

DFS årsmøde
Hotel Legoland marts 2019

DNA fragmentering i spermatozoer

Molekylærbiolog Anne Sofie Rex,
Aagaard Klinik, Virtus Health.
Odense Fertilitsklinik

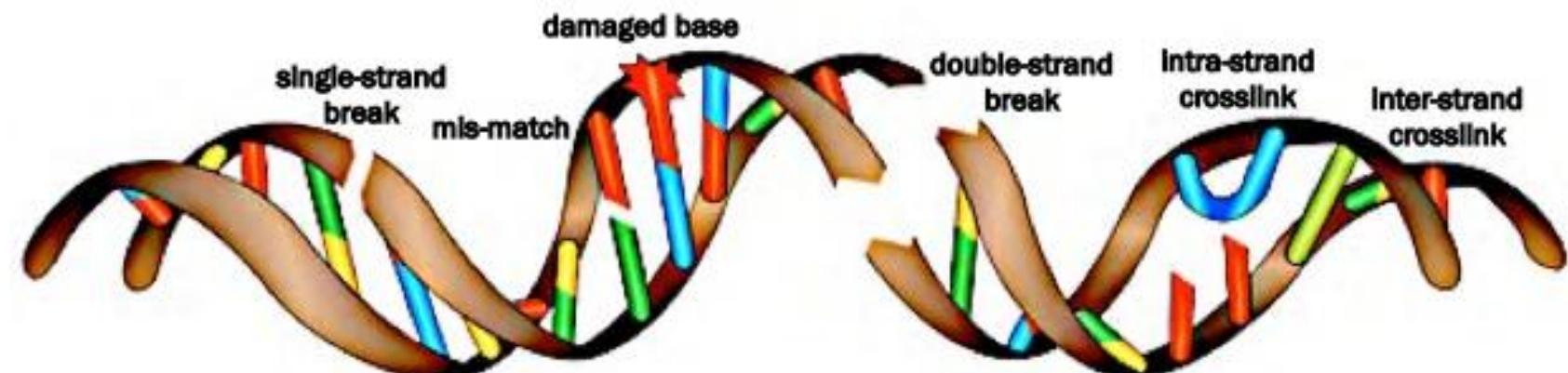
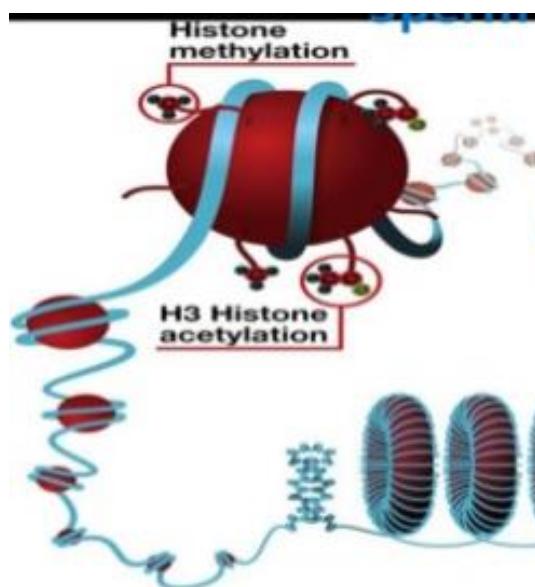


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Indhold:

- DNA fragmentering
 - Hvorfor er det relevant for os?
 - Betydning for fertilitet
 - Hvordan opstår det?
 - Hvordan måler vi det?
 - Hvad gør vi ved det?



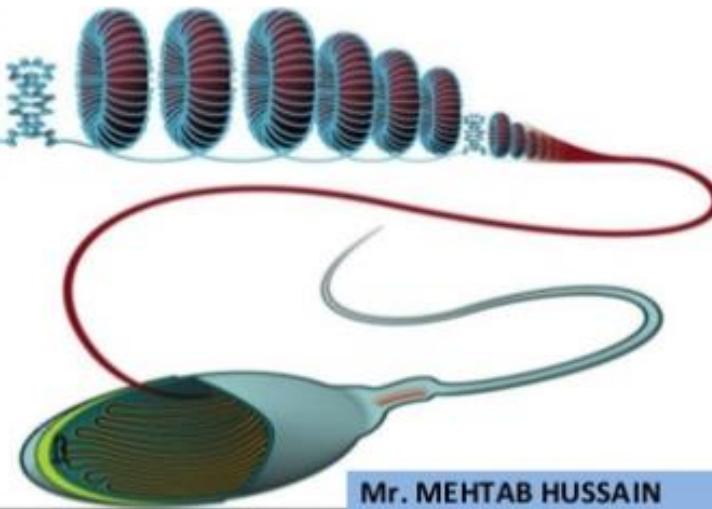
Esteves et al 2013; Alvarez and Gosálbez 2011; Ward 2011

