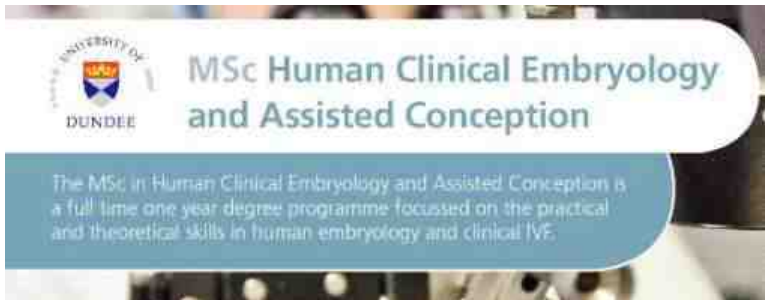
Updated from pdf contact c.barratt@Dundee.ac.uk


First for Biological Science in UK

# Declaration of COI

- Fully employed by University of Dundee (UoD).
- WHO paid honorarium to be Chair  of group and paid travel/accommodation/expenses.
- Editor in Chief of MHR
- Grant funding from MRC.
  - UoD Patent – sperm stimulation.
- Give occasional lectures that are company/society  sponsored e.g. Vertex : pay travel/accommodation/ expenses.
- Cambridge University Press – 2 edited books.
- I'm not on any company board, advisory board  or have a single share in anything or anybody.

# Is assessment of DNA damage any value?

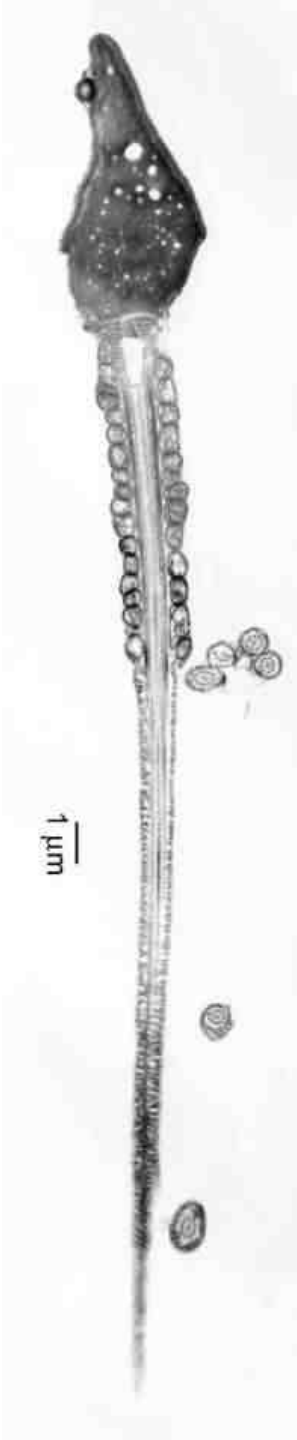
# Objectives

At the end of the presentation should be able to understand :

- Significant potential of DNA assessment
- Consensus of professional societies.
- Surprisingly - lack of  high quality comprehensive data on humans.
- Don’t throw baby out with bathwater

# A very sophisticated toolbox

- An optimally selected cell that finds its way to the egg - In vivo
- PLC Zeta1 (PLCZ1)
- ***High quality DNA***
- Centrosome
- Histone code
- RNA
- As yet unknown ……



# Examination of DNA testing not new
# It all started here ……

Evenson DP *et al* (1980) Science 210 1131-1133

## Relation of Mammalian Sperm Chromatin Heterogeneity to Fertility

Abstract. *Flow cytometry of heated sperm nuclei revealed a significant decrease in resistance to in situ denaturation of spermatozoal DNA in samples from bulls, mice, and humans of low or questionable fertility when compared with others of high fertility. Since thermal denaturation of DNA in situ depends on chromatin structure, it is assumed that changes in sperm chromatin conformation may be related to the diminished fertility. Flow cytometry of heated sperm nuclei may provide a new and independent determinant of male fertility.*

| Species | Condition | Mean $\alpha_t$ ± standard deviation | | Heated/ unheated ratio |
|---|---|---|---|---|
| | | Unheated sperm | Heated sperm | |
| Human | Proven fertility | 0.18 ± 0.01 | 0.29 ± 0.03 | 1.16 ± 0.11 |
| | Clinical samples | 0.20 ± 0.02 | 0.45 ± 0.11 | 2.25 ± 0.12 |
| Mouse | Control diet | 0.07 ± 0.03 | 0.15 ± 0.12 | 2.1 |
| | Zinc-deficient diet | 0.07 ± 0.03 | 0.28 ± 0.18 | 4.0 |
| Bull | High fertility | 0.10 ± 0.04 | 0.16 ± 0.12 | 1.6 |
| | Low fertility | 0.07 ± 0.04 | 0.41 ± 0.23 | 5.8 |

