Current State of the Art and Evidence in Andrology Testing

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Disclosures and Conflicts of Interest

- **Disclosure:**
  - **On ABU/AUA Written Exam Committee (paid)**
  - **Board member of ASRM**
  - **Chair of AUA Reproduction Urology Care Foundation**
  - **Chair of American Society of Andrology Public Affairs and Health Policy Committee**

- **Conflicts of interest**
  - **No financial involvement with Pharma or otherwise**

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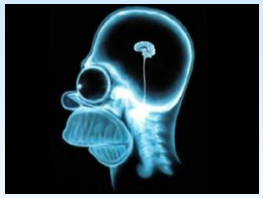


Sperm parameters – WHO 2010 values…

… “It’s just a number… right?”

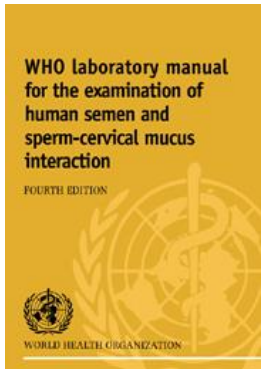
The Issue

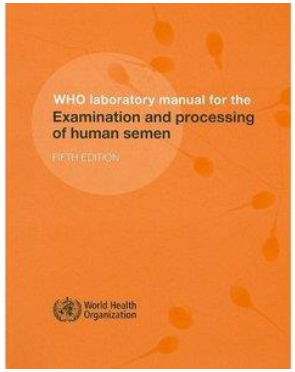
- **There is a complex relationship between semen analysis and pregnancy outcome**
- **Fertility and infertility are NOT defined by the semen analysis reference values alone**
- **BUT: Semen analysis parameters and reference values aim to provide evidence-based thresholds that aid the clinician in calculating the relative fertility of the patient through correlation with outcomes**
- **BUT: There are functional factors that are beyond just numbers**

Where have we been to see where we are going?

- **The World Health Organization (WHO) periodically releases manuals for laboratory examination of human semen:**

- **The first one was published in 1980, with subsequent updates in 1987, 1992, 1999 and now 2010**

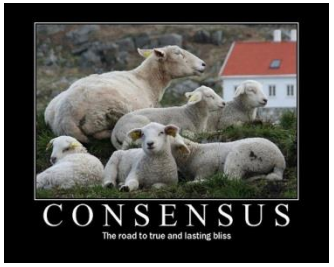




Semen Parameter	WHO 1980	WHO 1987	WHO 1992	WHO 1999	WHO 2010
Volume (cc)	-	≥2	≥2	≥2	**1.5**
Sperm conc (M)	20-200	≥20	≥20	≥20	**15**
Total Motility (%)	≥60	≥50	≥50	≥50	**39**
Morphology	80.5	≥50	≥30	>14*	**>4***
Leucocytes	<4.7	<1.0	<1.0	<1.0	**<1.0**

Before 2010:

- Up until 2010 the criteria were consensus based:
  - based on the clinical experience of investigators who have studied populations of healthy fertile men of unknown TTP
- Previous WHO manuals acknowledge the limitations by stating that each laboratory should determine its own reference values



How the 2010 WHO Reference Values Differ from Previous Versions:

- For the first time, multi-country data from recent fathers with known time-to-pregnancy (TTP)
- Evidence-based
- Standardized methods for semen analysis used according to the WHO manual
- Laboratories that practiced internal and external quality control.

BUT!

- Not an accurate representation of the fertile man across the globe…this was acknowledged by Cooper et al.
  - Only one center from southern hemisphere
  - Nothing from China, India, Africa, Middle East or South America
- Not clear how data was pooled from 5 studies
- Female age and fertility status were not considered
- A single sample semen sample was used to represent each man in the reference studies.
  - WHO recommends two to three samples to establish a baseline

BUT! (continued)

- **Not all studies used TTP as the end point**

- **Not all of the studies on morphology were conducted according to Kruger's strict criteria**

Study	Year [reference]	Country	TTP < 12 months clearly stated	Sperm morphology evaluation criterion	Overlapping authorship or collaboration among authors
Stewart *et al.**	2009 [6]	Australia	Yes	Tygerberg	Yes
Slama *et al.*	2002 [7]	France, Denmark, UK, Finland	Yes	David, Tygerberg	Yes
Swan *et al.**	2003 [8]	USA	No	Tygerberg	Yes
Jensen *et al.*	2001 [9]	France, Denmark, UK, Finland	Yes	David	Yes
Haugen *et al.**	2006 [10]	Norway	Yes	Tygerberg	No
Auger *et al.**	2001 [11]	France, Denmark, UK, Finland	No	modified David	Yes
Jørgensen *et al.*	2001 [12]	France, Denmark, UK, Finland	No	David	Yes
Bonde *et al.*	1998 [13]	Denmark	Yes	David	Yes

TTP = Time to pregnancy
UK = United Kingdom
*Studies contributing to data on sperm morphology.

Esteves S and Agarwal A. The Open Reproductive Science Journal 2011: 3; 7-15

Percentiles

- **Use of the cut-off of the lowest 5th percentile adequate?**

That is the question !

This is the Holy Grail of Andrology….every sperm is sacred but how many and which ones?

according to the strict (Tygerberg) criterion
†eosin-nigrosin staining

- **A certain number of functioning sperm are needed for normal physiology/fertilization….what is that?**

So how far have we come?

- **Only 5% of laboratories in the United Kingdom were compliant with the techniques set by the WHO guidelines for assessing sperm morphology[1].**

- **Keel et al[2]:**
  - **60% of laboratories indicated the criteria used for sperm morphology**
  - **77% reported sperm count**
  - **59% reported motility according to the WHO guidelines**
  - **35% of laboratories were either not familiar with the WHO manual or did not have a copy of it in their**

1.Riddell D, Pacey A, and Whittington K. Hum Reprod 2005; 12: 3441-5.
2. Keel BA, Stembridge TW, Pineda G, and Serafy NT Sr. Fertil Steril 2002; 78: 603-8.

Where are with WHO 1999 10 year later?

National semen analysis reference range reporting: adherence to the 1999 World Health Organization guidelines 10 years later

Heidi A. Penn, M.D.,[a] *Andrew Windsperger, M.D.,*[a] *Zachary Smith, B.S.,*[a] *Sijo J. Parekattil, M.D.,*[b] *Wayne W. Kuang, M.D.,*[c] *Peter N. Kolettis, M.D.,*[d] *and Ajay K. Nangia, M.B.B.S.*[a]

[a] Department of Urology, University of Kansas Medical Center, Kansas City, Kansas; [b] Department of Urology, University of Florida Gainesville, Gainesville, Florida; [c] Department of Urology, University of New Mexico, Albuquerque, New Mexico; and [d] Department of Urology, University of Alabama, Birmingham, Alabama

- **111 labs, 31 states**

Fertil Steril 2011;95:2320–3.

So how far have we come?

TABLE 2

Percentage of laboratories reporting semen parameters according to the 1999 WHO 4th edition manual reference values: comparison of ART vs. non-ART laboratories.

Parameter	ART	Non-ART	*P* value
No. laboratories	65	46	
Laboratories reporting all parameters recommended	21/65 (32)	5/46 (11)	.008
Laboratories reporting recommended concentration	62/65 (95)	31/46 (67)	.0004
Laboratories reporting recommended motility	50/65 (77)	28/46 (61)	.069
Laboratories reporting recommended morphology	26/65 (40)	5/46 (11)	.001

Note: Values are number (percentage).

Penn. Semen analysis reference range reporting. Fertil Steril 2011.

Male Factor Infertility

- **36% of ART cycles in the US now report male factor as a contributory diagnosis[1]**
- **This is an increase of 46% as compared to 1996[1]**
- **As male factor continues to become a more prevalent diagnosis for infertility, it is increasingly important that men are properly diagnosed**

1. Center for Disease Control. 2006 Assisted Reproductive Technology (ART) Report: Section 5—ART Trends, 1996–2006. www.cdc.gov/ART.

- **As updates to laboratory manuals occur the issue of defining male factor could potentially worsen:**
  - **in terms of national and international agreement by the community to use WHO reference ranges**
  - **criteria to define infertile versus sub-fertile men**
  - **thresholds to define treatment options.**

- **The controversy continues and ultimately large regional studies to define fertile ranges are needed and disseminated to labs.**

Will we see a decrease in the number of referrals for male infertility?

The effect of the new 2010 World Health Organization criteria for semen analyses on male infertility

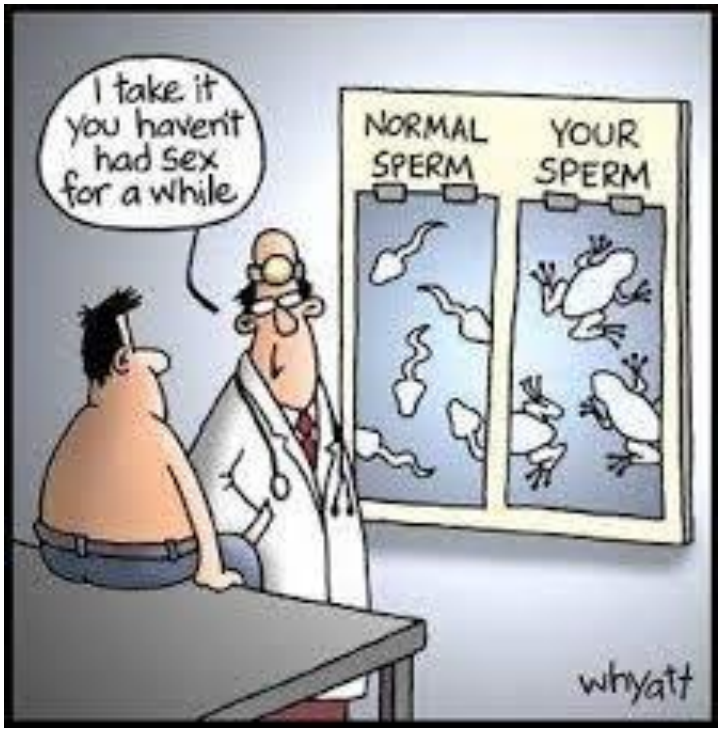
Katie S. Murray, D.O.,[a] Andrew James, M.D.,[a] James B. McGeady, M.D.,[b] Michael L. Reed, Ph.D.,[c] Wayne W. Kuang, M.D.,[b,d] and Ajay K. Nangia, M.B.B.S.[a]

[a] Department of Urology, University of Kansas Medical Center, Kansas City, Kansas; and [b] University of New Mexico, [c] Center of Reproductive Medicine of New Mexico, and [d] Southwest Fertility Center for Men, Albuquerque, New Mexico

Fertil Steril 2012;98:1428–31

- **A total of 184 men had at least two semen analyses**
- **A total of 501 men had one semen analysis**
- **Overall, 103 patients (15.1%) who had one or more parameter below the reference value on the original analysis were converted to having all parameters at or above the 2010 reference values.**

Morphology


I take it you haven't had sex for a while
NORMAL SPERM
YOUR SPERM
whyatt

- **15.7% to 19.3% of men would be reclassified as having normal morphology of greater than 4% from having been abnormal in the past i.e. less than 14%.**
  - **The change in this parameter in determining the use of ART, especially ICSI, is controversial**
  - **Many reproductive endocrinologists already determine the need for ICSI based on 4% normal morphology and not 14%.**

Sperm Morphology-what is the clinical use?