Esteves, 7

ANDROFERT, Referral Center for Male Reproduction



GALAXY Pharma Pvt. Ltd.
Karachi



Mr. MEHTAB HUSSAIN
Embryologist



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Tillæg – ikke erstatning!

- Konventionel analyse + analyse for DNA fragmentering
- Resultatet er uafhængig af de andre sædparametre
 - Evgeni et al. 2013
 - Spano et al. 2000

Hvor mange er påvirket af forhøjet mængde af DNA skader?

- Idiopatisk infertile: 25% *Oleszczuk et al. 2012*
- 20% af alle patienter, der ellers lever op til kriterierne for IUI *Bungum et al. 2011*
- 17% af IUI patienter, 16% af IVF patienter og 32% af ICSI patienter *Bungum et al. 2007*

Reducerer chancen for spontan graviditet

Sperm chromatin damage impairs human fertility

Marcello Spanò, Ph.D.,* Jens P. Bonde, Ph.D.,† Henrik I. Hjollund, Ph.D.,† Henrik A. Kolstad, Ph.D.,† Eugenia Cordelli, B.Sc.,* Giorgio Leter, and The Danish First Pregnancy Planner Study Team[‡]

ENEA CR Casaccia, Rome, Italy, and Aarhus University Hospital, Aarhus, Denmark

Objective: To examine the relationship between sperm chromatin defects, evaluated by the fluorescence (FCM) sperm chromatin structure assay (SCSA), and the probability of a pregnancy in a month (fecundability).

Design: Follow-up study.

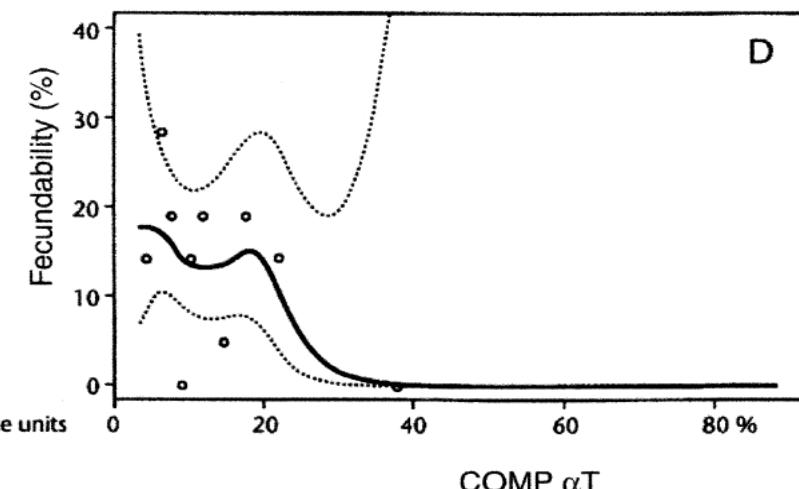
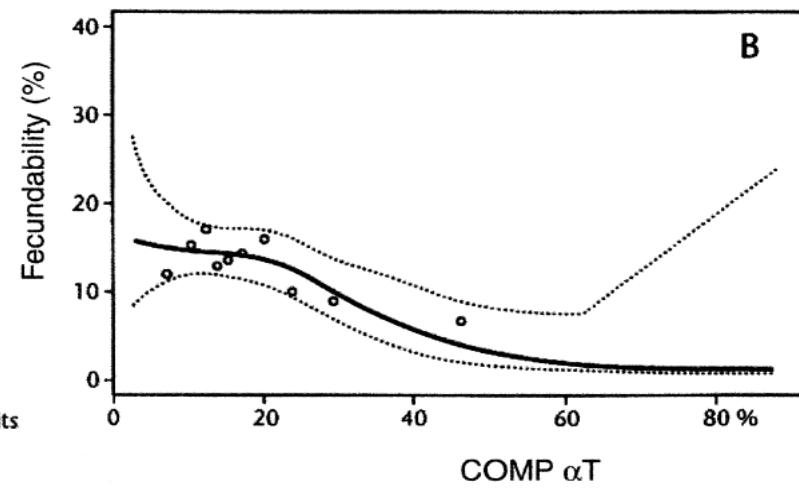
Setting: The Section of Toxicology and Biomedical Sciences, ENEA Casaccia, Rome, Italy, Department of Occupational Medicine, Aarhus University Hospital, Aarhus, Denmark.

