# Relevance of Sperm DNA Fragmentation

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# Relevance of Sperm DNA Fragmentation

1. Sperm DNA peculiarity
2. Etiology of DNA fragmentation (DF)
3. Test methods
4. Influence of laboratory techniques
5. DF and outcome in infertility treatments
6. Conclusions

# 1. Sperm DNA peculiarity

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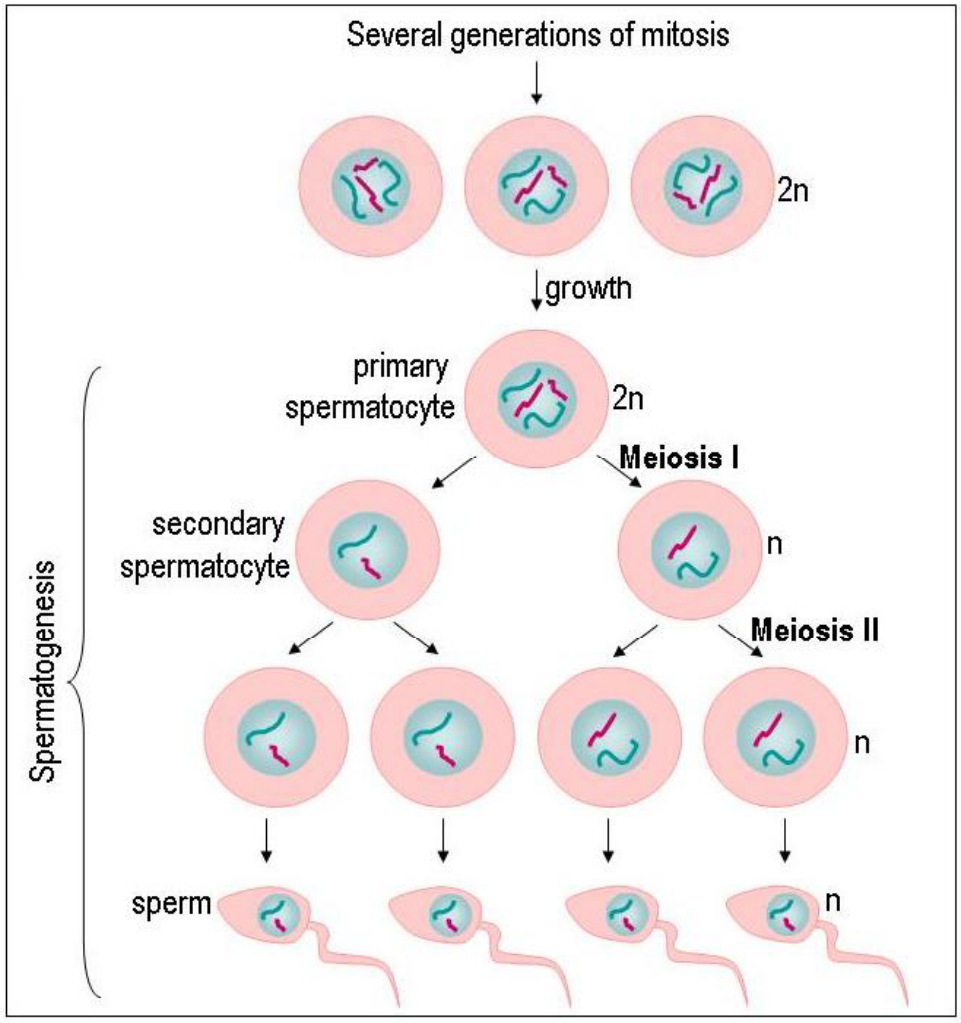
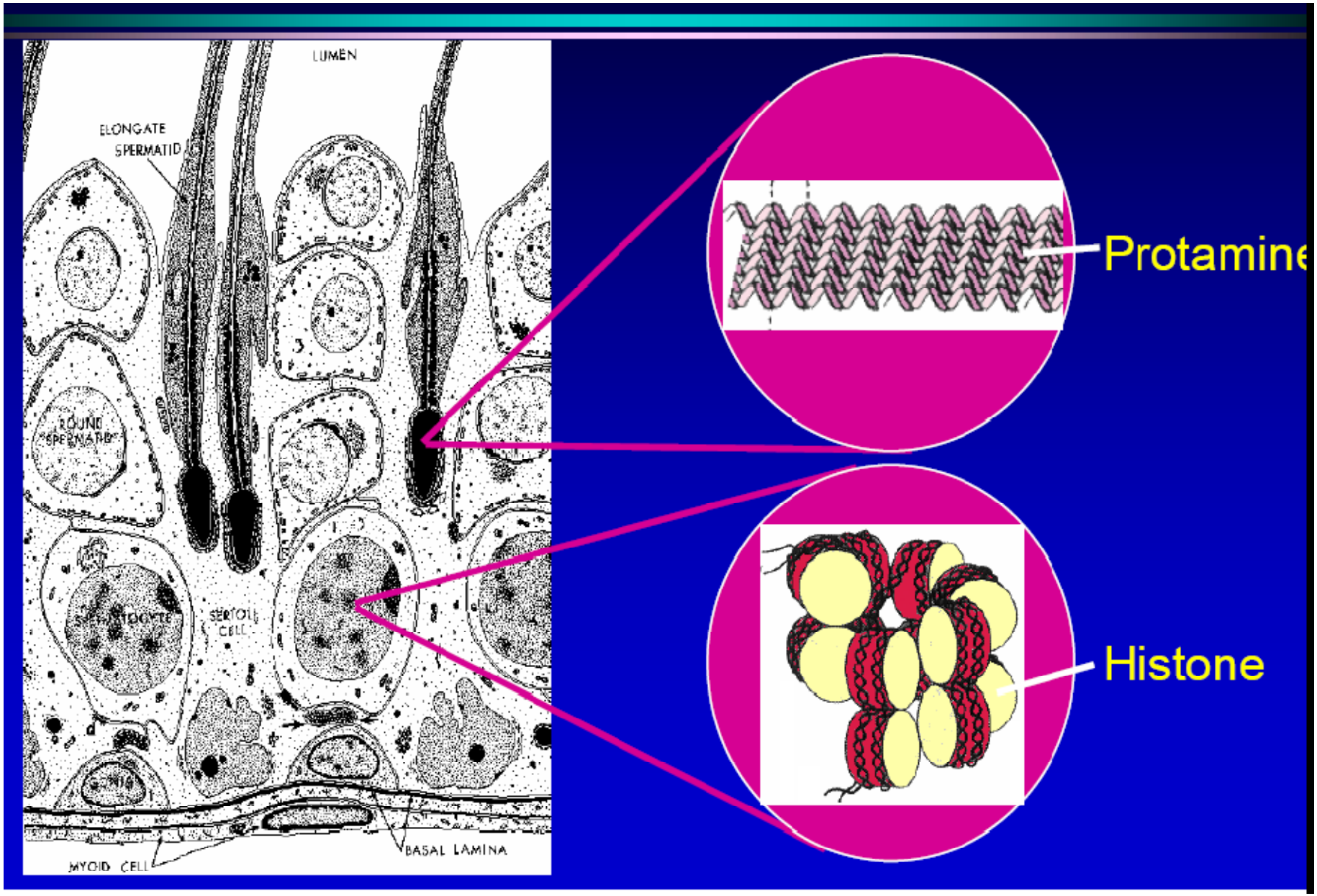



During spermiogenesis spermatids repackage their DNA with protamines, a small residue of histone-bound DNA is retained (15%).


LUMEN
ELONGATE SPERMATID
ROUND SPERMATID
SPERMATOCYTE
SERTOLI CELL
MYOID CELL
BASAL LAMINA
Protamine
Histone

# protamines

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- Are proteins with a high content of positively charged amino acids (48% arginine)
- Form a highly condensed complex with the sperm DNA (DNA has a strong negative charge)
- Incorporate cysteins
- Cysteins allow the formation of disulphide bonds between the protamines
- Therefore strongly stabilize the nucleoprotamine complex

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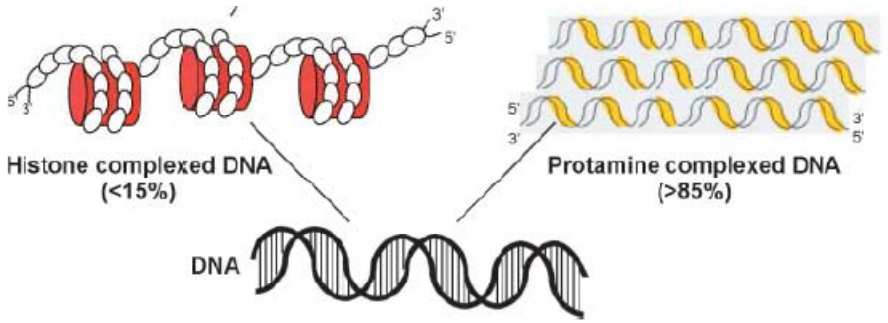
In mature sperm the DNA is

