INTEGRALNOŚĆ DNA PLEMNIKÓW

dr n wet R. Faúndez

Katedra Chorób Dużych Zwierząt z Kliniką

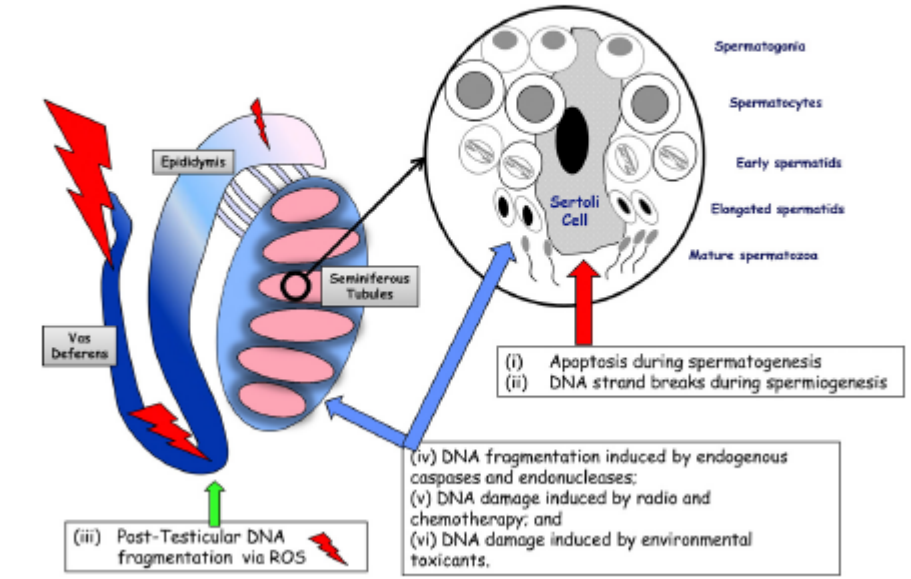
Zakład Rozrodu Zwierząt, Andrologii i Biotechnologii Rozrodu

Sperm DNA fragmentation: mechanisms of origin, impact on reproductive outcome, and analysis

Denny Sakkas, Ph.D., and Juan G. Alvarez, M.D., Ph.D, Fertility and Sterility Vol. 93, No. 4, March 1, 2010

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.

TABLE 1

Outcome results of TESA–ICSI in couples with repeated IVF failure and high levels of sperm DNA fragmentation in semen.

Characteristic	Value
Semen and case parameters	
Cases evaluated	68
Cases of TESA–ICSI performed	31
Mean DFI in semen (%)	39.4
Mean sperm concentration (million/mL)	44.7
Mean male age (y)	41.9
Outcome results	
Fertilization rate (%)	58 (20–100)
Number of embryos transferred	2.3
Clinical pregnancy rate (%)	40.0
Pregnancy rate in the first TESA–ICSI cycle (%)	93

Note: DFI = DNA fragmentation index; TESA–ICSI = testicular sperm extraction–intracytoplasmic sperm injection.

Sakkas. Sperm DNA fragmentation. Fertil Steril 2010.

TABLE 2

Pregnancy outcome in patients with high levels of sperm DNA fragmentation when treated with ejaculated compared with testicular sperm.

Sperm	Biochemical pregnancy rate (%)	Clinical pregnancy rate (%)	Implantation rate (%)	Miscarriage rate (%)
Ejaculated (n = 42)	6.90	13.79	6.56	75.0
Testicular (n = 30)	2.5	40.0	28.09	6.25
P value	NS	.035	.0021	.017

Note: All patients included in the study had a DNA fragmentation index value in semen by TUNEL >20%; statistical analysis by χ^2 test.

Sakkas. Sperm DNA fragmentation. Fertil Steril 2010.

Podsumowując, obecność fragmentacji DNA plemników została potwierdzona przez wiele artykuły w ciągu ostatniej dekady. Rozróżnienie pomiędzy plemnikami tych dotkniętych a nie wykazujących uszkodzonego DNA"" plemnika jest niezwykle istotne dla postępowania klinicznego tych pacjentów. W odniesieniu do technik wspomaganego rozrodu musimy identyfikować plemników uszkodzonych i wybrać populacji o" normalnych plemników". Wreszcie, obecność DNA sfragmentowanego w plemnikach jest, ma wpływ na zarodek i potomstwa. **Diagnozowanie stopnia fragmentacji DNA plemników jest dzisiaj koniecznością.**

TESTY OCENIAJĄCE INTEGRALNOŚĆ DNA

- **Ocena stabilności i kondensacji chromatyny**
  - AAB (Acidic Aniline Blue Stain)
- **Ocena integralności DNA**
  - SCSA (Sperm Chromatin Structure Assay)
  - Comet (SCGE-single cell gel electrophpresis)
  - TUNEL (terminal deoksynucleotidyl transferases dUTP and labeling)
  - DBD FISH (DNA Break Detection Fluorescent *in situ* Hybridization)
  - SCD (Sperm Chromatin Dispertion)

AAB (ACIDIC ANILINE BLUE STAIN)

- **Barwnik daje pozytywną reakcje dla lizyny; w konsekwencji niedojrzałe plemniki, których jądra histony bogate są w lizynę, wyróżniają się intensywnym** zabarwieniem

- **Wyniki testu wskazują na zaburzenia** protaminacji podczas spermatogenezy

- **Poziom kondensacji DNA jest skorelowany z obecnością złamań w łańcuchu DNA monitorowanych testem TUNEL i** Comet

TEST TUNEL
(TERMINAL DEOKSYNUCLEOTIDYL TRANSFERASES DUTP AND LABELING)

- **Test TUNEL ocenia stopień fragmentacji DNA**
- **Metoda bezpośrednia testu: dołączenie do wolnego końca 3'OH w łańcuchu DNA substratu** dUTP **znakowanego fluoresceiną (FITC –** fluorescein isothiocyanate)
- **Metoda pośrednia testu: do detekcji wbudowanego substratu** dUTP **używa się przeciwciało monoklonalne znakowane FITC** skierowane przeciwko substratowi
- **Wiązanie** dUTP **do końców DNA katalizuje terminalna** deoksynukleotydylotransferaza (TdT)

TEST TUNEL CD.

- **Obecność nacięć w łańcuchu DNA wyznakowanych sondą fluorescencyjną można obserwować w mikroskopie fluorescencyjnym, jako świecenie w obrębie główki plemnika (są** to tzw. komórki TUNEL-pozytywne)

- Kontrola pozytywna - dodatek DNA-zy **I indukującej fragmentację** DNA

- **W kontroli negatywnej nie dodaje się transferazy terminalnej - brak świecenia**

- **Analizę fluorescencji można wykonać ilościowo przy użyciu** cytometru **przepływowego. Ocenia się odsetek komórek TUNEL-**pozytywnych (o pofragmentowanym DNA) i TUNEL-negatywnych **(nie zawierających nacięć w łańcuchu DNA)**

