Genes and Infertility:
Where are we? Where are we going?

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Nataliance IVF center, Orléans, France

Context

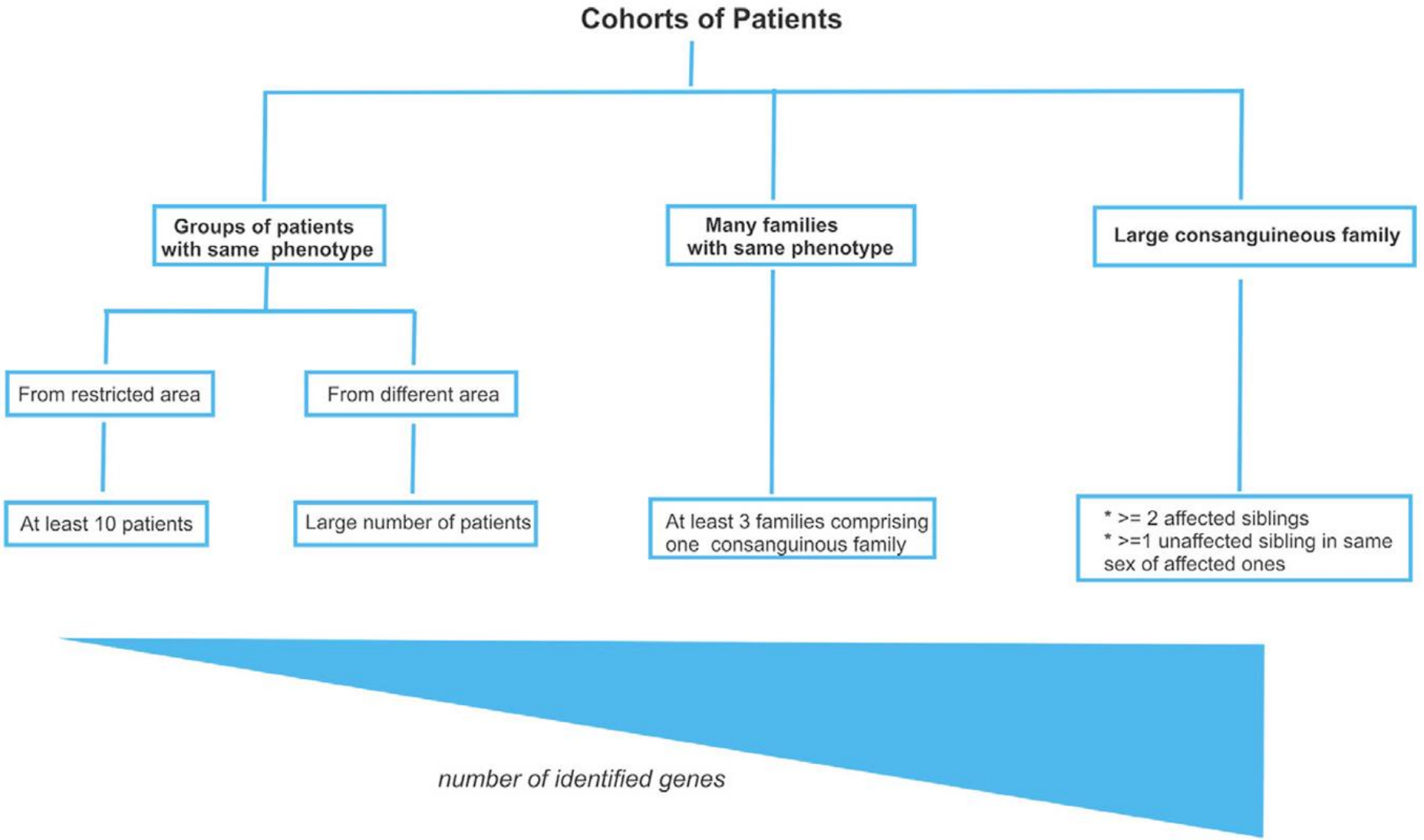
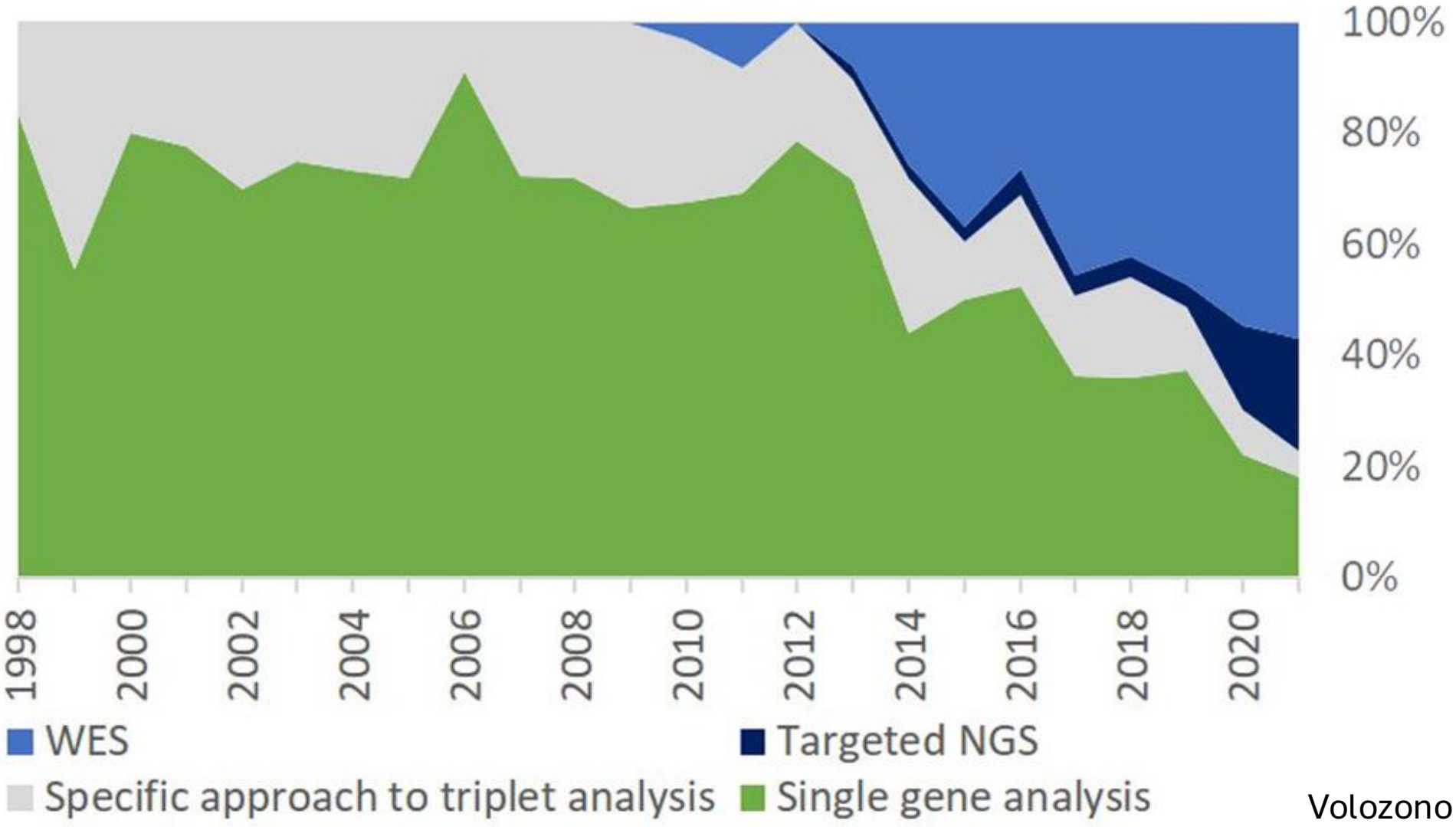


Figure 8.2 The different cohorts of patients that can be analyzed in the quest for infertility genes, the success in identifying genes depending on the cohort studied and the optimal number of patients to be analyzed. Cohorts can consist of groups of patients presenting the same phenotype, which can be possibly divided into two subgroups from a restricted area (where a founder effect is expected) or from different geographic locations. Family-based studies provide an alternative approach to identifying genes involved in infertility. The studies can be based on either numerous small families or one large family. Source: adapted from Okutman et al. [17] .

Viville et Okutman, 2023

Importance of NGS in the diagnostis of reproductive disorder



Volozonoka et al, 2022

NGS applications in Reproductive Medicine

- **Identify inheritable genetic conditions** (Carrier Screening): 3-4% of couples looking to conceive being at risk for highly penetrant severe conditions with childhood onset

- Anticipate late-onset medically actionnable conditions: 2-4% of individuals concerned > **especially relevant as some male and female infertility causes are risk factors for late-onset diseases (cardiac, cancer…) > careful to dfferentiate polygenic score from monogenic disease!**

- **IVF pharmacogenomics (Male and Female)**

- **Diagnosis of polygenic/monogenic infertility etiologies**

- **Anticipation of poor reproductive outcomes of genetic origin**

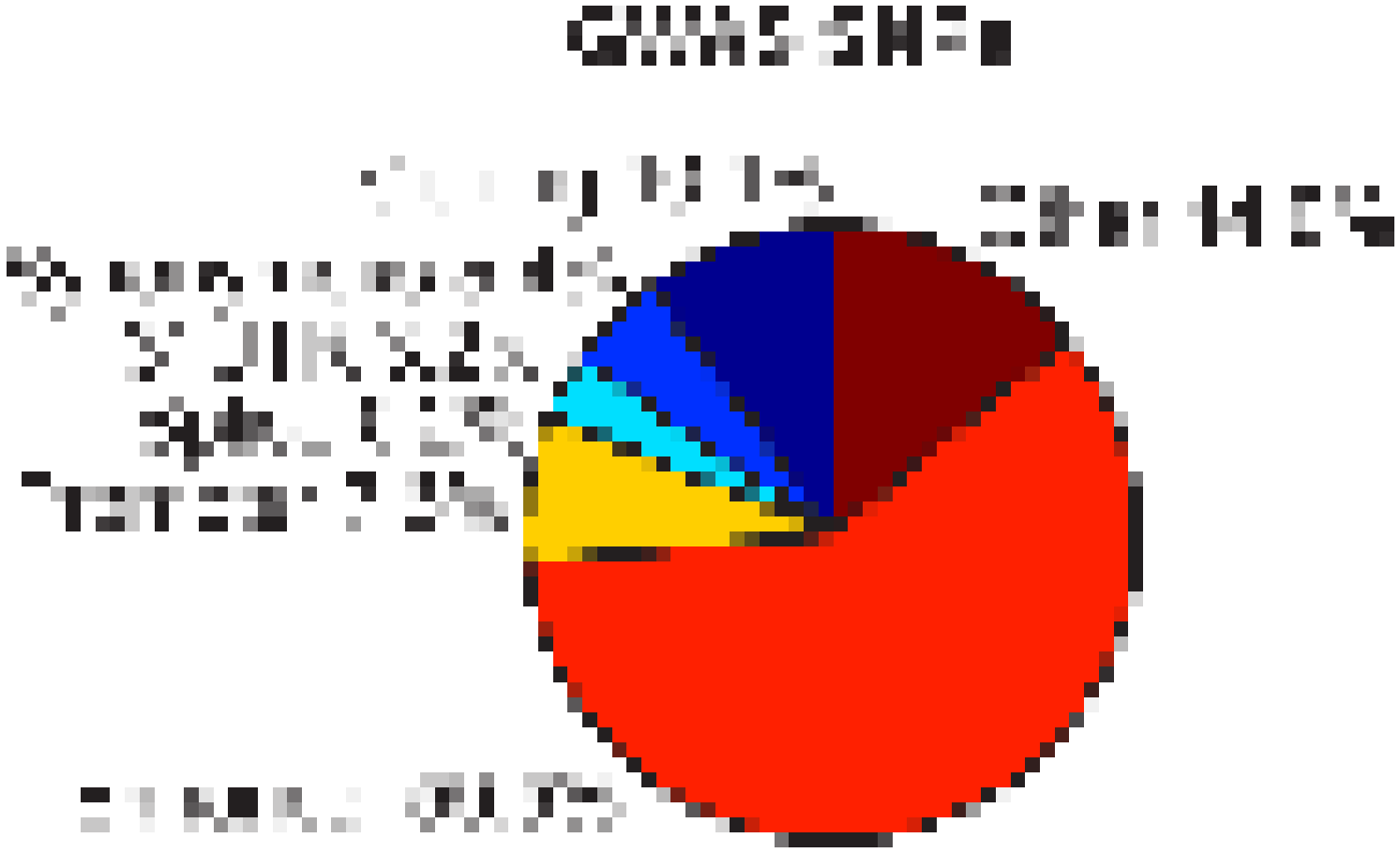
Capalbo et al, 2021

The issues with WES?

