

# Bilan génétique dans l'infertilité du couple

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# Quelles analyses génétiques pratiquées en 2017

## Le caryotype

Couple dont le conjoint à une altération de la spermatogenèse

Couple avec des FCS-R

Les IOP

## Analyse génétique ciblée

CFTR, FMR1, microdélétion du chromosome Y....

# Les couples infertiles

## Population de patients infertiles

	Nombre de patients	Azoospermie	Oligospermie
Retief et al 1984	496	14.1%	5.1%
Bourrouillou et al 1985	952	15.4%	6.9%
Van Asche et al 1996	Review	13.7%	4.6%

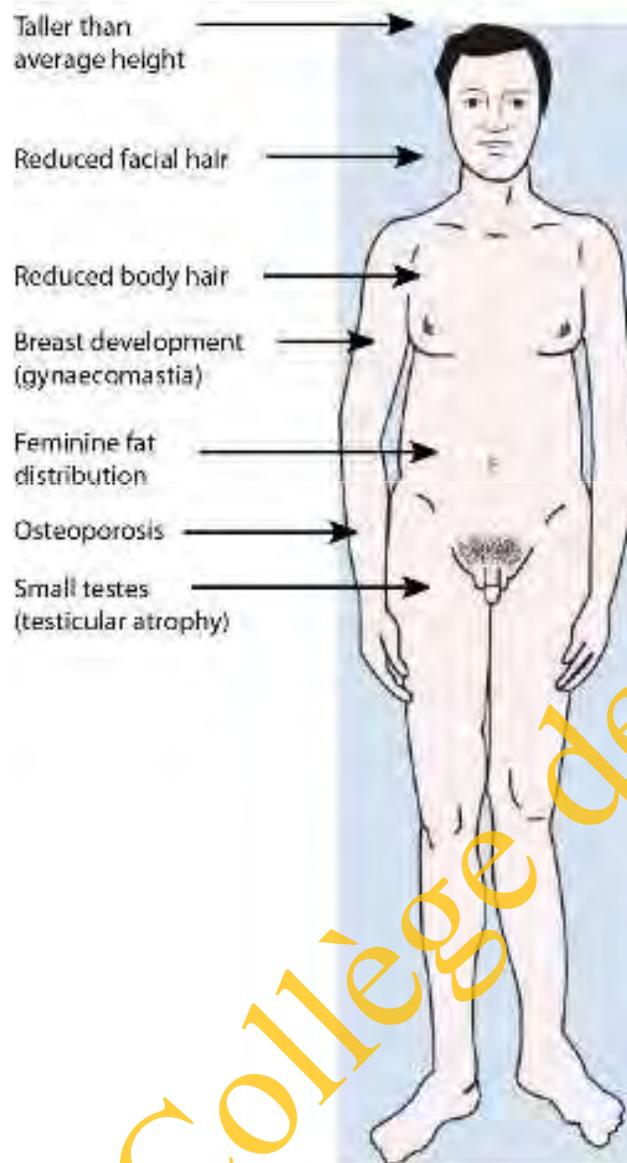
## Conjointes de patients infertiles

2% de remaniements chromosomiques

Gekas et al, 2000



# Syndrome de Klinefelter



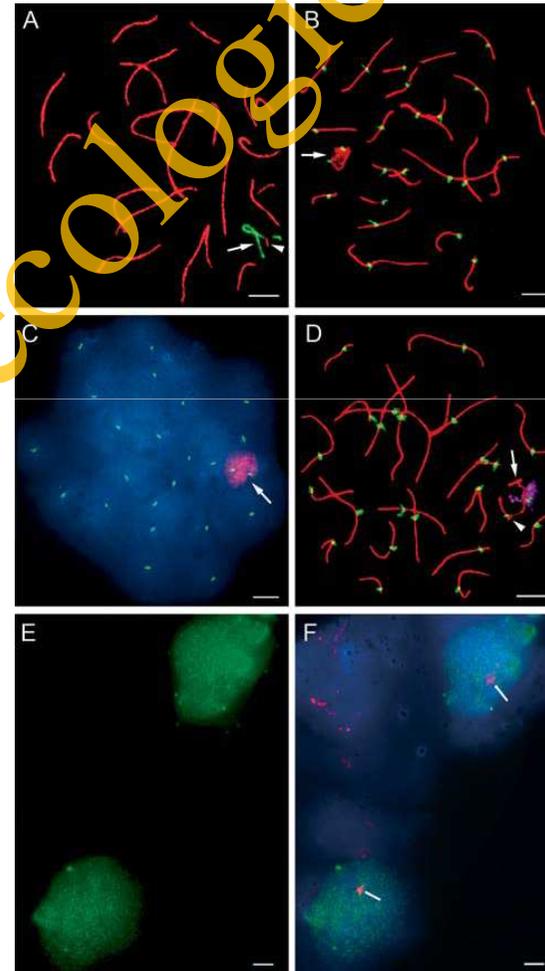
Fréquence: 1 sur 500-  
1000 naissances  
masculines

Moins de 50% des cas  
diagnostiqués

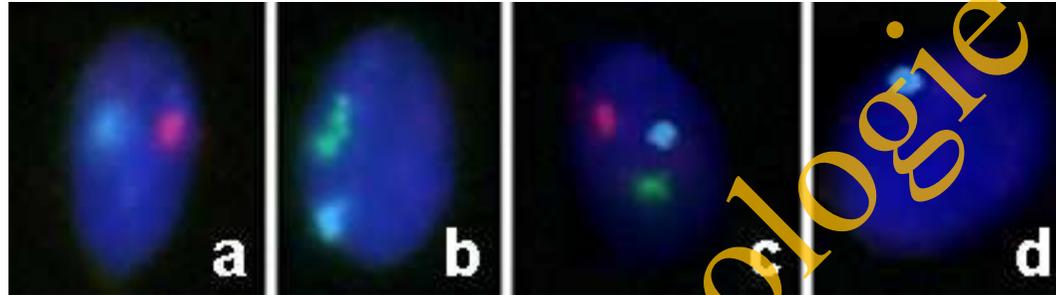
# Seules les cellules 46,XY sont aptes à faire une méiose

Focal spermatogenesis originates in euploid germ cells in classical Klinefelter patients

Sciurano et al, 2009



# Risque d'aneuploïdie et azoospermie



FISH sur spermatozoïdes

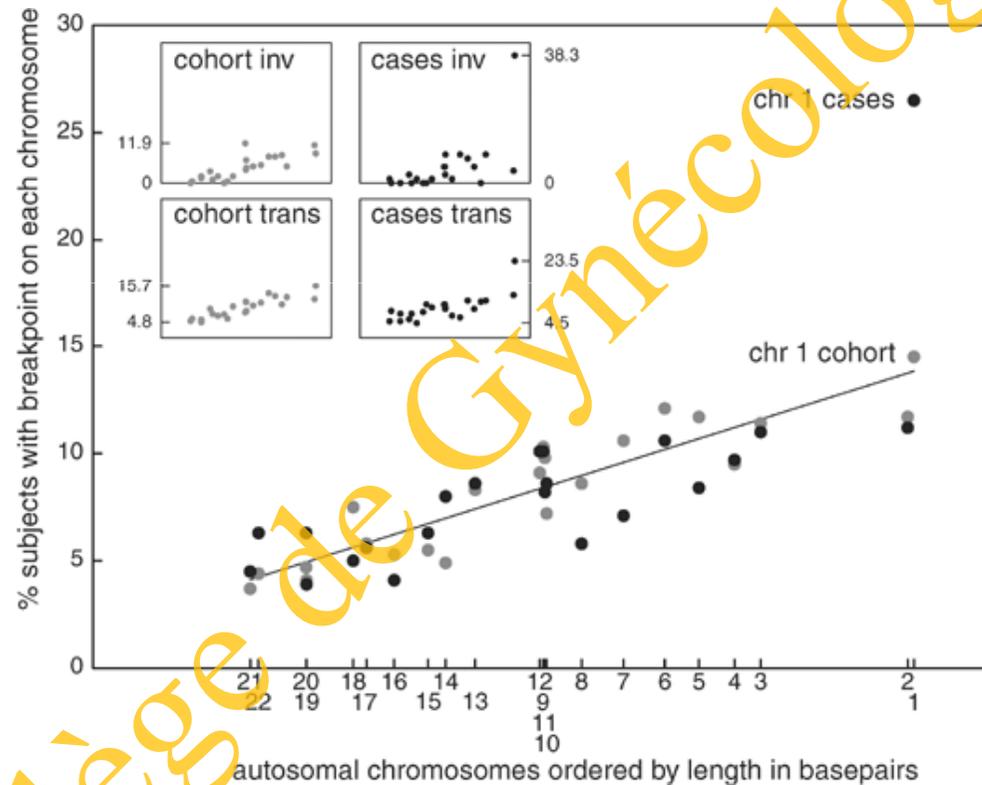
Groupe	Taux d'aneuploïdies spermatiques	Anomalies des gonosomes	Anomalies du chromosome 18
Patients Klinefelter (n=10)	<b>5,3% (n=977)</b>	<b>3,5%</b>	<b>1,4%</b>
Patients azoospermes sécrétoires (n=19)	<b>4.0% (n=2848)</b>	<b>2,4%</b>	<b>1,2%</b>
Patients azoospermes excrétoires (n=35)	<b>0,7% (n=25400)</b>	<b>0,5%</b>	<b>0,1%</b>
Témoins (n=11)	<b>0,6% (n=11178)</b>	<b>0,5%</b>	<b>0,1%</b>

Vialard et al. 2012

# Comment expliquer l'impact des translocations sur la spermatogenèse ?

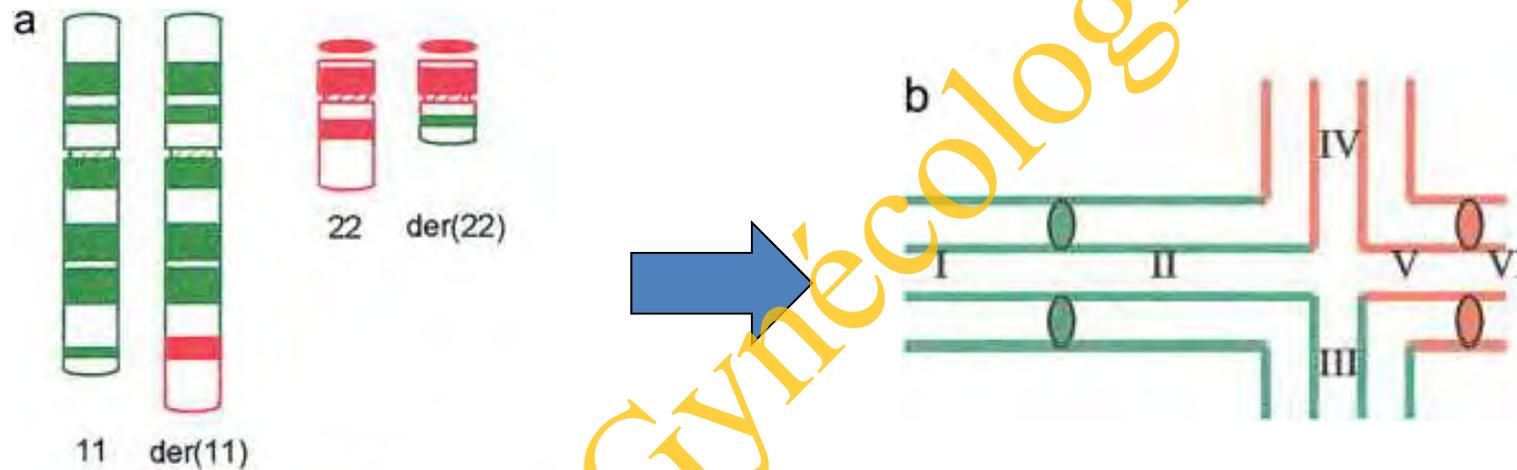
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# 1- inactivation d'un gène nécessaire pour la spermatogenèse



Bache et al, 2004

## 2: Translocation au stade pachytène

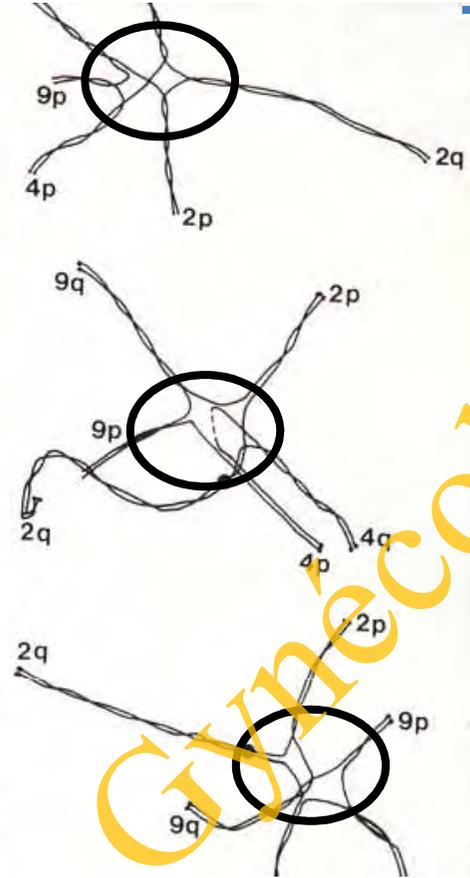
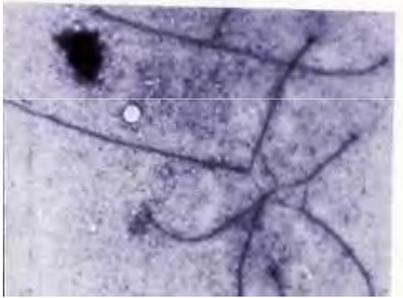
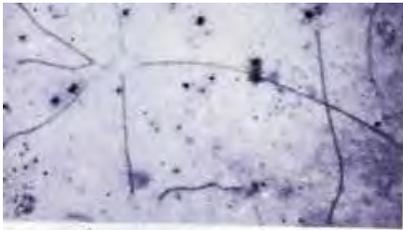


Encombrement ?

Interaction avec la vésicule sexuelle ?

Extension d'inactivation ?

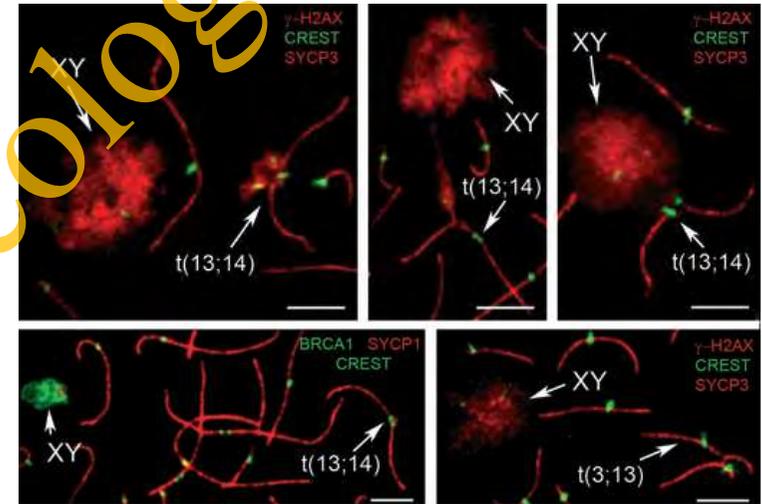
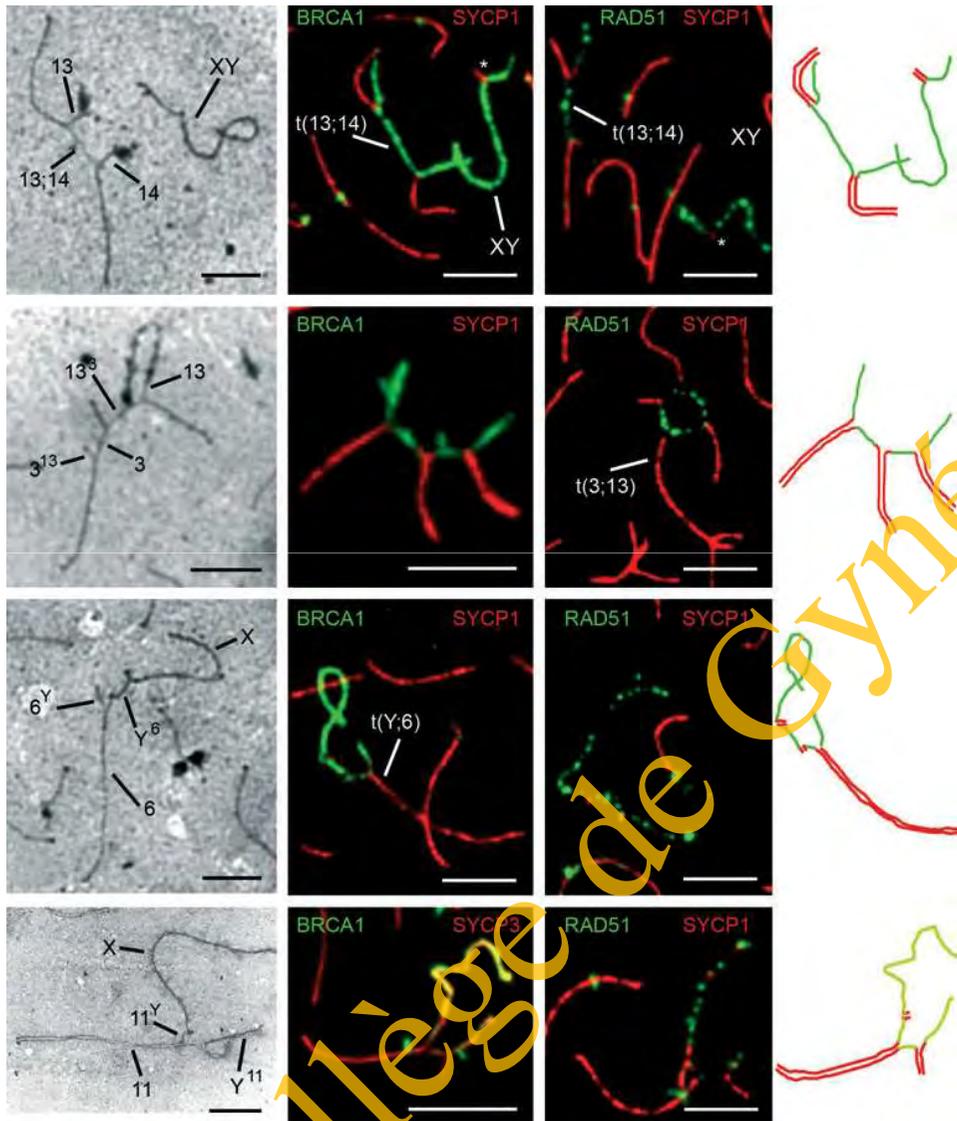
Les remaniements chromosomiques sont-ils tous associés au même risque d'altération de la spermatogenèse ?



