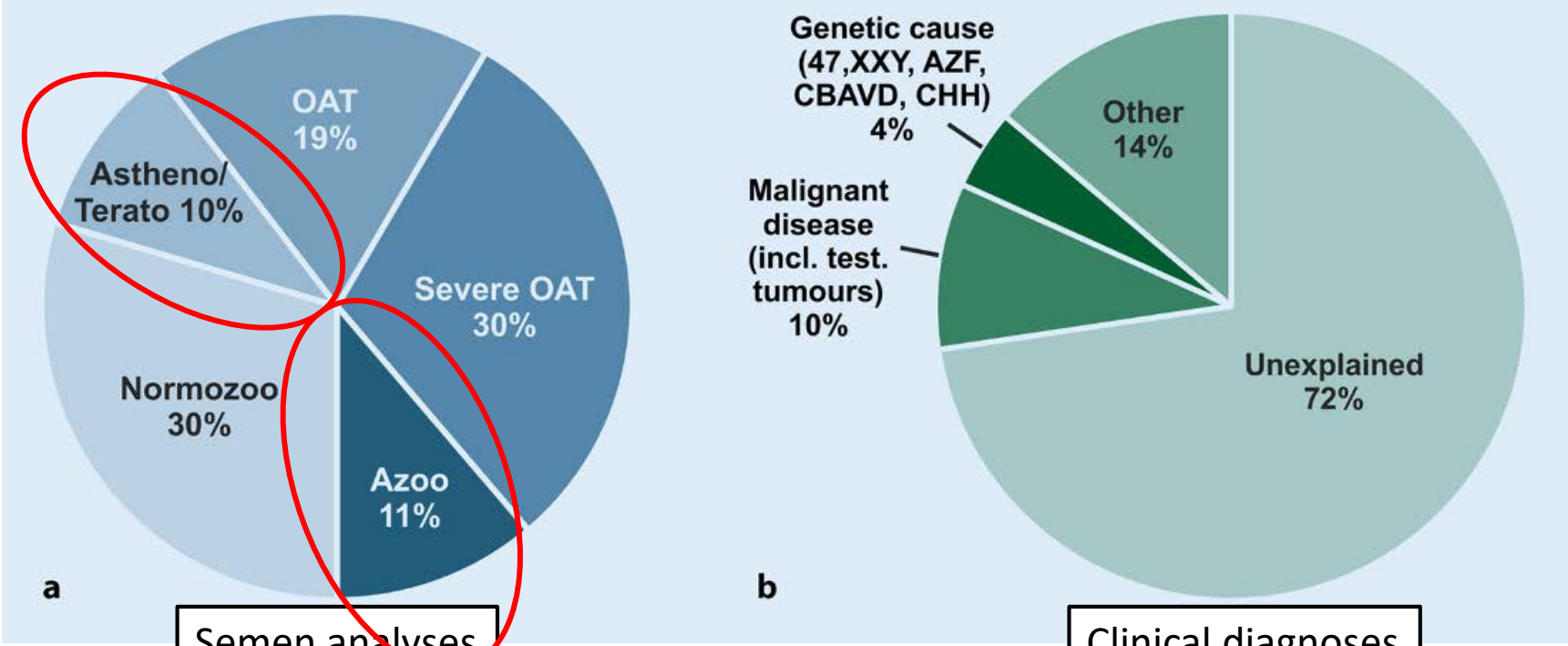
Pierre Jouannet
Université Paris Descartes

- Male infertility background
- Why gene editing should be considered to treat male inferility
- How gene editing could be used to treat male infertility

Second Human Genome Germline Editing commission meeting
London 14 November 2019

Male infertility background

26 091 men from infertile couples, Münster Andrology Centre (Tüttelmann *et al.* Medizinische Genetik 2018)



Semen analyses

Clinical diagnoses

Male infertility background

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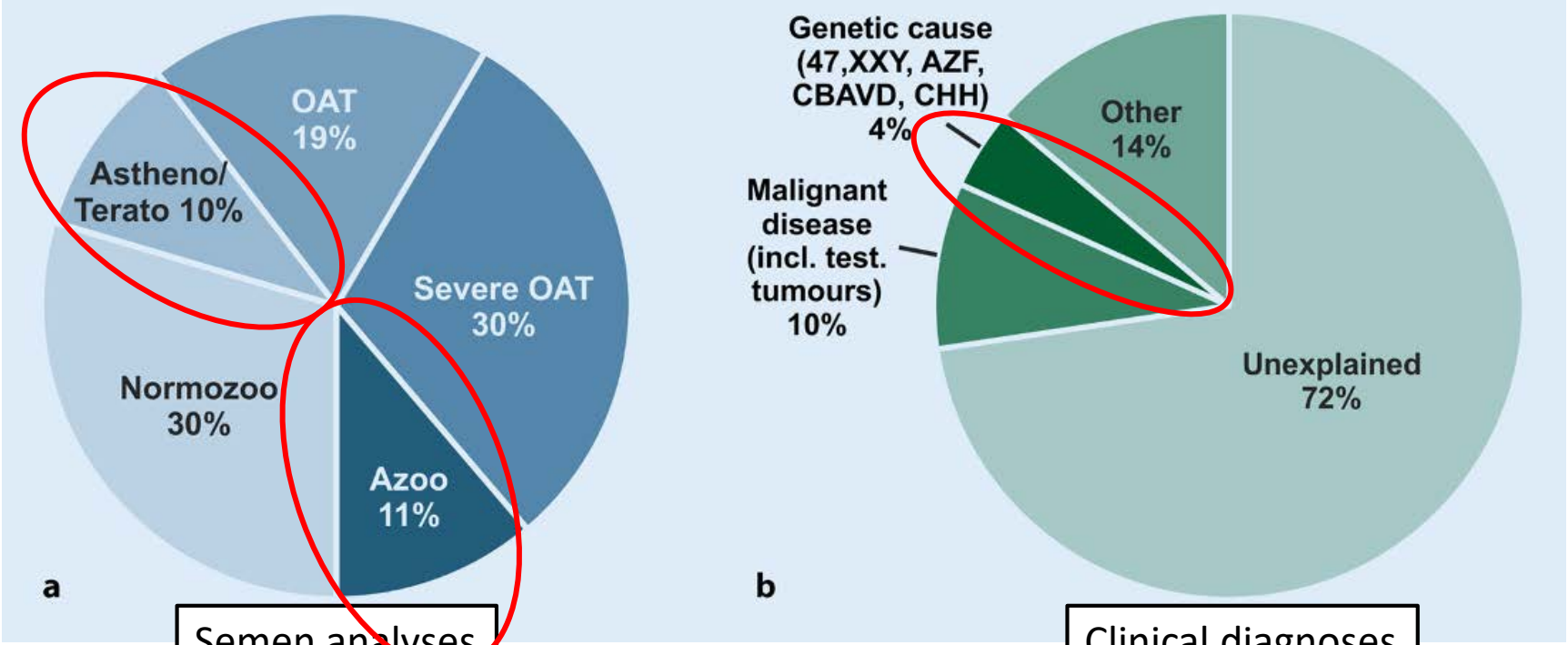


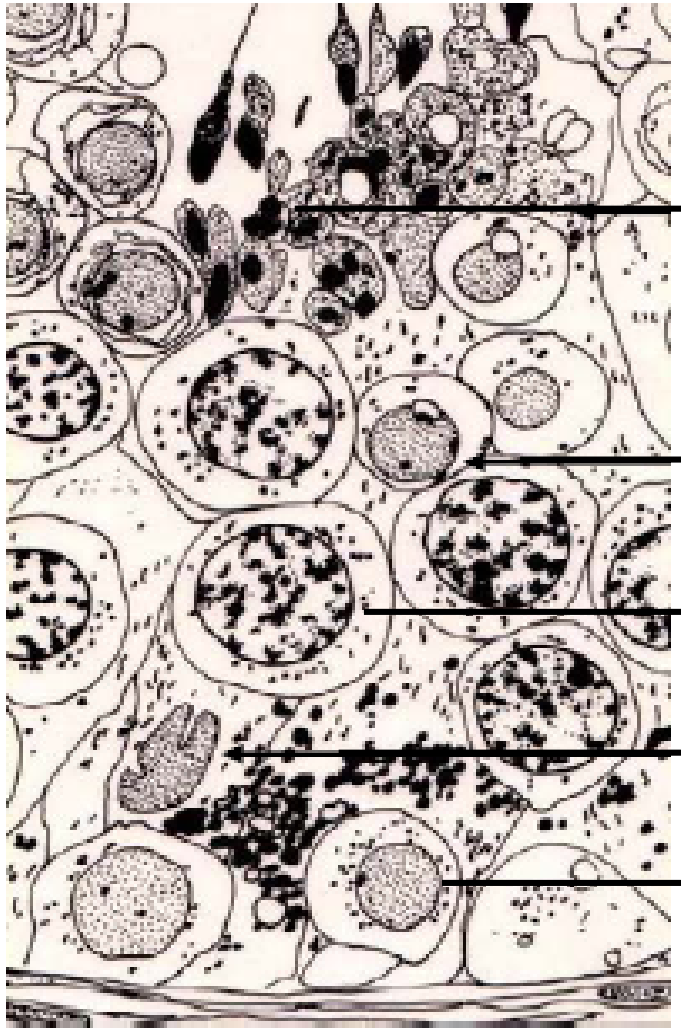
Semen analyses

Clinical diagnoses

Etiology
- Pretesticular
- Testicular → Spermatogenic failure
  - Genetic
    - Gene
    - Y deletion
    - Chromosome
  - Other (congenital, pathologies, iatrogenic…)
- Post testicular

