Primary Fertility Investigations and Male Infertility

Dr Bruno Radesic

Date: 16th May 2024

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Introducing

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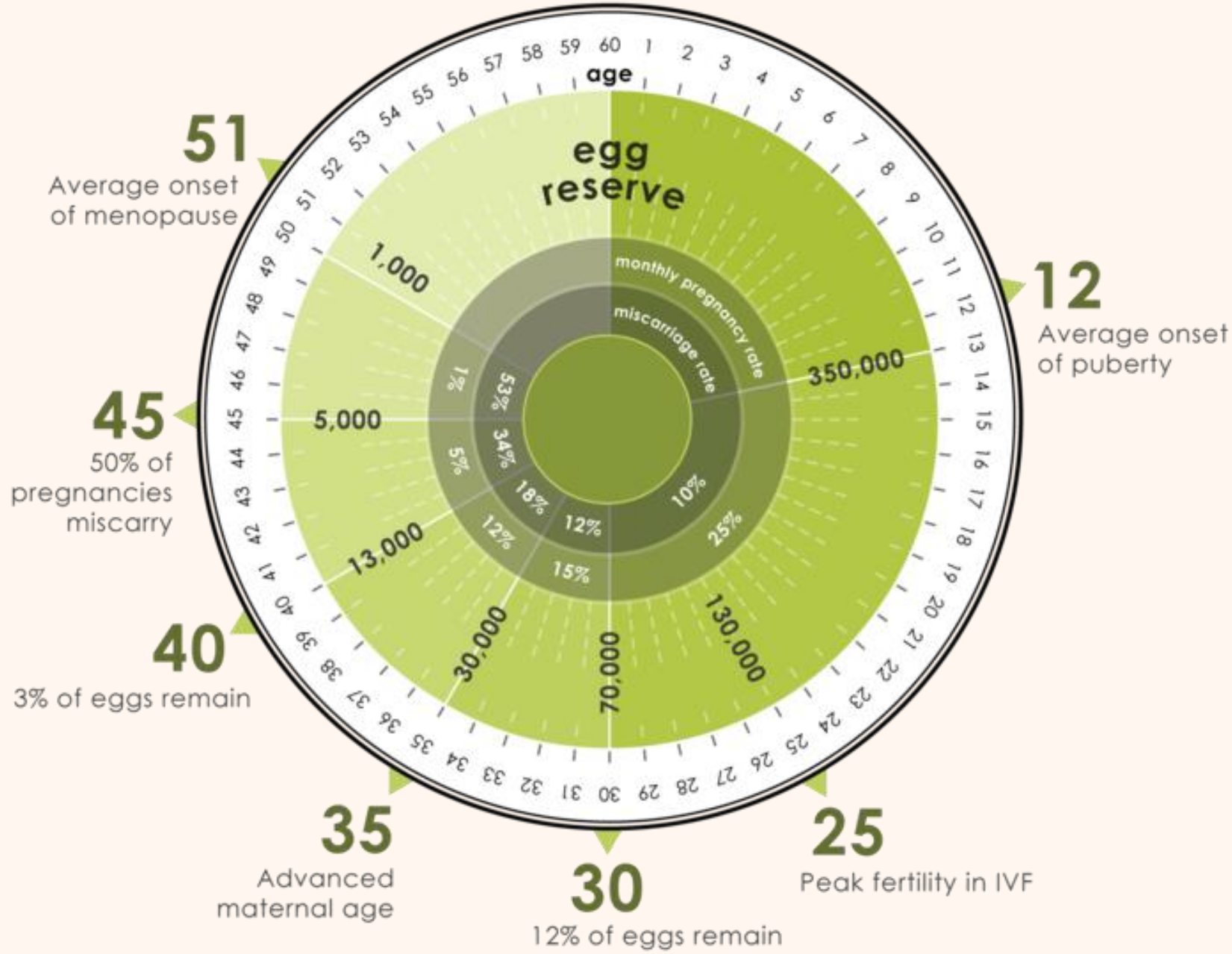

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Male Fertility/Infertility

AUA/ASRM Guideline

October 2020

Diagnosis and Treatment of Infertility in Men: AUA/ASRM Guideline

Peter N. Schlegel, MD; Mark Sigman, MD; Barbara Collura; Christopher J. De Jonge, PhD, HCLD(ABB); Michael L. Eisenberg, MD; Dolores J. Lamb, PhD, HCLD (ABB); John P. Mulhall, MD; Craig Niederberger MD, FACS; Jay I. Sandlow, MD; Rebecca Z. Sokol, MD, MPH; Steven D. Spandorfer, MD; Cigdem Tanrikut, MD, FACS; Jonathan R. Treadwell, PhD; Jeffrey T. Oristaglio, PhD; Armand Zini, MD

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Investigate Male Infertility

- **Rewarding (Simple investigation with big returns)**
  - **Common**
  - **Highly effective treatments available**
  - **Room for improvement - unlike egg, new sperm are made continuously into old age**

Causes	Frequency (%)
Sperm defects or dysfunction	30
Ovulation failure (amenorrhoea or oligomenorrhoea)	25
Tubal infective damage	20
Unexplained infertility	25
Endometriosis (causing damage)	5
Coital failure or infrequency	5
Cervical mucus defects or dysfunction	3
Uterine abnormalities (eg fibroids or abnormalities of shape)	1

15% of couple have more than one subfertility factors

Investigate Male Infertility

- Semen Analysis
  - Simple, non-invasive, inexpensive
  - Normal results excludes male factor in up to 90% (*** caution with interpretation*)

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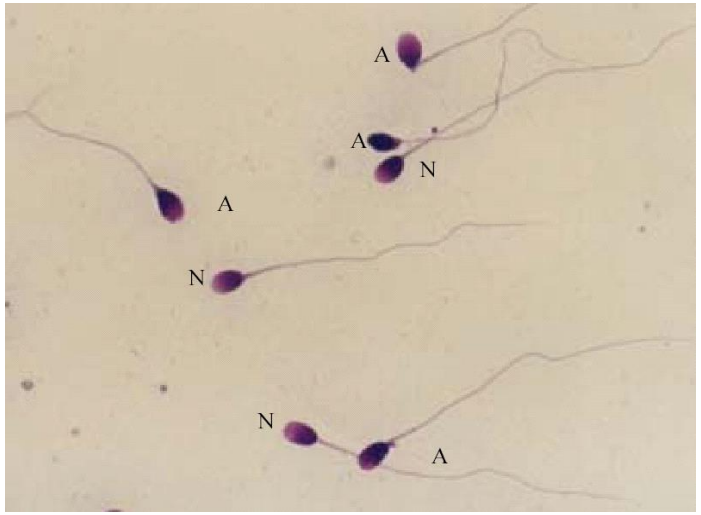
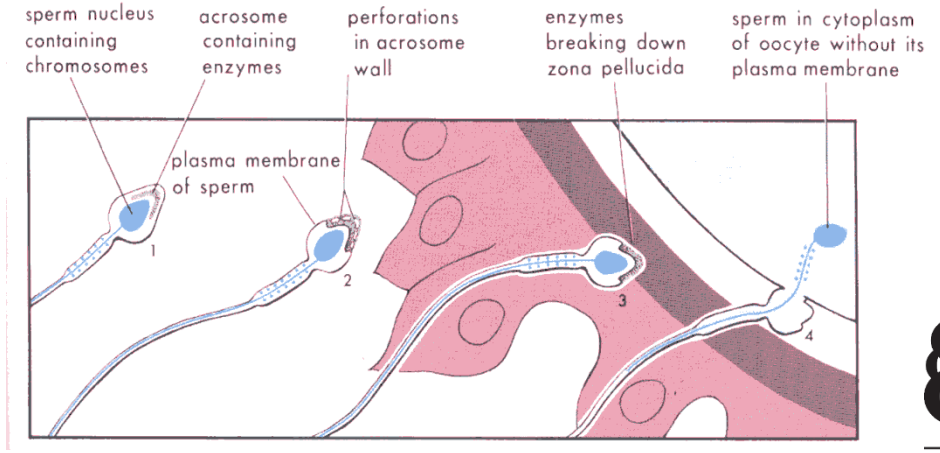
Fertilisation – concentration, motility and morphology





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Semen analysis ‘abnormal’

Lower reference limits (5th centiles and their 95% confidence intervals) for semen characteristics (11).

Parameter (units)	*N*	Centile		
		5	50	95
Total sperm number (10^6 per ejaculate)	1859	39	255	802
Sperm concentration (10^6 per ml)	1859	15	73	213
Total motility (PR + NP, %)	1781	40	61	78
Normal forms (%)	1851	4	15	44

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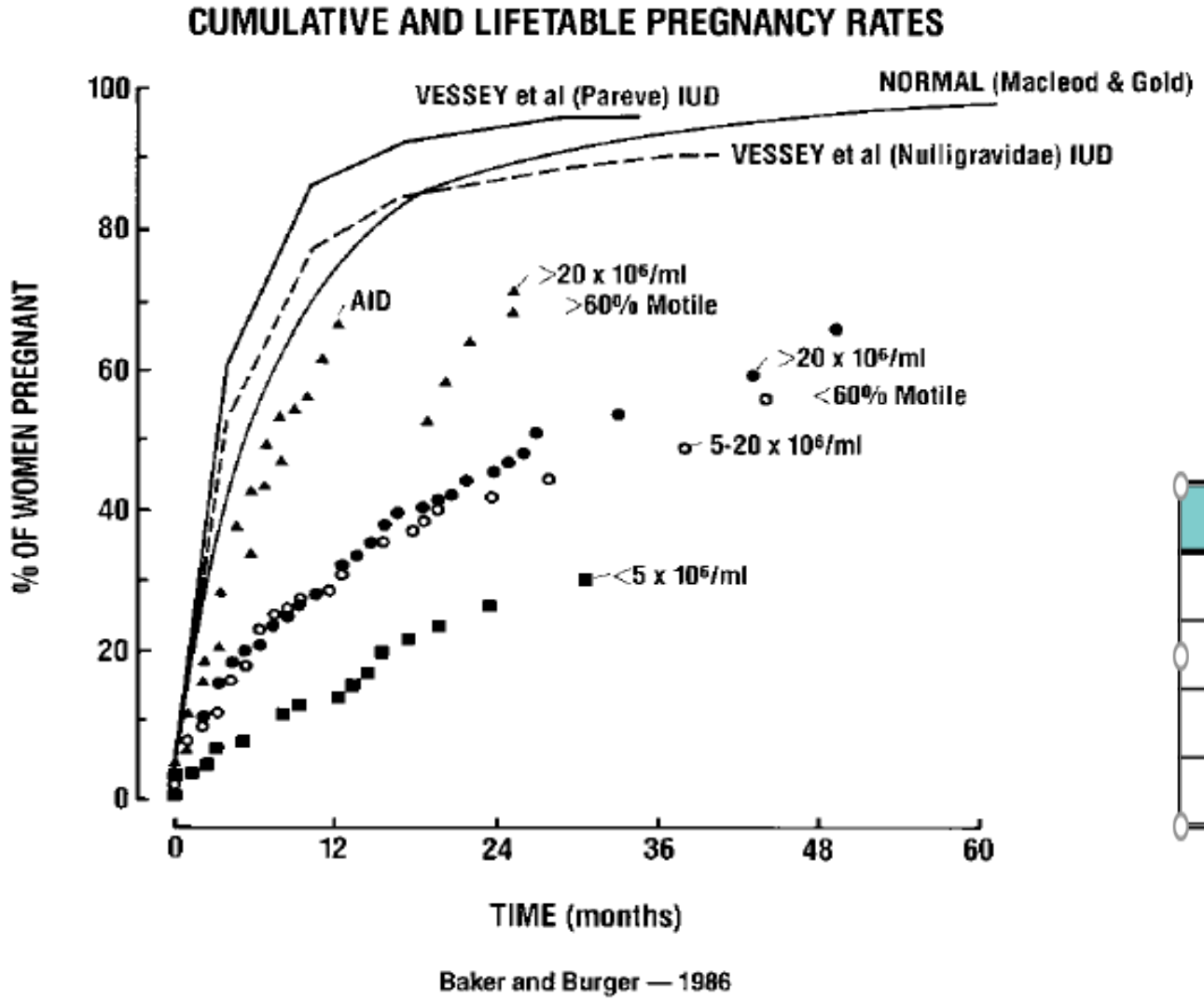
What does it mean doctor?



	Centiles		
	5	50	95
Sperm concentration (10^6/ml)	15	73	213
Progressive motility (PR, %)*	32	55	72
Normal forms (%)	4	15	44
Vitality (%)	58	79	91

Number of factors	Monthly chance	Mean Years to Pregnancy	% Pregnancy in 2 years
0	20%	0.3 (4m)	93.6
1	5%	2	63.8
2	1%	7	20.7
3	0.2%	40	4.7

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Male Investigations

- **History**
  - Hx of testicular disease: Cryptorchidism, torsion, trauma, infection, surgery.
  - Social Hx: smoking, heavy alcohol, recreational drugs, occupation, sleep
  - Med & surg Hx, Family Hx, Meds
  - Sexual Hx: Erection, penetration. ejaculation.

- **Endocrine: rare** - present with sexual dysfunction (<2%)
  - *Hypothyroidism*
  - *Hyperprolactinaemia*
  - hypogonadotropic hypogaonadism

Male Investigations

- **History**
  - Hx of testicular disease: Cryptorchidism, torsion, trauma, infection, surgery.
  - Social Hx: smoking, heavy alcohol, recreational drugs, occupation, sleep
  - Med & surg Hx, Family Hx, Meds
  - Sexual Hx: Erection, penetration. ejaculation.
- **Examination**
  - Body habitat
  - virilisation
  - Testicular
- **Investigations**
  - Semen analysis, ab
  - FSH, LH, Testosterone, SHBG
  - FBC, EUC, LFTs, Fasting glucose insulin homocysteine, TSH
  - Gentics (karyotype, Y-del, CF ect)
  - Testicular u/s, bone density scan

- **Obstructive Causes -** azoospermia
  - *iatrogenic*
  - *Congenital* non-junction or absence *CF* screening recommended (*CF and CFTR gene*), Congenital Bilateral Absence of the Vas Deferens (CBAVD)
  - bacterial *infection* (chlamydia trachomatis*)*
  - *Ejaculatory duct*

- **Primary Testicular Disease -** *most common* cause of male infertility
  - *66% unknown*
  - 20% testicular maldescent
  - 7% trauma and torsion
  - 5% Klinefelter's syndrome
  - 1% mumps orchitis
  - 1% chemo.ions