# Damaging the paternal genome (sperm) and effects on fertilisation, pregnancy and health

## Experimental data in animals

Numerous robust lines of evidence show negative effects on early embryo development, pregnancy outcome and live births from damage to paternal genome e.g. post implantation pregnancy loss (Delbes *et al.,* (2010) Mol Hum Reprod 16:14-22).

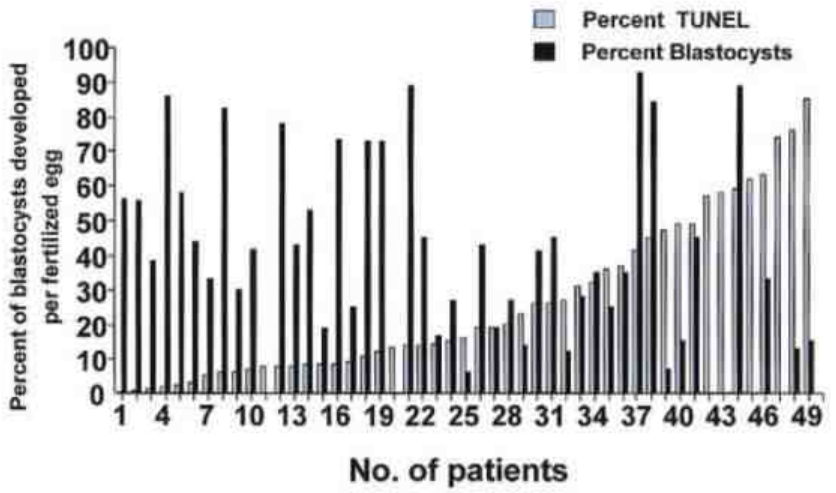
Interesting example negative effects of DNA fragmentation (from freezing) on health of offspring (survival, aberrant growth, premature aging, incidence of cancer) Fernandez-Gonzales et al., (2008) Biol Reprod 78: 761-772.

But : What about humans?

# DNA damage in sperm has a negative influence of blastocyst development

**FIGURE 2**

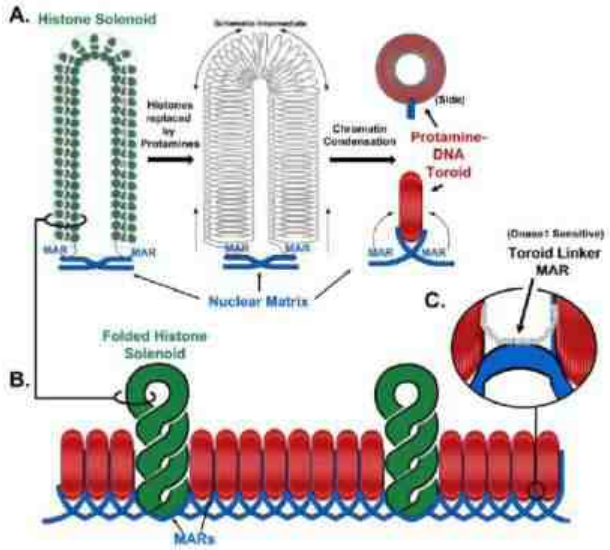
The percentage of blastocysts developed per fertilized egg and the level of TUNEL positivity in the spermatozoa prepared for insemination of individual patients (n = 49). The y axis represents the percentage of blastocyst development and TUNEL positivity.