90% of « pathogenic » SNP may be localized outside of coding regions

>interpretation issues

> Increased diagnostic yield WGS compared to WES

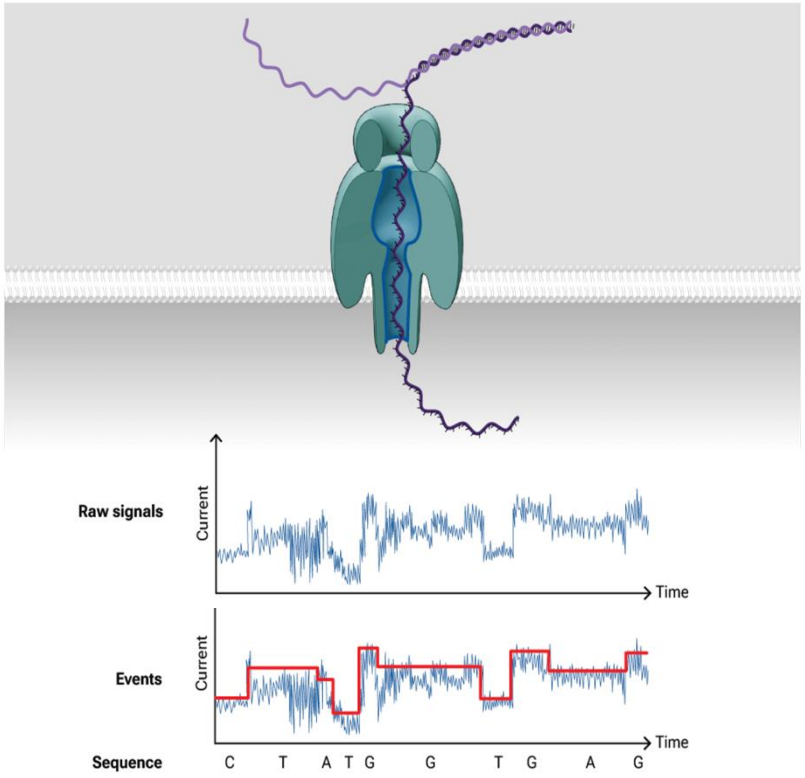


Genetic and Epigenetic Fine-Mapping of Causal Autoimmune Disease Variants

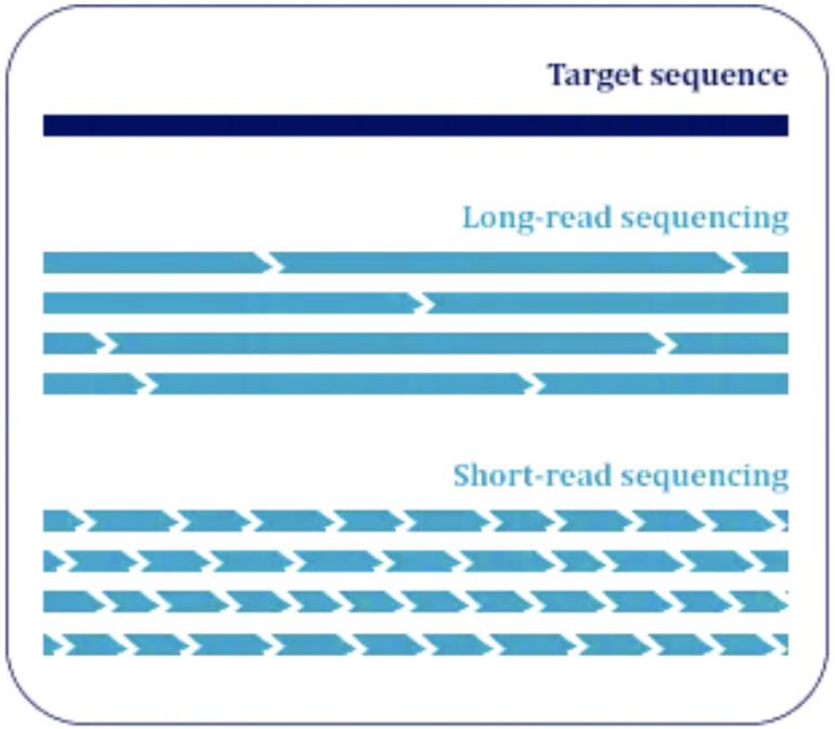
Farh et al, 2015

LRS: a new player in sequencing

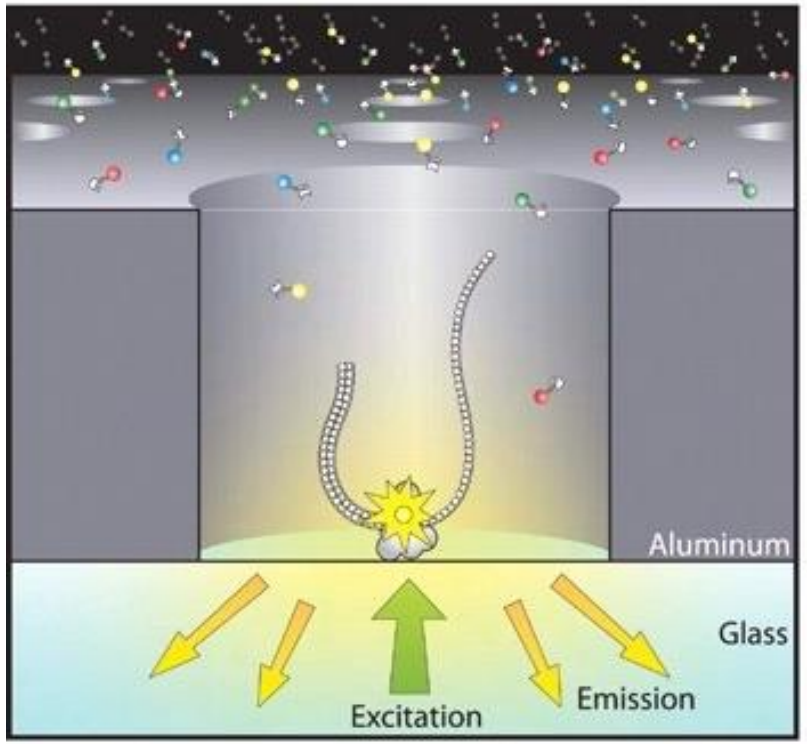
LRS:real-time sequencing of long (>100kb or ultralong DNA fragment (several megabases)



Oxford Nanopore Technologies (ONT)



SRS:massively parallel sequencing of small-insert size DNA libraries (<600bp)

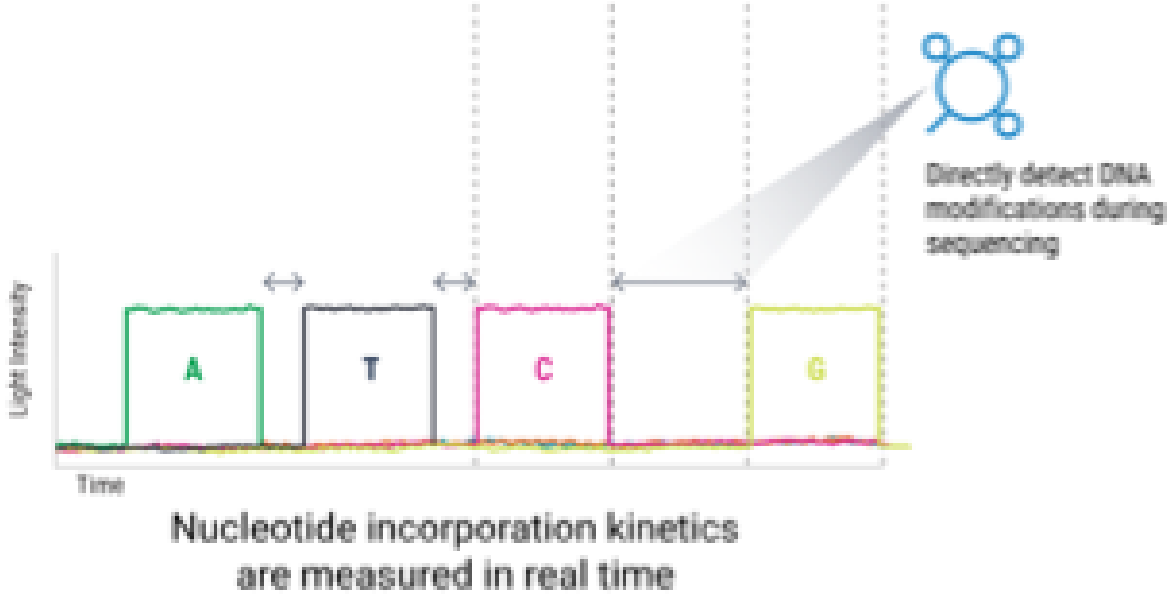


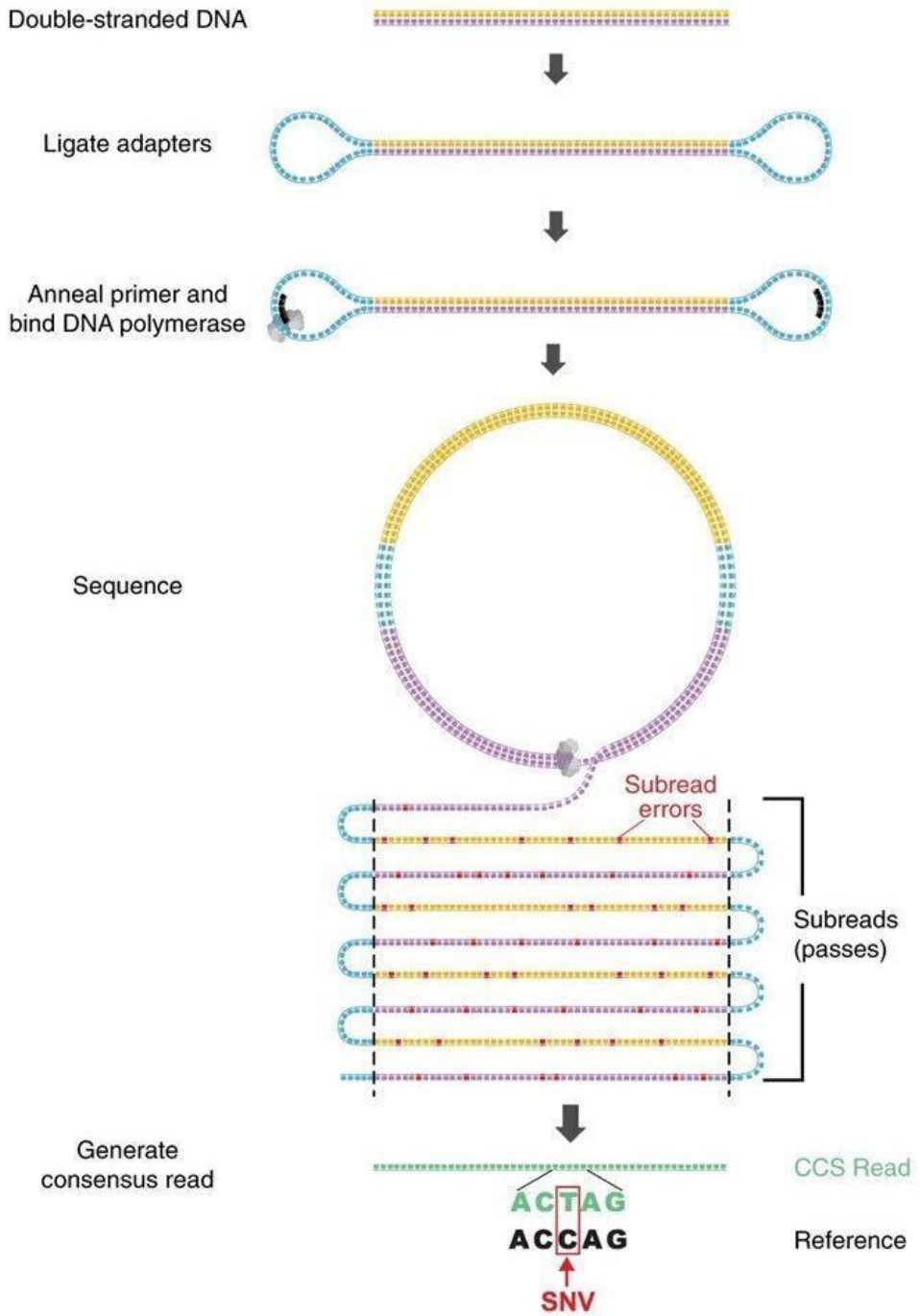
Zero-mode waveguides

Pacific Bioscience (PacBio)

Methylation analysis and HiFi Sequencing

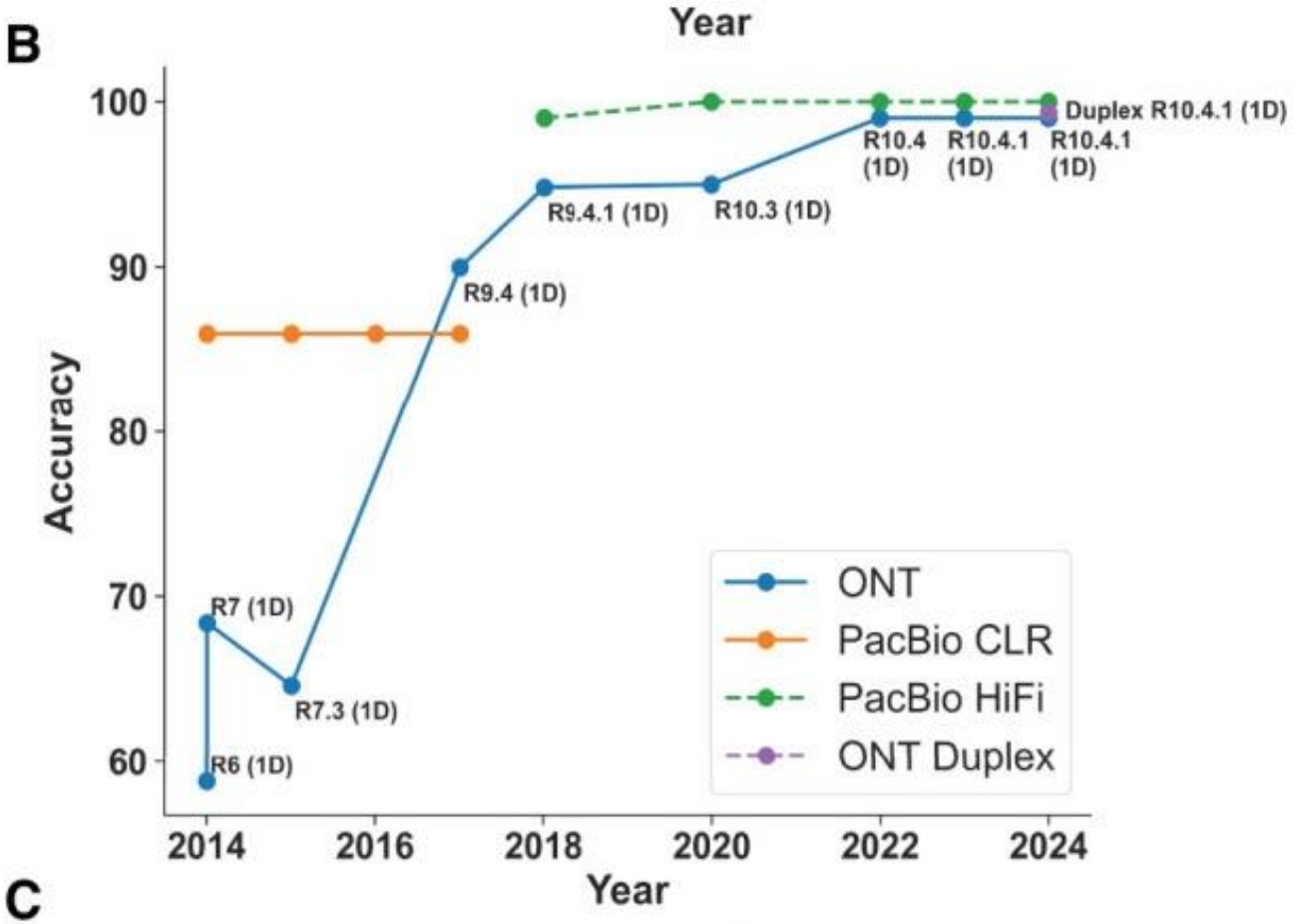
Methyl-cytosine





Source: Pacific Biosciences website

B



short reads often fail to span complex genomic regions while LRS can sequence DNA fragments ranging from kilobases to megabases in length

C

	SNVs	Indels	TRs	SVs	CNVs	Methylation	Phasing	Assembly
Short-Reads	+++	+++	++	+	++	+++**	+	+
Long-reads	+++	++	+++	+++	+++	+++	+++	+++

