Usefulness of Sperm Chromatin Tests in the Context of Infertility Treatment: IUI, IVF, ICSI

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Clinical Value of Sperm DNA Tests

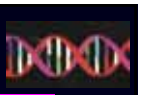
Overview

- **Etiology of sperm DNA damage**
- **Influence of sperm DNA damage on reproductive outcomes**
  - IUI
  - IVF
  - ICSI
- **Clinical value of sperm DNA tests**

Sperm DNA Damage: Etiology

Multi-factorial

Sperm DNA Damage: Etiology



Multi-factorial

Hormonal
Temperature
Toxins
Oxidants

Temperature
Toxins
Oxidants

Idiopathic
Genetic?
Developmental?

Sperm DNA Compaction: Evolution During Spermiogenesis

→ → → → → → Cytoplasmic Reduction → → → → → →

Histones → → → → → TP → → → → Protamines

→ → → → → Nuclear Compaction → → → → →

→ → → → → → Transient DNA nicks →→ → → → →

Marcon & Boissoneault *Biol Reprod* 2004

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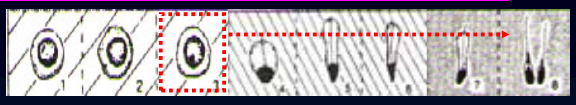


→ → → → → → Cytoplasmic Reduction → → → → → →

Histones → → → → → TP → → → → Protamines

→ → → → → Nuclear Compaction → → → → →

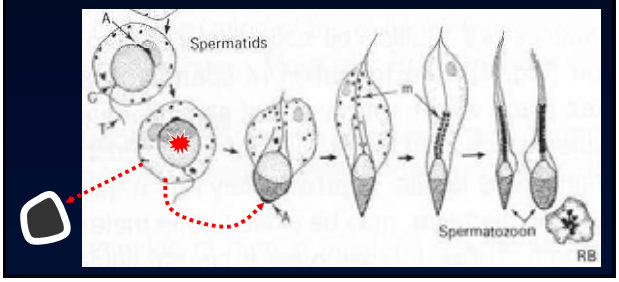
→ → → → → → Transient DNA nicks →→ → → → →

Marcon & Boissoneault *Biol Reprod* 2004

Sperm DNA Damage: Apoptosis

- The Concept of "Abortive apoptosis"
  - Cell enters and then escapes apoptosis

Sakkas *Reprod Biomed Online* 2003
Brinkworth & Nieschlag *Mutat Res* 2000



Sperm DNA Integrity

Why examine sperm DNA integrity?

1. **We need better markers of male fertility potential than conventional semen parameters**

→ To more accurately diagnose male infertility

Semen Analysis: World Health Organization (WHO) Guidelines

- **Conventional semen parameters are fair markers**
  - Exhibit a high degree of variability
  - Modest predictors of male fertility potential
- **Current WHO standards/thresholds fail to meet rigorous clinical and statistical standards**

WHO, 1999
Guzick et al, *NEJM* 2001
Menkveld et al, *Hum Reprod* 2001

Sperm DNA Integrity

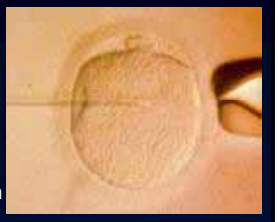
Why examine sperm DNA integrity?

2. To identify male fertility markers that can predict reproductive outcomes after ARTs (especially, IVF and ICSI)

- **conventional semen parameters are not predictive**

IVF/ICSI: A Revolutionary Treatment

- **Most significant advance in treatment of male infertility**
  - **Oligospermia**
  - **Obstructive azoospermia**
  - **Non-obstructive azoospermia**
- **Pregnancy rates: 30-50%**
  - **independent of semen quality**
- **IVF/ICSI is now an important part of the treatment algorithm**



Predictors of IVF/ICSI Outcome: Male Factors

- Presence of viable sperm (from any source)
  - **Nagy et al, *Hum Reprod* 1995**
  - **Creus et al, *Hum Reprod* 2000**
- Morphologically normal sperm
  - **De Vos et al, *Fertil Steril* 2003**
  - **Bartoov et al, *Fertil Steril* 2003**
- Sperm DNA integrity?

Sperm DNA Damage (Animal studies): Influence on IVF outcomes

- Sperm DNA damage was induced by gamma radiation
- Spermatozoa were then used in IVF cycles

Ahmadi & Ng, *J Exp Zool* 1999

	Gamma radiation dosage (GY)				
Parameter	0	5	10	50	100
Fertilization(%)		**53**	**64**	**60**	**59**	**61**
Blastocyst (%)		50	20	8	3	2
Live Fetus		34	21	0	-	-

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Fatehi et al, *J Androl* 2006 – Bovine Model

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Perez-Crespo et al, *J Androl* 2008 – Mouse Model – frozen-thawed sperm

Sperm DNA Integrity

Why examine sperm DNA integrity?