CYTOMETRIA PRZEPŁYWOWA

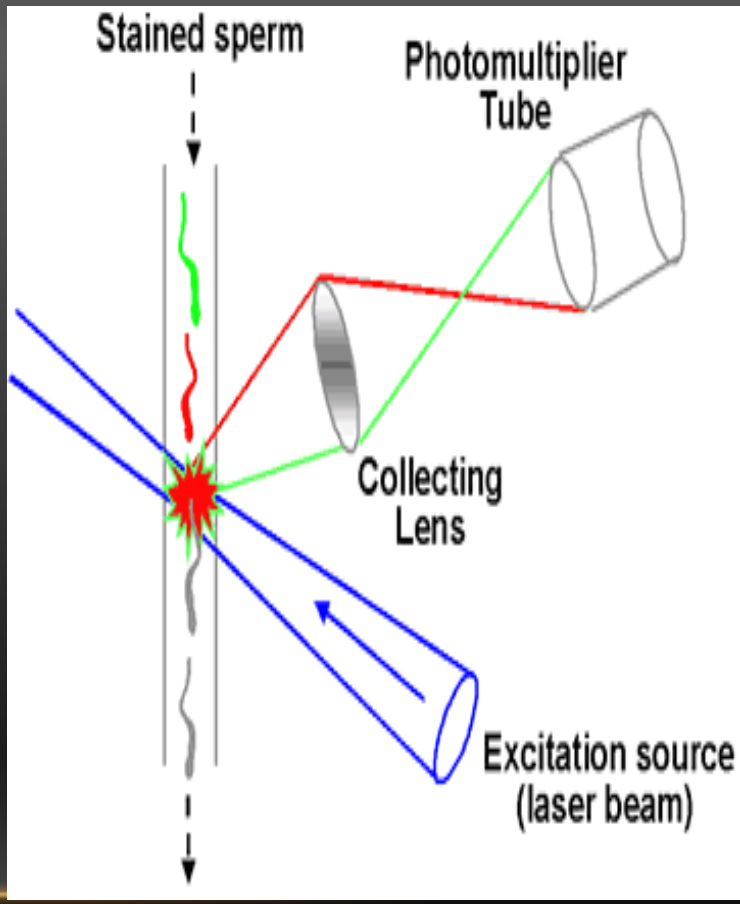
- Cytometria **przepływowa to metoda pomiaru pojedynczych komórek lub cząsteczek przepływających przez aparat w** strumieniu cieczy

- FACS (Fluorescence Activating Cell Sorting) metoda cytometrii **przepływowej rozszerzona o możliwość sortowania**

- **Mierzone sygnały**
  - **Rozproszenie światła przez badaną próbę**
  - **Intensywność fluorescencji składników komórki lub zastosowanych** barwników
  - **Absorbcja światła przez próbkę**
  - **Pomiary w czasie (użyte dla badania kinetyki, dynamicznych zachowań** populacji komórek)

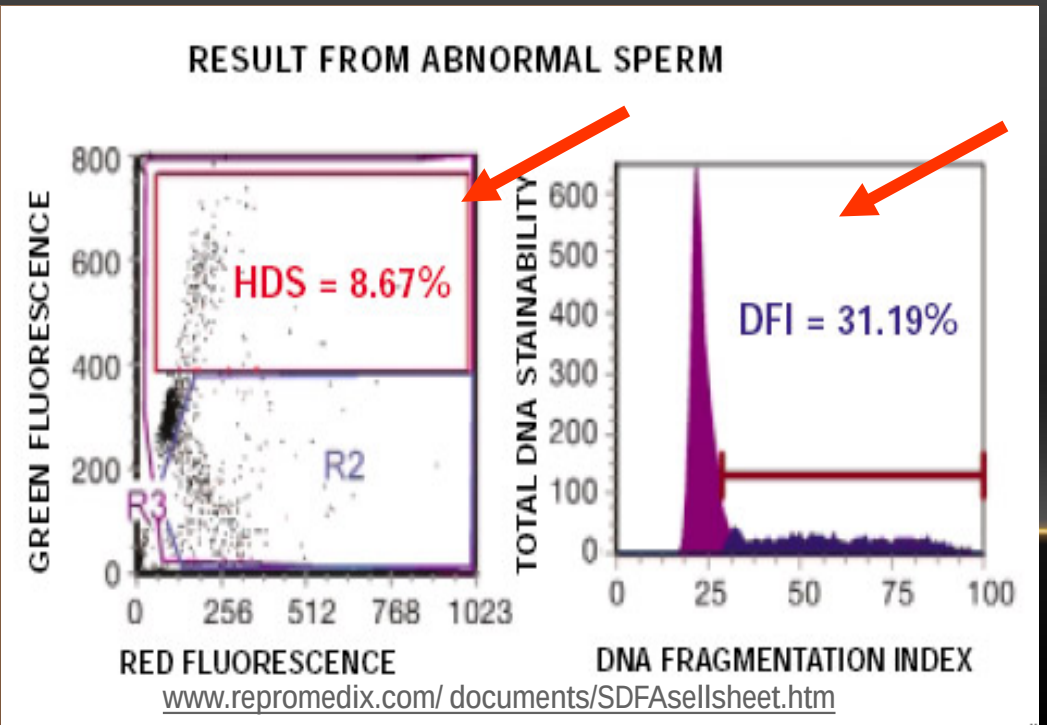
CYTOMETRIA PRZEPŁYWOWA CD.

- **Przedni detektor światła rozproszonego** (Forward Scatter Channel, FSC) **– ilość światła rozproszonego wzdłuż osi przebiegu światła wzbudzającego**
- **Boczny detektor światła rozproszonego** (Side Scatter Channel, SSC) **– ilość światła rozproszonego prostopadle do osi światła wzbudzającego**
- Detektory fluorescencji **– można używać kilka** barwników fluorescencyjnych lub **wyznakowanych przeciwciał**

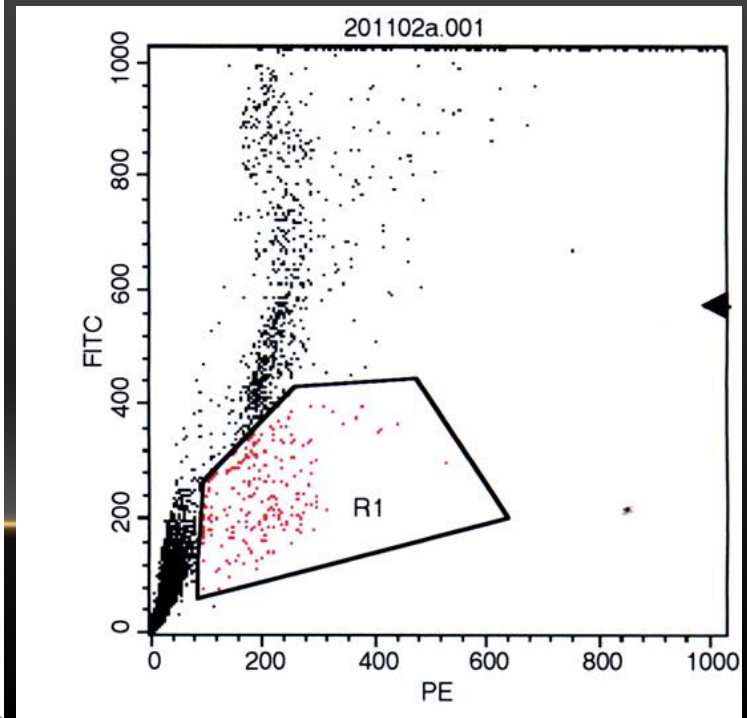


TEST SCSA
(SPERM CHROMATIN STRUCTURE ASSAY)