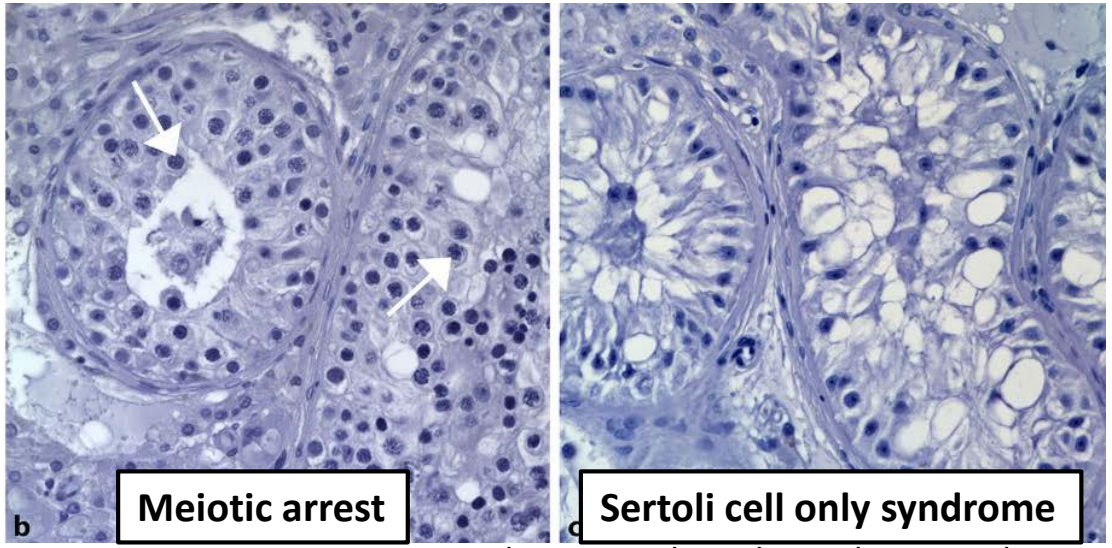
Spermatogenesis

Normal spermatogenis



AF Holstein *et al.* Reproductive Biology and Endocrinology 2003

Quantitative spermatogenic failure



Tüttelmann *et al.* Medizinische Genetik 2018

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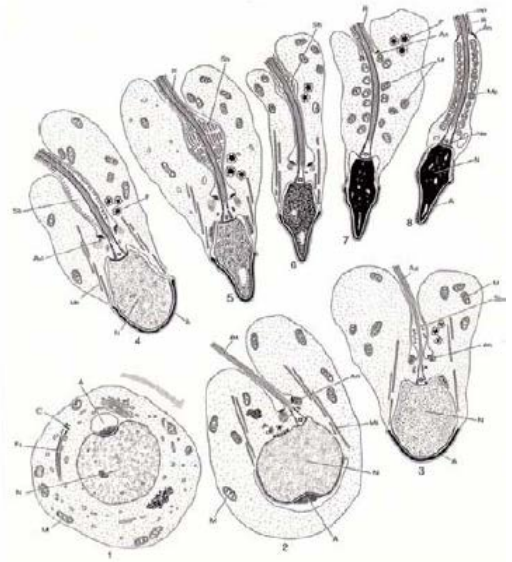
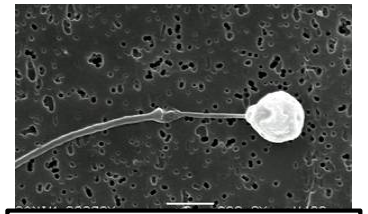


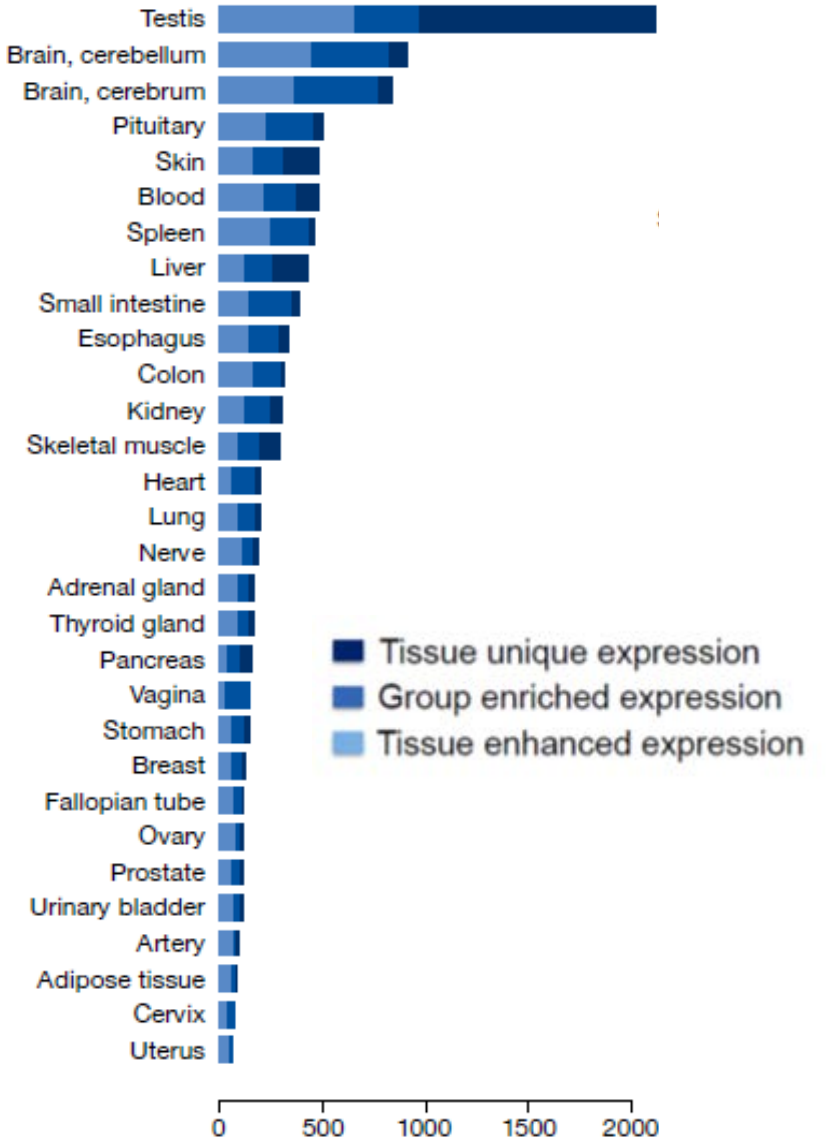

Macrozoospermia


Globozoospermia


Flagellar anomalies

C Arnoult

Genetic, testis function and male infertility



Number of protein-coding genes according to the Genome-based Tissue Expression (GTEx) consortium