- 85% protamine bound
- 15 % remains histone bound

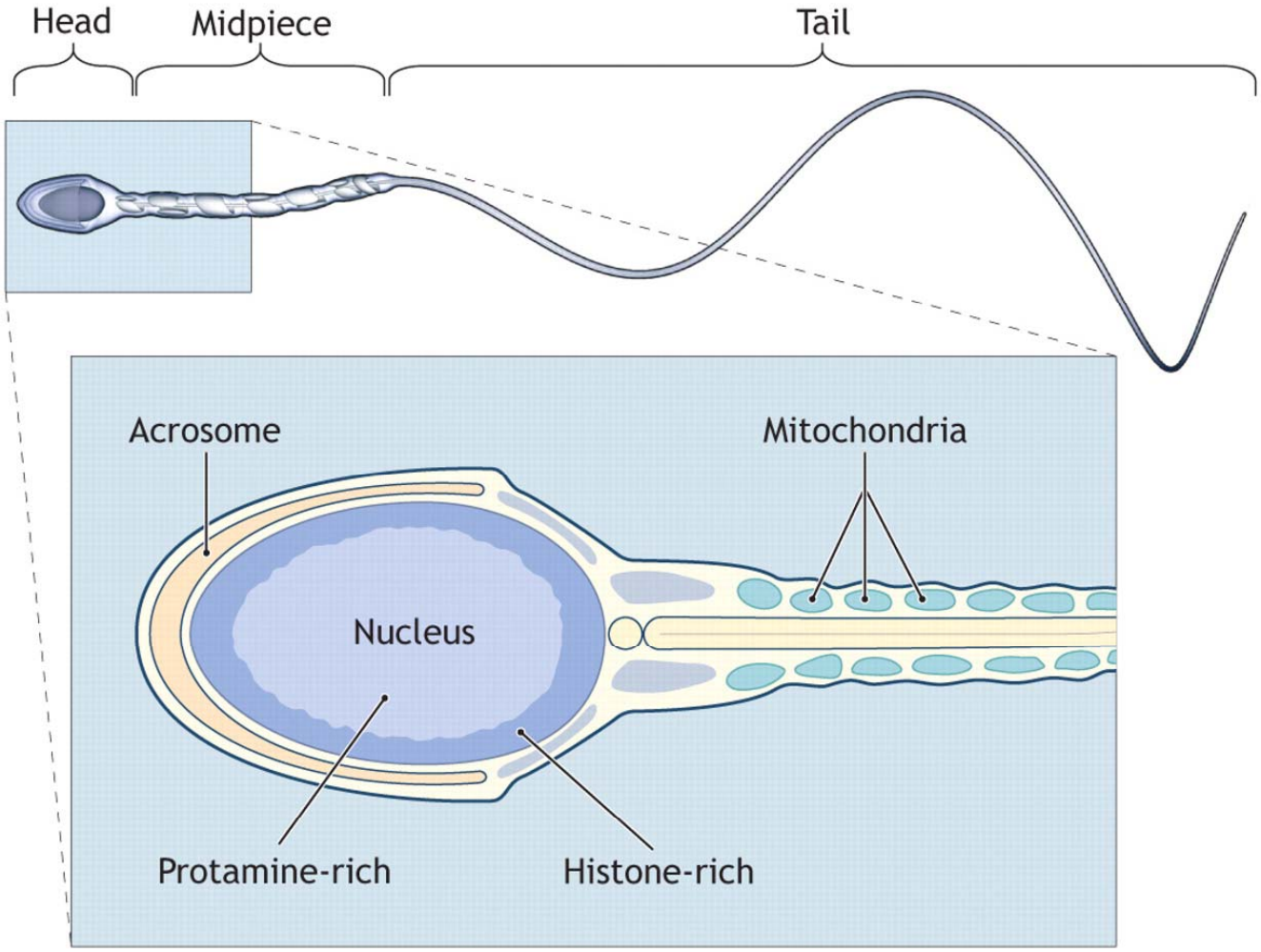



protamine deficiency: more susceptible for ROS

Infertile men have a higher histone:protamine ratio than fertile men
(Oliva 2006, Zhang 2006)

5-10% of infertile men have a complete protamine deficiency

# Fig. 1: The human sperm




**Zini, A. et al. CMAJ 2006;175:495-500**

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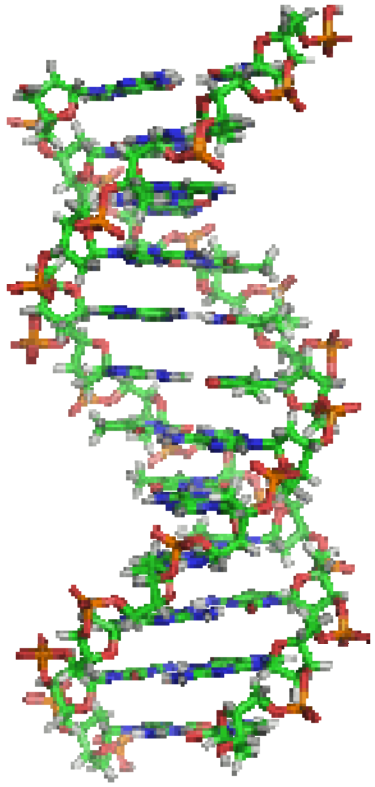
# 2. Etiology of DNA Damage

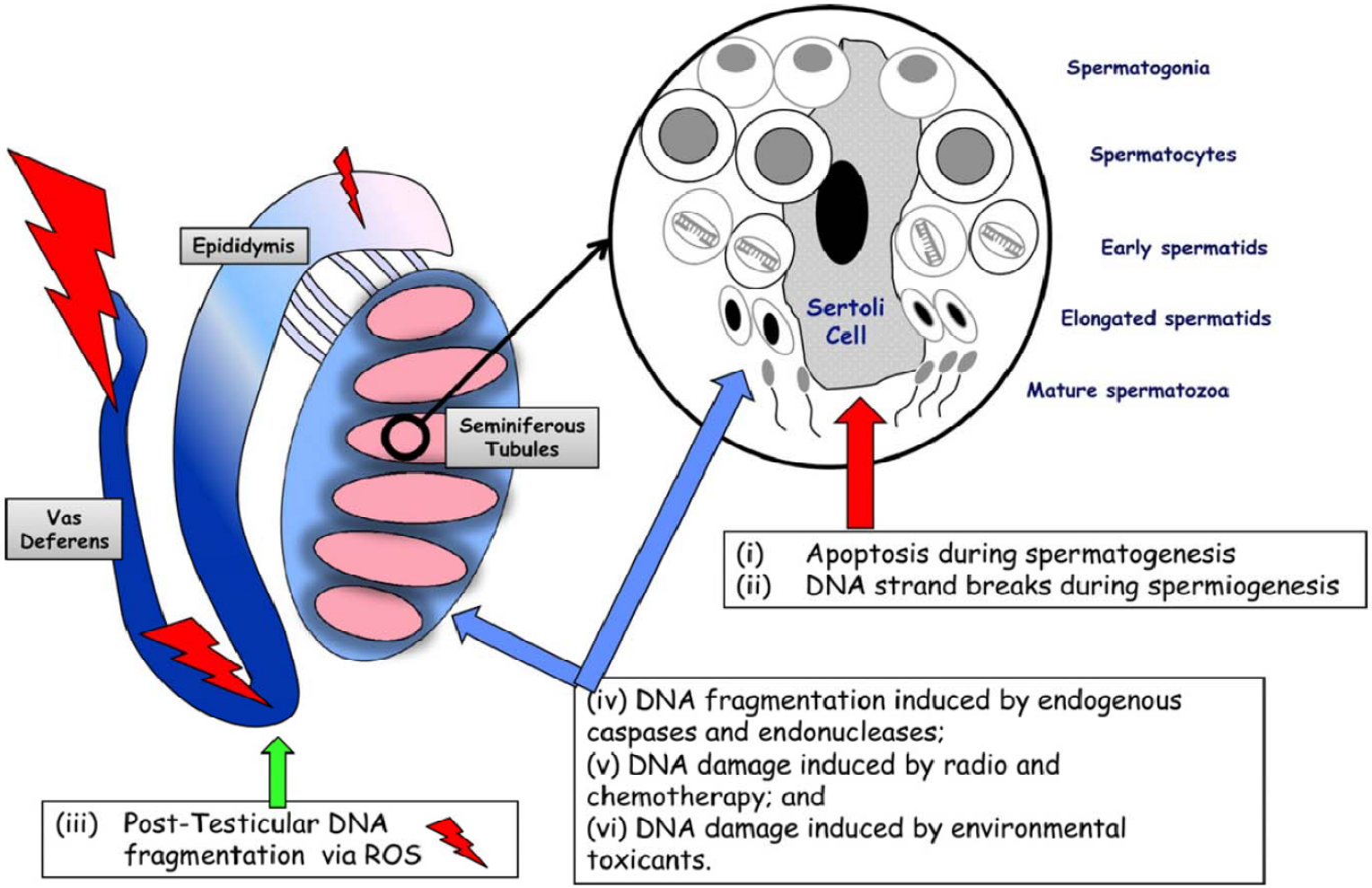
the etiology of sperm DNA damage is multifactorial

- In the testis during the process of spermatogenesis:
  - Apoptosis: screening mechanisms, that mark individual apoptotic sperms which causes phagocytosis of these cells, failed (double sb)
  - during remodeling of sperm chromatin the DNA is unwound through the induction of strand breaks (single sb), sperms with unrepaired strand breaks →semen.



## FIGURE 1

Major mechanisms of inducing DNA damage in spermatozoa during either the production or the transport of sperm cells: (i) apoptosis during the process of spermatogenesis; (ii) DNA strand breaks produced during the remodelling of sperm chromatin during the process of spermiogenesis; (iii) post-testicular DNA fragmentation induced, mainly by oxygen radicals, during sperm transport through the seminiferous tubules and the epididymis (increasing DNA damage is indicated by size of red flashes and gradient darkening in tract); (iv) DNA fragmentation induced by endogenous caspases and endonucleases; (v) DNA damage induced by radiotherapy and chemotherapy; and (vi) DNA damage induced by environmental toxicants.