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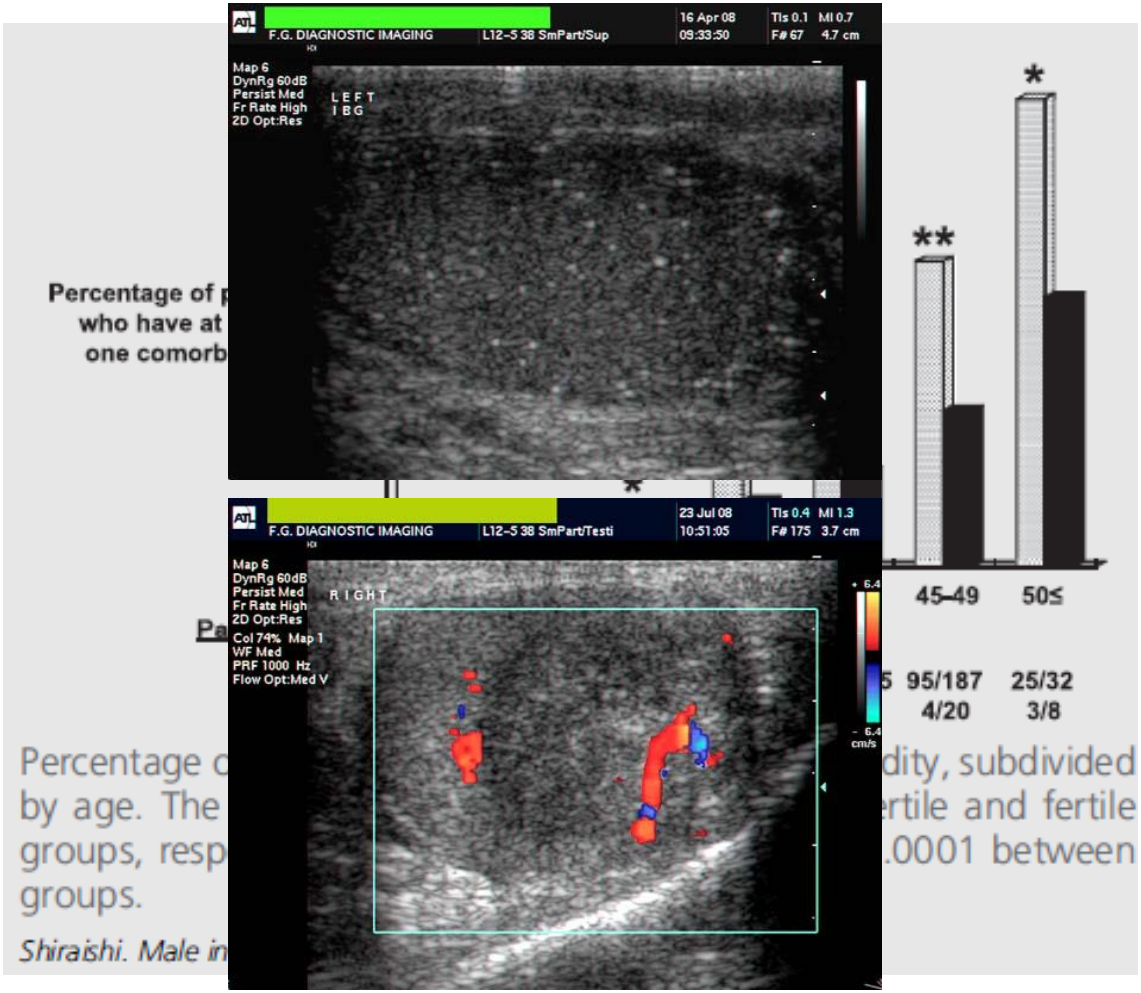
Male infertility - treatment

Options

- Treat correctable causes (lifestyles, toxins/drugs, hormone, inf) & health issues (cancer, T def)



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Spermatogenesis

Pathology Results: Mr [redacted]

Sem-F - 31/01/2012

Time collected	0935
Time examined	1155
Volume	2.0
Sperm Concentration	0

Motility
Rapidly progressive
Slowly progressive
Non progressive
Non motile

Normal forms

(Reference: WHO Laboratory Manual

Test,.SHBG/FAI - 10/04/2012

TESTOSTERONE,SHBG AND FAI

Date	25/02/12
Time F-Fast	1025 F
Lab ID	47568560
FSH	
LH	
Testosterone	* 46.3
SHBG	
FAI	

Supervising Pathologist: GC, NT

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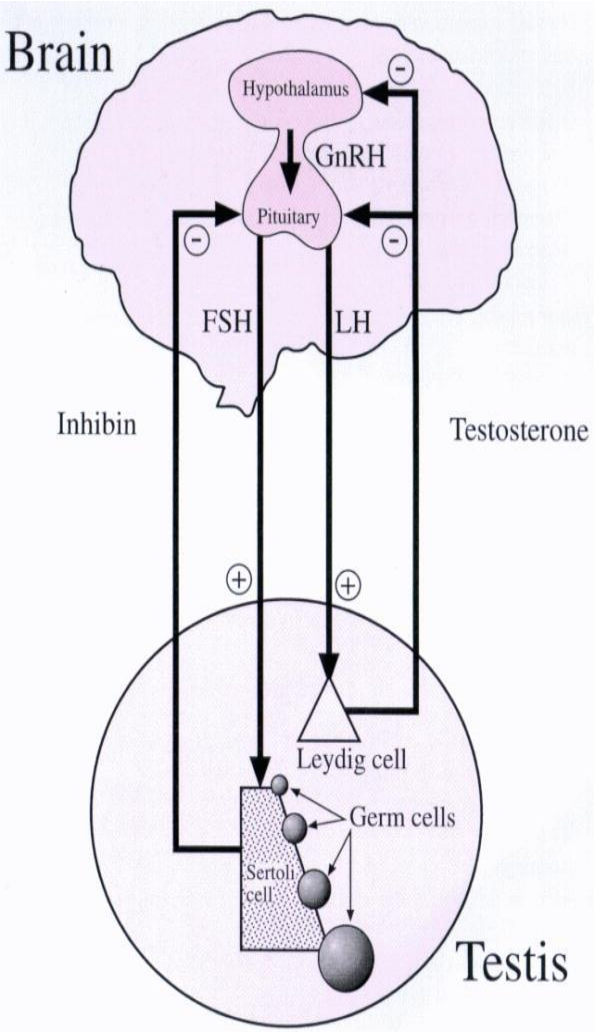
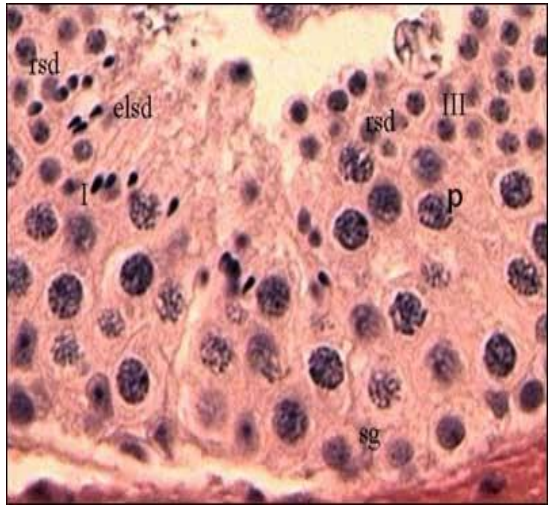
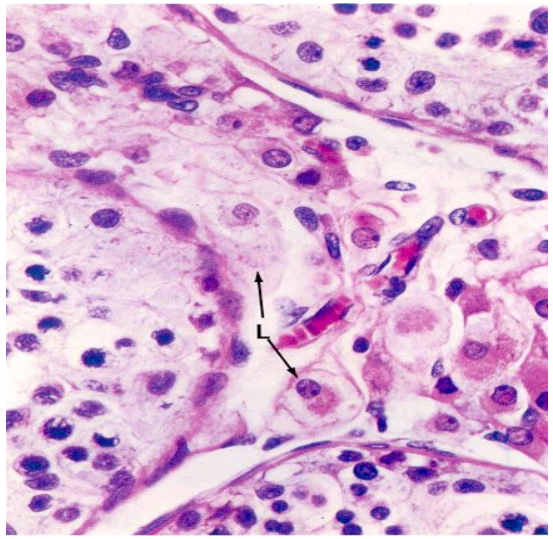
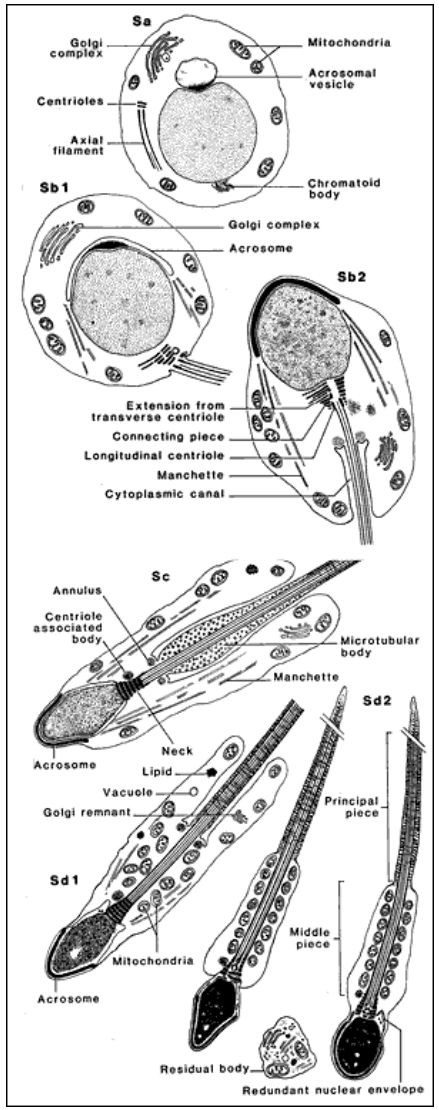
Spermatogenesis & Hormones









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Spermatogenesis

Pathology Results: Mr [redacted]

Sem-F -	31/01/2012	10/04/2012	16/04/2012		
Time collected	0935	0745	0754		
Time examined	1155	1425	0830		
Volume	2.0	* 1.0	* 1.0	mL	(2-6mL)
Sperm Concentration	0	22	28	10*6/mL	(>20x10*6/mL)
Motility					
Rapidly progressive		10	10	%	
Slowly progressive		25	15	%	
Non progressive		5	5	%	
Non motile		60	70	%	
Normal forms		23	22	%	

(Reference: WHO Laboratory Manual, 4th Edition 1999)

Test,.SHBG/FAI - 10/04/2012

TESTOSTERONE,SHBG AND FAI

Date	25/02/12	**10/04/12**	16/04/2012		
Time F-Fast	1025 F	**0820 F**			
Lab ID	47568560	**205130542**	212984244	Units	Range
FSH		1.6	1.6	U/L	(1.5 - 13.0)
LH		2.8	4.6	U/L	(2.0 - 10.0)
Testosterone	* 46.3	**14.8**	* 11.2	nmol/L	(11.5-32.0)
SHBG		**24**	25	nmol/L	(15-50)
FAI		**61.7**	45.8	%	(15-100)

Supervising Pathologist: GC, NT

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Spermatogenesis

DHM - Reference No: 276344931 Status: F

Patient: [redacted]
DOB: 19/07/1971
Address: 1/18-26 Romsey Street Waitara 2077
Ordered by: Dr Derek LOK on 09/05/2013
Copy to: Clinic Nurse Co-Ordinator
Collected: 28/09/2013 - 9:00 AM
Reported: 28/09/2013

TESTOSTERONE,SHBG AND FAI

Date	29/08/10	13/04/13
Time F-Fast	0915 F	0915 F
Lab ID	205046992	276063428
Testosterone	* 2.2	* 6.7
SHBG		25
FAI		26.8
FSH		2.5
LH		3.4
Oestradiol		146

SIVFANDR - Reference No: SYD03639 Status:

Patient: [redacted] **Linked by:** Dr Derek Lok
DOB: 19/07/1971 **Message:** sev triple know
Address: 1/18-26 Romsey Street Waitara 2077
Ordered by: Dr Derek LOK on 00/00/0000
Collected: 19/04/2013 - 12:00 AM **Notified by:** on 00/00/0000
Reported: 00/00/0000 **Message:**

GENEA - ANDROLOGY RESULTS

Test	Result	Units	Ref Range
SEMEN ANALYSIS			
Test Date	19/04/2013		
Ejaculate Volume	1.5	ml	>= 1.5
Sperm Concentration	1.7	million/ml	>= 15
Total Sperm Count	2.6	million/ejaculate	>= 39
Motility:			
Rapid	25	%	
Progressive	33	%	>=32
Motile	43	%	>=40
Motility Index	101		>120
Progression Rating	2		>=3
Morphology:			
Normal Forms	0	%	>=2 [GENEA] >=4 [WHO]
Head	100	%	
Neck/mid piece	47	%	
Tail	30	%	
Cytoplasmic droplets	0	%	
TDI	1.77		<2.0

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DHM - Reference No: 276344931 Status: F

Patient:	[redacted]	**Linked by:**	Jane Aziz
DOB:	19/07/1971	**Message:**	**No Action**
Address:	1/18-26 Romsey Street Waitara 2077		
Ordered by:	Dr Derek LOK on 09/05/2013		
Copy to:	Clinic Nurse Co-Ordinator		
Collected:	28/09/2013 - 9:00 AM	**Notified by:**	on 00/00/0000
Reported:	30/09/2013	**Message:**	

tara 2077
3

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Semen Analysis

Time collected	0900		
Time examined	0940		
Volume	2.5	mL	(2-6mL
Sperm Concentration	55	x10*6/mL	(>20x1

Motility

Rapidly progressive	30	%
Slowly progressive	30	%
Non progressive	20	%
Non motile	19	%

Normal forms	* 11	%

Comment on Lab ID 276344931

==========

:4/08/13 **# 28/09/13**
)920 **0900**
:77207228 **276344931**

Units	Range
16.7 **16.9** nmol/L	(9.5-28.0)
20 **20** nmol/L	(15-50)
83.5 **84.5** %	(15-100)
5.6 5.8 IU/L	(1.0 - 12)
5.8 6.6 IU/L	(0.6 - 12)
<50 70 pmol/L	(<160)

Aromatase Inhibitor

+ Lifestyle changes

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Male infertility - treatment

Options

- Treat correctable causes (lifestyles, toxin/drugs, hormone, inf) & health issues (cancer, T def)
- **Expectant & support**
- **Conventional medical and surgical treatment**s

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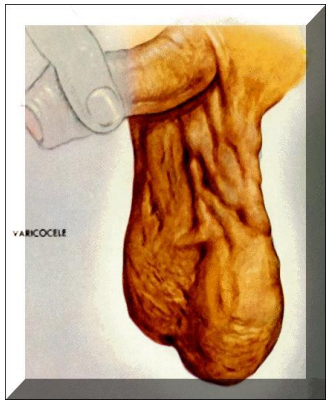
Surgical

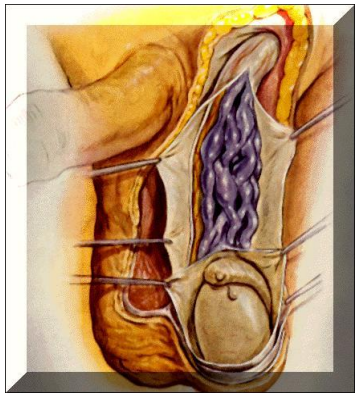
Preventative

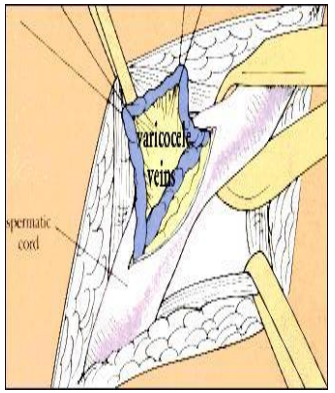
- Cryptorchidism

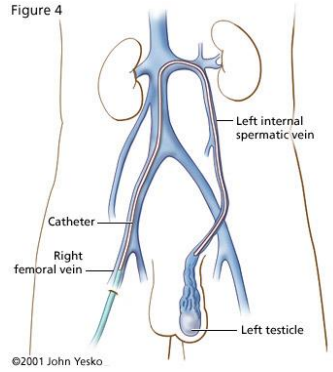
Severe oligo / azoospermia	Untreated	Pre-pubertal orchidopexy
Unilateral	50-70%	37%
Bilateral	100%	70%

- Varicocoele in adolescents









Treatment

- Varicocoele

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Surgical

Preventative

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Severe oligo / azoospermia	Untreated	Pre-pubertal orchidopexy
Unilateral	50-70%	37%
Bilateral	100%	70%

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Treatment

- Varicocoele

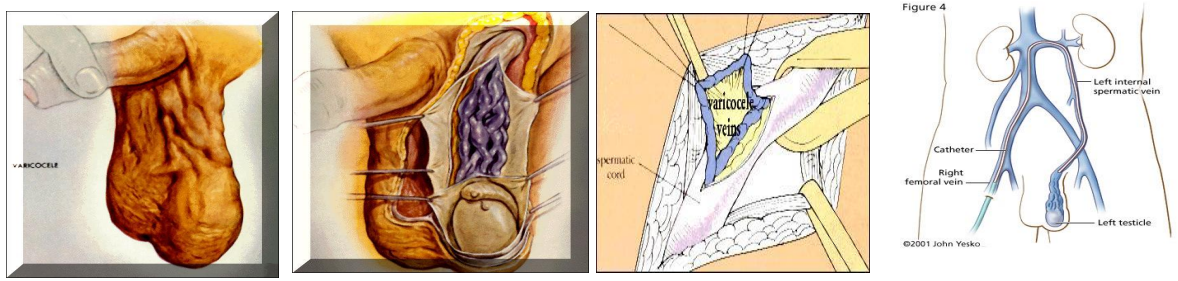
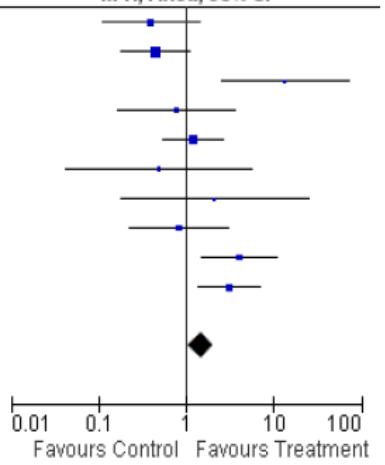


Figure 3. Forest plot of comparison: 1 Varicocele occlusion versus no treatment, outcome: 1.1 Pregnancy rate.