Keen and Moore J Pers Med 2015; M Uhlén *et al* Mol Syst Biol 2016

Oud *et al* Human Reprod 2019

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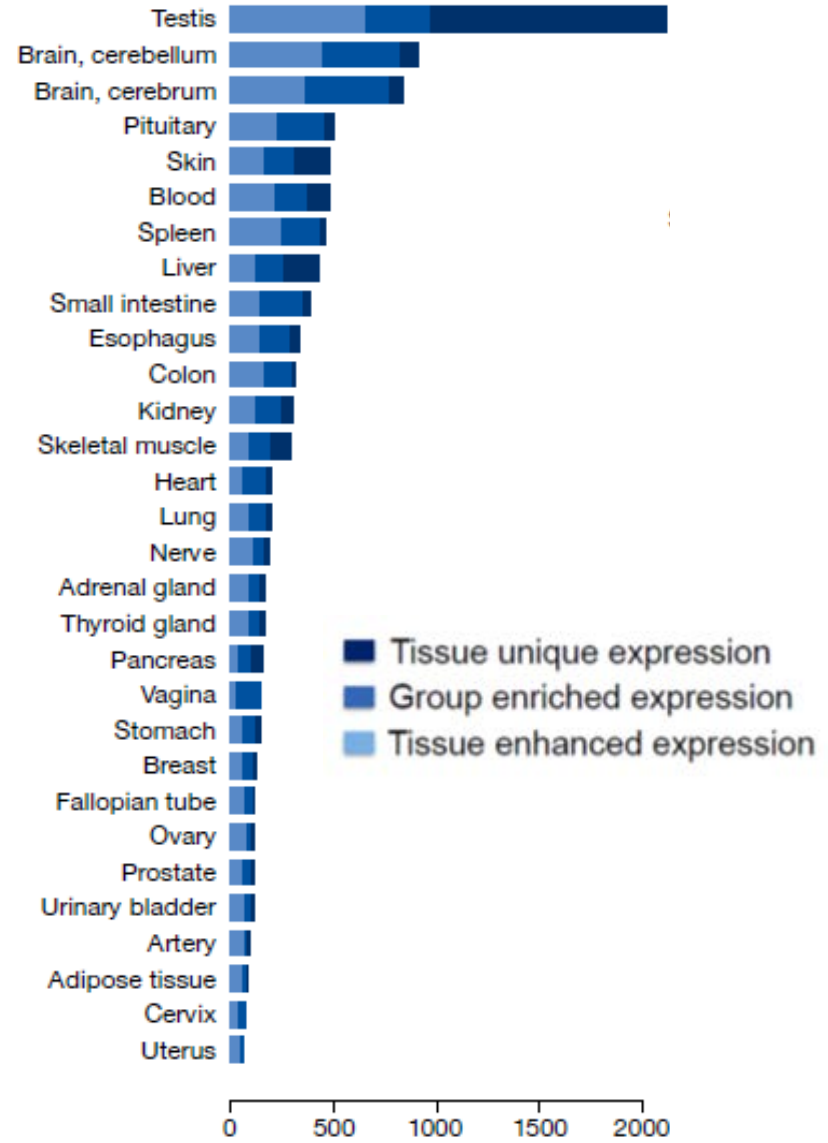

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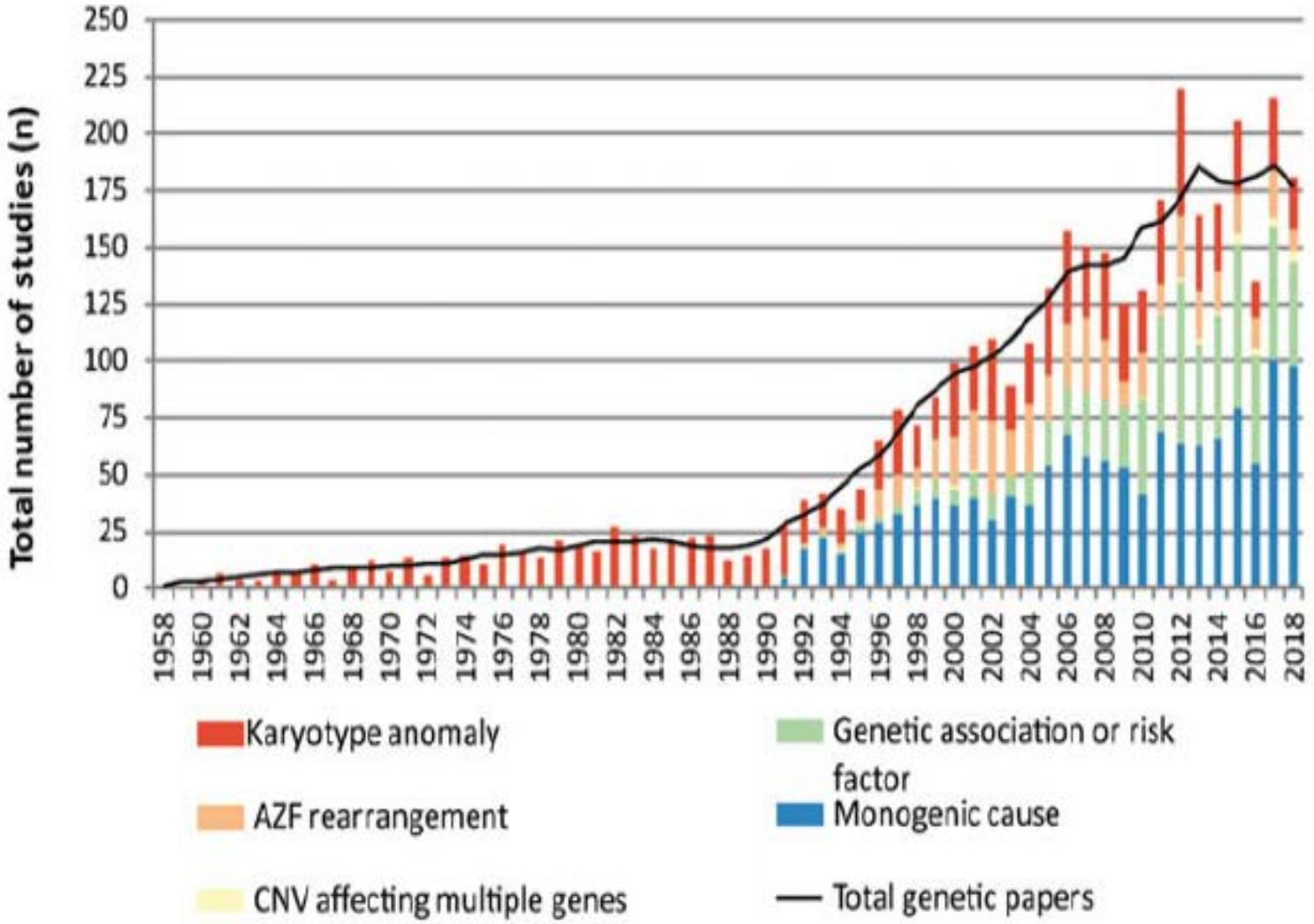
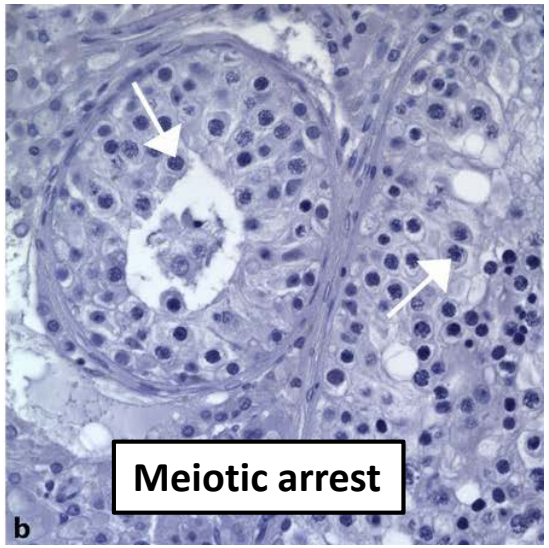
Genetic studies in Male infertility



Oud *et al* Human Reprod 2019

Single gene defect linked to quantitative spermatogenic anomalies

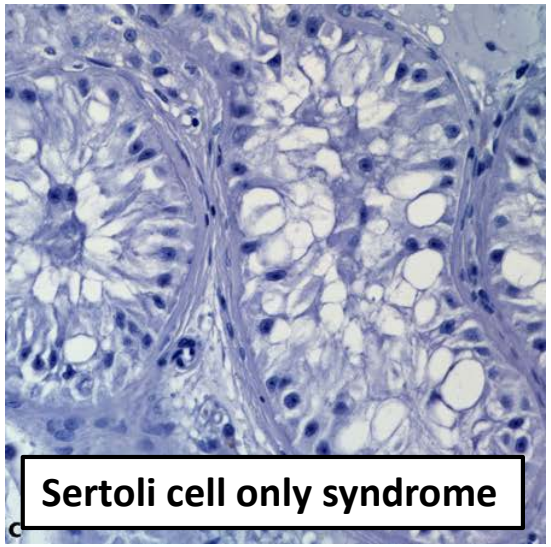


TEX11
Yatsenko *et al* N Engl J Med 2015

TEX15
Okutman *et al* Hum Mol Genet 2015

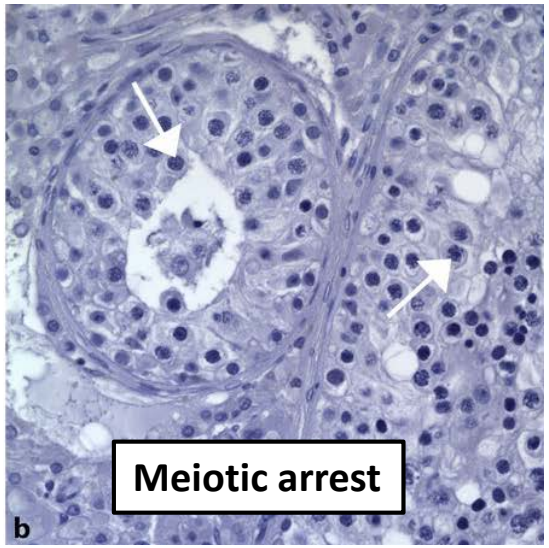
MCM8
Tenenbaum-Rakover *et al* J Med Genet 2015

