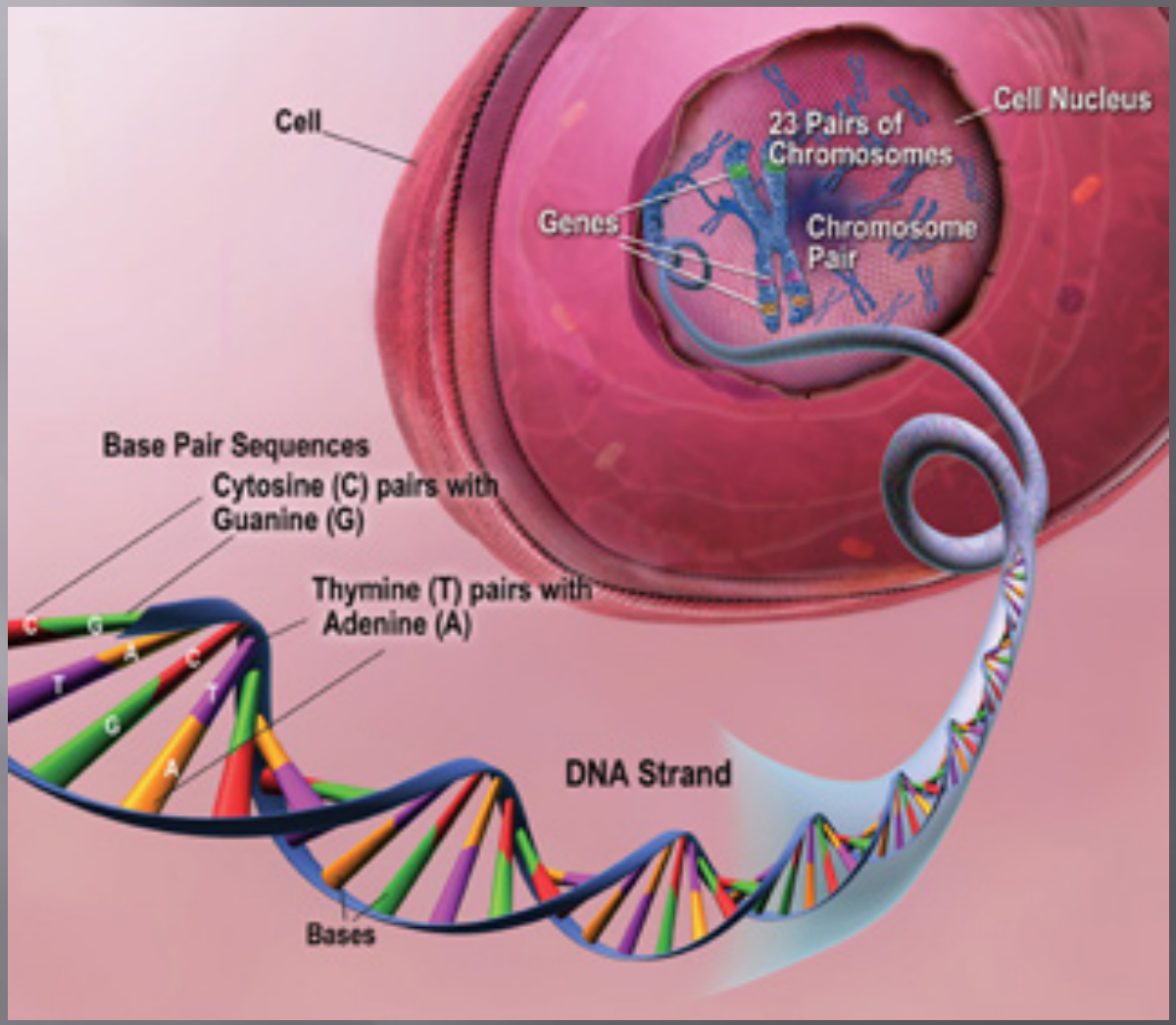
Η ΚΑΤΑΤΜΗΣΗ ΤΟΥ DNA ΤΩΝ ΣΠΕΡΜΑΤΟΖΩΑΡΙΩΝ

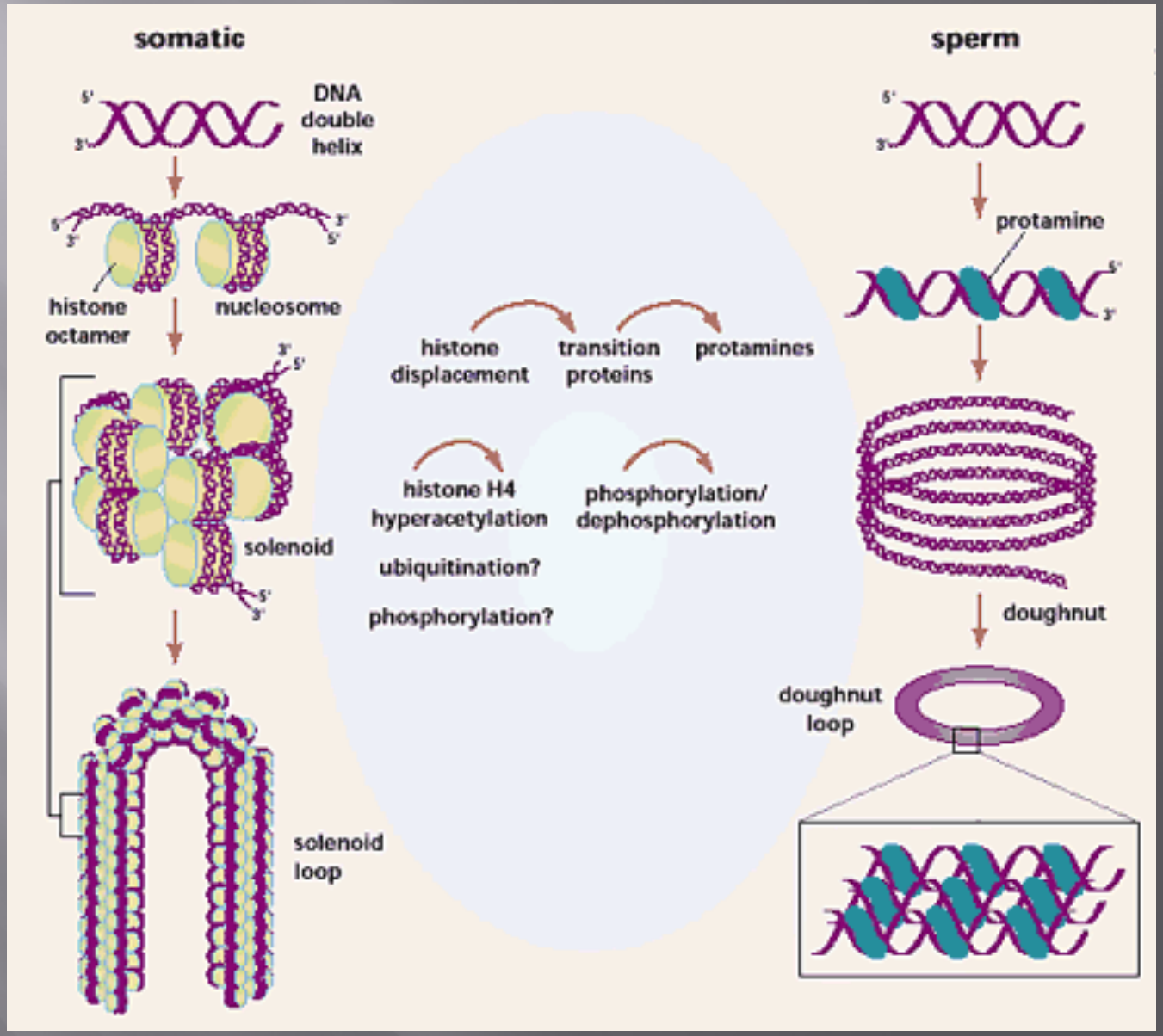
Θεοδοσία Ζεγκινιάδου, M.H.Sc., Ph.D.