*Sakkas. Sperm DNA fragmentation. Fertil Steril 2010.*

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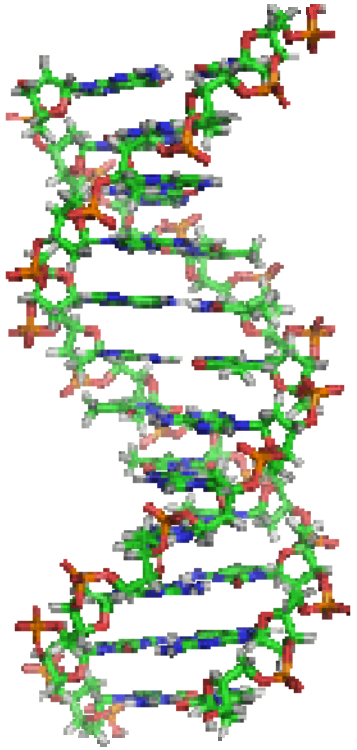
# Etiology of Sperm DNA Damage

- post-testicular:
  - during sperm transport through the seminiferous tubules and the epididymis
  - Varicocele
  - Genital tract infections (leukocytes)
  - Immature sperms (cytoplasmic droplet)
  - radio- and/or chemotherapy
  - Lifestyle factors (obesity, cell phones, nicotine), environmental toxicants
  - Laboratory factors



- Majority of DNA damage is associated with ROS (Aitken et al., 2010)

# ROS

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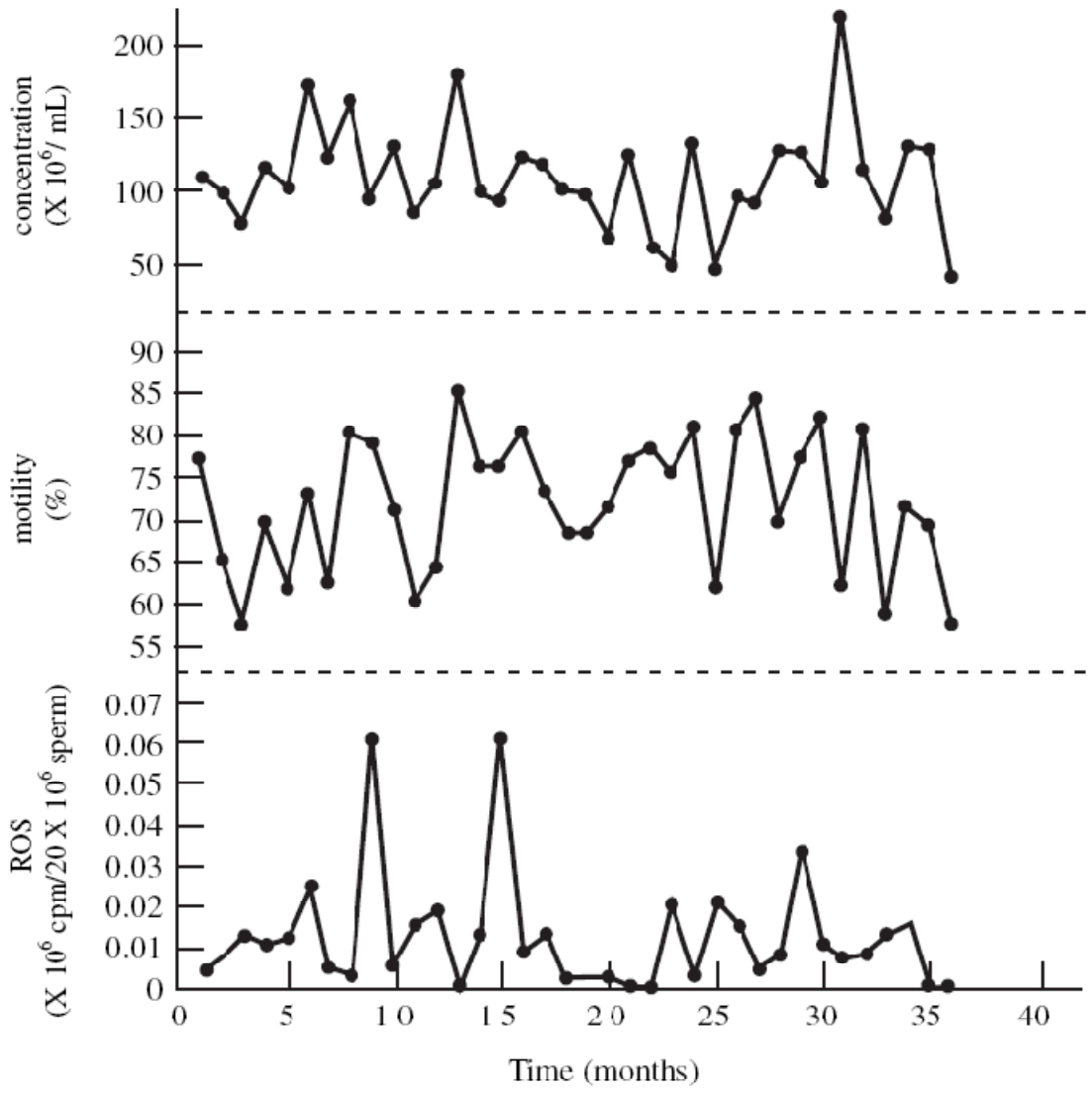
- Small levels are essential for normal sperm functions (capacitation, acrosome reaction, sperm-oocyte fusion (Sikka et al, 1995)
- Balance between ROS production and scavenging system is important
- in 25% of infertile men high ROS levels have been detected in their semen.

Variations in concentration, motility, and reactive oxygen species (ROS) in 36 semen samples provided by a single fertile donor over a period of 21 months.




*Desai. Correspondence. Fertil Steril 2010.*

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levels of ROS may fluctuat **within a fertile man** but do not affect sperm concentration and motility. This may be possible due to the presence of adequate antioxidant defense mechanismsin the present healthy individual.

this variations may have physiologic, seasonal, or lifestyle-related causes

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# 3. DNA Fragmentation Tests

## DFI: DNA Fragmentation Index %

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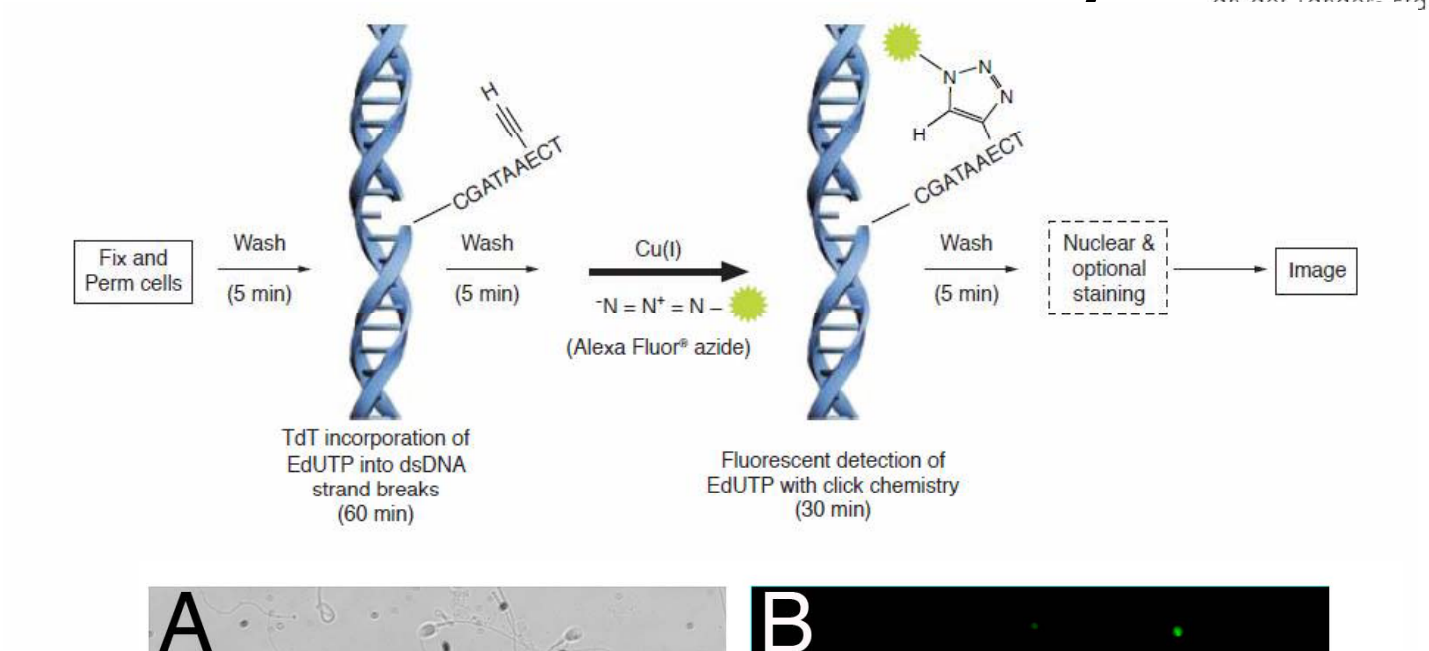
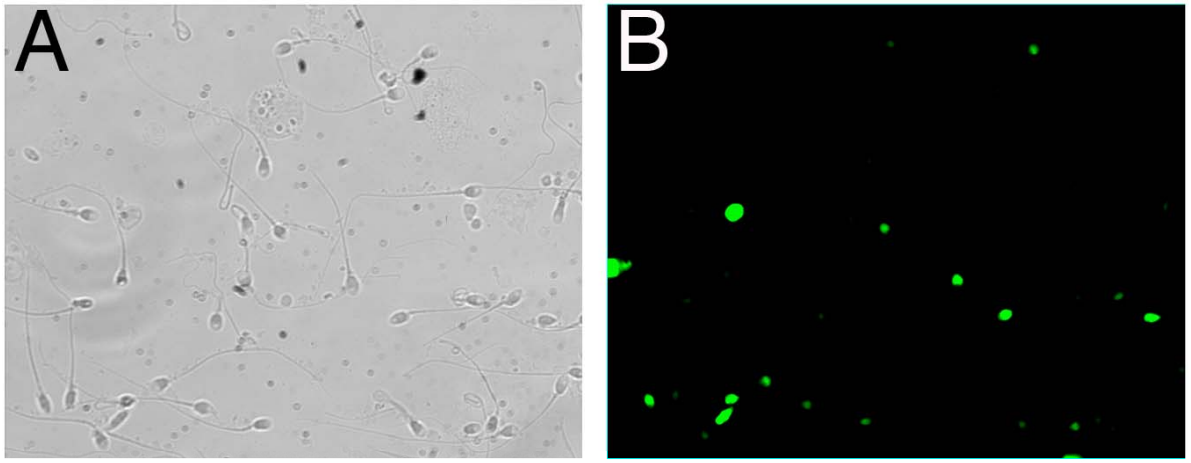
# DNA fragmentation - Tests

- DIRECT
  - TUNEL (terminal deoxynucleotydil transferase-mediated deoxyuridin triphosphate- nick-end labeling assay ) (s&dSB)
  - ISNT (in situ-nick translation)
  - Comet Assay at neutral pH (sdSB) (single cell gel electrophoresis
- INDIRECT (need denaturation of DNA)
  - SCSA (sperm chromatin structure assay)
  - SCD (sperm chromatin dispersion test, Halosperm Assay)
  - Comet Assay at acid or basic pH (sdSB)

# TUNEL assay




Labels single and double strand breaks, quantifies the incorporation of flourescent dUTP

# SCSA Test

## sperm chromatin structure assay

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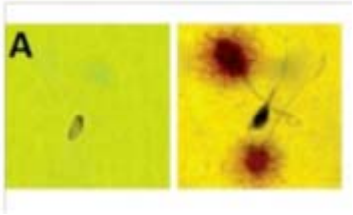
acid-induced denaturation of DNA followed by staining with AO.

is a flow-cytometric method, measures the metachromatic shift of AO fluorescence from green to red

- green (native DNA)
- red (denatured DNA).