3.To evaluate the influence of sperm DNA damage on the health of the IVF - ICSI child because:

- Natural barriers to fertilization are removed at ICSI
- Infertile men exhibit high levels of sperm DNA damage
- Pregnancy is possible despite high levels of DNA damage
- Experimental (animal) studies suggest that sperm DNA damage might adversely impact the health of the child

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Sperm DNA Damage and Fertility

Infertile men have higher levels of sperm DNA - Chromatin damage than fertile men

Chromatin Structure: Evenson et al, *Hum Reprod* 1999
…. Spano et al, *Fertil Steril* 2000
…. Zini et al, *Fertil Steril* 2001

DNA Fragmentation: Hughes et al, *Hum Reprod* 1996
…. Irvine et al, *J Androl* 2000

DNA Oxidation Sen & Ong, *Free Rad Biol Med* 2000

Protamine Deficiency: Gatewood et al, *J Biol Chem* 1990
…. Carrell & Liu, *J Androl* 2001
…. Zhang et al, *J Androl* 2006

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Sperm DNA Integrity: Influence on Health of the Offspring

- Mouse ICSI studies (fresh [N] and frozen-thawed spz [DFS])
- CD1 and B6D2F1 mouse strains

Fernandez-Gonzalez et al, *Biol Reprod* 2008

ICSI with DFS (compared to N sperm) →

- **Reduced embryo development**
- **Reduced number of live pups**
- **Development of atypical tumors in 33% of females (CD1)**
- **Reduced longevity (85% vs. 100% surviving at 25 weeks)**
- **Altered behavioral responses (“anxiety-like reactions”)**

Sperm DNA Damage: Practical Application

Are measures of sperm DNA damage associated with reproductive outcomes?

Systematic Review & Meta-analysis

Examined all studies on sperm DNA and…

- **IUI pregnancy**
- **IVF pregnancy**
- **ICSI pregnancy**
- **Pregnancy loss (after IVF and ICSI)**

Systematic Review & Meta-analysis

Diagnostic test

- **Sperm DNA integrity / damage**

Reproductive outcomes

- **Fertilization rate**
- **Embryo quality**
- **Pregnancy rate (clinical pregnancy)**
- **Pregnancy loss**

Systematic Review & Meta-analysis

Pregnancy

	Disease + (no preg)	Disease - (+ preg)
Test + (>cutoff)	a	b
DNA damage		
Test - (<cutoff)	c	d

Systematic Review & Meta-analysis

Pregnancy

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Test + (>cutoff)	a	b
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Systematic Review & Meta-analysis

	Disease + (no preg)	Disease - (preg)
Test + (>cutoff)	a (true + test)	b (false + test)
DNA damage		
Test - (<cutoff)	c (false - test)	d (true - test)

Sensitivity = a/(a+c) (true + test rate)
Specificity = d/(b+d) (true - test rate)
Odds Ratio = ad / bc (measure of assoc. b/n test and disease)
PPV (pos. predictive value)= a/(a+b)(disease prob if + test)
NPV (neg. predictive value)= d/(c+d)(no disease prob if - test)

Systematic Review & Meta-analysis

	Disease + (no preg)	Disease - (preg)
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Sperm DNA Damage and IUI Pregnancy

Study	n	Stimulation	Design
Duran,'02	154	mixed	prospective, mixed etiology
Muriel, '06	100	100%	prospective, mixed etiology
Bungum,'07	387	100%	prospective, consec, <40, UE Inf

Sperm DNA Damage and IUI Pregnancy

Study	n	%hDD	Preg	Assay	Cutoff*	Exclusion
Duran,'02	154	---	↓	TUNEL	4%	no 2 X 2 table
Muriel, '06	100	---	0	SCD	-----	no cutoff, no 2 X 2
Bungum,'07	387	17	↓	SCSA	30%	OK

Sperm DNA Damage and IUI Pregnancy

Study	n	%hDD	Sens	Spec	OR	(95% CI)
Bungum, '07	388	16	0.21	0.99	9.9	(2.37, 41.51)

Odds ratio = 9.9 (2.37, 41.51), $p < 0.001$
So, **sperm DNA damage has a significant effect on IUI PR**

Clinical Application?