*Seli. DNA damage in prepared ejaculated spermatozoa affects blastocyst development. Fertil Steril 2004.*

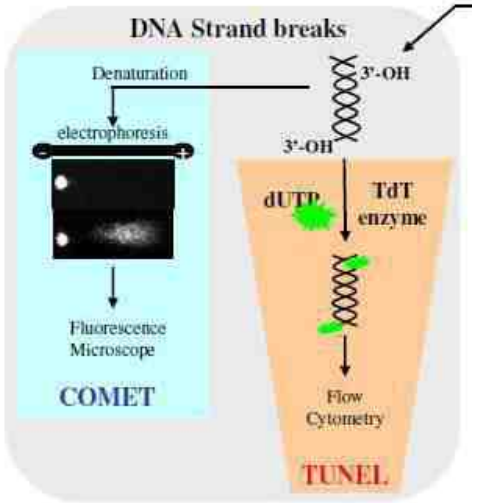
# A meta analysis in 2008……..

Ward (2010) Mol Hum Reprod 16, 30-6





Delbes *et al.,* (2010) Mol Hum Reprod 16, 14-22




*Special Issue*

*'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'*

*Collins et al (2008) Fertil Steril 89, 823-31*

# So where are we in 2016?

Several reviews of the literature (e.g. EHSRE) and clinical practice guidelines (ASRM, BFS) conclude DNA assessment not  clinically useful.

# ASRM 4 questions

- Does the DNA integrity test predict male fertility with natural conception ?
- Does the DNA integrity test predict pregnancy with IUI?
- Is DNA fragmentation predictive of pregnancy with IVF?
- Is DNA fragmentation predictive of pregnancy with ICSI?

LR of 5-10 and 0.1-0.2 create moderate changes in pre-test and post test probabilities and may be important.

# ASRM answers

- **Does the DNA integrity test predict male fertility with natural conception ?**

'there is fair level evidence (Level B) that increased DNA fragmentation is associated with reduced fertility, however, there is insufficient evidence (Level C) to use the test as a predictor of fertility since cut-points have not been clearly established and validated'

- **Does the DNA integrity test predict pregnancy with IUI?**

'there is insufficient evidence (Level C) to recommend the use of DNA integrity tests to predict pregnancy with IUI'

- **Is DNA fragmentation predictive of pregnancy with IVF?**

'there is insufficient evidence (Level C) to recommend routine use of DNA integrity testing for patients undergoing IVF'

- **Is DNA fragmentation predictive of pregnancy with IVF/ICSI?**

'there is insufficient evidence (Level C) to recommend routine use of DNA integrity testing for patients undergoing IVF/ICSI'

.

# ASRM Summary (2013)

1. Existing data do not support a consistent relationship between abnormal DNA integrity and reproductive outcomes
2. At present the results of sperm DNA integrity testing alone do not predict pregnancy rates achieved with IUI, IVF or ICSI. However, further research may lead to validation of the clinical utility of these tests.

**Recommendatio**n: ‘there is insufficient evidence to recommend the routine use of sperm DNA integrity tests in the evaluation and treatment of the infertile couple (Level B).

