Sperm Damage: Its Impact on Pregnancy

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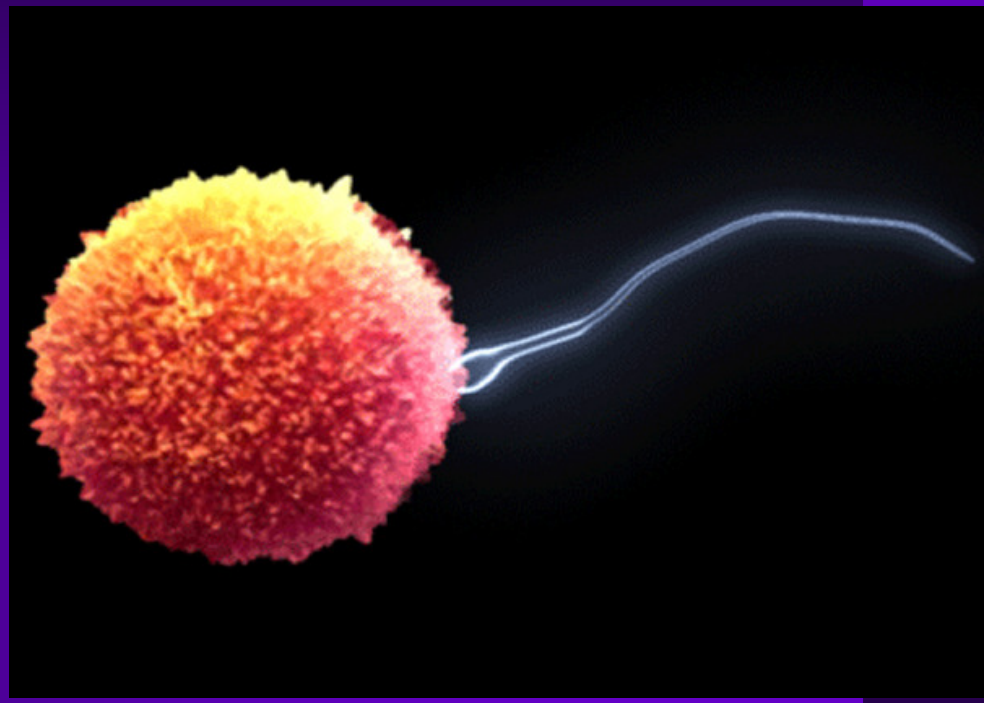
Sperm Damage

- Sperm contributes 50% of the genetic material of the new embryo



So, What?

- Implantation failure
- Miscarriage
- Off-spring abnormalities

Features of Sperm Damage

- Sperm quality
  - Morphology
  - Motility
- Sperm chromosome
  - Numerical abnormality
  - Structural abnormality
- Sperm chromatin fragmentation

Sperm Quality and RPL

Paper	Patients #	Recurrent pregnancy loss
Homonnai et al 1980	139	Higher sperm count and better morphology
Hill et al, 1994	98	No difference in sperm concentration and morphology compared to control
Sbracia et al 1996	120	No difference in sperm concentration, motility and morphology compared to control
Gill-Villa et al 2009	23	Worse morphology and motility

Sperm Membrane Integrity: The HOS test

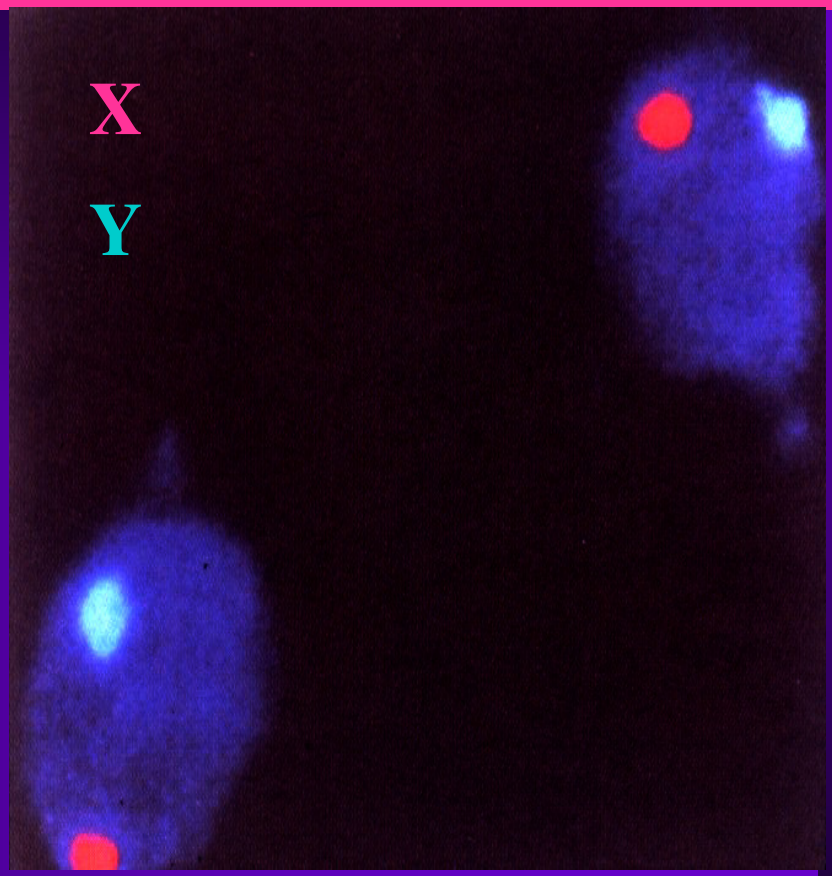
Paper	Patients #	RPL
Buckett etal, 1997	20	The HOS test scores are significantly lower compare to control
Patankar et al, 2001	25	The HOS test scores are significantly lower compare to control
Saxena et al 2008	35	The HOS test scores are significantly lower compare to control