- **Test wykorzystuje metachromatyczne właściwości AO (oranż akrydyny) do oceny wrażliwości DNA na kwaśną denaturację**
- **Różna wrażliwość na denaturację i dostępność barwnika wskazuje na obecność w nasieniu plemników o różnym stopniu upakowania chromatyny**



www.repromedix.com/ documents/SDFAsellsheet.htm



TEST SCSA

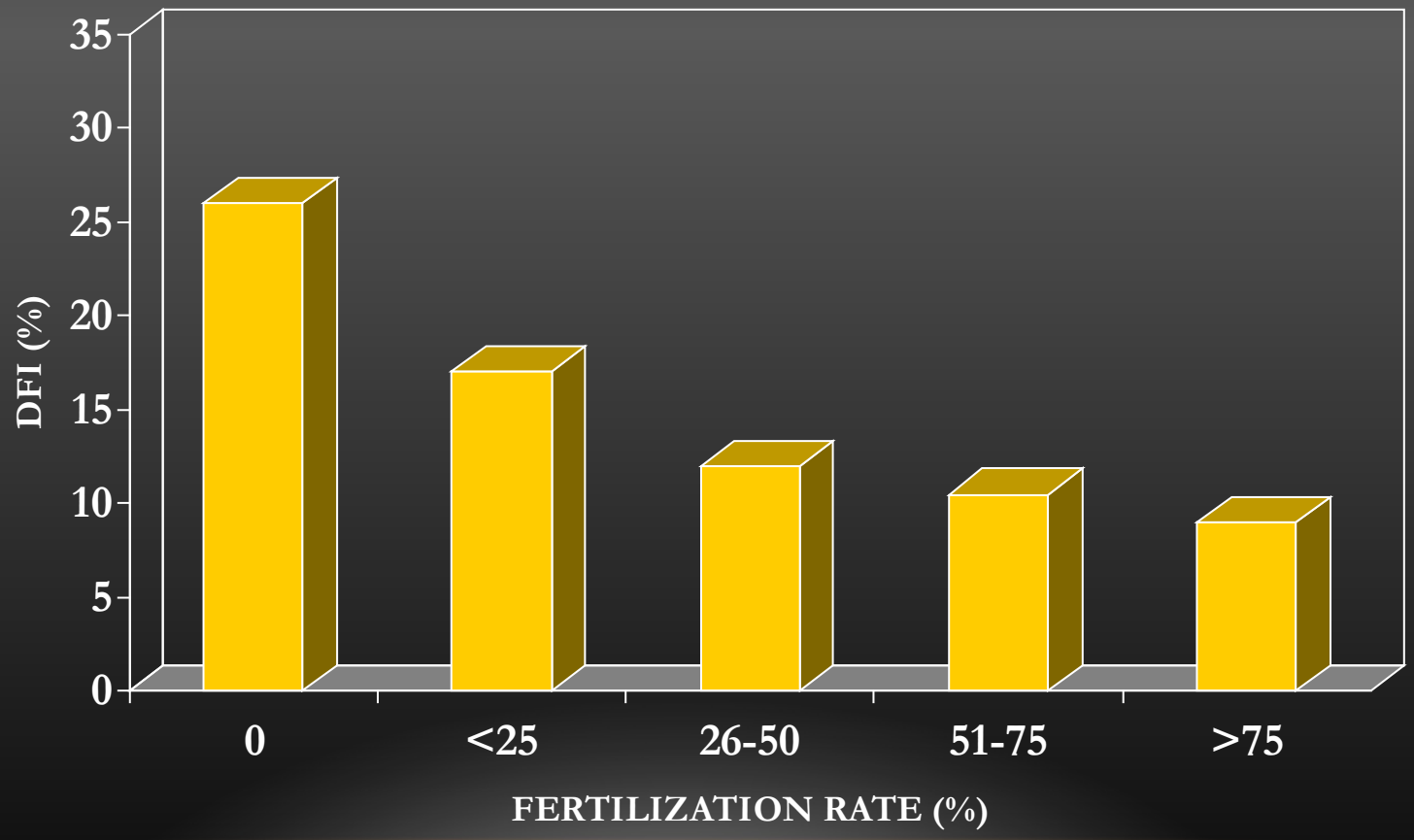
- **U mężczyzn, buhajów i myszy o niskiej płodności denaturacja DNA zachodzi w stopniu wyższym niż u samców o udowodnionej wysokiej płodności**

- 20-godzinne przechowywanie w temp. 5°C nasienia ogierów **o obniżonej płodności prowadzi do wzrostu wrażliwości chromatyny plemników na denaturację, podobny wpływ ma** przechowywanie nasienia knurów (72 godz, temp18°C)

- **W przypadku knurów skutkiem zmian stabilności chromatyny może być obniżenie liczebności miotów**

- **Wyniki testu SCSA korelują z wynikami zapłodnienia i liczebnością uzyskanych ciąż**

FRAGMENTACJA DNA PLEMNIKÓW A ODSETEK ZAPŁODNIEŃ PO ICSI



Benchabib, Fertil Steril, 2007

Parameters	ICSI	
	Sperm DNA fragmentation	
	<15%	>15%
Fertilization rate (%)	75,4	70,3
Arrested development (%)	4,2	18,2
Pregnency/transfer (%)	37,4	27,8
Pregnency/early transfer (%)	37,6	23,1
Pregnency/late transfer (%)	36,8	40,0
Miscarriage (%)	8,6	30,0

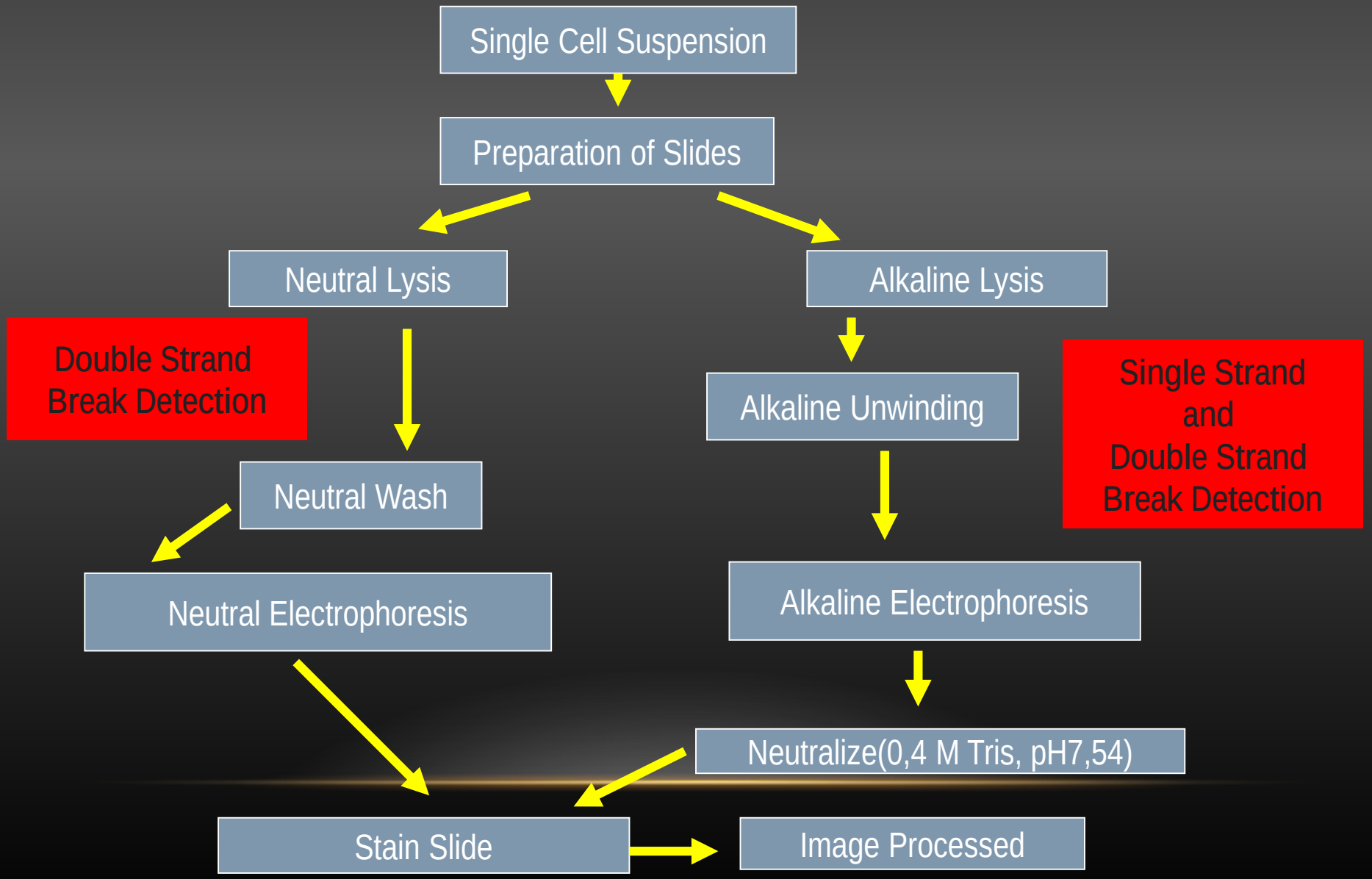
39% poronień występuje przy DFI>30% *(Evenson et al.)*