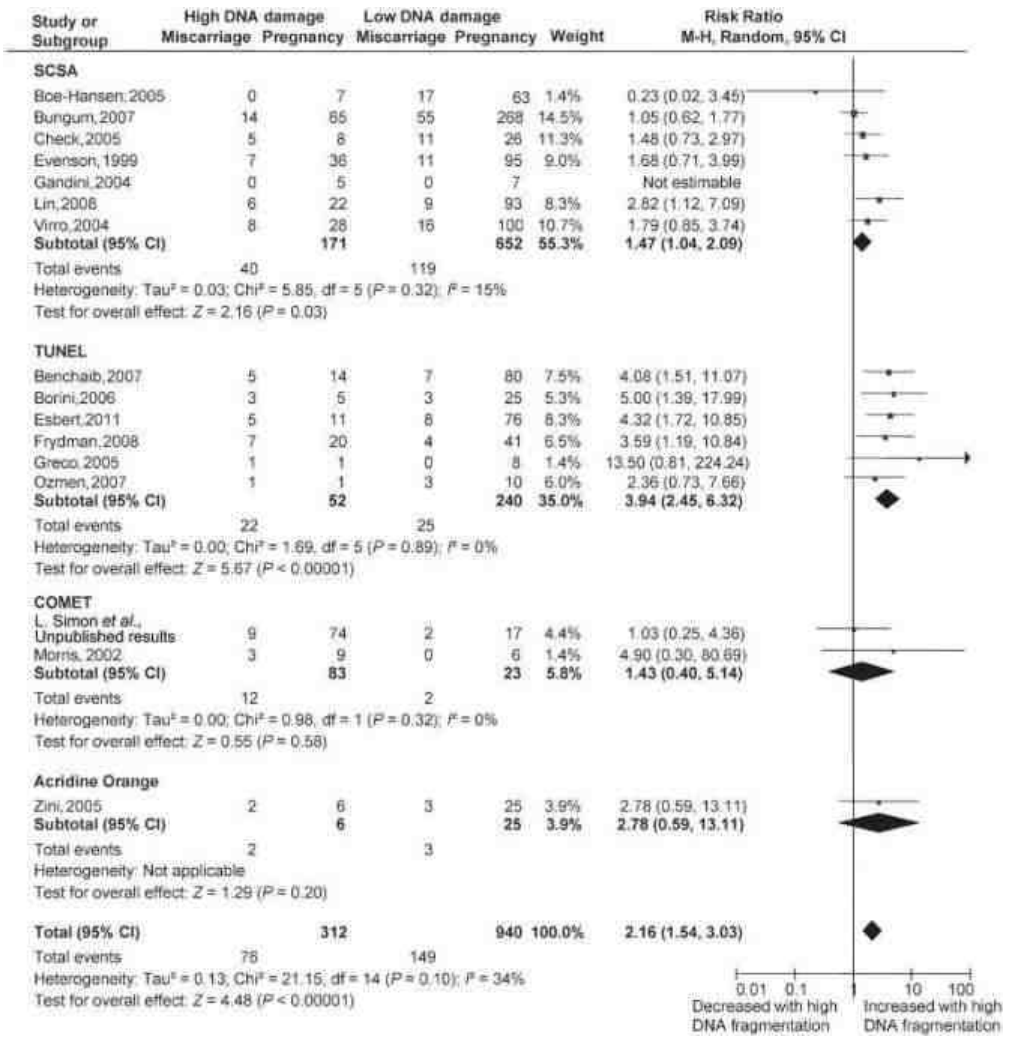
# BFS Guidance 2013

k. There is evidence of relationships between sperm DNA damage and either semen parameters and/or outcome of assisted conception. However reports conflict and depend largely on the laboratory test utilised. Results are unlikely to alter patient management. **GPP**

GPP = Good Practice Point

# However, miscarriage data is robust?

subgroup meta-analysis of assays used in studies comparing the effect of high DNA fragmentation versus low DNA fragmentation in sperm on miscarriage rates.