Standard Criteria of Sperm Quality

- Its correlation with spontaneous pregnancy loss remains controversial
- Incapable of predicting the risk of RPL
- The search for other specific quality markers continues
- Critique: strict criteria of sperm morphology were not applied in reported studies

Poor Sperm Morphology

- 76% of sperm cells had numerical abnormalities of chromosome 18, 21 and/ or sex chromosomes [1]
- ↑ chromatin fragmentation (60% v 21%) [2]



[1] Lewis-Jones & Aziz, 2003

[2] Tang et al, 2009

Pregnancy Loss

- 50% of PL remain clinically unexplained
- Genetic abnormalities are detectable in 50% of aborted material

Karyotyping of Spontaneous Abortions (Warhurton et al, 1991)

Karyotype	Number	Percent of Total (%)
Normal	1988	60.2
Autosomal trisomy	**645**	**19.5**
Sex chromosome trisomy	8	0.2
Mosaic trisomy	60	1.8
Double trisomy	35	1.1
Monosomy X	201	6.1
Mosaic monosomy (45,X/46,XX)	15	0.5
Triploidy total	**185**	**5.6**
Hypertriploidy	13	0.4
Tetraploidy	65	2
Hypertetraploidy	13	0.4
Balanced structural rearrangements	11	0.3
Unbalanced structural rearrangements	41	1.2
Others (double anomalies)	20	0.6
Total	3300	

The origin of chromosomal abnormalities in embryos

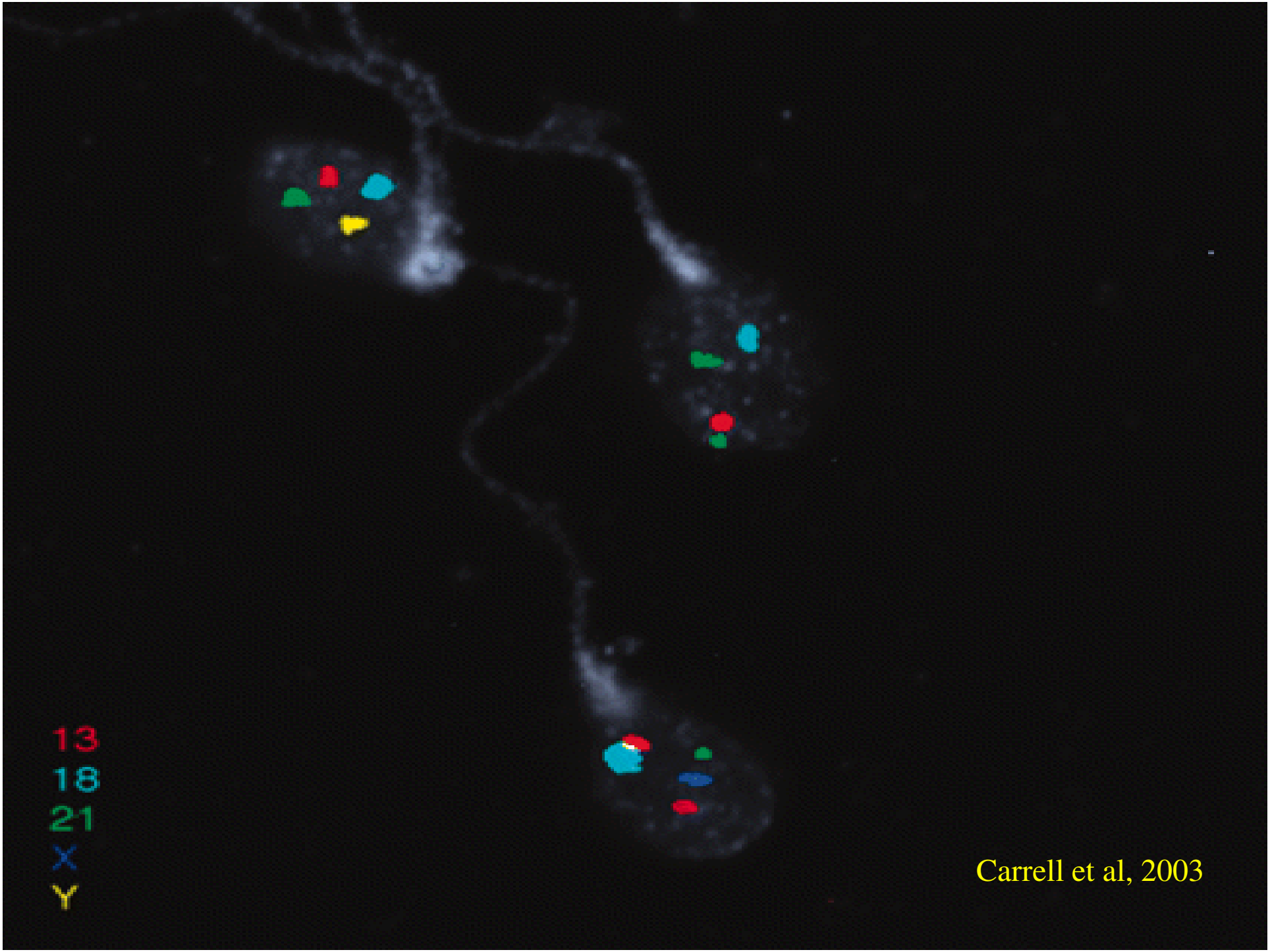
- Parents peripheral blood
- Arising *de novo* during early division
- Confined to gametes (Egg, sperm)