Study or Subgroup	Varicocele occlusion Events	Total	No treatment Events	Total	Weight	Odds Ratio M-H, Fixed, 95% CI
Nilsson 1979	4	51	8	45	13.9%	0.39 [0.11, 1.41]
Breznik 1993	13	38	22	41	24.7%	0.45 [0.18, 1.11]
Madgar 1995	15	25	2	20	1.6%	13.50 [2.55, 71.40]
Yamamoto 1996	3	45	4	47	6.5%	0.77 [0.16, 3.64]
Nieschlag 1995/1998	18	62	16	63	19.9%	1.20 [0.55, 2.65]
Grasso 2000	1	34	2	34	3.4%	0.48 [0.04, 5.61]
Unal 2001	2	21	1	21	1.6%	2.11 [0.18, 25.17]
Krause 2002	5	33	6	34	8.9%	0.83 [0.23, 3.05]
Dohle 2010	19	65	6	65	7.5%	4.06 [1.50, 10.99]
Abdel-Meguid 2011	24	75	10	75	12.0%	3.06 [1.34, 6.97]
Total (95% CI)		**449**		**445**	**100.0%**	**1.47 [1.05, 2.05]**
Total events	104		77			

Heterogeneity: $Chi^2 = 27.01$, $df = 9$ ($P = 0.001$); $I^2 = 67\%$
Test for overall effect: $Z = 2.23$ ($P = 0.03$)



Cochrane 2012

26. Clinicians should not recommend varicocelectomy for men with nonpalpable varicoceles detected solely by imaging. (Strong Recommendation; Evi-

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Surgical

Preventative

- Cryptorchidism

Severe oligo / azoospermia	Untreated	Pre-pubertal orchidopexy
Unilateral	50-70%	37%
Bilateral	100%	70%

- Varicocoele in adolescents

Treatment

- Varicocoele
- Ejaculatory duct cyst
- **Vasectomy reversal**

Authors	Year	# Pts	Patency rate	Preg rate
Cos et al	1983	87	75% (66/87)	46% (32/69)
Requeda	1983	47	80% (38/47)	46% (18/39)
Owen & Kapila	1984	475	93% (439/475)	82% (390/475)
Lee	1986	324	90% (292/324)	51% (165/324)
Silber*	1989	282	91% (258/282)	81% (228/282)
Belker et al	1991	1247	86% (865/1012)	52% (421/808)
Fox	1994	103	84% (86/103)	48% (31/64)
Total		**2565**	**88% (2044/2330)**	**62% (1285/2061)**

Surgical

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Preventative

- Cryptorchidism

Severe oligo / azoospermia	Untreated	Pre-pubertal orchidopexy
Unilateral	50-70%	37%
Bilateral	100%	70%

- Varicocoele in adolescents

Treatment

- Varicocoele
- Ejaculatory duct cyst
- Vasectomy reversal

ART – sperm retrieval

- Azoospermia
- Anejaculation
- Epididymal necrospermia / Sperm DNA fragme

Conventional Medical Treatment

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Medical therapy for semen defects not useful

Agents	RCT	Patients	OR	CI
HMG/rFSH	3	233	1.45	0.78-2.7
androgen	9/13	1025	1.02	0.72-1.44
anti-E2	6/11	459	1.33	0.78-2.28
kallikrein	4/16	459	0.92	0.4-2.28
Bromocriptine	3		No effect	

42. Clinicians should inform the man with idiopathic infertility that the use of SERMs has limited benefits relative to results of ART.

43. Clinicians should counsel patients that the benefits of supplements (eg, antioxidants, vitamins) are of questionable clinical utility in treating male infertility. Existing data are inadequate to provide recommendation for specific agents

Spermatogenesis – genetic variants/cryptic genetic factors

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Centiles	5	50	95
Sperm concentration (10^6/ml)	15	73	213
Total number (10^6/Ejaculate)	39	255	802
Normal forms (%)	4	15	44

Box 2 Genetic basis of human male infertility defects: spermatogenesis and sperm function

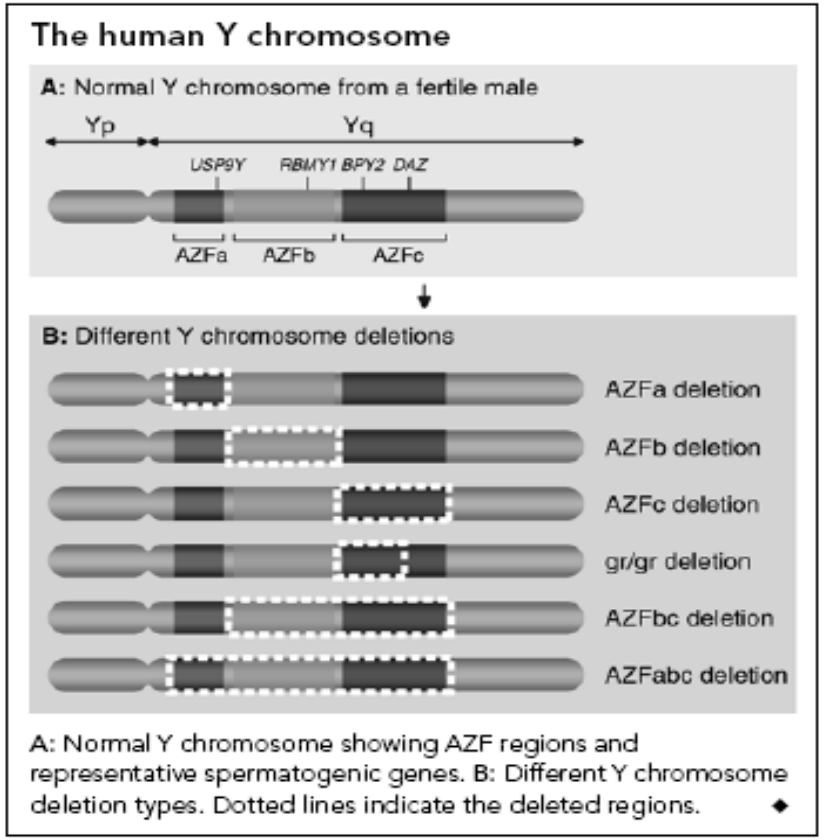
Gene defects identified in infertile male individuals with spermatogenesis or sperm function defects are listed below. The details of each gene and the associated phenotype are found in Supplementary Table 2. SNPs are shown in red. Some studies represent only a few individuals or case reports.

Abnormal spermatogenesis
ATM; ATMAC; DAZL; ERCC2; GTF2A1L; JUN; NLRP14; NRB0B1; POLG; PRM1; PRM2; SDHA; SOX8; XRCC1; YBX2
Azoospermia
APOB; ACSBG2; ART3; ATM; BOULE; BPY2; BRCA2; CDY1; CFTR; CREM; DAZ; DDX25; DDX3Y; DRFFY; ERCC1; ERCC2; FASLG; FHL5; FKBP6; HNRNPC; HSFY1; KLHL10; LAP3; MBOAT1; MEI1; MLH1; MLH3; MTR; NLRP14; PRDM16; RBMX; RBMY1A1; RBMY1F; SPATA16; SYCP1; SYCP3; TAF7L; TGIF2LX; TSPY; TSSK4; UBE2B; USP26; UTP14C; USP9Y; UTY; XPC; XPD; XRCC1; YBX2; ZNF230
Oligospermia
MT-ATP6; EGF; FASL; H19 and MEST; KLHL10; PIGA; PRM1; PRM2; SHBG; SDHA; TSSK4; UBE2B; VASA
Asthenozoospermia
AKAP3; AKAP4C; CATSPER2; DNMT3B; DHAH5; DNAH11; DNAL1; PDYN; GNA12; Mitochondrial DNA; *MTHFR; MT-ND4; PIGA; POLG; PPM1G; PRKAR1A; SHBG; SPAG16; TEKT1; TEKT2; TPN1; TPN2; TXNDC3;* T mt DNA haplotypes
Teratozoospermia
AURKC; PRM1; PVRL2; SPATA16; SP1
Oligoasthenozoospermia
JUND; mt-ND4; NALP14
Oligoasthenoteratozoospermia
MTRR; IL1B; SABP
Acrosome or fertilization
POIA3

DNA damage	Infertility
GSTM1	*AR; GSTM1 KIT; KITLG; IL1A; OAZ3; PRM1; TSPY; TSSK4; USP26; YBX2*

Varicocele effect
MT-ATP6; MT-ATP; CACNA1C; MT-CO1; MT-CO2; MT-ND3
Chromosome defect
Numerical sex chromosome (Klinefelter's; XXY–XXXXY) Structural chromosome (translocations, inversions or deletions) Y chromosome microdeletions, XX male or XY female
Systemic disorders affecting fertility

Kartagener's syndrome	Noonan (PTPN11)
Fanconi anemia (FANCA)	Sickle cell anemia (HBB)
Myotonic dystrophy (DMPK)	β-thalassemia



A: Normal Y chromosome showing AZF regions and representative spermatogenic genes. B: Different Y chromosome deletion types. Dotted lines indicate the deleted regions. ◆

Male infertility – Assisted Reproductive Treatment

Options

- Treat correctable causes (toxin, hormone, inf)
- Expectant & support
- Conventional medical and surgical treatments
- **Intrauterine insemination (IUI)**
- **IVF/ ICSI**
- Donor Sperm & artificial insemination
- Adoption

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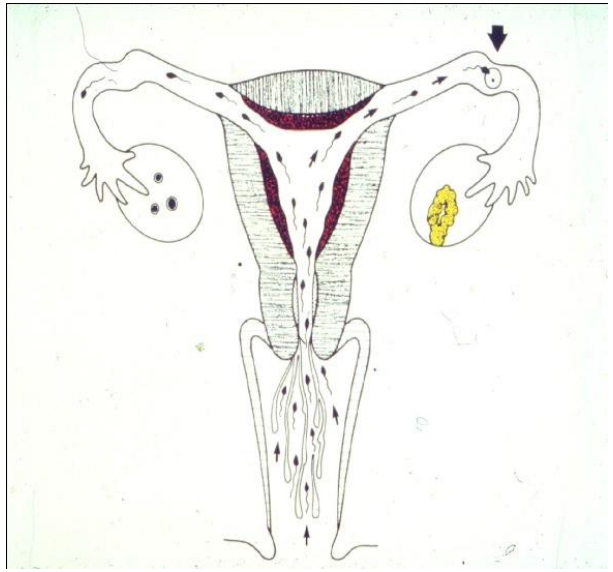
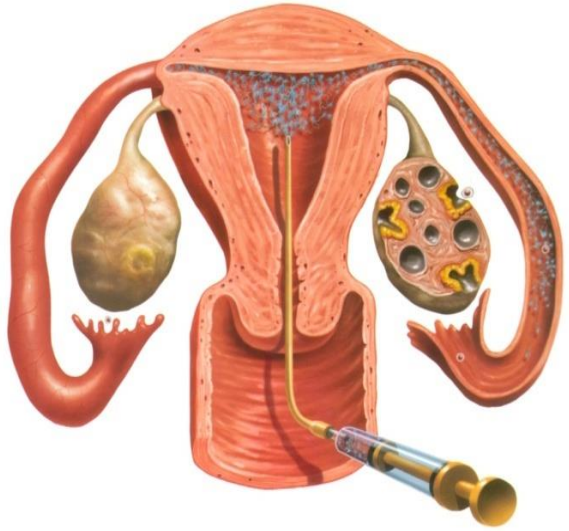
IUI of washed sperm

- May be useful as starting point for idiopathic infertility but less effective (half) in male infertility(3-10%)

- **Meta-analysis on IUI alone for male factor showed no benefit**
  - *Need => 5mil motile sperm*





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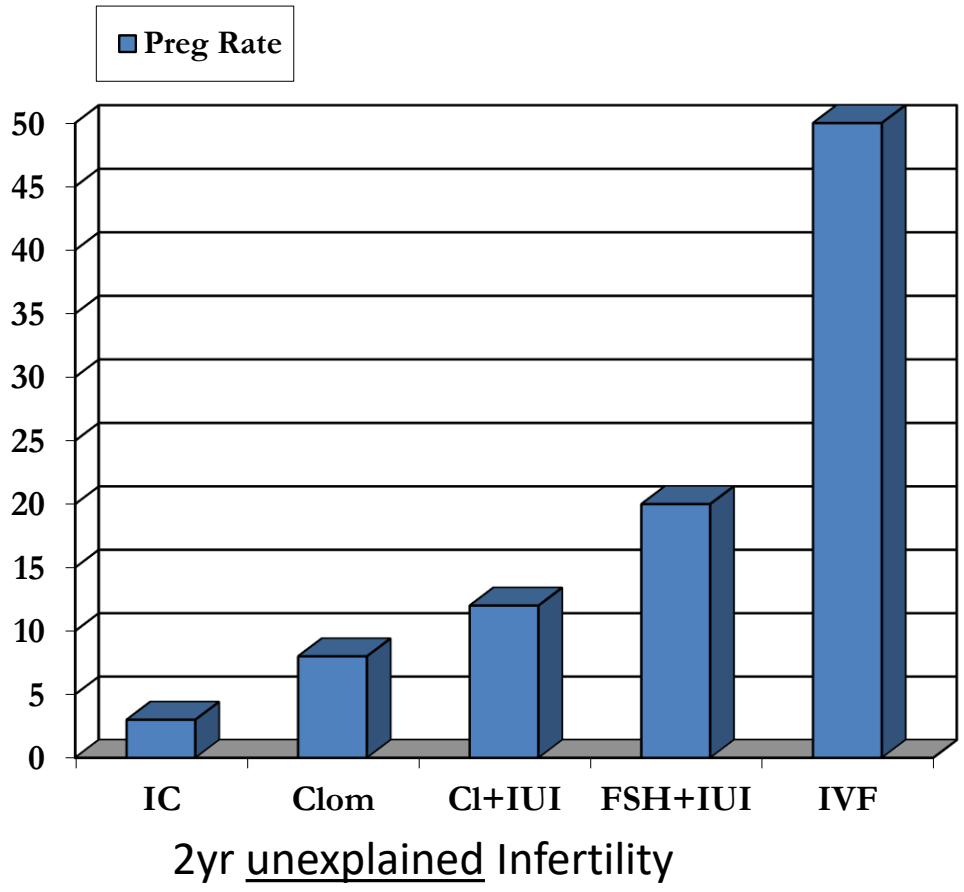
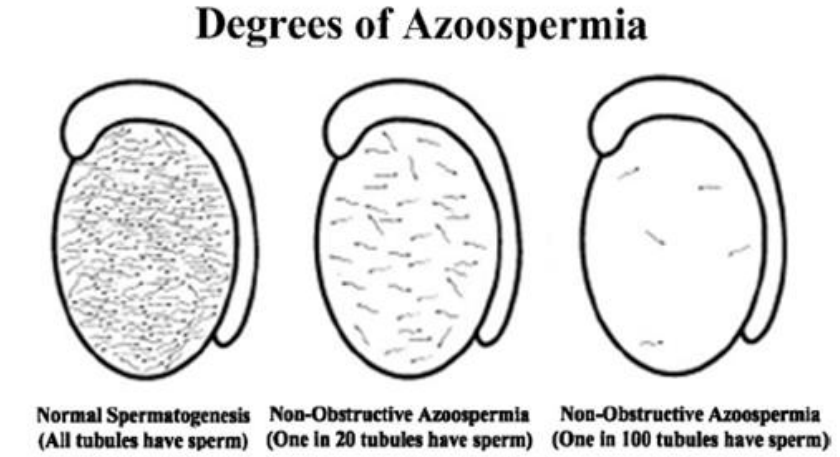
IVF and ICSI