# SCD or Halo Test

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The SCD is based on the principle that sperm with fragmented DNA fail to produce the dispersed DNA loops after acidic denaturation (halos)

Normal sperm produce a halo

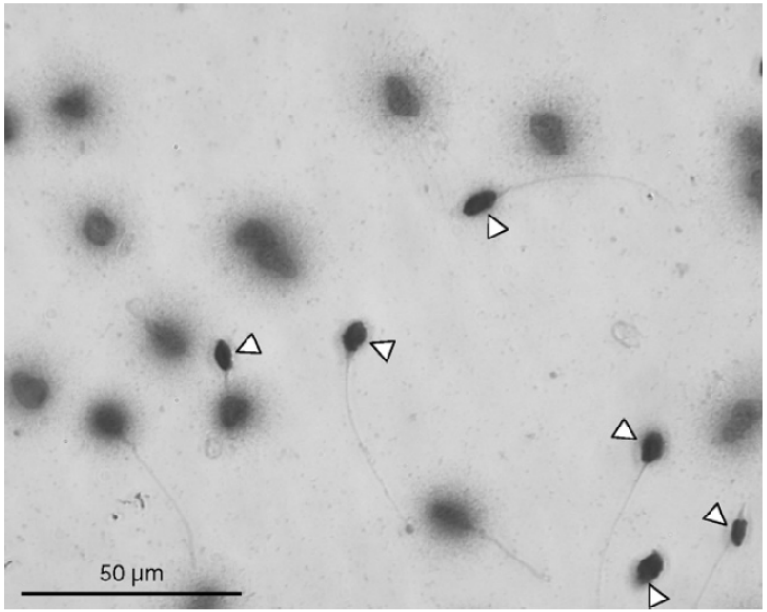


**Figure 3** Sperm chromatin dispersion (SCD) test in a patient showing approximately 30% strandbreak-positive spermatozoa in the raw semen. Arrow heads indicate spermatozoa with no or minor halos (i.e. those with strand breaks). Bar = 50 µm.

Ebner et al, 2011

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*Comparison of sperm DNA fragmentation in infertile men and fertile donors; values are mean ± SEM; different superscript lowercase letters show statistical difference (P < .05) within rows; different superscript capital letters show statistical difference within columns (P < .05)**

| | SCSA | TUNEL | SCD | ACT |
|---|---|---|---|---|
| Infertile men (n = 60) | $22.0 \pm 1.6^{aA}$ | $19.5 \pm 1.3^{aA}$ | $20.4 \pm 1.3^{aA}$ | $31.3 \pm 2.4^{bA}$ |
| Donors (n = 7) | $11.8 \pm 1.4^{aB}$ | $11.1 \pm 0.9^{aB}$ | $10.8 \pm 1.1^{aB}$ | $32.7 \pm 4.8^{bA}$ |

* SCSA indicates sperm chromatin structure assay; TUNEL, TdT-mediated-dUTP nick and labeling; SCD, sperm chromatin dispersion; and ACT, acridine orange staining technique.

Observed a strong relationship between the tests.

AO only coincided on values >30%, (technical problems of instinct colours, rapid fading, heterogenous staining of slides, also reported in other studies)

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**TABLE 1**

**Seminal characteristics of infertile patients and fertile men.**

| | Fertile men (n = 30) | Infertile patients (n = 60) | Total (n = 90) |
|---|---|---|---|
| Basic seminal parameters | | | |
| Age (y) | 33.8 ± 5.0 | 34.7 ± 4.0 | |
| Sperm concentration ($\times 10^6$/mL) | 64.9 ± 25.7 | 35.4 ± 25.3[d] | |
| Forward motility (%) | 58.5 ± 8.6 | 47.4 ± 9.7[d] | |
| Normal morphology (%) | 19.8 ± 4.1 | 14.3 ± 5.1[d] | |
| Sperm DNA fragmentation (%) | | | |
| SCD test | 13.6 ± 3.4[a] | 25.2 ± 10.2[d,b] | 21.4 ± 10.1[c] |
| TUNEL assay | 12.9 ± 3.0 | 22.8 ± 6.9[d] | 19.5 ± 7.5 |

*Note:* Values are mean ± SD.

[a] $P$=.155, compared with TUNEL assay.

[b] $P$=.001, compared with TUNEL assay.

[c] $P$=.000, compared with TUNEL assay.

[d] $P$<.001, Compared with fertile group.

*Zhang. Sperm DNA fragmentation. Fertil Steril 2010.*


4. Influence of lab procedures

# storage and sperm DFI

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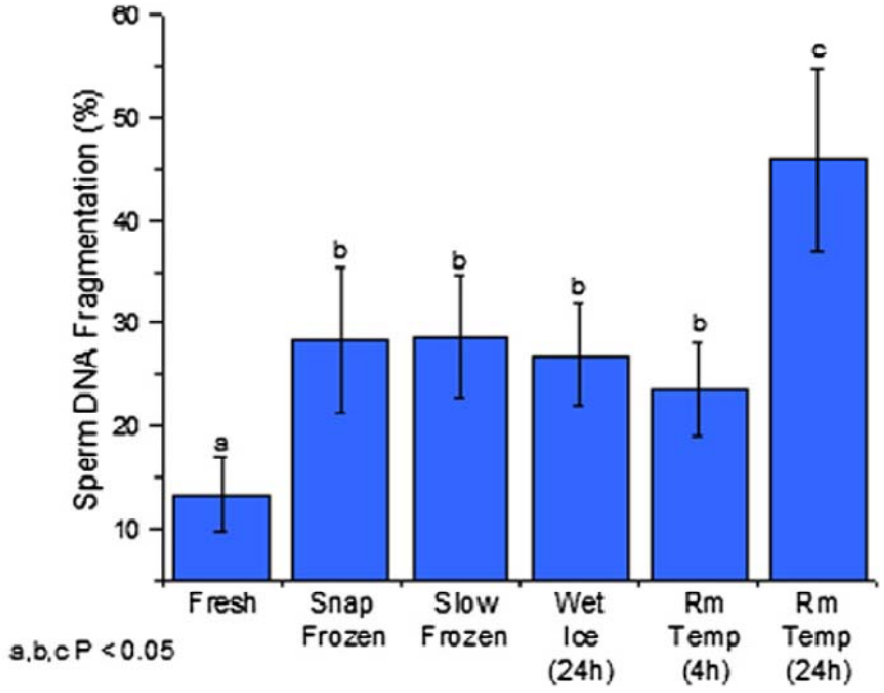
**FIGURE 2**

Levels of DNA fragmentation following treatment of sperm by different storage methods represented as mean ± standard error. There were no significant differences between freezing methods. The mean for all freezing methods was significantly less than for the 24-hour room temperature group.




*Jackson. Storage, processing, and sperm DNA integrity. Fertil Steril 2010.*

Unselected group

Our data indicate that any sample that will not be analyzed within 4 hours of collection should be frozen to prevent increasing DNA damage.

Among different methods of freezing, there was no statistically significant difference in the resultant amount of DNA fragmentation

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Relationships between fragmented DNA in fresh, frozen–thawed, and post-cryopreservation incubated testicular sperm.

| | DNA fragmentation (%) | |
|---|---|---|
| Time point of analysis | Mean ± S.E. | *P* value |
| Fresh | 10.6 ± 1.02 | — |
| 4-hour | 22.1 ± 3.49 | .052 |
| 24-hour | 19.1 ± 2.33 | .017 |
| Frozen–thawed | 16.5 ± 1.00 | .0001 |
| 4-hour post-thaw | 29.5 ± 1.45 | .00004 |
| 24-hour post-thaw | 30.4 ± 1.71 | <.00001 |

*Note:* *P* values are comparisons to fresh data; n = 34.

*Dalzell. Incubation of testicular sperm. Fertil Steril 2004.*

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Sperm motility and DNA denaturation before and after density-gradient centrifugation.

| | Sperm motility (%) | | | Sperm DNA denaturation (%) | | |
|---|---|---|---|---|---|---|
| Group | Whole semen | Processed | *P*-value[a] | Whole semen | Processed | *P*-value |
| Fertile men | 49 ± 7 | 71 ± 6 | 0.004 | 8 ± 2 | 9 ± 3 | 0.43 |
| Infertile men | 44 ± 3 | 56 ± 3 | 0.0005 | 15 ± 2 | 25 ± 3 | 0.0001 |

*Note:* Values are means ± SEM.

[a] Comparison is between whole and processed semen.