Incidence of Chromosomal Abnormalities

- Incidence of Somatic cell chromosomal abnormalities:
  - General population 0.7%
  - Parents of RPL: 1-4%
- Somatic cell analysis cannot assess the risk of errors arising de novo during germ cell maturation

Available Diagnostic Techniques

- Zona free hamster oocyte- sperm fusion assay (*Rudak et al, 1978*)
- FISH (Holmes and Martin, 1993)
  - Large number of sperm studied
  - Accuracy >98%
  - Expensive & time consuming
  - Automation is now available



Carrell et al, 2003

Future Diagnostic Technologies

- Array comparative genomic hybridization
  - Chips can analyse > 200,000 foci
  - Extending the ability of detecting smaller deletions, substitutions, duplications, translocations

Sperm Aneuploidy and RPL

Study	Patient #	Method	RPL
Rosenbusch & Sterzik , 1991	10	H. egg injection	Aneuploidy rate similar to control, ↑chromosome breaks
Giorlandino et al, 1998	2	FISH	↑ aneuploidy rate to 15%
Rubio et al, 2001	40	FISH	↑ sex chromosome disomy in 17.5% 0f patients
Carrel et al, 2003	24	FISH	Sex chromosome disomy doubled (2.77 Vv 1.19%)
Bernardini et al, 2004	20	FISH	25% of men has ↑ Aneuploidy (up to 15%) in
El-Hassan et al, 2005	14	FISH	Chromosomal abnormalities 16.5% v 4.6% in control

Correlating Sperm and Concepti Chromosomal Abnormalities

- 10% of all chromosomally abnormal concepti are of paternal origin [1]
- nearly 50% of XXY pregnancies were the result of paternal non-disjunction
- 10% of other trisomies were caused by paternal meiotic error.

[1] Bernardini et al, 2003

Diploidy Sperm and Miscarriage

- At least 8.5% of diandric triploid originates from diploid sperm [1]
- Most diandric triploids produced by Oligo-spermic men result from deploid sperm [2]
- 1/3 of male pronuclei after ICSI to treat infertile men are deploid [3]

1 Zaragoza et al, 2000
2 Egozcue et al 2002
3 Macas et al, 2001

Prognosis after Aneuploidy Miscarriage

- Is high frequency sperm aneuploidy a persistent feature? [1]
- Recurrence of same chromosomal abnormality in RPL patients with normal blood karyotype is sporadic [2]