- **Strict morphology originally used to predict fertilization for IVF**
- **Has been extrapolated to be a predictive factor for pregnancy outcome, both naturally and with ART**
- **Many recent studies refute this, especially with isolated teratozoospermia**
- **Should not base treatment solely on strict morphology**

Deveneau NE, Sinno O, Krauseb M, et al Fert Steril 2014

The Concerns:

- **Men may be classified as fertile by many providers especially in idiopathic cases**

- **This will affect reporting data for research or even demographics and outcomes e.g. to CDC/SART.**

- **This may under represent the cause and subsequent work up and treatment of male infertility in a couple e.g. varicocele**

- **Semen analysis alone is not an absolute marker of male infertility.**

- **Timeline of greater than 1 year and overall clinical picture still defines infertility and over-rides any semen analysis - abnormal or normal**

- **Providers should also appreciate that male factor may also still exist even with normal semen parameters, especially if functional sperm abnormalities are present**


Solutions
NEXT EXIT

Possible Solutions

- **Regional definition of normal fertility – would require a large study:**
  - **Stratified by female age range**
  - **Stratified by male age range**
  - **Race/ethnicity**
  - **?BMI**

- **Defining the odds of pregnancy by the percentile range not just lowest 5th percentile**

- **Regional definitions based on accurate data with ART outcomes…..what do we know now?**

Functional Testing:

- **Antisperm Antibodies**
- **Sperm DNA fragmentation**
- **Elevated oxidative stress (e.g. leucocytes)**
- **Viability**
- **Acrosome studies**
- **Genetic testing**

Antisperm Antibody Testing

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Many Clinicians do not Test for ASA-Why?

- Lack of standardized & universally accepted assay
- Unclear that results will change therapy
- No mechanistic explanation of how ASA decrease conception
- No consensus on clinical consequences of ASA

I disagree with that stance….

- **ASA can be a reason for unexplained infertility – male OR female…and is a definable cause**
- **ASA with delayed pregnancy post vas reversal with normal parameters**
- **If an IUI prep does not prep well with or without agglutination – a reason to suspect antibodies.**
- **Unsuccessful IUI or poor fertilization from IVF – may be ASA**
- **…We are looking for answers to explain the problem – immune/ASA is part of that work up.**

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Ohl DA and Naz RK. Urology 1995; 46: 591-602

TABLE I. ***Indications for antisperm antibody testing***

Infertility and at least one of the following
Identifiable risk factors (see text)
Abnormal semen analysis, especially
Clumping/agglutination of sperm
Low motility
Shaking-in-place motility
Poor sperm viability
Abnormal postcoital test, including
Low numbers of sperm in mucus
Poor motility
Shaking-in-place motility
Abnormal in vitro cervical mucus penetration test
Failed or low fertilization during in vitro fertilization
Abnormal sperm penetration assay
Unexplained infertility after male and female evaluation

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Ohl DA and Naz RK. Urology 1995; 46: 591-602

Mechanism of ASA

- **12% of infertile men have ASA – serum, seminal plasma, direct to sperm**

- **Antibodies are primary/“idiopathic” or secondary – due to a known cause: exposure of autoantigens**

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Hendry WF et al; BJU 1977: 44; 757

Known Associations

Obstruction:	Trauma
Vasectomy and reversal	Coital
Idiopathic epididymal obstruction	Torsion
Ejaculatory duct obstruction	Testis Biopsy
CBAVD	Oral, rectal exposure
Inflammation	**Thermal**
Orchitis	Varicocele
STIs	Cryptorchism
Prostatitis	Hot tubs, baths
Cancer	
Genetic	
Thymic maldevelopment	
HLA-B28 haplotype	

The limitations of the test:

?

- What type of antibody matters?
- What test is most “accurate”?
- What degree of binding and to what matters?
- What epitopes matter?
- Why is the test so polyclonal in this day and age with no advancement in the science?

- Valid concerns but I still use the test while thinking about the above issues in unexplained cases or known associations

Comparing ASA Results

"The confusion over the role of ASA in infertility…reflects the inadequacies of the current diagnostic techniques."

Chiu WW-C, Chamley LW. Clinical associations and mechanisms of action of antisperm antibodies. *Fert Steril* 2004; 83:529-535.

1. Different tests give different results for the same specimen
2. Test results sensitive to specific methodology, e.g., sperm preparation effect on surface ASA
3. What is positive? Different cut-off values constituting a positive test
4. Tests are polyclonal: test for ASA in general, but variable effect of each ASA on fertility

Types of ASA Tests

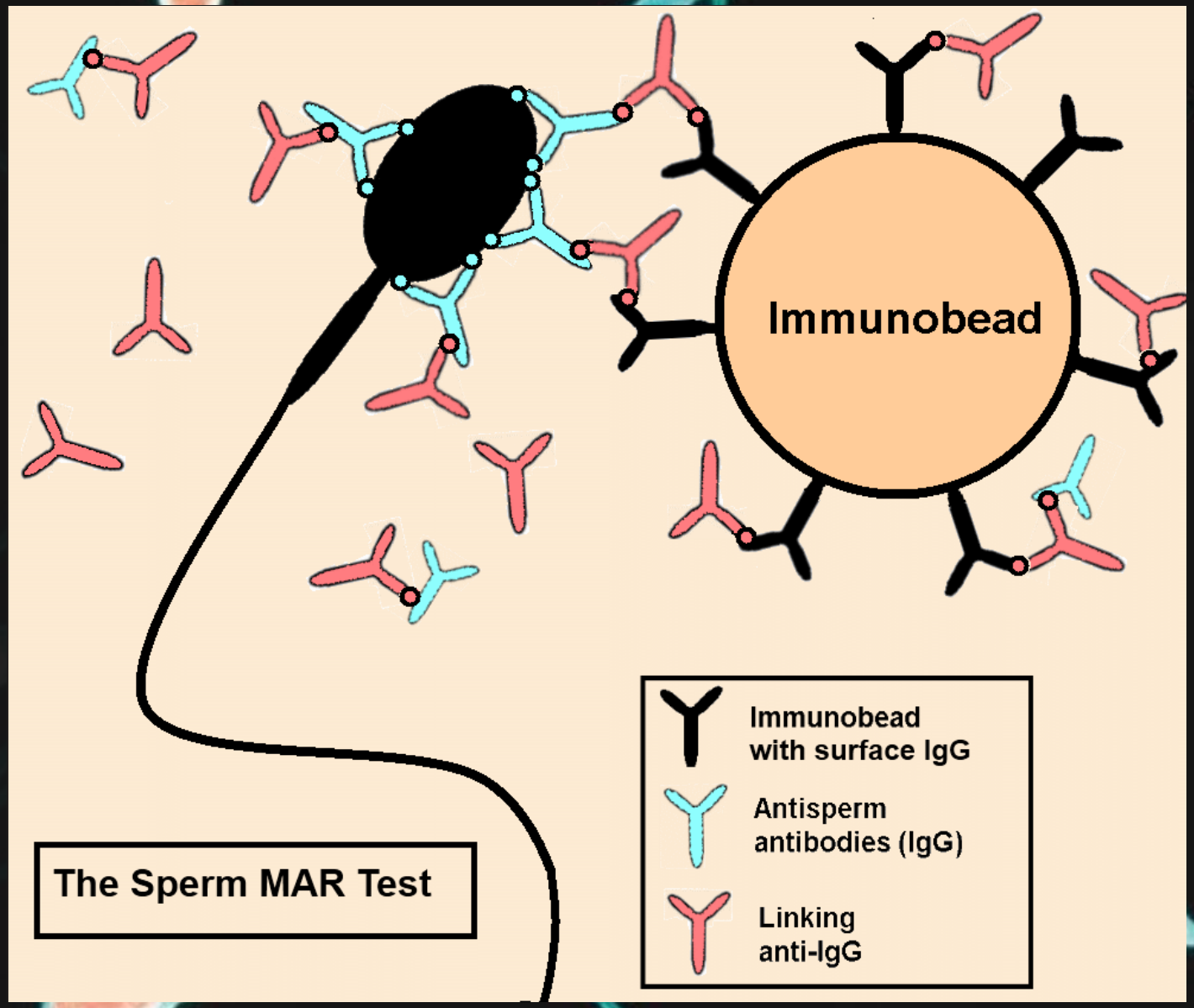
Test	What is tested?	Used clinically?	
Direct Immunobead Test (IBT)	Sperm	Yes	Uses washed sperm
Mixed Agglutination Reaction (MAR)	Sperm	Yes	Sperm in semen
Indirect IBT or MAR test	Fluids	Yes	Donor sperm treated with fluid
Tray Agglutination Test (TAT)	Fluids	No longer	Donor sperm treated Agglutination detected
Sperm Immobilization Test (SIT)	Fluids	No	Only detects ASA that fix complement
ELISA	Fluids	Yes	Sperm Ags on a plate Nonspecific & internal Ags recognized
Flow Cytometry	Sperm	Not yet	Nonmotile and motile sperm used

If unfixed, washed, motile sperm are used, results equivalent, regardless of the probe (immunobead, fluorescence, enzyme)

Haas GG, D'Cruz OJ, DeBault LE. Comparison of the indirect immunobead, radiolabled and immunofluorescence assays for immunoglobulin G serum antibodies to human sperm. *Fert Steril* 1991; 55:377-388.