Asynapsis



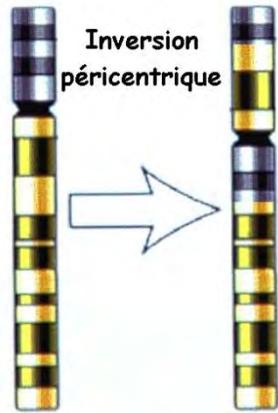
Appariement avec la vésicule sexuelle



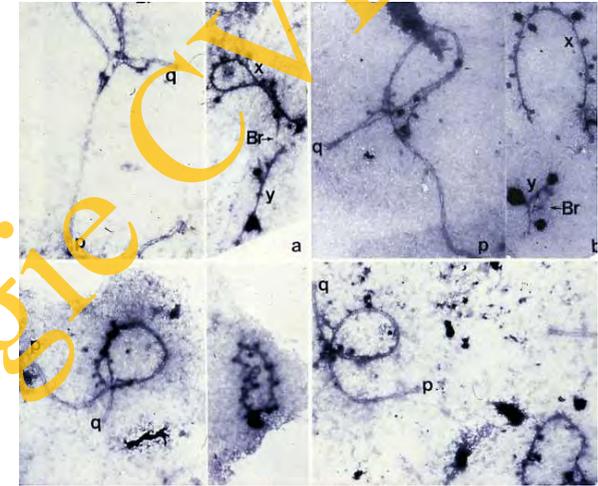
Collège de Gynécologie CVL

Sciurano et al, 2011

18<sup>ème</sup> journée Orléannaises, 7 avril 2017



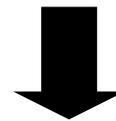
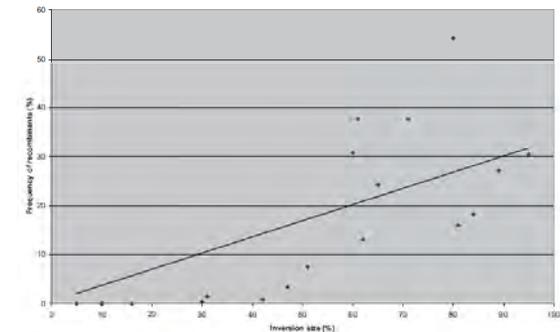
# Inversion péricentrique



**Risque fonction de** (Anton et al. 2005, Morel et al 2006):

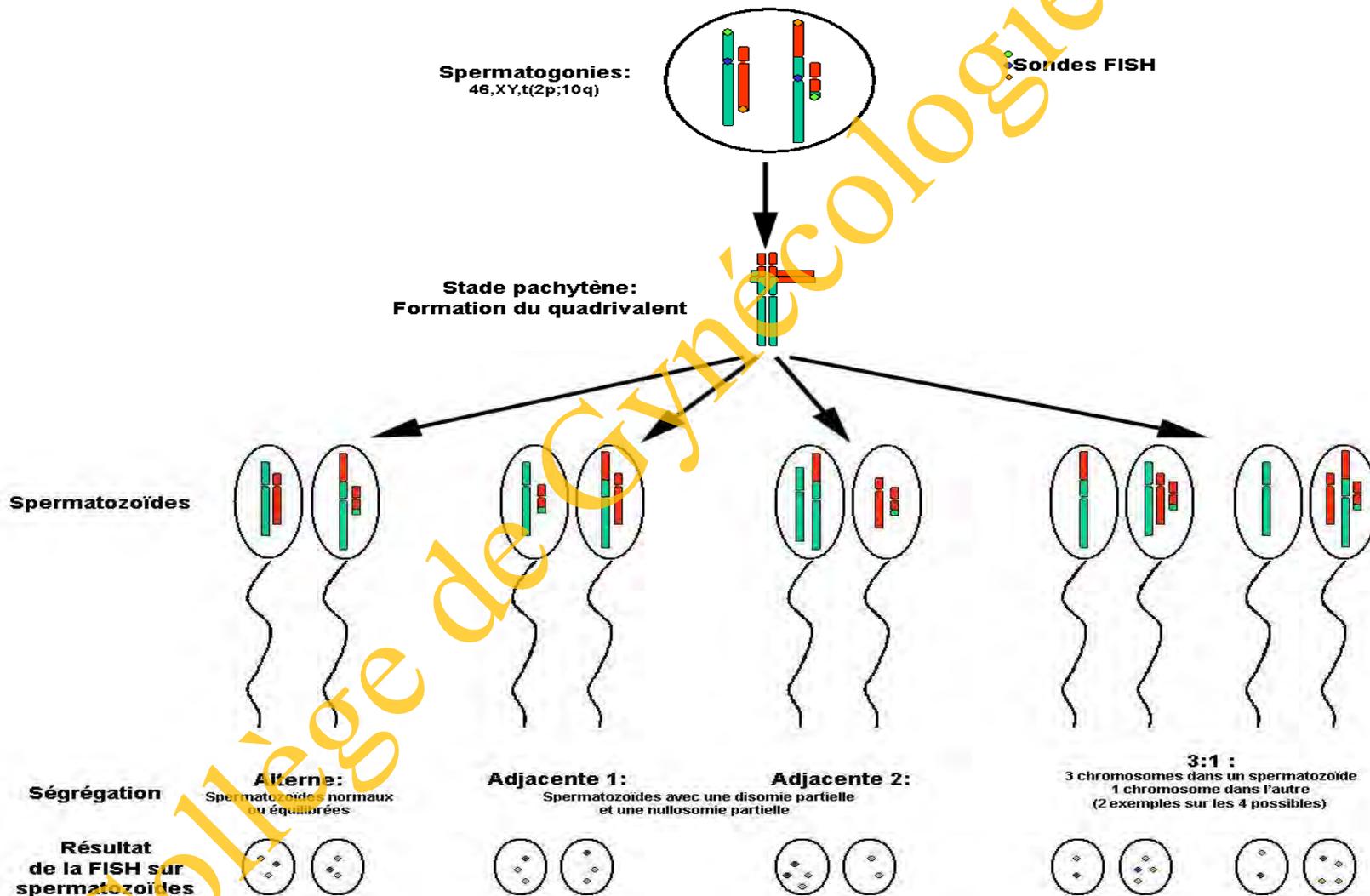
- La taille de l'inversion
- Sa proportion au sein du chromosome

taille supérieure à 100 Mb  
proportion supérieure à 50%



**Augmentation du risque de mal-ségrégation.**

# Quel est le risque pour la descendance s'il y a des gamètes ?



# Intérêt d'une évaluation en FISH des spermatozoïdes

- Quel est le taux de déséquilibre ?
- Ces déséquilibres sont ils viables ou non ?
- Conséquences cliniques importantes
  - Déséquilibre non viable = Echec d'implantation
  - Déséquilibre viable à terme = Enfant avec retard mental et polymalformé
  - Cas intermédiaire = Fausse couche spontanée
- Aide au conseil génétique aux patients
  - Discuter le type de prise en charge
  - Diagnostic pré implantatoire ? (DPI)
  - Diagnostic pré natal ? (DPN)

# La FISH sur spermatozoïdes



Ségrégation	Alterne	Adjacente 1	Adjacente 2	3:1	Autres
Viable à terme	Oui	Non	Non	Non	Non
Fréquence	55.0%	30.2%	5.8%	7.4%	1.6%
Fréquence à terme	100%				

## ICSI envisageable

Ségrégation	Alterne	Adjacente 1	Adjacente 2	3:1	3:1
Viable à terme	Oui	Oui	Non	Oui	Non
Fréquence	45.0%	25.0%	5.0%	10.0%	15.0%
Fréquence à terme	56.2%	31.2%		12.5%	

## Orientation vers le DPI

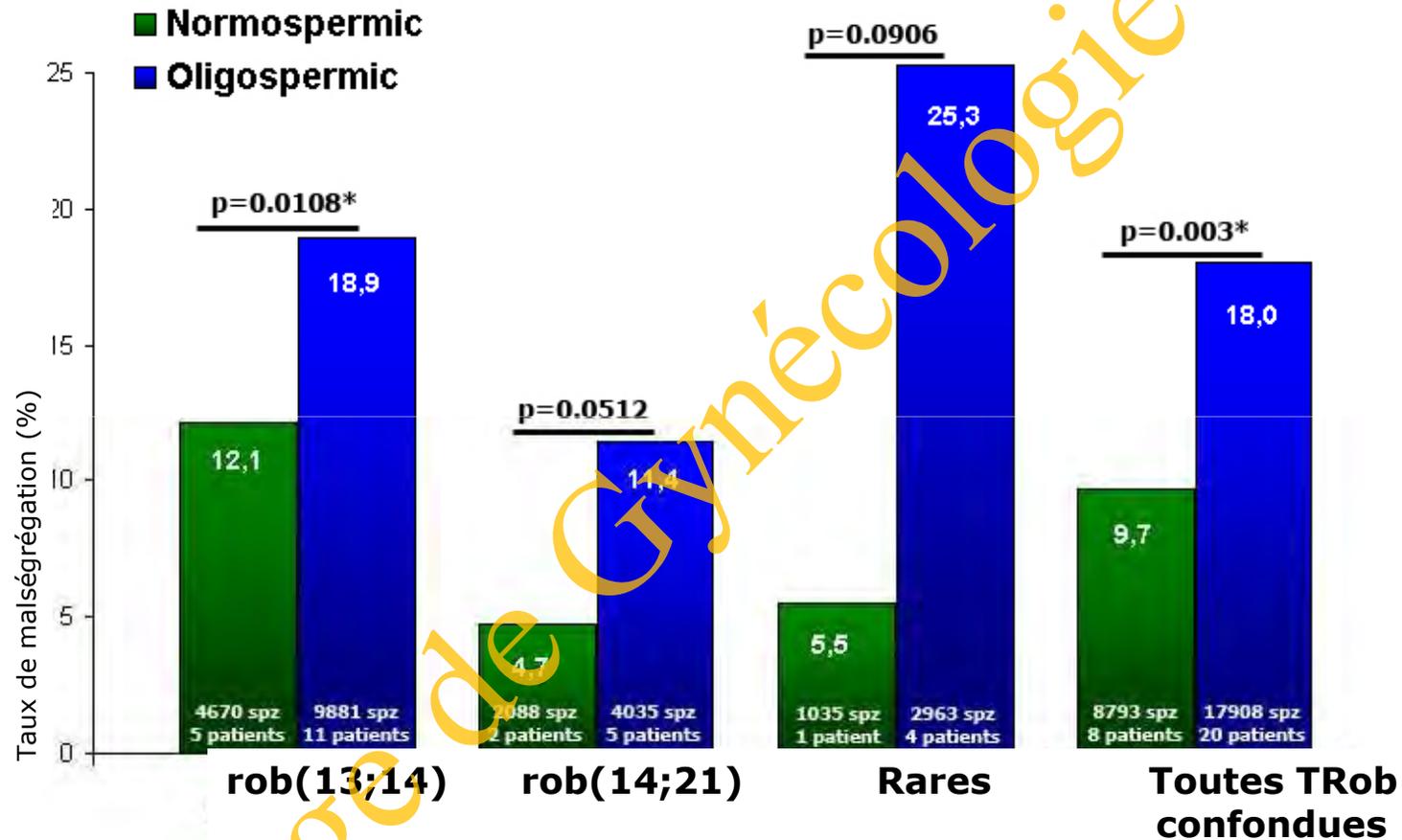
18<sup>ème</sup> journée Orléannaises, 7 avril 2017

# Valeur prédictive du taux de malségrégation et succès en DPI

Translocation	Pregnancy	No. of embryos replaced	Abnormal embryos (%/n)	Abnormal sperm (%)
46,XY,t(1;6)(p22;p21.3)	No	0	100 (5/5)	62.4
46,XY,t(2;18)(q11.2;q21.1)	No	1	75 (6/8)	69.0
46,XY,t(1;18)(p36;q21)	No	1	80 (4/5)	70.8
46,XY,t(3;4)(q12;p15.2)	No	1	94 (16/17)	76.1
46,XY,t(6;9)(p12;q13)	No	1	80 (4/5)	76.4
46,XY,t(11;22)(q23;q11)	No	2	82 (9/11)	77.5
46,XY,t(15;22)(q22;q13)	No	0	100 (4/4)	81.0

Escudero et al, 2003

# Numération & Risque chromosomique de la translocation robertsonnienne

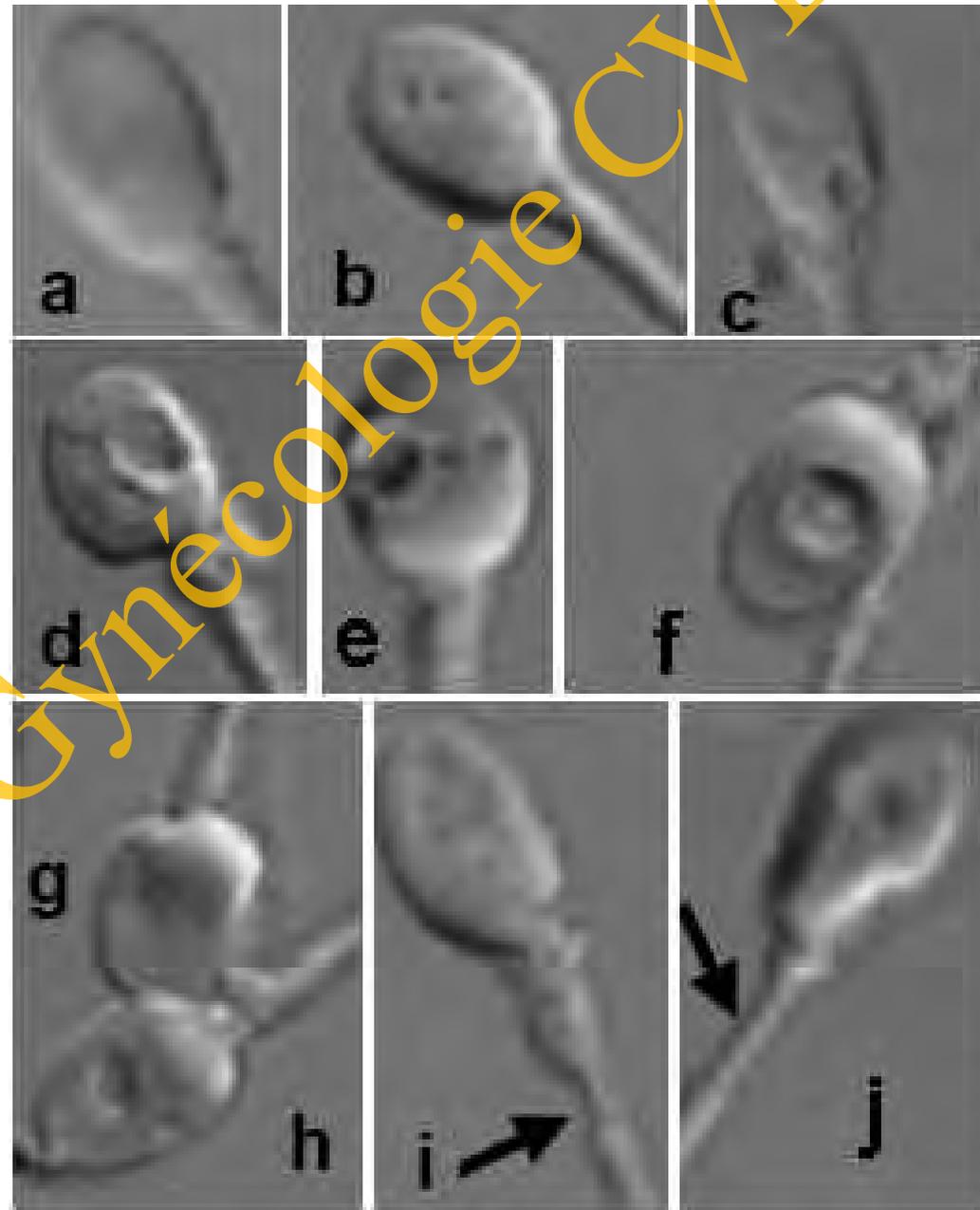


→ Le taux de malségrégation chez les patients porteurs d'une TRob diffère selon la numération spermatique

Ferfour et al, 2012

La sélection des spermatozoïdes à fort grossissement pourrait-elle diminuer la fréquence des aneuploïdies spermatiques ?