1 Robinson et al, 2001
2 Bernardini et al,2004

AZF*c* Deletions and RPL

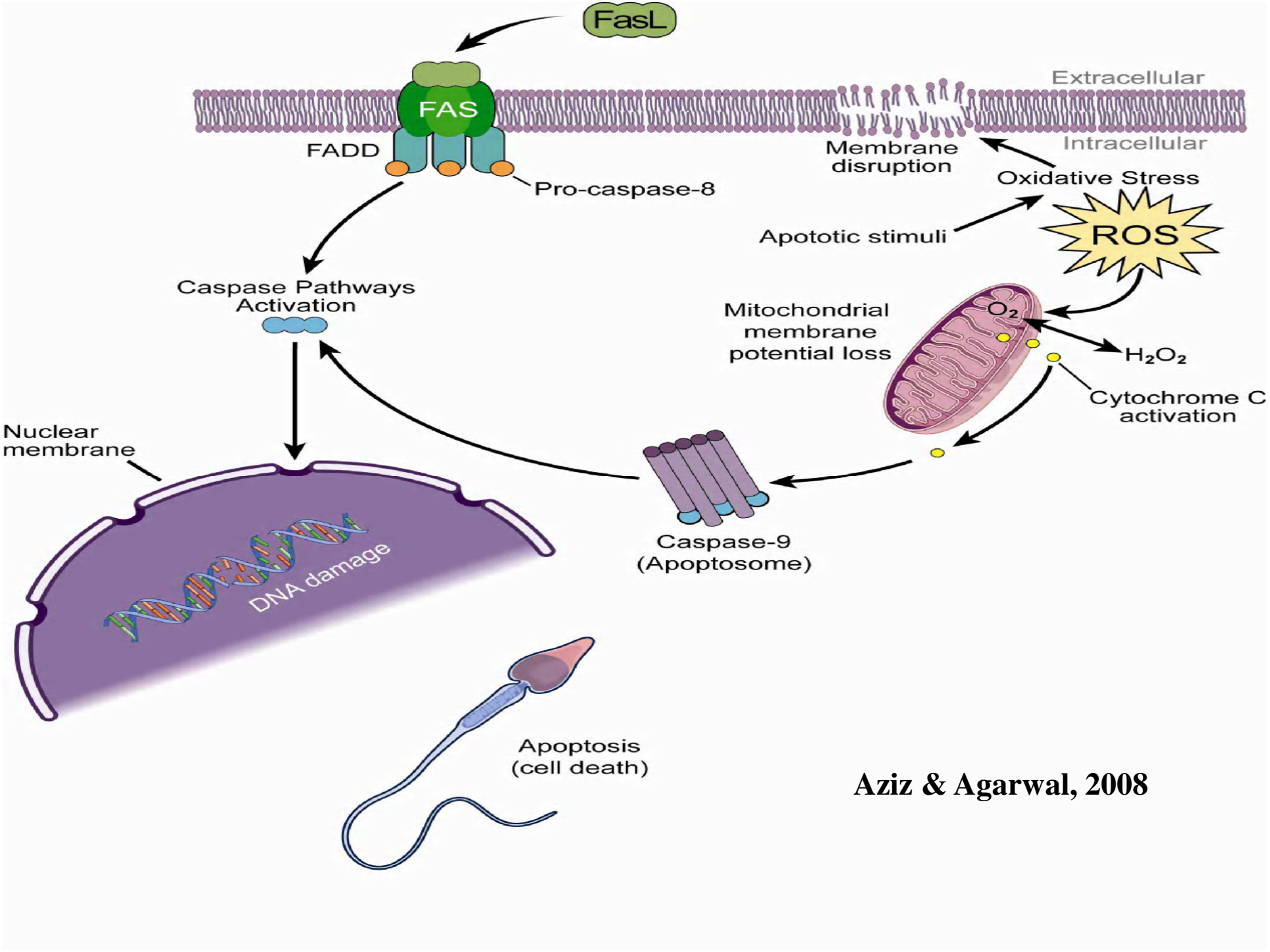
- The prevalence was much higher (82%) in men from RPL couples than from fertile or infertile couples (20%) [1]
- Y-chromosome microdeletion testing may be considered in the evaluation of RPL couples when all other tests fail to reveal the aetiology [1]

1 Dewan et al, 2006

Sperm Apoptosis

- ↑ apoptotic sperm% in RPL patients compared to fertile men (38% v 12%) [1]
- Sperm apoptosis may be causative of RPL in some patients [1]

1 Carrell et al, 2003



Aziz & Agarwal, 2008

Oxidative Sperm Damage

- >50% of male partners of RPL patients have
  - Higher lipid peroxidation of sperm cell membrane in RPL and/ or [1]
  - ↑ DNA fragmentation [1, 2]
- Antioxidants rich food or antioxidant treatment improved pregnancy outcome [3]