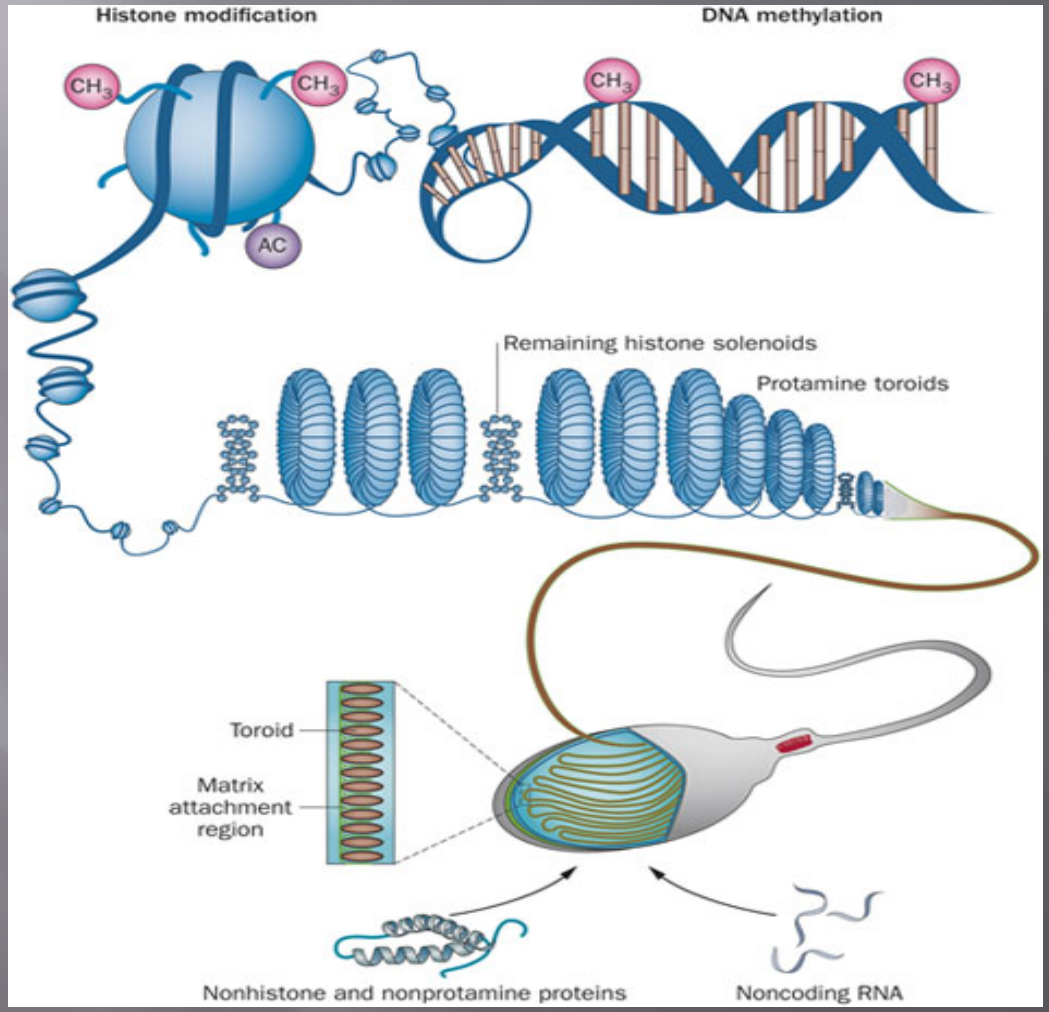

Cell
Cell Nucleus
23 Pairs of Chromosomes
Genes
Chromosome Pair
Base Pair Sequences
Cytosine (C) pairs with Guanine (G)
Thymine (T) pairs with Adenine (A)
DNA Strand
Bases

Η συμπύκνωση της χρωματίνης

Στο DNA του σπερματοζωαρίου οι ιστόνες αντικαθίστανται από προταμίνες - P1 και P2, με αποτέλεσμα :