*Zini. Semen quality and DNA integrity. Fertil Steril 2000.*

**The potential detrimental effect of density gradient centrifugation on sperm DNA integritiy is related to initial semen quality (Zini)**

Sperms of infertile patients are more susceptible to external influences

| Autor | Factor | |
|---|---|---|
| Peer et al (2007) | Incubation at 37 °C (2 hrs) | ↑ vacuolated nuclei |
| | Incubation at 21 °C (2hrs) | no sign. changes |
| | Incubation at 37 °C (21 hrs) | ↑ vacuolated nuclei like 2hrs |
| Zini et al (2000) | After Sperm processing | |
| | Fertile men | No increased DNA denaturation |
| | Infertile men | Increased DNA denaturation |
| Muratori et al (2003) | Swim-up selected sperms | |
| | normospermic | No increase in DNA fragmentation during long term incubation |
| | teratospermic | Increasing DNA fragmentation during long term incubation |
| Donnelly et al (2000) | After percoll preparation (normo and astheno) | Signif less DNA fragmented sperms |
| Lewis (2004) | Incubations of testicular sperms (37 °C) | 24 hrs optimal for development of motility-<br>But 50% more DNA fragm |
| | Frozen thawed Tese | ↑ DNA fragm |
| | Incub of frozen thawed Tese | ↑ DNA fragm |
| Huszar et al (2004) | Overnight shipping simulated by storage in 2-4 °C for 24hrs | DNA integrity remained unchanged |

# Sperm processing and DFI

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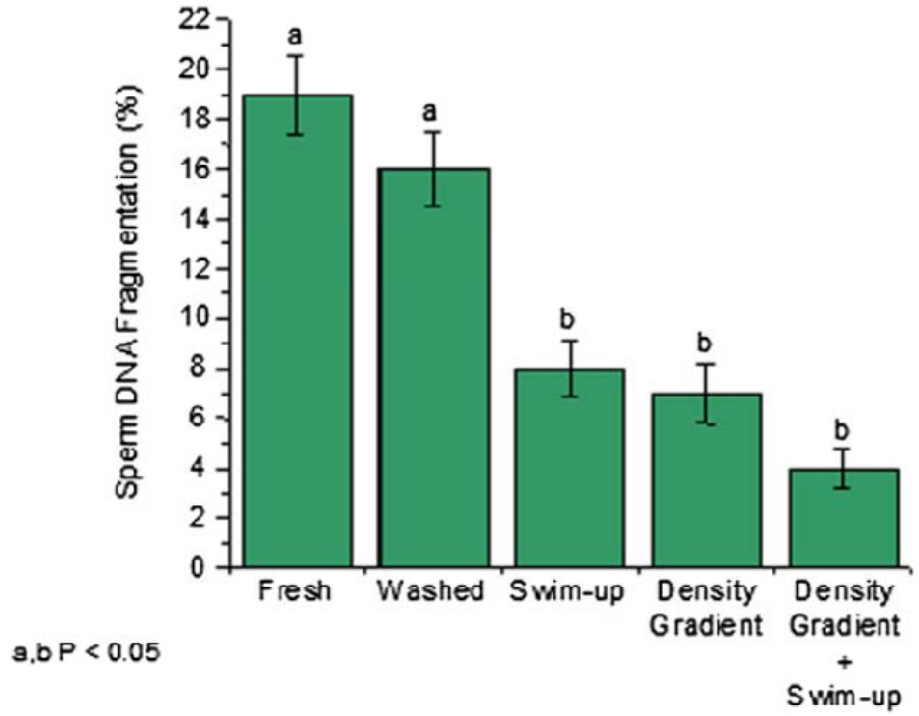
**FIGURE 3**

Levels of DNA fragmentation following treatment of sperm by different separation methods represented as mean ± standard error. The mean for swim-up, density gradient centrifugation, and density gradient centrifugation + swim-up groups was significantly less than for fresh and wash groups.




*Jackson. Storage, processing, and sperm DNA integrity. Fertil Steril 2010.*

- DFI Levels immediately following processing were significantly lower for swim-up, density gradient and DG &SU than for fresh and washed semen samples.

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# Easy sperm processing technique allowing exclusive accumulation and later usage of DNA-strandbreak-free spermatozoa

T Ebner [a,*], O Shebl [a], M Moser [a], RB Mayer [a], W Arzt [b], G Tews [a]

**Abstract** Sperm DNA fragmentation is increased in poor-quality semen samples and correlates with failed fertilization, impaired preimplantation development and reduced pregnancy outcome. Common sperm preparation techniques may reduce the percentage of strandbreak-positive spermatozoa, but, to date, there is no reliable approach to exclusively accumulate strandbreak-free spermatozoa. To analyse the efficiency of special sperm selection chambers (Zech-selectors made of glass or polyethylene) in terms of strandbreak reduction, 39 subfertile men were recruited and three probes (native, density gradient and Zech-selector) were used to check for strand breaks using the sperm chromatin dispersion test. The mean percentage of affected spermatozoa in the ejaculate was 15.8 ± 7.8% (range 5.0–42.1%). Density gradient did not significantly improve the quality of spermatozoa selected (14.2 ± 7.0%). However, glass chambers completely removed 90% spermatozoa showing strand breaks and polyethylene chambers removed 76%. Both types of Zech-selectors were equivalent in their efficiency, significantly reduced DNA damage ($P < 0.001$) and, with respect to this, performed better than density gradient centrifugation ($P < 0.001$). As far as is known, this is the first report on a sperm preparation technique concentrating spermatozoa unaffected in terms of DNA damage. The special chambers most probably select for sperm motility and/or maturity.



90% of patients showed no strandbreaks after processing

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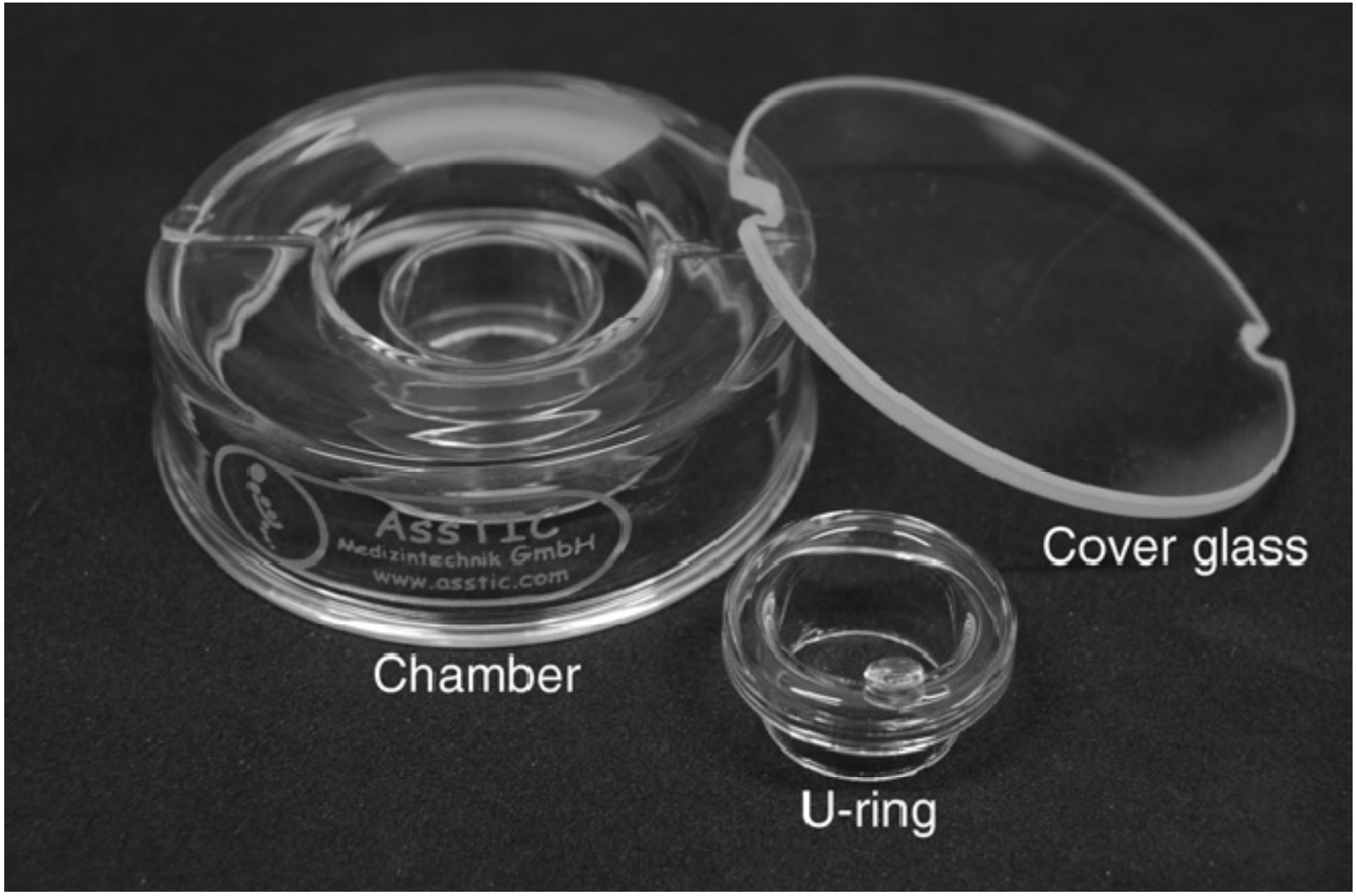


Ebner et al, 2010

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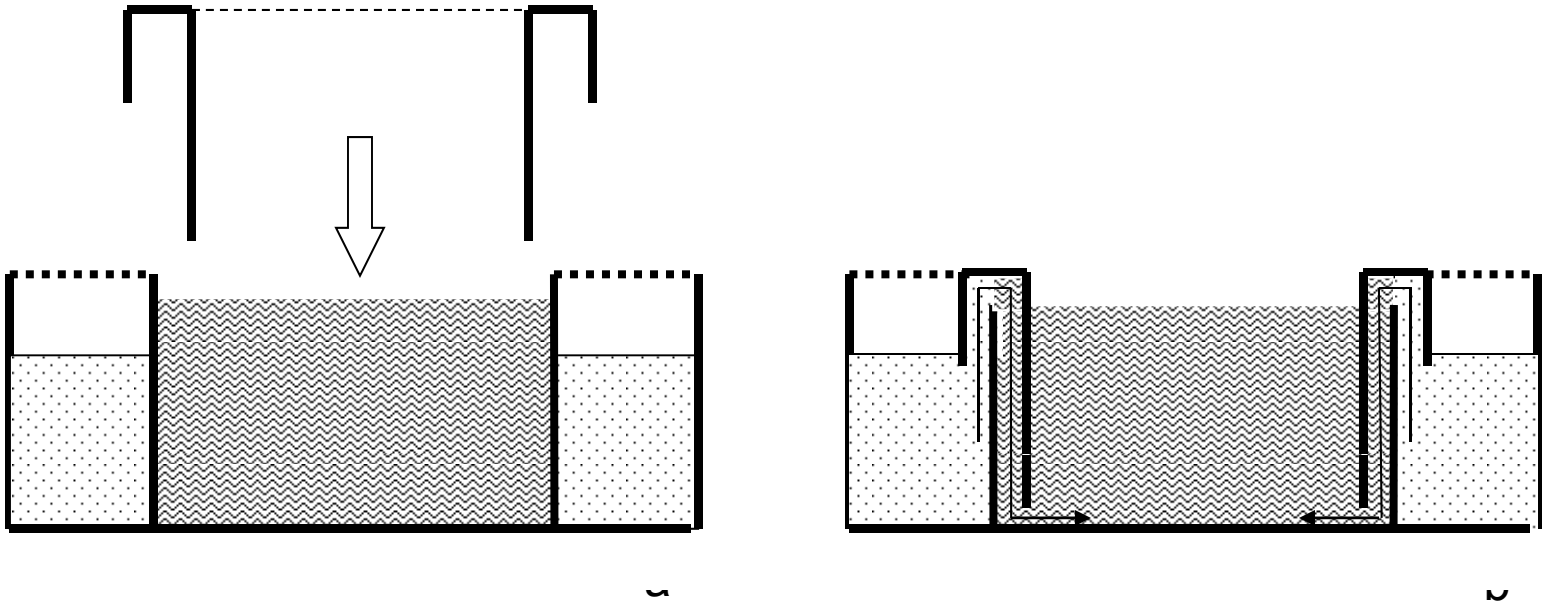

