Ο ρόλος της δοκιμασίας κατακερματισμού DNA σπερματοζωαρίων στην πρόγνωση της γονιμοποιητικής ικανότητας του σπέρματος

Μια εξέταση χωρίς αξία;

Χαράλαμπος Γ. Θωμάς

Χειρουργός Ουρολόγος

M.Sc., FECSM


I SAMUEL 17:47
THE LORD DOESN'T SAVE BY USING A SWORD OR A SPEAR. AND EVERYONE WHO IS HERE WILL KNOW IT.
THE BATTLE BELONGS TO THE LORD
HE WILL HAND ALL OF YOU OVER TO US.
BA BIBLICALLY ACCURATE
www.CrossroadsKidsClub.net
GOD'S STORY OF DAVID AND GOLIATH IN I SAMUEL 17:47 WITH DAVID AND GOLIATH IN ONE CORNER THE PHILLISTINES AND THE OTHER CORNER THE ISRAELITES FEATURING KING SAUL WEAPON OF CHOICE 5 SMOOTH STONES WITH MY TRUSTY SLINGSHOT WITH ONE LION A BIG OL' BROWN BEAR AND A FEW SHEEP WATCHING FROM AFAR
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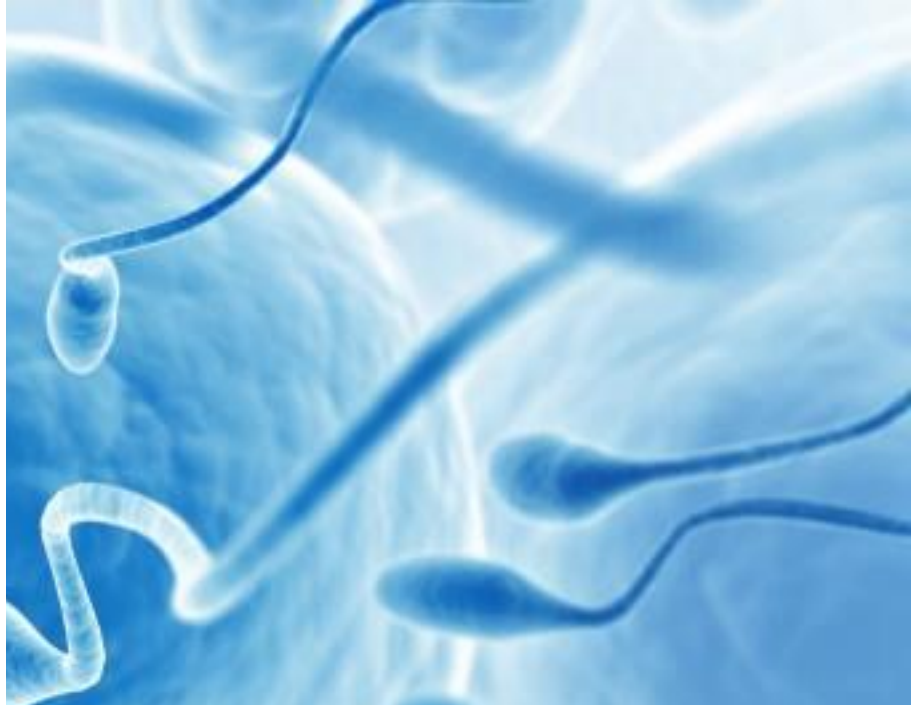
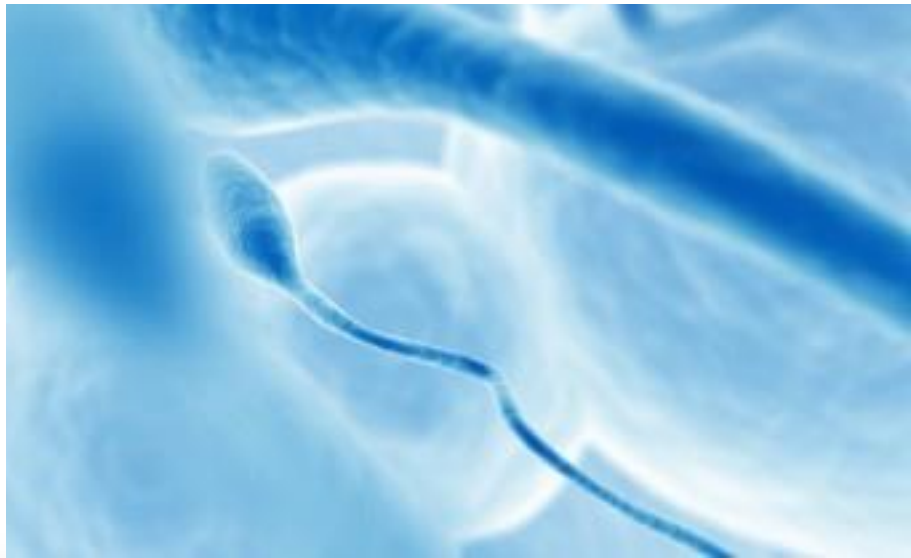
Guidelines on Male Infertility