- ο πυρήνας να γίνεται πιο συμπαγής οπότε το DNA προστατεύεται από εξωτερικούς παράγοντες (Kosower et al 1992)
- να μεταφέρεται με αυτή την μορφή στο ωάριο (Balhorn et al., 2000)


somatic
sperm
DNA
double
helix
histone
octamer
nucleosome
solenoid
solenoid
loop
histone
displacement
transition
proteins
protamines
histone H4
hyperacetylation
ubiquitination?
phosphorylation?
phosphorylation/
dephosphorylation
protamine
doughnut
doughnut
loop


Histone modification
DNA methylation
CH3
CH3
CH3
CH3
AC
Remaining histone solenoids
Protamine toroids
Toroid
Matrix attachment region
Nonhistone and nonprotamine proteins
Noncoding RNA

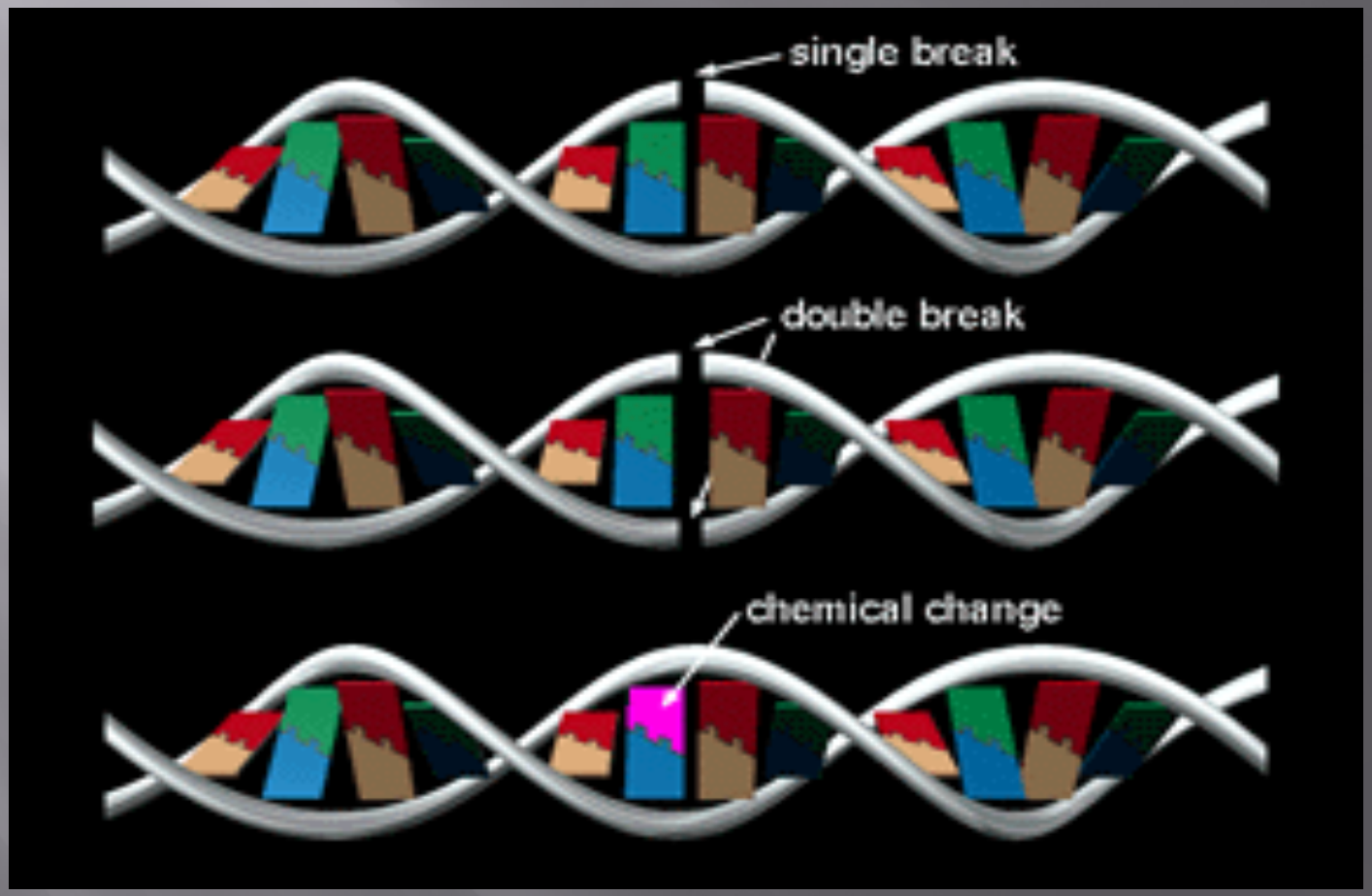
Προταμίνες και ιστόνες

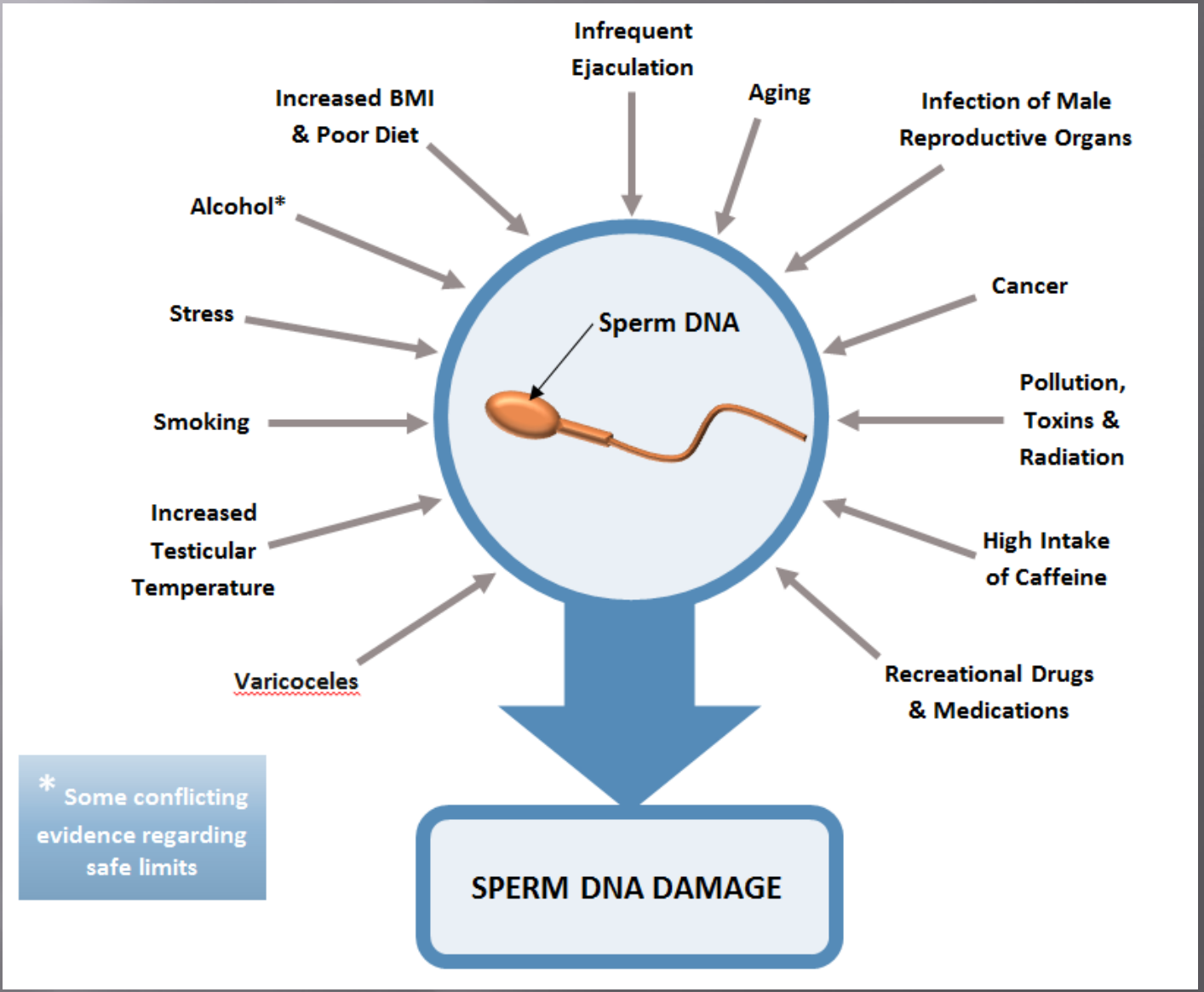
- Ένα ποσοστό της τάξης του 5–15% του DNA του σπερματοζωαρίου είναι συνδεδεμένο με ιστόνες (Tanphaichitr et al., 1978; Wykes and Krawetz, 2003)

- Το DNA που είναι συνδεδεμένο με ιστόνες περιλαμβάνει γονίδια που είναι σημαντικά για την κυτταρική διαφοροποίηση και την ανάπτυξη του εμβρύου στα πρώτα στάδια

….. suggests that histone bound DNA in sperm cells is associated with gene families that are important for cell differentiation and early embryo patterning ….
(Hammoud et al., 2009)

Αιτίες που προκαλούν την κατάτμηση




Infrequent Ejaculation
Increased BMI & Poor Diet
Aging
Infection of Male Reproductive Organs
Alcohol*
Cancer
Stress
Sperm DNA
Pollution, Toxins & Radiation
Smoking
Increased Testicular Temperature
High Intake of Caffeine
Varicoceles