SYCE1
Maor-Sagie *et al* J Assist Reprod Genet 2015



FANCM
Kasak *et al* Am J Hum Genetic 2018

Single gene defect linked to quantitative spermatogenic anomalies



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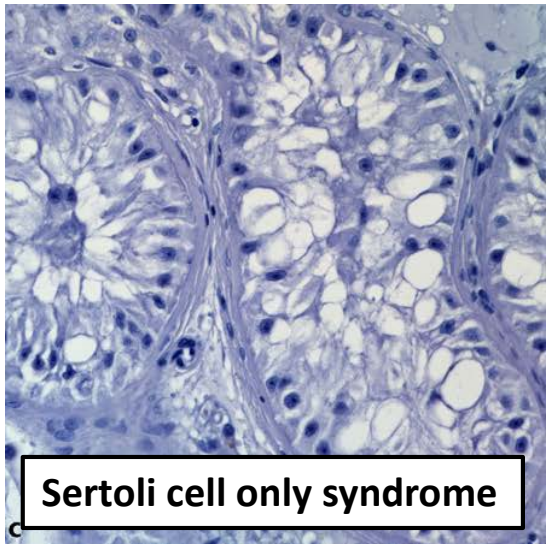

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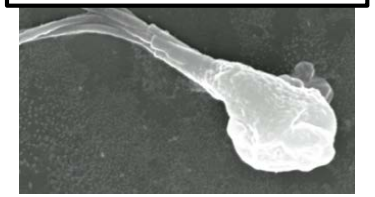
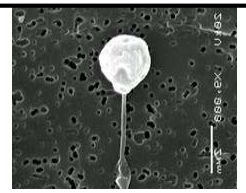
Single gene defect linked to Qualititative spermatogenic anomalies

Macrozoospermia



AURKC
Ben Khelifa *et al* Mol Hum Reprod 2011

Globozoospermia



DPY19L2
Harbuz *et al* Am J Hum Genet 2011

Acrosome biogenesis and azoospermia

SPINK2
Kherraf *et al* EMBO Mol Med. 2017

Multiple morphological abnormalities of the sperm flagella

DNAH1
CFAP43
CFAP44
CFAP69
WDR66
FSIP2
ARMC2
CFAP70
QRICH2
TTC21A
SPEF2
CFAP65
TTC29

Nsota Mbango *et al*
Basic Clin Androl. 2019 (Review)

Why gene editing should be considered to treat male infertility

When the etiology of spermatogenesis failure is a single gene defect

If no sperm is available for IVF/ICSI

Indeed alternatives, as donor insemination or adoption, may be proposed to the infertile couple

But in some countries alternatives are not available

Prevalence of genetic cause of infertility in North-African men

Constantine-Algeria (599 men 2011-2012)

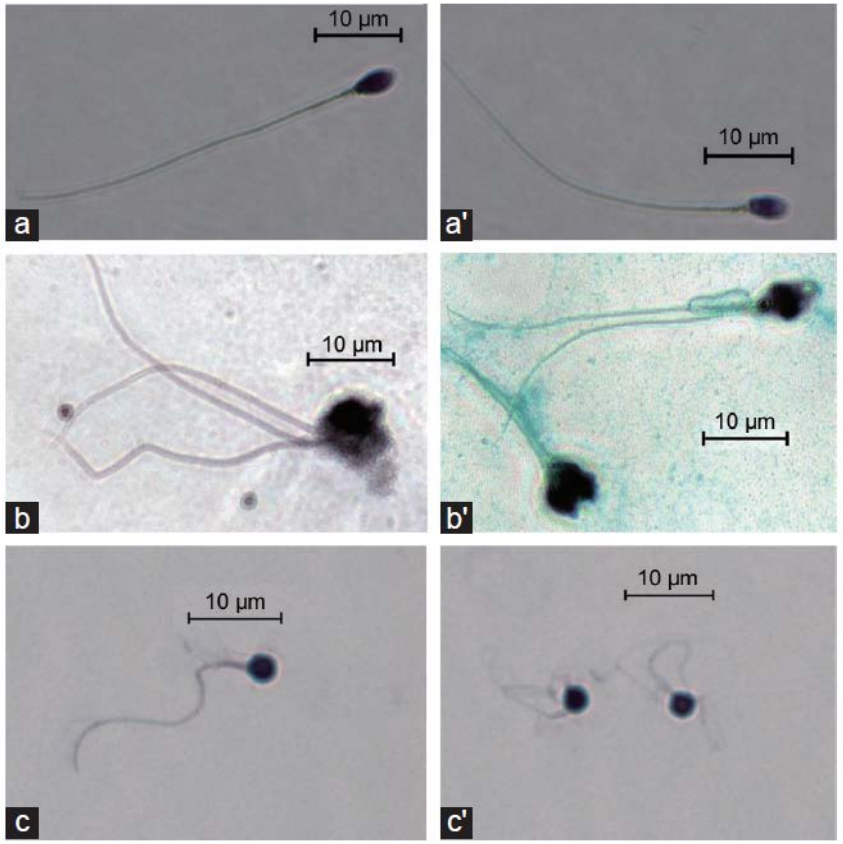


Figure 1: Photographs of representative spermatozoa from (a, a') a normal donor, (b, b') a patient with macrozoospermia (with a homozygous aurora kinase C c.144delC mutation) and (c, c') a patient with globozoospermia (with a homozygous *DPY19L2* deletion).

Homozygous *AURKC* mutation: 2.7 % of men with abnormal semen analysis
Homozygous *DPY19L2* deletion: 1.2 % of men with abnormal semen analysis

Ounis *et al*, Asian J of Andrology, 2015

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Gene therapy as treatment of male infertility is not a new concept

Editing gene based therapy for male infertility is increasingly suggested

De Jonge CJ, Barratt CL (USA)
The future of reproductive cellular engineering in male infertility.
Urol Clin North Am. 2002

Kojima Y, Kurokawa S, Mizuno K, Umemoto Y, Hayashi S, Kohri K (Japan)
Gene transfer to sperm and testis: future prospects of gene therapy for male infertility.
Curr Gene Ther. 2008

Parrington J, Coward K, Gadea J (United Kingdom)
Sperm and testis mediated DNA transfer as a means of gene therapy.
Syst Biol Reprod Mes 2011

Smith RP, Lipshultz LI, Kovac JR (USA)
Stem cells, gene therapy, and advanced medical management hold promise in the treatment of male infertility.
Asian J Androl. 2016

Mulder CL, Zheng Y, Jan SZ, Struijk RB, Repping S, Hamer G, van Pelt AM. (The Netherlands)
Spermatogonial stem cell autotransplantation and germline genome editing: a future cure for spermatogenic failure and prevention of transmission of genomic diseases
Hum Reprod Update. 2016

Darbey A, Smith LB. (United Kingdom-Australia)
Deliverable transgenics & gene therapy possibilities for the testes
Mol Cell Endocrinol. 2018

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Gene therapy as treatment of male infertility is not a new concept

At present there is no efficient and safe procedure applicable to human infertility treatment

However the ongoing experiments and technical developments of male germ cell editing and manipulation in animal models allow to look ahead how gene editing could be used to treat male infertility