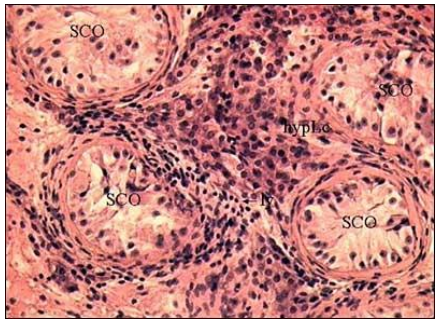


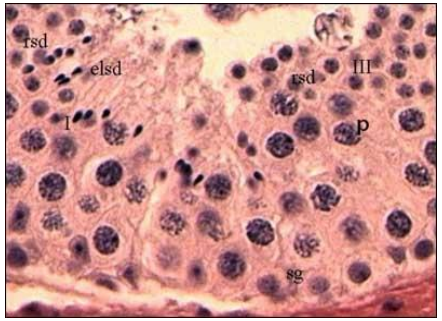
2yr unexplained Infertility

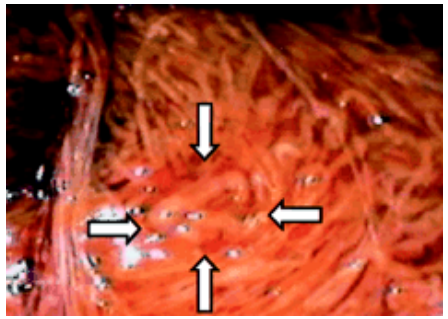
Azoospermia & sperm extraction

- 0.5% of men, 2% of infertile couples, **8%** of the cases of male infertility (BMJ 291:1693; BJU 56:422)
- 40% obstructive & **60% non-obst** (HR 200015:2356); <2% correctable (hypogonadotrophic, ejaculatory duct cyst)
- Sperm retrieval far more likely with open biopsy then needle aspiration (e.g. **43% vs 11%** - Friedler et al 97; **43% vs 7%** Tournaye 99)
- May require >10 and up to 14 biopsies (average 4.5, those with single bx <30% sperm recovery) (Osted et al Urology 1998:52:692-7)
- **Microdissection TESE** less tissue removed (4.7mg vs 56mg) lower bleeding complication (13-30 vs 58%) and higher **sperm recovery rates (47-63%** vs 30-45%) (Amer et al 83, Schlegel & Li 98)



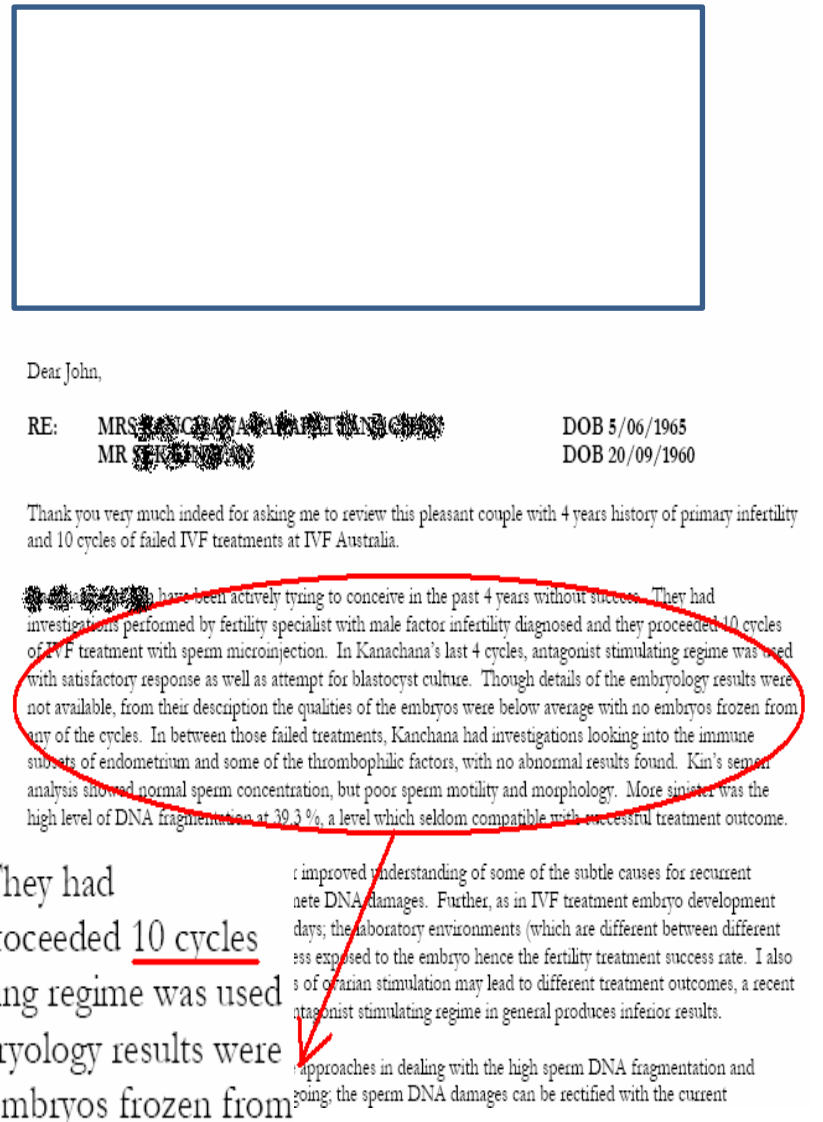






Case: Multiple IVF failures

- F42, M47
- Primary Infertility 4 yrs
- **Male factor**; Female age
- **ICSI** x 10 cycles (Aug 04 – May 07) no preg/mc



Dear John,

RE: MRS [redacted] DOB 5/06/1965
MR [redacted] DOB 20/09/1960

Thank you very much indeed for asking me to review this pleasant couple with 4 years history of primary infertility and 10 cycles of failed IVF treatments at IVF Australia.

[redacted] have been actively tyring to conceive in the past 4 years without success. They had investigations performed by fertility specialist with male factor infertility diagnosed and they proceeded 10 cycles of IVF treatment with sperm microinjection. In Kanachana's last 4 cycles, antagonist stimulating regime was used with satisfactory response as well as attempt for blastocyst culture. Though details of the embryology results were not available, from their description the qualities of the embryos were below average with no embryos frozen from any of the cycles. In between those failed treatments, Kanchana had investigations looking into the immune subsets of endometrium and some of the thrombophilic factors, with no abnormal results found. Kin's semen analysis showed normal sperm concentration, but poor sperm motility and morphology. More sinister was the high level of DNA fragmentation at 39.3 %, a level which seldom compatible with successful treatment outcome.

improved understanding of some of the subtle causes for recurrent
nete DNA damages. Further, as in IVF treatment embryo development
days; the laboratory environments (which are different between different
ess exposed to the embryo hence the fertility treatment success rate. I also
s of ovarian stimulation may lead to different treatment outcomes, a recent
ntagonist stimulating regime in general produces inferior results.

approaches in dealing with the high sperm DNA fragmentation and
going; the sperm DNA damages can be rectified with the current

[redacted] have been actively tyring to conceive in the past 4 years without success. They had investigations performed by fertility specialist with male factor infertility diagnosed and they proceeded 10 cycles of IVF treatment with sperm microinjection. In [redacted]'s last 4 cycles, antagonist stimulating regime was used with satisfactory response as well as attempt for blastocyst culture. Though details of the embryology results were not available, from their description the qualities of the embryos were below average with no embryos frozen from any of the cycles. In between those failed treatments, [redacted] had investigations looking into the immune subsets of endometrium and some of the thrombophilic factors, with no abnormal results found. [redacted]'s semen analysis showed normal sperm concentration, but poor sperm motility and morphology. More sinister was the high level of DNA fragmentation at 39.3 %, a level which seldom compatible with successful treatment outcome.

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Male treatment: IUI, IVF & ICSI

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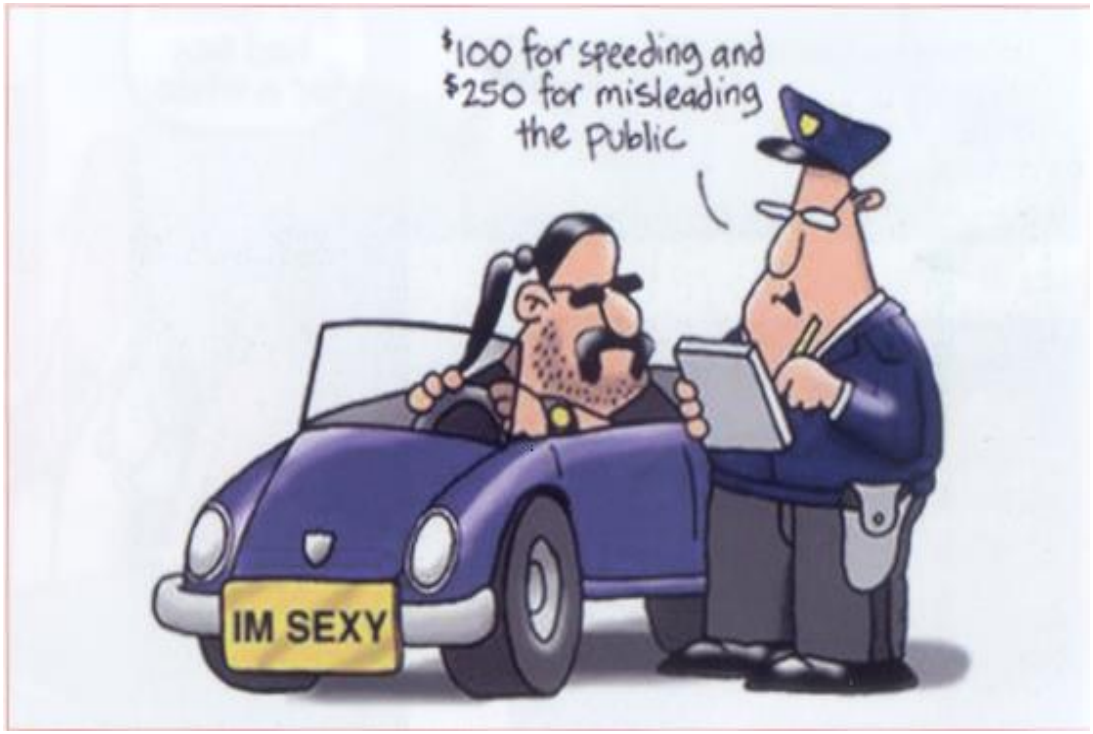
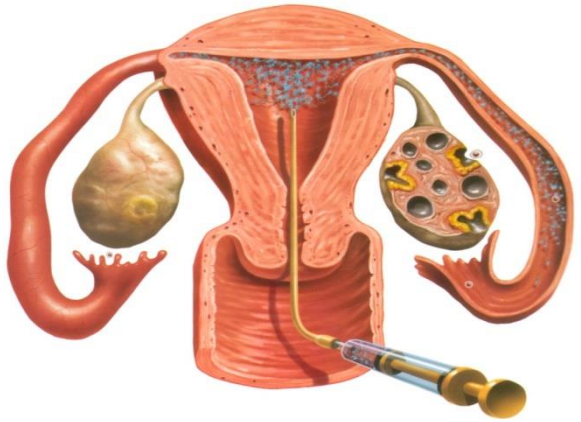
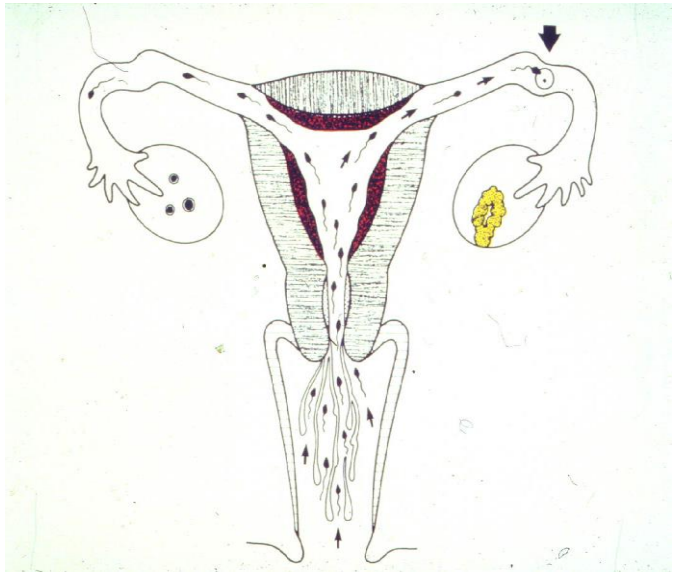
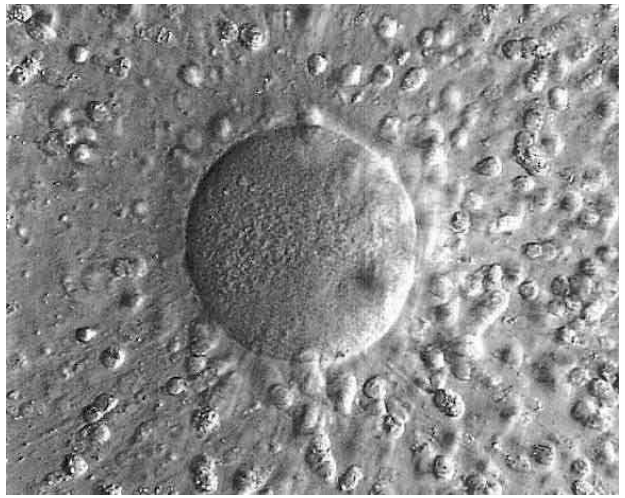
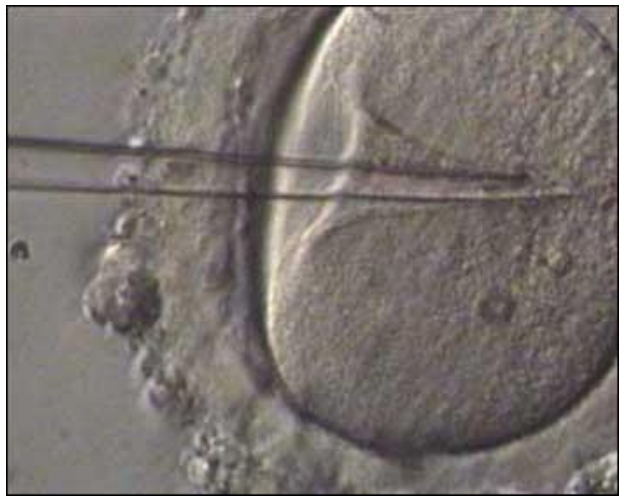
Aim: Restore
low fertilisation