Recreational Drugs & Medications
* Some conflicting evidence regarding safe limits
SPERM DNA DAMAGE

Ενδογενείς παράγοντες

- 1) Ελαττωματική συμπύκνωση της χρωματίνης
- 2) Ελεύθερες ρίζες
- 3) Διαφυγή από το μονοπάτι της απόπτωσης

1) Ελαττωματική συμπύκνωση της χρωματίνης

- Η αλλαγή των ιστονών από τις προταμίνες γίνεται με την βοήθεια της τοποισομεράσης (topoisomerase) και μπορεί να δημιουργηθούν θραύσεις στην αλυσίδα του DNA πιθανά λόγω "torsional stress"

(Boissonneault, 2002; Kwan et al., 2003)

(Sakkas et al., 1999; Marcon and Boissonneault, 2004)

- Αν δεν είναι πλήρης η αλλαγή τότε θα εμφανιστούν θραύσεις στην μία είτε και στις 2 αλυσίδες του DNA στα ώριμα σπερματοζωάρια

(Marcon and Boissonneault, 2004)

2) Οξειδωτικό στρες

2) Οξειδωτικό στρες

Journal of Andrology, Vol. 33, No. 6, November/December 2012

Review

Reactive Oxygen Species and Sperm Function—In Sickness and In Health

R. JOHN AITKEN,* KEITH T. JONES,*† AND SARAH A. ROBERTSON‡

*From the *Priority Research Centre for Reproductive Biology, Faculty of Science and IT and the †Faculty of Health, University of Newcastle, Callaghan, Australia; and the ‡Robinson Institute, School of Paediatrics and Reproductive Health, University of Adelaide, Adelaide, Australia.*

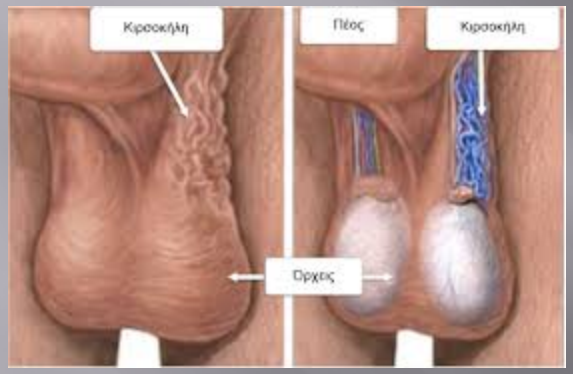
3) Διαφυγή από την απόπτωση

Sakkas D, Seli E, Bizzaro D, Tarozzi N, Manicardi GC.

Abnormal spermatozoa in the ejaculate: abortive apoptosis and faulty nuclear remodelling during spermatogenesis.

Reprod Biomed Online 2003;7:428-432.