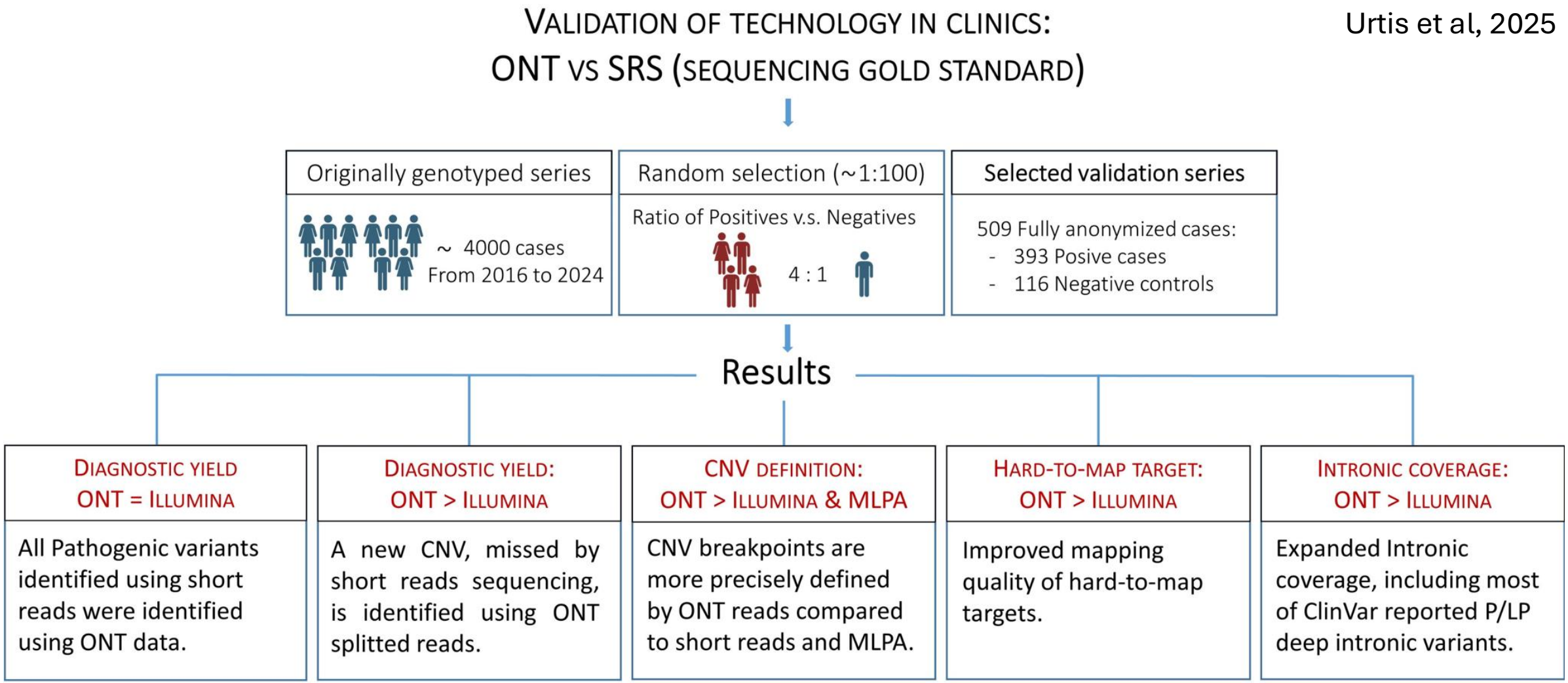
\+ Minimum performance +++ Maximum performance

Nature Method of the Year 2023

Nature Methods, 2023

Clinical Validation study: LRS Vs SRS

Urtis et al, 2025



Increasing Explanation rate

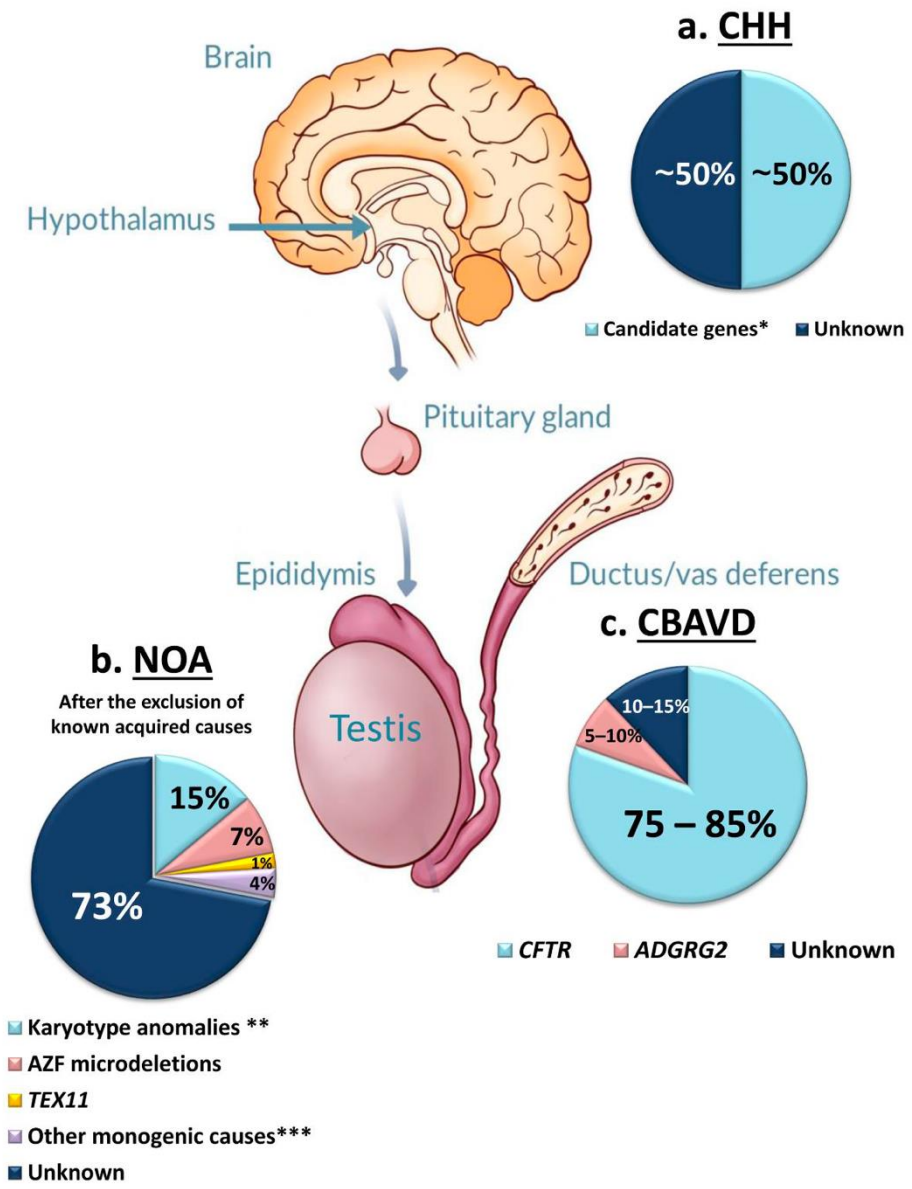
Technology	Genetic variation captured	Explanation rate	References
Karyotype	Chromosomal abnormalities	~5%	De Vries et al. 2005
Chromosomal microarray analysis	Copy number variants (CNVs) >50 kb	~10%	Clark et al. 2018
Short-read whole exome	SNVs and indels, some large exonic variants	~30–40%	De Ligt et al. 2012; Chung et al. 2023
Short-read whole genome	SNVs, indels, some large variants	~40%	Gilissen et al. 2014; Chung et al. 2023
HiFi long-read whole genome	SNVs, SVs, CNVs, phasing, translocations, inversions, repeat expansions, methylation	>50%	Farrow et al. 2024*