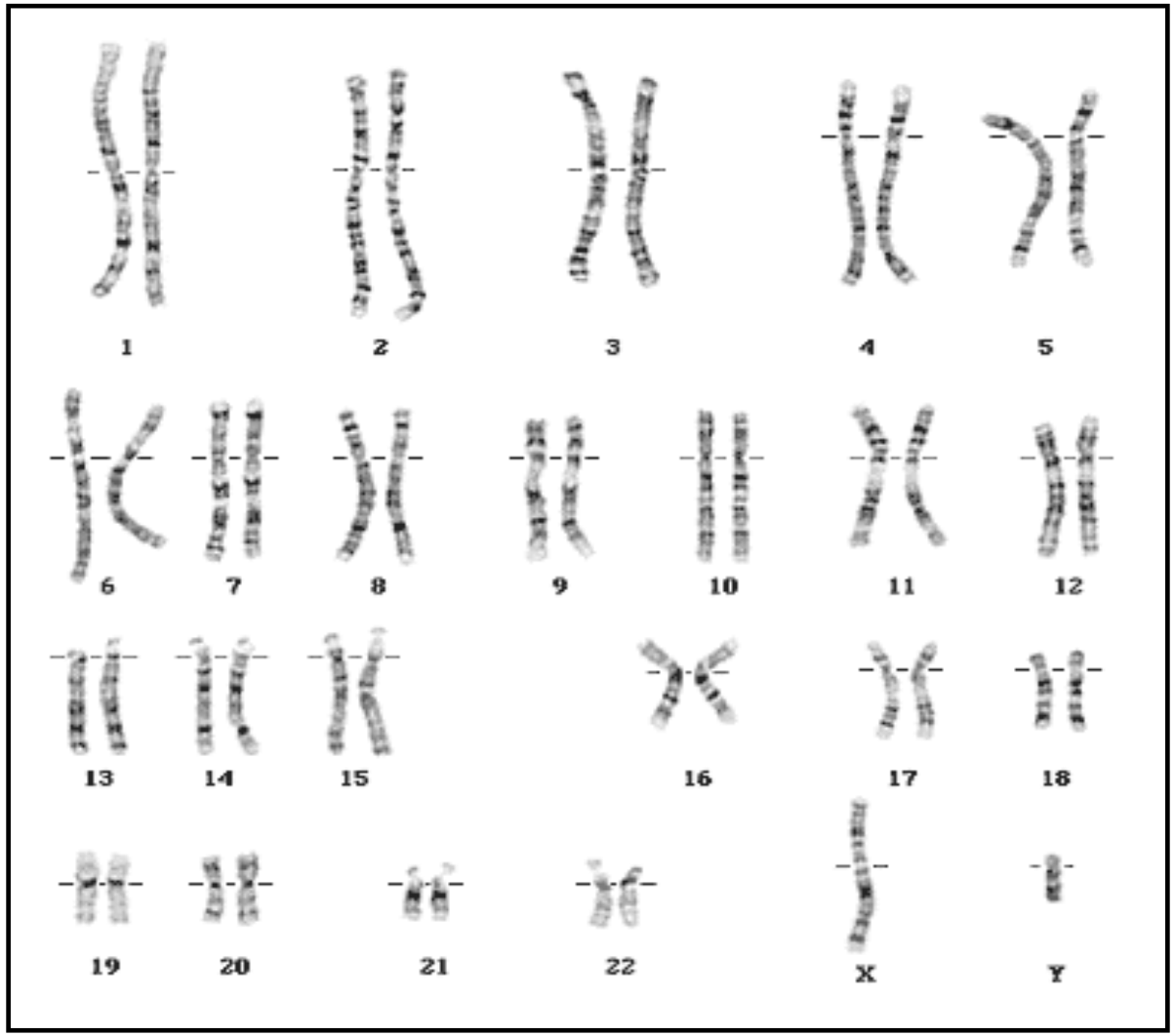








Reproduction – What is in the nutshell?



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Primary testicular disease - genetics

Incidence chromosomal aberrations in infertile compared to the fertile population

Numerical
50 x more 47XXY
4x more 47XYY
60x more 46XX
20x more 46X derY

Structural
8.5 x more Robertsonian translocations
5 x more reciprocal translocations
8 x more inversions
3 x more additional maker chromosomes

Hum Reprod 14 (Suppl 1) 24-27

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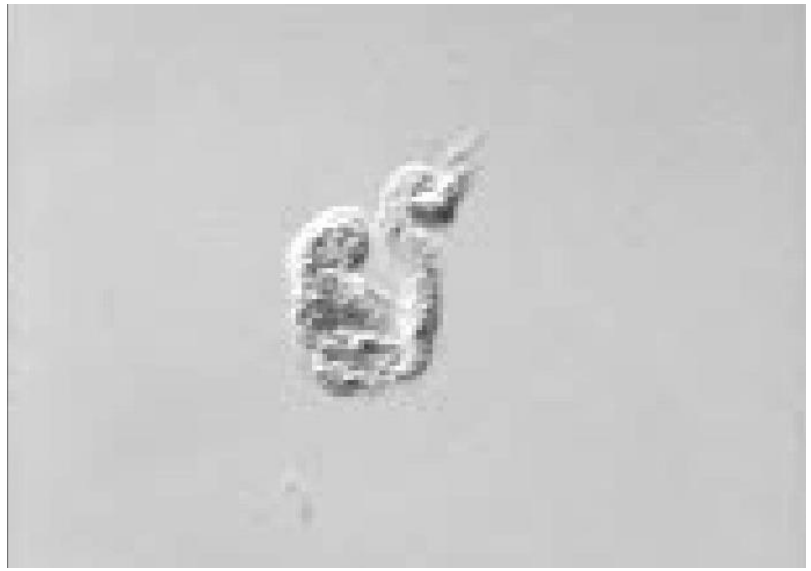
Preimplantation Genetic Diagnosis

Day 5 *(~128 cells)* **biopsy**
2-9 cells from trophectoderm



- Mutation Testing (PCR)
- Translocations
- Aneuploidy Screening
  - Reproductive Failure
  - Elective
- HLA matching

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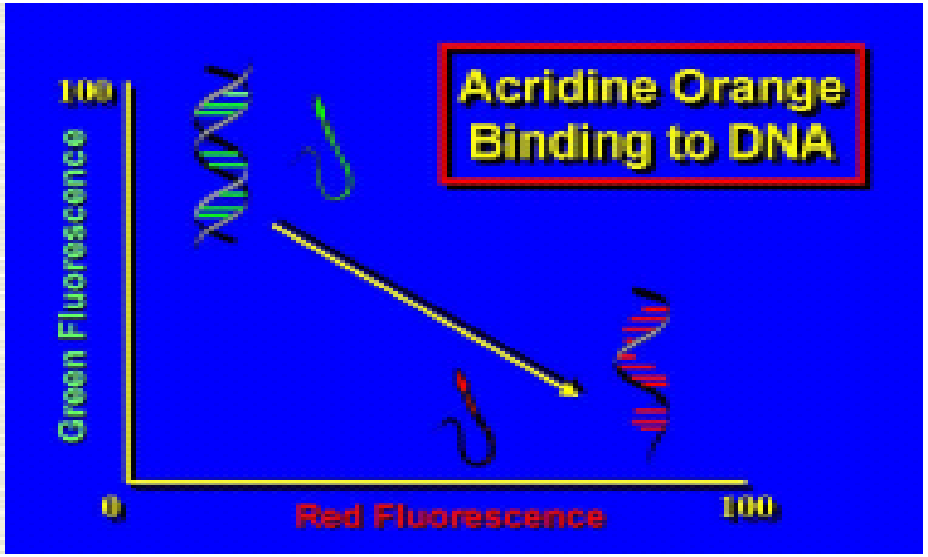
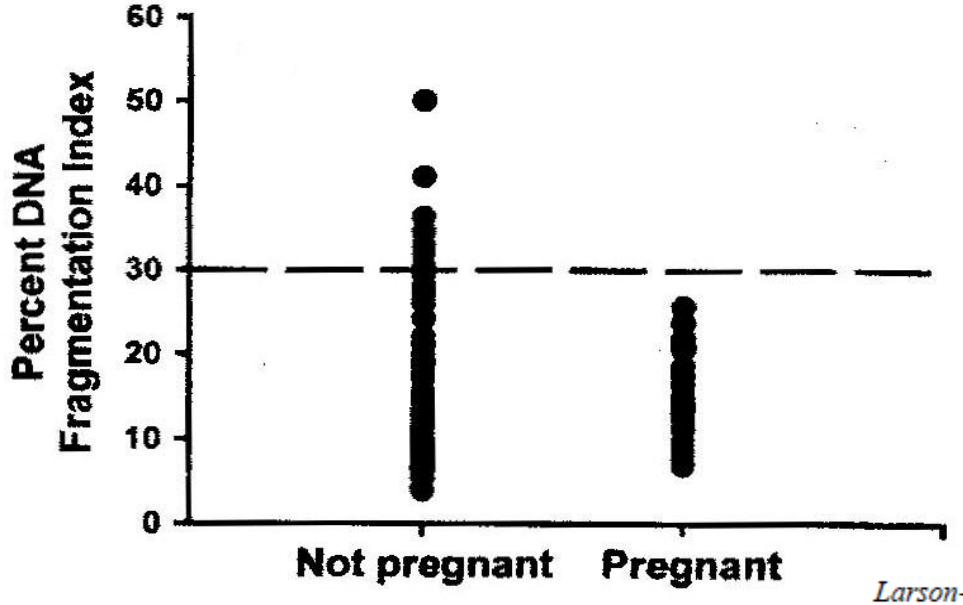
Sperm DNA Integrity Chromatin Structure Assay (SCSA)



- Sperm Chromatin Structural Assay (SCSA)
- Sperm Chromatin Dispersion Assay (SCD)
- Terminal Deoxyuridine Nick End Labelling (TUNEL)
- Comet Assay



- Acridine Orange Assay

Larson-Cook. Assisted reproduction and sperm DNA damage. Fertil Steril 2003.

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Sperm DNA damages – ART outcomes

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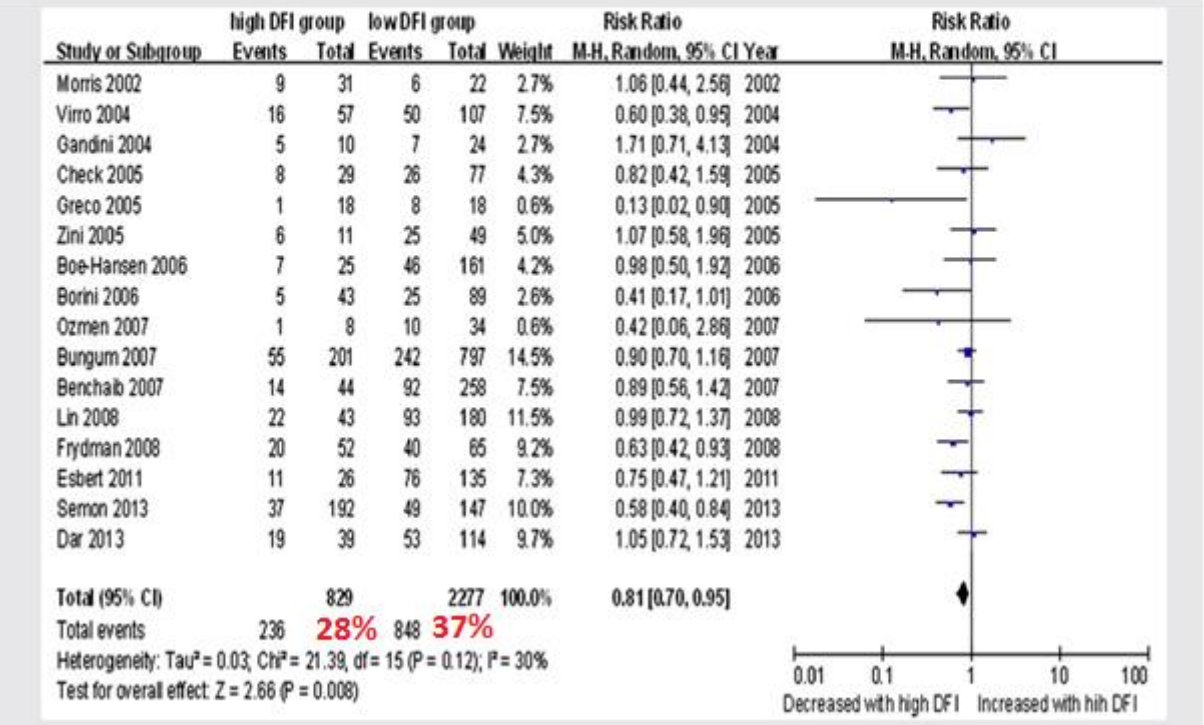
Whether sperm deoxyribonucleic acid fragmentation has an effect on pregnancy and miscarriage after in vitro fertilization/intracytoplasmic sperm injection: a systematic review and meta-analysis

Jing Zhao, M.D., Qiong Zhang, M.D., Yonggang Wang, M.D., and Yanping Li, M.D.