Εξωγενείς παράγοντες



Ηλικία και κατάτμηση

Alshahrani *et al. Reproductive Biology and Endocrinology* 2014, **12**:103
http://www.rbej.com/content/12/1/103

REPRODUCTIVE BIOLOGY AND ENDOCRINOLOGY

RESEARCH Open Access

Infertile men older than 40 years are at higher risk of sperm DNA damage

Saad Alshahrani[1,2], Ashok Agarwal[1*], Mourad Assidi[3,4,5], Adel M Abuzenadah[3,4,5], Damayanthi Durairajanayagam[1,6], Ahmet Ayaz[1], Rakesh Sharma[1] and Edmund Sabanegh[1]

Κιρσοκήλη και κατάτμηση

Asian Journal of Andrology (2016) 18, 239–245

www.asiaandro.com; www.ajandrology.com

Open Access

INVITED REVIEW

Male Fertility

Summary evidence on the effects of varicocele treatment to improve natural fertility in subfertile men

Bruno C Tiseo[1], Sandro C Esteves[2], Marcello S Cocuzza[1]

Ακτινοβολία και κατάτμηση

Sperm quality and DNA damage in men from Jilin Province, China, who are occupationally exposed to ionizing radiation

D.D. Zhou[1,2], J.L. Hao[3], K.M. Guo[4], C.W. Lu[3] and X.D. Liu[1]

[1]Key Laboratory of Radiobiology (Ministry of Health), School of Public Health, Jilin University, Changchun, China
[2]Department of Radiology, First Hospital of Jilin University, Changchun, Jilin Province, China
[3]Department of Ophthalmology, First Hospital of Jilin University, Changchun, Jilin Province, China
[4]Department of Andrology, First Hospital of Jilin University, Changchun, Jilin Province, China

Καρκίνος και κατάτμηση

Hindawi Publishing Corporation
BioMed Research International
Volume 2016, Article ID 7893961, 8 pages
http://dx.doi.org/10.1155/2016/7893961

Research Article

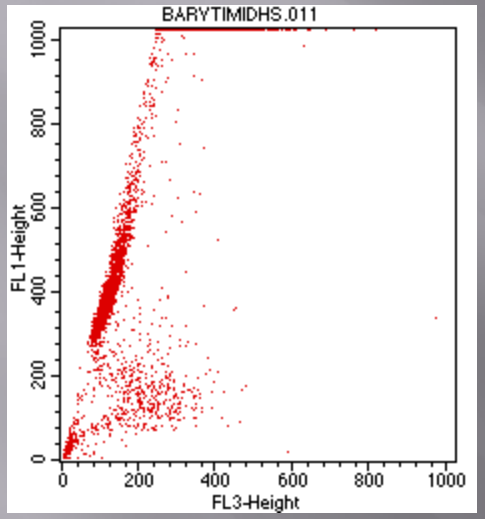
Sperm DNA Fragmentation Index and Hyaluronan Binding Ability in Men from Infertile Couples and Men with Testicular Germ Cell Tumor

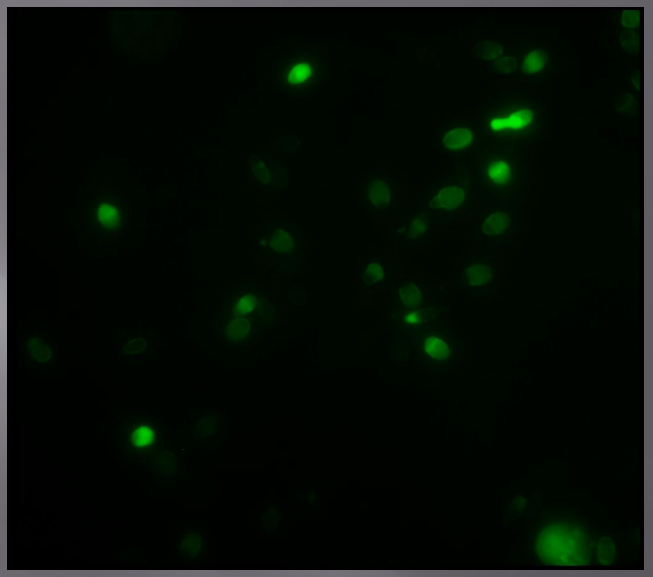
Katarzyna Marchlewska,[1] Eliza Filipiak,[1] Renata Walczak-Jedrzejowska,[1] Elzbieta Oszukowska,[2] Slawomir Sobkiewicz,[3] Malgorzata Wojt,[3] Jacek Chmiel,[3] Krzysztof Kula,[1] and Jolanta Slowikowska-Hilczer[1]

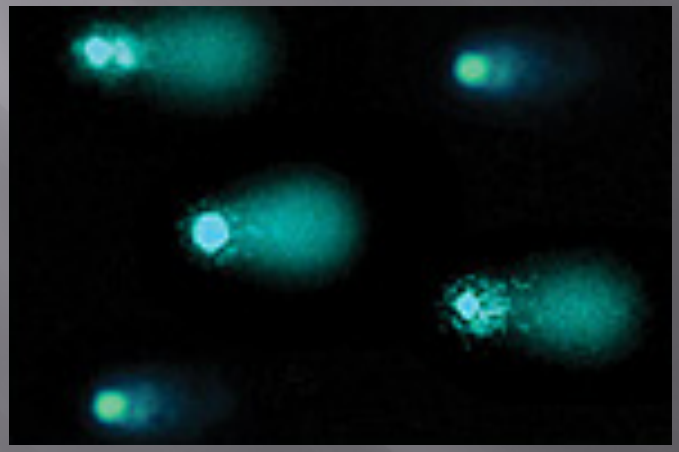
[1]Department of Andrology and Reproductive Endocrinology, Medical University of Lodz, Lodz, Poland
[2]II Clinic of Urology, Medical University of Lodz, Lodz, Poland
[3]Infertility Clinic, Salve Medica, Lodz, Poland