Positive predictive value: 97% no PR (**3% PR**)
Negative predictive value: **24% PR**

Sperm DNA Damage and IVF Outcomes

Study	n	Design	Fem - Selection	Fertilization
Filatov '99	176	not specified	none	0
Host, '00…	175	prospective, consecutive	none	↓
Tomlinson,'01	140	not specified	none	0
Tomsu, '02	40	prospective	<40 yo	0
Morris, '02	20	retrospective	<40 yo	0
Henkel, '03	208	prospective	none	0
Gandini, '04	12	prospective	none	0
Huang, '05	217	retrospective	none	↓
Boe-Hansen,'06	139	prospective	fsh<10	NA
Borini, '06	82	not specified	none	↓
Bakos, '07	45	not specified	none	↓
Benchaib, '07	84	prospective	none	0
Bungum, '07	388	prospective, consecutive	<40 yo, fsh<12	0
Lin, '07	137	prospective	<40, fsh<10	0
Frydman,'07	117	prospective	<38, fsh<15	0
Total	1809	15 studies		

Sperm DNA Damage and IVF Outcomes

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Filatov '99	176	not specified	none	0
Host, '00…	175	prospective, consecutive	none	↓
Tomlinson,'01	140	not specified	none	0
Tomsu, '02	40	prospective	<40 yo	0
Morris, '02	20	retrospective	<40 yo	0
Henkel, '03	208	prospective	none	0
Gandini, '04	12	prospective	none	0
Huang, '05	217	retrospective	none	↓
Boe-Hansen,'06	139	prospective	fsh<10	NA
Borini, '06	82	not specified	none	↓
Bakos, '07	45	not specified	none	↓
Benchaib, '07	84	prospective	none	0
Bungum, '07	388	prospective, consecutive	<40 yo, fsh<12	0
Lin, '07	137	prospective	<40, fsh<10	0
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Total	1809	15 studies		

Sperm DNA Damage and IVF Fertilization

Study	n	Design	Fem - Selection	Fertilization
Filatov '99	176	not specified	none	0
Host, '00…	175	prospective, consecutive	none	↓
Tomlinson,'01	140	not specified	none	0
Tomsu, '02	40	prospective	<40 yo	0
Morris, '02	20	retrospective	<40 yo	0
Henkel, '03	208	prospective	none	0
Gandini, '04	12	prospective	none	0
Huang, '05	217	retrospective	none	↓
Boe-Hansen,'06	139	prospective	fsh<10	NA
Borini, '06	82	not specified	none	↓
Bakos, '07	45	not specified	none	↓
Benchaib, '07	84	prospective	none	0
Bungum, '07	388	prospective, consecutive	<40 yo, fsh<12	0
Lin, '07	137	prospective	<40, fsh<10	0
Frydman,'07	117	prospective	<38, fsh<15	0
Total	1809	15 studies		

Sperm DNA Damage and IVF Outcomes

Study	n	Design	Female age	Fertilization
Filatov '99	176	not specified	not controlled	0
Host, '00…	175	prospective, consecutive	controlled	↓
Tomlinson,'01	140	not specified	controlled	0
Tomsu, '02	40	prospective	controlled	0
Morris, '02	20	retrospective	not cont, <40	0
Henkel, '03	208	prospective	not controlled	0
Gandini, '04	12	prospective	controlled	0
Huang, '05	217	retrospective	not controlled	↓
Boe-Hansen,'06	139	prospective	not cont, fsh<10	NA
Borini, '06	82	not specified	controlled	↓
Bakos, '07	45	not specified	not controlled	↓
Benchaib, '07	84	prospective	controlled	0
Bungum, '07	388	prospective, consecutive	controlled	0
Lin, '07	137	prospective	controlled	0
Frydman,'07	117	prospective	controlled	0
Total	1809	15 studies		

Sperm DNA Damage and IVF Pregnancy

Study	n	PREG	Assay	Cutoff	Cutoff Justification
Filatov '99	176	↓	CC*	50%	Based on fertile population
Host, '00…	175	↓	TUNEL	4%	Based on fertile population
Tomlinson,'01	140	↓	ISNT	----	no cutoff
Tomsu, '02	40	↓	Comet	----	no cutoff
Morris, '02	20	0	Comet	----	no cutoff
Henkel, '03	208	0	TUNEL	37%	Best CO from ROC analysis
Gandini, '04	12	0	SCSA	27%	Based on Evenson 2000, 2002
Huang, '05	217	0	TUNEL	10%	Not justified
Boe-Hansen,'06	139	↓	SCSA	27%	Based on Evenson 2000, 2002
Borini, '06	82	↓	TUNEL	10%	Based on Benchaib 2003
Bakos, '07	45	↓	TUNEL	----	no cutoff
Benchaib, '07	84	↓	TUNEL	15%	Based on IVF-ICSI results
Bungum, '07	388	↓	SCSA	30%	Based on Evenson 2000, 2002
Lin, '07	137	↓	SCSA	27%	Based on Evenson 2000, 2002
Frydman,'07	117	↓	TUNEL	35%	Median value