How gene editing could be used to treat male infertility

Germ cells are available

Gene editing in vivo

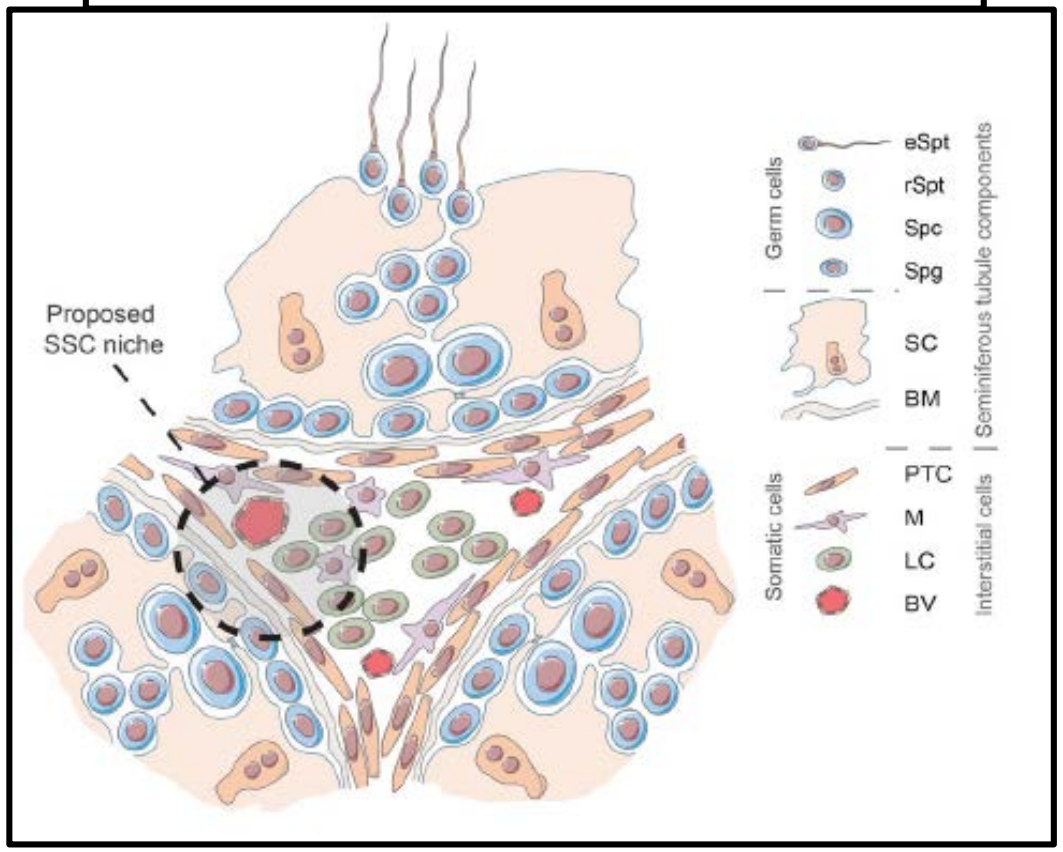
Gene editing in vitro → Spermatogenesis in vivo

Gene editing in vitro → Spermatogenesis in vitro

No germ cell available

Germ cell gene editing In vivo

The testicular microenvironment

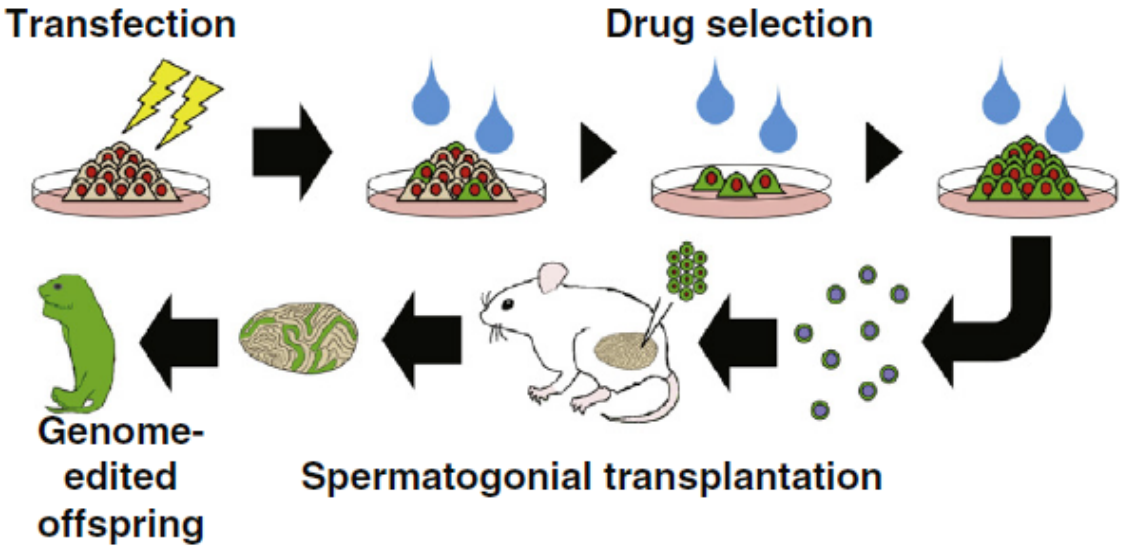


Oliver and Stukenborg Andrology 2019

Mice: offspring +

- In vivo transfection of testicular germ cells and transgenesis by using the mitochondrially localized jellyfish fluorescent protein gene
  Huang *et al* FEBS Letters 2000
- Transgenic mice produced by retroviral transduction of male germ line stem cells in vivo.
  Kanatsu-Shinohara *et al* Biol Reprod. 2004
- In Vivo genetic manipulation of spermatogonial stem cells and their microenvironment by Adeno-Associated Viruses
  Watanabe *et al* Stem Cell Reports 2018

Germ cell gene editing In vitro with spermatogenesis in vivo



Genetic selection *in vitro*

Kanatsu-Shinohara et al., 2005b
Transgenic mice were derived from GSCs transfected with a plasmid vector carrying an *Egfp* expression construct and drug resistance gene

Kanatsu-Shinohara et al., 2006a
KO mice were produced by gene targeting and random integration in GSCs

Wu et al., 2015
Genome-edited mice were produced by CRISPR-Cas9 technology in GSCs

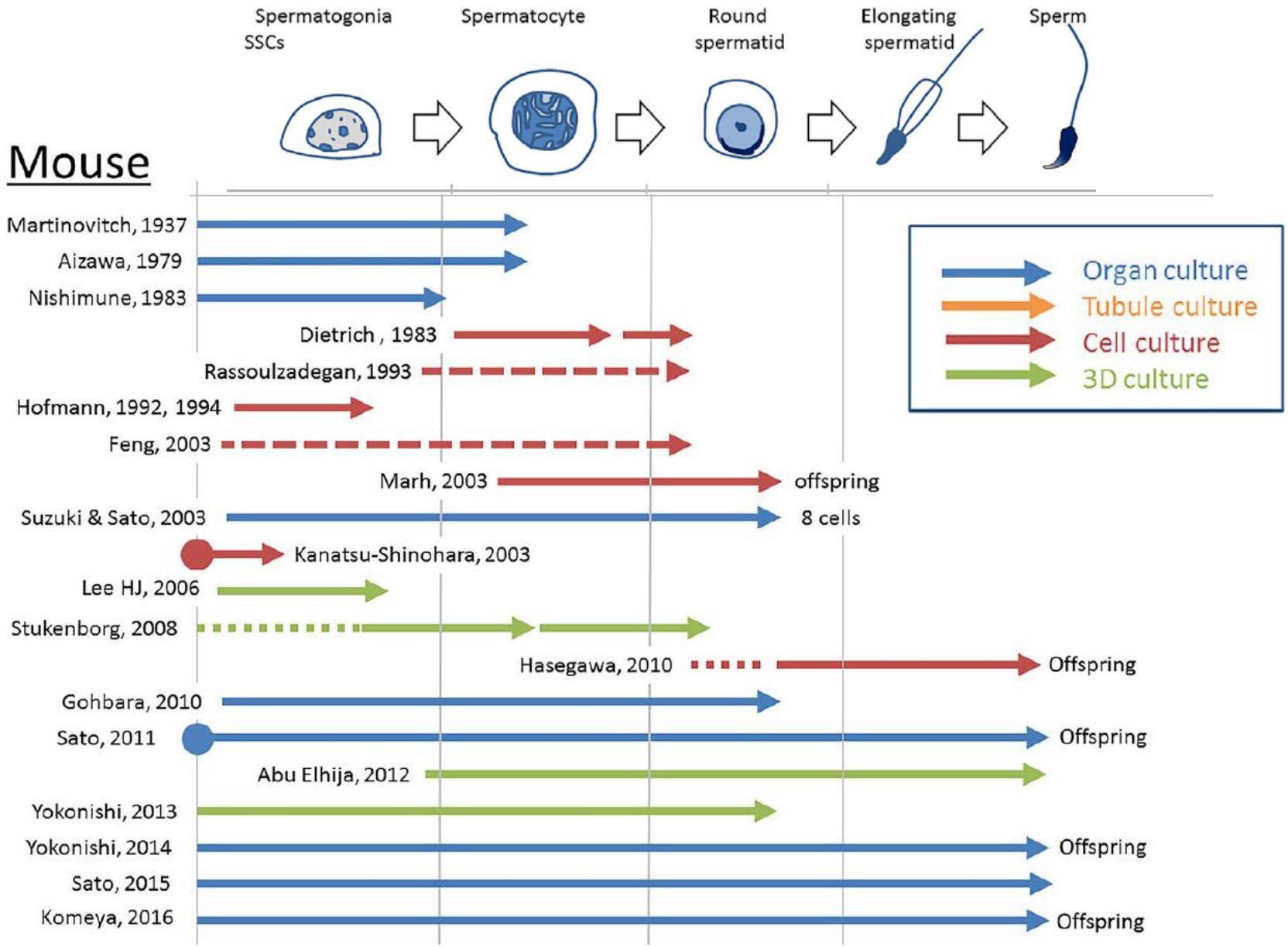
Chapman et al., 2015
Genome-edited rats were produced by CRISPR-Cas9 technology in GSCs