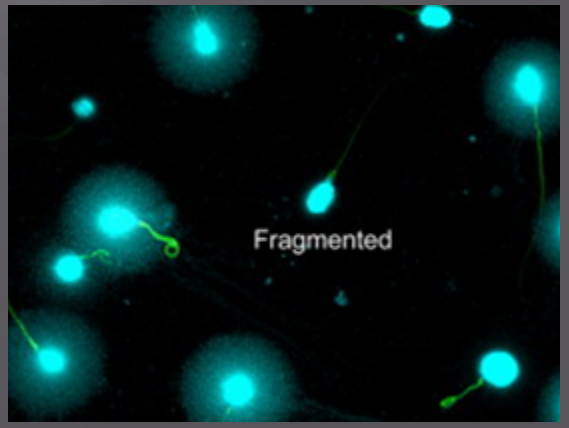
Εργαστηριακές μέθοδοι

1. SCSA – Sperm chromatin structure assay
2. Tunel – terminal deoxy nucleotide transferase mediated
3. Comet – single cell gel electrophoresis
4. SCD – Sperm chromatin dispersion


BARYTIMIDHS.011
FL1-Height
FL3-Height
0
200
400
600
800
1000






Fragmented

Εργαστηριακές μέθοδοι

Όλες οι μέθοδοι ανιχνεύουν θραύσεις στην μία ή και στις δύο αλυσίδες του DNA

- Οι Tunel και Comet μετρούν άμεσα την διακοπή στο DNA

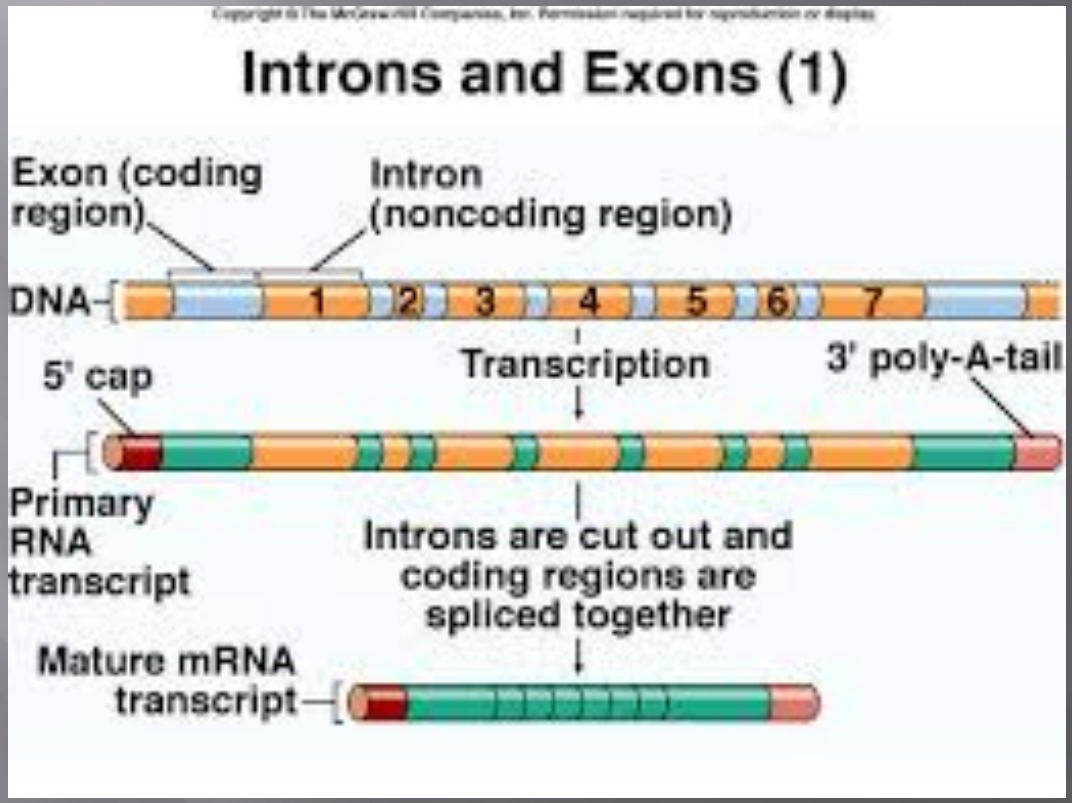
- Οι SCSA και SCD μετρούν την επιδεκτικότητα του DNA στην μετουσίωση

Εργαστηριακές μέθοδοι Γενικά

SCSA-Tunel-Comet-SCD

- Όλες οι μέθοδοι υπολογίζουν το ποσοστό των σπερματοζωαρίων με «σπασμένο DNA» και συγκρίνουν το αποτέλεσμα με κάποιο όριο, υποθέτοντας ότι όσο μεγαλύτερο το ποσοστό τόσο μεγαλύτερη και η βλάβη στο δείγμα