Sermondade et al, 2006

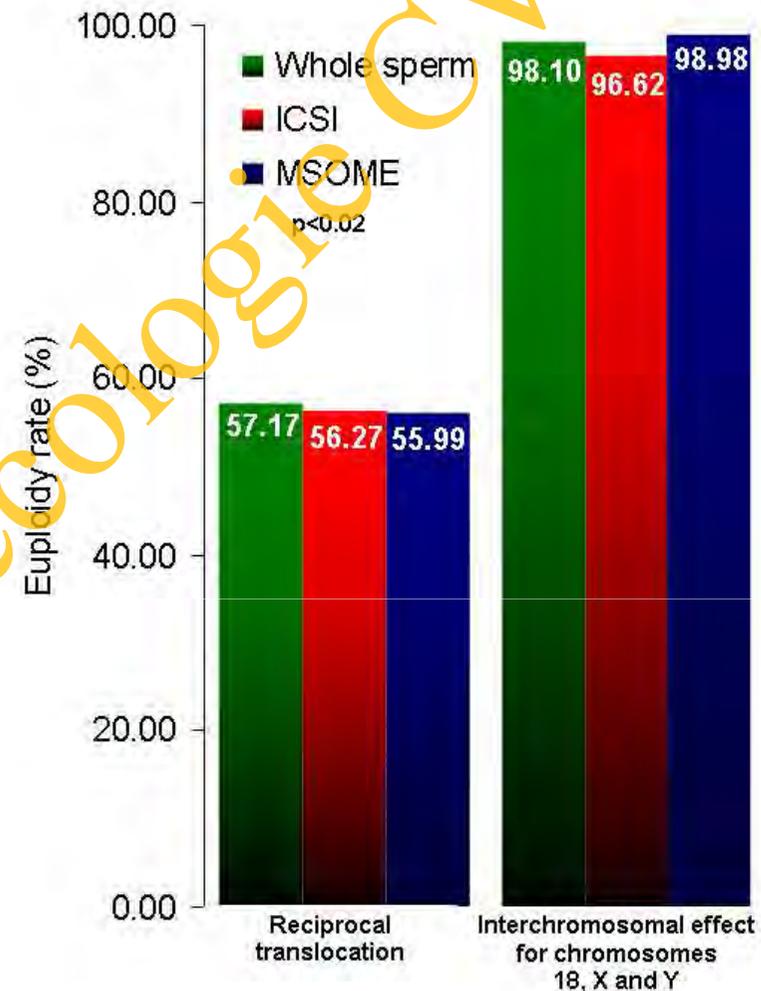


**TABLE 3**  
Sperm fluorescence in situ hybridization (FISH) results in (A) reciprocal translocation carriers and (B) Robertsonian translocations carriers.

MSOME score <sup>a</sup>	A					
	P1	P3	P4	P5	P6	P8
	Class I: 0 Class II: 30 Class III: 70	Class I: 10 Class II: 55 Class III: 35	Class I: 10 Class II: 50 Class III: 40	Class I: 25 Class II: 50 Class III: 25	Class I: 10 Class II: 60 Class III: 30	Class I: 5 Class II: 60 Class III: 35
Alternate segregation						
NS	50%	44.40%	52.60%	47.80%	37.10%	44%
Class I	X	43.40%	53%	59.60%	23.40%	40%
Class II	43.30%	53.50%	64%	55%	35.50%	46%
Class III	50%	38.30%	41.20%	51.70%	39.10%	44.7
Adjacent I						
NS	42%	25.30%	25.20%	31.30%	29.40%	28.40%
Class I	X	26.60%	24.70%	25.40%	40%	40%
Class II	46.70%	30.30%	11% <sup>b</sup>	25.90%	38.70%	26%
Class III	43.30%	36.7%	30.20%	20.90%	34.80%	36.80%
Adjacent II						
NS	0	6.80%	5.40%	7.60%	12%	10.80%
Class I	X	20% <sup>b</sup>	5.10%	8.50%	16.70%	10%
Class II	0	8.40%	9%	8.6%	19.30%	12%
Class III	0	10%	4.60%	8.80%	13%	10.50%
3:1						
NS	8%	23.70%	16.80%	13.30%	21.50%	16.70%
Class I	X	10% <sup>b</sup>	17.20%	6.50%	20%	10%
Class II	10%	7.80% <sup>b</sup>	16%	10.50%	6.40% <sup>b</sup>	16%
Class III	6.70%	15%	9.30%	18.80%	13.10%	8%
MSOME score <sup>a</sup>	B					
	P2	P7	P9			
	Class I: 15 Class II: 55 Class III: 30	Class I: 5 Class II: 55 Class III: 40	Class I: 5 Class II: 55 Class III: 45			
Alternate segregation						
NS	86.60%	81.20%	70%			
Class I	84.90%	80.40%	X			
Class II	87.30%	82.50%	87%			
Class III	82.40%	80.80%	73.70%			
Adjacent segregation						
NS	11.40%	18.80%	28%			
Class I	15.10%	19.60%	X			
Class II	12.70%	17.40%	12% <sup>b</sup>			
Class III	17.60%	19.20%	24.40%			
3:0						
NS	0	0	2%			
Class I	0	0	X			
Class II	0	0	0			
Class III	0	0	0			

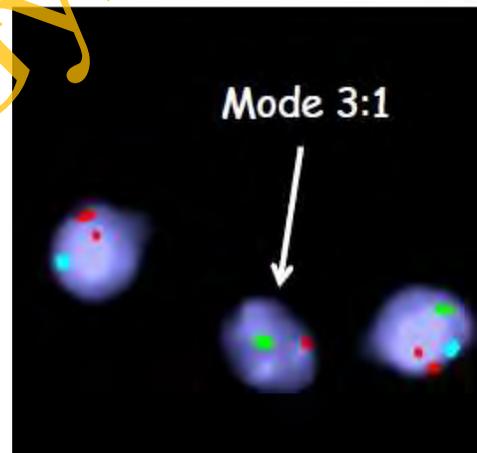
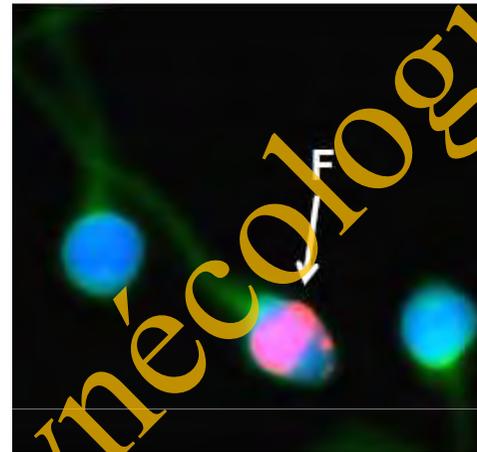
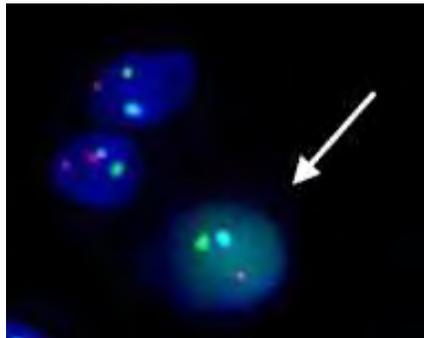
Note: In both cases, the percentages of the three classes of spermatozoa (MSOME score) is given for each patient. The frequency of the different segregation modes is given in each class of sperm cells and in nonselected spermatozoa as controls. NS – nonselected spermatozoa; X – no class I spermatozoa in this patient.  
<sup>a</sup> Percentages of each class at  $\times 6,100$  magnification.  
<sup>b</sup>  $P < .05$ . Indicates some statistically significant differences between nonselected and selected spermatozoa but without biological significance.  
 Cassuto. Sperm FISH in translocation carrier. Fertil Steril 2011.

Cassuto et al, 2011



Chelli et al, 2013

# Ségrégation chromosomique et Fragmentation de l'ADN



		Balanced	Unbalanced
P1	Segregation	47.7 %	52.3 %
	TUNEL-positive	0.5 %	2 % * ( $p=0.003$ )
P2	Segregation	30 %	70 %
	TUNEL-positive	5.4 %	8.9 % * ( $p=0.015$ )
P3	Segregation	44.8 %	55.2 %
	TUNEL-positive	2.3 %	4.9 % * ( $p=0.003$ )
P4	Segregation	70 %	30 %
	TUNEL-positive	2.5 %	6.5 % * ( $p=0.01$ )
P5	Segregation	80.7 %	19.3 %
	TUNEL-positive	1.1 %	3.6 % * ( $p=0.001$ )
P6	Segregation	97.9 %	2.1 %
	TUNEL-positive	4.2 %	14.3 % * ( $p=0.004$ )

Taux de fragmentation moyen spermatozoïdes équilibrés: **5.0%**  
spermatozoïdes déséquilibrés: **13.3%**

Rouen et al, 2013

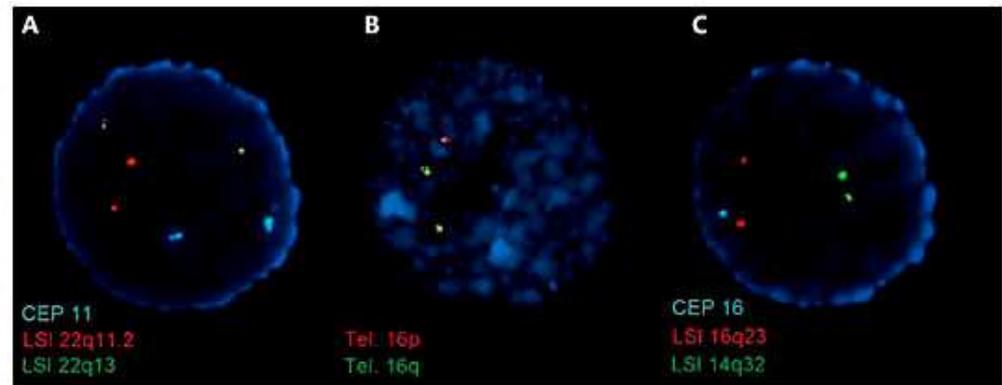
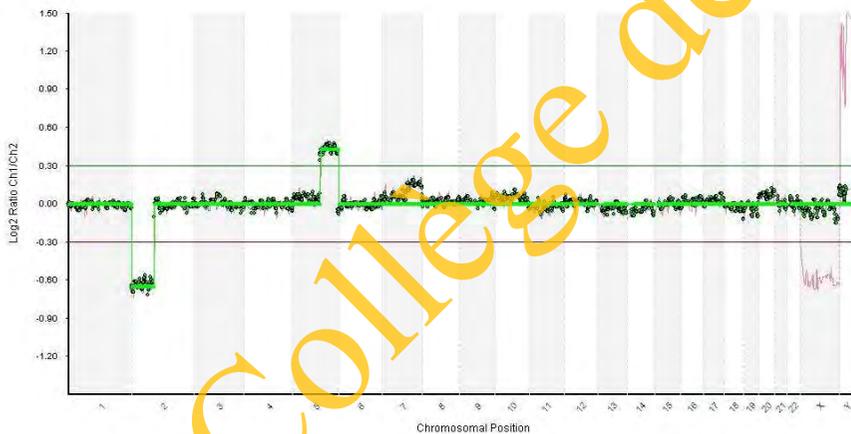
Perrin et al, 2013

# Le diagnostic préimplantatoire

**Table I** Ten years of PGD Consortium data.

	Cycles to OR	No. embryos biopsied	No. embryos transferred (mean/ET)	Embryo transfer procedures	Clinical pregnancy rate (per OR and per ET)
Single genes	4733	27 980	7035 (1.9)	3727	22% per OR 29% per ET
Structural chromosome abnormalities	4253	27 068	4775 (1.7)	2731	17% per OR 26% per ET
Sexing X-linked	1167	7317	1598 (1.8)	880	19% per OR 26% per ET
Aneuploidy	16 806	90 404	21 543 (1.8)	12 071	19% per OR 27% per ET
Social sexing	671	4285	993 (2.0)	492	21% per OR 29% per ET

OR, oocyte retrieval; ET, embryo transfer procedure.

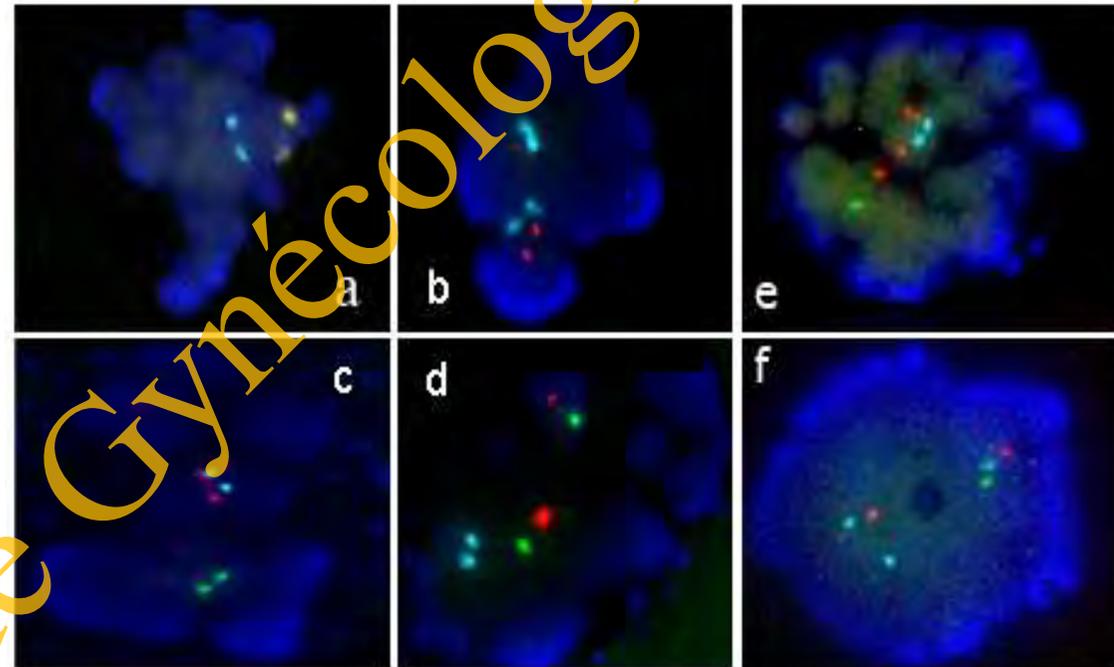
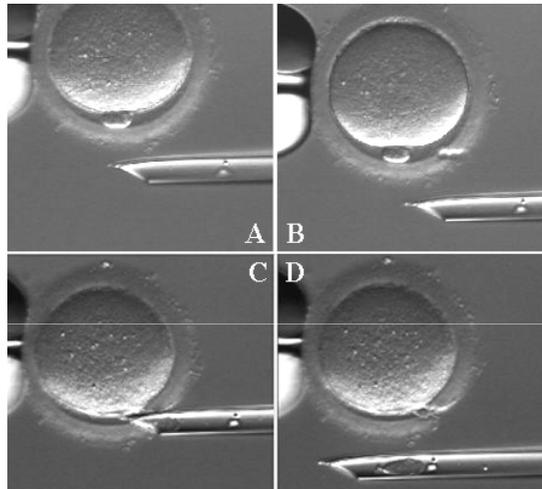


# Risque chromosomique en fonction du sexe

Stage (references)	Total	Mode		Mode	
		Alternate	%	Adjacent	%
<b>Female 13/14</b>					
Polar body/oocyte (1,2)	81	52	64	27	33
Preimplantation embryos (3,4)	63	42	67	30	32
Prenatal diagnosis (5,6,7,8)	276	273	99	3	1
<b>Male 13/14</b>					
Spermatocytes (9,10,11,12,13)	29 433	25 539	87	3689	13
Preimplantation embryos (3,4)	124	105	85	17	14
Prenatal diagnosis (5,6,7,8)	123	122	99	1	1
<b>Female 14/21</b>					
Polar body/oocyte (1,2)	93	53	57	38	41
Preimplantation embryos (4)	49	31	63	18	37
Prenatal diagnosis (5,7,8)	308	177	57	31	15
<b>Male 14/21</b>					
Spermatocytes (11,13,14,15)	228 63	20 122	88	2557	11
Preimplantation embryos (3,4)	63	60	95	3	5
Prenatal diagnosis (5,7,8)	74	73	99	1	1

Bint et al 2011

# L'étude du 1<sup>er</sup> globule polaire



## Exemple de Globules polaires analysés

a : ségrégation adjacente 2

b : ségrégation adjacente 1

c et d : ségrégation alterne avec en d les 2 dérivés chromosomiques

e : GP avec CO déséquilibré

f : GP avec CO équilibrés

# Les données sur Poissy

## Translocation Réciproque

Hommes: 50,7% (47 patients)

Femmes: 70.3% (165 ovocytes , 18 patientes)

## Translocation Robertsonienne

Hommes: 15,4% (31 patients)

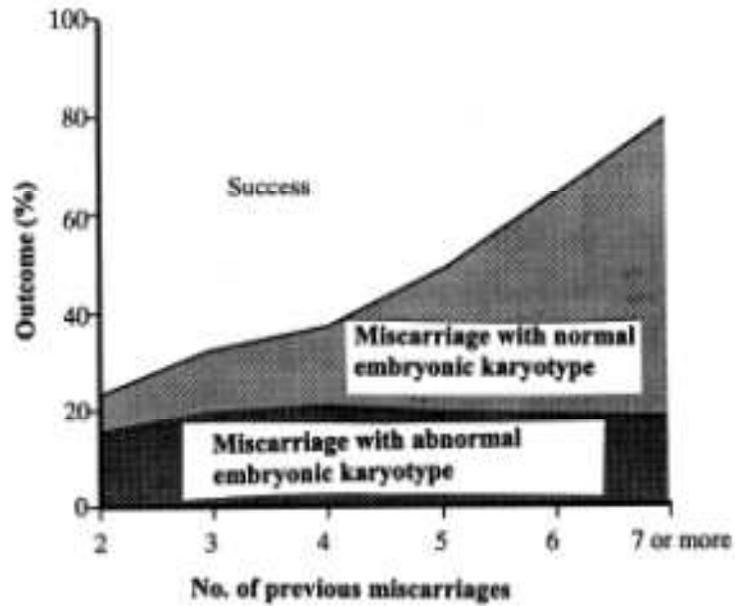
Femmes: 42,5% (133 ovocytes , 10 patientes)