Study or Subgroup	high DFI group Events	Total	low DFI group Events	Total	Weight	Risk Ratio M-H, Random, 95% CI	Year
Morris 2002	9	31	6	22	2.7%	1.06 [0.44, 2.56]	2002
Virro 2004	16	57	50	107	7.5%	0.60 [0.38, 0.95]	2004
Gandini 2004	5	10	7	24	2.7%	1.71 [0.71, 4.13]	2004
Check 2005	8	29	26	77	4.3%	0.82 [0.42, 1.59]	2005
Greco 2005	1	18	8	18	0.6%	0.13 [0.02, 0.90]	2005
Zini 2005	6	11	25	49	5.0%	1.07 [0.58, 1.96]	2005
Boe-Hansen 2006	7	25	46	161	4.2%	0.98 [0.50, 1.92]	2006
Borini 2006	5	43	25	89	2.6%	0.41 [0.17, 1.01]	2006
Ozmen 2007	1	8	10	34	0.6%	0.42 [0.06, 2.86]	2007
Bungum 2007	55	201	242	797	14.5%	0.90 [0.70, 1.16]	2007
Benchaib 2007	14	44	92	258	7.5%	0.89 [0.56, 1.42]	2007
Lin 2008	22	43	93	180	11.5%	0.99 [0.72, 1.37]	2008
Frydman 2008	20	52	40	65	9.2%	0.63 [0.42, 0.93]	2008
Esbert 2011	11	26	76	135	7.3%	0.75 [0.47, 1.21]	2011
Semon 2013	37	192	49	147	10.0%	0.58 [0.40, 0.84]	2013
Dar 2013	19	39	53	114	9.7%	1.05 [0.72, 1.53]	2013
Total (95% CI)		829		2277	100.0%	0.81 [0.70, 0.95]	
Total events	236	28%	848	37%			

Heterogeneity: $Tau^2 = 0.03$; $Chi^2 = 21.39$, df = 15 (P = 0.12); $I^2 = 30\%$

Test for overall effect: Z = 2.66 (P = 0.008)

Risk Ratio M-H, Random, 95% CI

0.01 0.1 1 10 100

Decreased with high DFI Increased with hih DFI

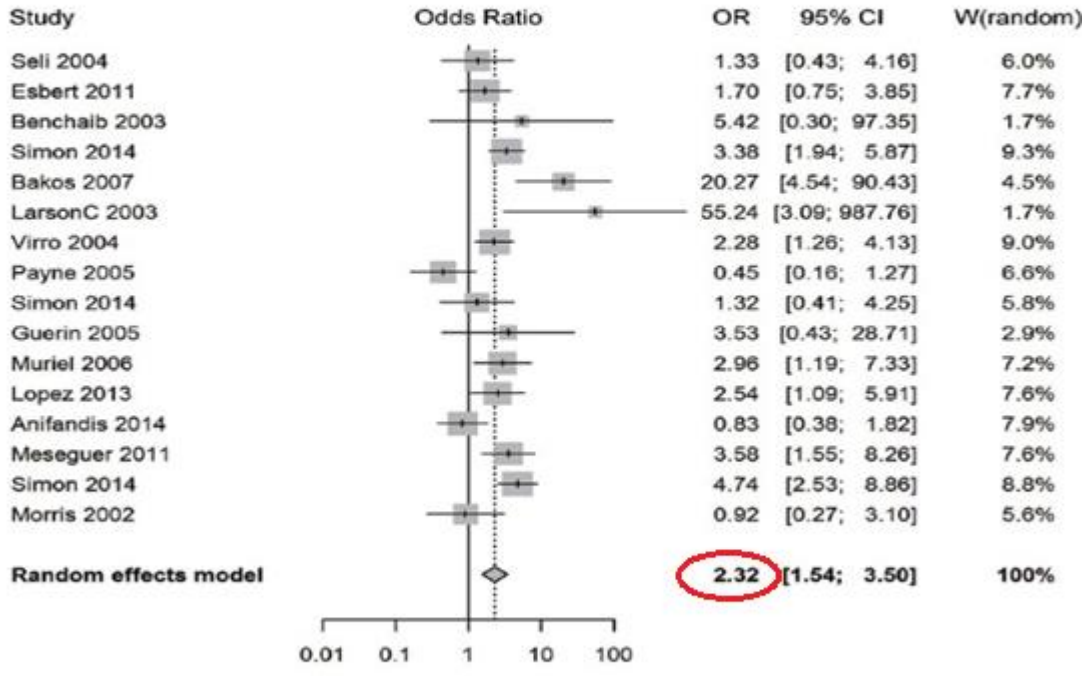
Forest plot showing the results of meta-analysis of studies comparing the effect of high sperm DNA damage and low sperm DNA damage on pregnancy after IVF/ICIS.

Fertility and Sterility® Vol. 102, No. 4, October 2014

Sperm Biology

A systematic review and meta-analysis to determine the effect of sperm DNA damage on *in vitro* fertilization and intracytoplasmic sperm injection outcome

Luke Simon[1,*], Armand Zini[2,*], Alina Dyachenko[2], Antonio Ciampi[2], Douglas T Carrell[1,]



Study	Odds Ratio	OR	95% CI	W(random)
Seli 2004		1.33	[0.43; 4.16]	6.0%
Esbert 2011		1.70	[0.75; 3.85]	7.7%
Benchaib 2003		5.42	[0.30; 97.35]	1.7%
Simon 2014		3.38	[1.94; 5.87]	9.3%
Bakos 2007		20.27	[4.54; 90.43]	4.5%
LarsonC 2003		55.24	[3.09; 987.76]	1.7%
Virro 2004		2.28	[1.26; 4.13]	9.0%
Payne 2005		0.45	[0.16; 1.27]	6.6%
Simon 2014		1.32	[0.41; 4.25]	5.8%
Guerin 2005		3.53	[0.43; 28.71]	2.9%
Muriel 2006		2.96	[1.19; 7.33]	7.2%
Lopez 2013		2.54	[1.09; 5.91]	7.6%
Anifandis 2014		0.83	[0.38; 1.82]	7.9%
Meseguer 2011		3.58	[1.55; 8.26]	7.6%
Simon 2014		4.74	[2.53; 8.86]	8.8%
Morris 2002		0.92	[0.27; 3.10]	5.6%
Random effects model		**2.32**	**[1.54; 3.50]**	**100%**

0.01 0.1 1 10 100

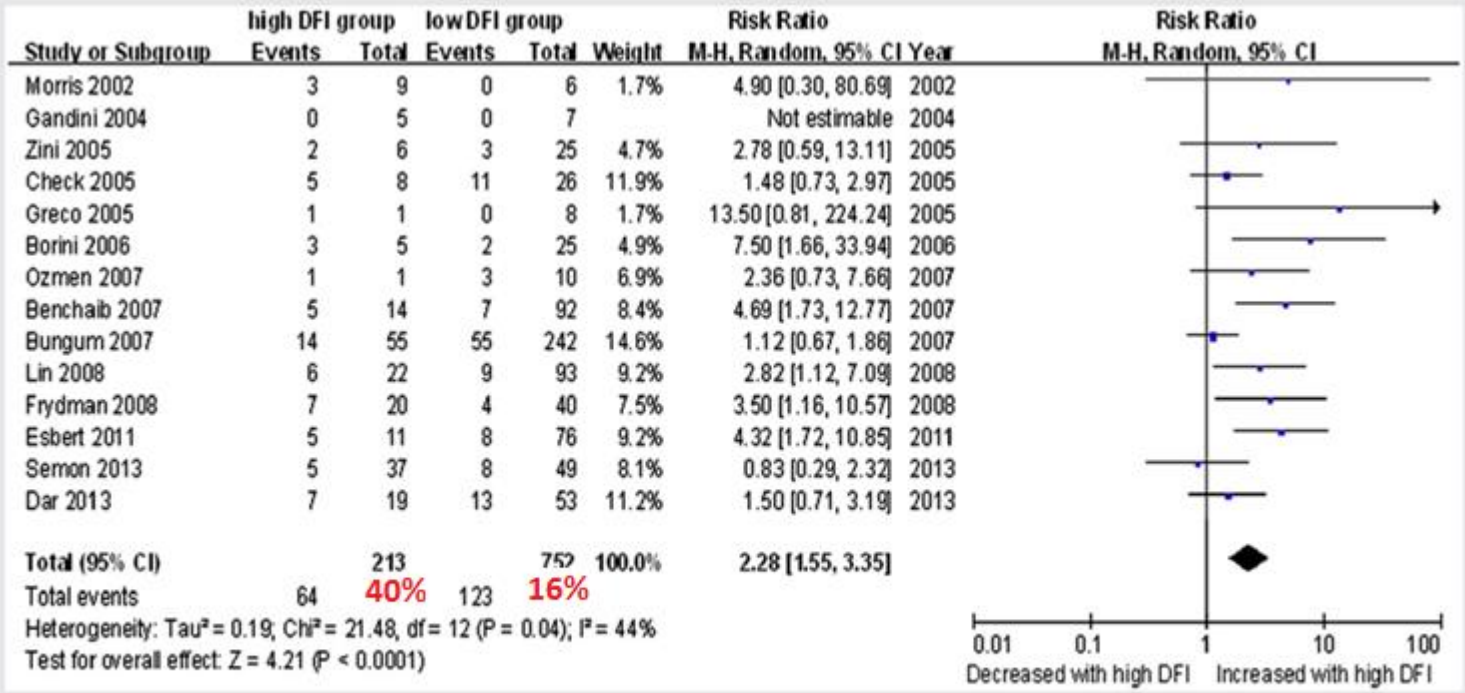
Figure 2: Forest plot of odds ratio to determine the negative effect of sperm DNA damage on clinical pregnancy outcome. (c) following "Mixed" type o

Asian Journal of Andrology (2017) 19, 80–90

Sperm DNA damages – ART miscarriages

Whether sperm deoxyribonucleic acid fragmentation has an effect on pregnancy and miscarriage after in vitro fertilization/intracytoplasmic sperm injection: a systematic review and meta-analysis

Jing Zhao, M.D., Qiong Zhang, M.D., Yonggang Wang, M.D., and Yanping Li, M.D.



Study or Subgroup	high DFI group Events	Total	low DFI group Events	Total	Weight	Risk Ratio M-H, Random, 95% CI	Year
Morris 2002	3	9	0	6	1.7%	4.90 [0.30, 80.69]	2002
Gandini 2004	0	5	0	7		Not estimable	2004
Zini 2005	2	6	3	25	4.7%	2.78 [0.59, 13.11]	2005
Check 2005	5	8	11	26	11.9%	1.48 [0.73, 2.97]	2005
Greco 2005	1	1	0	8	1.7%	13.50 [0.81, 224.24]	2005
Borini 2006	3	5	2	25	4.9%	7.50 [1.66, 33.94]	2006
Ozmen 2007	1	1	3	10	6.9%	2.36 [0.73, 7.66]	2007
Benchaib 2007	5	14	7	92	8.4%	4.69 [1.73, 12.77]	2007
Bungum 2007	14	55	55	242	14.6%	1.12 [0.67, 1.86]	2007
Lin 2008	6	22	9	93	9.2%	2.82 [1.12, 7.09]	2008
Frydman 2008	7	20	4	40	7.5%	3.50 [1.16, 10.57]	2008
Esbert 2011	5	11	8	76	9.2%	4.32 [1.72, 10.85]	2011
Semon 2013	5	37	8	49	8.1%	0.83 [0.29, 2.32]	2013
Dar 2013	7	19	13	53	11.2%	1.50 [0.71, 3.19]	2013
Total (95% CI)		213		752	100.0%	2.28 [1.55, 3.35]	
Total events	64	40%	123	16%			

Heterogeneity: Tau² = 0.19; Chi² = 21.48, df = 12 (P = 0.04); I² = 44%
Test for overall effect: Z = 4.21 (P < 0.0001)

Forest plot showing the results of meta-analysis of studies comparing the effect of high sperm DNA damage and low sperm DNA damage on miscarriage after IVF/ICSI.

Fertility and Sterility® Vol. 102, No. 4, October 2014

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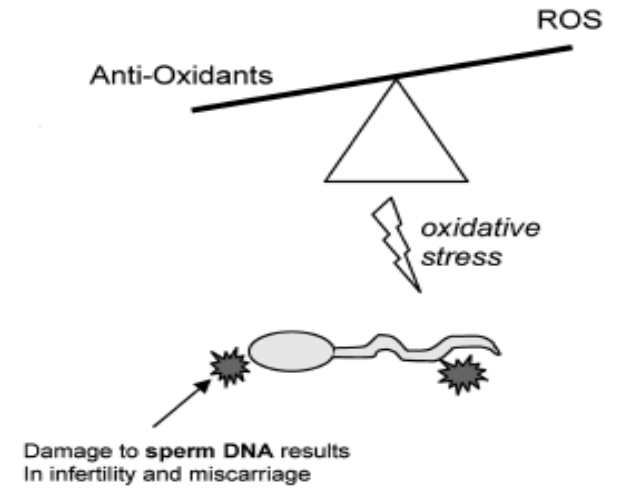
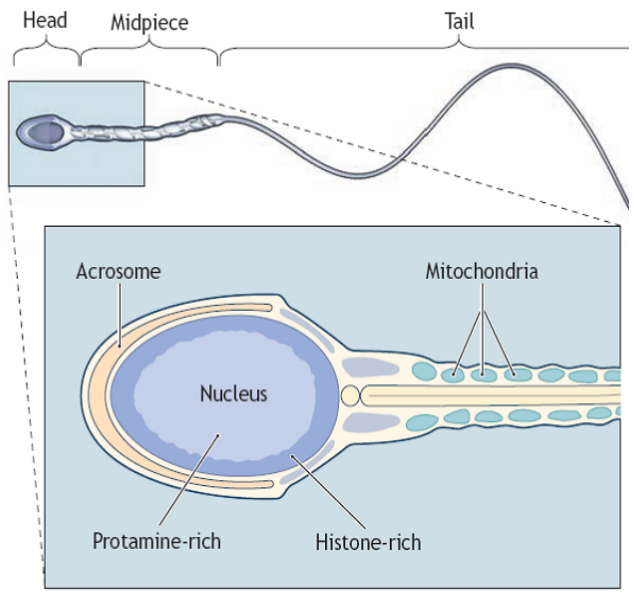
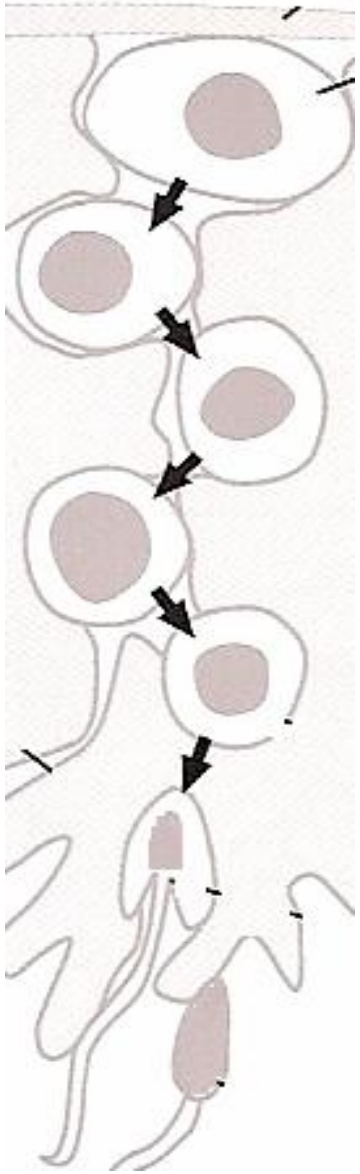
Sperm DNA damages

- **Oxidative damages**
  - Sperm are unable to repair DNA damage induced by oxidative stress, because they lack the required cytoplasmic enzyme systems (lost through sperm maturation) to perform the repair.
  - High reactive oxygen species (free radicals) can be found in fried foods, alcohol, tobacco smoke, pesticides and air pollutants, obesity/inflammation.
  - Sperm damage has been identified in 30-80% of infertile men[2,3,4]
- **Susceptibility**
  - Sperm defects – loss of tight packaging of the DNA
- **Outcomes – egg qualities**
  - The oocyte/egg is capable of repairing low levels of sperm DNA damage. However, if not repaired sufficiently, damaged genes may be passed on to the offspring or increase risk of miscarriage







Sperm DNA damages

- **Susceptibility**
  - Sperm defects – loss of tight packaging of the DNA
- **Oxidative damages**
  - Excessive ROS
  - Deficient anti-oxidants
- **Poor outcomes**
  - Inadequate oocyte repairing capability

- **Treatment options**
  - **Improving spermatogenesis**
  - **Lower ROS exposures**
  - **Antioxidants**
  - Shorten sperm transit time
  - Obtain sperm before damage – testicular sperm
  - Select undamaged sperm
    - Sperm washing
    - Sperm sorter
    - PICSI
    - High magnification ICSI

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Antioxidants

Australian and New Zealand Journal of Obstetrics and Gynaecology 2007; **47:** 216–221

Original Article

A randomised control trial examining the effect of an antioxidant (Menevit) on pregnancy outcome during IVF-ICSI treatment

Menevit active capsule
Lycopene 6 mg
Vitamin E 400 IU
Vitamin C 100 mg
Zinc 25 mg
Selenium 26 µgm
Folate 0.5 mg
Garlic 1000 mg
Palm oil (vehicle)

H. W. G. BAKER D. EDGAR Letters to the Editor

indicated in the discussion that the reports are inconsistent. It is peculiar that no data are reported on change in semen analysis or TUNEL results for the trial patients. Was there no change?