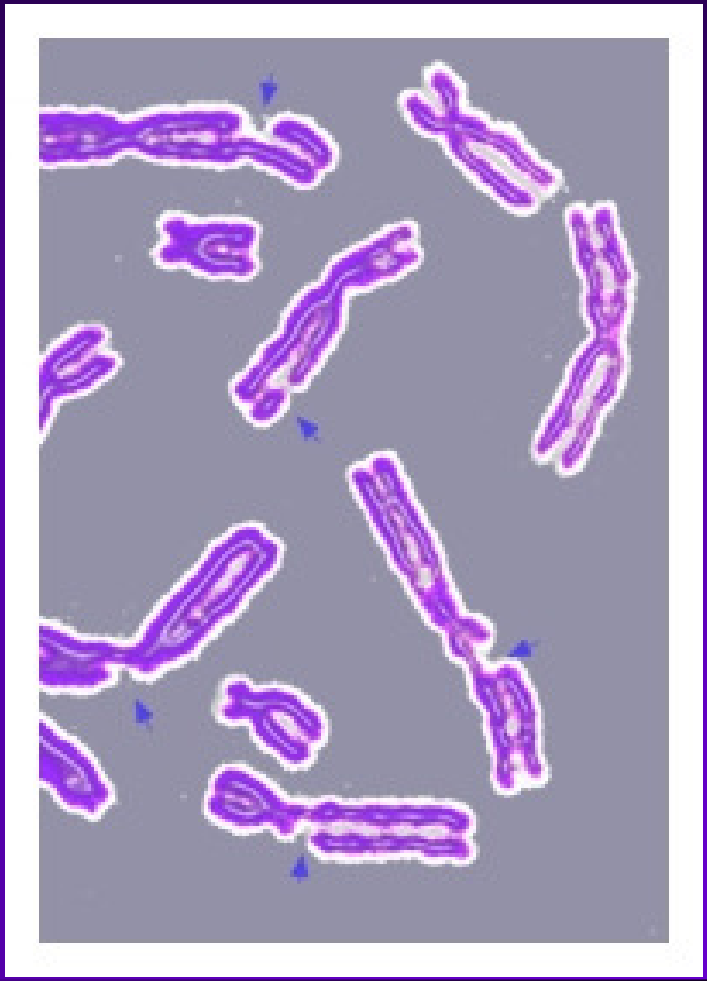
[1] Gill-Villa et al, 2009a
2 Carrell et al 2009
3 Gill-Villa et al, 2009b

Naturally Occurring Breaks

- DNA breaks occur naturally in elongating sperm
- Normally, Topo ll ligates breaks
- Sperm is at disadvantage to repair the damage
- Oocytes and early embryos are capable of repairing the breaks in the male genetic material

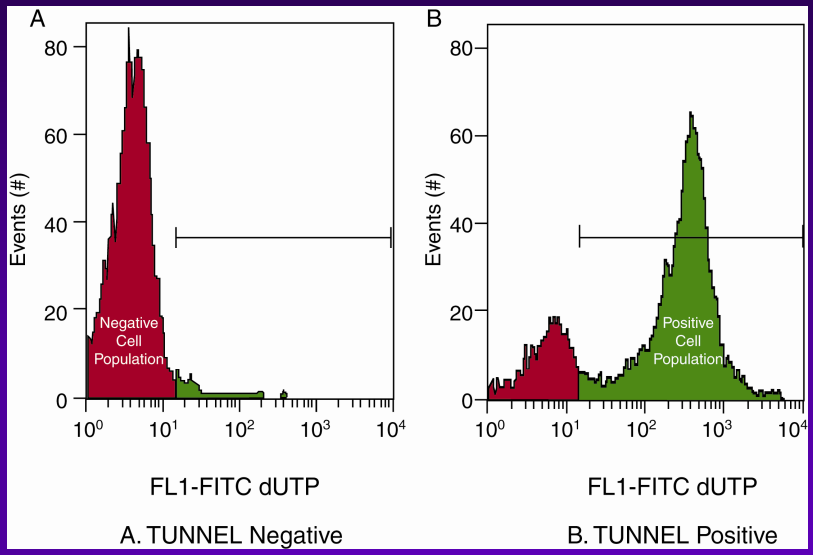
Sperm Chromatin Fragmentation

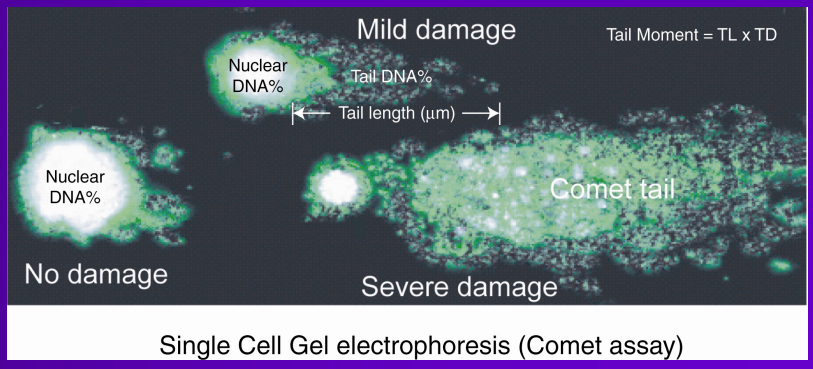
- Single and double DNA strands breaks
  - Hallmark of apoptosis
  - Oxidative stress
  - Abnormalities in the processes of recombination and protamination



Evaluation of Sperm Chromatin Fragmentation

- Chromatin Structure Assay
- TUNEL
- Comet assay (single cell electrophoresis





Single Cell Gel electrophoresis (Comet assay)

DNA Fragmentation Testing

- Correlates well with reproductive outcome
- Not fully standardized
  - Reagents used
  - Exposure time
  - Time since ejaculation
  - Fraction of ejaculation