# Cytogénétique et fausses couches à répétition

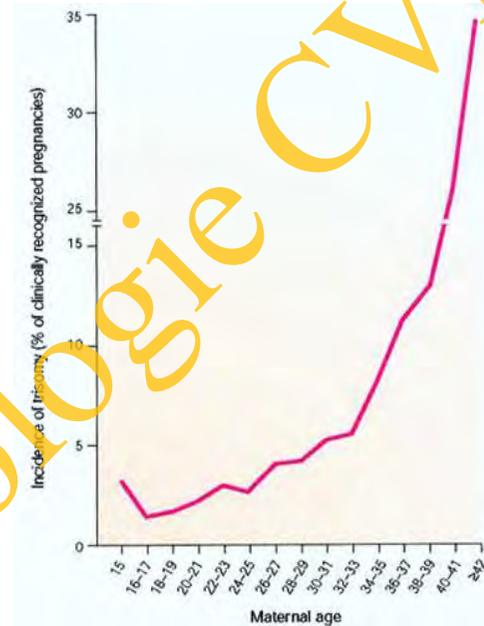
**Pourquoi:** Les anomalies chromosomiques induisent des remaniements déséquilibrés

**Chez qui:** Les 2 membres du couple

**Quelle prise en charge:** Fonction de différents critères



Ogawasara et al, 2000



Hassold et al, 2001

Nombre de fausses couches antérieures				
Âge	2	3	4	5
20	92	90	88	85
25	89	86	82	79
30	84	80	76	71
35	77	73	68	62
40	69	64	58	52
45	60	54	48	42

Brigham et al, 1999

# Faut il analyser le produit de fausses couches ?

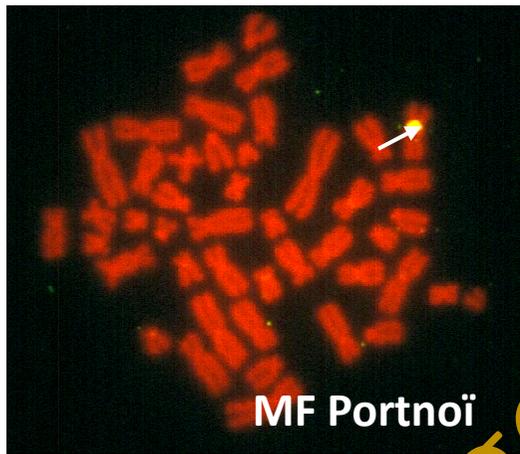
TAT	DR-A	Fetal skin or muscle-A	CVS or unidentified tissue origin-B	DR-B
24h			Microsatellite analysis (MCC exclusion) MCC → STOP MCC + or triploidy → STOP	11% (Triploidies)
48h	75% (Aneuploidies)		Abnormal → STOP Normal → CMA	75% (Aneuploidies)
2-3 weeks	22% (mosaics <50%, structural rearrangements, polyploidies)		Abnormal → STOP Normal → CMA	11% (mosaics <50%, structural rearrangements, polyploidies)
3-5 days	6% (Cryptic unbalances and low mosaics)		Abnormal → STOP Normal → CMA	6% (Cryptic unbalances and low mosaics)

TAT= Turn around time; DR= Detection rate; MCC= maternal cell contamination

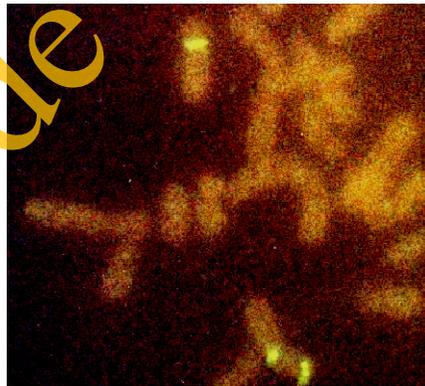
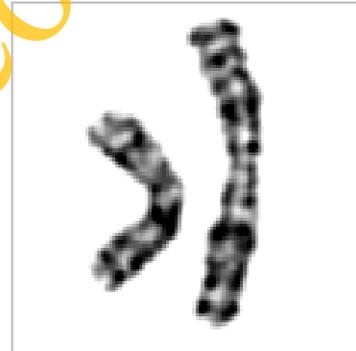
Grati et al, 2013

# Les insuffisances ovariennes prématurées

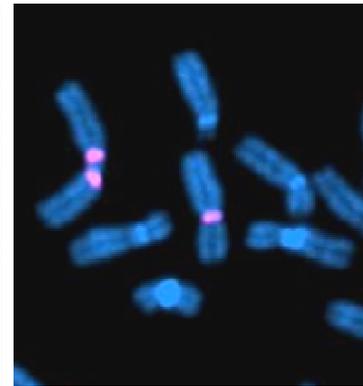
**Le caryotype:** 10% de remaniements chromosomiques  
Anomalies du chromosome X: 45,X, etc...



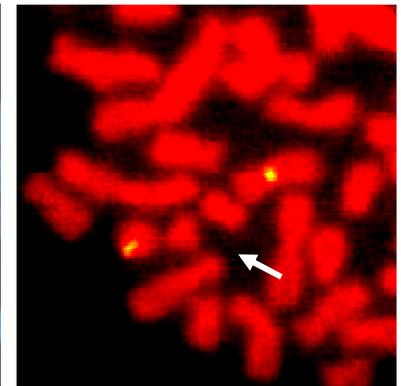
45,X



46,X,del(Xp)



46,X,i(Xq)

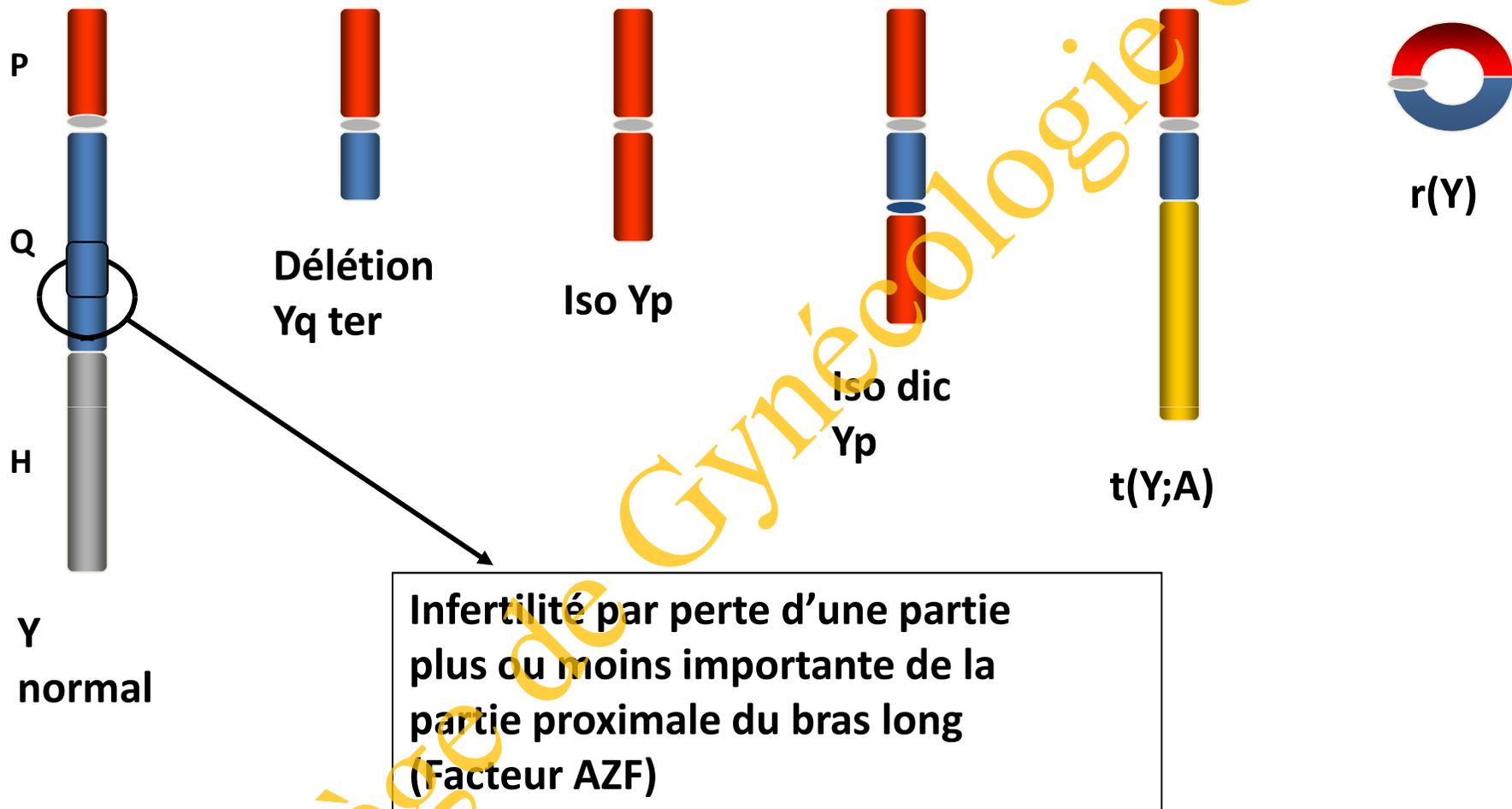


46,X,r(X)

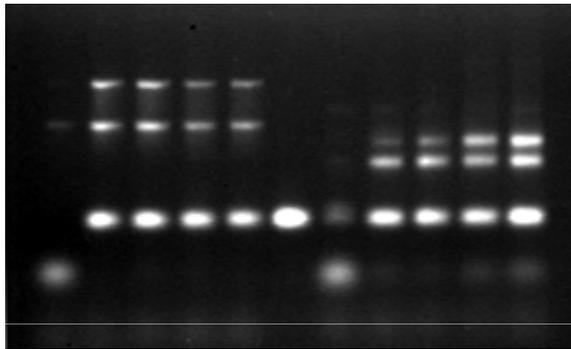
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# Analyse génique ciblée

# Chromosome Y et fertilité



# Microdélétion du chromosome Y

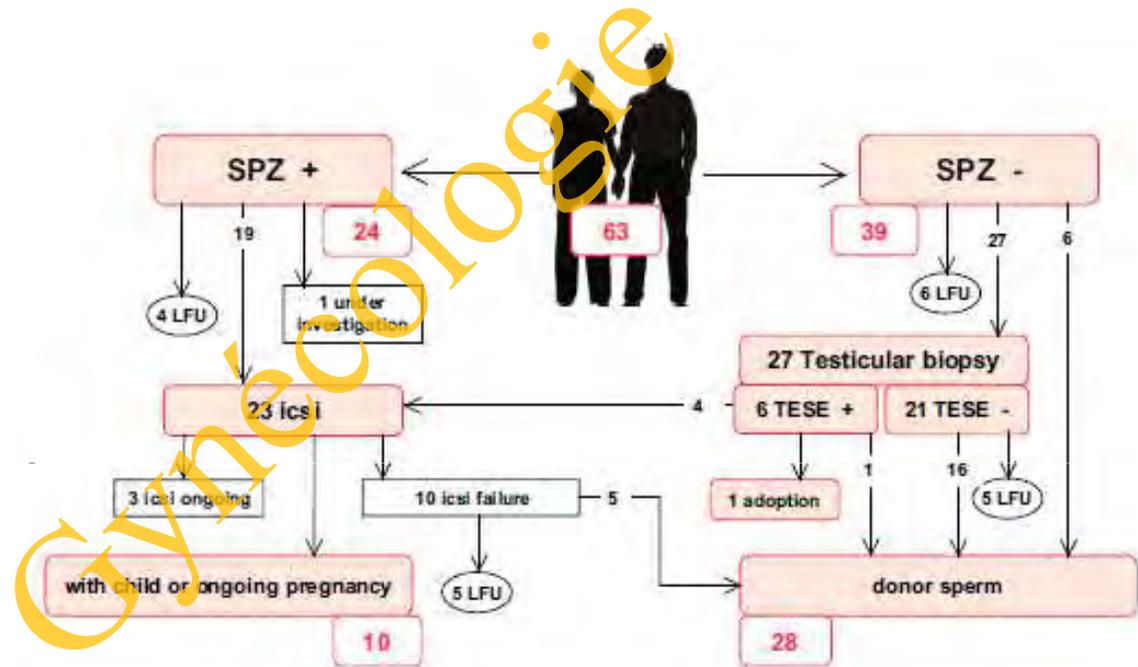


3 loci AZF

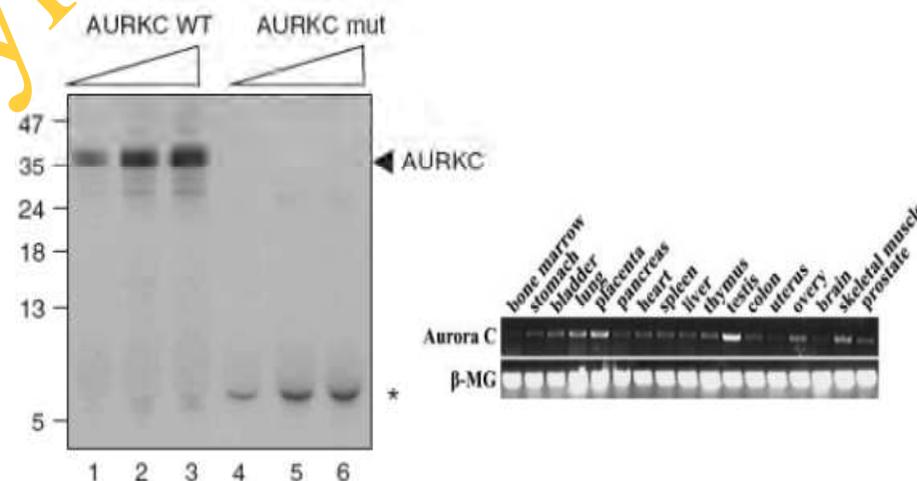
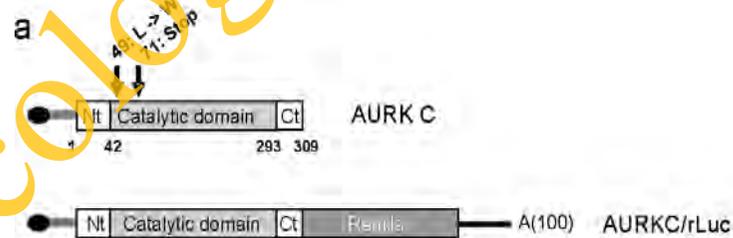
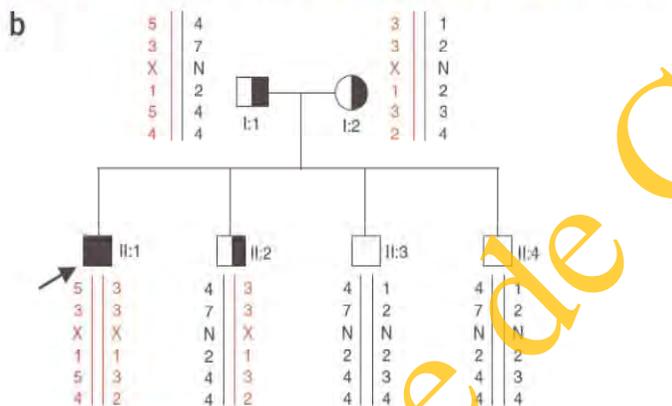
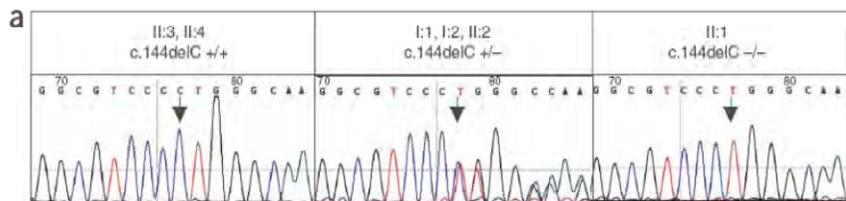
**AZFa**  
intervalle 5  
SCO

**AZFb**  
intervalles 5-6  
Arrêt en méiose

**AZFc**  
intervalle 6  
phénotype variable



# Aurora Kinase C et macrocéphalie spermatique



Expression d'Aurora C par RT-PCR

Dieterich et al. 2007

# Classification des macrocéphalies spermatisques.

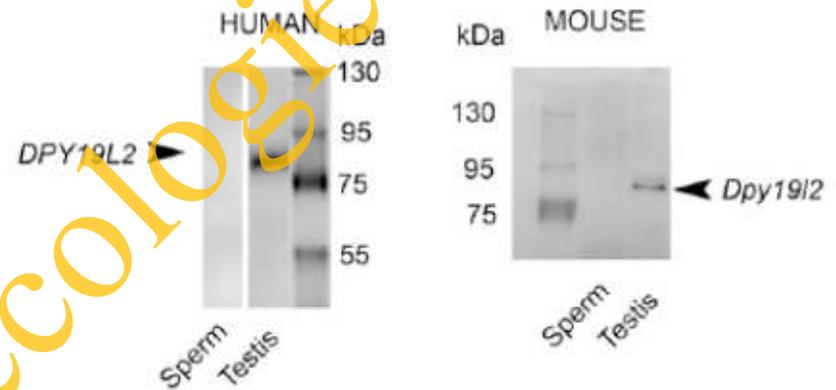
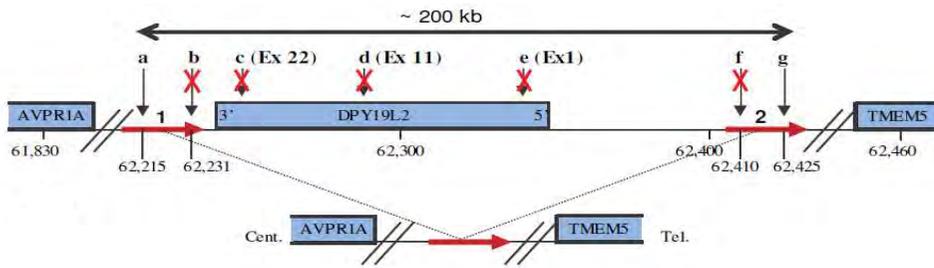
**Table 3** Proposed classification for semen containing large-headed spermatozoa and ART possibilities decision