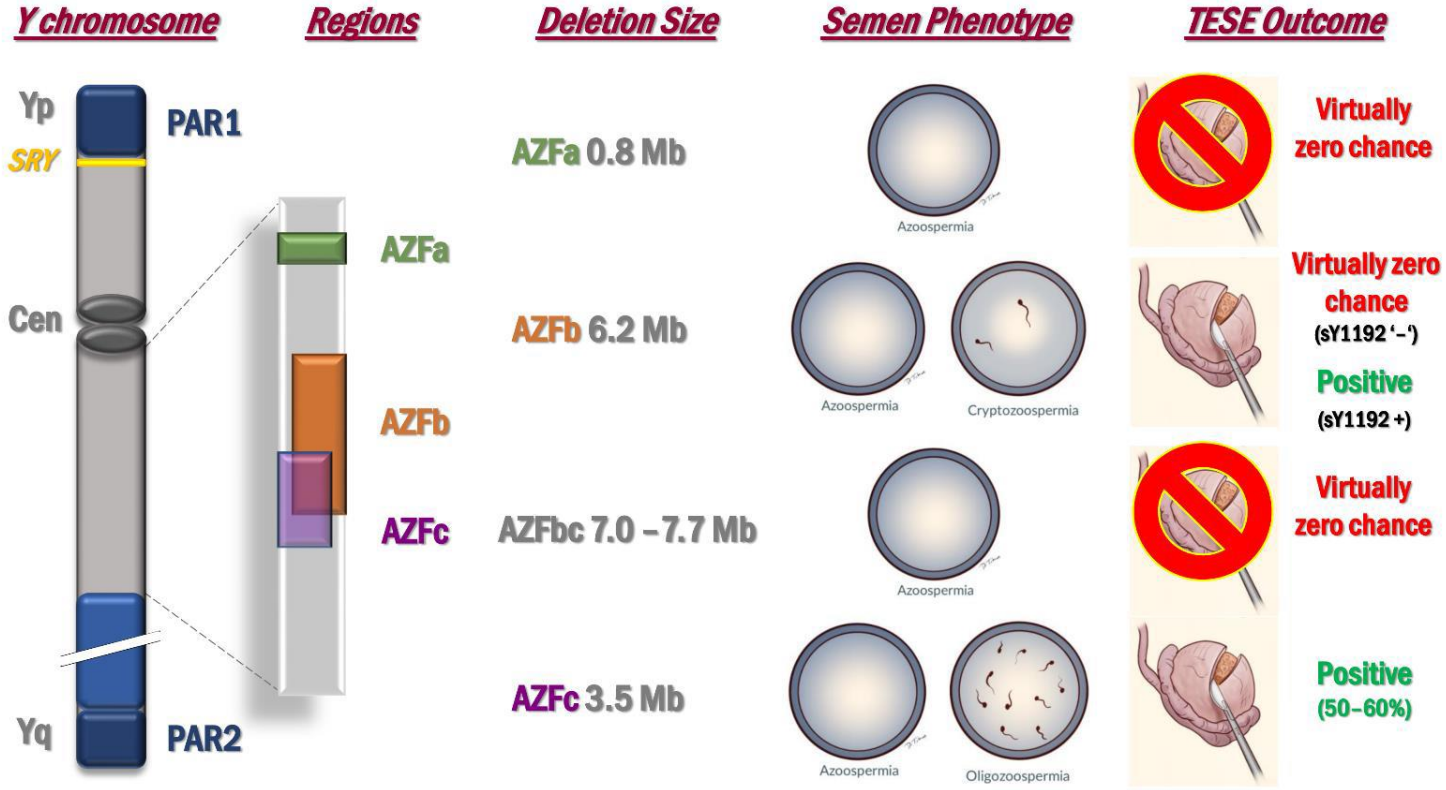
Source: Pacific Biosciences website

Genetic origin of infertility

Male

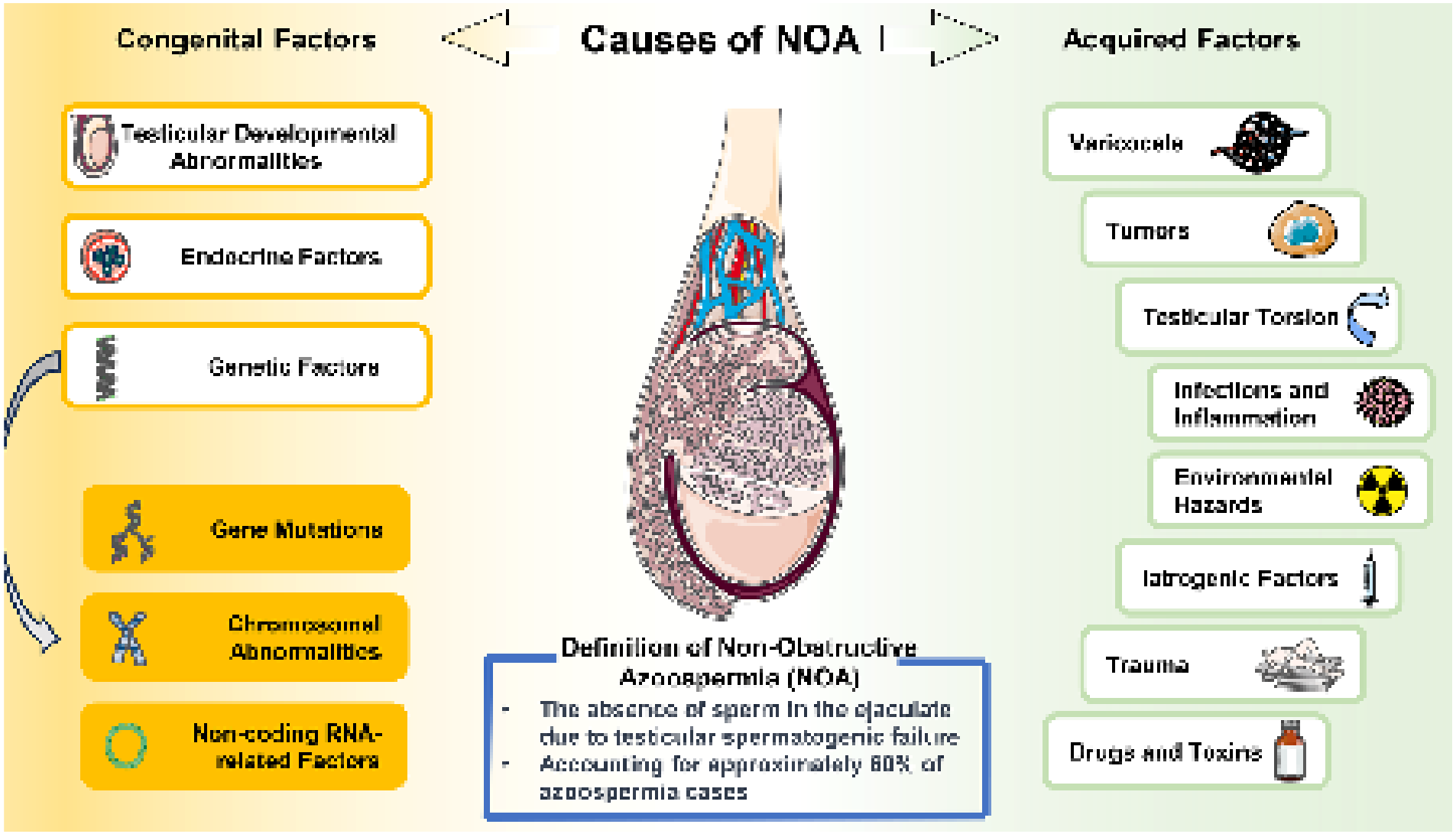
Genetic origin of azoospermia





Cioppi et al, 2021

NOA



Wang et al 2025

Impact of epigenetic disruption in NOA

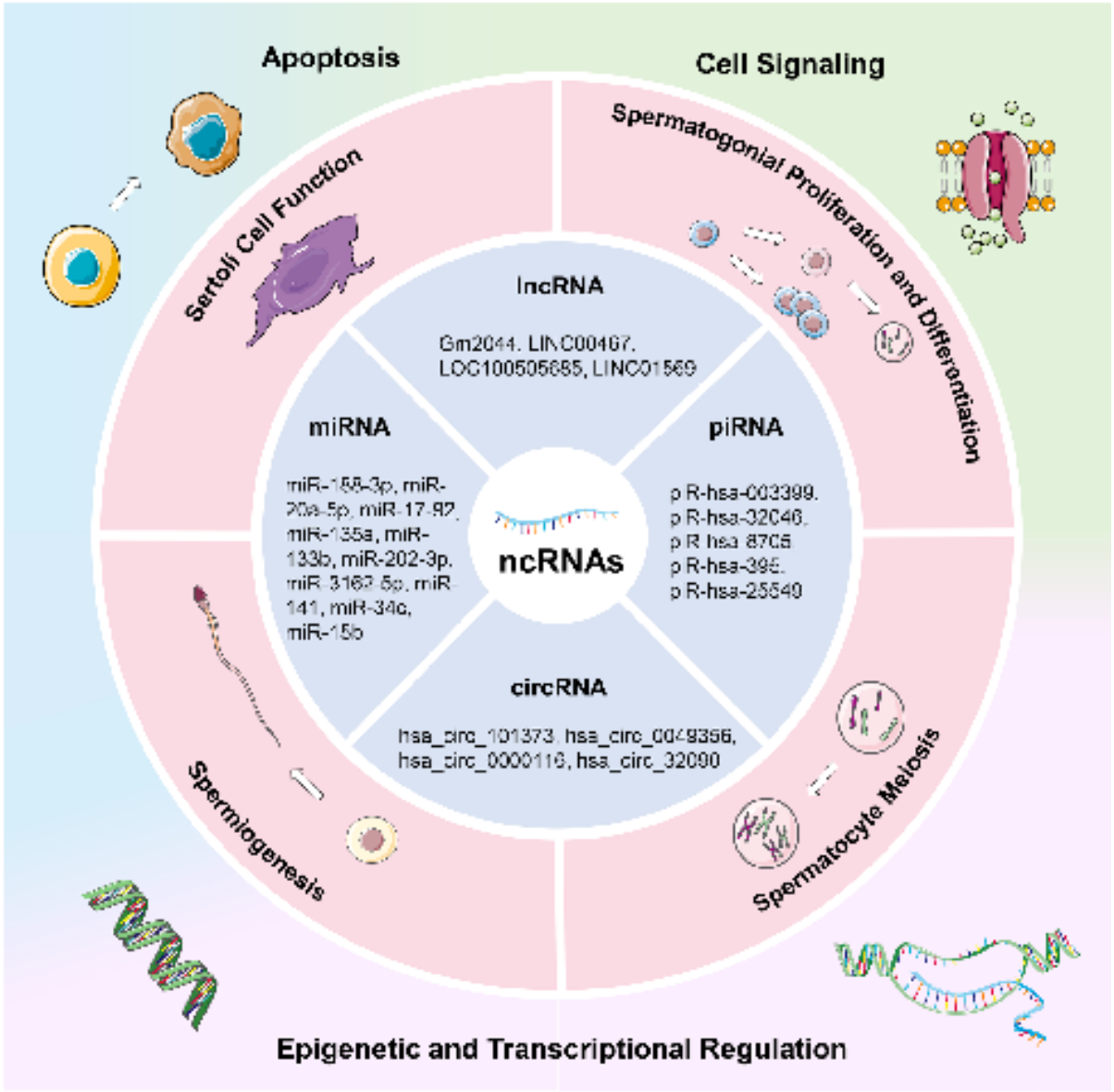
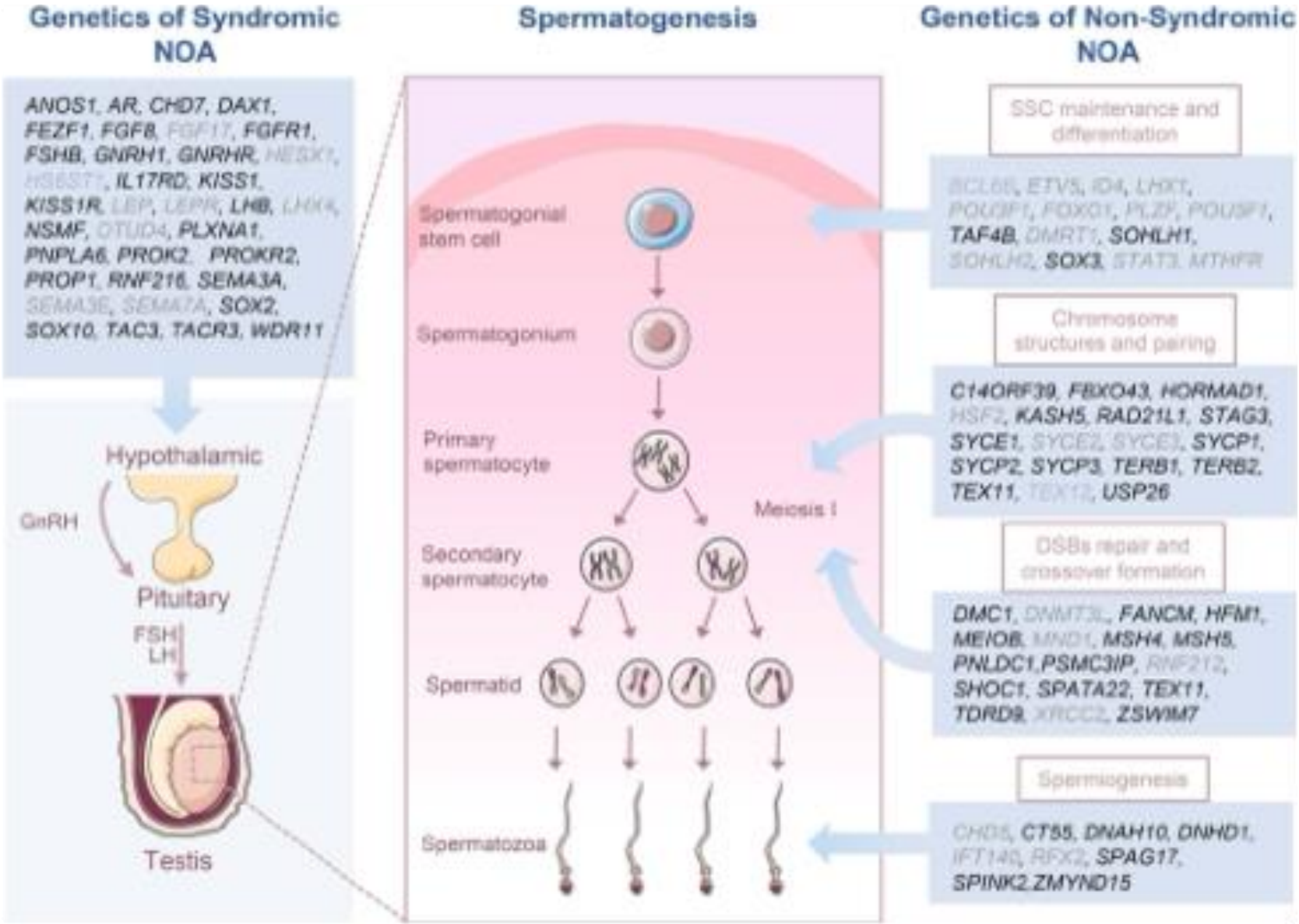


Fig. 4 Roles of ncRNAs in spermatogenesis

Wang et al 2025

Gene variants associated with NOA: prognosis?



Genotypic heterogeneity poses difficulties in clinical interpretation. For instance, the same *SYCE1* mutation (c.689_690del) has been associated with diverse phenotypes, ranging from complete spermatocyte arrest to residual spermatogonia

In addition, many reported m tions lack functional validation, limiting their clinical utility.

Wang et al 2025

Other clinical manifestations of pathogenic variants in male infertility

Teratozoospermia	Globozoospermia	*SPATA16* (609856)
		DPY19L2 (613893)
	Macrocephaly Acephalic sperm	*AURKC* (603495)
		BRDT (602144)
		PMFBP1 (618085)
		CEP112 (618980)
		SUN5 (613942)
		TSGA10 (607166)
	Multiple morphologic abnormalities of the flagella (MMAF)	*DNAH8* (603337)
		DZIP1 (608671)
		CFAP58 (619029)
		DNAH1 (603332)
		CFAP43 (617558)
		CFAP44 (617559)
		CFAP69 (617949)
		FSIP2 (615796)
		WDR66 (618146)
		AK7 (615364)
		SPEF2 (610172)
		DNAH2 (603333)
		TTC29 (618735)
		CEP135 (611423)
		ARMC2 (618424)
		QRICH2 (618304)
Asthenozoospermia		*CATSPER1* (606389)
		GALNTL5 (615133)*
		SLC26A8 (608480)
		DNAH17 (610063)
		EIF4G1 (600495)*
		SPAG17 (616554)*
		AKAP4 (300185)*
Total fertilization failure		*PLCZ1* (608075)

ACTL9
ACTL7A
IQCN
PFN3

Sperm Head Shaping Disruption?

Viville et Okutman, 2023

Sperm Head Shaping Disruption

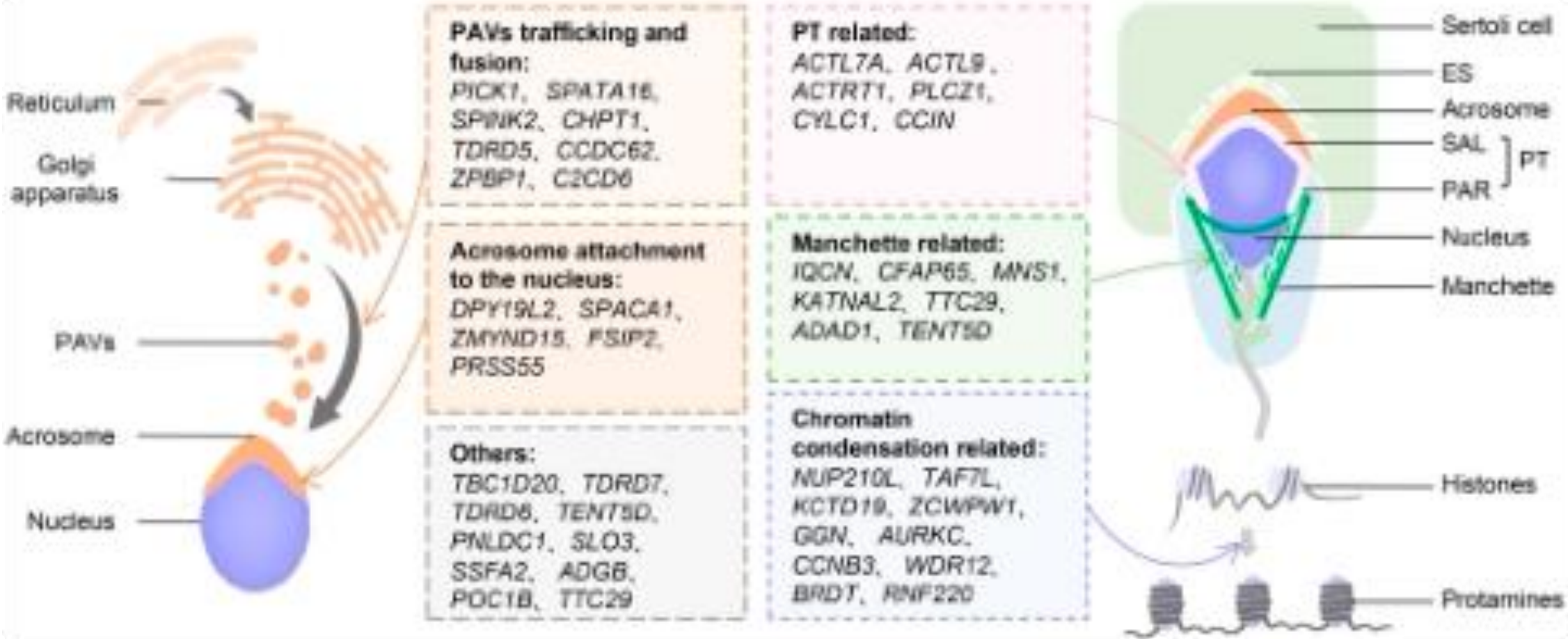
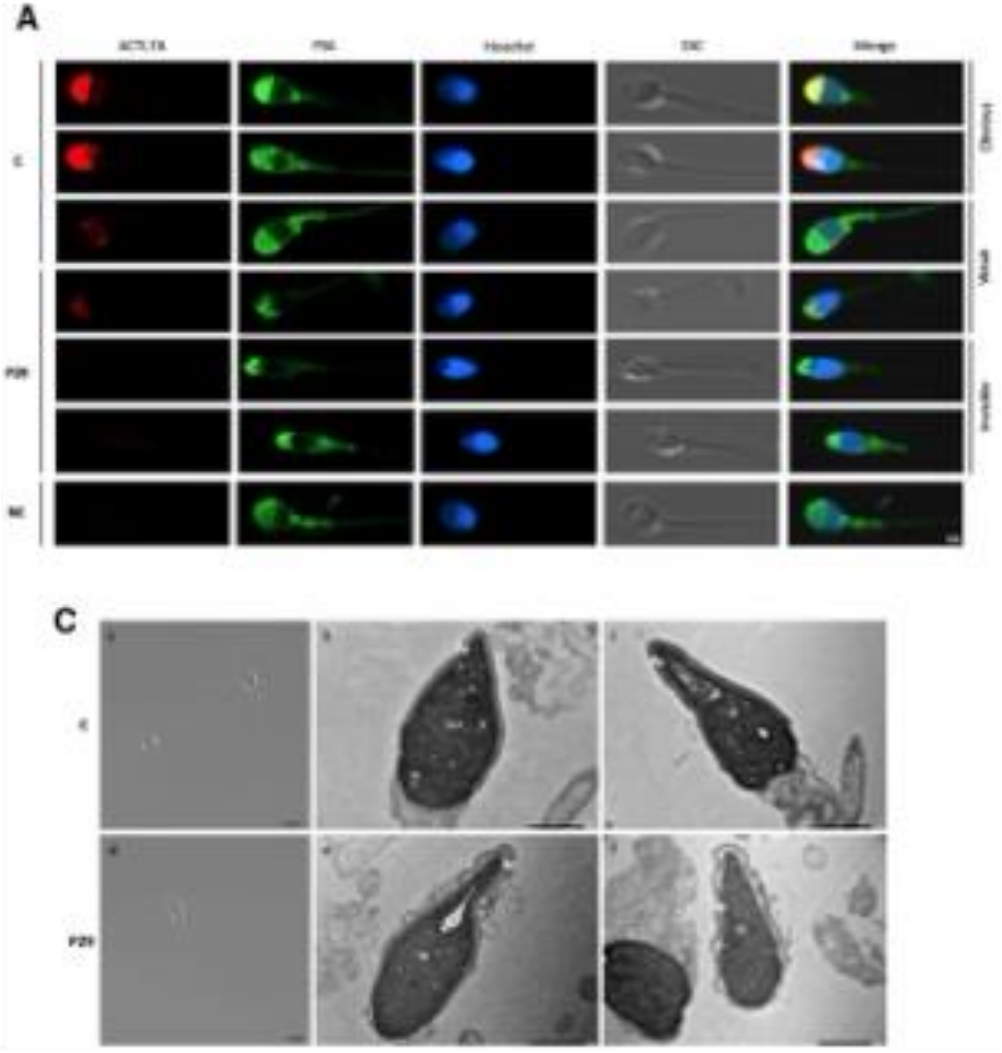


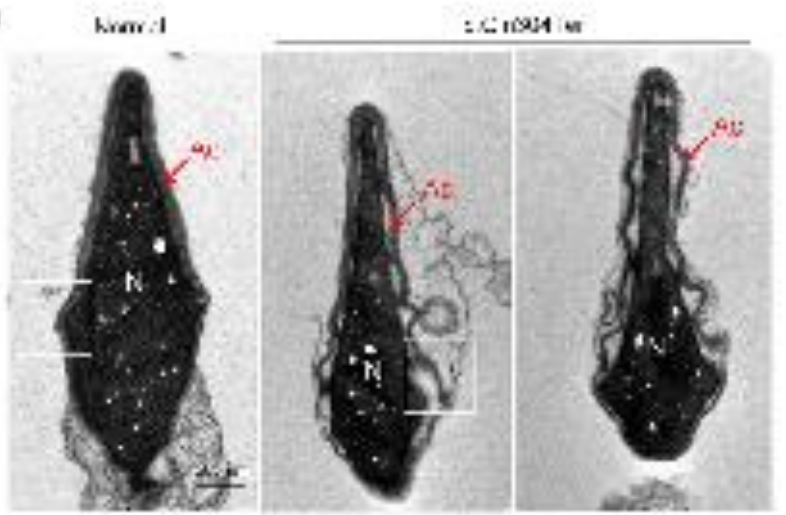
Figure 4. Sperm head shaping-related genes associated with human male infertility. Homozygous, compound heterozygous, or hemizygous mutations in these genes have been reported to cause male infertility in humans. ES, ectoplasmic specialization; PAR, postacrosomal region; PAVs, proacrosomal vesicles; PT, perinuclear theca; SAL, subacrosomal layer.