Sato et al., 2015b
Knock-in mice were produced by TALEN technology in GSCs

Takashima and Shinohara, Stem Cell Res, 2018

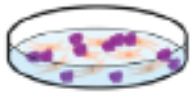
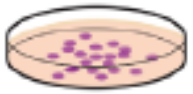
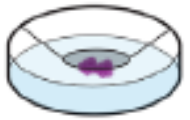
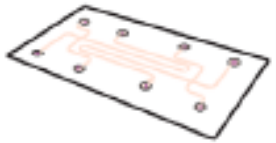
Spermatogonial stem cell transplantation into Rhesus testes regenerates spermatogenesis producing functional sperm
Hermann et al Cell Stem Cell 2012
Donor spermatogenesis in de novo formed seminiferous tubules from transplanted testicular cells in rhesus monkey testis
Shetty et al Hum Reprod 2018

In vitro spermatogenesis



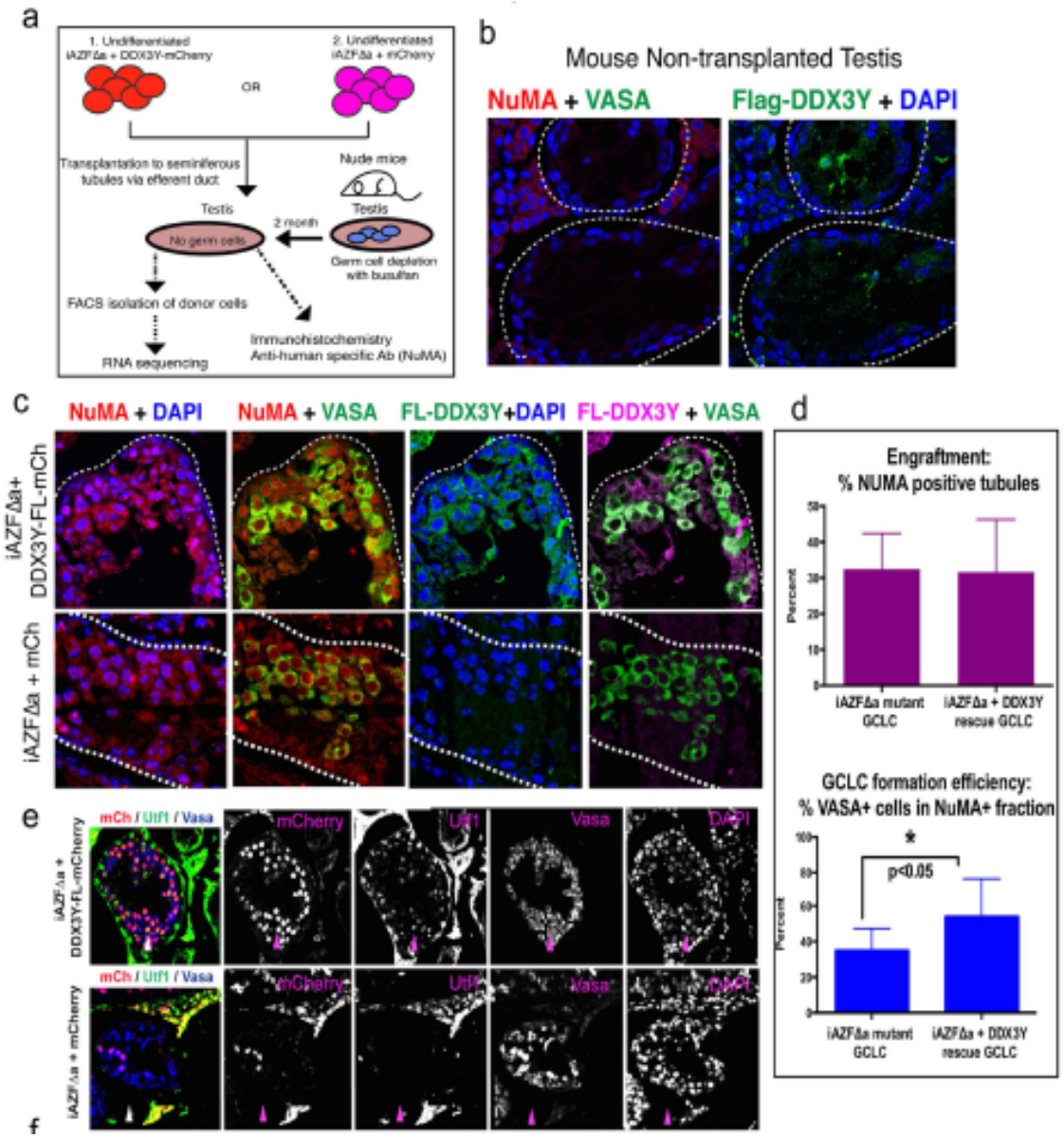
Komeya *et al* Reprod Med Biol. 2018

Human in vitro spermatogenesis

		Complete spermatogenesis	Functional sperm	Somatic cell function	Tissue reorganisation	Potential culture limitations	Selected references
2D-models a) Enriched SSCs b) Single-cell tissue digest		✓[a] & ✗[b]	✓[a] & ✗[b]	✗	✗[a] & (✓[b])	a) No somatic cells b) No 3D tissue structure	a) Yang et al. 2014 b) von Kopylow et al. 2018
3D-models	3D gel matrix 	✓	✓	✗	✗	Lacking full somatic cell complement (only germ cell/ Sertoli cell aggregates)	Sun et al. 2018
	Air–liquid interphase 	✗	✗	✓	N/A	Incomplete somatic cell maturation	de Michele et al. 2018
	Hanging drop 	✓	✗	✓	(✓)	Limited reorganisation, immortalised somatic cells	Pendergraft et al. 2017
	Bioreactor 	✓	✗	✗	N/A	Poor yield	Perrard et al. 2016

Oliver and Stukenborg Andrology 2019

No germ cell available



DDX3Y gene rescue of a Y chromosome AZFa deletion restores germ cell formation and transcriptional programs

Y chromosome AZFa deletion → Sertoli cell only (DDX3Y and USP9Y are missing)

- Stable iPSC lines with AZFa deletions were generated,
- Deleted iPSC cells were complemented with DDX3 (Talen)
- Xenotransplantation into nude mouse seminiferous tubules
- Formation of germ cell like cells (GCLCs)
- Expression of UTF1, a prospermatogonial protein
- Transcriptomic fate close to prospermatogonia

DDX3Y expression is sufficient to drive germ cell differentiation on an AZFa-deleted background