Light microscopy for large-headed spermatozoa	Proposal of percentage of spermatozoa with large heads	Proposal of FISH on selected spermatozoa with a normal head size	Additional analysis proposal	Classification	ART possibility decision
Irregular head shape and multiple flagella	100 %	Not recommend	Screen for AURK mutations	Type I	Intracouple ART contraindicated
	<100 %	No euploid spermatozoa	Screen for AURK mutations	Type IIA	Intracouple ART contraindicated
		Presence of euploid spermatozoa	Screen for AURK mutations?	Type IIB	ICSI + PGD if available
Normal head shape and a single flagella	>10 % <sup>a</sup>	Normal level of aneuploid spermatozoa	Normal sperm chromatin condensation for spermatozoa with normal size head	Type IIIA	ICSI with spermatozoa with normal size head
		Normal level of aneuploid spermatozoa	Normal sperm chromatin condensation	Type IIIB	Intracouple ART
		High level of aneuploid spermatozoa	FISH	Type IIIC	ICSI + PGD if available

Classification of sperm with enlarged head according to light microscopy evaluation, percentage of spermatozoa with large heads, FISH studies, additional analysis performed, and ART (assisted reproductive technology). <sup>a</sup>Guthauser et al., 2013 [26]

Guthauser et al. 2016

# DPY19L2 et globozoospermie



Harbuz et al. 2011

Anomalie de DPY19L2 entraîne un mauvais accochage du noyau et de l'acrosome

Pierre et al, 2012

Anomalie de DPY19L2: rôle majeur dans les globozoospermie

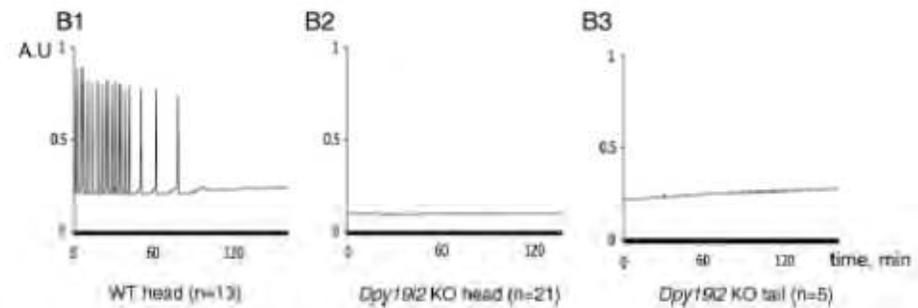
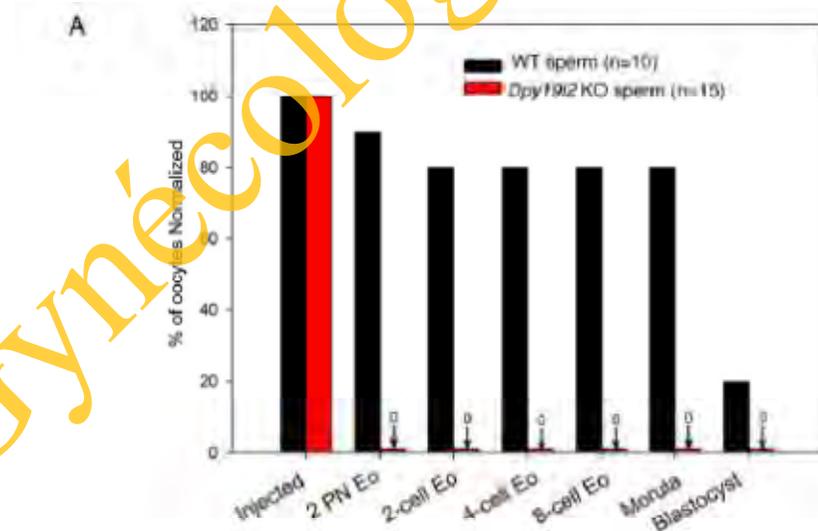
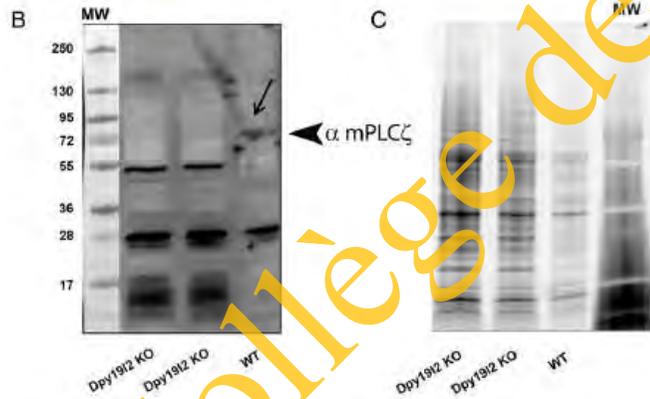
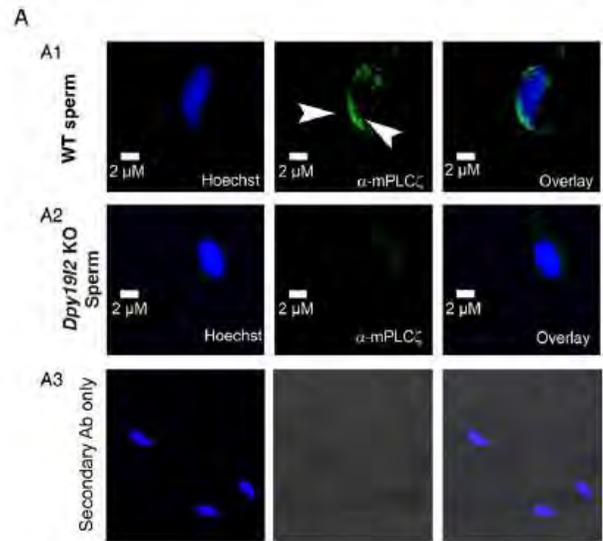
Mutation décrite

Coutton et al, 2012

Patients Hétérozygotes composites

Zhu et al, 2013

# DPY19L2 et défauts d'activation ovocytaire



Escoffier et al 2015

# Anomalies du gène CFTR et infertilité masculine

**Table 1.** Series review of F508del, R117H and IVS8-Tn frequencies in patients with CBAVD.

Series	$\Delta F508$ (%)	5T (%)	R117H (%)	Others <sup>a</sup> n (%)	Proportion of identified alleles (%)	Reference
<i>Caucasian</i>						
German (106)	26	13	11.3	43 (50)	80.3	Dork <i>et al.</i> (1997)
Spanish (110)	18	23	4	35 (38)	83	Casals <i>et al.</i> (2000)
French (800)	22	19	4.4	134 (34)	79.4	Claustres <i>et al.</i> (2000)
Canadian (134)	20	21	6	12 (15)	62	Zielenski <i>et al.</i> (1995); Mak <i>et al.</i> (1999)
<i>Non-Caucasian</i>						
Egyptian (20)	2.5	43.7	0	na	na	Lissens <i>et al.</i> (1999)
Turkish (51)	2.9	19.6	0	25 <sup>b</sup> (50)	72.5	Dayangac <i>et al.</i> (2004)
Taiwanese (27)	0	44.4	0	1 <sup>c</sup> (50)	na	Wu <i>et al.</i> (2004)
Japanese (19)	0	29	0	3 (18)	47	Anzai <i>et al.</i> (2003)

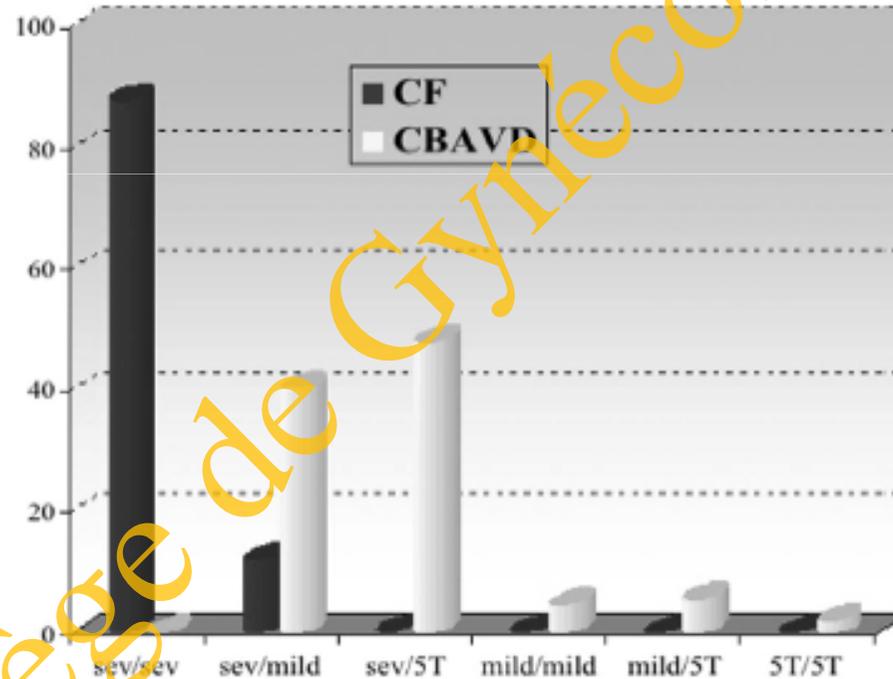
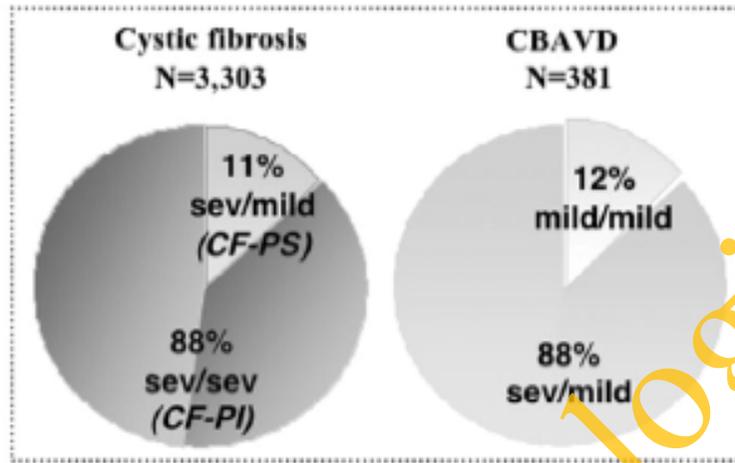
<sup>a</sup>Number of other different mutations identified and percentage of total alleles.

<sup>b</sup>Mutation D1152H was the most prevalent, accounting for 14.7% of alleles.

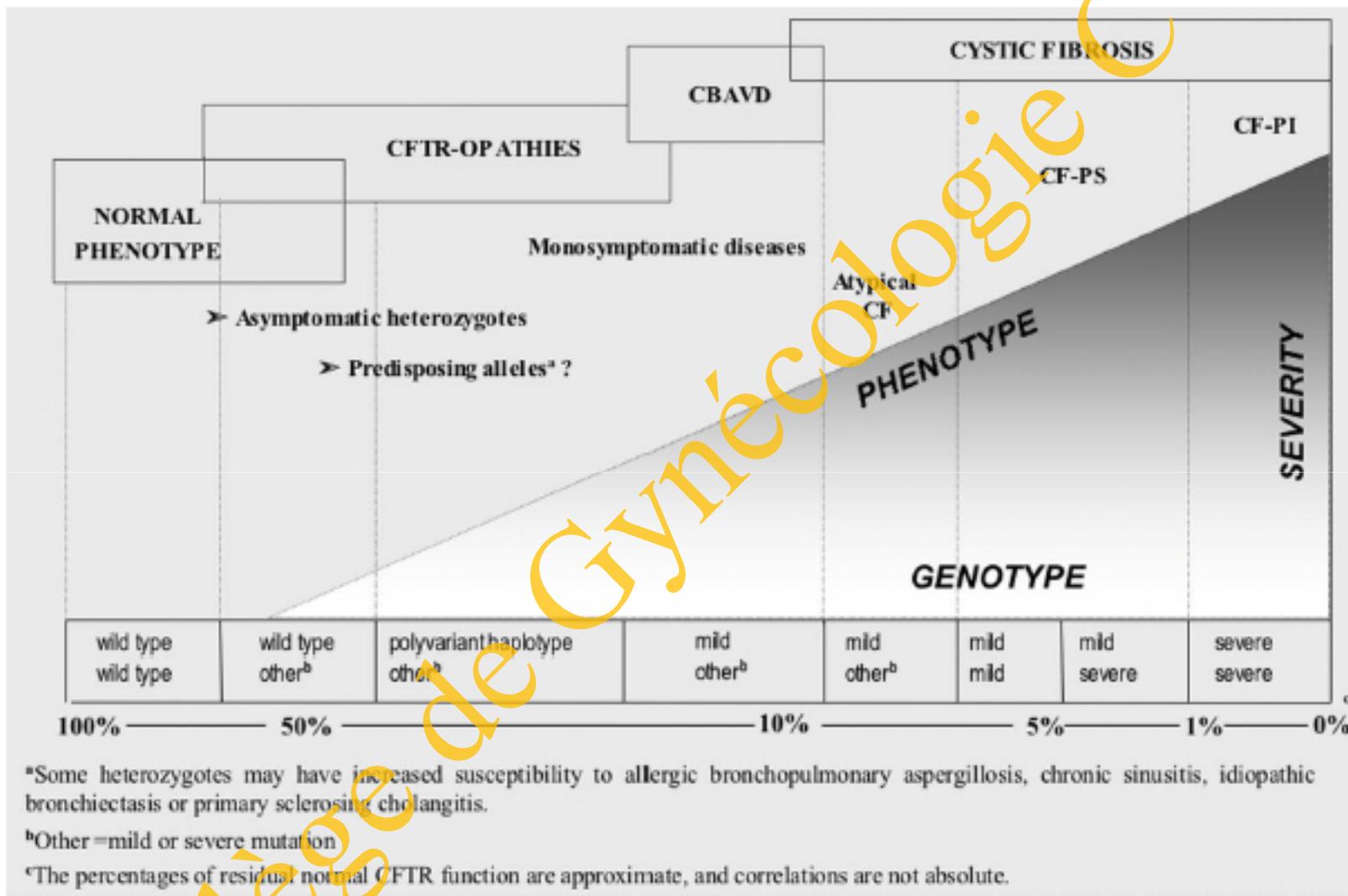
<sup>c</sup>Mutation Q1352H was the most prevalent, accounting for 13% of alleles.

na = not available.

Claustres et al. 2005

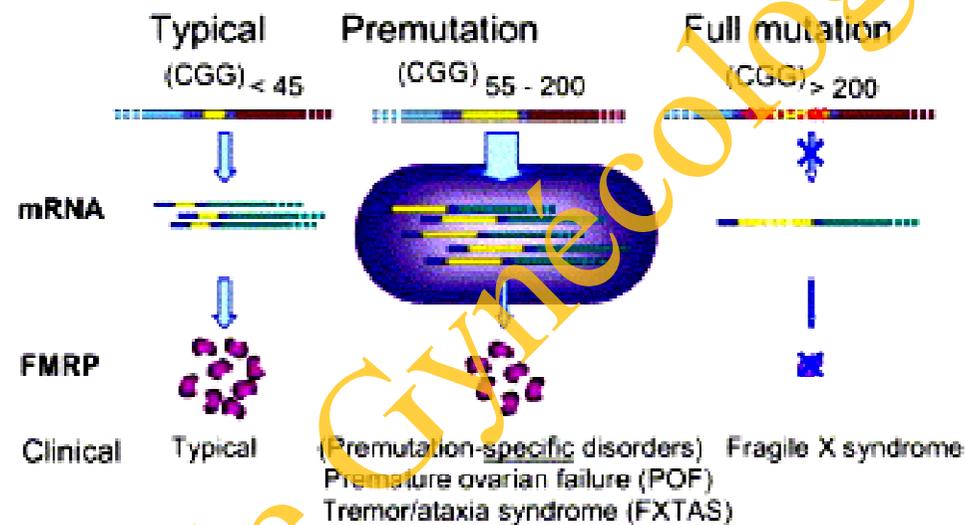


Claustres et al. 2005



Claustres et al. 2005

# Altération du gène FMR1(Xq27.3) et IOP



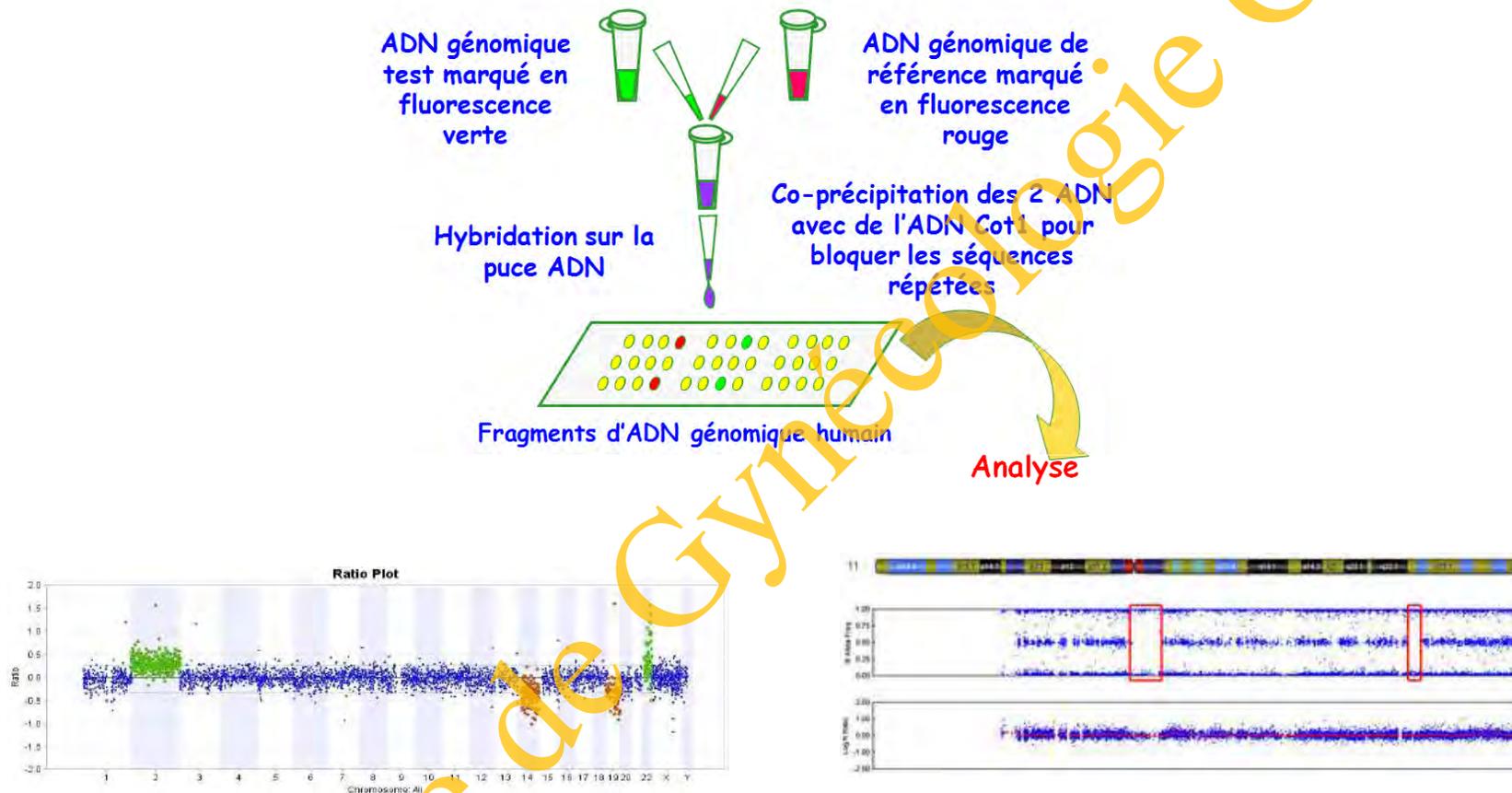
**20% des porteuses de la prémutation ont une insuffisance ovarienne prématurée**