Patient(s): Two hundred fifteen Danish first pregnancy planners with no previous knowledge of capability.

Intervention(s): None.

Main Outcome Measure(s): Semen samples were collected at enrollment to measure semen volume, concentration, motility, and morphology (by microscopy), as well as chromatin susceptibility to acid-induced partial denaturation by the FCM SCSA. Time to pregnancy was evaluated during the follow-up period. Demographic, medical, reproductive, occupational, and lifestyle data were collected via questionnaire. Fecundability was correlated with SCSA-derived parameters.

Result(s): Fecundability declines as a function of the percentage of sperm with abnormal chromatin structure.



Signifikante forskelle i DFI mellem gruppen af par med:

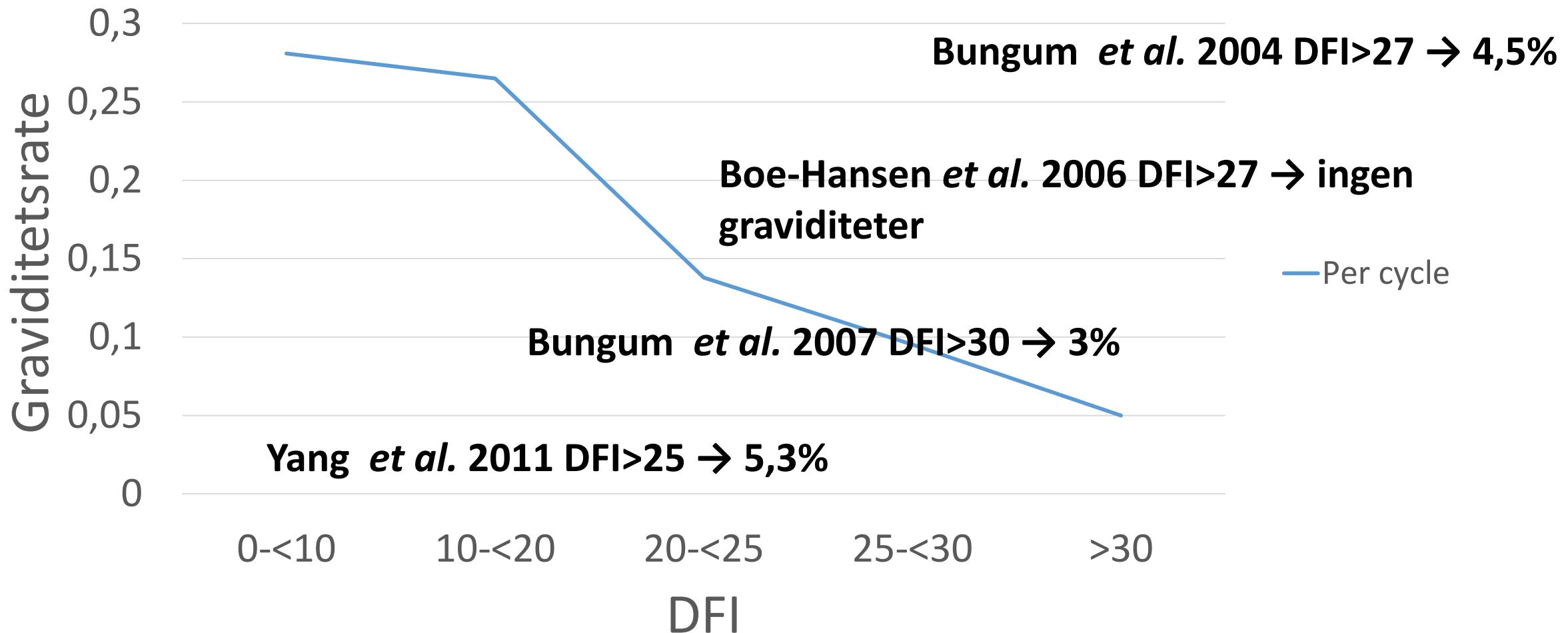
1. Graviditet indenfor 1-3 mdr.