Sperm Chromatin Fragmentation

- High Ch. F (>30%) Correlated with
  - Poor sperm morphology and motility
  - ↓ fertilization and implantation rate
  - ↓ developmental rate to blastocyst stage
  - ↑ spontaneous abortion rate [1]
  - Unexplained RPL [2]

1 Lin et al. 2007
2 Carrell et al, 2003

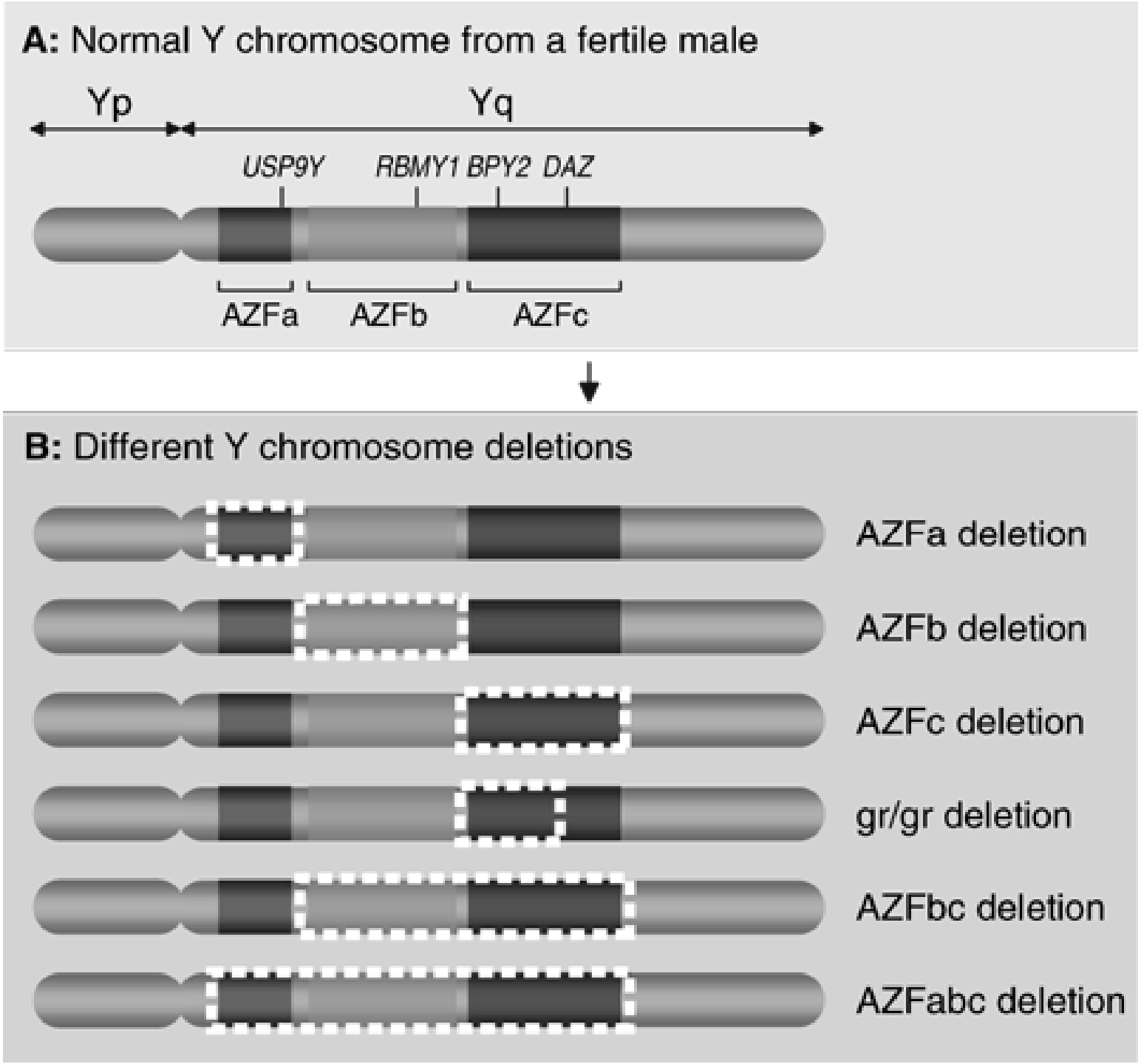
Age and Sperm Chromatin Fragmentation

- Increased by age which may explain
- ↑ spontaneous early pregnancy loss among older men (>45 compared to <25) [1]
- The ↑ is noted even if the female is > 40 [2]

1 Salama et al, 2005
2 Kleihaus et al, 2006

Conclusions

- Sperm damage influences pregnancy outcome
- Newer techniques of assessing sperm integrity to uncover possible causes of unexplained pregnancy loss
- Strategies to improve pregnancy outcome by reversing sperm damage are being developed


A: Normal Y chromosome from a fertile male
Yp
Yq
USP9Y
RBMY1
BPY2
DAZ
AZFa
AZFb
AZFc
B: Different Y chromosome deletions
AZFa deletion
AZFb deletion
AZFc deletion
gr/gr deletion
AZFbc deletion
AZFabc deletion