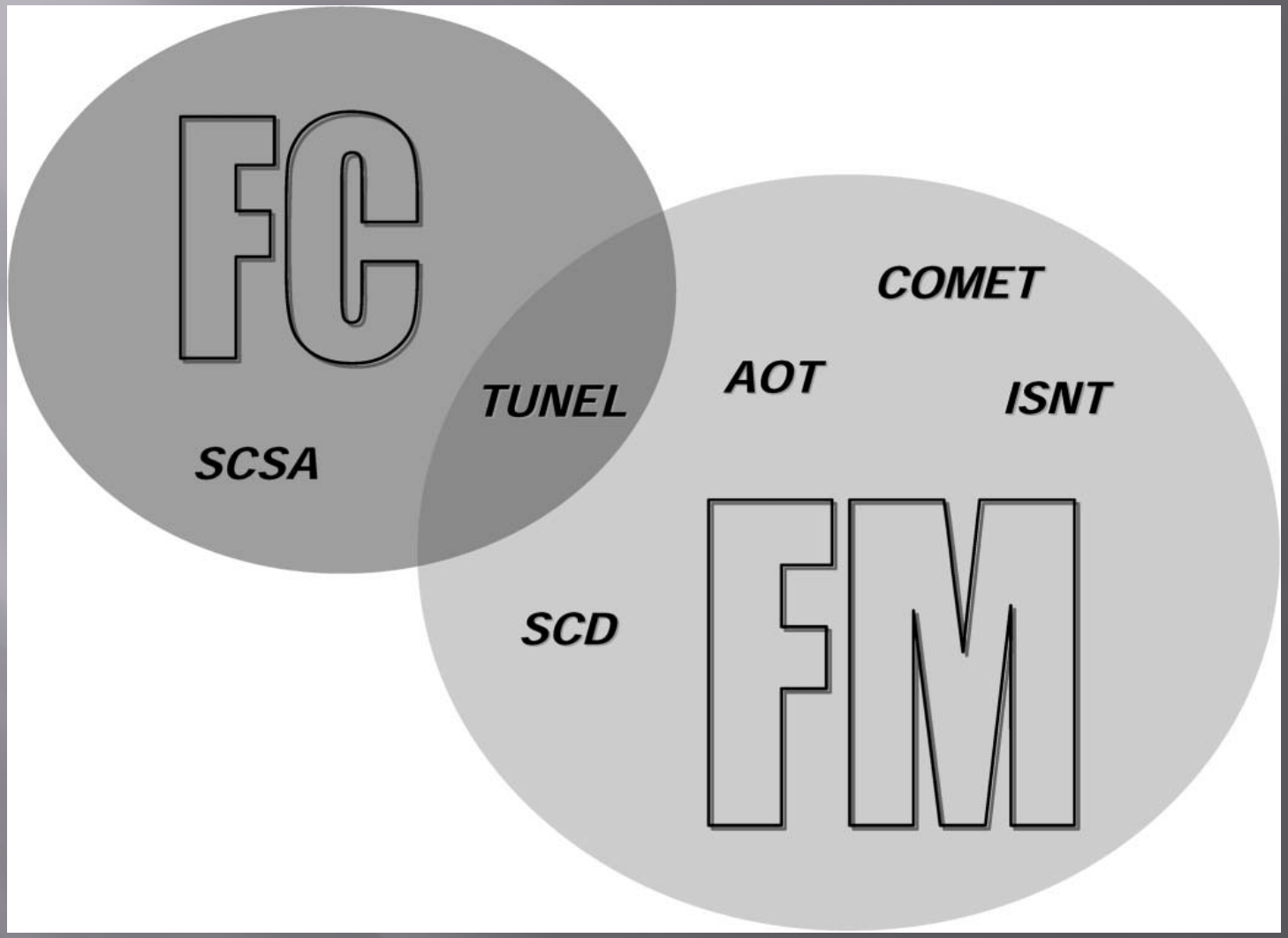
Εσόνια και εξόνια



Εργαστηριακές μέθοδοι Γενικά

- Δεν ξεχωρίζουν επίσης αν είναι σε εσόνιο ή εξόνιο
- Οι μέθοδοι δεν μπορούν να ξεχωρίσουν τα γονίδια στα οποία υπάρχει το σπάσιμο.


Reproduction, Fertility and Development
Asian Journal of Andrology
family building
Journal of ANDROLOGY
THERIOGENOLOGY
BIOLOGY of REPRODUCTION
Official Journal of the Society for the Study of Reproduction
FERTILITY TODAY
The AGING MALE
infertility
THE JOURNAL of UROLOGY
JOURNAL OF ASSISTED REPRODUCTION AND GENETICS
Human Reproduction
ESHRE 2006
Prague
SCSA®
DIAGNOSTICS
A Vital Step in Screening Male Fertility


FC
SCSA
TUNEL
COMET
AOT
ISNT
SCD
FM

Ολικός ή επιλεγμένος πληθυσμός?

- Tomlinson MJ, Moffatt O, Manicardi GC, Bizzaro D, Afnan M, Sakkas D. Interrelationships between seminal parameters and sperm nuclear DNA damage before and after density gradient centrifugation: implications for assisted conception.

Hum Reprod 2001;16:2160-2165

Σπερματοζωάρια ή ορχικός ιστός ?

- Greco E, Scarselli F, Iacobelli M, et al. Efficient treatment of infertility due to sperm DNA damage by ICSI with testicular spermatozoa. Hum Reprod 2005;20:226-230.
- Nicopoullos JD, Ramsay JW, Almeida PA, Gilling-Smith C. Assisted reproduction in the azoospermic couple. BJOG 2004;111:1190-1203.

Έλεγχος κατάτμησης Πότε ???

- Έλεγχος ανδρικής γονιμότητας
- Ανεξήγητη υπογονιμότητα
- Αποτυχία σε IUI
- Αποτυχία στο IVF
- Αποβολές

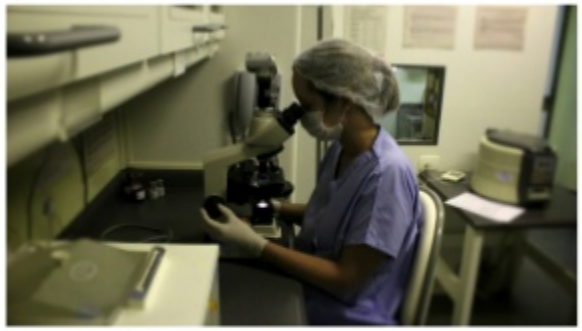
Κατάτμηση και φυσική σύλληψη

- Irvine DS, Twigg JP, Gordon EL, Fulton N, Milne PA, Aitken RJ. DNA integrity in human spermatozoa: relationships with semen quality. J Androl 2000;21:33-44.
- Zini A, Bielecki R, Phang D, Zenzes MT. Correlations between two markers of sperm DNA integrity, DNA denaturation and DNA fragmentation, in fertile and infertile men. Fertil Steril 2001;75:674-677.

- Highly abnormal (>30%) DNA fragmentation rates have also been identified in ~8% of infertile men with a normal semen analysis;

Zini A, Bielecki R, Phang D, Zenzes MT. Correlations between two markers of sperm DNA integrity, DNA denaturation and DNA fragmentation, in fertile and infertile men. Fertil Steril 2001; 75: 674–7

Frequency of Elevated SDF in Men with Normal Semen Analysis (WHO 2010)



Androfert 2013; N=860; SCD test; cutoff value of 20%

ORIGINAL ARTICLE

Correspondence: Krzysztof Oleszczuk,
Reproductive Medicine Centre, Skåne University
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Malmö 205 02, Sweden.
E-mail: krzysztof.oleszczuk@med.lu.se

Keywords:
SCSA, IVF, ICSI, fertilization, GQE, pregnancy,
miscarriage, live births

Sperm chromatin structure assay in prediction of in vitro fertilization outcome

K. Oleszczuk, A. Giwercman and M. Bungum

Reproductive Medicine Centre, Skåne University Hospital, Lund University, Malmö, Sweden

…..The results suggest that ICSI might be a preferred method of in vitro treatment in cases with high DFI…

Clinical utility of SDF tests

I.ASRM, 2013

1. Does SDF test predict male fertility with natural conception?

✓Fair evidence (Level B) that increased SDF is associated with reduced fertility

✓Insufficient evidence (Level C) to use the test as a predictor of fertility since cut-points have not been clearly established and validated.