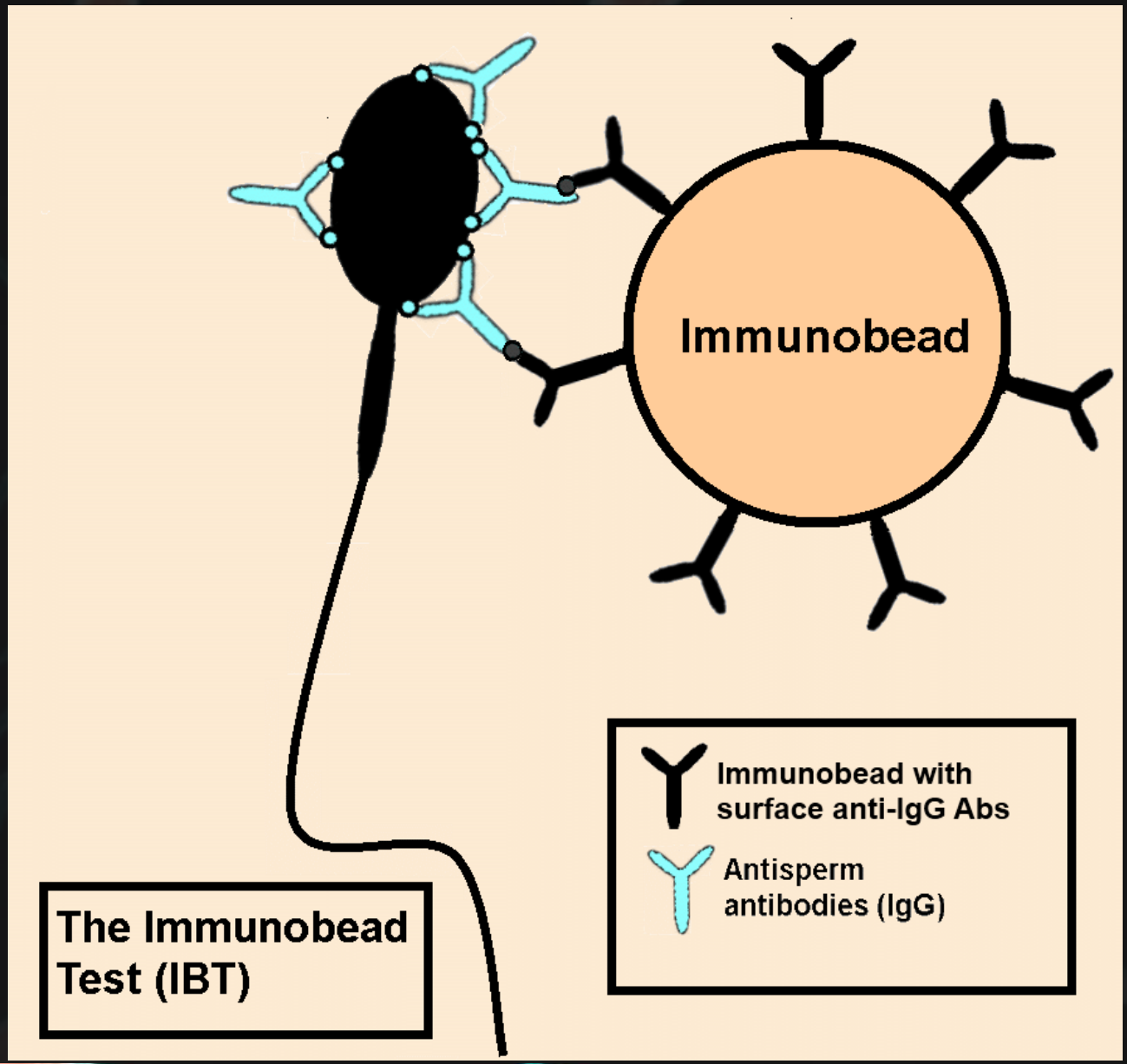
Sperm MAR Test (mixed antiglobulin reaction)

- Immunobeads coated with IgG are added to whole semen
- In the cartoon the sperm is coated with IgG ASA
- Linker anti-IgG antibodies are added & bind to IgG on bead & sperm
- Motile sperm with linked beads are counted



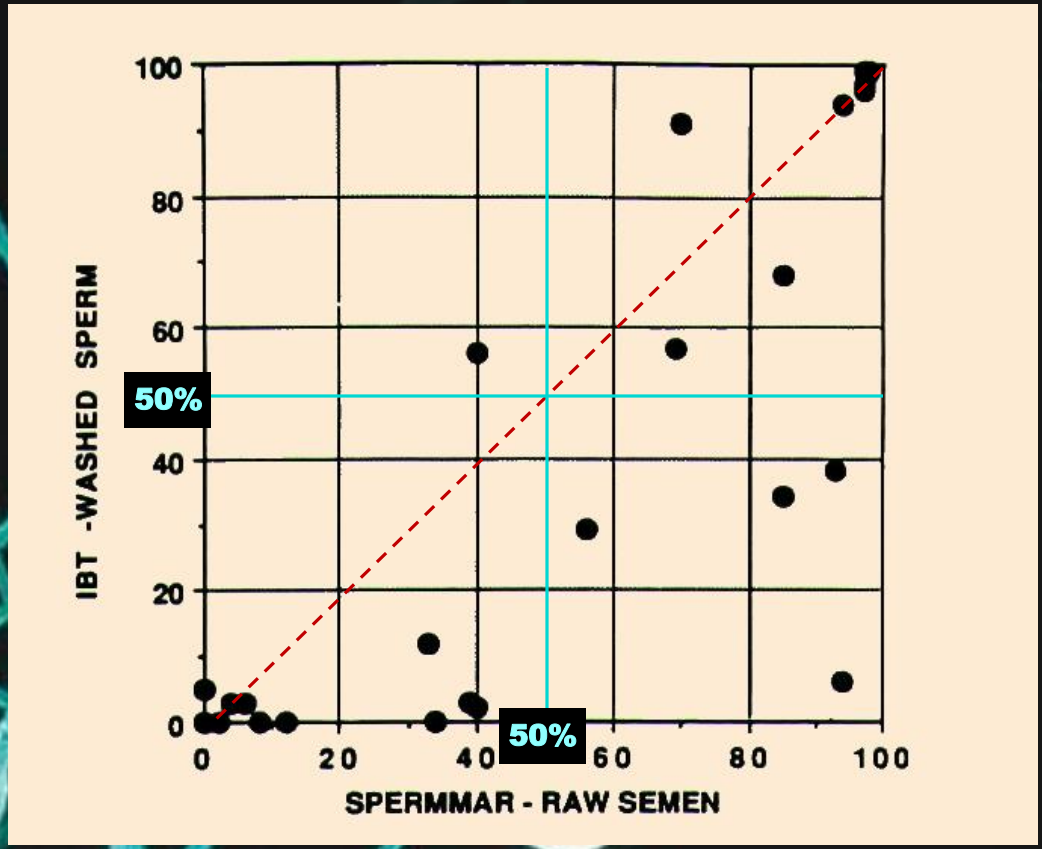
Immunobead Test (IBT)

- Immunobeads with bound anti-IgG antibodies are added to washed sperm
- The beads bind directly to the IgG ASA on the sperm
- Motile sperm with bound beads are scored
- This requires more time for washing the patient & control sperm



IBT versus SpermMAR?

- The two tests agree reasonably well & each is appropriate for routine testing
- Note that the IBT tends to give lower values, likely because some Ag recognized by SpermMAR are adsorbed Ags that are removed by washing



- Adsorbed proteins can be from accessory glands; some are important in sperm transport & capacitation

Figure from: Hellstrom WJG, Samuels SJ, Waits AB, Overstreet JW (1989) A comparison of the usefulness of SpermMar and Immunobead tests for the detection of antisperm antibodies. *Fertility and Sterility*, 52: 1027–1031.

Scoring ASA Tests-Cut off Value

- **The consensus cut-off value for clinical significance is 50% of sperm having ASA**
- **There are few clinical data to support this value, but it is the value recommended by WHO, 1999; 2010**

WHO *Laboratory manual for the examination of human semen and sperm-cervical mucus interaction*, 4th ed. Cambridge, UK: Cambridge University Press, 1999.

WHO laboratory manual for the examination & processing of human semen, 5th ed. Cambridge, UK: Cambridge University Press, 2010.

% Sperm with immunobeads	IUI Pregnancy Rate in 12 cycles
> 50%	15.3%
< 50%	66.7%

Ayvaliotis B, Parslow JM, Hargreave TB, Hendry WF (1985) Conception rates in couples where auto-immunity to sperm is detected. *Fertil Steril* 43:739-742.

- **However, researchers and clinical laboratories use cut-off values from 10% to 50%**
- **Probably not the %sperm with ASA but what epitopes are ASA bound**

Variation in ASA Cutoff Values

	n	Assay	ASA cutoff
de Almeida et al, 1989	15	IBT	**70%**
Rahah et al, 1992	36	IBT & MAR	**20%**
Lahteenmaki, 1993	156	IBT & MAR	**1%**
Acosta et al, 1994	67	IBT	**10%**
Pagidas et al, 1994	435	IBT	**10%**
Sukcharoen & Keith, 1995	167	IBT	**20%**
Vazquez-Levin et al., 1997	18	MAR	**20%**
Vijisic et al, 2005	52	IBT	**20%**
Clarke, 2006	89	IBT	**80%**
Van Weert et al, 2008	473	MAR	**20%**
Nagy et al, 1995	1822	MAR	**80%**
Lahteenmaki et al, 1995	49	MAR	**10%**
Clarke et al, 1997	179	IBT	**80%**
Mercan et al, 1998	207	IBT & MAR	**30%**
Check et al, 2000	93		**50%**
Esteves et al, 2007	351	IBT	**50%**

Data from: Zini A, Fahmy N, Belzile E, Ciampi A, Al-Hathal N, Kotb A. Antisperm antibodies are not associated with pregnancy rates after IVF and ICSI. *Hum Reprod* 2011; 26:1288-1295.

Serum or Seminal ASA most relevant?

Women - serum?

Men – seminal plasma/direct?

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Serum/Semem

- **In women:**
  - **Uterine/serum may be more relevant?**
  - **Local – cervical mucus**

- **In men:**
  - **Less significance for serum – vasectomy/reversal data**

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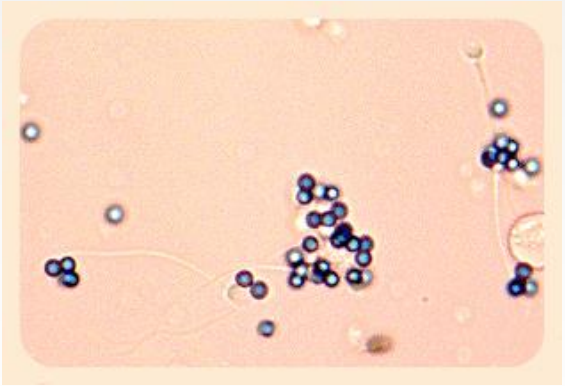
Isotypes of ASA – Relevance in Decision Making?

- **Ig A:**
  - **In seminal plasma and virtually never in serum – produced local and secretory in genital tract**
  - **Generated by local antigen inoculation**
  - **Most clinically relevant**



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Hass GG et al. Fertil Steril 1984; 42: 606-613
Uehling DT. Fertil Steril 1971; 22: 769-773

Isotypes (continued)

- **Ig G: – most common**
  - **Primarily an transudate/exudate from serum**
    - **Only 1% of serum IgG observed in seminal plasma**
    - **Most produced from systemic antigen inoculation**
  - **Produced locally in the male genital tract in situ**

- **IgM:**
  - **Large pentomer – prevents transudation into seminal fluid but have been found**
  - **Role in infertility most likely limited**

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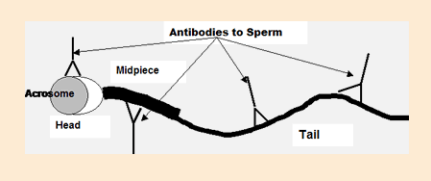
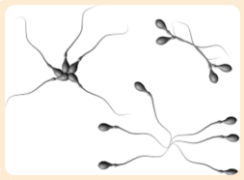
Rumke P. Clin Exp Immunol 1974; 17: 287-297
Bronson RA. J Reprod Immunol 1999; 45: 159-183

Is there any correlation for ASA location?

- ASA on sperm is poorly correlated to ASA in blood serum
- ASAs in cervical secretions are poorly correlated to ASA in blood serum
- Location on the sperm the ASA are located has significance – head binding vs tail
- Variation in results for one patient over time-patients with ASA have “flares” and remissions





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Mandelbaum SL et al. Fertil. Steril 1987;47; 644–651.