AZF*b* Deletions and RPL

- Seven of the 43 men (16%) from couples with RPL had microdeletions in 1 or more of the AZF loci segments studied [1]
- Deletion at locus AZF*b* was found in all 7
- Microdeletion in AZF region may be a possible etiologic factor of RPL

1 Karaer et al, 2008

Structural Abnormalities

- Y-chromosome microdeletions
- Balanced translocations

Diagnosing Sperm Chromatin Fragmentation

- Chromatin Structure Assay
- TUNEL (terminal deoxynucleotidyl transferase-mediated dUDP nick-end labeling)
- Comet assay (single cell electrophoresis)

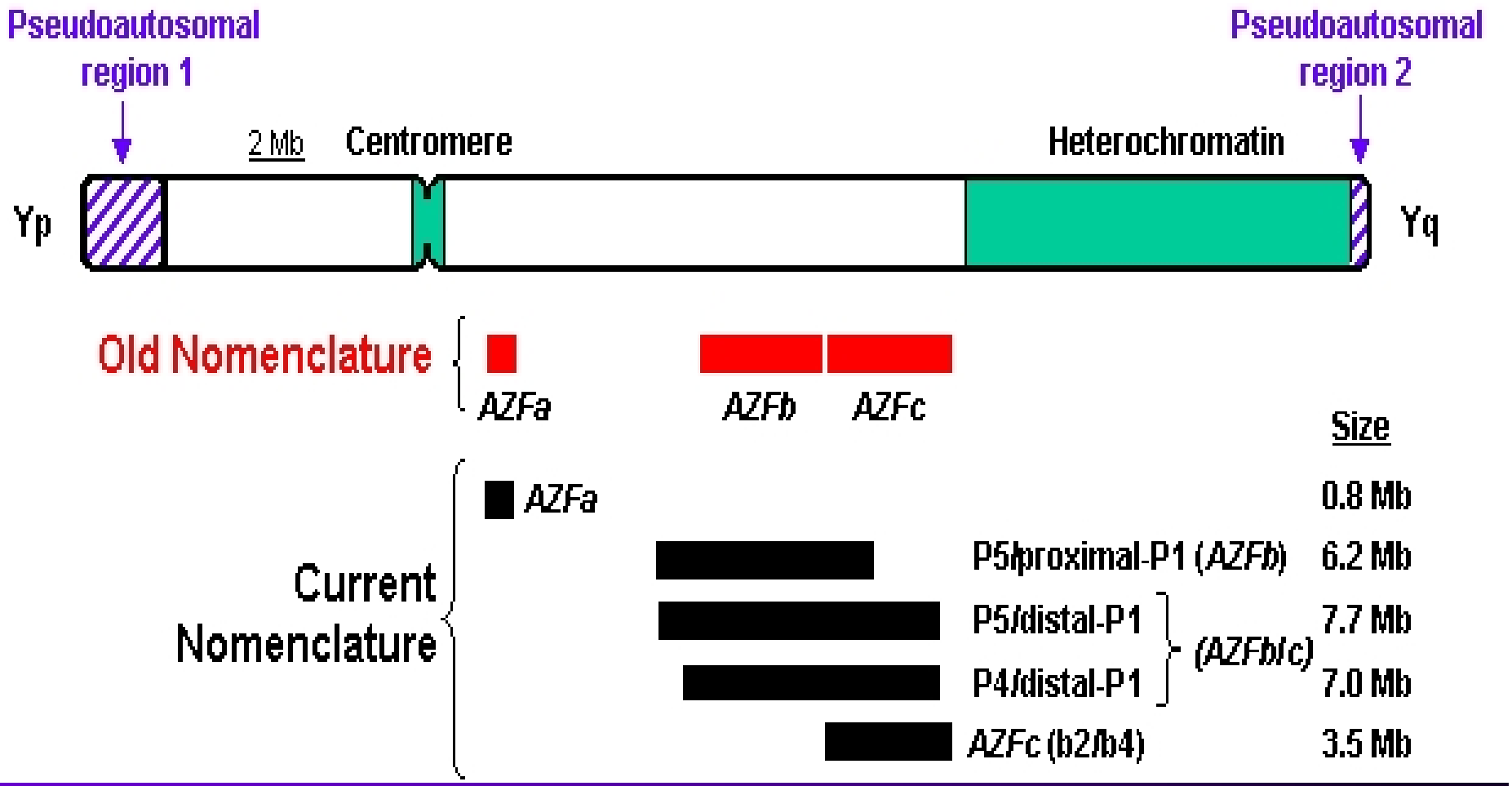
Sperm Quality and Function

- Sperm morphology
- Sperm motility
- leukozoospermia
- Hypo-osmotic swelling (HOS) test
- Chromatin decondensation