*Chromatin compaction (by flow cytometry)

Systematic Review & Meta-analysis

	Pregnancy: Disease + (no preg)	Pregnancy: Disease - (+ preg)
Test + (>cutoff)	a	b
DNA damage		
Test - (<cutoff)	c	d

Sperm DNA Damage and IVF Pregnancy

Study	n	PREG	Assay	Cutoff	Cutoff Justification
Filatov '99	176	↓	CC*	50%	Based on fertile population
Host, '00175	↓	TUNEL	4%	Based on fertile population	
~~Tomlinson,'01~~	~~140~~	~~↓~~	~~ISNT~~	~~----~~	~~no cutoff~~
~~Tomsu, '02~~	~~40~~	~~↓~~	~~Comet~~	~~----~~	~~no cutoff~~
~~Morris, '02~~	~~20~~	~~0~~	~~Comet~~	~~----~~	~~no cutoff~~
Henkel, '03	208	0	TUNEL	37%	Best CO from ROC analysis
~~Gandini, '04~~	~~12~~	~~0~~	~~SCSA~~	~~27%~~	~~Too small (no DFI>20%)~~
Huang, '05	217	0	TUNEL	10%	Not justified
Boe-Hansen,'06	139	↓	SCSA	27%	Based on Evenson 2000, 2002
Borini, '06	82	↓	TUNEL	10%	Based on Benchaib 2003
~~Bakos, '07~~	~~45~~	~~↓~~	~~TUNEL~~	~~----~~	~~no cutoff~~
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*Chromatin compaction (by flow cytometry)

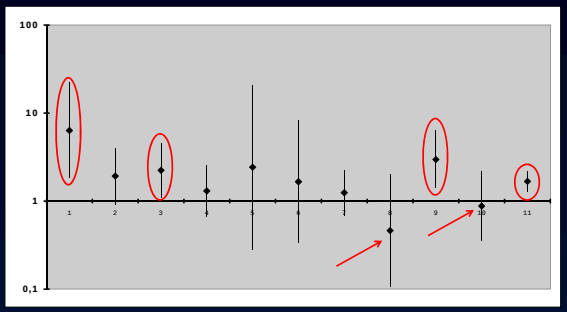
Sperm DNA Damage and IVF Pregnancy

Study	n	%hDD	Sens	Spec	OR	(95% CI)
Filatov, '99	**176**	**41**	**0.46**	**0.88**	**6.33**	**(1.82, 22.08)**
Host, '00…	175	30	0.34	0.79	1.92	(0.92, 4.04)
Henkel, '03	208	69	0.35	0.81	**2.24**	**(1.09, 4.58)**
Huang, '05	217	19	0.22	0.83	1.30	(0.66, 2.56)
Boe-Hansen,'06	139	5	0.06	0.97	2.43	(0.28, 20.83)
Borini, '06	82	16	0.17	0.89	1.66	(0.33, 8.28)
Lin, '07	137	16	0.15	0.83	0.88	(0.35, 2.19)
Benchaib, '07	84	10	0.07	0.86	0.46	(0.11, 2.00)
Bungum, '07	388	16	0.17	0.86	1.24	(0.69, 2.26)
Frydman,'07	117	44	0.58	0.68	**2.97**	**(1.39, 6.32)**
Total	1723	23%	0.19	0.84		

Test for Homogeneity: ($p > 0.1$)

Fixed Effects Model:
Combined **Odds ratio = 1.67** (1.27, 2.20), $p < 0.01$

Sperm DNA Damage and IVF Pregnancy



Sperm DNA Damage and IVF Pregnancy

Fixed Effects Model:
Combined **Odds ratio = 1.67** (1.27, 2.20), $p < 0.01$

Clinical Application?

Positive predictive value (**PPV** median): 74% no PR (**26% PR**)
Negative predictive value (**NPV** median): **34% PR**

Clinical significance of an 8% difference in PR?