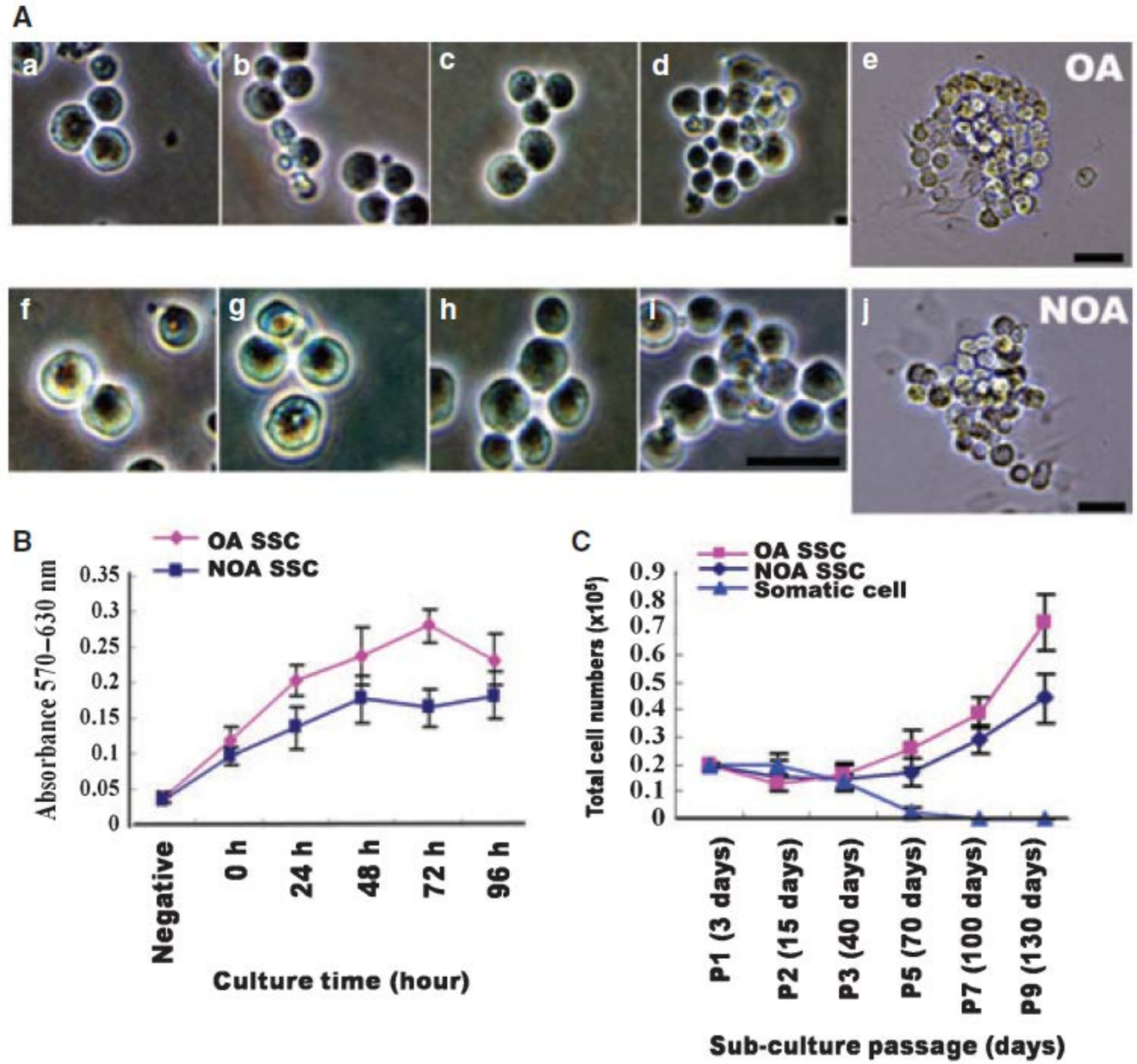
Ramathal *et al.* Sci Rep. 2015

Conclusion

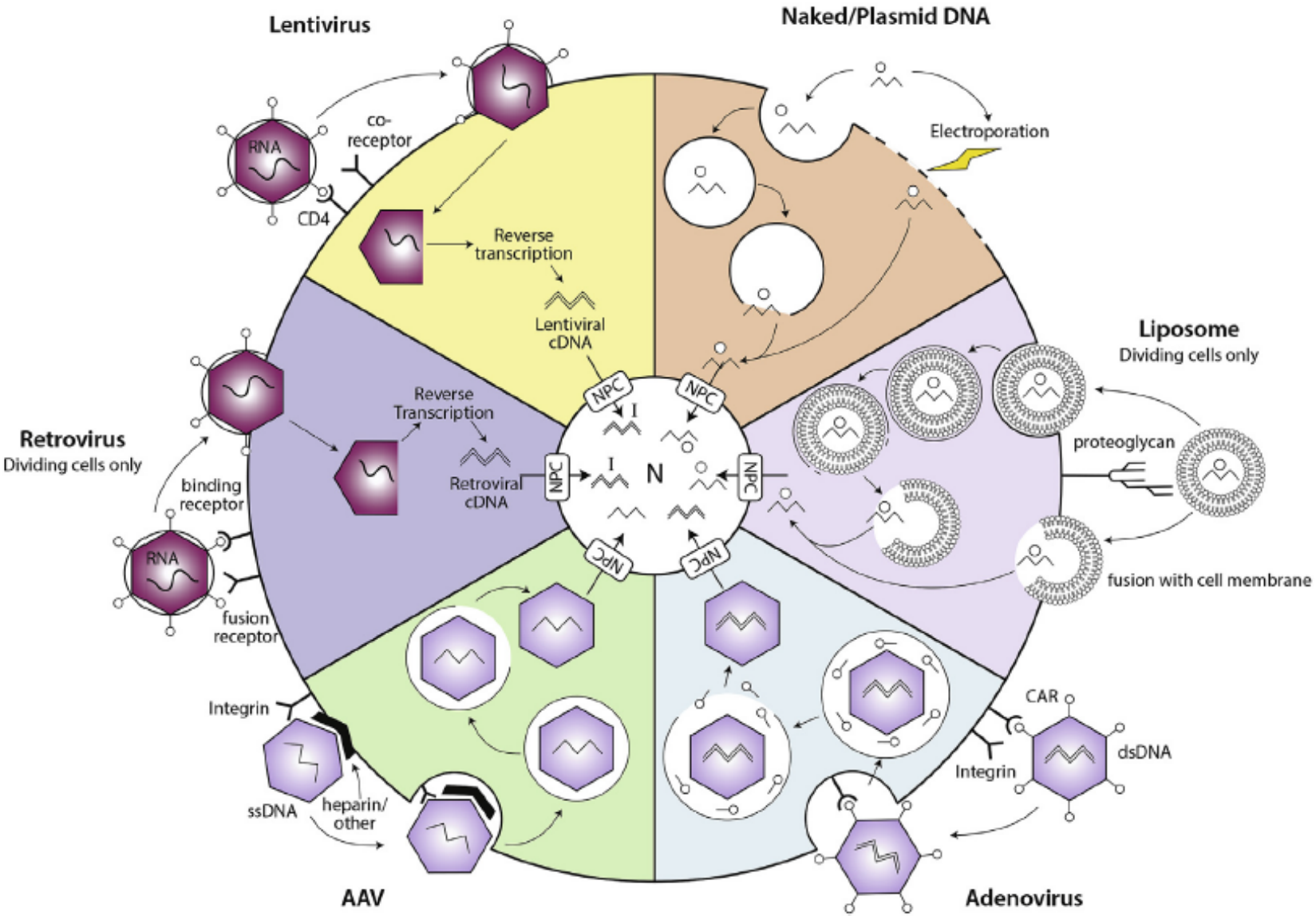
Spermatogenic failure and male infertility are (and will be) more and more explained by (single) gene defects.

Could editing these genes be a resonsible treatment of male infertility in the future?

Probably yes when it will be possible to control the safe and efficient production and maturation of edited germ cells and sperm in vivo or in vitro



Lim et al Cell Prolif. 2010



Darbey and Smith Mol Cell Endocrinol. 2018

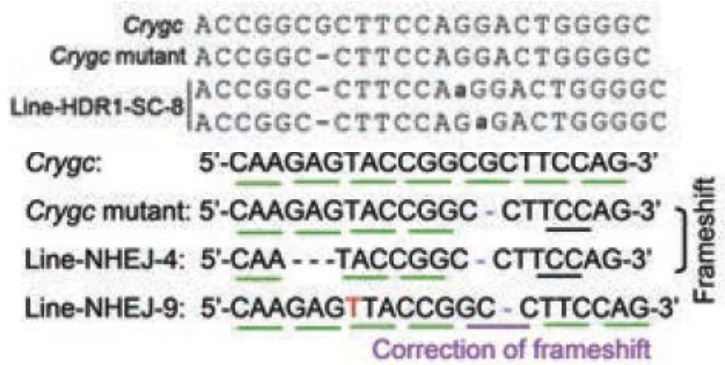
Cell Research (2015) 25:67-79.

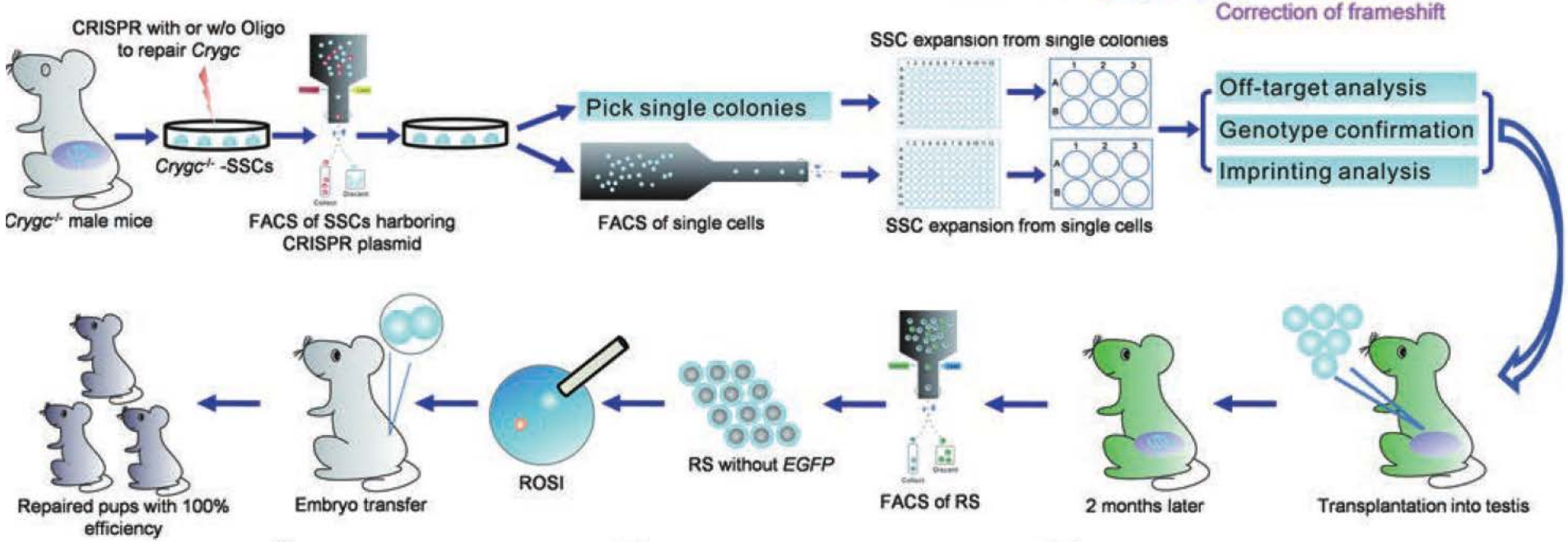
www.nature.com/cr

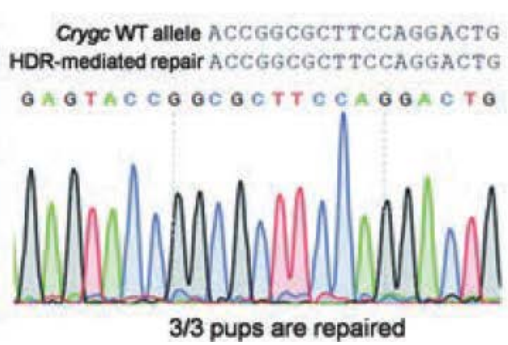
ORIGINAL ARTICLE

Correction of a genetic disease by CRISPR-Cas9-mediated gene editing in mouse spermatogonial stem cells