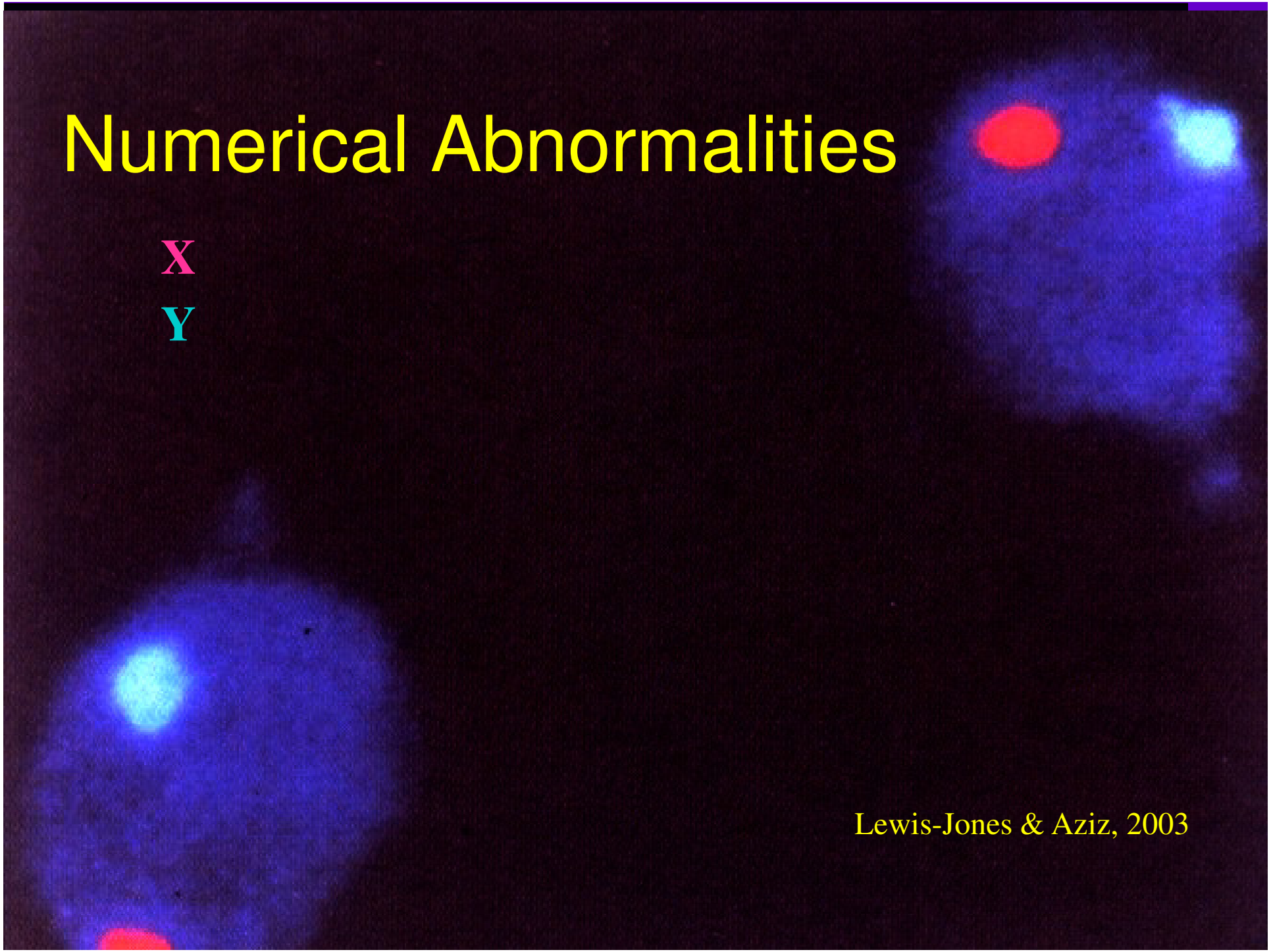
The Y Chromosome

- Contains the SRY gene, which triggers embryonic development as a male
- Contain other genes needed for normal sperm production.

AZF Loci


Pseudoautosomal region 1
Pseudoautosomal region 2
2 Mb
Centromere
Heterochromatin
Yp
Yq
Old Nomenclature
AZFa
AZFb
AZFc
Size
Current Nomenclature
AZFa
0.8 Mb
P5/proximal-P1 (AZFb)
6.2 Mb
P5/distal-P1
7.7 Mb
(AZFb/c)
P4/distal-P1
7.0 Mb
AZFc (b2/b4)
3.5 Mb

Numerical Abnormalities



Lewis-Jones & Aziz, 2003

RPL and Chromatin Decondensation [1]

- A significant decrease in the capacity of nuclear chromatin to decondense in vitro
- When sperm were examined with E/M:
  - ↑ defects of chromatin condensation and
  - irregular nuclei with ↑ vacuoles

[1] Gopalkrishnan et al, 2000