2. Graviditet indenfor 4-12 mdr. ($p = 0,01$)

3. Ingen graviditet ($p = 0,001$)

Evenson *et al.* 1999

Graviditetsrater



Sædprøven

Total på 54 mio. sædceller/ml

Prog. motile 22 mio./ml

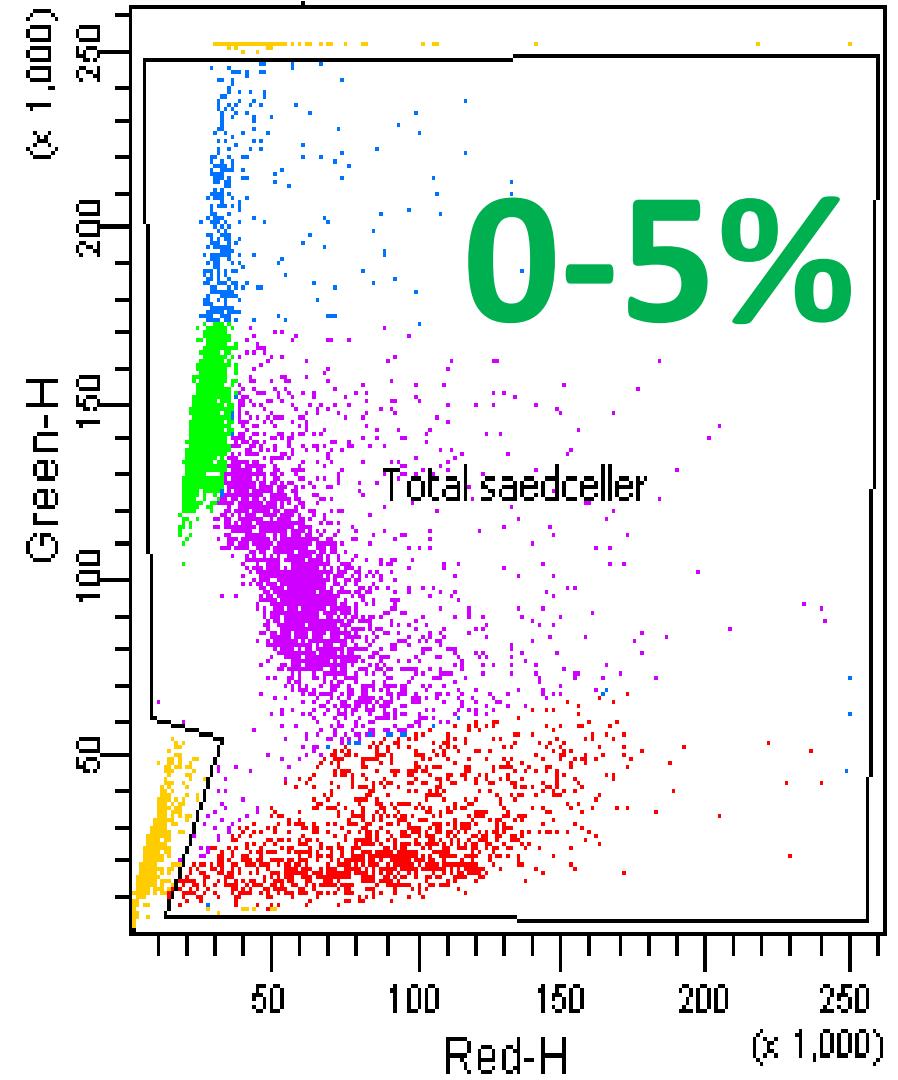
Normal volumen

Normal morfologi



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~~21%~~



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IVF/ICSI

- Faldende fertiliseringsrate for IVF som DFI stiger
- Ingen påvirkning af fertiliseringsrate efter ICSI som DFI stiger.