A.Jungwirth (Chair), T. Diemer, G.R Dohle, A. Giwercman,
Z. Kopa, C.Krausz, H. Tournaye



3C.4.3 ***DNA fragmentation in spermatozoa***

There is increased DNA damage in spermatozoa from men with oligozoospermia. This increase is associated with reduced chances of natural conception and an increased chance of early pregnancy loss [84].



European
Association
of Urology

WHO laboratory man

Examination and p of human semen

FIFTH EDITION

World Health Organization

2.15.2 Classification of abnormal sperm morphology

Human semen samples contain spermatozoa with different kinds of malformations. Defective spermatogenesis and some epididymal pathologies are commonly associated with an increased percentage of spermatozoa with abnormal shapes. The morphological defects are usually mixed. Abnormal spermatozoa generally have a lower fertilizing potential, depending on the types of anomalies, and may also have abnormal DNA. Morphological defects have been associated with increased DNA fragmentation (Gandini et al., 2000), an increased incidence of structural chromosomal aberrations (Lee et al., 1996), immature chromatin (Dadoune et al., 1988) and aneuploidy (Devillard et al., 2002; Martin et al., 2003). Emphasis is therefore given to the form of the head, although the sperm tail (midpiece and principal piece) is also considered.

The following categories of defects should be noted (see Fig. 2.13).

- Head defects: large or small, tapered, pyriform, round, amorphous, vacuolated (more than two vacuoles or >20% of the head area occupied by unstained vacuolar areas), vacuoles in the post-acrosomal region, small or large acrosomal areas (<40% or >70% of the head area), double heads, or any combination of these.
- Neck and midpiece defects: asymmetrical insertion of the midpiece into the head, thick or irregular, sharply bent, abnormally thin, or any combination of these.
- Principal piece defects: short, multiple, broken, smooth hairpin bends, sharply angulated bends, of irregular width, coiled, or any combination of these.
- Excess residual cytoplasm (ERC): this is associated with abnormal spermatozoa produced from a defective spermatogenic process. Spermatozoa characterized by large amounts of irregular stained cytoplasm, one third or more of the sperm head size, often associated with defective midpieces (Mortimer & Menkveld, 2001) are abnormal. This abnormal excess cytoplasm should not be called a cytoplasmic droplet (Cooper, 2005).

Comment 1: Cytoplasmic droplets (membrane-bound vesicles on the midpiece at the head–neck junction) are normal components of physiologically functional human spermatozoa. If swollen, they may extend along the length of the midpiece, as observed by phase-contrast, differential-interference-contrast and X-ray microscopy of living cells in semen, cervical mucus and medium (Abraham-Peskir et al., 2002; Fetic et al., 2006).