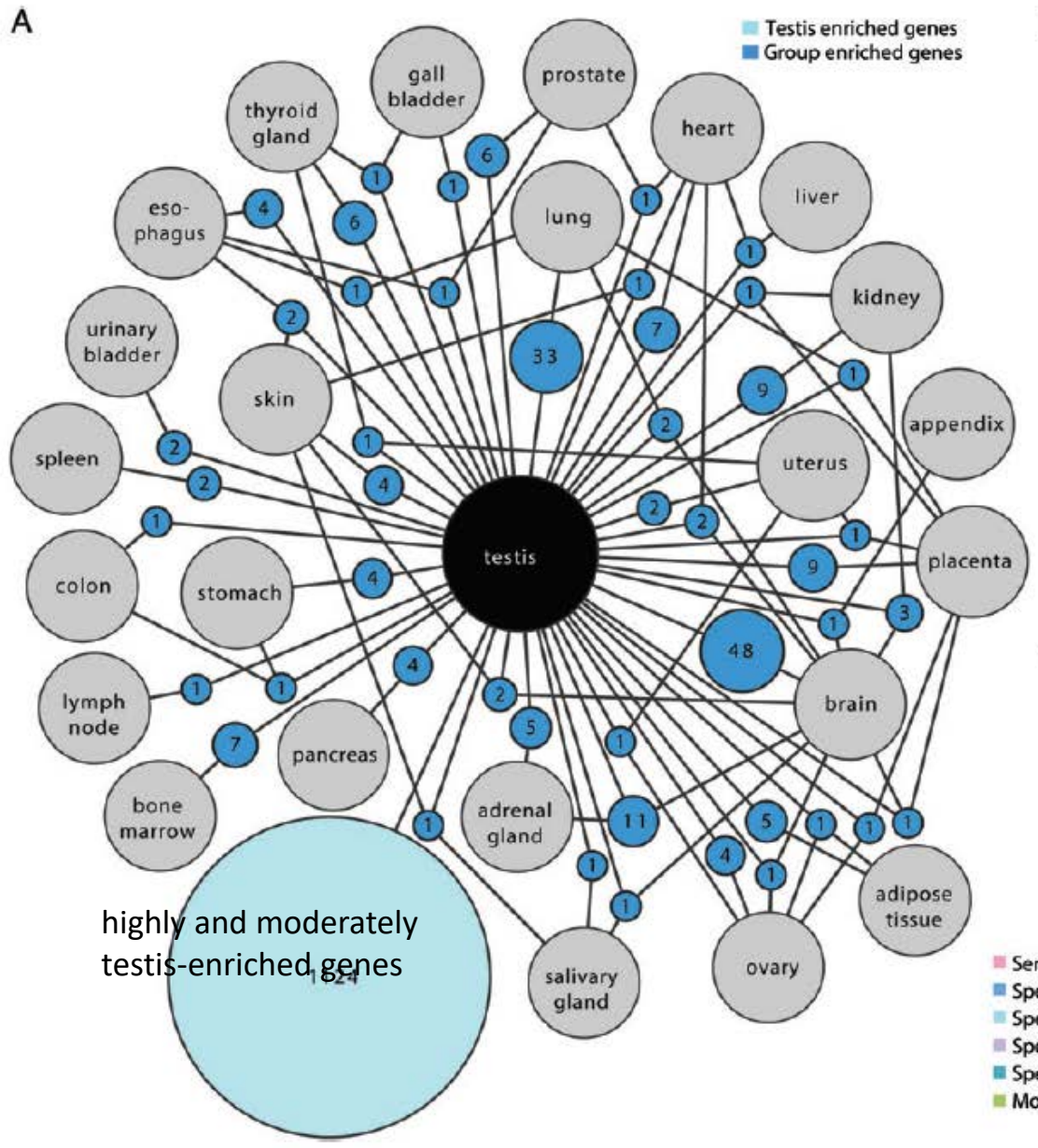
Yuxuan Wu[1, 2, *], Hai Zhou[1, 2, 3, *], Xiaoying Fan[4, *], Ying Zhang[2, 5, *], Man Zhang[1, 2, *], Yinghua Wang[1, 2], Zhenfei Xie[1, 2], Meizhu Bai[1, 2, 6], Qi Yin[1, 2], Dan Liang[1, 2], Wei Tang[7], Jiaoyang Liao[1, 2], Chikai Zhou[1, 2], Wujuan Liu[1, 2], Ping Zhu[4], Hongshan Guo[4], Hong Pan[1, 2], Chunlian Wu[3], Huijuan Shi[8], Ligang Wu[2, 5], Fuchou Tang[4], Jinsong Li[1, 2, 6]





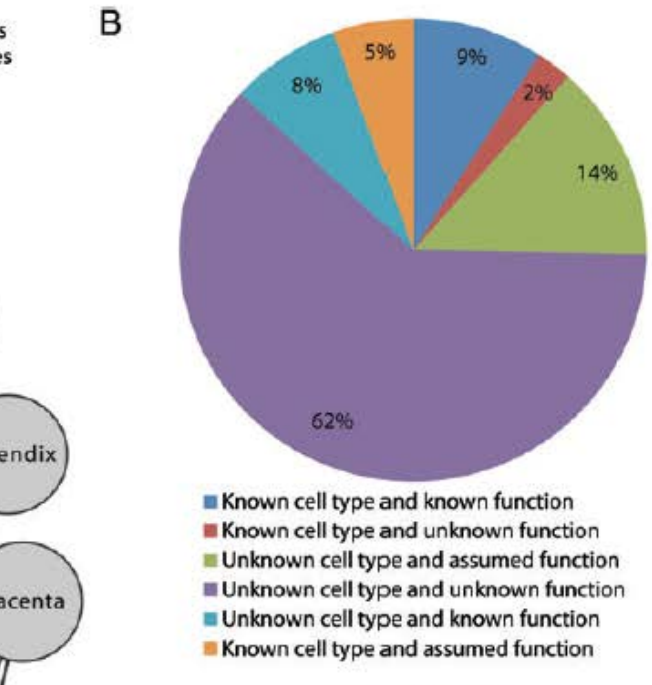


Classification of the genes expressed in testis

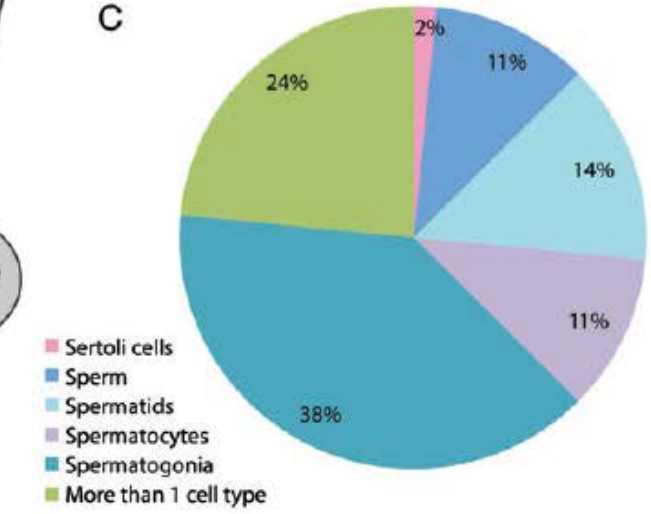


highly and moderately testis-enriched genes

Network plot of the testis-enriched genes showing the relation of group-enriched genes



Known cell type and function of the 364 highly testis-enriched genes



Cell type localization based on protein profiling using immunohistochemistry for 62 of the previously uncharacterized highly testis-enriched genes

D. Djureinovic et al Mol Human Reprod 2014