Sperm DNA Damage and ICSI Outcomes

Study	n	Design	Fem - Selection	Fertilization
Hammadeh, '96	61	prospective	none	0
Host, '00…	61	prospective, consecutive	none	0
Virant-Klun, '02	183	prospective	none	↓
Morris, '02	40	retrospective	<40 yo	0
Henkel, '03	54	prospective	none	0
Gandini, '04	22	prospective	none	0
Huang, '05	86	retrospective	none	↓
Check, '05	104	not specified, IVF failure	none	N/A
Zini, '05	60	prospective, consecutive	<40	0
Boe-Hansen,'06	47	prospective	fsh<10	N/A
Borini, '06	50	not specified	none	0
Muriel, '06	85	prospective	none	↓
Benchaib, '07	218	prospective	none	0
Bungum, '07	223	prospective, consecutive	<40 yo, fsh<12	0
Lin, '07	86	prospective	<40, fsh<10	0
Bakos, '07	68	not specified	none	0
Total	1450	16 studies		

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Bakos, '07	68	not specified	not controlled	0
Total	1450	16 studies		

Sperm DNA Damage and ICSI Pregnancy

Study	n	PREG	Assay	Cutoff	Cutoff Justification
Hammadeh, '96	61	↓	A-Blue	29%	ROC analysis
Host, '00…	61	0	TUNEL	4%	Based on fertile population
Virant-Klun, '02	183	0	AO	56%	Based on Liu & Baker 1992
Morris, '02	40	0	Comet	-----	No cutoff
Henkel, '03	54	↓	TUNEL	24%	ROC analysis
Gandini, '04	22	0	SCSA	30%	Based on Evenson 2000, 2002
Huang, '05	86	0	TUNEL	4%	Not justified
Check, '05	104	0	SCSA	30%	Based on Evenson 2000, 2002
Zini, '05	60	0	SCSA	30%	Based on Evenson 2000, 2002
Boe-Hansen,'06	47	0	SCSA	27%	Based on Evenson 2000, 2002
Borini, '06	50	↓	TUNEL	10%	Based on Benchaib 2003
Muriel, '06	85	0	SCD	----	No cutoff
Benchaib, '07	218	↓	TUNEL	15%	Based on ART results (10%,'03)
Bungum, '07	223	0	SCSA	30%	Based on Evenson 2000, 2002
Lin, '07	86	0	SCSA	27%	Based on Evenson 2000, 2002
Bakos, '07	68	↓	TUNEL	35%	Cannot construct 2 x 2 table
Total	1450				

Sperm DNA Damage and ICSI Pregnancy

Study	n	PREG	Assay	Cutoff	Cutoff Justification
Hammadeh, '96	61	↓	A-Blue	29%	ROC analysis
Host, '00…	61	0	TUNEL	4%	Based on fertile population
Virant-Klun, '02	183	0	AO	56%	Based on Liu & Baker 1992
Morris, '02	40	0	Comet	-----	No cutoff
Henkel, '03	54	↓	TUNEL	24%	ROC analysis
Gandini, '04	22	0	SCSA	30%	Based on Evenson 2000, 2002
Huang, '05	86	0	TUNEL	4%	Not justified
Check, '05	104	0	SCSA	30%	Based on Evenson 2000, 2002
Zini, '05	60	0	SCSA	30%	Based on Evenson 2000, 2002
Boe-Hansen,'06	47	0	SCSA	27%	Based on Evenson 2000, 2002
Borini, '06	50	↓	TUNEL	10%	Based on Benchaib 2003
Muriel, '06	85	0	SCD	----	No cutoff
Benchaib, '07	218	↓	TUNEL	15%	Based on ART results (10%,'03)
Bungum, '07	223	0	SCSA	30%	Based on Evenson 2000, 2002
Lin, '07	86	0	SCSA	27%	Based on Evenson 2000, 2002
Bakos, '07	68	↓	TUNEL	35%	Cannot construct 2 x 2 table
Total	1450				

Sperm DNA Damage and ICSI Pregnancy

Study	n	PREG	Assay	Cutoff	Cutoff Justification
Hammadeh, '96	61	↓	A-Blue	29%	ROC analysis
Host, '00…	61	0	TUNEL	4%	Based on fertile population
~~Virant-Klun, '02~~	~~183~~	~~0~~	~~AO~~	~~56%~~	~~Sub-optimal test~~
~~Morris, '02~~	~~40~~	~~0~~	~~Comet~~	~~-----~~	~~No cutoff~~
Henkel, '03	54	↓	TUNEL	24%	ROC analysis
Gandini, '04	22	0	SCSA	30%	Based on Evenson 2000, 2002
Huang, '05	86	0	TUNEL	4%	Not justified
Check, '05	104	0	SCSA	30%	Based on Evenson 2000, 2002
Zini, '05	60	0	SCSA	30%	Based on Evenson 2000, 2002
Boe-Hansen,'06	47	0	SCSA	27%	Based on Evenson 2000, 2002
Borini, '06	50	↓	TUNEL	10%	Based on Benchaib 2003
~~Muriel, '06~~	~~85~~	~~0~~	~~SCD~~	~~----~~	~~No cutoff~~
Benchaib, '07	218	↓	TUNEL	15%	Based on ART results (10%,'03)
Bungum, '07	223	0	SCSA	30%	Based on Evenson 2000, 2002
Lin, '07	86	0	SCSA	27%	Based on Evenson 2000, 2002
~~Bakos, '07~~	~~68~~	~~↓~~	~~TUNEL~~	~~35%~~	~~Cannot construct 2 x 2 table~~
Total	1450				