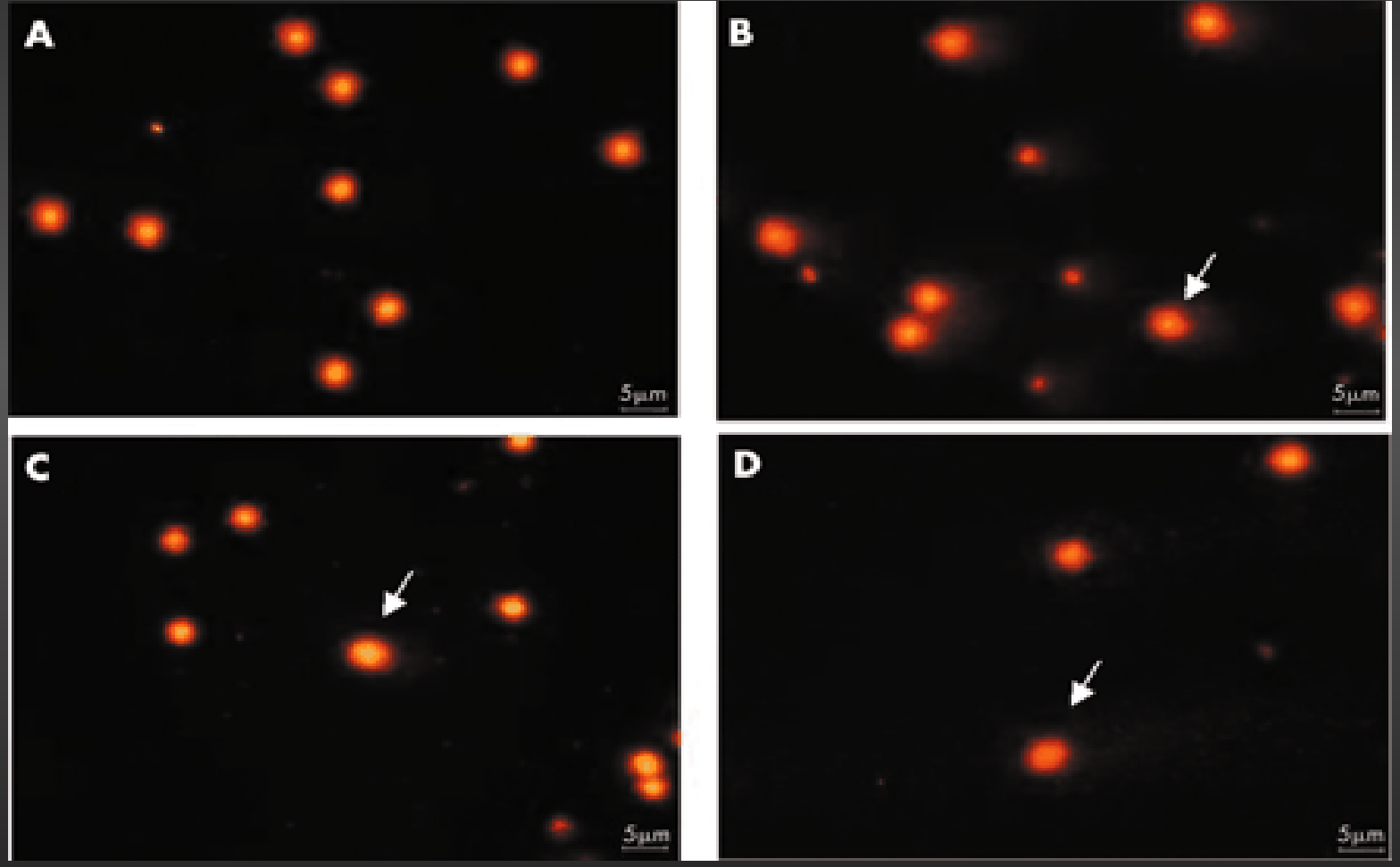
31% przypadków poronień występuje przy użyciu do ICSI nasienia cryptozoospermicznego i OAT *(Sanchez et al.)*

TEST COMET
(SCGE - SINGLE CELL GEL ELECTROPHPRESIS)

- Wykorzystywany do badania fragmentacji DNA plemników
- **Wyniki uzyskane są skorelowane z rozwojem zarodków uzyskanych na drodze zapłodnienia in vitro, także z wynikami** testów SCSA i TUNEL
- **Do oceny wyników stosuje się mikroskop fluorescencyjny; wymaga doświadczenia na etapie analizy i interpretacji** wyników
- **Możliwość analizy komputerowej**

TEST COMET CD.