Comment 2: Cytoplasmic droplets are osmotically sensitive and are not well preserved by routine air-drying procedures (Chantler & Abraham-Peskir, 2004; Cooper et al., 2004). They are not obvious in stained preparations, where they may appear as small distensions of the midpiece. Cytoplasmic droplets are less than one third the size of the sperm head in fixed and stained preparations (Mortimer & Menkveld, 2001) and are not considered abnormal.

Χαρακτηριστικά μιας ιδανικής εξέτασης:

1. Εύκολα αναπαραγώγιμη
2. Μεγάλη ευαισθησία
3. Μεγάλη ειδικότητα
4. Μεγάλη θετική/αρνητική προγνωστική αξία
5. Ελάχιστη υποκειμενικότητα χρήστη (bias)
6. Χαμηλό κόστος

Review Article

Human Sperm DNA Fragmentation and its Correlation with Conventional Semen Parameters

Evangelini Evgeni [1, 2*], Konstantinos Charalabopoulos [2], Byron Asimakopoulos [2]

1- Seminology Laboratory, Athens, Greece
2- Laboratory of Physiology, School of Medicine, Democritus University of Thrace, Thrace, Greece

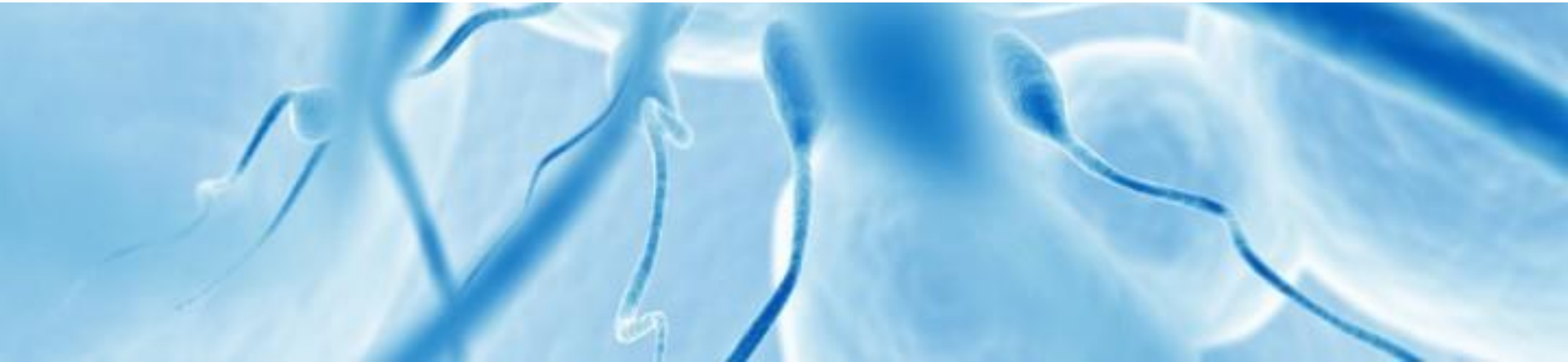


Table 2. Methods of evaluation of sperm DNA fragmentation and sperm chromatin integrity

Sperm DNA fragmentation		
Method	**Advantages**	**Disadvantages**
SCD	• Technically simple • Precise • Highly reproducible • Inexpensive • Not requiring special equipment • Test results correlate with SCSA	• Time-consuming • Labor intensive (microscopic evaluation of at least 500 spermatozoa) • Training required to avoid technician subjectivity
SCSA	• Rapid evaluation of a large number of spermatozoa (~5,000) • Rapid assessment of many samples • Flexibility in routine laboratory practice (also used in frozen samples) • Highly reproducible • Correlations with the results of other methods evaluating different types of DNA damage (TUNEL, COMET) • Application in environmental studies • Sensitive • Statistically robust • DFI: unique reference limits associated with fertility prognosis • HDS: provides information on chromatin condensation, associated with sperm cell immaturity	• High cost equipment is required • Precision is based on the evaluation of a large number of spermatozoa • Reference sample is required for flow cytometer calibration • The evaluation of partially stained spermatozoa reduces the objectivity • Does not reflect a distinct physiological process • Indirect evaluation of the actual fragmentation of the DNA • Result interpretation can be difficult
TUNEL	• Assessment of a small number of spermatozoa (~200) • The use of bright field microscopy may reduce the cost • Effective even in low concentration samples (*eg.* testicular biopsy) • Reference sample is not required	• Time consuming (~3 hours of laboratory time per assay) • Not clear correlation between suggested reference limits and prognosis in ART • Immature spermatozoa are not evaluated (*eg.* high HDS cells of SCSA) • High intra-assay and inter-laboratory variability