**Robinson L et al. Hum. Reprod. 2012;27:2908-2917**



human reproduction

# Does recent meta analysis shed light?

Reproductive BioMedicine Online (2015) 30, 120-127

www.sciencedirect.com
www.rbmonline.com

REVIEW

## The effect of sperm DNA fragmentation on live birth rate after IVF or ICSI: a systematic review and meta-analysis

A Osman *, H Alsomait, S Seshadri, T El-Toukhy, Y Khalaf

# Remarkably low powered studies.

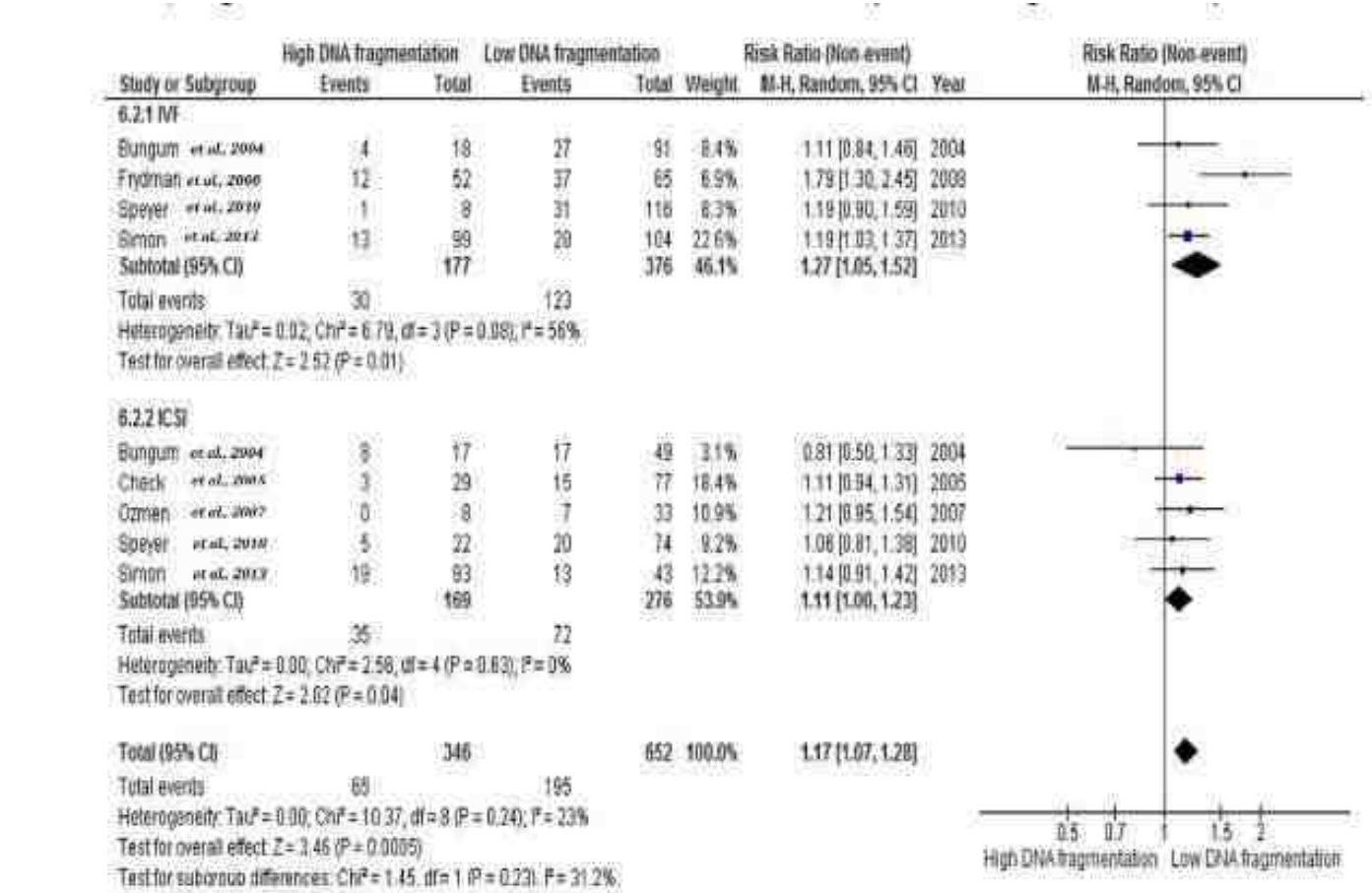



**Figure 2** Live birth rate in high and low sperm DNA fragmentation groups. ICSI = intracytoplasmic sperm injection.

# So where can we go from here? [look back]

# Sperm DNA: organization, protection and vulnerability: from basic science to clinical applications. A position report.

Recommendations

.

1. Our knowledgebase is dramatically improving although fundamental questions remain. [e.g. chromatin structure and changes in development and female/epigenome/what happens in the egg/how does the egg respond/what type of damage]

2. ***What do the tests measure and how robust are they? Lack of standardisation.***

3. More animal model experiments are required. Their usefulness needs to be emphasised and used to address basic questions.

4. ***Primary Clinical data in the field remains limited e.g. 1 IUI trial.***

5. Follow up of children born from ART is [pitiful]

Barratt et al., (2010) Hum Reprod 25, 25(4):824-38

# *Recommendation 2*
# *What test do we use and when?*

- What do they really assess?

- Repeatability, reliability and robustness. Do we need different/more quantitative tests? More robust assays

- Additional to semen assessment as routine initial investigation- [greater predictive value] – essential [odds ratio>15]. Example of progressive motility (Simon and Lewis 2011).

- What proportion of sample should we assess (semen/SU)? Does this tell us something different?