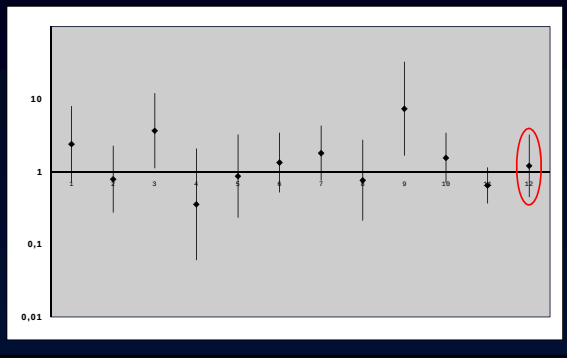
Sperm DNA Damage and ICSI Pregnancy

Study	n	%DD	Sens	Spec	OR	(95% CI)
Hammadeh, '96	61	44	0.50	0.70	2.40	(0.72, 7.96)
Host, '00…	61	59	0.57	0.38	0.79	(0.28, 2.25)
Henkel, '03	54	48	0.68	0.63	3.67	(1.12, 12.0)
Gandini, '04	22	41	0.31	0.44	0.36	(0.06, 2.08)
Huang, '05	86	57	0.64	0.50	1.80	(0.76, 4.27)
Zini, '05	60	18	0.17	0.81	0.87	(0.23, 3.22)
Check, '05	104	28	0.29	0.76	1.34	(0.52, 3.43)
Boe-Hansen,'06	47	38	0.36	0.57	0.76	(0.21, 2.72)
Borini, '06	50	60	0.71	0.75	7.36	(1.67, 32.4)
Benchaib, '07	218	17	0.19	0.87	1.55	(0.70, 3.41)
Bungum, '07	223	33	0.29	0.61	0.65	(0.37, 1.14)
Lin, '07	86	24	0.26	0.77	1.21	(0.45, 3.23)
Total	**1074**	**39%**	**0.33**	**0.70**		

Test for Homogeneity: P > 0.1

Fixed Effects Model:
Combined **Odds ratio = 1.20** (0.91, 1.59), P >0.05

Sperm DNA Damage and ICSI Pregnancy



Sperm DNA Damage and ICSI Pregnancy

Fixed Effect Model:

Combined **Odds ratio = 1.20** (0.91, 1.59), P >0.05

Clinical Application?

Sperm DNA damage has no significant effect on pregnancy rates after ICSI

Sperm DNA Damage and IVF or ICSI

What about the mixed IVF and ICSI studies?

Sperm DNA Damage and IVF or ICSI

Study	n	Design	Fem - Selection	Fertilization
Larson-C, '03	89	retrospective	none	0
Virro, '04	249	retrospective	none	0
Seli, '04	49	prospective, consecutive	none	↓
Payne, '05	100	prospective	none	↓
Meseguer, '07	38	prospective	none	0
Velez de la C,'08	622	prospective, consecutive	none	↓
Tavalaee, '09	92	prospective	none	0
Total	1239	7 studies		

Sperm DNA Damage and IVF or ICSI

Study	n	Design	Female age	Fertilization
Larson-C, '03	89	retrospective	controlled	0
Virro, '04	249	retrospective	controlled	0
Seli, '04	49	prospective, consecutive	controlled	↓
Payne, '05	100	prospective	controlled	↓
Meseguer, '07	38	prospective	controlled	0
Velez de la C,'08	622	prospective, consecutive	controlled	↓
Tavalaee, '09	92	prospective	controlled	0
Total	1239	7 studies		

Sperm DNA Damage and IVF or ICSI

Study	n	PREG	Assay	Cutoff	Cutoff Justification
Larson-C, '03	89	↓	SCSA	27%	Based on Evenson 2000, 2002
Virro, '04	249	↓	SCSA	30%	Based on Evenson 2000, 2002
Seli, '04	49	0	TUNEL	20%	not justified
Payne, '05	100	0	SCSA	27%	Based on Evenson 2000, 2002
Meseguer, '07	38	↓	DNAox	27%	Based on fertile population
Velez de la C,'08	622	0	SCD	18%	Regression model
Tavalaee, '09	92	0	SCD	-----	------
Total	1239	7 studies			