COMET	
• Quantifies the actual DNA damage of each examined spermatozoon (strand breaks) • More sensitive in alkaline conditions (identifies both single and double DNA strand breaks) • Correlates well with TUNEL and SCSA	• Special software required • Experience in data collection and interpretation required • Special equipment required (electrophoresis unit connected to fluorescence microscope) • Difficult to standardize (high intra- assay and inter-laboratory protocol variability) • Time consuming Alkaline method: • Possible overestimation of DNA breaks due to induced conversion of alkali-labile sites into breaks • Does not provide clear distinction between fertile normospermic and infertile normospermic/asthenozoospermic men Neutral method: • low sensitivity • no reference limits correlating test results and prognosis in fertility potential
DNA ladder	
• Detects apoptotic spermatozoa in relation to low molecular weight DNA molecules present • Low molecular weight DNA-bearing spermatozoa correlate with TUNEL positive spermatozoa	• Radioactive stains are required to observe the characteristic 'ladder' forms
DNA-break detection FISH	
• DNA fragmentation assessed directly in spermatozoa using genomic probes	• New method • Test results not adequately validated yet
Sperm chromatin integrity	
Aniline/Toluidine blue staining	
• DNA-protein interaction better evaluated in comparison to SCSA • Assessment of a small number of spermatozoa • Inexpensive • Applied with bright field microscopy • Test results correlate with TUNEL, SCSA, COMET	• Precision dependent on staining efficiency • Inter-laboratory variability not tested
Chromomycin A3	
• Negative correlation with fertilization rates in IVF	• Technically demanding • Current application only in research protocols • Inter-laboratory variability not tested

	Εντατική εργασία	Ακριβός εξοπλισμός	Υποκειμενικότητα αναλυτή	Επικύρωση & τυποποίηση
TUNEL	++++	+++	++	++
SCSA	++	++++	+	++++
Comet	++++	++	+++	++
SCD	+	+	++	+++

- ✓ Ημιποσοτική εκτίμηση της γενικότερης κατάστασης του DNA χωρίς να προσφέρει ενδείξεις για το πόιές αλληλουχίες έχουν υποστεί βλάβη (διαφορετικό επίπεδο βλάβης-δεν προσδιορίζεται η φύση και η αιτιολογία της βλάβης) χωρίς να έχουμε πάντα συγκρίσιμα αποτελέσματα.
- ✓ Ποικιλότητα στη μεθοδολογία και στα κριτήρια που χρησιμοποιούνται για την ανάλυση του σπέρματος (εκτίμηση αριθμού-χρώσεις, ακόμη και η υιοθέτηση ή μη των κριτηρίων της WHO.
- ✓ Ποιοτικός έλεγχος των εργαστηρίων.
- ✓ Έλλειψη ομοιομορφίας στους ελεγχόμενους πληθυσμούς.

The clinical utility of sperm DNA integrity testing

The Practice Committee of the American Society for Reproductive Medicine

American Society for Reproductive Medicine, Birmingham, Alabama

Sperm DNA damage is more common in infertile men and may contribute to poor reproductive performance. However, current methods for evaluating sperm DNA integrity do not reliably predict treatment outcomes, and no treatment for abnormal DNA integrity has proven clinical value. (Fertil Steril® 2008;90:S178–80. ©2008 by American Society for Reproductive Medicine.)