Wittenberger et al, 2006

**Est-ce suffisant à l'aire des  
analyses plus fines du génome  
comme le NGS et l'ACPA**

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# Analyse chromosomique sur puce à ADN



## Oligo-array

Les séquences sont des oligomères (30 ac nucléique)

## SNP array

Variation nucléotidiques

# Qu'est ce que le NGS

Techniques permettant un séquençage dit « à haut débit »

Accessibilité réduite

Quelques définitions:

Whole génome

Exome

Ciblé ou panel

# Les applications en infertilité

Identification des altérations génétiques

Etiologie

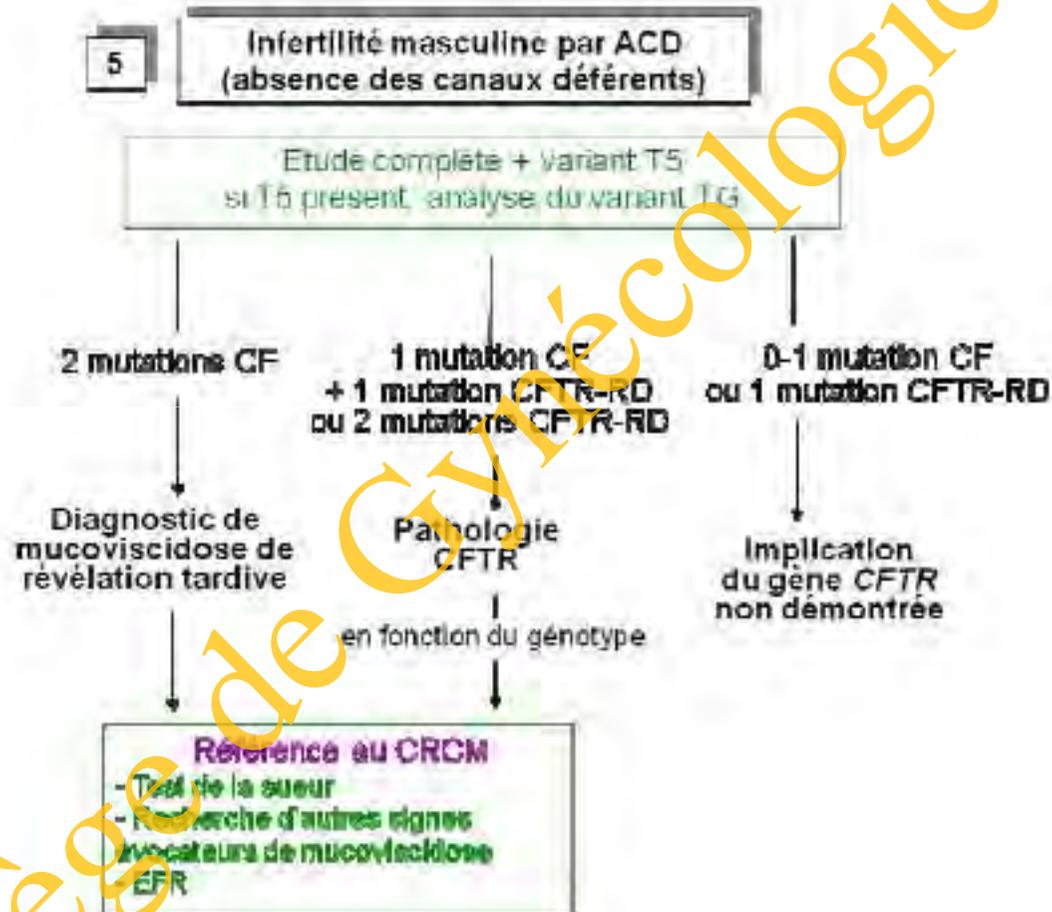
Recherche

Traitement

Utilisation en cas de DPI-An

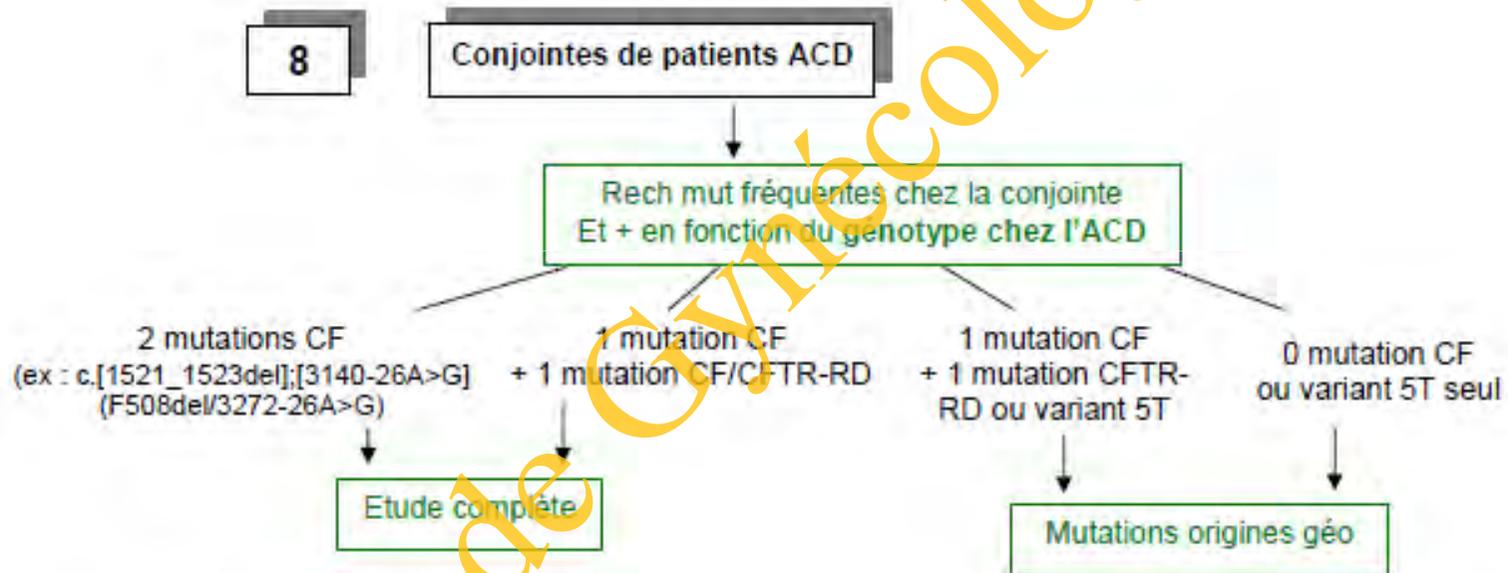
En préconceptionnel

# Un exemple: ABCD



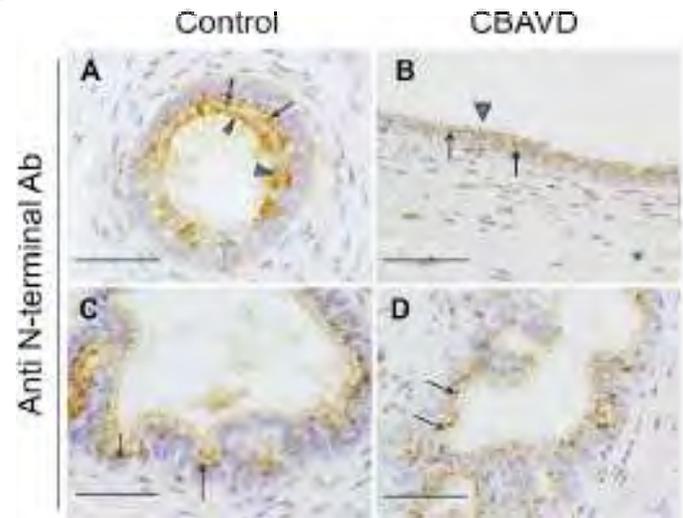
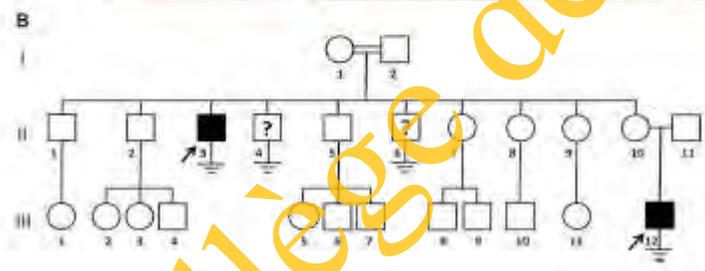
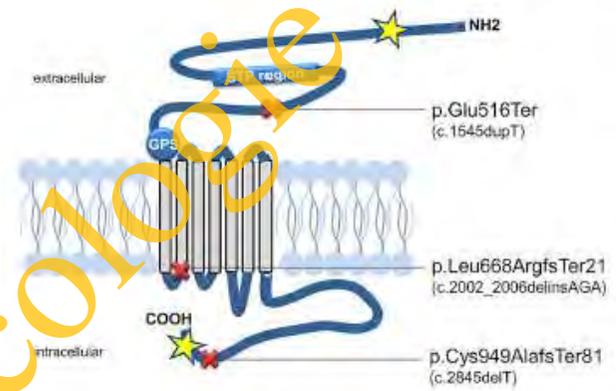
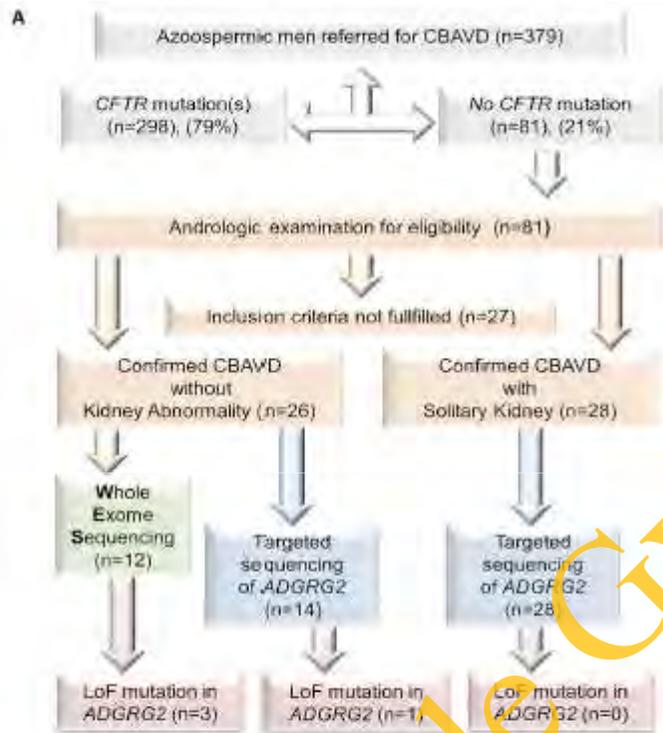
GenMucoFrance, 2013

# Un exemple: ABCD



GenMucoFrance, 2013

# Un exemple: ABCD



Patat et al, 2016

# Quelle stratégie pour l'ABCD

**1- Recherche des mutations classiques de la mucoviscidose**

**2- Etude complète du gène CFTR (ABCC7)**

**3- Etude du gène ADGRG2**

**4- Faire un panel de gènes**

CFTR + ADGRG2 + Autres gènes ?

# Les altérations de la spermatogenèse

## Un exemple: l'azoospermie

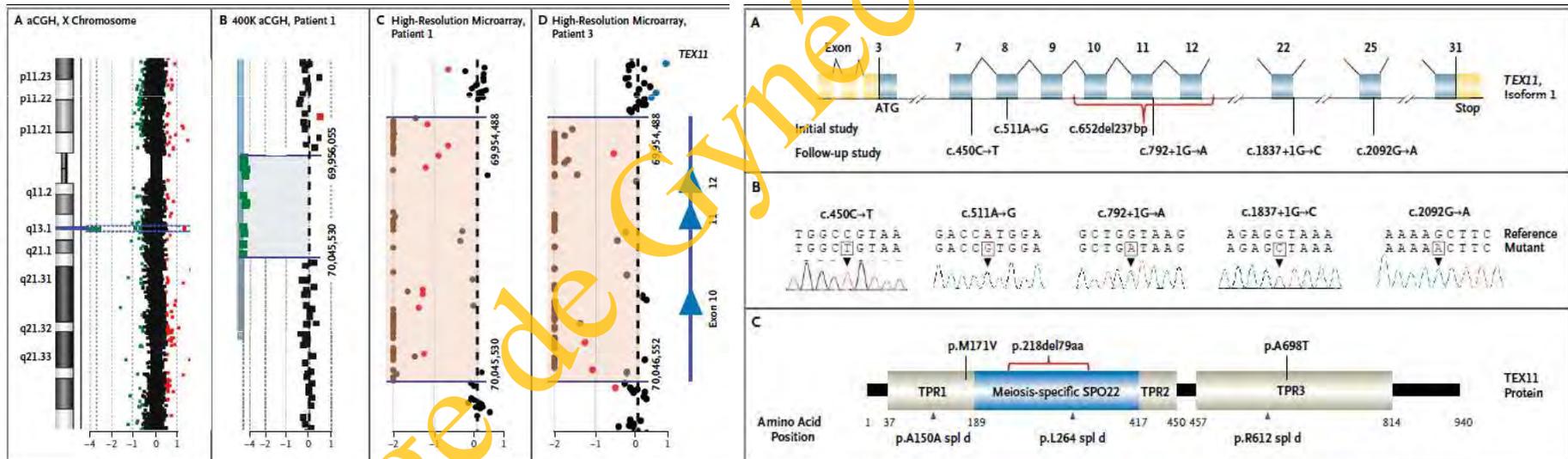
- Les anomalies chromosomiques
- Microdélétion du chromosome Y

**Est-ce suffisant aujourd'hui ?**

# L'analyse chromosomique sur puce à ADN

ORIGINAL ARTICLE

## X-Linked *TEX11* Mutations, Meiotic Arrest, and Azoospermia in Infertile Men



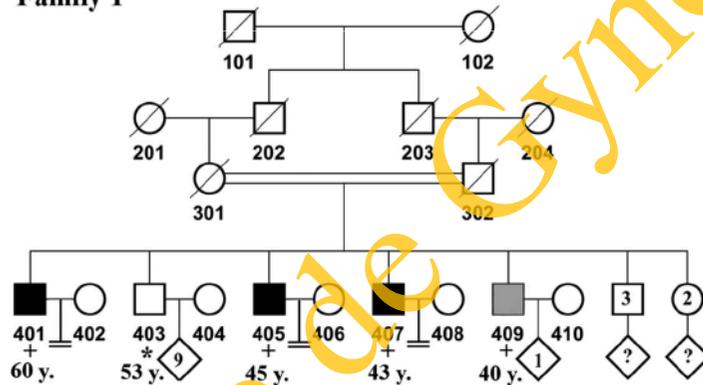
N ENGL J MED 372;22 NEJM.ORG MAY 28, 2015

# NGS et arrêt de maturation

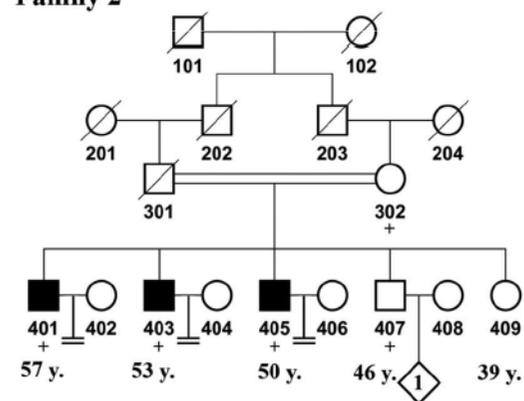
Truncating mutations in *TAF4B* and *ZMYND15* causing recessive azoospermia

Ayhan Ö. et al. *J Med Genet* 2014;51:239–244.