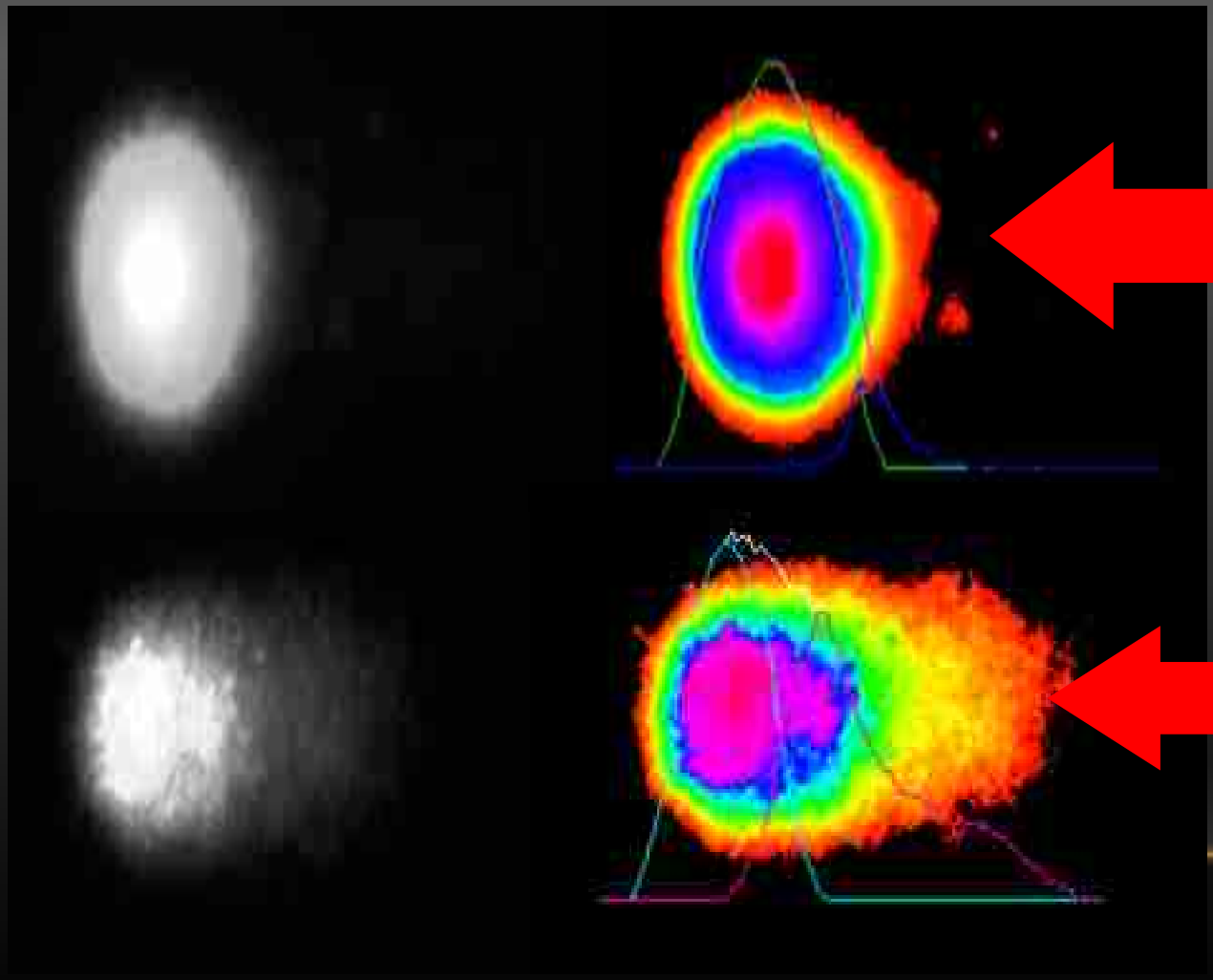




Ryc. W przypadku występowania uszkodzeń DNA zaobserwować można obraz przypominający kometę. Głowę komety stanowi obszar jądra komórkowego, natomiast ogon fragmenty DNA, które wyemigrowały podczas elektroforezy. Im większe uszkodzenia tym ogon jest dłuższy i zawiera więcej DNA. (A) Grupa kontrolna: plemniki o nieuszkodzonym DNA. (B) Plemniki o niskiej fragmentacji DNA. **(C) Plemniki o średniej fragmentacji DNA. (D) Plemniki o największej fragmentacji** DNA.

http://oem.bmjjournals.com/cgi/content/full/61/12/999/F1

TEST COMET CD.



Obraz DNA:
komórki kontrolne
9% DNA w ogonie
(lewa strona widok rzeczywisty,
prawa z oprogramowania do analizy)