TABLE 2

Effect of sperm DNA integrity test results on reproductive outcomes.

Study population	No. of studies	Odds ratio (95% confidence interval) for Achieving pregnancy[a]
Normal couples	2	2.08 (0.23–19.0)
IVF	3	1.06 (0.27–4.25)
ICSI	3	1.07 (0.39–2.93)
IVF and ICSI	6	1.62 (0.96–2.72)

Note: OR = odds ratio; 95% CI = 95% confidence interval.

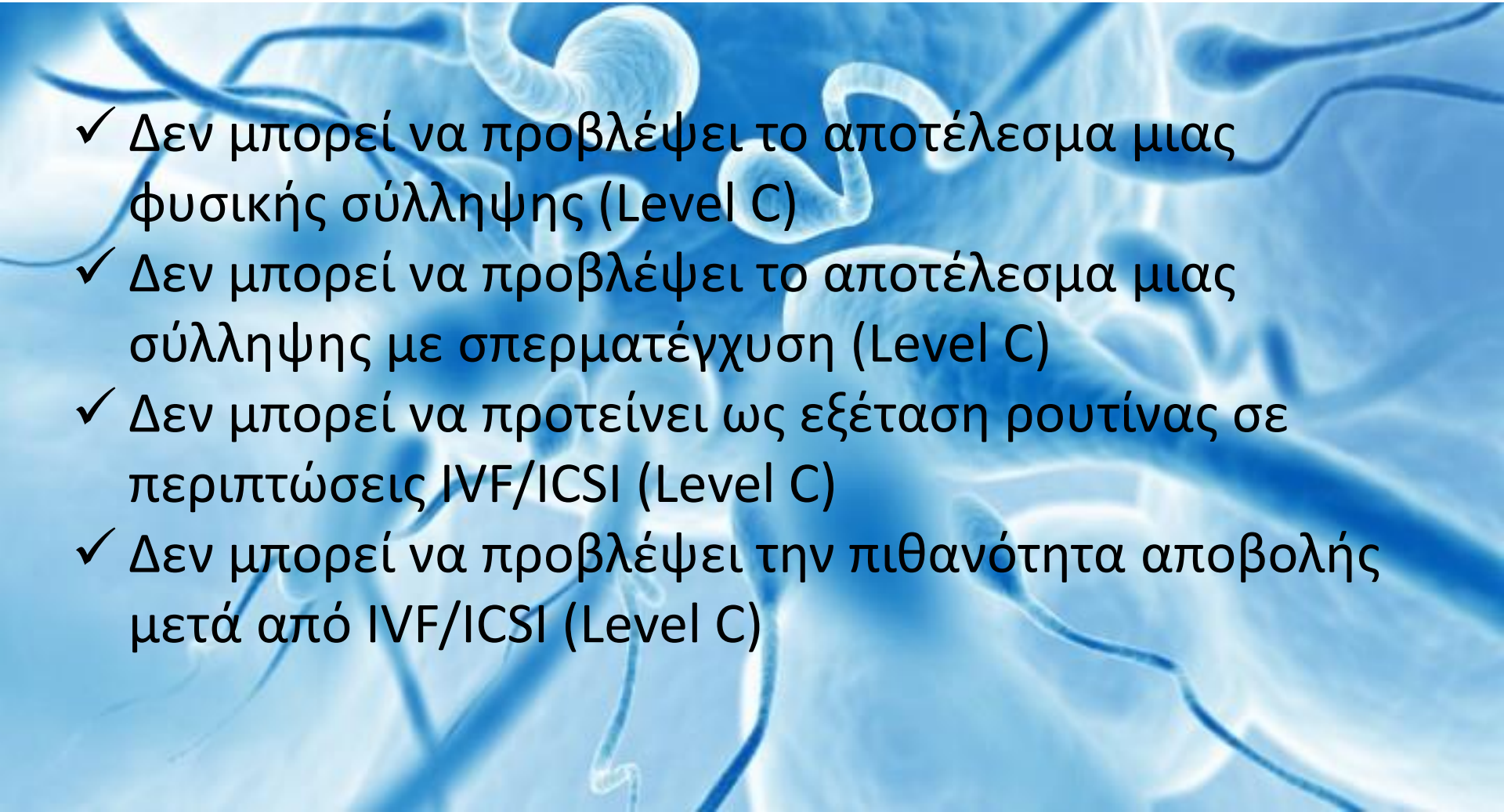
[a] OR >1 indicates pregnancy rate higher using sperm with normal DNA integrity.