Family 1



Family 2



# Quelle stratégie pour l'azoospermie avec blocage de maturation

## Diagnostic

- Réalisation d'un caryotype
- Recherche de microdélétion du chromosome Y

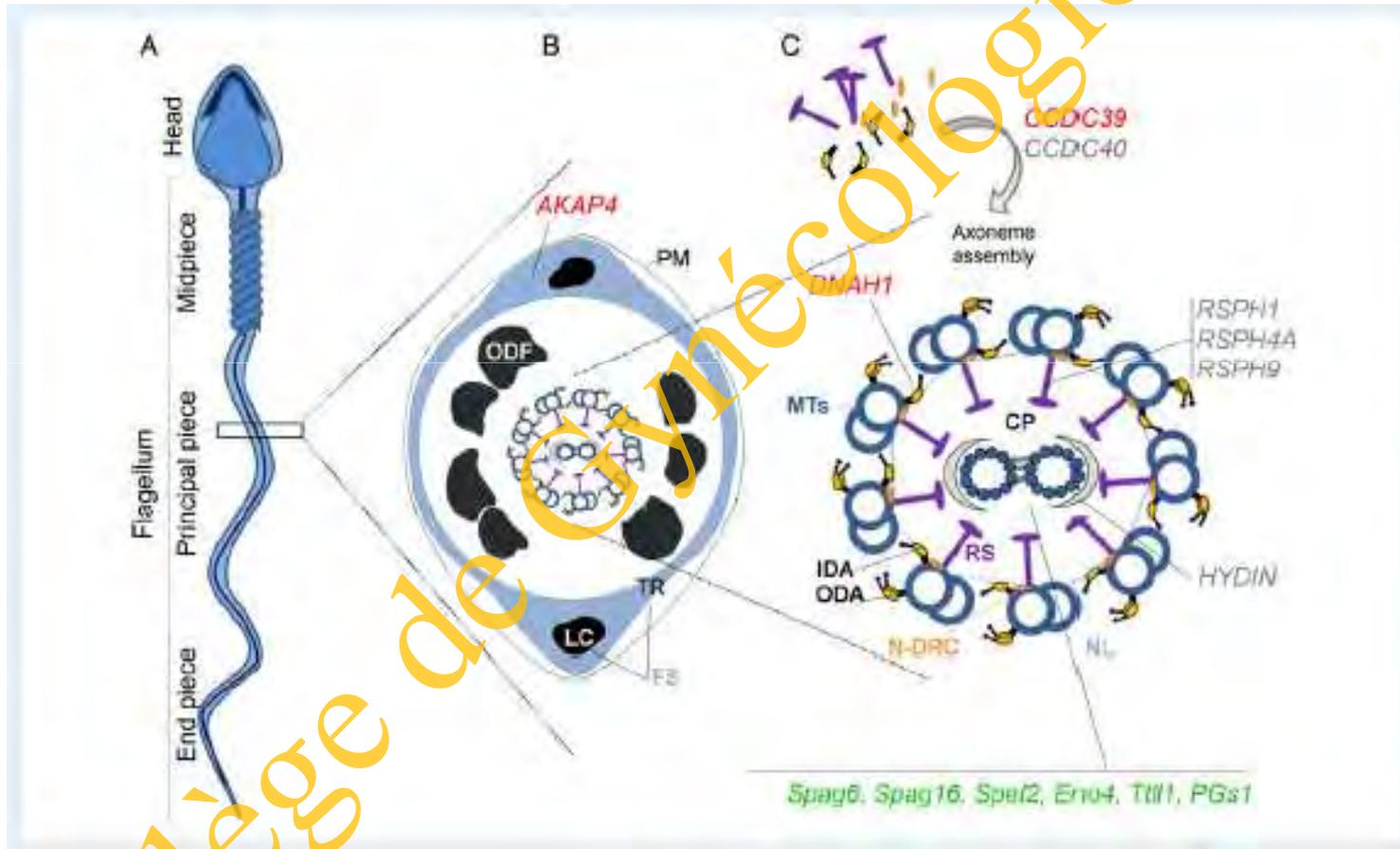
## Recherche

- Analyse de TEX11
- Exome

## Thérapeutique

- Thérapie génique ....

# Anomalies flagellaires et génétique



Coutton et al, 2015

# Les insuffisances ovariennes prématurées: ESHRE guidelines

**Table II Summary of diagnostic workup for POI.**

Test	Implications	
	Positive test	Negative test
Genetic/chromosomal		
Karyotyping (for diagnosis of Turner syndrome)	Refer to endocrinologist, cardiologist and geneticist	A second analysis of the karyotype in epithelial cells (in case of high clinical suspicion)
Test for Y-chromosomal material	Discuss gonadectomy with the patient	
Fra-X	Refer to geneticist	
Autosomal genetic testing <sup>a</sup>		
Antibodies <sup>b</sup>		
ACA/21OH antibodies	Refer to endocrinologist	Re-test in case of clinical signs or symptoms
TPO-Ab	Test TSH every year	

Fra-X, fragile X; TPO-Ab, thyroid antibodies; ACA, adrenocortical antibodies.

<sup>a</sup>Not at present indicated in women with POI, unless there is evidence suggesting a specific mutation (e.g. BPES).

<sup>b</sup>POI of unknown cause or if an immune disorder is suspected.

Anderson et al, 2016

# Les insuffisances ovariennes prématurées

**Table 1.** Human Genes Known to Cause POI When Mutated

Gene (Ref)	Role of the Gene	Inheritance	Phenotype
RARS2 (151)	Charges mitochondrial tRNA-ala with alanine during mitochondrial translation	AR	Ovariohypoplasia + POI
RBBP2 (175)	RNA-binding protein with a prokaryotic-like DNA-proteinase	XLB	POI
RSE (161)	Ratase transcription factor required to signal immunity	AR	Autoimmune polyglandular syndrome type 1 + POI
ATM (82)	Rise in cellular responses to genomic damage	AR	Ataxia telangiectasia + POI
BLM (82)	DNA helicase, regulates homologous recombination	AR	Bloom syndrome + POI
BMPS1 (108)	Member of TGF- $\beta$ superfamily, regulates hematopoiesis	XLB	POI
BMPS1B (64)	Transmembrane serine/threonine kinase involved in endochondral bone formation and embryogenesis	AR	Acromesomelic chondrodysplasia + POI
CTCF (148)	mDNA heliase, required for mDNA replication and maintenance	AR	Perovai syndrome + POI
PRM2 (136)	Enzyme necessary for the synthesis of GDP-mannose	AR	Congenital disorder of glycosylation + POI
CLPP (152)	Component of a mitochondrial ATP-dependent proteolytic complex, required for unfolded protein response	AR	Perovai syndrome + POI
CSB-PCBD3 (92)	Pulsive role in cellular responses to genomic damage	AR	POI
CYP7A1 (134)	Monooxygenase, key enzyme in the steroidogenic pathway that produces progesterone, mineralocorticoids, glucocorticoids, androgens, and estrogens	AR	POI
CYP17A1 (136)	Monooxygenase, key enzyme responsible for the biosynthesis of estrogens	AR	POI, fetal masculinization
EIF2B1 (101)	GTP exchange factor that is essential for protein synthesis	AR	Ovarioleukodystrophy + POI
EIF2B4 (101)	GTP exchange factor that is essential for protein synthesis	AR	Ovarioleukodystrophy + POI
EIF2B5 (101)	GTP exchange factor that is essential for protein synthesis	AR	Ovarioleukodystrophy + POI
EIF4EB1 (105)	Nucleocytoplasmic shuttling protein responsible for the nuclear import of EIF4E	AR	POI
FANCA (87)	Required for S phase of the growth cycle and after exposure to DNA crosslinking agents	AR	Fanconi anemia + POI
FANCC (87)	Required for S phase of the growth cycle and after exposure to DNA crosslinking agents	AR	Fanconi anemia + POI
FANCD1 (87)	Required for S phase of the growth cycle and after exposure to DNA crosslinking agents	AR	Fanconi anemia + POI
FOXP2 (64)	Crucial role in brain development and maintenance and female fertility	AD	Speech apraxia + POI
FMR1 (168)	RNA-binding protein that associates with polyribosomes, putative role in translation	XLB (premutational)	POI
FSHR (125)	Receptor for FSH, required for folliculogenesis	AR	POI
GALT (155)	Responsible for the conversion of galactose 1-phosphate (gal-1P) and UDP-glucose into glucose 1-phosphate and UDP-galactose	AR	Galactosemia + POI
HARS2 (150)	Charges mitochondrial tRNA-lys with lysine during mitochondrial translation	AR	Perovai syndrome + POI
HFM1 (75)	Required for normal progression of homologous recombination and proper synapsis between homologous chromosomes	AR	POI
HSD17B4 (133)	Involved in fatty acid $\beta$ -oxidation and steroid metabolism	AR	Perovai syndrome + POI
LARS2 (149)	Charges mitochondrial tRNA-lys with lysine during mitochondrial translation	AR	Perovai syndrome + POI
LMNA (65)	Structural protein components of the nuclear lamina, a protein network underlying the inner nuclear membrane	AR	Progeria + POI
MCM8 (88)	Required for homologous recombination-mediated repair of double-strand breaks	AR	POI
MCM9 (89)	Required for homologous recombination-mediated repair of double-strand breaks	AR	POI
NANCS3 (97)	Suppression of apoptosis	AR	POI
NBN (86, 236)	Required for the repair of double-strand breaks	AR	Nijmegen breakage syndrome + POI, infertility
NCOX1 (117)	Homeobox gene involved in regulation ofocyte-specific genes	AR	POI
NOG (109, 110)	Signaling molecule involved in promoting somite patterning during embryogenesis	AD	Proximal symphalangism + POI
NR5A1 (50)	Transcription factor required for sex development, steroidogenesis, and fertility	AR	POI (DSD in male)

(Continued)

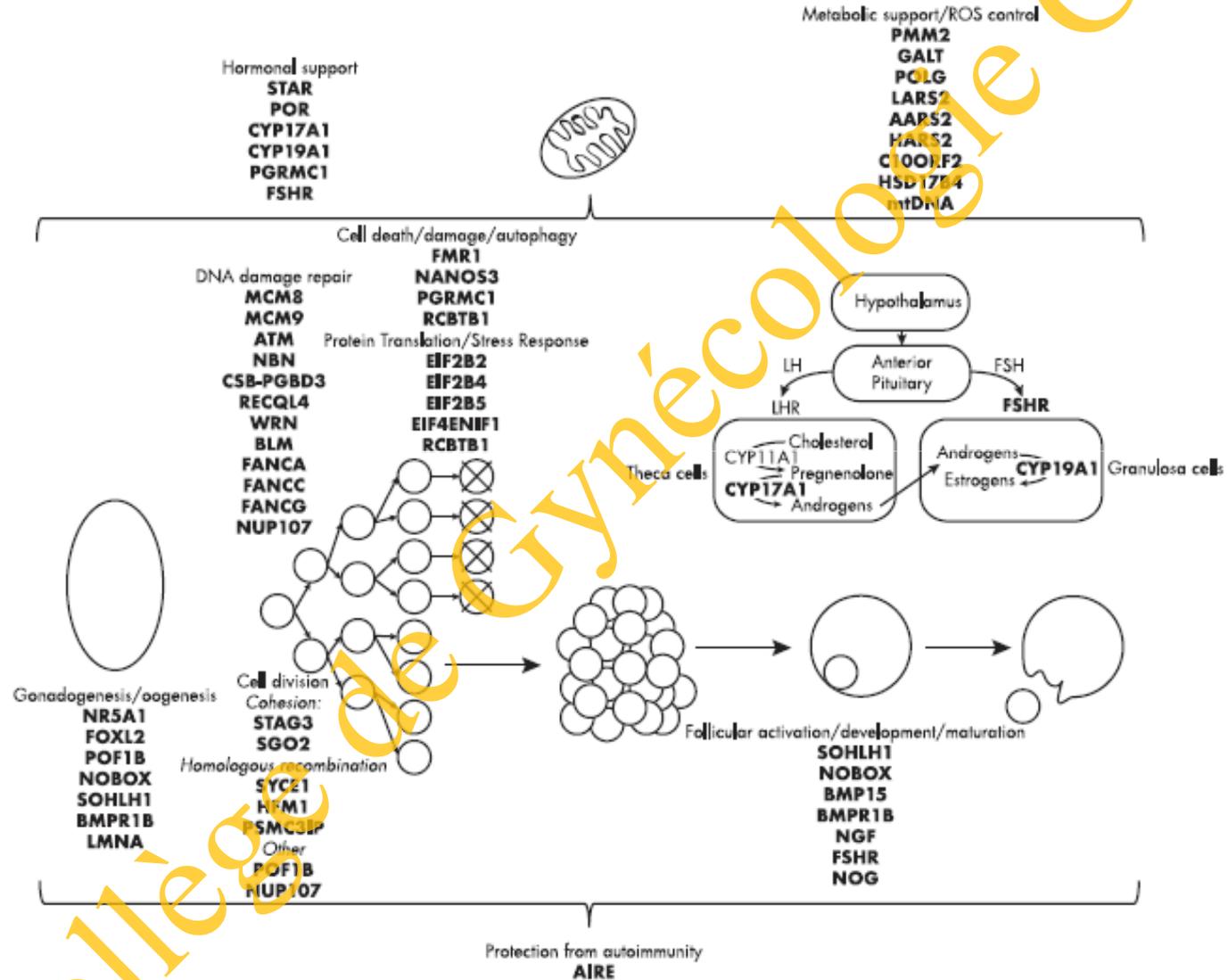
**Table 1.** Continued

Gene (Ref)	Role of the Gene	Inheritance	Phenotype
NUP133 (77)	Nucleoporin protein involved in transport between cytoplasm and nucleus, putative role in meiotic/mitotic progression and/or DNA damage	AR	POI (XX gonadal dysgenesis)
PERM1 (91)	Binds and activates P53 protein for steroidogenesis, responsible for antiapoptotic action of progesterone	AD	POI
PCF11 (93)	Involved in muscle actin filaments, possible role in germ cell division	XLB	POI
PCNA (142, 147)	Helicase that synthesizes new mDNA and corrects mDNA errors	AR, AD	Progressive ovarian insufficiency and Parkinsonism + POI
PSM-SE (95)	Role in muscle reorganization, coactivator of nuclear hormone receptors	AR	POI, XX ovarian dysgenesis
RFX1 (102)	Ubiquitinase, likely regulates oxidative stress	AR	POI, renal dystrophy, intellectual disability
RFC1 (84)	DNA helicase that unwinds double-stranded DNA, involved in DNA repair	AR	Rothmund-Thomson syndrome + POI
SCD2 (67)	Maintains the integrity of the cohesion complex, required for mitosis	AR	POI
SOX4 (121)	Gonadal transcription factor required for folliculogenesis	AR	POI
STAG3 (66)	Subunit of cohesin, required for proper pairing and segregation of chromosomes during meiosis	AR	POI
STAR (134)	Required for conversion of cholesterol to progesterone	AR	Congenital hypoadrenal hypoplasia + POI
SYCE1 (71)	Part of the synaptonemal complex that physically links homologous chromosomes	AR	POI
WRN (83)	DNA helicase that unwinds double-stranded DNA, involved in DNA repair	AR	Werner syndrome + POI

Abbreviations: AR, autosomal recessive; AD, autosomal dominant; XLB, X-linked recessive; XLB, X-linked dominant; DSD, disorder of sex development. Gene symbol, role, inheritance pattern, patient phenotype, and relevant references are indicated.

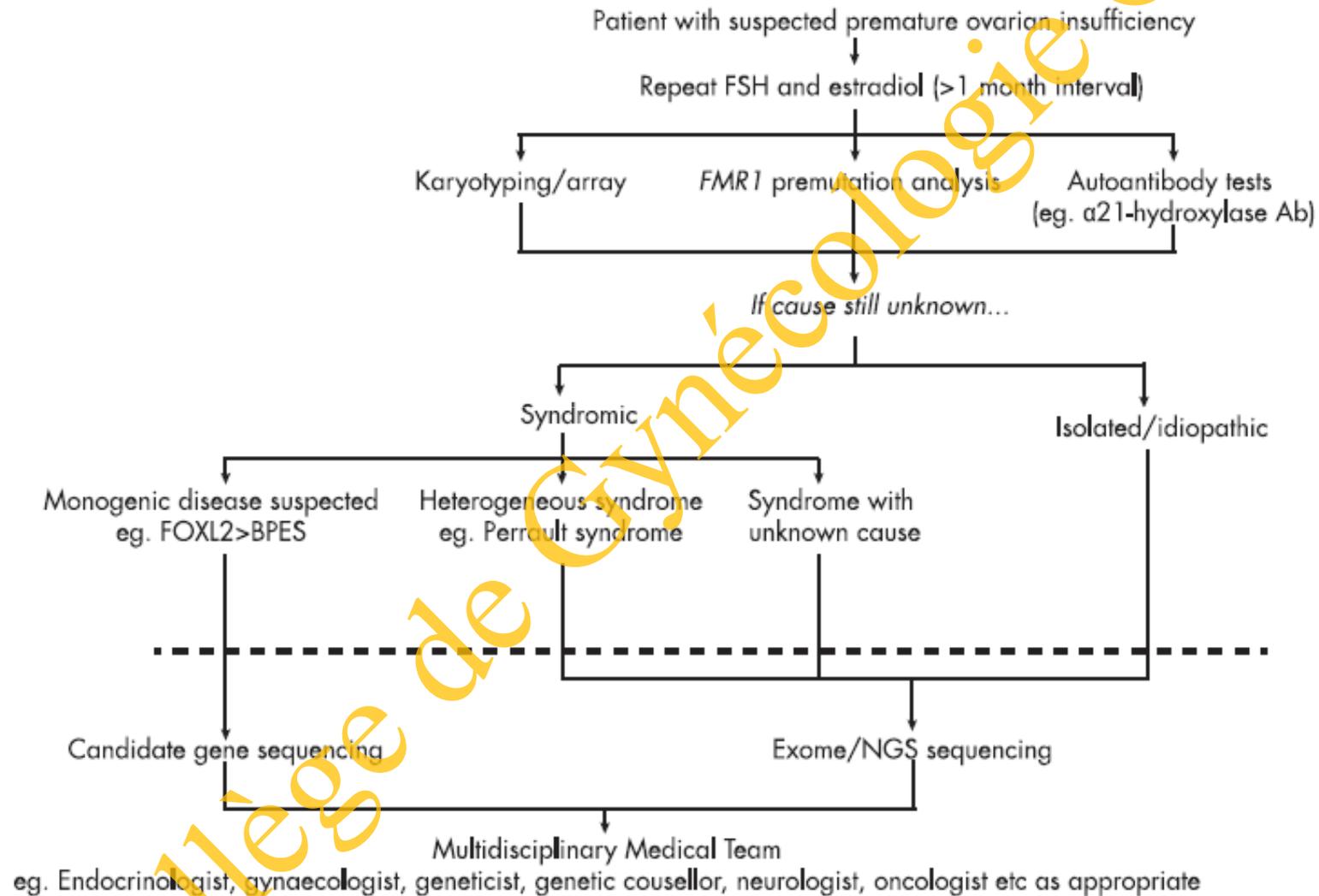
Tucker et al, 2016

# Les insuffisances ovariennes prématurées



Tucker et al, 2016

# Les insuffisances ovariennes prématurées



Tucker et al, 2016

# NGS et dépistage préconceptionnel

**La thérapie génique est elle aujourd'hui opérationnelle ?**

**Pourquoi faut il attendre un accident ?**

**Existe-t-il un moyen technique de contourner ces obstacles ?**

# NGS et dépistage préconceptionnel

**Réalise t'on un dépistage génétique préconceptionnel aujourd'hui ?**

**Caryotype dans de nombreuses populations**

**Dépistage de la drépanocytose chez les patients d'origine Africaine**

**Dépistage de la Thalassémie**

**Etc....**

# NGS et dépistage préconceptionnel

## Pour quelle(s) population(s) ?

**Les patients pris en charge en AMP**

**Les dons de gamètes**

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# NGS et dépistage préconceptionnel

## Quelles pathologies recherchées ?

**Les plus fréquentes**  
**Les plus invalidantes**  
**Sans traitement à l'heure actuelle**

La mucoviscidose, l'amyotrophie spinale, le maladie de Steinert, le syndrome de l'X fragile, les muco-polysaccharidoses, etc....