Swim-up for maximum 2 hours

Ebner et al, 2010

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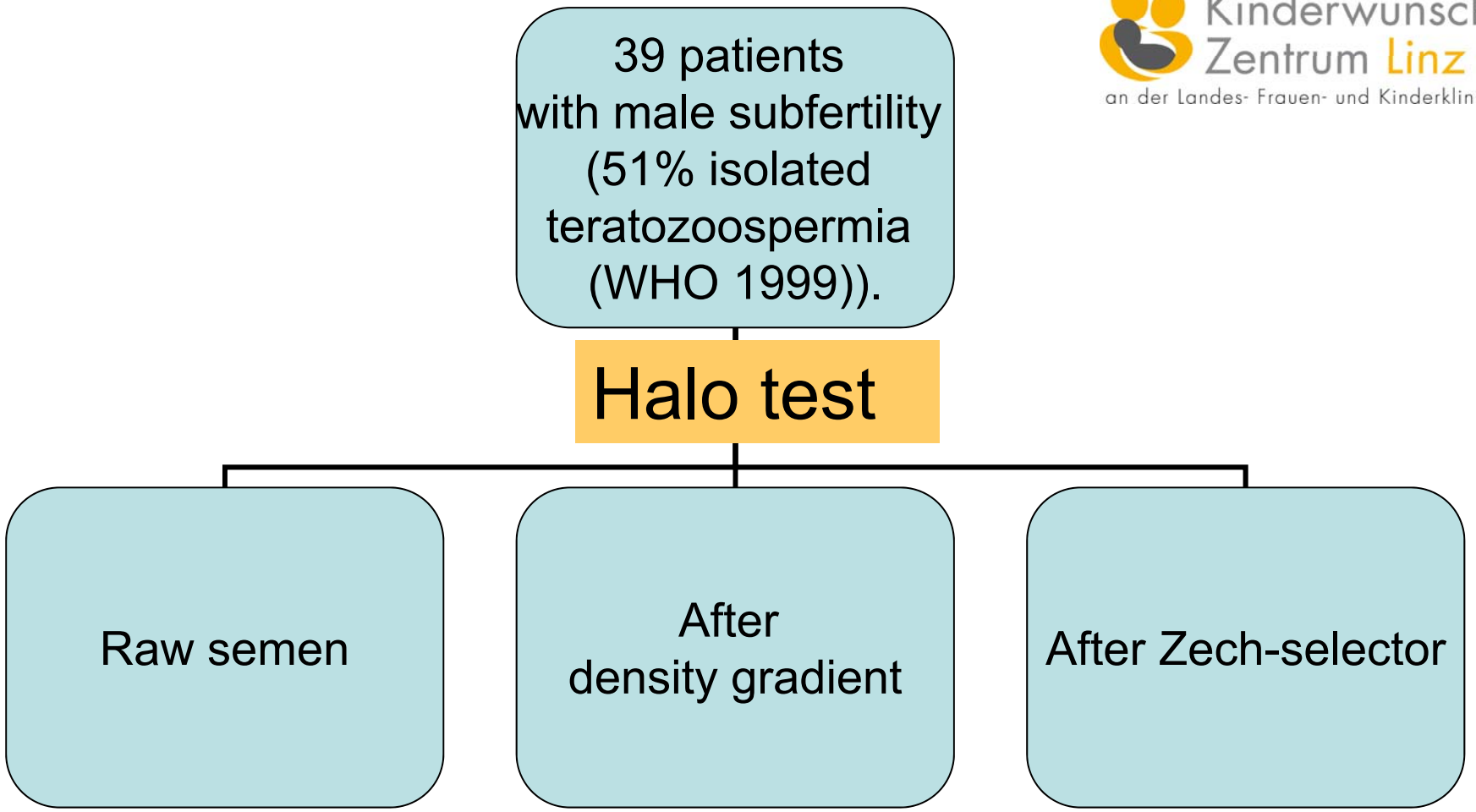


Ebner et al, 2011

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| | DFI | motility |
|---|---|---|
| Raw semen | 15.8 ± 7.8% | 36.6 ± 19.4 % a and b |
| Density gradient | 14.2 ± 7.0% | not investigated |
| Zech Selector | 0.4 ± 1.1% | 100% a |

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- Motility seems to be the utmost factor, since the selector separates spermatozoa according to their motilty and not to their morphology.
- This is supported by the literature Ramos and Wetzels, 2001, Van den Berg et al 1998) and by our own observation, that the sperm swimming close to the surface of the supernatant show significantly reduced rates of DF after density centrifugation and swim-up.
- Simply overlaying a sperm sample with medium, (w/o centrifugation) could lead to similar results.

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## Why do fast progressive sperms show no sign of DF?

- Both nuclear and mitochondrial DNA can be harmed by strand breaks
- Mitochondrial DNA could cause alterations in ATP production, which is a prerequisite for optimal sperm motility
- Deletions within mitochondrial DNA have been associated with reduced sperm motility (Ozmen et al, 2007)

- It could be possible, that grade a spermatozoa could be neither harmed by nuclear or mitochondrial DNA damage
- In the selector grade b spermatozoa cannot overcome the capillary gap between the outer and the inner ring in 2 hours due to their reduced energy and forward movement.

# Correlation of sperm parameters and DFI

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| | Yes | No |
|---|---|---|
| Oligozoospermia | Burallo et al 2004<br>Host et al 1999<br>Tomlinson 2001 | Gandini et al. 2000 |
| Teratozoospermia | Host et al 1999<br>Muratori et al 2003<br>Trisini et al 2004<br>Tomlinson 2001 | Chan et al. 2001<br>Donnely et al 2001 |
| Asthenozoospermia | Giwercman et al 2003<br>Irvine et al 2000<br>Mahfouz 2010<br>Varghese ez al 2009 | ?? |

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# 5. Is DFI testing relevant for fertility assessment

# DFI and IUI

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**The predictive value of sperm chromatin structure assay (SCSA) parameters for the outcome of intrauterine insemination, IVF and ICSI**

M.Bungum[1,4], P.Humaidan[1], M.Spano[2], K.Jepson[3], L.Bungum[1] and A.Giwercman[3]

**Sperm DNA integrity assessment in prediction of assisted reproduction technology outcome**

M.Bungum[1,2,5], P.Humaidan[1], A.Axmon[3], M.Spano[4], L.Bungum[1], J.Erenpreiss[2] and A.Giwercman[2]

- with a DFI < 27% the chances for pregnancy are significantly higher than in patients with a DFI > 27%

# Fertility potential, threshold levels

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**Evenson et al (1999), Spano (2000):**

If >30% sperm have abnormal chromatin: fertility is hampered independent of sperm number, morphology and motility.

**Evenson et al (2002**):

Fertility potential according to DFI fraction:

Excellent <15%

Good 15-24%

Fair 25-30%

poor >30%

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Achievement of pregnancy:

– 84% of men with DFI of <15% achieved a pregnancy during the first 3 months

– 10% of men with a DFI<30% achieved a pregnancy during months 4-12

– 20% of men with a DFI > 30%  never achieved a pregnancy

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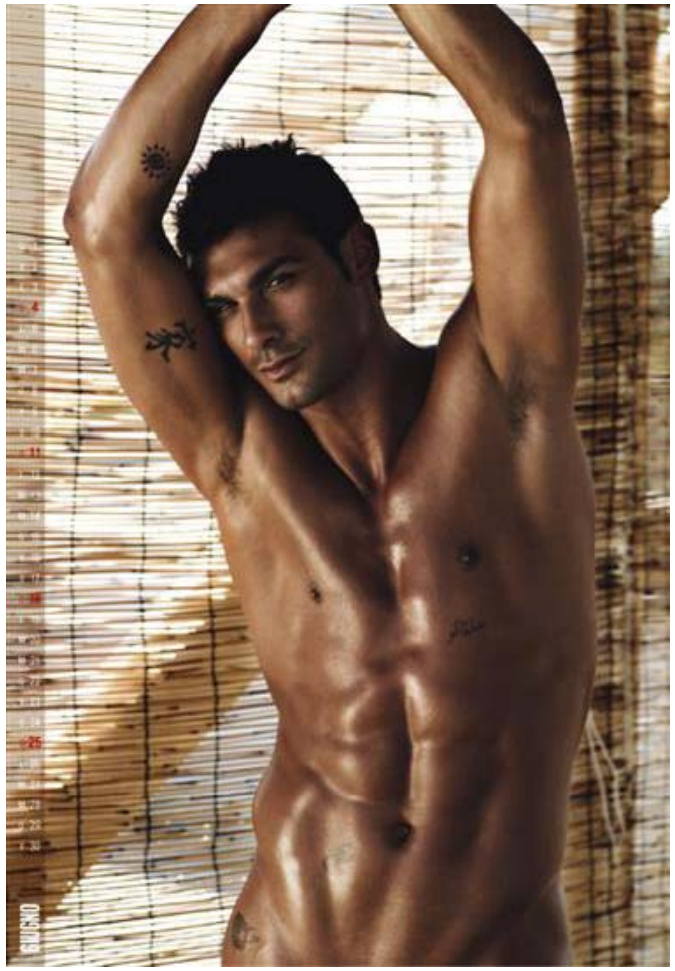
- in the general population:
  1-4% of men have a DFI > 30%.
- Infertility patients
  – a DFI > 30% have
    - IUI patients: 7%
    - IVF patients: 16%
    - ICSI patients: 33%