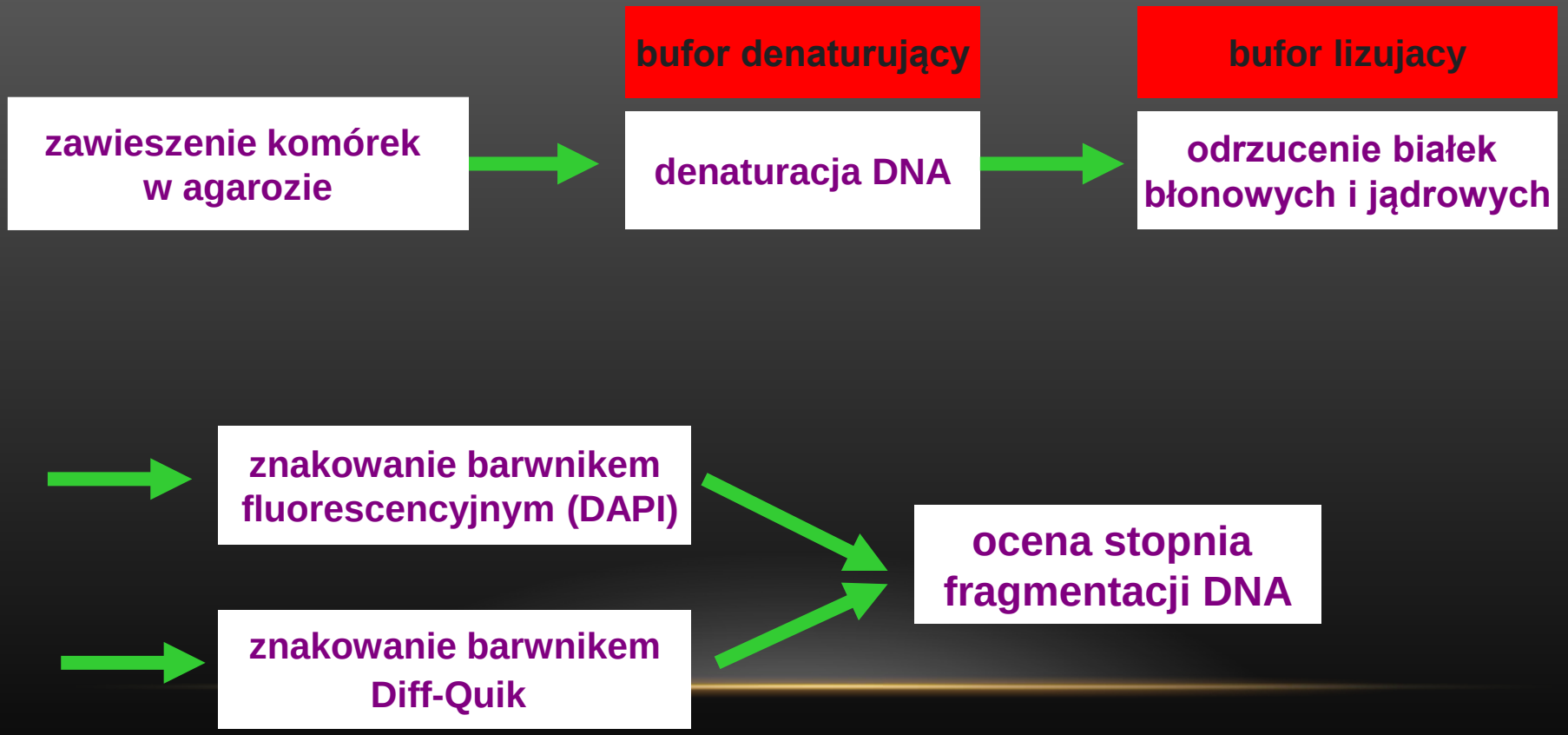
Obraz DNA:
50% DNA w ogonie

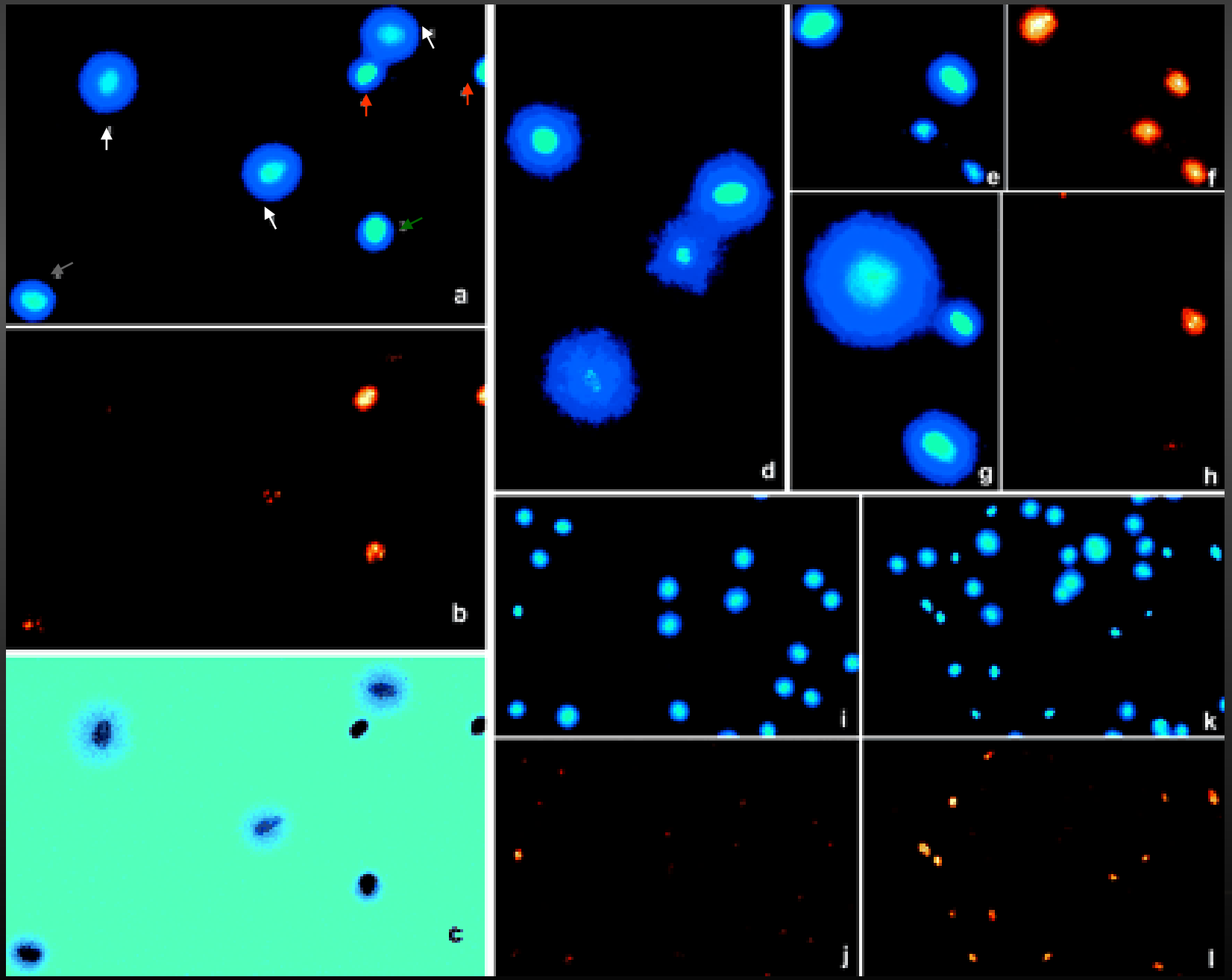
DNA BREAK DETECTION FLUORESCENT *IN SITU* HYBRIDIZATION TEST (DBD FISH)

- **Test ocenia obecność złamań w łańcuchu DNA pojedynczych** komórek utrwalonych w agarozie
- **Po odrzuceniu nukleoprotein stosuje się bufor alkaliczny do rozplecenia podwójnej nici DNA w miejscach obecności złamań w łańcuchu DNA**
- **Złamania w pojedynczej nici DNA wizualizuje się wyznakowaną fluorescencyjnie sondą genomową**
- Fluorescencja pojedynczej komórki jest proporcjonalna do liczby **złamań w pojedynczej nici DNA**

TEST SCD

(SPERM CHROMATIN DISPERTION)

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OCENA FRAGMENTACJI DNA JAKO WSKAŹNIK POWODZENIA ART

- SCSA - >30% DFI
- DFI<27%,HDS<10% wzrost odsetka udanych inseminacji
- TUNEL – 12 - 36,5%
- Test SCD >36%
- Ryzyko zatrzymania rozwoju zarodka po ICSI wzrasta gdy DFI>15%*
- **Odsetek ciąż (ICSI+IVF) 35,7% gdy DFI<15%, 31,8 gdy DFI>15%***
- **Odsetek ciąż (ICSI) 37,4% gdy DFI<15%, 27,8% gdy DFI>15%***
- Poronienia: 37,5% gdy DFI>15%, 8,8% gdy DFI<15%*
- **M. Benchabib, J. Lornage, C. Mazoyer et al.. Fert. Ster. Vol87, No. 1, January 2007*

FRAGMENTACJA DNA – „KONSEKWENCJE”!!!

- **Obniżona zdolność do zapłodnienia** *in vivo:*
  - **przyłączenie do komórek nabłonka jajowodu**
  - hiperaktywacja **i łączenie się z** *zona pellucida*

- **Obniżona możliwość** dekondensacji **materiału genetycznego**
- **Nieprawidłowy podział zygoty i wczesny rozwój zarodka**
- **Częstsze wczesne i spontaniczne poronienia**
- **Podwyższone ryzyko uszkodzenia genomu wynikające z braku** mechanizmów naprawczych DNA
- Ryzyko przeniesienia wad genetycznych na potomstwo

SCD

Mgr inż. Katarzyna Jaroszek
Laboratorium Embriologii Klinicznej
InviMed Europejskie Centrum Macierzyństwa
Warszawa