He et al, 2025

Sperm Head Shaping Disruption



ACTL9/ACTLA7 variants
Barberan et al, 2024



IQCN variant
(Dai et al, 2022

Standard sperm morphology is not informative

- **PLCz expression and distribution alteration**
- **AOA is effective**

ICSI outcomes?

GOOD w/o AOA

ZPBP1
FSIP2
ADGB
SLO3
TENT5D
WDR12

POOR even with AOA

Meiosis related genes:

NUP210L
KCTD19
CCNB3
ZMYND15
FBXO43
AURKC

Important disruption of the manchette…

AOA needed

Disruption of PLCz expression or localization

DPY19L2
PLCz
ACTL9
ACTLA7
ACTRT1
CCIN
IQCN
TDRD5
SSFA2
TAF7L

He et al, 2025

The case of PLCz variants in classical IVF

Table 1: Itemized *in vitro* fertilization data for two men carrying phospholipase C zeta 1 variants

Patient	*Number of oocytes retrieved*	*Insemination method*	*Number of oocytes inseminated*	*Fertilization outcomes*	*Number of good-quality embryos*	*Number of blastocysts obtained*	*Number of blastocysts transferred*	*Number of pregnancies*
Patient 1								
Cycle 1	13	IVF	13	MPN: 10 MI/GV: 3	NA	NA	NA	NA
Cycle 2	10[a]	ICSI + AOA	5	2PN: 5	5	5	2	1
Patient 2								
Cycle 1[b]	5	IVF	5	MPN: 3 0PN: 2	0	0	NA	NA
Cycle 2[c]	10	IVF	5	MPN: 4 0PN: 1	0	0	NA	NA
		ICSI	5	0PN: 5	0	0	NA	NA
Cycle 3[d]	4	ICSI + AOA	3	2PN: 1 1PN: 2	3	2[e]	0	NA
Cycle 4[d]	5	ICSI + AOA	3	2PN: 2 3PN: 1	2	2	1	1

[a]Two mature oocytes were cryopreserved. [b]Second wife. [c]Third wife. [d]Fourth wife. [e]One blastocyst derived from a 2PN embryo was determined to be a complex aneuploid embryo, and the other from a 1PN embryo was determined to be a parthenogenetic embryo. IVF: *in vitro* fertilization; ICSI: intracytoplasmic sperm injection; AOA: artificial oocyte activation; PN: pronucleus; MPN: multiple pronuclei; MI: metaphase of meiosis II; GV: germinal vesicle; NA: not applicable

MPN + High spermatozoa fixation on ZP (same phenotype for a patient with PFN3 variants (Clamart) >Treatment = ICSI + AOA

Che et al, 2024

Loss of profilin3 impairs spermiogenesis by affecting acrosome biogenesis, autophagy, manchette development and mitochondrial organization

Naila Umer[1], Lena Arevalo[1], Sharang Phadke[1,3], Keerthika Lohanadan[4], Gregor Kirfel[4], Dominik Sons[5], Sophia Denise[6], Walter Witke[6], Hubert Schorle[1*].

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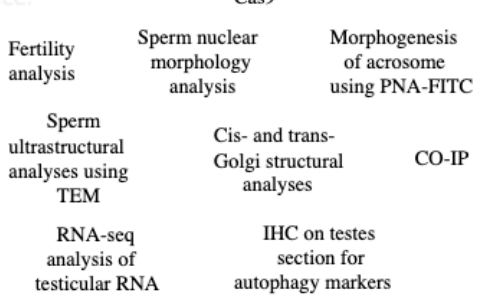
BACKGROUND

Profilins (PFNs) are key regulatory proteins for actin polymerization in cells and are encoded in mouse and humans by four Pfn genes. PFNs are involved in cell mobility, cell growth, neurogenesis and metastasis of tumor cells. The testes specific PFN3 is localized in the acroplaxome-manchette complex of developing spermatozoa. To understand the role of PFN3 in male fertility, we used CRISPR/Cas9 to generate Pfn3 deficient mice. $Pfn3^{-/-}$ males are sub-fertile displaying type II-globozoospermia.

METHODOLOGY



RESULTS

Abnormal sperm head morphology of **$Pfn3^{-/-}$** sperms

Nuclear morphology analysis showed that sperms of $Pfn3^{+/-}$ (Cluster 2) and $Pfn3^{-/-}$ mice (cluster 2-4) displayed shortened hook area, altered circularity and irregular head-tail junction coupling.

Impaired acrosome biogenesis in **$Pfn3^{-/-}$** mice

PNA-FITC revealed Acrosome Biogenesis is impaired in all three phases of development (Golgi, Cap and Acrosome) of $Pfn3^{-/-}$ testis (Fig A-I).

TEM showed detached and abnormal acrosome covering in of $Pfn3^{-/-}$ sperms (Fig J).

Disrupted Golgi network in **$Pfn3^{-/-}$** mice

GM130 and TGN46 IF staining revealed disrupted cis- and trans- Golgi network in $Pfn3^{-/-}$ testis (Fig A-F).

Disturbed AMPK/mTOR signaling leads to inhibited autophagy in **$Pfn3^{-/-}$** mice

RNA-Seq analysis showed upregulation of Trim27 (data not shown here), which leads to higher levels of mTOR resulting in lower AMPK and ATG2a protein levels mediated inhibition of autophagy in $Pfn3^{-/-}$ testis. As a consequence, autophagic flux stalls, indicated by accumulation of LC3B and SQSTM1 (Fig A-C).

Co-IP revealed PFN3-TRIM27 interaction

From whole testis lysate a PFN3-specific antibody pulled down TRIM27.

Specificity of this interaction by using reciprocal antibodies on WB (Fig A-B).

DISCUSSION

Loss of Pfn3 disturbs the morphology of Golgi sub-domains resulting in perturbance of Golgi derived vesicles. Loss of PFN3 leads to deregulation of autophagy markers which seem to lead to the disruption of acrosome formation in Pfn3 deficient germ cells (Fig A-B).

Abnormal manchette development in **$Pfn3^{-/-}$** spermatozoa

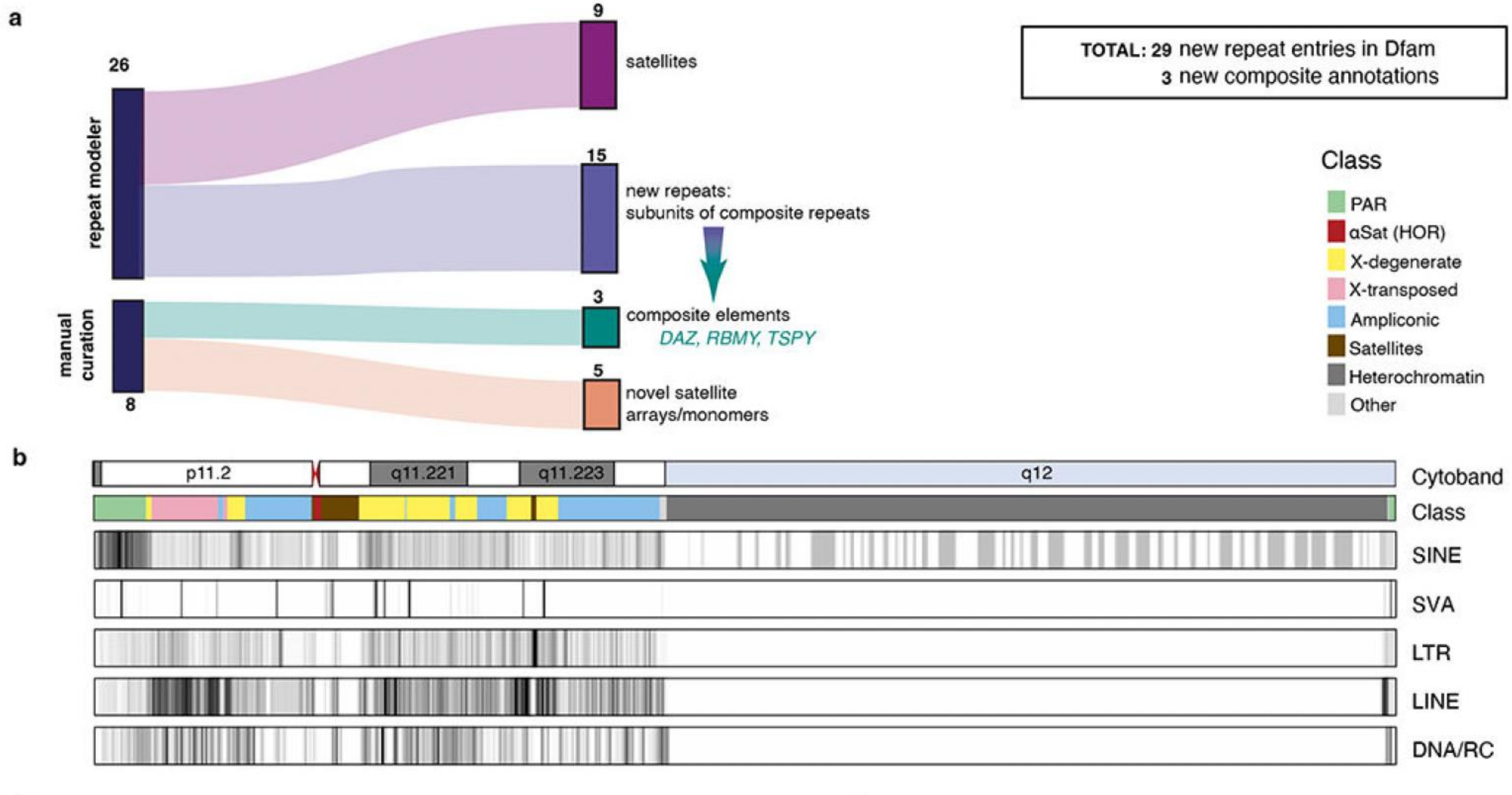
$Pfn3^{-/-}$ mice showed abnormal manchette developme from step 8 to step 13, which results in abnormal hea morphology compared to WT (Fig A-C).

$Pfn3^{-/-}$ sperms displayed flagellar deformities

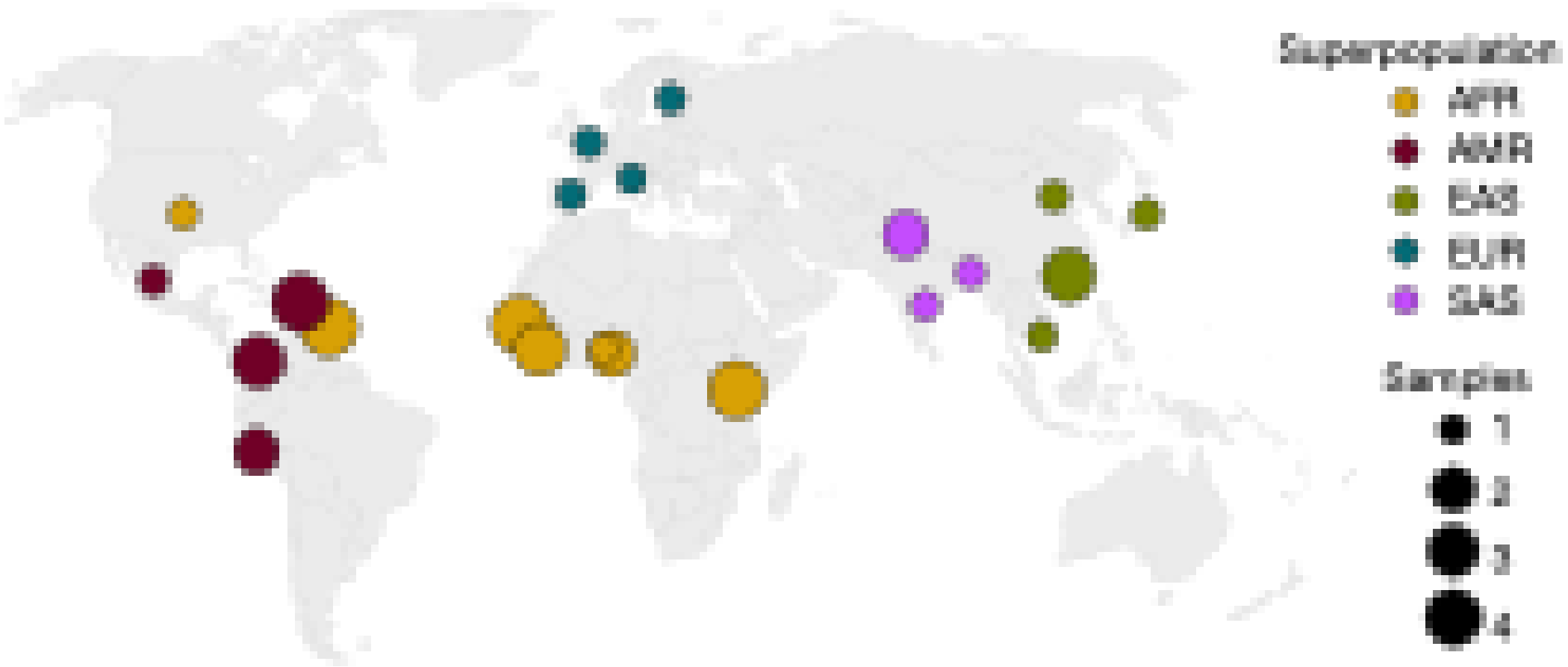
$Pfn3^{-/-}$ mice showed mitochondrial disorganizati using MitoRed and TEM (Fig A-J). SEM showe cytoplasmic droplets (Fig K-N). Fig O showed flagell deformities of 50% sperm cells.