Value of Serum Antisperm Antibodies in Diagnosing Obstructive Azoospermia

Richard Lee, Marc Goldstein,* Brant W. Ullery, Joshua Ehrlich, Marc Soares, Renee A. Razzano, Michael P. Herman, Mark A. Callahan, Philip S. Li, Peter N. Schlegel and Steven S. Witkin

From the The Center for Male Reproductive Medicine and Microsurgery, Department of Urology and Cornell Institute for Reproductive Medicine (RL, BWU, JE, MS, MPH, PSL, PNS, MG), Department of Public Health (RAR, MAC), and Department of Obstetrics and Gynecology, Division of Immunology (SSW), Weill Cornell Medical College, and The Population Council, Center for Biomedical Research (PNS, MG), New York, New York

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Dilemmas

- **How long do you pursue natural conception; IUI; and/or conventional IVF in "unexplained" infertility…when it may actually be explained i.e. positive ASA?**

- **Is early use of ICSI the correct treatment for ASA infertility and thereby cost effective in these situations?**

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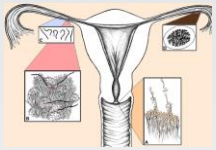
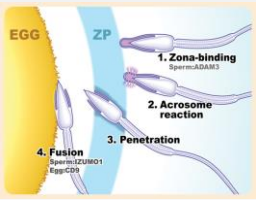
ASA – Functional Evidence

- **Some men with ASA will have normal fertility with intercourse, IUI or IVF BUT overall, ASA inhibits every sperm function:**



  - **Penetration of cervical mucus**
  - **Sperm storage in the oviduct (where they normally reside for up to 3 days awaiting the oocyte)**
  - **Binding to the ZP**
  - **The acrosome reaction (an absolute requirement for fertilization)**
  - **Fusion with the oolemma**
  - **Post-fusion events are less clear (which is why ICSI is very successful).**



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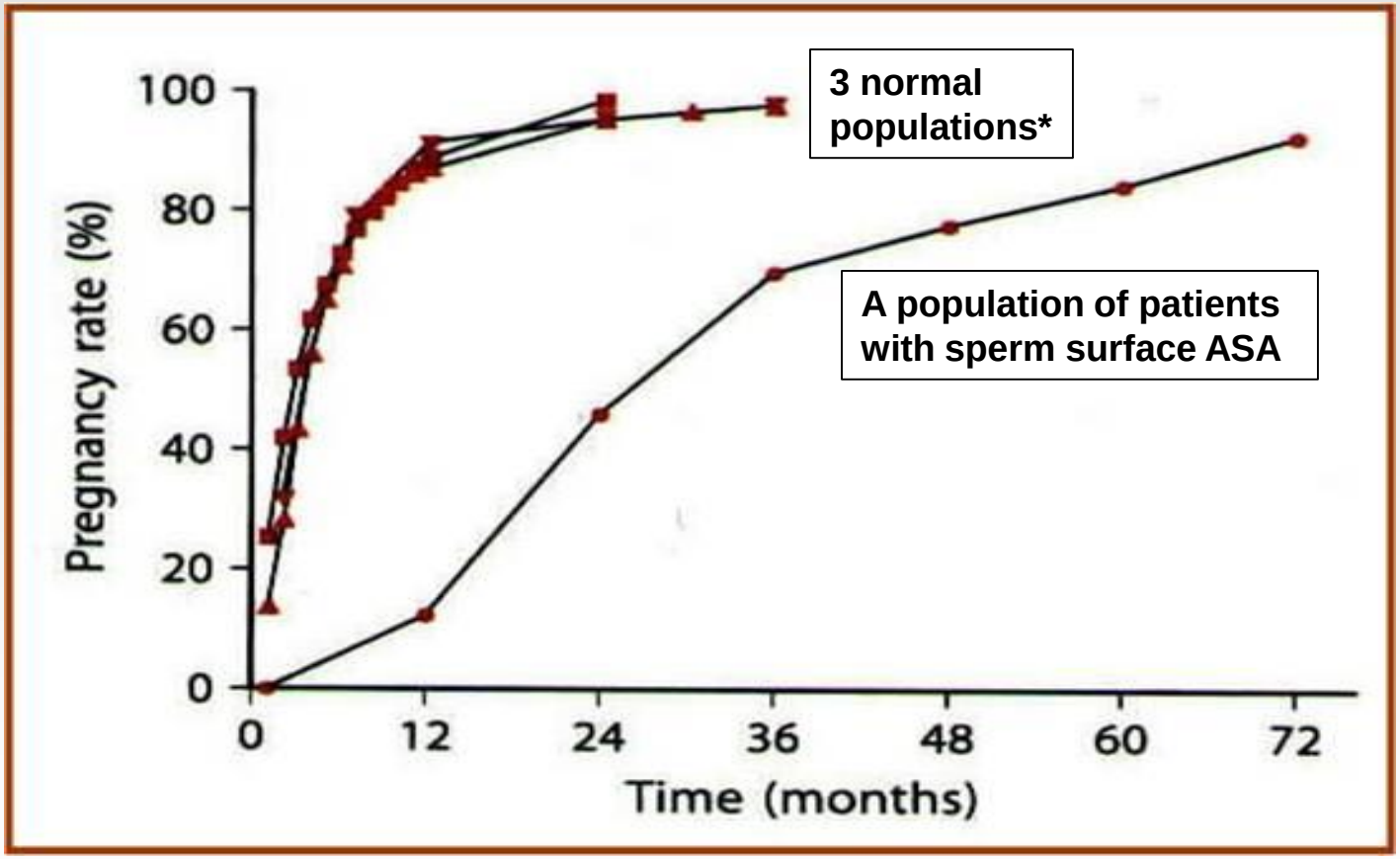
Cumulative Spontaneous Pregnancy Rates



*Normal populations are from 1) Canadian church registries from 17th and 18th centuries, 2) Population in NY in 1950"s, 3) Population in Germany in in the 1990's

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Figure from: Nieschlag E, Behre HM. Andrology 2nd Ed. 2001. Springer New York.
ASA data from: Abshagen K, et al. Fertil Steril 1998; 70:355-356

ASA and IUI Outcomes

- **If cervical ASA – IUI may be possible*.**
- **There are NO CONTROLLED PROSPECTIVE STUDIES OF IUI IN TREATING IMMUNE INFERTILITY**
- **IUI for head binding shown to be worse than tail binding****

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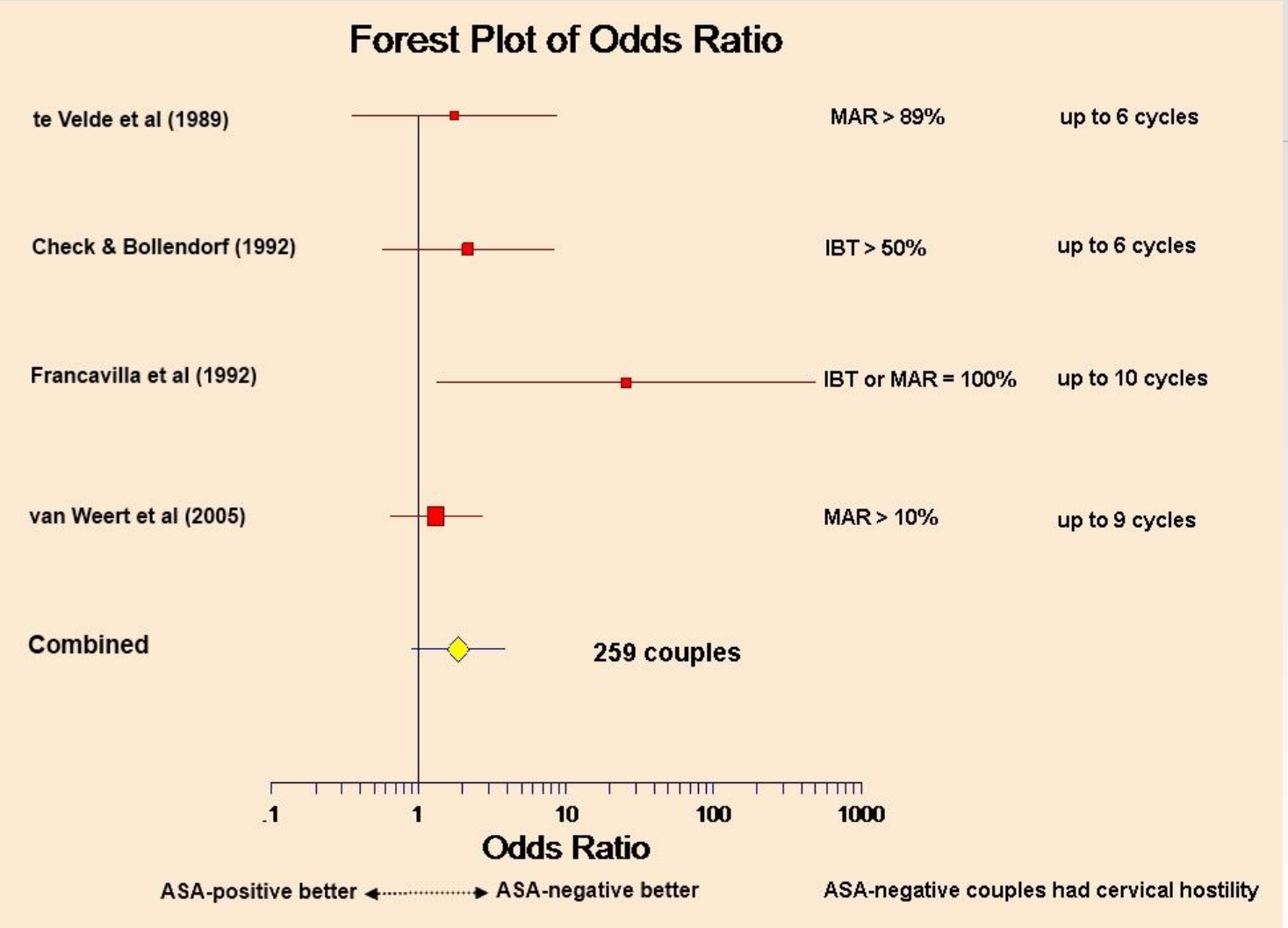
*Bronson RA. J Reprod Immunol 1999; 45: 159-83
**Margalloth EJ et al. Fertil Steril 1988; 50: 441-446



Combined OR= 1.85 (0.88-3.88)
(NS – but a trend to significance)

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Courtesy of Dr Erma Drobnis

EFFECT OF TREATING ANTIBODY-COATED SPERM WITH CHYMOTRYPSIN ON PREGNANCY RATES FOLLOWING IUI AS COMPARED TO OUTCOME OF IVF/ICSI

J. H. CHECK, W. HOURANI, M. L. CHECK, V. GRAZIANO, and E. LEVIN

University of Medicine/Dentistry of New Jersey, Robert Wood Johnson Medical School at Camden, Cooper Hospital/University Medical Center, Department of Obstetrics/Gynecology, Division of Reproductive Endocrinology/Infertility, Camden, New Jersey, USA

Table 1. Comparsion of two treatment modalities for males with 100% of their sperm demonstrating antisperm antibodies

	Chymotrypsin treated group (n = 17)	IVF with ICSI treated group (n = 25)
Number of cycles	47	38
Number of pregnancies	5	11
Pregnancy rate/cycle	10.6%	28.9%
Pregnancy rate/patient	29.4%	44%
Average number of cycles/patient	2.7	1.5
Miscarriages	0 (0%)	1 (9.0%)