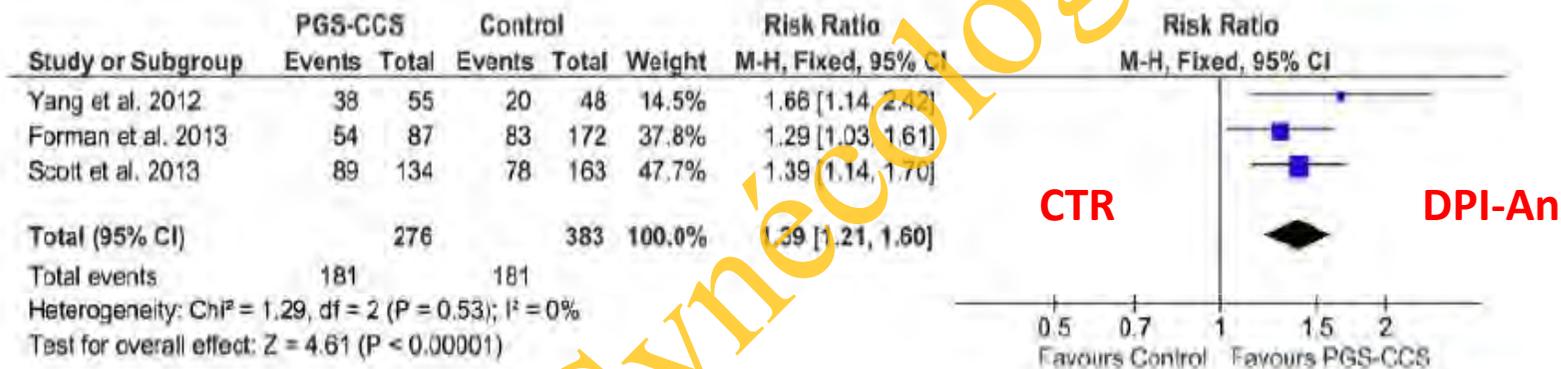
# **DPI-An:** **Les techniques d'analyses**

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# DPI-An et PR: Résultats d'une méta-analyse

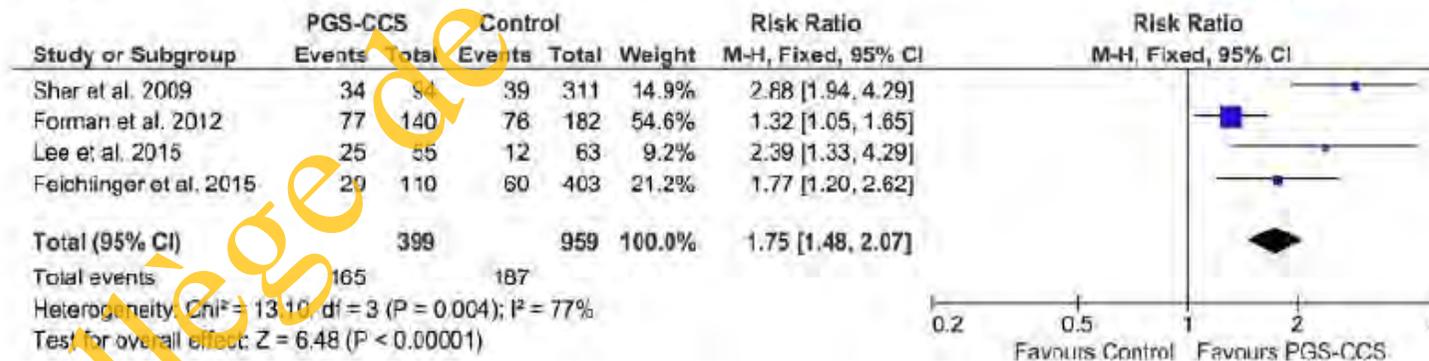
## Sustained implantation rate (> 20 weeks gestation)

RCTs



## Sustained implantation rate (> 20 weeks gestation)

Observational



Dadouh et al 2015

# Pourcentage de cycles avec au moins 1 embryon euploïde

	Donneuse	<35	35-37	38-40	41-42	>42
<b>1-3 embryons</b>	81	78	71	55	36	23
<b>4-6 embryons</b>	98	96	93	85	70	49
<b>7-10 embryons</b>	100	99	98	94	79	62
<b>&gt;10 embryons</b>	100	100	100	98	92	88
<b>Total</b>	97	92	86	74	54	33

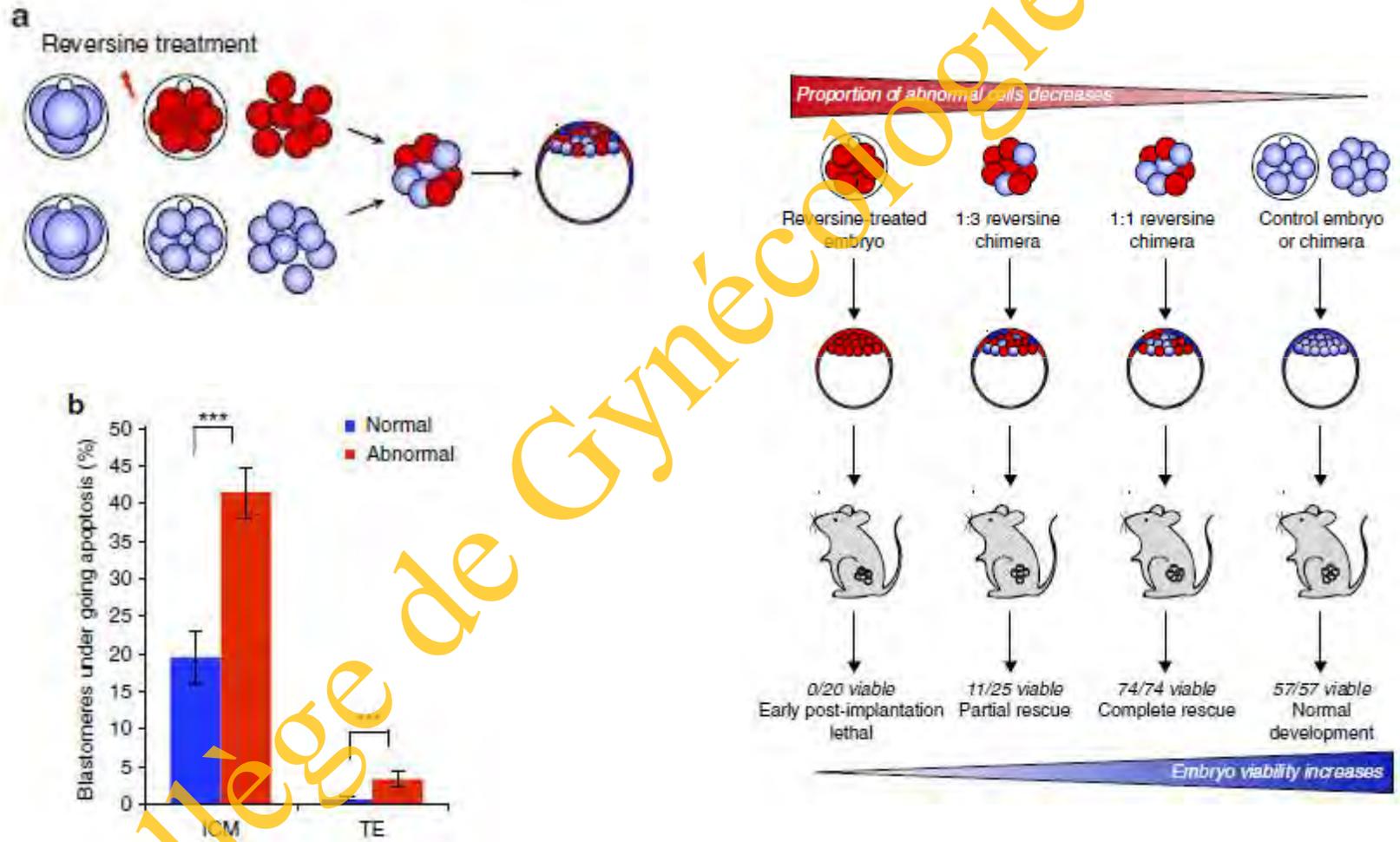
# PGS et mosaïque: qu'en faire ?

**Table 1. Clinical Outcomes of Single Mosaic Blastocysts Transferred.\***

Patient No.	Chromosomal Constitution	Mosaicism† percent	Karyotype‡	Clinical Outcome
1	arr(4)x1,(10)x1	40	46,XX	Baby healthy at birth
2	arr(6)x1,(15)x1	50	46,XX	Baby healthy at birth
3	arr(2)x1	40	46,XX	Baby healthy at birth
4	arr(2)x1	35	46,XY	Baby healthy at birth
5	arr(5)x1	50	46,XX	Baby healthy at birth
6	arr(5)x1,(7)x1	40	46,XX	Baby healthy at birth
7	arr(11)x1,(20)x3,(21)x3	30	NA	No pregnancy
8	arr(1)x1,(6)x3,(10)x3,(12)x3,(13)x3,(14)x3,(21)x3	50	NA	No pregnancy
9	arr(3)x1,(10)x3,(21)x3	35	NA	No pregnancy
10	arr(1)x3	50	NA	Biochemical pregnancy§
11	arr 9p21.2q34.3(26,609,645-40,499,711)x3	45	NA	Biochemical pregnancy§
12	arr(15)x3	30	NA	No pregnancy
13	arr(18)x1	50	NA	No pregnancy
14	arr(18)x1	50	NA	No pregnancy
15	arr(18)x1	40	NA	No pregnancy
16	arr(4)x1	50	NA	No pregnancy
17	arr(5)x3	40	NA	No pregnancy
18	arr 10q21.3q26.3(67,216,644-134,326,648)x3	50	NA	No pregnancy

Greco et al. 2015

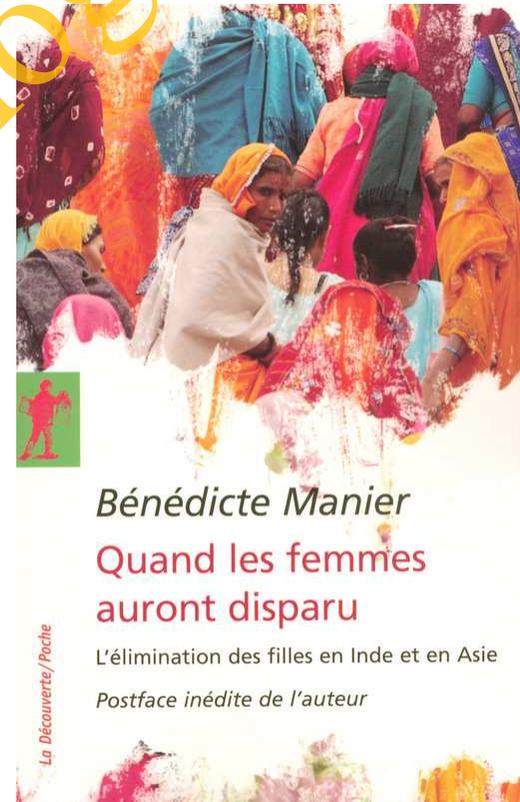
# Mosaïque embryonnaire et survie cellulaire



Boston et al. 2016

# DPI-An et NGS

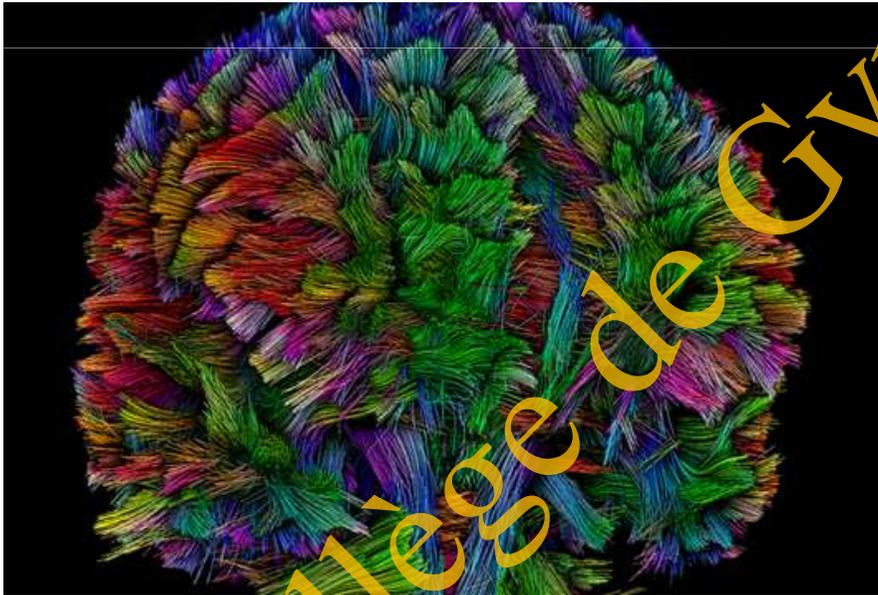
## A quand le séquençage de tout le génome ?



# DPI-An et NGS

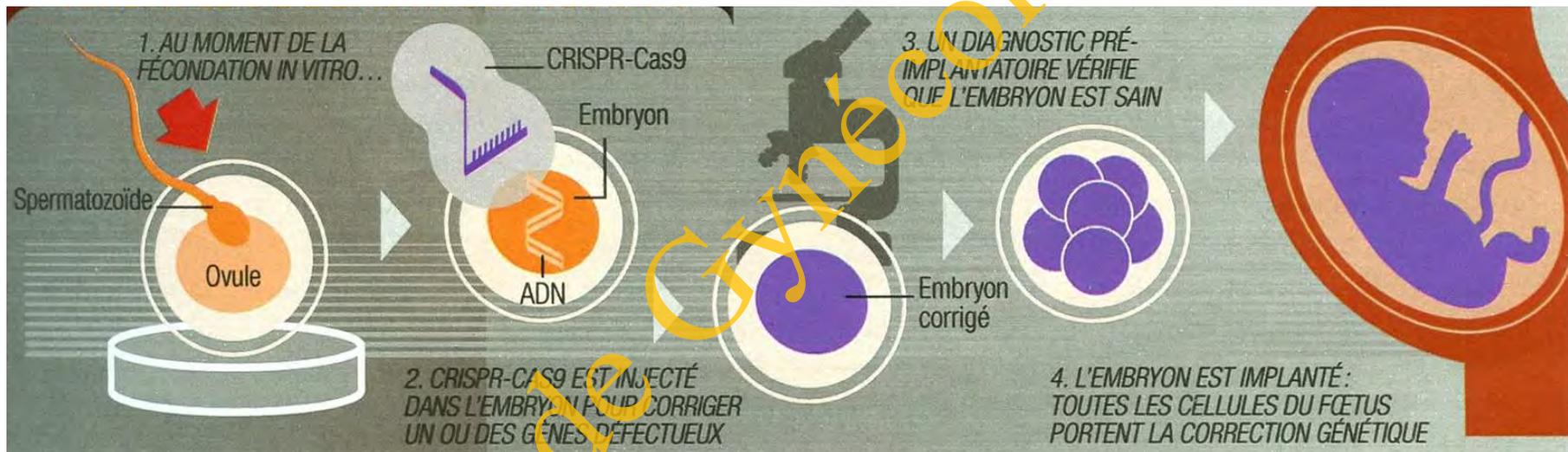
## A quand le séquençage de tout le génome ?

### QI et sélection embryonnaire



# DPI-An et NGS

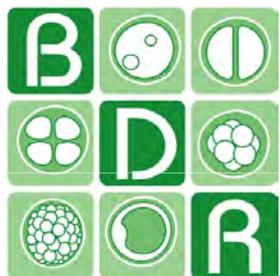
## A quand la correction du génome ?



Collège



Clinique  
Pasteur-Lanroze



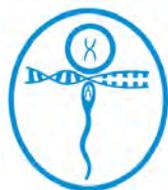
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ST-QUENTIN-EN-YVELINES



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