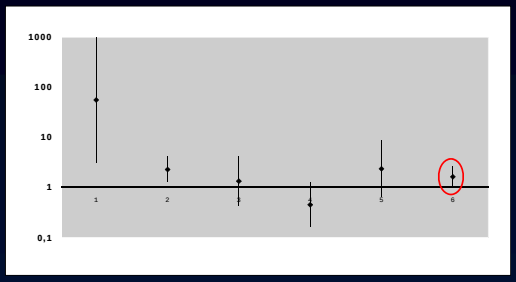
Sperm DNA Damage and IVF or ICSI

Study	n	PREG	Assay	Cutoff	Cutoff Justification
Larson-C, '03	89	↓	SCSA	27%	Based on Evenson 2000, 2002
Virro, '04	249	↓	SCSA	30%	Based on Evenson 2000, 2002
Seli, '04	49	0	TUNEL	20%	not justified
Payne, '05	100	0	SCSA	27%	Based on Evenson 2000, 2002
Meseguer, '07	38	↓	DNAox	27%	Based on fertile population
~~Velez de la C,'08~~	~~622~~	~~0~~	~~SCD~~	~~18%~~	~~Cannot construct 2x2 table~~
~~Tavalaee, '09~~	~~92~~	~~0~~	~~SCD~~	~~-----~~	~~Cannot construct 2x2 table~~

Sperm DNA Damage and IVF or ICSI

Study	n	PREG	Assay	Cutoff	Cutoff Justification
Larson-C, '03	89	↓	SCSA	27%	Based on Evenson 2000, 2002
Virro, '04	249	↓	SCSA	30%	Based on Evenson 2000, 2002
Seli, '04	49	0	TUNEL	20%	not justified
Payne, '05	100	0	SCSA	27%	Based on Evenson 2000, 2002
Meseguer, '07	38	↓	DNAox	27%	Based on fertile population
Total	525	5 studies			

Sperm DNA Damage and IVF or ICSI



Fixed Effects Model:
Combined Odds ratio = 1.63 (1.03, 2.59), p < 0.05

Sperm DNA Damage and Pregnancy Loss after IVF and/or ICSI

Study	ART	Cycles	Preg	P-Loss	RISK*	Comment
Virro, '04	Mixed	---	---	---	↑	No 2 x 2 table
Check, '05	ICSI	104	34	47%	↑	Failed >2 IVF Rx
Zini, '05	ICSI	60	31	16%	↑	PL after CP
Borini, '06	IVF	82	18	6%	↑	PL after CP & BP
Borini, '06	ICSI	50	12	25%	↑	PL after CP & BP
Benchaib, '07	IVF	84	26	13%	↑	PL after CP
Benchaib, '07	ICSI	218	68	13%	↑	PL after CP
Lin, '07	IVF	137	81	12%	↑	PL after CP
Lin, '07	ICSI	86	44	12%	↑	PL after CP
Frydman,'07	IVF	117	59	19%	↑	PL after CP
Bungum, '07	IVF	388	148	22%	0	PL after BP
Bungum, '07	ICSI	223	106	22%	↑	PL after BP
Total	**1549**	**617**				

*Risk of Pregnancy loss with DNA damage

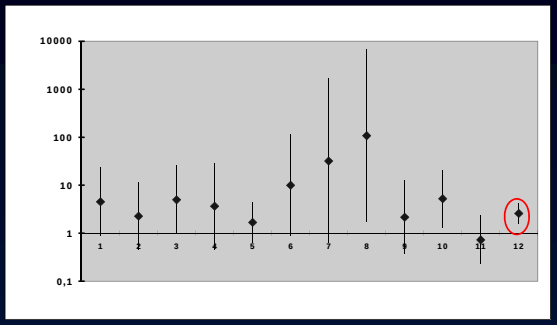
Sperm DNA Damage and Pregnancy Loss (All) after IVF and/or ICSI

Study	ART	P-Loss	Ab Test	Sens	Spec	OR	(95% CI)
Check, '05	ICSI	47%	24%	0.63	0.83	2.27	(0.45, 11.59)
Zini, '05	ICSI	16%	19%	0.33	0.85	3.67	(0.46, 29.42)
Borini, '06	IVF	6%	11%	0.91	0.94	32.0	(0.62, 1663)
Borini, '06	ICSI	25%	25%	0.97	0.99	108.0	(1.73, 6729)
Benchaib, '07	IVF	15%	15%	0.50	0.91	10.0	(0.87, 114.8)
Benchaib, '07	ICSI	12%	15%	0.30	0.88	3.51	(0.89, 23.28)
Lin, '07	IVF	9%	15%	0.17	0.86	2.56	(0.44, 15.03)
Lin, '07	ICSI	18%	23%	0.40	0.83	5.00	(0.97, 25.77)
Frydman,'07	IVF	19%	32%	0.37	0.75	5.25	(1.31, 21.11)
Bungum, '07	IVF	24%	14%	0.19	0.85	0.73	(0.23, 2.33)
Bungum, '07	ICSI	29%	40%	0.24	0.63	1.69	(0.63, 4.49)