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**Human Reproduction Vol.21, No.8 pp. 2061–2064, 2006**
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# Intra-individual variation in sperm chromatin structure assay parameters in men from infertile couples: clinical implications

**J.Erenpreiss[1,2,5], M.Bungum[1,3], M.Spano[4], S.Elzanaty[1], J.Orbidans[2] and A.Giwercman[1]**

| | 1.Test | 2.Test | 1.Test | 2.Test |
|---|---|---|---|---|
| DFI % | >30% | <30% | <30% | >30% |
| Patients | | 37 % | | 27 % |

Retrospective study on 282 patients

**TABLE 1**

**Methodological features: studies of the association between sperm DNA fragmentation and pregnancy.**

| Study | Treatment | Assay | Normal range | Cycles | Pregnancy outcome | Outcome rates (%) |
|---|---|---|---|---|---|---|
| Boe-Hanson et al., 2006 (46) | IVF | SCSA | DFI <27% | 139 | Clinical | 28 |
| | ICSI | SCSA | DFI <27% | 47 | Clinical | 30 |
| Borini et al., 2006 (52) | IVF | TUNEL | <10% | 82 | Clinical | 22 |
| | ICSI | TUNEL | <10% | 50 | Clinical | 24 |
| Bungum et al., 2007 (27) | IVF | SCSA | DFI <30% | 388 | Delivery | 28 |
| | ICSI | SCSA | DFI <30% | 223 | Delivery | 38 |
| Check et al., 2005 (47) | IVF | SCSA | DFI <30% | 106 | Ongoing | 17 |
| Gandini et al., 2004 (48) | ICSI | SCSA | DFI <30% | 22 | Full term | 41 |
| Host et al., 2000 (53) | IVF | TUNEL | ≤4% | 175 | Biochemical | 29 |
| | ICSI | TUNEL | ≤4% | 61 | Biochemical | 34 |
| Huang et al., 2005 (54) | IVF | TUNEL | ≤4% | 217 | Pregnancy | 55 |
| | ICSI | TUNEL | ≤4% | 86 | Pregnancy | 51 |
| Larson et al., 2000 (24) | IVF, ICSI | SCSA | DFI <27% | 24 | Pregnancy | 29 |
| Larson-Cook et al., 2003 (25) | IVF, ICSI | SCSA | DFI <27% | 89 | Clinical | 31 |
| Payne et al., 2005 (49) | IVF, ICSI | SCSA | DFI <27% | 94 | Clinical | 33 |
| Seli et al., 2004 (14) | IVF, ICSI | TUNEL | <20% | 49 | Clinical | 47 |
| Virro et al., 2004 (50) | IVF, ICSI | SCSA | DFI <30% | 249 | Ongoing | 41 |
| Zini et al., 2005 (51) | ICSI | SCSA | DD ≤30% | 60 | Clinical | 52 |

*Note:* DD, sperm DNA denaturation; DFI, DNA fragmentation index; SCSA, sperm chromatin structure assay; TUNEL, terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate-biotin nick end labeling assay.

*Collins. Sperm DNA integrity tests. Fertil Steril 2008.*

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# Do sperm DNA integrity tests predict pregnancy with in vitro fertilization?

*John A. Collins, M.D.,*[a] *Kurt T. Barnhart, M.D.,*[b] *and Peter N. Schlegel, M.D.*[c]

**Conclusion(s):** The small but statistically significant association between sperm DNA integrity test results and pregnancy in IVF and ICSI cycles is not strong enough to provide a clinical indication for routine use of these tests in infertility evaluation of men. It is possible that yet to be determined subgroups of infertile couples may benefit from sperm DNA integrity testing. (Fertil Steril® 2008;89:823–31. ©2008 by American Society for Reproductive Medicine.)

Negative Influence of DNA damage on

| Author | n | Assay | Fertilization | Embryo quality | Pregnancy | pregnancy loss | P-value |
|---|---|---|---|---|---|---|---|
| Lopes et al. (1998) | 131 | TUNEL | Yes | No | na | na | <0.05 |
| Sun et al. (1997) | 143 | TUNEL | Yes | Yes | na | na | <0.01 |
| Tomlinson et al. (2001) | 140 | NT | No | No | Yes | na | <0.05 |
| Morris et al. (2002) | 60 | Comet | No | No | No | No | |
| Tomsu et al. (2002) | 40 | Comet | No | Yes | Yes | na | <0.05 |
| Virant-Klun et al. (2003) | 183 | AO | Yes | Yes | No | No | <0.05 |
| Benchaib et al. (2003) | 104 | TUNEL | Yes | No | Yes | na | <0.05 |
| Larson-Cook et al. (2003) | 89 | SCSA | No | No | Yes | na | <0.01 |
| Bungum et al. (2004;2007;2008) | 1296 | SCSA | na | na | No | No | |
| Gandini et al. (2004) | 34 | SCSA | No | No | No | No | |
| Henkel et al. (2004) | 249 | TUNEL | No | nd | Yes | nd | <0.05 |
| Seli et al. (2004) | 49 | TUNEL | No | Yes | No | na | <0.05 |
| Virro et al. (2004) | 249 | SCSA | No | Yes | Yes | No | <0.01 |
| Huang et al. (2005) | 303 | TUNEL | Yes | na | No | na | <0.05 |
| Payne et al. (2005) | 100 | SCSA | Yes | No | No | No | <0.05 |
| Borini et al. (2006) | 132 | TUNEL | na | na | Yes | Yes | <0.01 |
| Muriel et al. (2006) | 85 | SCD | Yes | Yes | No | na | <0.05 |
| Frydman et al. (2008) | 117 | TUNEL | No | No | Yes | Yes | <0.001; <0.01 |
| Lin et al. (2008) | 223 | SCSA | No | No | No | Yes | <0.05 |
| Avendano et al. (2010) | 36 | TUNEL | na | Yes | Yes | na | <0.001; <0.05 |
| Simon et al. (2010) | 360 | Comet | Yes | Yes | Yes | No | <0.05 |
| Speyer et al. (2010) | 347 | SCSA | No | No | Yes | No | <0.01 |
| Meseguer et al. (2011) | 210 | SCD | na | na | Yes | No | <0.05 |

n: number of cycles

Table follows reviews of **Sharma et al (2004)**, **Zini and Libman (2006)** and **Zini and Zigman (2009)**.

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23 papers that investigated the influence of DNA damage on

| | **Fertilization** | **Embryo quality** | **Pregnancy** | **Pregnancy loss** |
|---|---|---|---|---|
| Total nr.of papers | **19** | **18** | **21** | **12** |
| **YES** | **8** | **8** | **12** | **3** |
| **NO** | **11** | **10** | **9** | **9** |

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# DFI and pregnancy loss

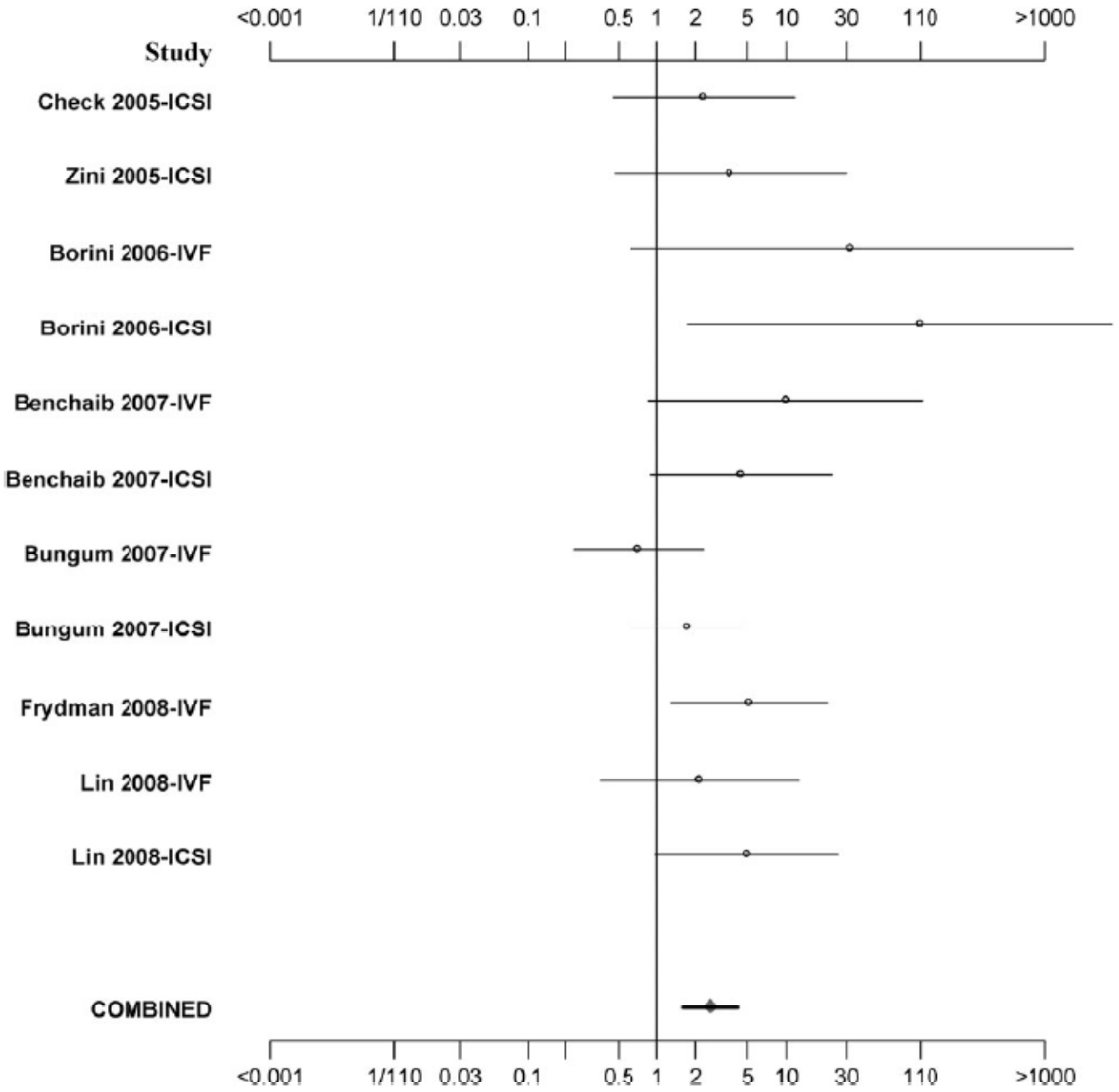


**Figure 1:** Forest plot depicting odds ratio (OR) and 95% confidence interval (CI) of the 11 studies and the combined OR from the meta-analysis