2008 The Royal Australian and New Zealand College of Obstetricians and Gynaecologists; **48:** 124–127

ICSI in cases of sperm DNA damage: beneficial effect of oral antioxidant treatment

Human Reproduction Vol.20, No.9 pp. 2590–2594, 2005

Ermanno Greco[1], Stefania Romano[1], Marcello Iacobelli[1], Susanna Ferrero[1], Elena Baroni[1], Maria Giulia Minasi[1], Filippo Ubaldi[1], Laura Rienzi[1] and Jan Tesarik[2,3]

[1]Centre for Reproductive Medicine, European Hospital, Via Portuense 700, 00149 Rome, Italy and [2]MAR&Gen, Molecular Assisted Reproduction and Genetics, Gracia 36, 18002 Granada, Spain

[3]To whom correspondence should be addressed. E-mail: cmendoza@ugr.es

BACKGROUND: Most studies examining the use of ICSI for cases of elevated sperm DNA fragmentation report poor pregnancy and implantation rates. ICSI with testicular sperm samples has recently been suggested for these cases. Here we test a less invasive approach based on oral antioxidant treatment prior to ICSI with ejaculated spermatozoa. METHODS: Thirty-eight men with an elevated (≥15%) percentage of DNA-fragmented spermatozoa in the ejaculate were treated with antioxidants (1 g vitamin C and 1 g vitamin E daily) for 2 months after one failed

men with an elevated (≥15%) percentage of DNA-fr

ted spe

antioxidants (1 g vitamin C and 1 g vitamin E daily) fo

RESUL

ICSI a

pregnancy (48.2% versus 6.9%) and implantation (19.6% versus 2.2%) rates was observed after the antioxidant treatment as compared with the pretreatment ICSI outcomes. CONCLUSIONS: Oral antioxidant treatment appears to improve ICSI outcomes in those patiens with sperm DNA damage, in whom this treatment reduces the percentage of damaged spermatozoa.

Table II. Comparison of basic sperm parameters and the incidence of DNA fragmentation in the antioxidant-responsive group before and after the treatment period[a]

Time of analysis	Sperm concentration ($\times 10^6$/ml)	Sperm motility (%)	Normal sperm forms (%)	TUNEL-positive spermatozoa (%)
Before treatment	17.9 ± 16.3	40.6 ± 24.8	10.5 ± 8.3	24.0 ± 7.9
After treatment	18.3 ± 17.9[b]	39.9 ± 19.0[b]	9.6 ± .4[b]	8.2 ± 4.3[c]

[a]Data are mean ± SD.
[b]$P > 0.05$.
[c]$P < 0.001$.

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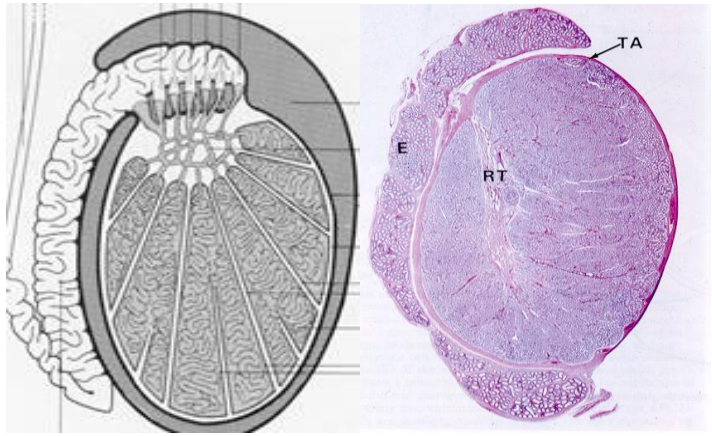
Shorten sperm - transit time



Abnormally high ROS levels during sperm passage through epididymis in between antioxidant protection of Sertoli cytoplasm and seminal plasma
(Evenson FS 2003)

While normal spermatozoa do produce small amounts of superoxide radicals, production is many times more in dead or defective spermatozoa and white blood cells (Ford 1990).

Serial daily ejac x 4 days reduced DFI by 25%
Gosalbez et al FS 11

Reduction occurred in 90% of those affected
Pons et al JARG 13

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Sperm DNA damages

- **Oxidative damages**
  - Excessive ROS
  - Deficient anti-oxidants
- **Susceptibility**
  - Sperm defects – loss of tight packaging of the DNA
- **Poor outcomes**
  - Inadequate oocyte repairing capability

- **Treatment options**
  - Improving spermatogensis
  - Lower ROS exposures
  - Antioxidants
  - **Shorten sperm transit time**
  - **Obtain sperm before damage – testicular sperm**
  - Select undamaged sperm
    - Sperm washing
    - Sperm sorter/microfluidics
    - PICSI
    - High magnification ICSI

Testicular sperm

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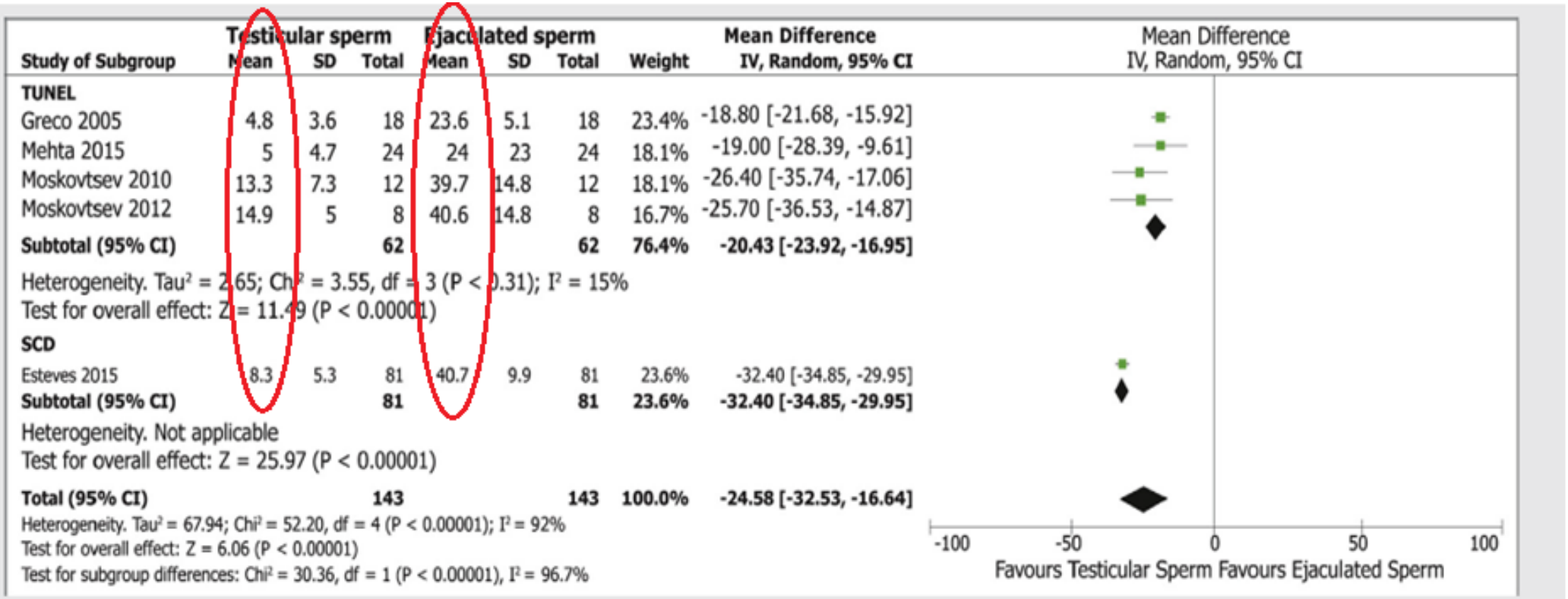
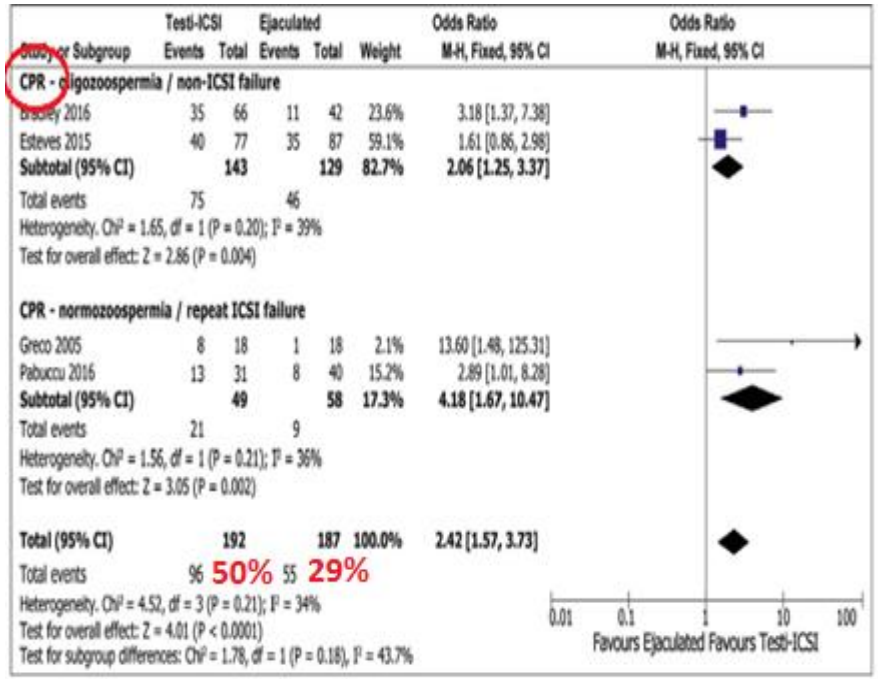


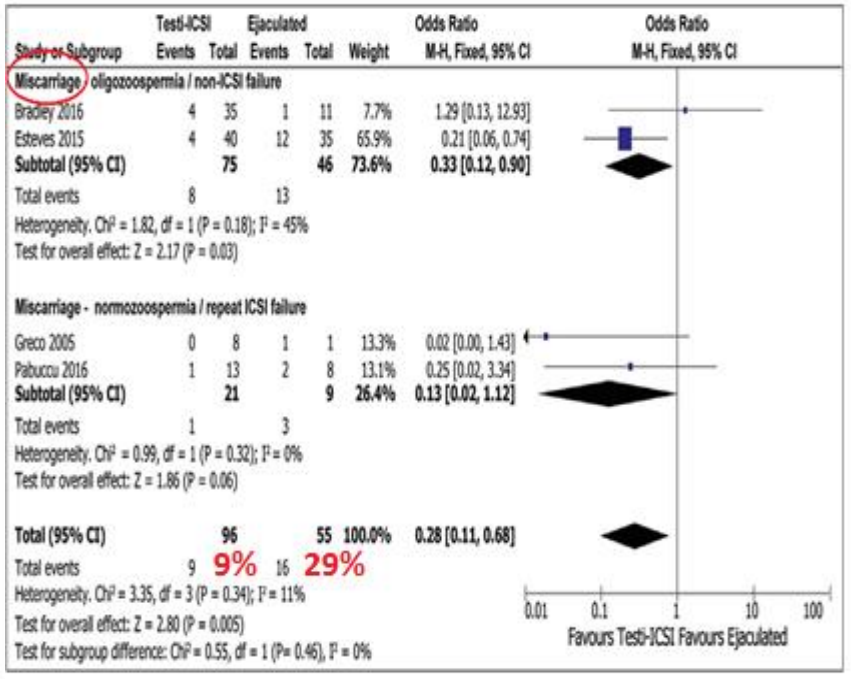
Forest plot showing mean difference for sperm DNA fragmentation (SDF) rates between testicular and ejaculated sperm in men with high SDF, including subgroup analysis according to SDF assay (TUNEL and sperm chromatin dispersion [SCD]). CI = confidence interval; IV = inverse variance.

Esteves. Testicular sperm for ICSI in high-SDF patients. Fertil Steril 2017.

Testicular sperm

Reproductive outcomes of testicular versus ejaculated sperm for intracytoplasmic sperm injection among men with high levels of DNA fragmentation in semen: systematic review and meta-analysis





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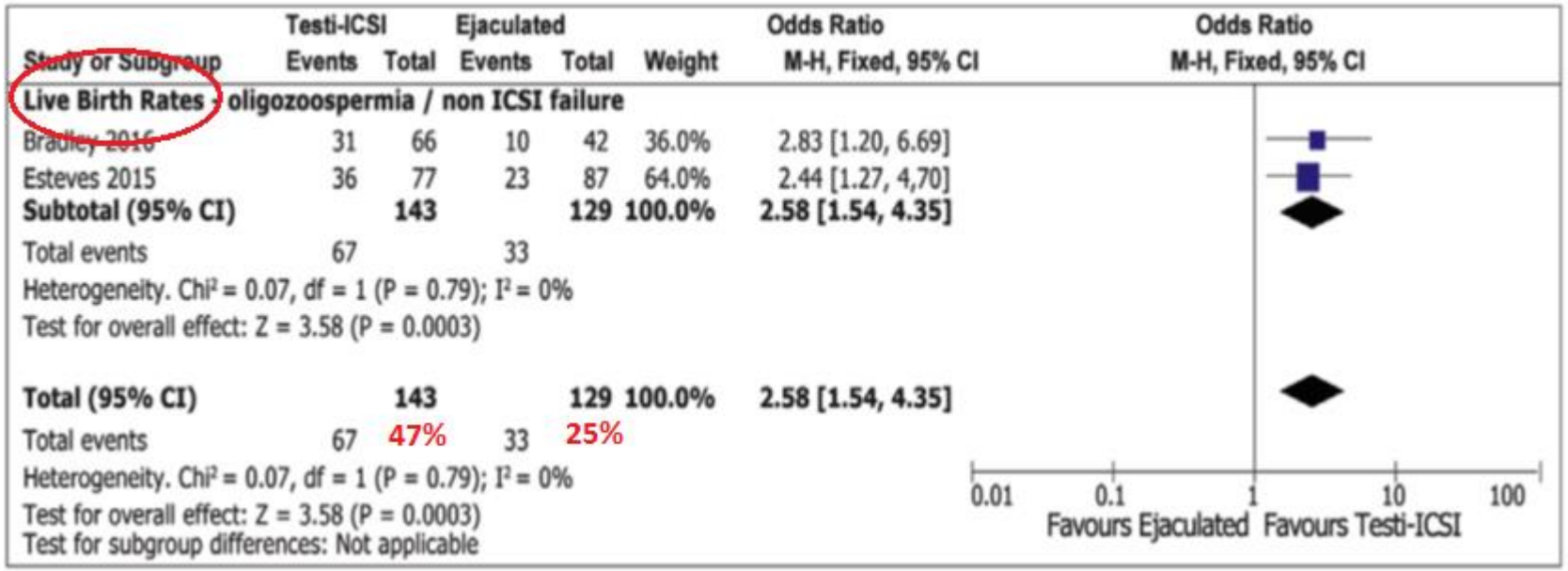

Esteves. Testicular sperm for ICSI in high-SDF patients. Fertil Steril 2017.