.

Y chromosome sequence obtained with LRS



Combined use of ONT and HiFi to sequence multiple Y chromosome of different geographic origin

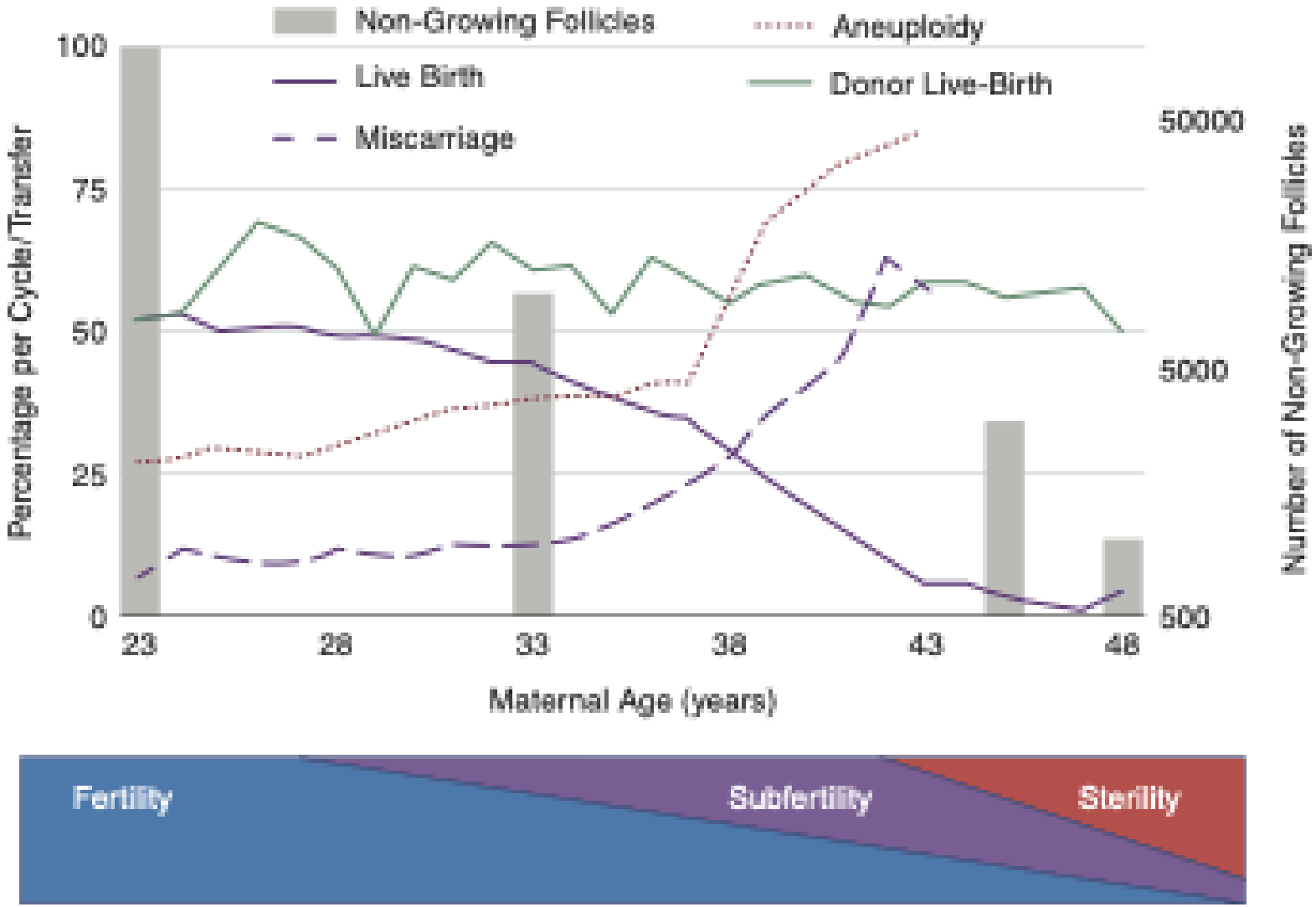


Hallast et al, 2023

Genetic origin of infertility

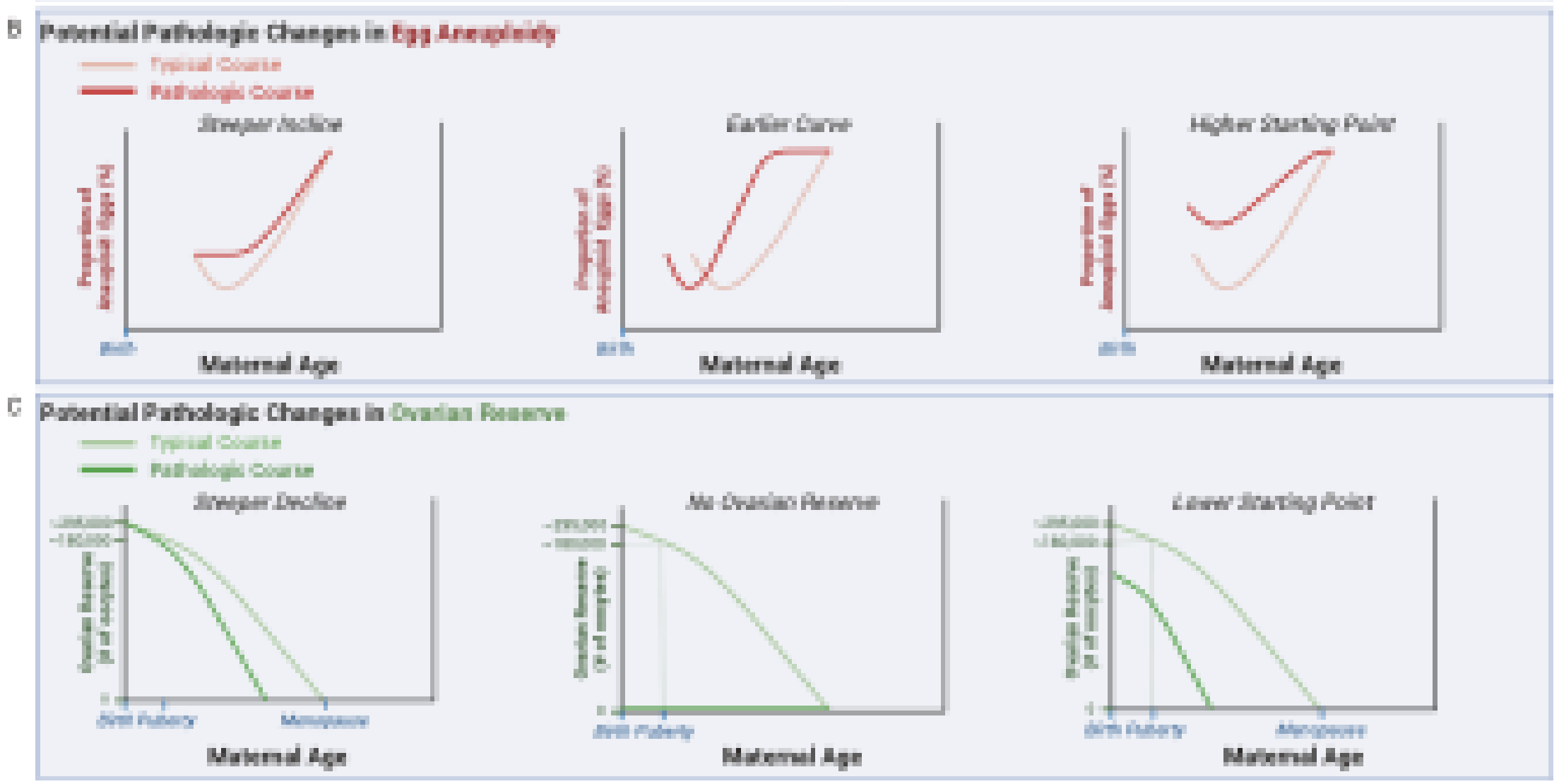
Female

Impact of gene variants on reproductive lifespan?



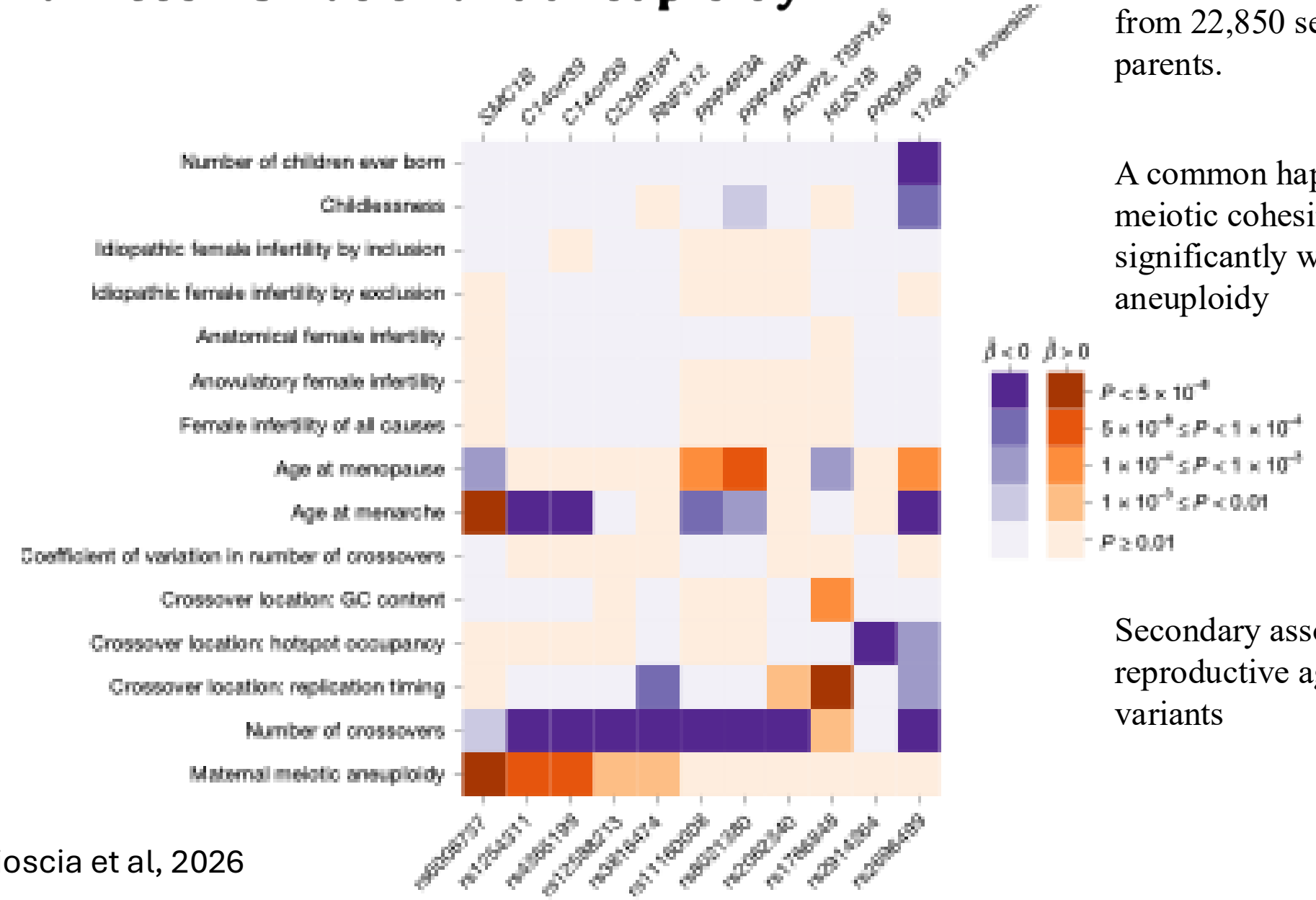
Shaw et al, 2022

Impact of gene variants on reproductive lifespan?



Biswas et Schindler, 2024

Common variation in meiosis genes shapes human recombination and aneuploidy



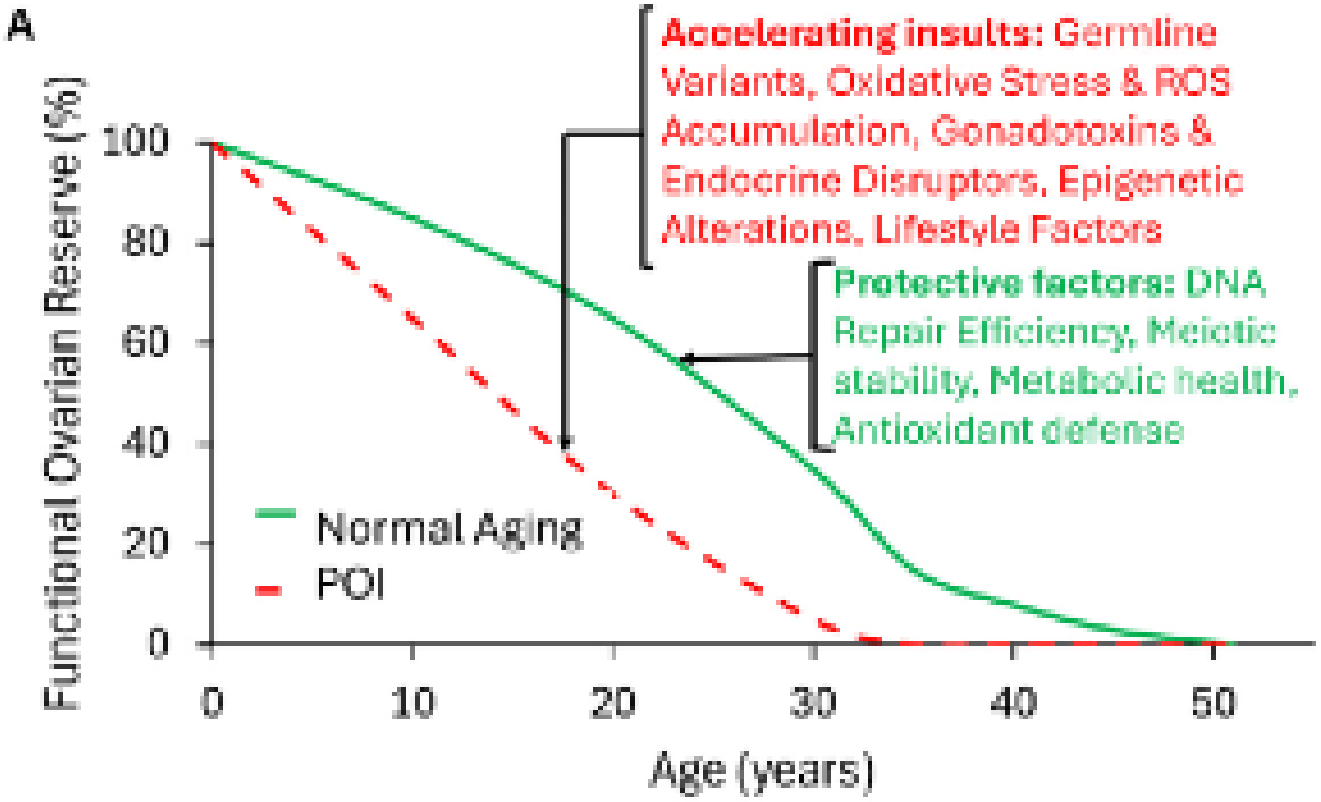
139,416 in vitro fertilized embryos from 22,850 sets of biological parents.