Test for Homogeneity: Q = 12.462 with 10 degrees of freedom (p = 0.255)

Fixed Effects Model:
Combined **Odds ratio = 2.48** (1.52, 4.04), p < 0.0001
Random Effects Model:
Combined **Odds ratio = 2.69** (1.51, 4.78), p < 0.0001

Sperm DNA Damage and Pregnancy Loss (All) after IVF and/or ICSI



Sperm DNA Damage and Pregnancy Loss after IVF and/or ICSI

Pregnancy Loss (All definitions)
Combined **Odds ratio = 2.48** (1.58, 4.04), $p < 0.0001$

Clinical Application?

Positive predictive value (**PPV** median): **37% PL**
Negative predictive value (**NPV** median): 90% no PL (**10% PL**)

Sperm DNA Damage and Pregnancy Loss after IVF and/or ICSI

Pregnancy Loss (All)
Combined **Odds ratio = 2.48** (1.58, 4.04), $p < 0.0001$

Pregnancy Loss (IVF, 5 studies)
Combined **Odds ratio = 2.17** (1.02, 4.60), $p < 0.05$

Pregnancy Loss (ICSI, 5 studies)
Combined **Odds ratio = 2.73** (1.43, 5.20), $p < 0.05$

Summary of Findings

Sperm DNA damage and …
IUI pregnancy: strong negative impact (OR = 9.9)
IVF pregnancy: modest negative impact (OR = 1.6)
ICSI pregnancy: no effect
IVF-ICSI pregnancy loss: moderate impact (OR = 2.5)

Explanation of Findings …
IUI pregnancy: strong effect on *in vivo* reproduction
IVF pregnancy: modest early effect (?selection process)
ICSI pregnancy: no early effect (?selection process)
IVF-ICSI pregnancy loss: moderate late effect on embryo-fetal development

Sperm DNA Damage: Practical Application

What is the potential clinical utility of these assays?

3 Clinical Scenarios:

1. Infertile couples with mild male factor
2. Infertile couples with severe male factor
3. Infertile couples with pregnancy loss post-IVF

Sperm DNA Damage: Practical Application

1. Infertile couples with mild male factor:

IUI: Positive predictive value: 97% no PR (**3% PR**)
Negative predictive value: **24% PR**

If +test → IVF or ICSI (ICSI slightly better)

If +test → Increased risk of PL with IVF or ICSI

But prevalence of +test (17%) and sensitivity (20%) are low
Clinical decision based on 1 valid IUI study

Sperm DNA Damage: Practical Application

2. Infertile couples with severe male factor:

ICSI (or possibly IVF)

If +test → IVF or ICSI (ICSI slightly better)

If +test → Increased risk of PL with IVF or ICSI

Test result has little impact on treatment options but may help estimate risk of pregnancy loss

Sperm DNA Damage: Practical Application

3. Infertile couples with pregnancy loss post-IVF

Test Characteristics:

Median prevalence of a + test is 25-30%
Median sensitivity 40% → many other causes for PLoss
Median specificity 85 % → + test points to male factor in PL

If +test → Increased risk of PL with IVF or ICSI

Evaluate the male & correct any male factor

Sperm DNA Damage: Treatment Options?

- Minimize exposure to gonadotoxins, hyperthermia
  - E.g. smoking, medications, saunas, hot-tubs
- Vitamin (antioxidant) supplementation
  - Vitamins E, C, selenium, folate, zinc

    Fraga et al, *PNAS* 1992 — Greco et al, *J Androl* 2005
    Menezo et al, *RBM Online* 2007 — Silver et al, *J Androl* 2005
- Antibiotics for semen infection
- Varicocelectomy
  - Sperm DNA damage decreases after varicocele repair

    Zini et al, *Hum Reprod* 2005 — Werthman et al, *Fertil Steril* 2007
    Chen et al, *J Urol* 2008
- ICSI with testicular sperm
  - 18 couples: 2 failed ICSI & >15% sperm DNA damage (TUNEL)
  - TESE/ICSI clinical pregnancy rate: 44% (8/18)

    Greco et al, *Hum Reprod* 2004