Oleszczuk *et al.* 2012

- Fertilisationsraterne efter IVF/ICSI er ikke påvirket af DFI.

Virro *et al.* 2004

- Mindre forskel på fertiliseringsraterne efter IVF eller ICSI ved højt DFI

Cissen *et al.* 2016

- Graviditetsrater efter ICSI ikke påvirket af DFI, men moderat påvirket ved IVF behandling

Zini *et al.* 2008

Risiko for abort

- Signifikant større risiko for at miste graviditeten igen, hvis DFI er forhøjet.

- 808 IVF og 741 ICSI, 640 graviditeter hvoraf 122 blev mistet.

Zini *et al.* 2008

- 2969 par , 1252 graviditeter hvoraf 225 blev mistet.

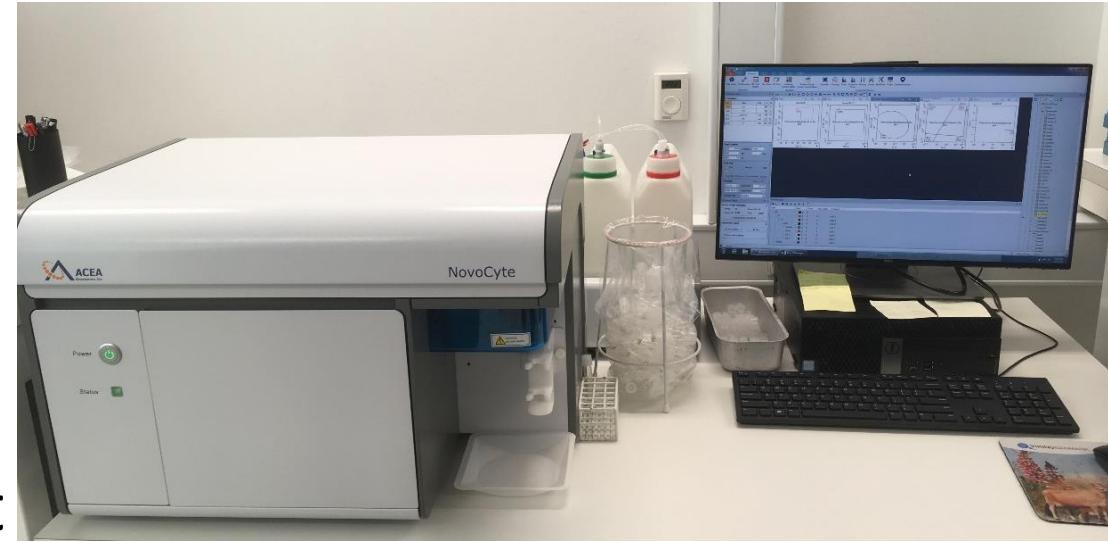
Robinson *et al.* 2012

Gestationslængde eller fødselsvægt ikke påvirket af DFI.

Bungum *et al.* 2011

Metoder til analysen

- Sperm chromatin dispersion (SCD) test
- TUNEL
- Sperm chromatin structure assay (SCSA)
- Alkaline Comet Assay
- Neutral Comet Assay
- Acridine Orange Test (AOT)



- Chohan *et al.* 2006
- Ribas-Maynou *et al.* 2013
- Evenson *et al.* 2002

VIGTIGT – Reference og god kvalitetskontrol

J Assist Reprod Genet (2016) 33:291–300
DOI 10.1007/s10815-015-0635-7

TECHNOLOGICAL INNOVATIONS



Terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) assay using bench top flow cytometer for evaluation of sperm DNA fragmentation in fertility laboratories: protocol, reference values, and quality control

Rakesh Sharma¹ · Gulfam Ahmad^{1,2} · Sandro C. Esteves³ · Ashok Agarwal¹

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Abstract

Purpose The purpose of this study is to provide a detailed protocol and quality control steps for measuring sperm DNA fragmentation (SDF) by terminal deoxynucleotidyl transferase deoxyuridine triphosphate (dUTP) nick end labeling (TUNEL) assay using a new bench top flow cytometer, determine the reference value of SDF, and assess sensitivity, specificity, and distribution of SDF in infertile men and controls

distribution of different cutoff values in controls and infertile patients were calculated.