2. Does SDF test predict pregnancy with IUI?

Insufficient evidence (Level C) to recommend the use of SDF tests to predict pregnancy with IUI.

3. Is SDF predictive of pregnancy with in IVF?

✓Insufficient evidence (Level C) to recommend routine use of SDF testing for patients undergoing IVF.

4. Is SDF predictive of pregnancy with IVF and ICSI?

✓Insufficient evidence (Level C) to recommend routine DNA integrity testing for patients undergoing IVF/ICSI.

5. Is SDF predictive of pregnancy loss?

✓ Insufficient evidence (Level C) to recommend routine DNA integrity testing to predict pregnancy loss.

- **Recommendation**

There is insufficient evidence to recommend the routine use of SDF testing in evaluation and treatment of infertile couple {level C}

Guideline

Clinical utility of sperm DNA fragmentation testing: practice recommendations based on clinical scenarios

Ashok Agarwal[1], Ahmad Majzoub[2], Sandro C. Esteves[3], Edmund Ko[4], Ranjith Ramasamy[5], Armand Zini[6]

[1]American Center for Reproductive Medicine, Cleveland Clinic, Cleveland, OH, USA; [2]Department of Urology, Glickman Urological and kidney Institute, Cleveland Clinic, Cleveland, OH, USA; [3]ANDROFERT, Andrology and Human Reproduction Clinic, Referral Center for Male Reproduction, Campinas, SP, Brazil; [4]Department of Urology, Loma Linda University, Loma Linda, California, USA; [5]Department of Urology, University of Miami, Miami, Florida, USA; [6]Department of Urology, McGill University, Montreal, Canada

Correspondence to: Dr. Ashok Agarwal. Professor and Director, American Center for Reproductive Medicine, Cleveland Clinic, Mail Code X-11, 10681 Carnegie Avenue, Cleveland, OH 44195, USA. Email: AGARWAA@ccf.org.

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Unexplained Infertility	
High SDF is found in men with normal semen analysis	Saleh *et al.* (7); Oleszczuk *et al.* (69)
SDF is an independent predictor of male fertility status	Bungum *et al.* (70); Oleszczuk *et al.* (69)
SDF levels can predict the likelihood of natural pregnancy	Evenson *et al.* (6)

Recurrent pregnancy loss	
High SDF is associated with greater incidence of abortion	Ford *et al.* (71); Khadem *et al.* (72); Absalan *et al.* (73)

Transl Androl Urol 2016;5(6):935-950

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Varicocele	
Significant association between SDF and varicocele has been detected	Zini and Dohle (63); Esteves *et al.* (23)
Varicocelectomy improves percentage of SDF resulting in improved pregnancy rates	Zini and Dohle (63); Smit *et al.* (64); Ni *et al.* (65)
Little is known about the effect of low grade varicocele on SDF. High SDF has been reported in clinical varicocele, particularly grades 2 and 3; improvement of SDF in all grades of varicocele have been reported after varicocelectomy	Sadek *et al.* (67); Ni *et al.* (65); Krishna Reddy *et al.* (68)

Lifestyle risk factors	
Age, obesity, smoking and environmental/occupational exposures have detrimental effects on SDF	Shi *et al.* (86); Bosch *et al.* (87); Sloter *et al.* (88); Yang *et al.* (89); Elshal *et al.* (90); Tunc *et al.* (91); Rybar *et al.* (92); Kort *et al.* (93); Wijesekara *et al.* (94); Sanchez-Pena *et al.* (95); Rahman *et al.* (96)

Transl Androl Urol 2016;5(6):935-950

Guideline

Clinical utility of sperm DNA fragmentation testing: practice recommendations based on clinical scenarios

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Recurrent IUI failure	
High SDF is associated with lower IUI pregnancy rates	Duran *et al.* (74); Bungum *et al.* (70)

IVF and ICSI failures	
SDF modestly affect IVF pregnancy rates	Zini and Sigman (75); Osman *et al.* (76); Jin *et al.* (77)
SDF does not affect ICSI pregnancy rates	Zini and Sigman (75); Zhao *et al.* (78)
High SDF is associated with greater incidence of abortion in both IVF and ICSI	Zini and Sigman (75); Zini *et al.* (79); Simon *et al.* (80); Lin *et al.* (81); Robinson *et al.* (82)
Testicular sperm have lower SDF than ejaculated sperm	Moskovtsev *et al.* (83); Greco *et al.* (84); Esteves *et al.* (24)
Higher IVF/ICSI success rates with testicular sperm	Esteves *et al.* (24); Greco *et al.* (84); Pabuccu *et al.* (85)

Transl Androl Urol 2016;5(6):935-950

Should sperm DNA fragmentation testing be included in the male infertility work-up?

Sheena EM Lewis *

Centre for Public Health, Queen's University, Belfast BT12 6 BJ, Northern Ireland, UK
* *E-mail address:* s.e.lewis@qub.ac.uk.

Will the results change practice? Will testing add to the information already provided when taking a medical history?

Yes. Clearly, those men who have high levels of sperm DNA damage…

Should sperm DNA fragmentation testing be included in the male infertility work-up?

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There are patients with normal semen parameters and high DNA-F; does this mean all men should be tested during work-up?

Again, the answer must be **yes**…

Should sperm DNA fragmentation testing be included in the male infertility work-up?

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Centre for Public Health, Queen's University, Belfast BT12 6 BJ, Northern Ireland, UK
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Is DNA testing better than any previous andrological test?

Yes. It is, however, being withheld from clinical use…

ΕΥΧΑΡΙΣΤΩ