- When to test in patients pathway :
  - Secondary to initial analysis – if so only on abnormal (take account of female)?
  - Tertiary - Just for ART – more predictive [of failure] for IUI, IVF or ICSI.
  - Diagnostic tests can be used as replacement, triage or add-on with their usefulness being dependent on a large number of factors (Bossuyt *et al.* 2006).

- What do we do if high level damage (counselling, AO)?

- Does result change the patient pathway?

# Summary

*'A fundamental challenge in ascertaining the clinical relevance of any novel fertility assay is to learn from past promises, both kept and not, and progress forward. We believe the field has never been better prepared for this challenge. It is our sincere expectation that the clinical utility of DNA damage assessment will be fully realized'.*

De Jonge and Barratt

# Don’t take my word for it….

Reproductive BioMedicine Online (2015) 30, 111–112

www.sciencedirect.com
www.rbmonline.com

ELSEVIER

EDITORIAL

## Are we ready to incorporate sperm DNA-fragmentation testing into our male infertility work-up? A plea for more robust studies

Unfortunately, after all the studies that have been published looking at the value of DNA-F testing for diagnosis and prognosis for infertility patients, it remains impossible to recommend its routine use. It remains unclear whether DNA-F is that magic test that will allow us to counsel our male-factor patients with confidence. Additional studies with

# Summary

Now you should be able to understand :

- Significant potential of DNA assessment
- Consensus of professional societies.
- Surprisingly - lack of  high quality comprehensive data on humans.
- **Don’t throw baby out with bathwater**

Reproductive BioMedicine Online (2015) 30, 120–127

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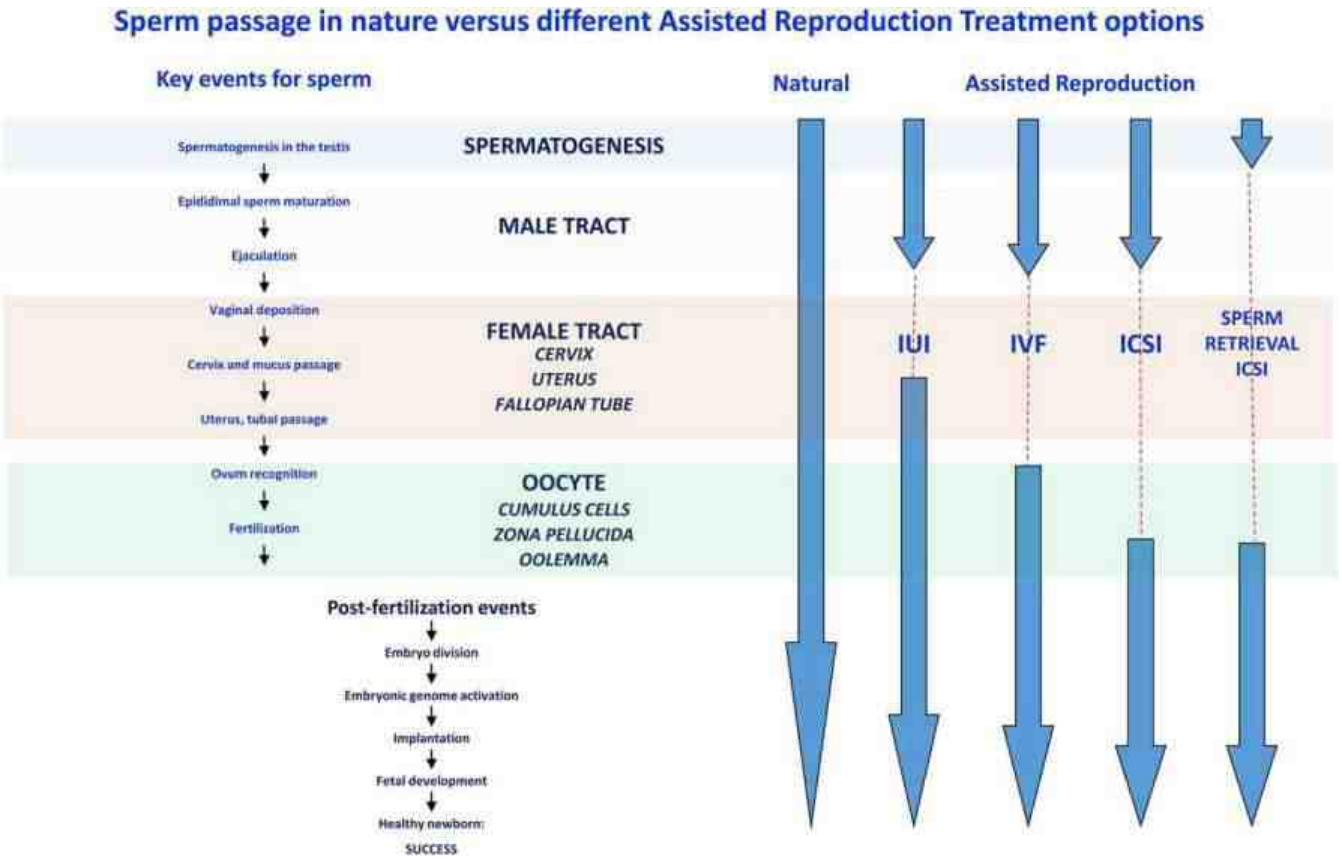
REVIEW