Results The reference value of SDF by TUNEL assay was 16.8 % with a specificity of 91.6 % and sensitivity of 32.6 %. The positive and negative predictive values were 91.4 and 33.1 %, respectively. The upper limit of DNA damage in infertile men was significantly higher (68.9 %) than that in the controls (19.6 %).

Implementation of an in-house flow cytometric analysis of DNA fragmentation in spermatozoa.

*Anne Sofie Rex^{1, 2, 3}, Chunsen Wu^{1, 3}, Jørn Aagaard², Jens Fedder^{1, 3}

¹Centre of Andrology & Fertility Clinic, Odense University Hospital, 5000 Odense; Denmark; ² Aagaard Gynaecological Clinic, 8200 Skejby, Aarhus; Denmark; ³ Research Unit of Gynaecology and Obstetrics, Faculty of Health, University of Southern Denmark, 5000 Odense, Denmark.

*Corresponding author. For contact: annesofierex@gmail.com

Abstract

An increased amount of DNA fragmentation in the spermatozoa (SDF) is linked to male infertility. The Sperm Chromatin Structure Assay (SCSA) has been used for analysis of SDF. However, the current software (SCSASoft*) linked to this assay is expensive and often located within larger diagnostic centres.

In this study, we present a protocol for using other types of software than SCSASoft* to determine the SDF index (DFI) with clinical relevance.

This protocol is engineered after collecting and analysing 254 samples from fertility patients and sperm donors over a 15-months' period. DFI is analysed using a strict protocol where the spermatozoa are treated with a strong acid (pH 1.2) followed by acridine orange. DFI is determined by a standard flow cytometric software, FACSDiva 6.1.3. Analysis of the outcome of

IN REVIEW

Infektioner

Kost

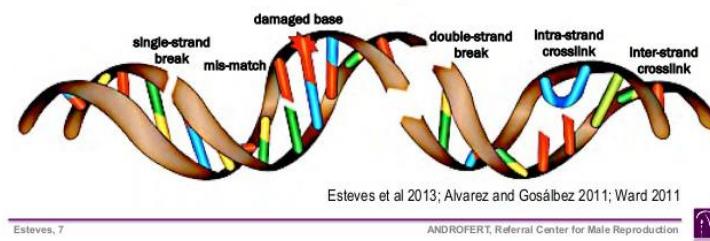
Rygning

Feber

Alder

Overvægt

Forlænget
ophold i
testiklerne



?

Spermatogenesen

Ubalance i
antioxidanter

FSH

Varicocele

Hvad gør vi ved det?

- Stoppe med rygning
- Kostomlægning
 - Salas-Huetos *et al.* 2017
 - Wright *et al.* 2014
- Hyppige ejakulationer
 - Lu *et al.* 2018
 - Pons *et al.* 2013
 - Agarwal *et al.* 2016
 - Hanson *et al.* 2018
 - Uppangala *et al.* 2015

- FSH
 - DFI ↓ Graviditet ↑
 - Garolla *et al.* 2017
 - Muratori *et al.* 2018
- Urinprøve for infektioner
 - Klamydia og Mycoplasma
 - Gallagos *et al.* 2008
 - Sellami *et al.* 2014
 - Moazenchi *et al.* 2018
- Varicocele
 - Associeret med DFI
 - Varicocelectomi → DFI ↓
 - Roque & Eszteves 2018
- Antioxidanter
 - Gual-Frau *et al.* 2015
 - Greco *et al.* 2005
 - Gil-Villa *et al.* 2009
 - Martines-Soto *et al.* 2016
- Mulige bivirkninger
 - Ménézo *et al.* 2007
 - Lewis *et al.* 2013
- IMSI

Hvor skal vi passe på

- Sammenligne på tværs af metoder
- Cut-off værdier
- Skal bruges som negativ prædiktor for graviditet og IKKE en positiv.

Opsummering

Måling af DNA fragmentation kan være et tillæg til konventionel sædanalyse, der laves i klinikkerne.

Flere metoder kan benyttes.

- Man kan vejlede i forhold til:

- Rygning
- Kost
- Hyppige ejakulationer

- Man kan undersøge yderligere:

- FSH
- Infektioner
- Varicocele
- Antioxidanter
- Anden fert. behandling (eks. IMSI)

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