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Case: K(42) & SK(47)

- Aug 2014 – May 2017 (**age 38-41**)
  - 10 IVF cycles
  - Embryo qualities poor, no embryo avail for freezing
  - No preg / Mc

- Aug 2017 (age 42)
  - Dx and Correction of high sperm DNA damages (DFI 39.3%)
  - 6 of 6 oocytes fertilised by ICSI
  - 5 blastocysts (grade 1–2):
    - 1 transferred fresh – preg – live birth June 08
    - 2 cryopreserved
    - single FET – live birth 10
    - 1 remains

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Making babies – Male perspectives

Rewarding (Simple investigation with big returns)

- Common
- Highly effective treatments available
- Room for improvement - unlike egg, new sperm are made continuously into old age

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Q & A

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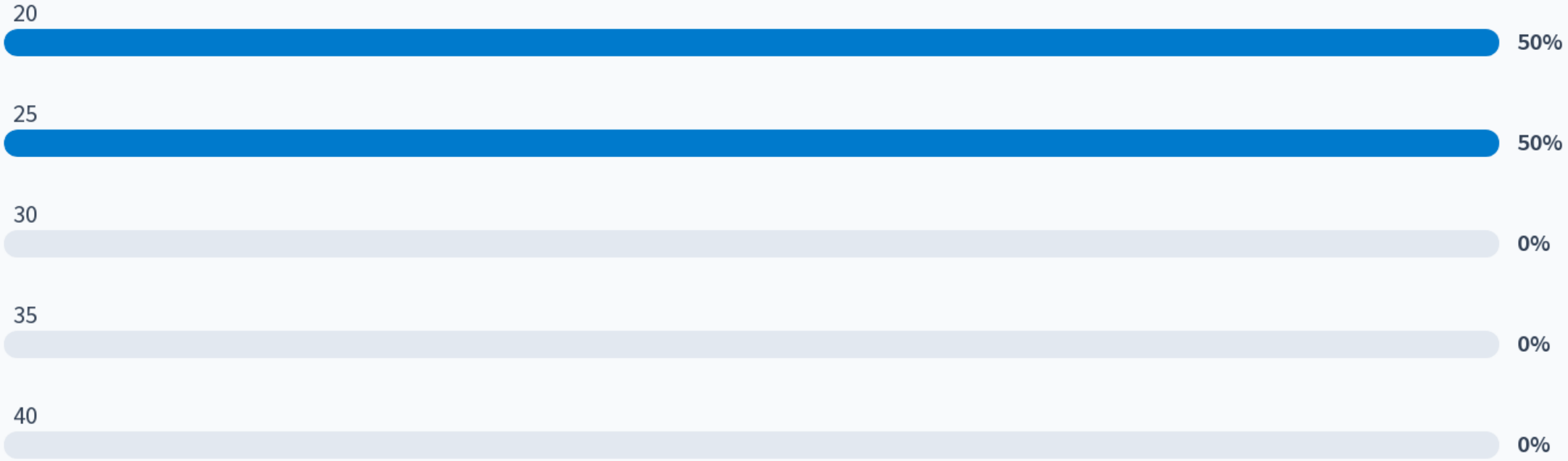
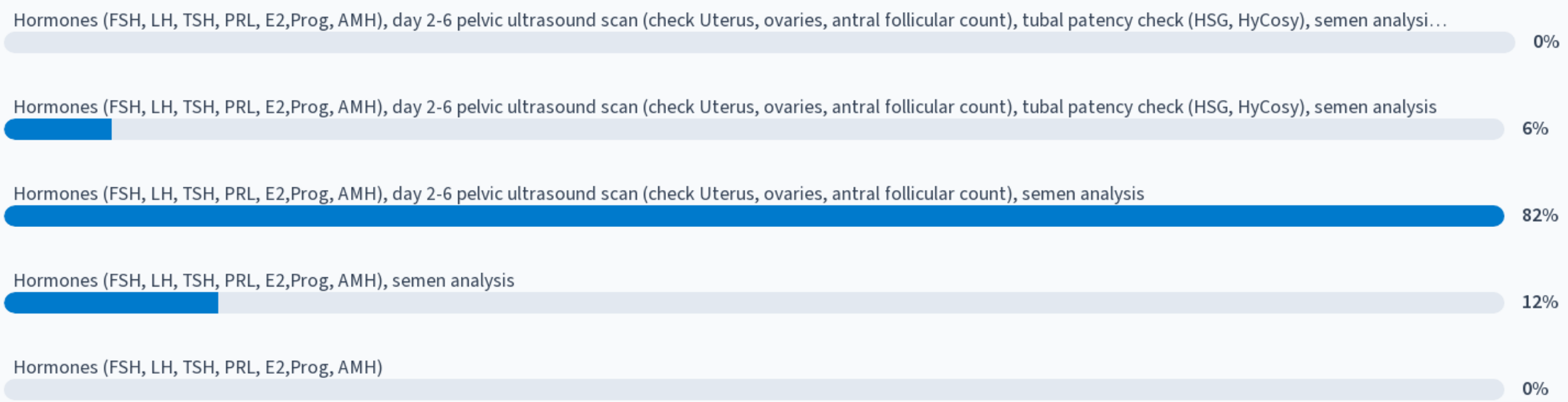
What is most fertile age for women?



Which are first line fertility investigations for couple?

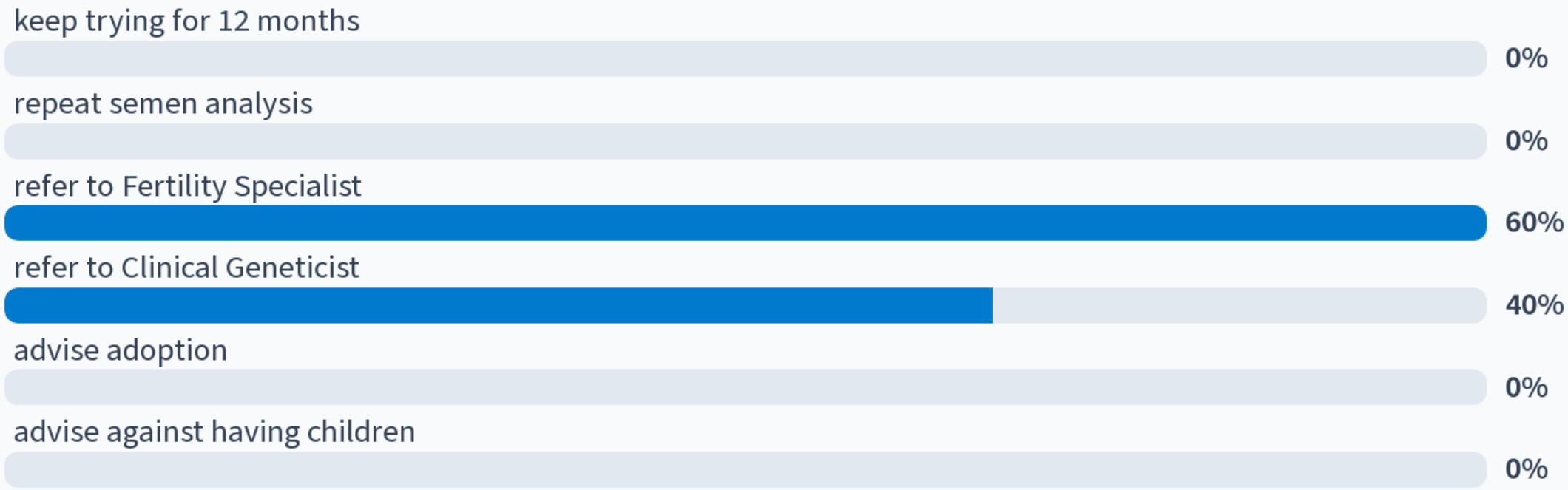


Couple seeks your advice on fertility after 12 months of not using contraception. How long would you advise couple to keep trying naturally at female age 40y + semen analysis (total sperm numbers 30mil , total motility 50%, morph 5%)?

Couple seeks your advice on fertility after 12 months of not using contraception. How long would you advise couple to keep trying naturally at female age 35y (AMH at 25percentile) + semen analysis (total sperm numbers 60mil, forward motility 32%, morph

Trying for pregnancy 1 year. Female age 28y, all investigations are normal. Karyotype 47, XXY, Azospermia, age 28. Your advice to the couple?

Trying for pregnancy 1 year. Female age 25y, all investigations are normal. Karyotype mos 46, X, idic (Y) (q11.21) (47) / 45,X (13), semen analysis (azospermia). Your advice to the couple?



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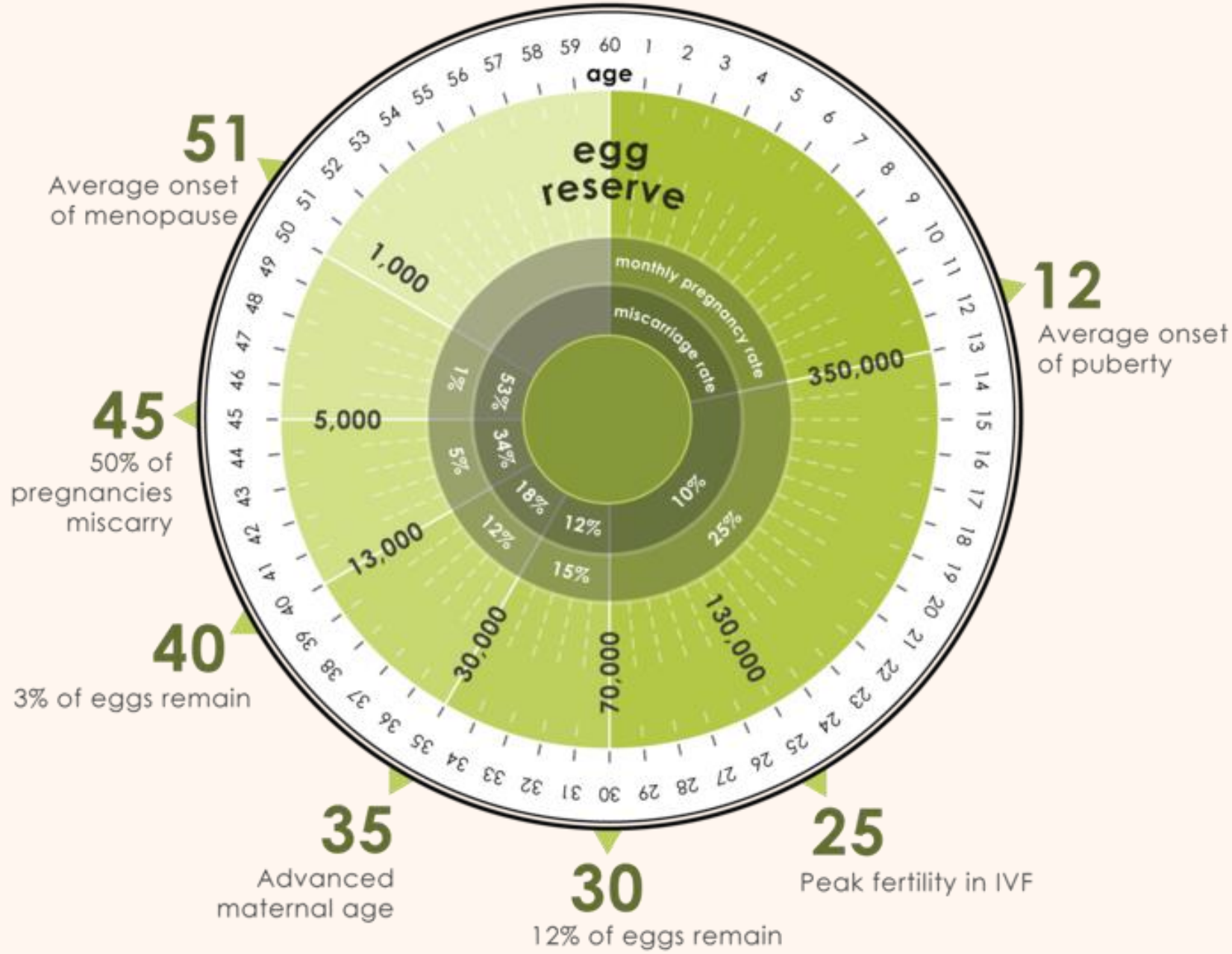

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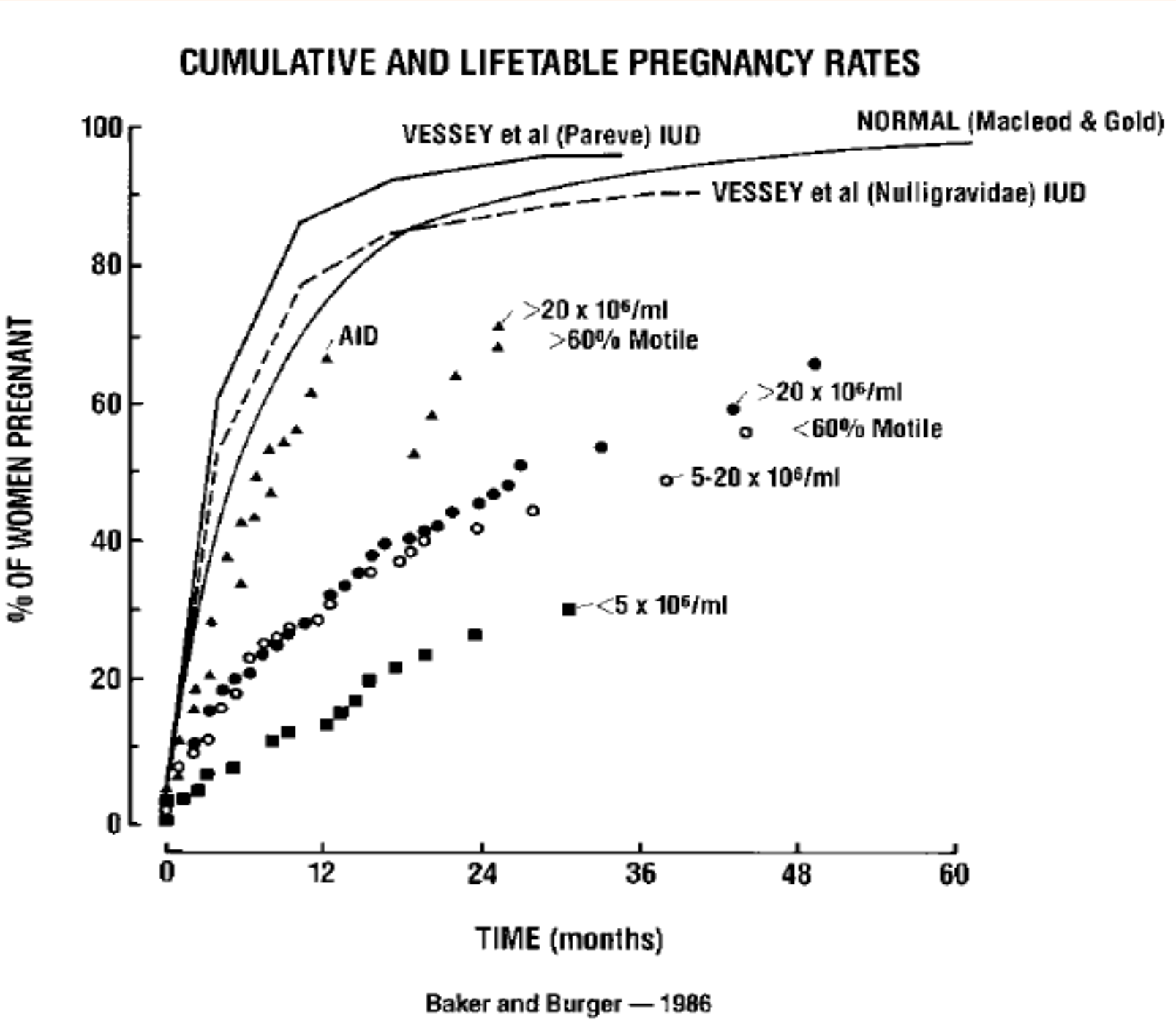
Number of factors	Monthly chance	Mean Years to Pregnancy	% Pregnancy in 2 years
0	20%	0.3 (4m)	93.6
1	5%	2	63.8
2	1%	7	20.7
3	0.2%	40	4.7



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This patient has a mosaic karyotype with a cell line with an isodicentric Y chromosome with two copies of the Y p arm (78%) and a 45,X cell line (12%).

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