# The effect of sperm DNA fragmentation on live birth rate after IVF or ICSI: a systematic review and meta-analysis

A Osman *, H Alsomait, S Seshadri, T El-Toukhy, Y Khalaf

**Abstract** A systematic review and meta-analysis was conducted to evaluate the relationship between the extent of sperm DNA damage and live birth rate (LBR) per couple and the influence of the method of fertilization on treatment outcome. Searches were conducted on MEDLINE, EMBASE and Cochrane Library. Six studies were eligible for inclusion in the meta-analysis. Overall, LBR increased signficantly in couples with low sperm DNA fragmentation compared with those with high sperm DNA fragmentation (RR 1.17, 95% CI 1.07 to 1.28; $P = 0.0005$). After IVF and intracytoplasmic sperm injection (ICSI), men with low sperm DNA fragmentation had significantly higher LBR (RR 1.27, 95% CI 1.05 to 1.52; $P = 0.01$) and (RR 1.11, 95% CI 1.00 to 1.23, $P = 0.04$), respectively. A sensitivity analysis showed no statistically significant difference in LBR between low and high sperm DNA fragmentation when ICSI treatment was used (RR 1.08, 95% CI 0.39 to 2.96; $P = 0.88$). High sperm DNA fragmentation in couples undergoing assisted reproduction techniques is associated with lower LBR. Well-designed randomized studies are required to assess the role of ICSI over IVF in the treatment of men with high sperm DNA fragmentation.

# A comparison of sperm passage in nature versus different assisted reproductive technologies (ART).




**Denny Sakkas et al. Hum. Reprod. Update 2015;21:711-726**



human reproduction update

# New data continually appearing

**Human Reproduction, Vol.29, No.11 pp. 2402–2412, 2014**
Advanced Access publication on September 8, 2014 doi:10.1093/humrep/deu228

human reproduction ORIGINAL ARTICLE *Embryology*

## Paternal influence of sperm DNA integrity on early embryonic development

**L. Simon[1], K. Murphy[1], M.B. Shamsi[1], L. Liu[1], B. Emery[1], K.I. Aston[1], J. Hotaling[1], and D.T. Carrell[1,2,3,*]**

[1]Andrology and IVF Laboratory, Department of Surgery (Urology), University of Utah, Salt Lake City, UT 84108, USA [2]Department of Obstetrics and Gynecology, University of Utah, Salt Lake City, UT 84108, USA [3]Department of Human Genetics, University of Utah, Salt Lake City, UT 84108, USA

Effect of sperm DNA damage during embryogenesis

**Table IV Comparison between sperm DNA damage and implantation rate.**

| | Low | Intermediate | High | *P*-value |
|---|---|---|---|---|
| Implantation rate % (*n*) | 65.0 (60) | 55.3 (114) | 33.3 (123) | <0.001 |
| Female age ≤35 years % (*n*) | 60.4 (48) | 56.6 (83) | 44.8 (87) | <0.001 |
| Female age >35 years % (*n*) | 83.3 (12) | 51.6 (31) | 5.6 (36) | <0.001 |

The chi-square statistic was used to calculate the *P*-value. *P*-values compare the implantation rate between the sperm DNA damage categories.