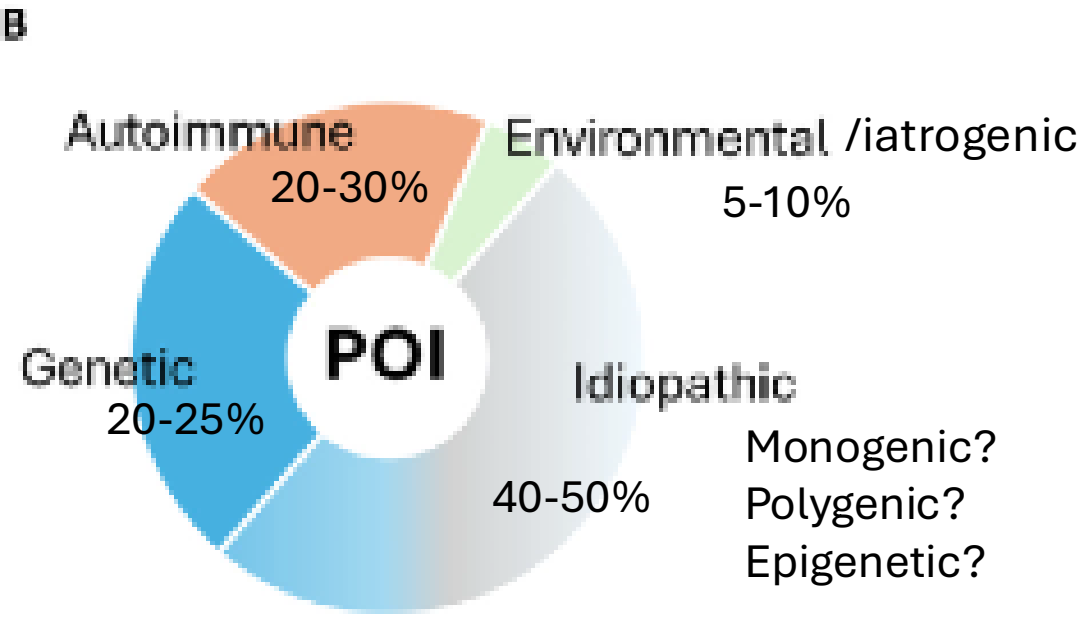
A common haplotype spanning the meiotic cohesin SMC1B is associated significantly with maternal meiotic aneuploidy

Secondary association with reproductive ageing traits for other variants

Carioscia et al, 2026

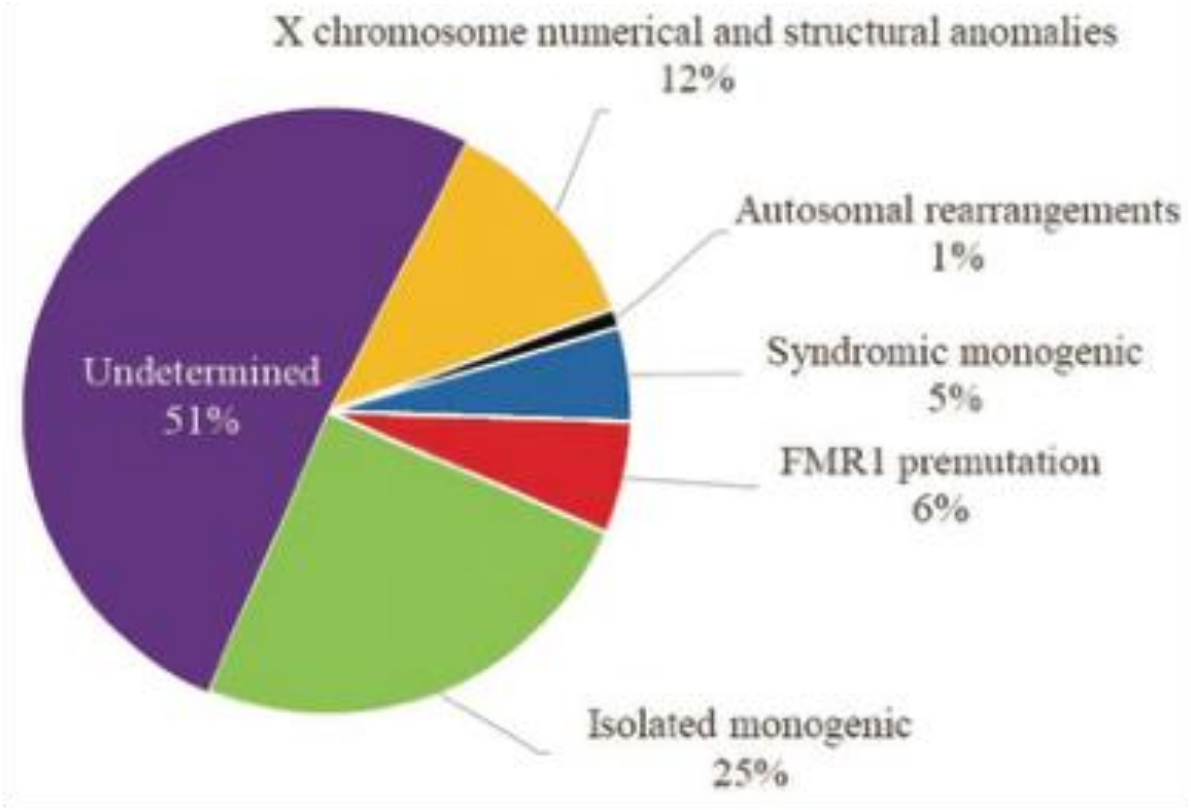
Extreme cases: POI/POF





Abujaber et al, 2026

Extreme cases: POI/POF of genetic origin



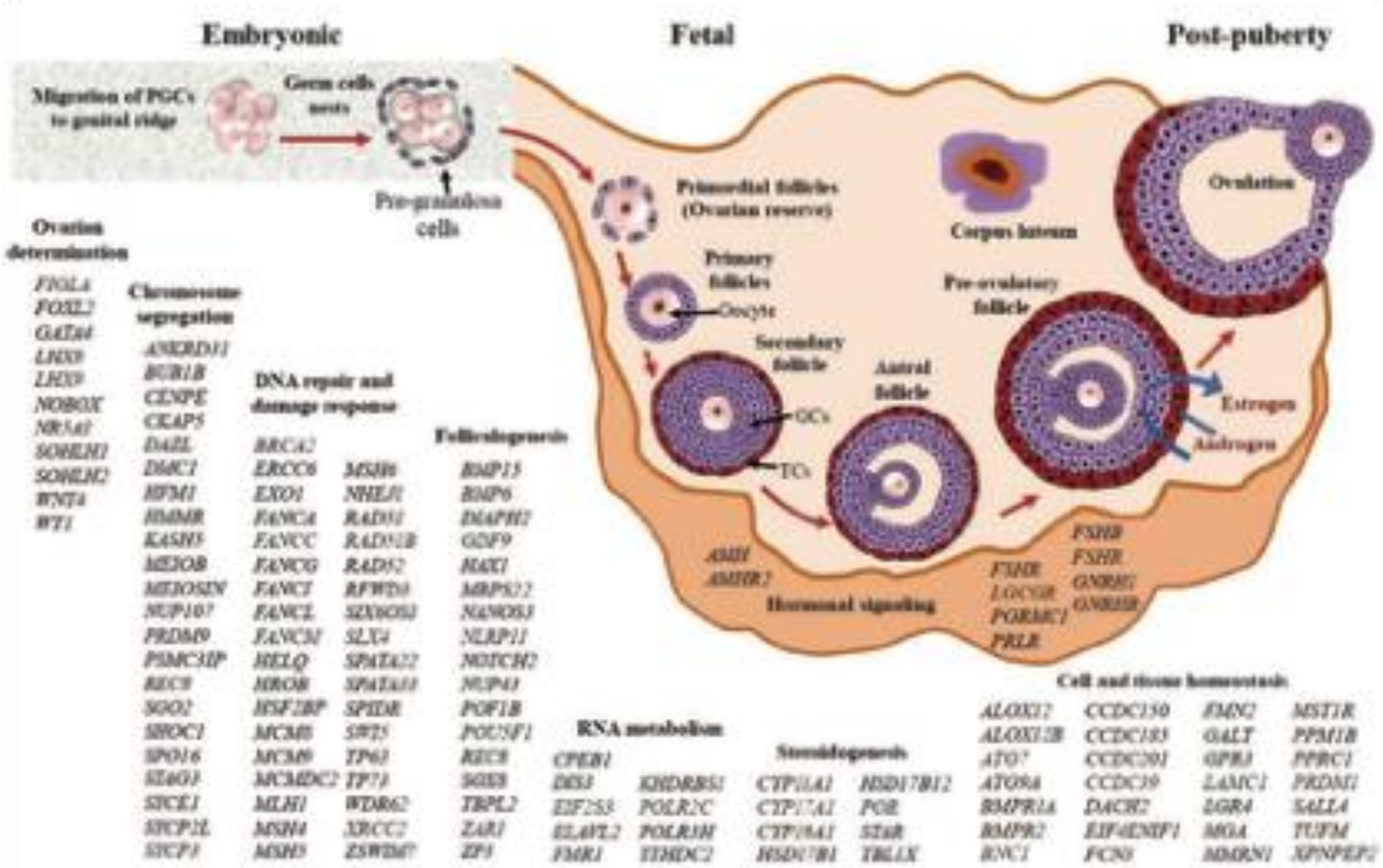
Genetic causes of POI: SNV, triplet repeat or Chromosomal alterations

X chromosome alterations accounts for 12% of POI cases (eg. Turner, partial X deletions, X-autosome translocation)

Xq13-q21 and Xq24-q27 regions are critical for POI > not explained by gene disruption to date: IMPACT OF NON CODING REGIONS?

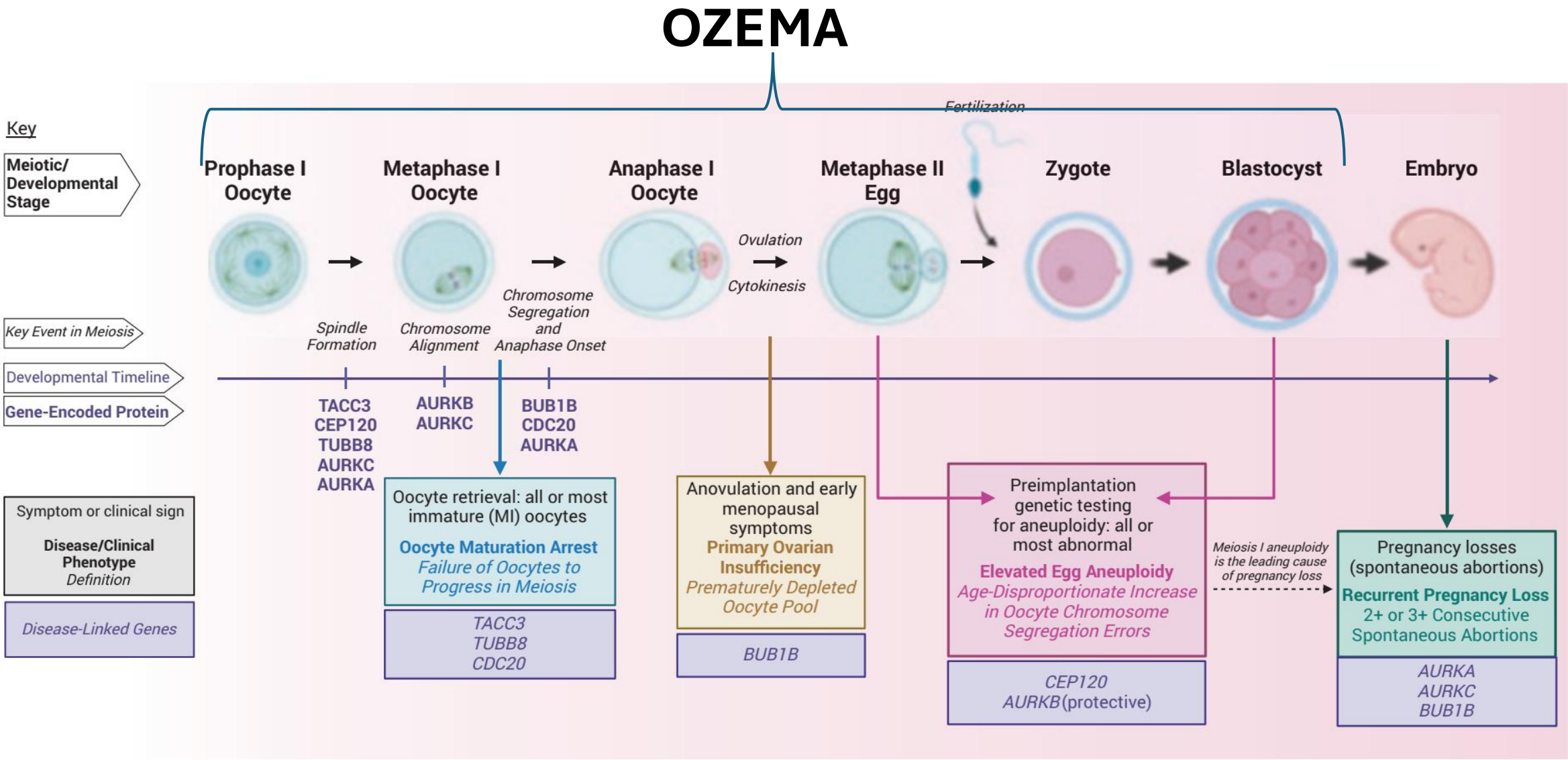
Yatsenko and Rajkovic, 2026

Extreme cases: POI/POF: Pathogenic SNV



Yatsenko and Rajkovic, 2026

Extreme cases: OZEMA and EEA



Biswas et Schindler, 2024

Diet and metabolic status
High Fat/High Sugar, red meat
Negative energy balance (weight loss diet)
Insulin resistance
Folate rich Diet
Mediterranean Diet

Food supplements
Vitamin B complex (OCM), Vit D
Melatonin, Inositols
NMN, CoQ10-MitoQ, resveratrol, quercetin

Chromosomal aberrations
Pathogenic SNV
« OZEMA/EEA phenotype » and others

Toxic exposure
Air pollution, Trace elements, heavy metals, pesticides, EDs
Smoking/alcohol

Female Age

IVF culture failure
Poor Oocyte Quality/poor or no embryo development
Dealing with uncertainty!