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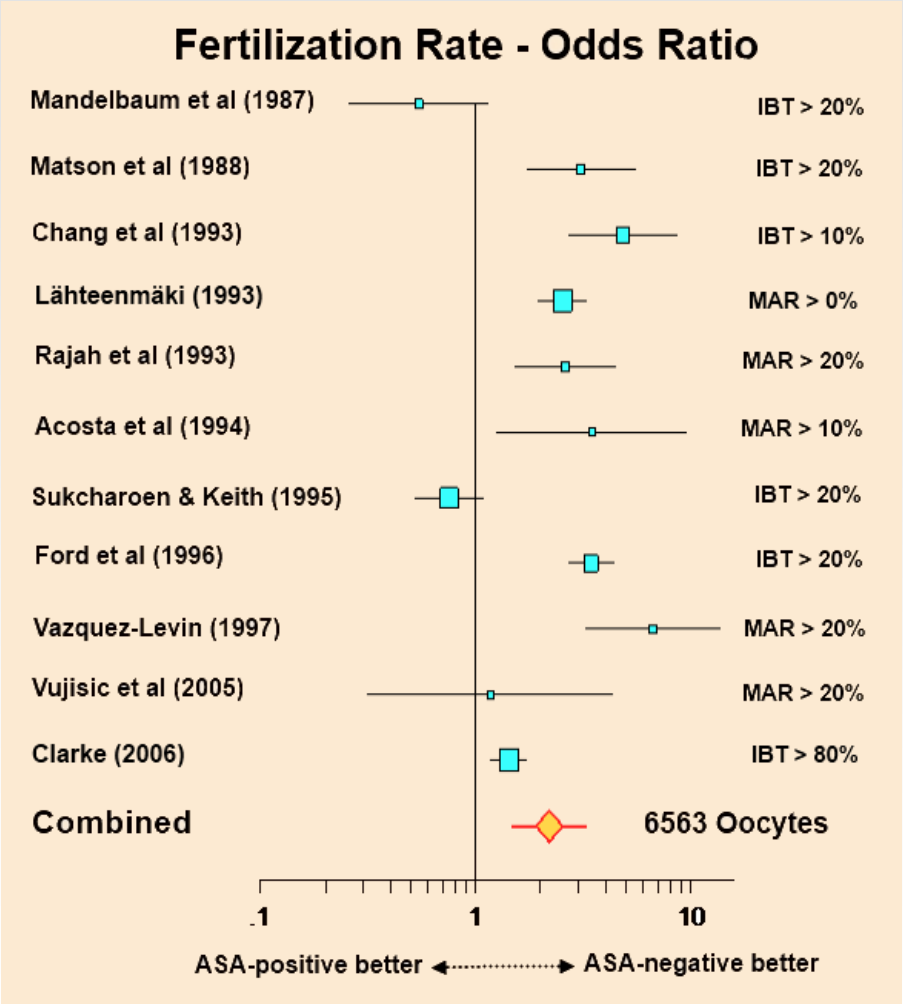
ASA and IVF Fertilization Rate Outcomes



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ASA and IVF-ICSI Outcomes

Antisperm antibodies are not associated with pregnancy rates after IVF and ICSI: systematic review and meta-analysis

Armand Zini [1,*], Nader Fahmy [1], Eric Belzile [2], Antonio Ciampi [2,3], Naif Al-Hathal [1], and Ahmed Kotb [1]

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Human Reproduction 2011; 26: 1288-1295

Craig Niederberger said it best…

- **"My concern with the study is that an odds ratio for pregnancy failure of 1.00 for ICSI is different than that of 1.22 for IVF, with IVF being worse; it is just that the number of included studies did not achieve statistical significance. It is a common problem with meta-analyses that by mixing together differing studies we may be throwing out the baby with the bathwater."**

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J Urol. 2012 Mar;187:995-996

ASA and IVF-ICSI Outcomes

- **ICSI leads to similar fertilization and pregnancy rates in ASA positive and negative cases**
  - **Approx 78% for ASA+ vs 69% for ASA-**

- **Concern about embryo quality with ASA positive sperm**
  - **e.g. ICSI with sperm with >80% MAR binding. 38% preg loss in ASA+ vs 0% in ASA-**

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Lähteenmäki, A et al. Hum. Reprod 1995;10; 2824–2828.
Nagy ZP et al. Human Reprod 1995; 10; 1775-1780

FISH and Male Infertility

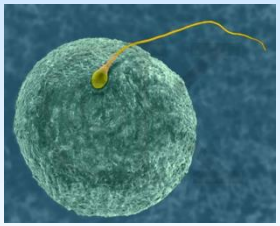
- Fluorescent in situ hybridization (FISH) detects sperm aneuploidy, even in men with normal karyotype
- May explain reproductive failure, including recurrent miscarriage and failed fertilization
- Numerous clinical syndromes found to be related to abnormal FISH
- Some authors advocate sperm FISH prior to sperm retrieval (NOA), as well as in couples with unexplained failed IVF cycles

Carrel DT. The clinical implementation of sperm chromosome aneuploidy testing: pitfalls and promises. J Androl 29:124–133 2008.

Sperm DNA testing

- DNA fragmentation: Tests available and how do they differ and what value/limitations do they have?
  - Predictive for failed fertilization/poor implantation/recurrent miscarriages?

Introduction



- Sperm with DNA damage take a longer time to conceive. (Spano 2000)
  - But ultimately these sperm are able to fertilize. (Lopes 1998, Gandini 2004, Aitken 1998)

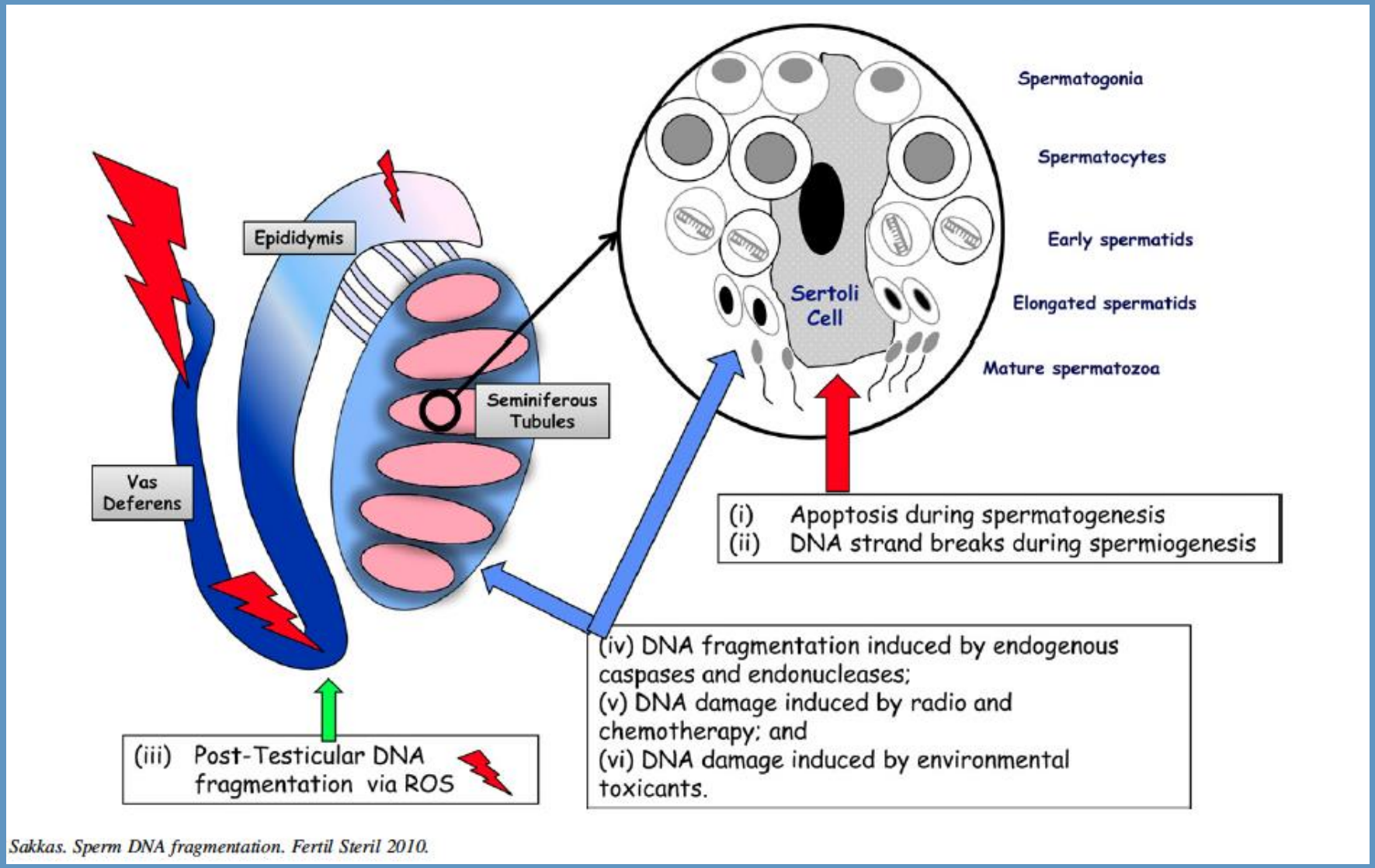
- Both oocyte and embryo are equipped with mechanisms to repair some paternal DNA anomalies. (Wells 2005, Gasca 2007)
  - depends on the quality of the oocyte
  - impacted dramatically by increasing female age.

- Sperm DNA quality is increasingly being linked to paternal age. (Wyrobek 2006)
  - This may further exacerbate the decrease in pregnancy rate observed in women of advanced age. (Belloc 2008)

- Sperm have few repair mechanisms. (Aitken 2006)

Lopes S. Fertil Steril 1998; 69: 528–32.
Gandini L. Hum Reprod 2004; 19:1409–17.
Aitken RJ. Biol Reprod 1998; 59: 1037–46

How does sperm DNA damage occur?



Sakkas. Sperm DNA fragmentation. Fertil Steril 2010.