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Advance Access publication on August 29, 2008
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# Sperm DNA damage is associated with an increased risk of pregnancy loss after IVF and ICSI: systematic review and meta-analysis

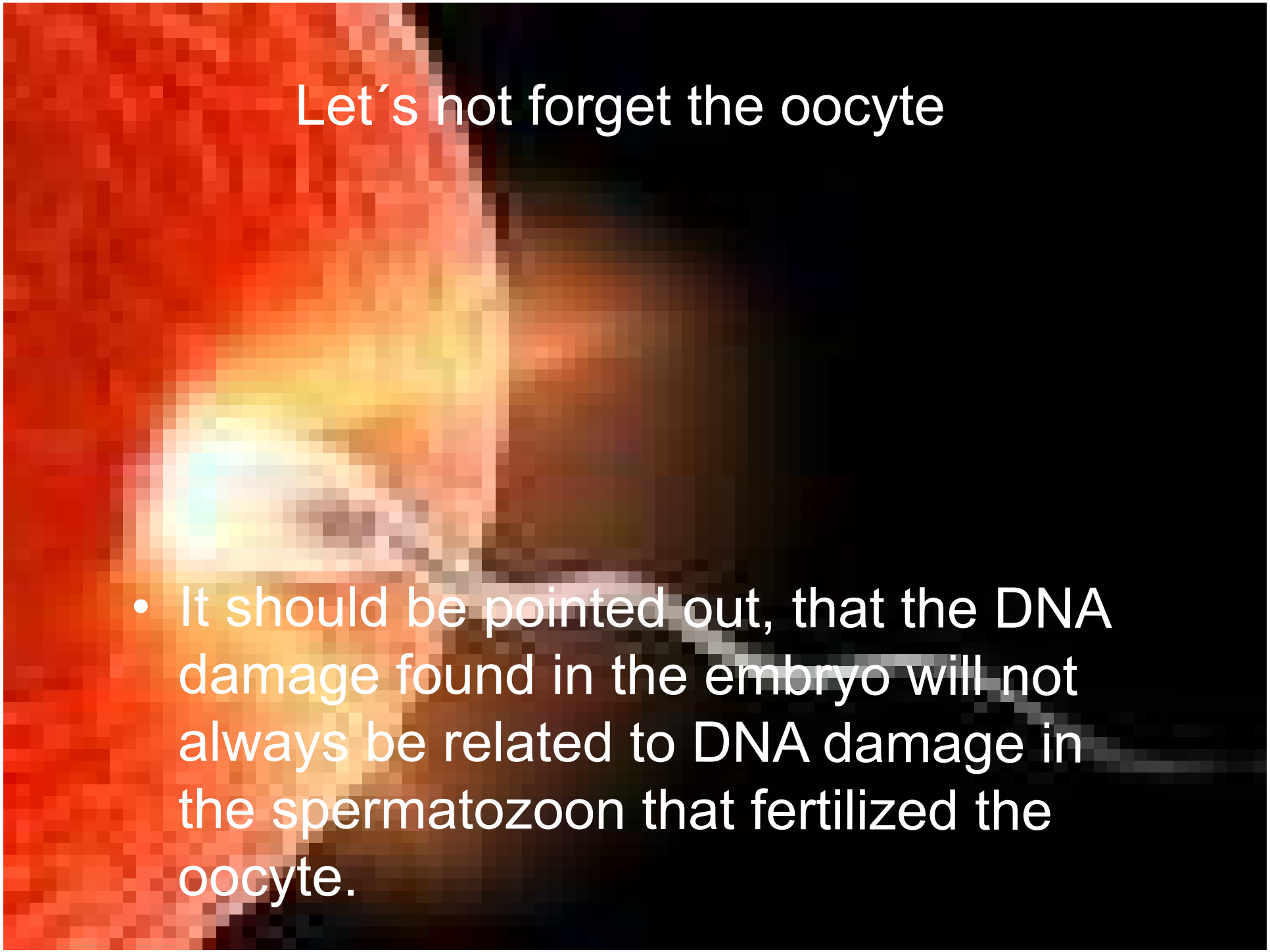
Armand Zini[1,4], Jason M. Boman[1], Eric Belzile[2] and Antonio Ciampi[2,3]

CONCLUSIONS

sperm DNA damage is associated with a significantly increased risk of pregnancy loss after IVF and ICSI.

The data provide a clinical indication for the evaluation of sperm DNA damage prior to IVF or ICSI and a rationale for further investigating the association between sperm DNA damage and pregnancy loss.

# Let´s not forget the oocyte



- It should be pointed out, that the DNA damage found in the embryo will not always be related to DNA damage in the spermatozoon that fertilized the oocyte.

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# Effect of sperm DNA fragmentation on pregnancy outcome depends on oocyte quality

*Marcos Meseguer, Ph.D.,*[a] *Rebeca Santiso, Ph.D.,*[b,c] *Nicolas Garrido, Ph.D.,*[a] *Sandra García-Herrero, Ph.D.,*[a] *Jose Remohí, M.D.,*[a] *and Jose Luis Fernandez, M.D.*[b,c]

**Patient(s):** Two hundred ten male partners of couples undergoing in vitro fertilization (IVF) or first intracytoplasmic sperm injection (ICSI) cycles with fresh or thawed sperm with the women's own or donated oocytes.

When oocytes from infertile patients were employed, DF had a statistically significant negative impact on chance of pregnancy.

For every 10% increase in DF, the probability of not achieving pregnancy increased by 1.31.

When donated oocytes were employed, DF did not have a statistically significant effect

Fertility and Sterility® Vol. 95, No. 1, January 2011

# Conclusions I

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- Infertile men have higher DF than fertile men
- Sperms of infertile men are more susceptible to damage (ROS)
- sperm with damaged DNA can successfully fertilize - but  may cause de novo mutations in the offspring. (despite the ability of the oocyte and embryo to repair some damage)

  → causes concern about the safety of ICSI

- Extensive DF might not be overcome by oocyte repair mechanisms.
- Oocyte repair capacity cannot be measured.

# Conclusions II

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- Small but significant association between DF and pregnancy – but- no indication for routine use in male evaluation (Collins)
- ? Indication in failed IVF, several pregnancy losses, several failed IUI´s
- More studies needed to identify subgroups that would merit from DF tests
- Apply careful sperm processing method, so not to enhance DFI
- ICSI: use methods to pick out sperms with reduced risk for DF


Thank you for your attention

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**TABLE 1**

**Standard sperm parameters, sperm %DFI, and sperm %HDS in the four subgroups of semen samples.**

| Subgroup (infertile patients!) | N | A | O | T | *P* value |
|---|---|---|---|---|---|
| n | 31 | 30 | 10 | 16 | |
| Sperm concentration ($\times 10^6$/mL) | 89.3 ± 56.3[a] | 72.8 ± 64.9 [a] | 10.3 ± 5.5[b] | 53.3 ± 55.3 [a] | <.0001 |
| Progressive sperm motility (%) | 63.6 ± 8.7 [a] | 31.8 ± 12.0[b] | 37.6 ± 19.5[b] | 32.3 ± 16.6[b] | <.0001 |
| Normal forms (%) | 7.7 ± 1.9[a] | 7.0 ± 2.0[a] | 7.4 ± 1.8[a] | 2.0 ± 0.8[b] | <.0001 |
| Abnormal heads (%) | 90.1 ± 3.5[a] | 91.8 ± 2.3[a] | 91.5 ± 2.1[a] | 95.3 ± 3.1[b] | <.0001 |
| Abnormal necks (%) | 11.6 ± 5.2 | 14.2 ± 6.2 | 13.9 ± 4.4 | 19.6 ± 15.2 | .065 |
| Abnormal tails (%) | 6.7 ± 4.2[a] | 9.8 ± 6.7[a,b] | 8.3 ± 5.6[a,b] | 11.3 ± 6.5[b] | .016 |
| Index of teratozoospermia | 1.24 ± 0.08[a] | 1.32 ± 0.15[a,b] | 1.29 ± 0.12[a,b] | 1.37 ± 0.14[b] | .0023 |
| Sperm %DFI | 13.8 ± 9.9 | 17.3 ± 12.2 | 21.9 ± 21.5 | 15.7 ± 12.2 | .73 |
| Sperm %HDS | 4.4 ± 2.8[a] | 4.3 ± 3.0[a] | 5.7 ± 4.6[a,b] | 7.8 ± 5.7[b] | .015 |

*Note:* N = normozoospermia; A = asthenozoospermia; O = oligozoospermia and oligoasthenozoospermia; T = teratozoospermia, asthenoteratozoospermia, and oligoasthenoteratozoospermia. Values are means ± SD.

[a,b] Different letters indicate significant difference between subgroups (Kruskal-Wallis one-way analysis of variance on ranks).

*Zini. Sperm head morphology and DNA stainability. Fertil Steril 2009.*

Infertile Patients

HDS: high DNA stainability: a measure of nuclear chromatin compaction

Sperm head abnormalities may in part be due to incomplete sperm chromatin condensation

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# Is sperm dna damage associated with IVF embryo quality? A systematic review.

Zini A, Jamal W, Cowan L, Al-Hathal N.
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28 studies (8 IVF, 12 ICSI and 8 mixed IVF-ICSI studies) that evaluated the relationship between sperm DNA damage and embryo quality.

3226 treatment cycles (1033 IVF and 873 ICSI, 1320 mixed IVF-ICSI cycles)

CONCLUSIONS: This systematic review indicates that the evaluable studies are heterogeneous and that overall, there is no consistent relationship between sperm DNA damage and embryo quality and/or development.