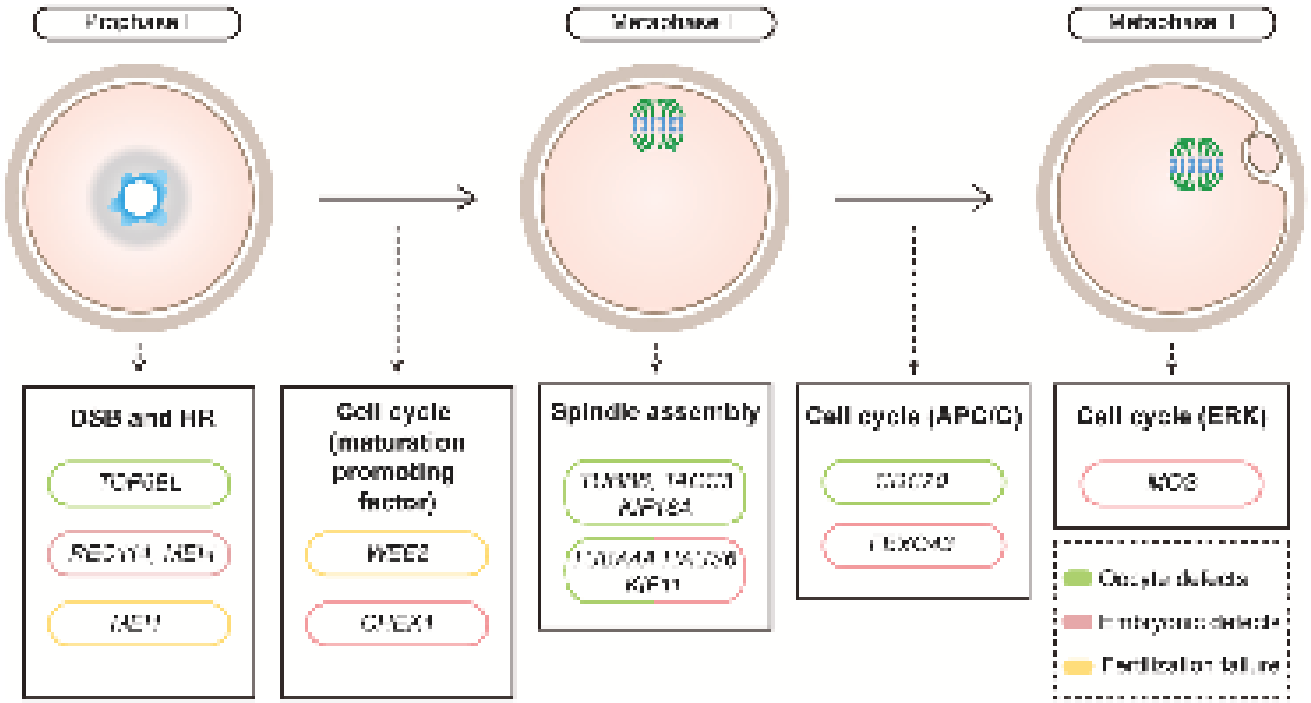
Lab protocols
Culture and manipulation conditions
Fertilization techniques
Fertilization timing (hpt)
Improvement of culture media/systems
Experimental interventions

Adjuvant therapy
GH
GCSF
DHEA
rapamycin

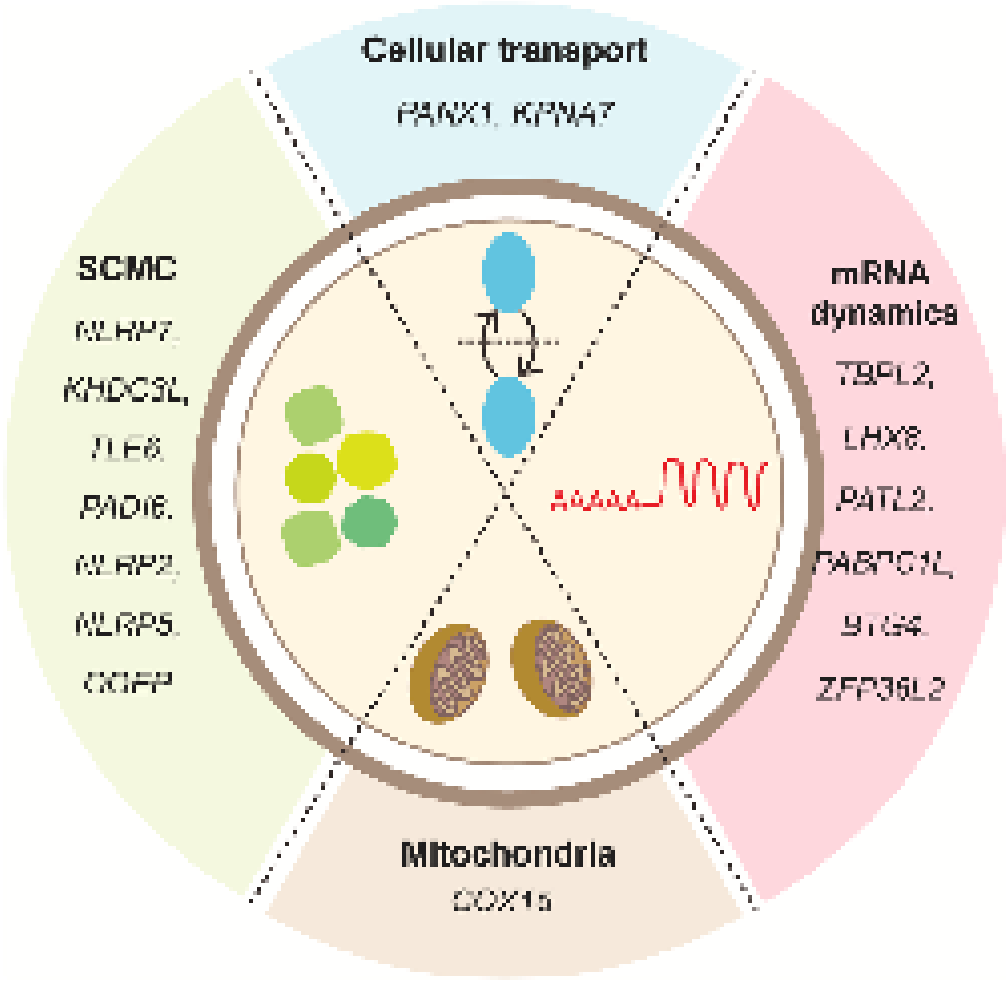
Stimulation regimens
Pretreatment (E2, GnRH agonist)
Poseidon categories stim adaptation
Gn type and dose, oral agents (LTZ, CC)
Sequential LH?
Trigger type (eg. Dual trigger)

Conditions associated with infertility and potential treatments
PCOS, Endometriosis, Tubal factors, Endocrinopathy…

OZEMA/EEA



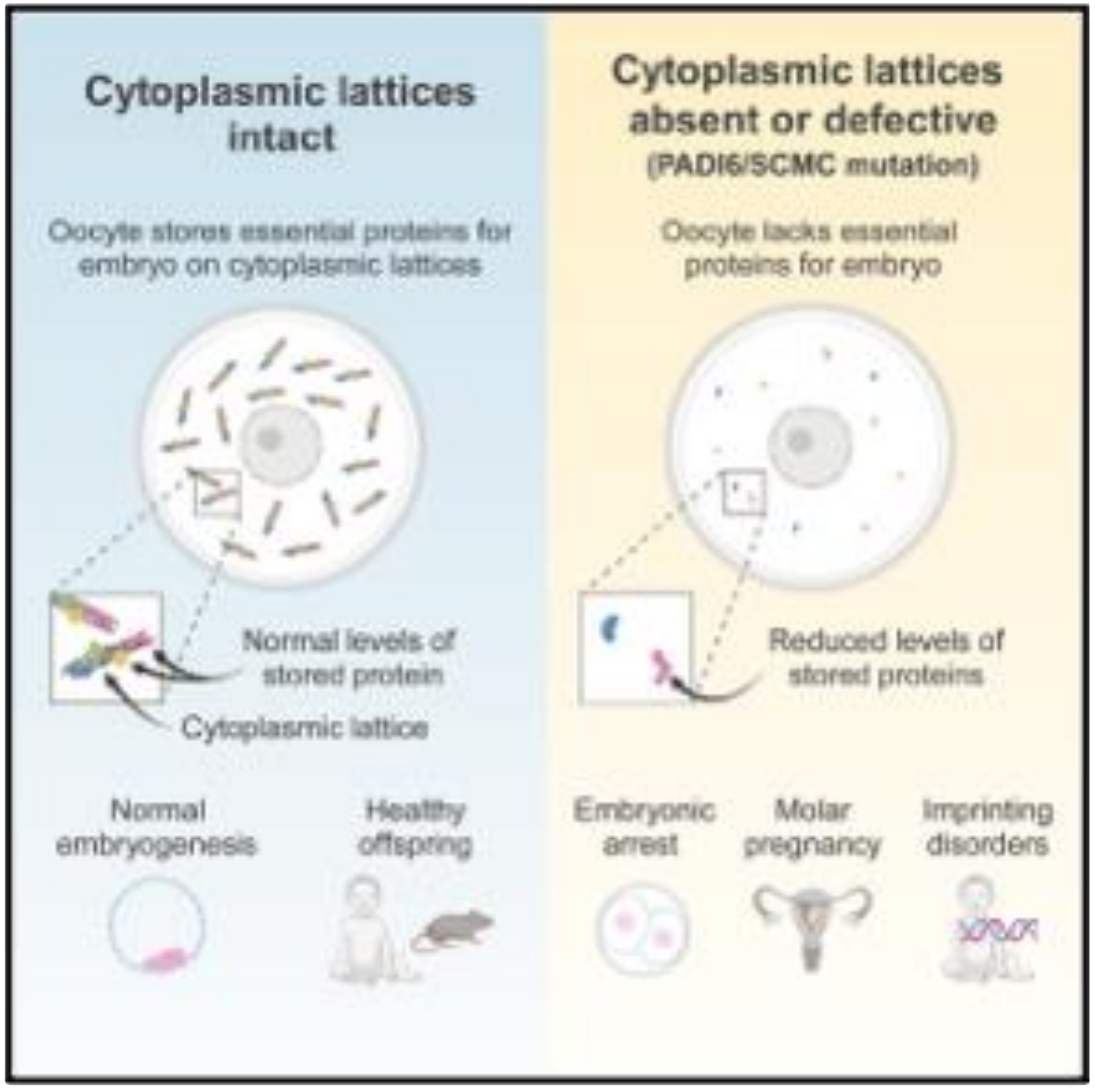
Nuclear maturations defects



Cytoplasmic maturation defects

Gu et al, 2025

SCMC variants and the risk of Molar pregnancy and imprinting disorder



Jentoft et al, 2023

EFS

LHCGR (POI/DOR)
ZP1
ZP2
ZP3

OMA

TUBB8 >145 variants
PATL2
TRIP13
TBPL2 (POI/DOR)
TACC3 (undetectable spindle)
ZFP36L2
PABPC1L
LHX8
CDC23
MAD2L1BP
DLGAP5
COX15 (ferroptosis)

Elevated Egg Aneuploïdy/RIF/PL

MEI1
CEP120
AURKA/C?
RNF212B
BUB1B (POI/DOR)
KIF20A
KIF18A
SYCP3 (RPL)

Fertilization failure

WEE2
PATL2
TUBB8
CDC20
ASTL (may conceive via ICSI)
TLE6 (SCMC)
ZP1, 2 and 3
ZAR1 (MARDO)
MEI1

Oocyte Death Syndrom

PANX1 (mature oocyte and/or zygote spontaneous degeneration)
COX15?

Early embryo developmental arrest/PED

TUBB8
CDC20
TBPL2 (POI/DOR)
TLE6 (SCMC)
PADI6 (SCMC) (hydatidiform moles)
NLRP 2/5/7 (SCMC) (recurrent hydatidiform mole)
CHECK1/BTG4 (Zygotic arrest, Cleavage Failure)
NLRP14 (SCMC)
KHDC3L (SCMC) (recurrent hydatidiform mole)
OOEP (SCMC) (multilocus imprinting disturbances)
FBXO43
MOS (possible large PB1)
MEI1 (aneuploidy,RIF, RPL and recurrent hydatidiform mole), MEI4
REC114 (MPN,complete hydatidiform mole)
ZFP36L2
KPNA7
CEP120
KIF11
CKAP5 (abnormal spindle), DLGAP5
RNF212B
TUB4A, TUBA1C
COX15

Understanding mechanisms is a prerequisite to treatment, prognosis and prevention:

- IVM+Coding RNA
- IVM+Gene editing/gene therapy
- *Mitochondrial replacement therapy (Spindle transfer…)*
- AOA
- Ferrostatin-1 (Cox15)
- Egg donation
- TESE or no TESE / sperm donation
- Anticipate late-onset medically actionnable conditions (eg. Cancer for some NOA or POI associated variants in homologous recombination and meiosis genes (Allen-Brady et al, 2025)

Take-Home Messages

Increased performance of new sequencing and cytogenomic technologies such as LRS and OGM, progressively introduced from research to clinical practice.

Combination of sequencing technologies (LRS, SRS) and/or cytogenomic tools already enables complex clinical situations management (RM, risk of recurrence of germline de novo mutations…)

LRS may increase diagnostic output of poor reproductive outcomes or unexplained severe infertility **> toward increased explainability, individualized prognosis, treatment or prevention.**

Increasing data availability and use of sequencing technologies **calls for increasing counselling and interpretation rules**

Risk of inappropriate conclusions on incidental findings (VUS, ethnicity…)

Functional *in vitro* experiments and/or in vivo mouse models, **testing impact of variants are mandatory for new genes variants**

Need consensus on indications, interpretation and specialized consultations with MDs able to deal with uncertainty!

Statements on genetic testing in failed female reproduction

- The composition of genetic testing panels requires a clear distinction of the differences between causative genes, risk factors and genes only showing a positive association with certain reproductive failure phenotypes.
- The interpretation of sequence variants should follow existing guidelines (e.g. those of Richards et al. (2015)), taking into account disease inheritance patterns and mutational mechanisms.
- A patient's clinical genetic diagnosis can be made solely from characterized genes with an established gene–disease clinical validity association; variants in genes with limited or no evidence should be interpreted with great caution.
- Effort should be made in research and clinics to elucidate the inheritance patterns of putative causative alleles in female reproductive failure, at least through analyses of parental loci.

Volozonoka et al, 2022

What we want to avoid: unnecessary IVF cycles

Table 1. Patient A: Summary of cycle type and outcomes from 2020 to 2023

Cycle type	Outcome	N (%)
Autologous IVF	total oocytes retrieved	74
	mature oocytes	52 (70.2)
	2 pronuclear fertilization	28 (53.8)
	total blastocysts (days 5–7)	1 (100)
	euploid blastocysts	0 (0)
Spontaneous pregnancy or IUI	total pregnancies	12
	losses	12 (100)

IUI, intrauterine insemination; IVF, *in vitro* fertilization.

Darko et al, 2025

23yo, G12P0, 5 IVF cycles > variant in the RNF212B gene

When do we prescribe WGS in these case?
Need specialized consultations and guidelines/consensus+++

Thank you