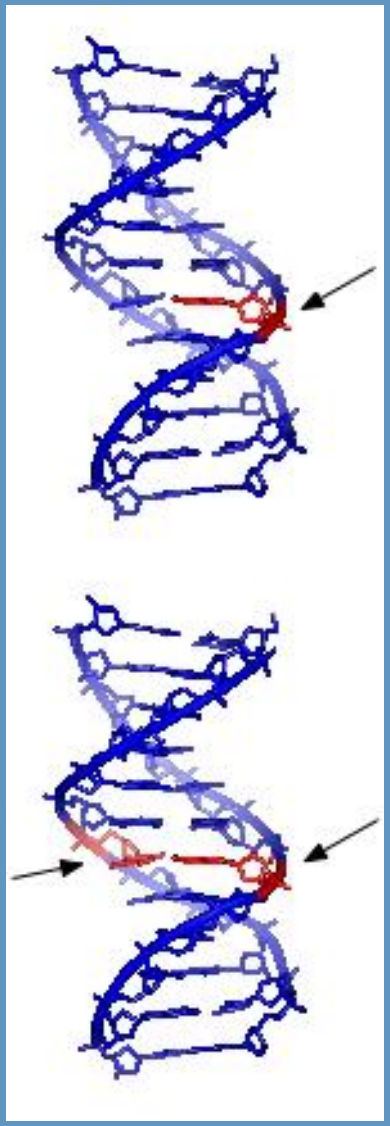
Oxidative stress

- Oxidative stress has long been implicated as the major etiological factor in sperm DNA damage.
- Reactive oxygen species (ROS): need some but not too much.
- Oxidative stress leads to base modifications, which may lead to discrete DNA strand breaks. (Croteau 1997)

Croteau, D.L. Journal of Biological Chemistry, 272, 1997 25409–25412.
Kodama, H. Fertility and Sterility, 68,1997, 519–524.
Shen, H.M.. Journal of Andrology, 20, 1999, 718–723

Type of DNA damage: single vs double-stranded

- Single-stranded DNA damage:
  - Better prognosis, easier to repair
  - Caused by:
    - Unrepaired DNA nicks generated during chromatin remodeling
    - Oxygen radical-induced damage
- Double-stranded DNA damage:
  - Caused by:
    - Apoptosis
    - Hydrolysis by caspases and endonucleases
    - Oxygen radical-induced DNA damage through the activation of caspases and endonucleases.
      - Damage depends on levels of antioxidant enzymes present in the lumen of the epididymis. (Britan 2006)



Britan A. Cell Tissue Res 2006;324:301–10.

DNA Fragmentation Tests

- Different assays measure different aspects of sperm DNA and chromatin:
  - Degree of DNA fragmentation, protamination, DNA denaturation.

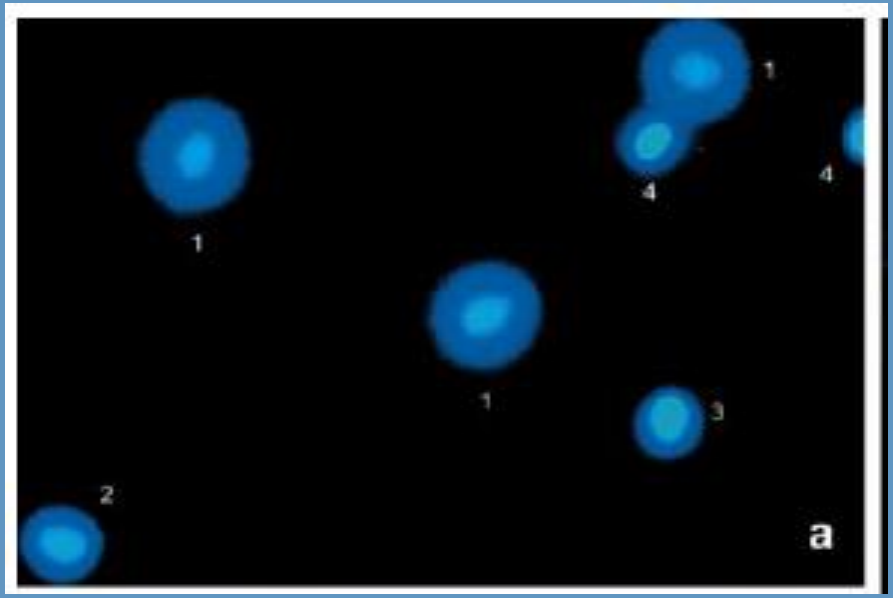
- Assay conditions can greatly influence the accessibility of the dye or enzyme to the sites of damaged DNA and, therefore, impact on the final results.
  - Reagents themselves can alter the reactions
  - The concentration of reducing agents can alter sperm nuclear decondensation
  - Sample preparation and handling (centrifugation, prolonged incubation) can impact the test results.

- Assays do not identify the DNA fragmentation in an individual cell.

Sperm Chromatin Dispersion

- Measure the rate at which denatured single stranded DNA form from native double stranded DNA.
- Sperm with fragmented DNA fail to produce the characteristic halo of dispersed DNA loops that is observed in sperm with nonfragmented DNA.
- Assessed by fluorescence and brightfield microscopy.

- >30%- poor fertility prognosis



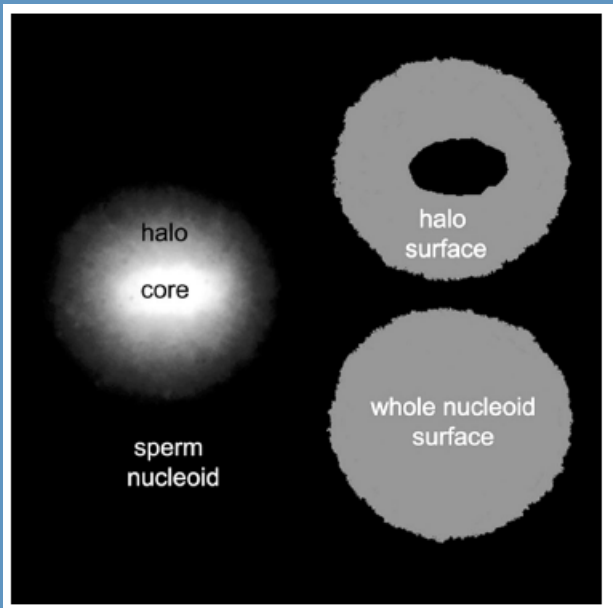
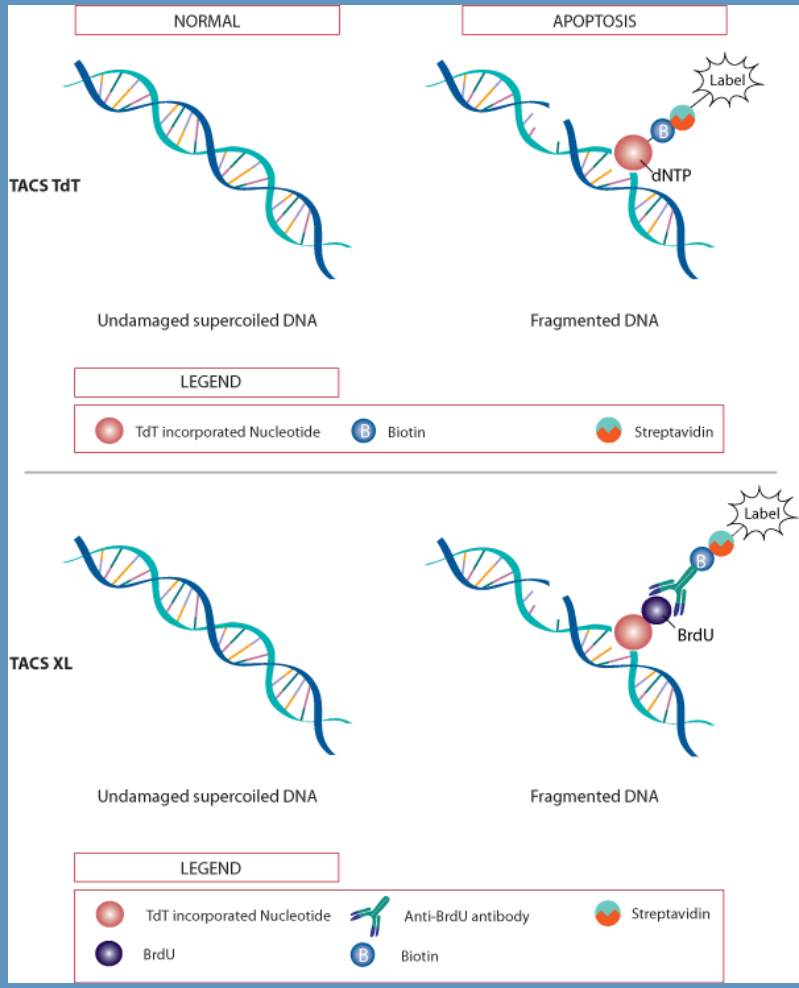
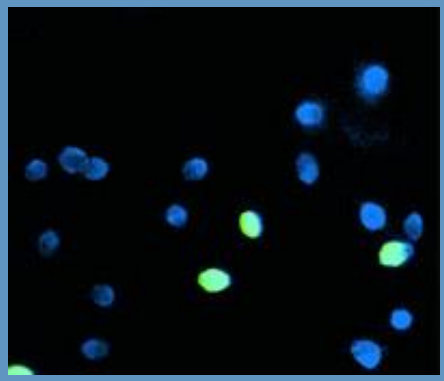


Figure 1. Demonstration of how the relative halo size is obtained by digital image analysis (DIA). The DAPI (4′,6-diamidino-2-phenylindole) stained sperm nucleoid (blue fluorescence, left) contains a central core and a peripheral halo of dispersed DNA loops. Using DIA software, the relative halo size is obtained from the halo surface (upper right) and the whole nucleoid surface (lower right). Dividing the halo surface by the respective whole nucleoid surface, we obtain the relative halo size as it relates to the nucleoid.

TUNEL

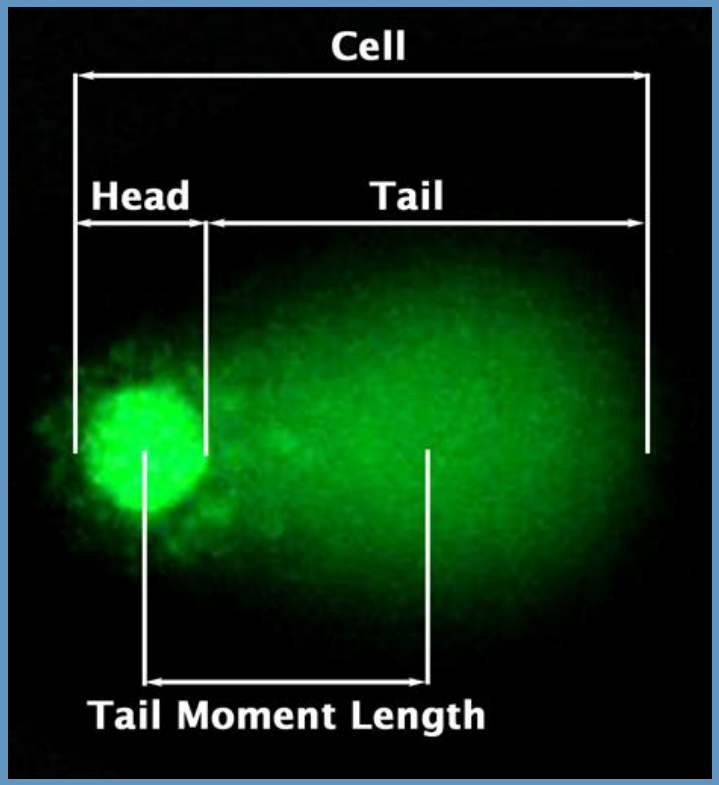
- Terminal deoxynucleotidyl transferase dUTP nick end labeling
- DNA fragmentation detected by labeling the terminal end of nucleic acids.
- Nicks in the DNA are identified by TdT, an enzyme that will catalyze the addition of a fluorescent nucleotide marker.
- Threshold for subfertility variable: 4-20%





Comet

- Detects DNA damage at the level of the individual sperm
- Allows for quantitative measurement of DNA damage.
- Cells are lysed with detergent and salt to form nucleoids containing loops of DNA.
- Electrophoresis → look at the pattern of DNA migration through the gel, observed by fluorescence microscopy.
- The intensity of the comet tail relative to the head reflects the number of DNA breaks.
- More sensitive than other tests.