ASRM Practice Committee. Sperm DNA integrity testing. Fertil Steril 2008.

The clinical utility of sperm DNA integrity testing: a guideline

The Practice Committee of the American Society for Reproductive Medicine

American Society for Reproductive Medicine, Birmingham, Alabama



- ✓ Δεν μπορεί να προβλέψει το αποτέλεσμα μιας φυσικής σύλληψης (Level C)
- ✓ Δεν μπορεί να προβλέψει το αποτέλεσμα μιας σύλληψης με σπερματέγχυση (Level C)
- ✓ Δεν μπορεί να προτείνει ως εξέταση ρουτίνας σε περιπτώσεις IVF/ICSI (Level C)
- ✓ Δεν μπορεί να προβλέψει την πιθανότητα αποβολής μετά από IVF/ICSI (Level C)

REVIEW

Sperm DNA damage: how relevant is it clinically?

Victor E. Beshay and Orhan Bukulmez

- ✓ Γενικώς το επίπεδο του DFI δεν επηρεάζει το αποτέλεσμα της ICSI.
- ✓ Οι κλινικές μελέτες μέχρι στιγμής υποφέρουν από χαμηλή αναπαραγωγιμότητα, διαφορετικούς ουδούς DFI και μη καταληκτικά συμπεράσματα όσον αφορά το αποτέλεσμα μιας εγκυμοσύνης.
- ✓ Έλλειψη μιας κοινά αποδεκτής μεθόδου προσδιορισμού του DFI με μια cut-off τιμή που να προσφέρει σημαντικά προγνωστικά οφέλη.
- ✓ Πολλές στρατηγικές έχουν διαμορφωθεί για τη μείωση του DFI, όμως καμμιά τεχνική δεν επιτρέπει την επιλογή σπερματοζωαρίων με ανέπαφο DNA με 100% επιτυχία.

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DEBATE

Sperm DNA fragmentation testing: To do or not to do?

Ο μέγιστος περιορισμός του DFI είναι η αδυναμία χρήσης του εξετασθέντος δείγματος για κλινική χρήση.

ΣΥΜΠΕΡΑΣΜΑΤΙΚΑ:

- Απαιτούνται μεγάλες κλινικές μελέτες για την πλήρη ενσωμάτωση της εξέτασης στην καθημερινή πρακτική.

Lin MH Fertil Steril 2008

- Οι στατιστικές μεταβλητές (ειδικότητα, ευαισθησία, πιθανότητα εμφάνισης) δεν επιτρέπουν ευθεία συσχέτιση με την πιθανότητα κύησης μετά από IVF ή ICSI.
- Αναγνώριση υποομάδων που θα μπορούν να ωφεληθούν.
- Απαιτείται σχεδιασμός και δημιουργία δοκιμασιών που θα μπορούν να αναγνωρίζουν και να απομονώνουν σπερματοζωάρια με ανέπαφο DNA.
- Μέχρι να αποκτήσουμε διαγνωστικές εξετάσεις με στανταρισμένο πρωτόκολλο, συγκεριμένους ουδούς που θα καθορίζουν την κλινική συσχέτιση και την προγνωστική αξία δεν μπορεί να χρησιμοποιηθούν στην καθημερινή κλινική πράξη.


Ευχαριστώ για την προσοχή σας!!!