SCSA

100 IVF or ICSI cycles

TABLE 5

Number of couples and pregnancy rates for different values of DFI and HDS.

	Number	Pregnancy rate (%)
DFI (%)		
0–≤9	23	8.7
9.01–≤18	32	40.6
18.01–≤27	21	33.3
27.01–≤36	11	54.5
>36	8	37.6
HDS (%)		
0–5	15	40.0
5.01–≤10	50	32.0
10.01–≤15	15	40.0
15.01–≤20	7	28.6
>20	8	12.5

Payne. Redefining SCSA and ART outcomes. Fertil Steril 2005.

Payne JF, et al. Fertil Steril. 2005 Aug;84(2):356-64.

Predictive value of sperm DFI testing

- The predictive value of DNA fragmentation testing is likely the sum of many factors:
  - Percent of sperm with DNA damage
  - Extent of DNA damage per spermatozoon
  - Whether there is combined nucleotide damage and DNA fragmentation
  - Ability of the oocyte to repair DNA damage in the fertilizing sperm
  - Type of sperm DNA fragmentation test used
  - Sperm processing in ART
  - Oocyte number
  - Oocyte quality

Outcomes

- Numerous studies have shown that higher DNA fragmentation rates are associated with impaired fertility:
  - longer times to conceive (Spano et al., 2000)
  - impaired embryo cleavage (Morris et al., 2002)
  - higher miscarriage rates (Evenson et al., 1999)
  - increased risk of pregnancy loss after both IVF and ICSI (Zini 2008)

- The impact of sperm DNA damage on ART outcomes decreases with invasiveness:
  - SP > IUI > IVF > ICSI (least useful in ICSI) (Collins 2008, Zini 2009)
  - Hypothesis: ICSI is able to bypass genetic (and functional) defects. (Ozmen 2007, Bungum et al., 2008)

Spano, M.. Fertility and Sterility, 2000 73, 43–50.
Morris, I.D. Human Reproduction, 2002 17, 990–998.
Evenson, D.P.. Human Reproduction, 1999 14, 1039–1049.
Zini, A. Human Reproduction, 2008 23, 2663–2668.
Collins, J.A.Fertility and Sterility, 2008 89, 823–831.
Zini, A. Journal of Andrology, 2009 30, 219–229.
Ozmen, B.Reproductive Biomedicine Online, 2007 14, 384–395.
Bungum, M. Human Reproduction, 2008 23, 4–10.

Spontaneous Pregnancy & IUI

- Spontaneous Pregnancy:
  - Prolonged time to pregnancy (Evenson 1999, Giwercman 2010, Loft 2003, Spano 2000)
  - Failure to achieve a natural pregnancy (OR = 7.01, p < 0.001; Table 1)

Table 1. Selected Diagnostic Properties of Studies on Sperm DNA Damage and Natural Pregnancy.

Study	*n*	Assay	%hDD	Sens	Spec	PPV	NPV	OR	(95% CI)
Evenson et al. 1999	144	SCSA	7	0.19	0.96	0.60	0.81	6.54	(1.72, 24.92)
Spano et al. 2000	215	SCSA	13	0.23	0.96	0.86	0.55	7.59	(2.54, 22.67)
Giwercman et al. 2010	257	SCSA	12	0.21	0.96	0.83	0.58	6.82	(2.52, 18.47)

Abbreviations: %hDD = proportion of samples with high sperm DNA damage; Sens = sensitivity; Spec = specificity; PPV = positive predictive value; NPV = negative predictive value; OR = odds ratio; SCSA = sperm chromatin structure assay.

- IUI:
  - Lower IUI pregnancy rates (Bungum 2007, Duran 2002, Muriel 2006)
  - OR = 9.9 (p < 0.001)

Evenson, D.P.. Hum Reprod 1999, 14:1039–1049.
Giwercman, A. Int J Androl 2010, 33:e221–e227.
Loft, S. Hum Reprod 2003,18:1265–1272.
Spano, M. Fertil Steril 2000, 73:43–50.
Bungum, M. Hum Reprod 2007, 22:174–179.
Duran, E.H. Hum Reprod 2002, 17:3122–3128.
Muriel, L. Hum Reprod 2006, 21:738–744.

IVF & DFI

- Zini et al. meta-analysis: Sperm DNA damage is associated with lower IVF pregnancy rates.
  - Combined OR 1.70 ($p < 0.05$)

Table 2. Selected Diagnostic Properties of 11 Studies on Sperm DNA Damage and Pregnancy After IVF.

Study	*n*	Assay	%hDD	Sens	Spec	PPV	NPV	OR	(95% CI)
Filatov et al. 1999	176	CC	41	0.46	0.88	0.96	0.21	6.34	(1.82, 22.08)
Host et al. 2000	175	TUNEL	30	0.34	0.79	0.77	0.37	1.92	(0.92, 4.04)
Henkel et al. 2003	208	TUNEL	69	0.35	0.81	0.81	0.35	2.24	(1.09, 4.58)
Huang et al. 2005	217	TUNEL	19	0.22	0.83	0.50	0.57	1.30	(0.66, 2.56)
Boe-Hansen et al. 2006	139	SCSA	5	0.06	0.97	0.86	0.29	2.43	(0.28, 20.83)
Borini et al. 2006	82	TUNEL	16	0.17	0.89	0.85	0.23	1.66	(0.33, 8.28)
Lin et al. 2008	137	SCSA	16	0.15	0.83	0.45	0.51	0.88	(0.35, 2.19)
Benchaib et al. 2007	84	TUNEL	10	0.07	0.86	0.50	0.32	0.46	(0.11, 2.00)
Bungum et al. 2007	388	SCSA	16	0.17	0.86	0.71	0.34	1.24	(0.69, 2.26)
Frydman et al. 2008	117	TUNEL	44	0.58	0.68	0.64	0.35	2.97	(1.39, 6.32)
Tarozzi et al. 2009	82	CMA3	17	0.22	0.97	0.97	0.28	10.86	(0.62, 191.5)

Abbreviations: %hDD = proportion of samples with high sperm DNA damage; Sens = sensitivity; Spec = specificity; PP[illegible]edictive value; NPV = negative predictive value; OR = odds ratio; CC = chromatin compaction; TUNEL = terminal deoxynucleotidyl transferase-mediated dUTP nick end-labeling; SCSA = sperm chromatin structure assay; CMA3 = chromomycin A3.

Zini A, Systems Biology in Reproductive Medicine, 2011, 57: 78–85

ICSI & DFI

- Zini et al. meta-analysis: Sperm DNA damage is not related to ICSI pregnancy rates
  - Combined OR 1.15 (p = 0.65)
- The careful selection of the sperm and embryo during ICSI may negate the adverse effect of sperm DNA damage on reproductive outcomes.

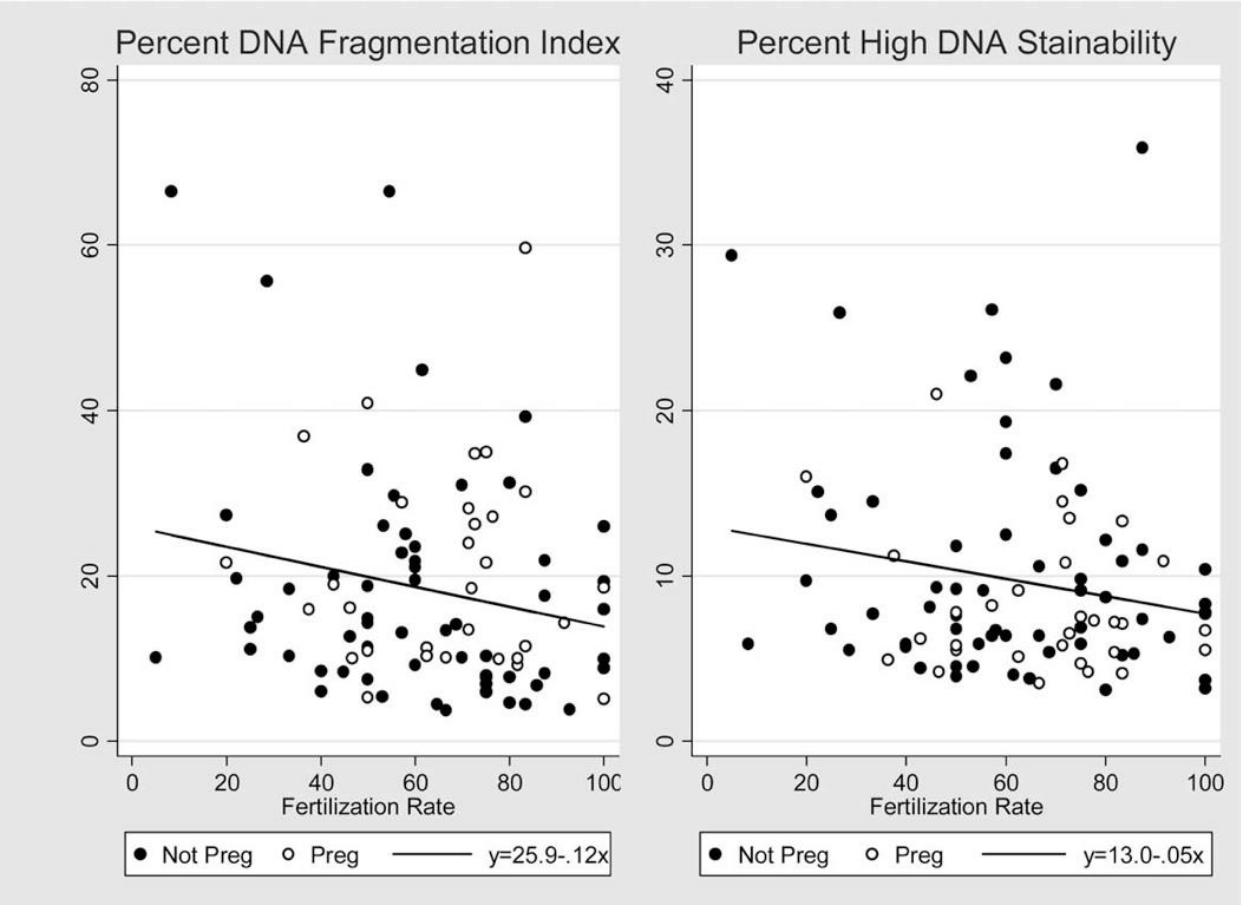
Table 4. Selected Diagnostic Properties of 14 Studies on Sperm DNA Damage and Pregnancy After ICSI.

Study	*n*	Assay	%hDD	Sens	Spec	PPV	NPV	OR	95% CI
Hammadeh et al. 1996	60	ABlue	44	0.50	0.71	0.82	0.35	2.40	(0.72, 7.96)
Host et al. 2000	61	TUNEL	59	0.57	0.38	0.58	0.36	0.79	(0.28, 2.25)
Henkel et al. 2003	54	TUNEL	48	0.68	0.63	0.79	0.50	3.67	(1.12, 12.0)
Gandini et al. 2004	22	SCSA	41	0.31	0.44	0.44	0.31	0.36	(0.06, 2.08)
Huang et al. 2005	86	TUNEL	57	0.64	0.50	0.55	0.60	1.80	(0.76, 4.27)
Zini et al. 2005	60	SCSA	18	0.17	0.81	0.46	0.51	0.87	(0.23, 3.22)
Check et al. 2005	104	SCSA	28	0.29	0.76	0.72	0.34	1.34	(0.52, 3.43)
Boe-Hansen et al. 2006	47	SCSA	38	0.36	0.57	0.67	0.28	0.76	(0.21, 2.72)
Borini et al. 2006	50	TUNEL	60	0.71	0.75	0.90	0.45	7.36	(1.67, 32.4)
Benchaib et al. 2007	218	TUNEL	17	0.19	0.87	0.72	0.37	1.55	(0.70, 3.41)
Bungum et al. 2007	223	SCSA	33	0.29	0.61	0.52	0.37	0.65	(0.37, 1.14)
Lin et al. 2008	86	SCSA	24	0.26	0.77	0.52	0.52	1.21	(0.45, 3.23)
Micinski et al. 2009	50	SCSA	35	0.40	0.85	0.91	0.28	3.73	(0.74, 18.77)
Tarozzi et al. 2009	50	CMA3	56	0.49	0.27	0.61	0.18	0.34	(0.09, 1.29)

Abbreviations: %hDD = proportion of samples with high sperm DNA damage; Sens = sensitivity; Spec = specificity; P[illegible]dictive value; NPV = negative predictive value; OR = odds ratio; ABlue = aniline blue; TUNEL = terminal deoxynucleotidyl transferase-mediated dUTP nick end-labeling; SCSA = sperm chromatin structure assay; CMA3 = chromomycin A3.

SCSA and Fertilization Rate

100 IVF or ICSI cycles



Payne JF, et al. Fertil Steril. 2005 Aug;84(2):356-64.

Pregnancy loss after IVF/ICSI

- Zini et al. meta-analysis: Sperm DNA damage is related to pregnancy loss after IVF and ICSI
  - Combined OR 2.48 ($p < 0.0001$)

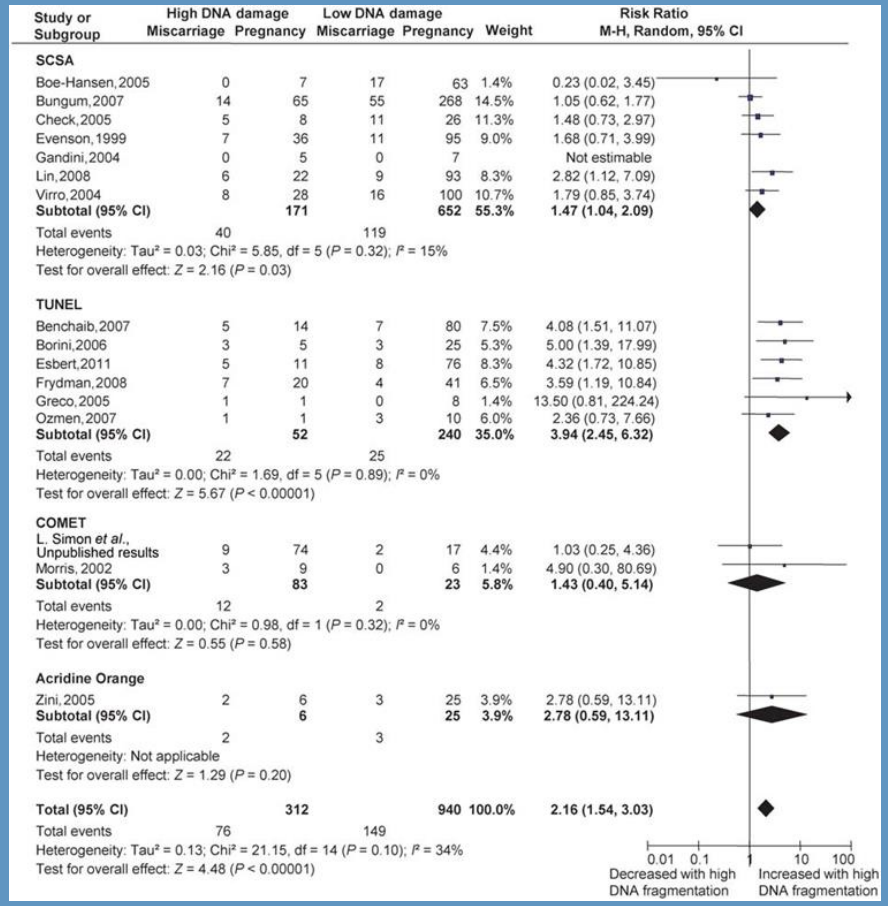
Table 6. Selected Diagnostic Properties of Studies on Sperm DNA Damage and Pregnancy Loss (PL) After IVF and IVF/ICSI.

Study	*n*	ART	Assay	PL (%)	Ab Test* (%)	Sens	Spec	PPV	NPV	OR (95% CI)
Check et al. 2005	104	ICSI	SCSA	47	24	0.31	0.83	0.63	0.58	2.27 (0.45, 11.59)
Zini et al. 2005	60	ICSI	SCSA	16	19	0.40	0.85	0.33	0.88	3.67 (0.46, 29.42)
Borini et al. 2006	82	IVF	TUNEL	6	11	0.91	0.94	0.50	0.99	160 (0.18,144708)
Borini et al. 2006	50	ICSI	TUNEL	25	25	0.97	0.99	0.97	0.99	2700 (0.38, 2×10^{7})
Benchaib et al. 2007	84	IVF	TUNEL	15	15	0.50	0.91	0.50	0.91	10.0 (0.87, 114.8)
Benchaib et al. 2007	218	ICSI	TUNEL	12	15	0.38	0.88	0.30	0.91	4.54 (0.89, 23.28)
Bungum et al. 2007	388	IVF	SCSA	24	14	0.11	0.85	0.19	0.76	0.73 (0.23, 2.33)
Bungum et al. 2007	223	ICSI	SCSA	19	40	0.50	0.63	0.24	0.84	1.69 (0.63, 4.49)
Frydman et al. 2008	117	IVF	TUNEL	19	32	0.64	0.75	0.37	0.90	5.25 (1.31, 21.11)
Lin et al. 2008	137	IVF	SCSA	10	17	0.29	0.84	0.17	0.92	2.16 (0.37, 12.72)
Lin et al. 2008	86	ICSI	SCSA	18	23	0.50	0.83	0.40	0.88	5.00 (0.97, 25.77)

Abbreviations: ART = assisted reproductive technology; PL = pregnancy loss; Ab Test = proportion of abnormal sperm DNA test amongst documented pregnancies; Sens = sensitivity; Spec = specificity; PPV = positive predictive value; NPV = negative predictive value; OR = odds ratio.

- No difference in the OR according to the type of ART (IVF or ICSI).
- Possible cause: impaired embryo/blastocyst development associated with sperm DNA.

Miscarriage Rates



Robinson L. Hum Reprod. 2012 Oct;27(10):2908-17.

Epididymal vs ejaculated vs TESA

- DNA damage is significantly lower in the seminiferous tubules compared with cauda epididymis or ejaculated sperm. (Suganuma 2005, Steele 1999, Greco 2005)
- The use of testicular sperm in couples with repeated pregnancy failure in ART and high sperm DNA fragmentation in semen → a significant increase in PRs in these couples. (Greco 2005, Alvarez 2008)
  - Eliminates the burden of sperm DNA repair by the oocyte
- However, testicular sperm may not always solve the problem…
  - DNA damage may also occur in the seminiferous tubules by apoptosis or be due to defects in chromatin remodeling during spermiogenesis.

Suganuma R. Hum Reprod 2005;20:3101–8.
Steele EK. Mol Hum Reprod 1999;5:831–5.
Greco E. Hum Reprod 2005;20:226–30.
Alvarez J. Argentina de Andrologıa 2008;5.

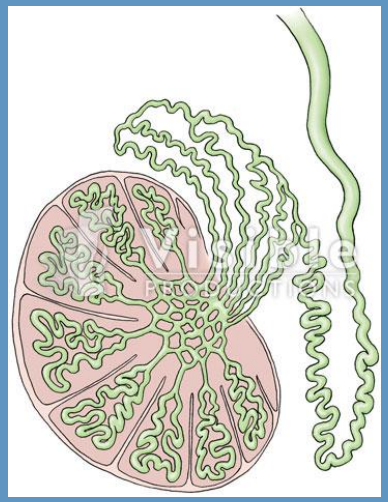
Epididymal vs ejaculated vs TESA

- The rate of aneuploidy: testicular sperm > ejaculated sperm.
  - Concerns about ICSI sperm with a higher rates of chromosomal abnormalities.
  - Possibly due to selective elimination of aneuploid sperm during passage through the epididymis. (Egozcue 2005)
  - However, this data is from studies using sperm from severe oligospermic or azoospermic men.
  - These men may have higher aneuploidy at baseline as compared with normospermic men.

- Using testicular sperm in couples with high levels of sperm DNA fragmentation, PRs were higher and miscarriage rates lower- than when using ejaculated sperm. (Borini 2006)

Egozcue J. Cytogenet Genome Res 2005;111:337–42.
Borini A. Hum Reprod 2006;21: 2876–81.

- Greco et al.:
  - Men with TUNEL measured DFI >15%
  - Failed IVF with ejaculated sperm
  - IVF with testicular sperm
  - Clinical PR of 44.4% (vs- 0% with ejaculated spermatozoa)



Greco E. Hum Reprod 2005; 20:226–30.

So, Do All Roads lead to ICSI with High DFI?

- Problem with high DFI
  - Consistency with each SA
  - What threshold?
  - Pregnancies do happen with high DFI – natural /IUI/IVF

- BUT….we can't really fix high DFI in most cases
  - Treat ROS/WBC/know toxins…maybe
  - Testicular sperm over ejaculated
  - MVI – no benefit really
  - Aren't a lot of labs moving to all ICSI to prevent any chance of failed fertilization...controversial

Applications?…the real question

- Infertile couples who present to with:
  - a history of longstanding infertility
  - repeated IVF failure
  - recurrent miscarriages

Alvarez J. Revista Argentina de Andrologıa 2008;5.

Screening for DNA Fragmentation

Spontaneous Pregnancy:

- The prevalence of a positive test in first pregnancy planners is low (<10%) and 17% of couples with a positive test will achieve a pregnancy, indiscriminate sperm DNA testing in this context is not advised.

Screening in Mild Male-Factor (IUI Candidates):

- More studies are needed before routine DNA fragmentation testing is recommended prior to IUI.

Screening in Severe Male-Factor (IVF Candidates):

- Couples with sperm DNA damage may choose to proceed to ICSI, where pregnancy rates are independent of the test result.
- The clinical value of an 11% difference in pregnancy rates (34% vs. 23%) is modest and it may be hard to justify routine testing.
- However, clinicians may want to test select couples (e.g., with failed IVF) so as to better counsel these couples in future ART cycles.

Thank you!