SCD

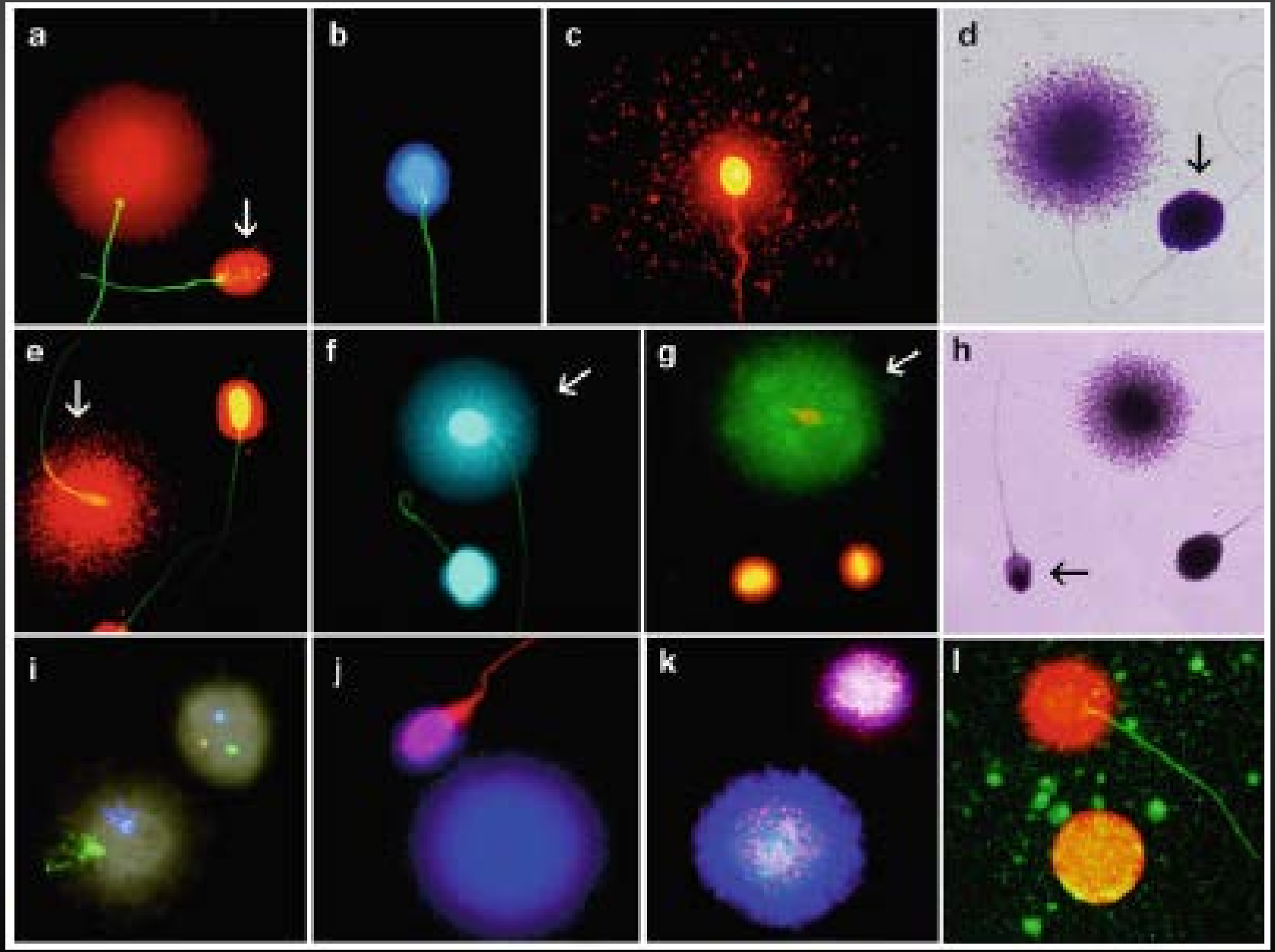
application

keep dry

Halosperm® Kit from Halotech Dna, SL is a simple test that allows assessment of sperm DNA fragmentation in humans.

Principle of the method:

The method is based on the Sperm Chromatin Dispersion (SCD) test (Fernández et al., J. Androl 24: 59-66, 2003; Fertil Steril 84: 833-842, 2005). Intact unfixed spermatozoa (fresh, frozen/unthawed, diluted samples) are immersed in an inert agarose microgel on a pretreated slide. An initial acid treatment denatures DNA in those sperm cells with fragmented DNA. Following this, the lysing solution removes most of the nuclear proteins, and in the absence of massive DNA breakage produces nucleoids with large halos of spreading DNA loops, emerging from a central core. However, the nucleoids from spermatozoa with fragmented DNA either do not show a dispersion halo or the halo is minimal.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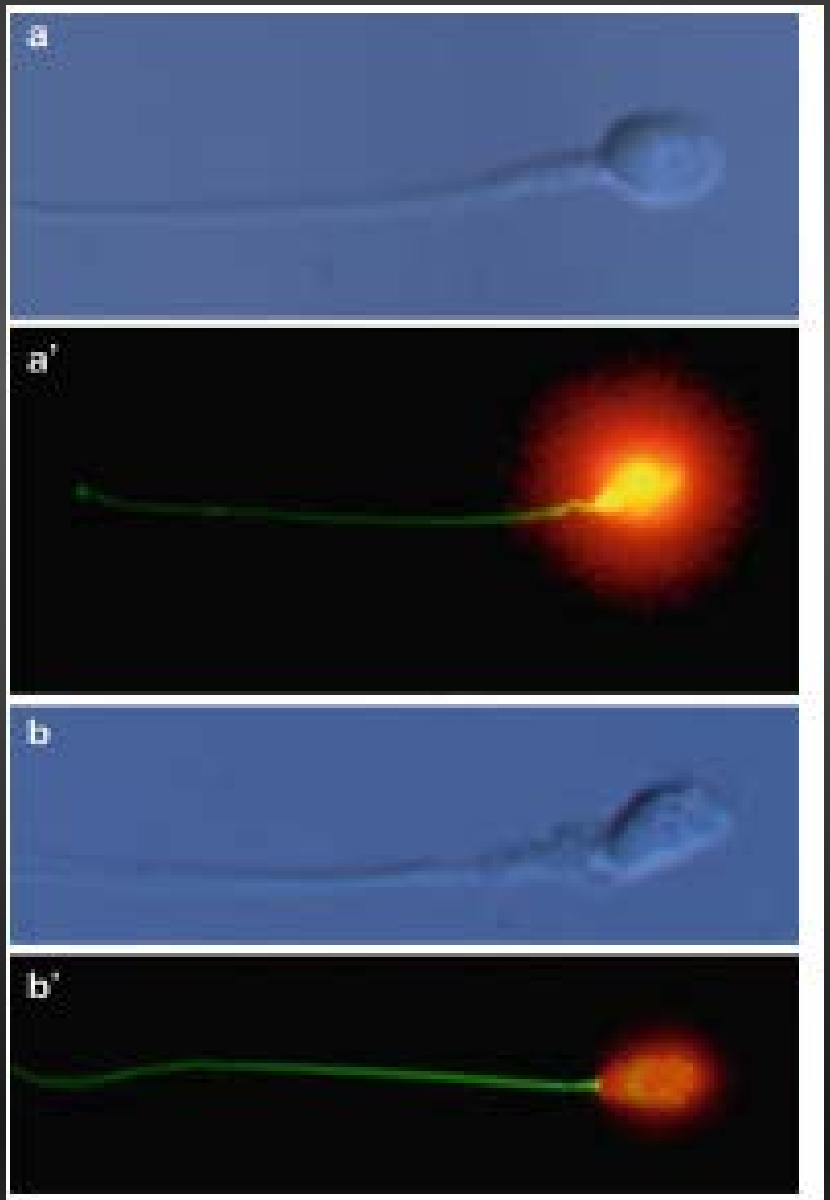


Fig. 10.3 High magnification selected sperm (a, b) and the characterization of SDF in the same sperm (a', b'). The SCD test allows the direct assessment of the DNA status and the sperm morphology

halotech dna

halosperm®

Comparison of sperm DNA fragmentation tests

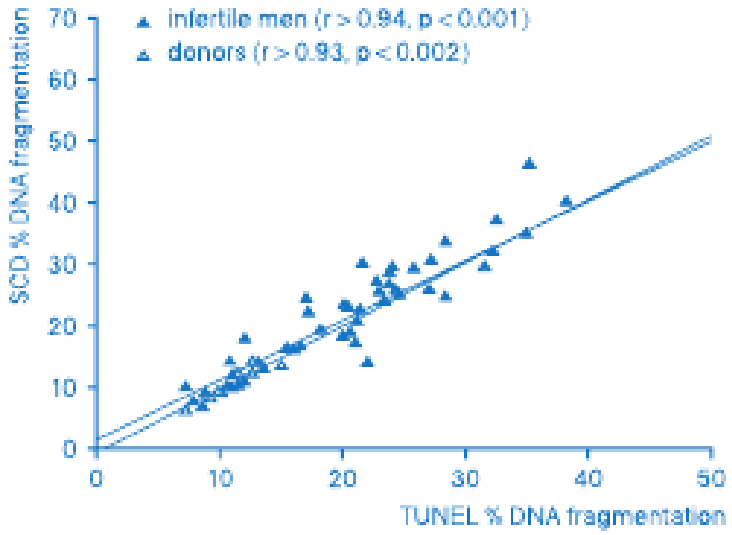
In order to evaluate sperm DNA fragmentation, Halotech has developed the **Halosperm®** kit: a fast and simple in vitro diagnostic kit based on the Sperm Chromatin Dispersion (SCD) technique first described by Fernández et al., 2003. Here is how our kit compares to other techniques available on the market:

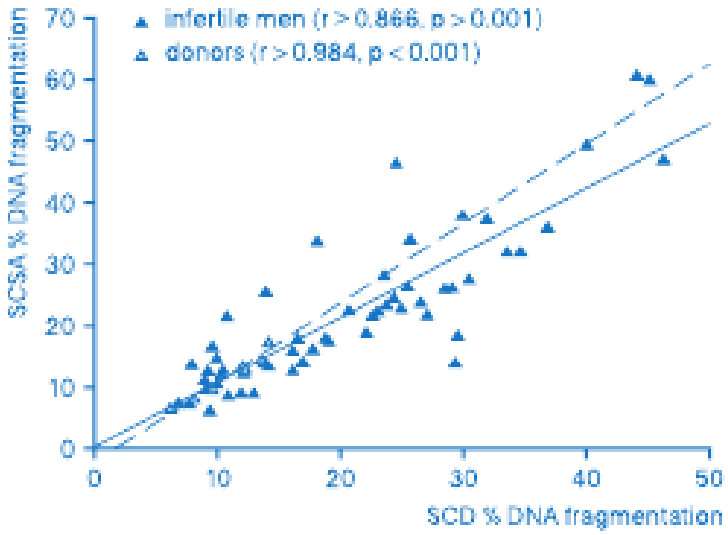
Underlying principles of the main sperm DNA fragmentation tests

ASSAY	PRINCIPLE	MEASURED PARAMETER	DETECTION METHOD
TUNEL	Addition of labeled dUDP nucleotides with terminal deoxynucleotidyl transferase to both SS and DS DNA breaks. Template-independent	% cells with labeled DNA	Fluorescence or bright field microscopy / Flow cytometry
COMET	Electrophoresis of sperm cells reveals fragmented DNA. Alkaline conditions denature DNA to reveal SS and DS DNA breaks. Neutral conditions reveal mostly DS breaks	% cells with migration tails containing fragmented DNA (also length of tail, % of DNA in tail)	Fluorescence microscopy
SCSA	Mild acid treatment denatures DNA with DS or SS breaks. Acridine orange binds DNA and fluoresces green with DS DNA (non-denatured) and red with SS DNA (denatured)	DNA Fragmentation Index (DFI): cells with red fluorescence divided by total number of cells (red +green). Expressed as %	Flow cytometry
SCD	Mild acid denaturation of DNA and lysis of protamines creates a chromatin decondensation halo around sperm head when DNA is intact and no halo when DNA is damaged	% cells with small or absent decondensation halo	Bright field microscopy

TUNEL, TdT (Terminal deoxynucleotidyl Transferase) –mediated dUDP nick-end labeling. SCSA, Sperm Chromatin Structure Assay. SCD, Sperm Chromatin Decondensation test, SS, Single-Stranded. DS, Double-Stranded. (Tarozzi et al., 2007, Zini and Sigman, 2009)–

The results obtained with Halosperm® are comparable to those of TUNEL and SCSA

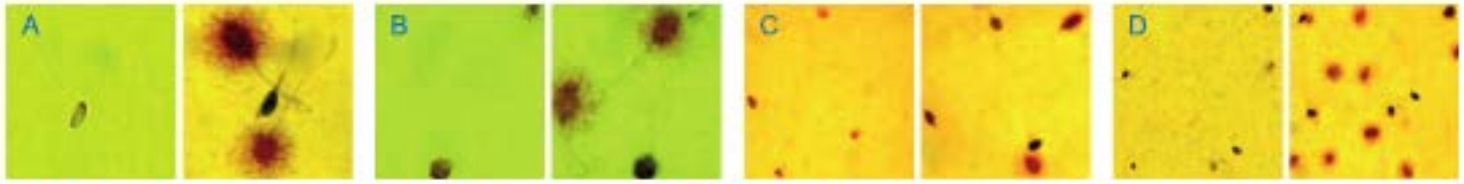




Sperm DNA fragmentation was analyzed in fertile donors (n=7, solid line) and infertile patients (n=60, dotted line). The correlations between the techniques (left, SCD and TUNEL, and right, SCD and SCSA) were analyzed using Student's t-test and are shown above each graph. (Adapted from: Chohan et al., 2006)

halosperm®

The same sperm cells are stained using TUNEL and Halosperm®



Sperm cells were embedded onto a slide and processed using the TUNEL assay followed by the SCD test. (A,B,C,D – left picture) Before Diff-quik staining, TUNEL positive sperms were stained brown. (A,B,C,D – right picture) After Diff-quik staining, TUNEL-positive sperms showed no halo and TUNEL-negative sperms showed a large halo. Pinhead sperm cells with no halo (Arrow) could not be observed with the TUNEL assay before Diff-quik staining. (Adapted from: Zhang et al., 2009).

Comparison of advantages and disadvantages for each technique

ASSAY	MAIN ADVANTAGES	MAIN DISADVANTAGES
TUNEL	1. Can perform on few sperm 2. For certain protocols use of fluorescence microscopy or flow cytometry may not be necessary	1. Thresholds not standardized 2. Variable assay protocols 3. Not specifically designed for human spermatozoa 4. Labor intensive
COMET	1. Sensitive 2. Can perform on few sperm	1. Requires complicated imaging software 2. Variable assay protocols 3. Unclear thresholds
SCSA	1. Many cells rapidly examined 2. Most published data	1. Expensive equipment necessary 2. Small variations in lab conditions affect results 3. Calculations involve qualitative decisions

(Adapted from Zini and Sigman, 2009)

List of competitive advantages offered by SCD technique - Halosperm® kit

1. Has been specifically designed for use with human spermatozoa and unlike some other techniques, there exists only one standard protocol that is highly reproducible. For instance, it was recently demonstrated that small variations in crucial steps of the TUNEL assay can greatly affect measures of sperm DNA fragmentation (Muratori et al., 2009).
2. Is one of the most economical techniques to measure sperm DNA fragmentation on the market.
3. Is the fastest technique available on the market. The turnaround time is under 1 hour, and multiple slides can be processed at a time.
4. Is the simplest and easiest technique available on the market. Only basic laboratory equipment is needed to perform the test, and slides can be analyzed with any bright field light microscope.
5. Can be used with few sperm cells, such as in cases of oligozoospermia.
6. Is a highly versatile tool that can also be used in research in conjunction with a number of other techniques, e.g. labeling for 8-oxoguanine to measure oxidative stress, FISH to measure chromosomal aberrations or immunocytochemistry to investigate protein status,...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Kit to analyze sperm dna fragmentation

halomax®

HALOTECH DNA SL
Parque Científico de Madrid
PTM C/Santiago Grisolía, 2
28760 Tres Cantos
Madrid (Spain)
T. +34 91 571 3598
F. +34 91 567 1557

Kit for DNA fragmentation analysis in *Canis familiaris* spermatozoa (10 determinations)

KIT CONTAINS

- 10 precoated slides
- 10 vials with low melting agarose
- 1 flask with 100 ml of lysing solution. **Work under continuous air removal or cover trays during incubation step.**

BACKGROUND

The **SPERM-HALOMAX®** kit from **HALOTECH DNA SL** bursts into the market as a pioneering product for the analysis of fragmentation levels in spermatozoa both in domestic and experimental animals.

SPERM-HALOMAX® is based on the differential response of fragmented and unfragmented spermatozoa nuclei to a protein depletion treatment. The extraction of nuclear proteins from spermatozoa containing fragmented DNA releases DNA fragments between two breakage points. Sperm nuclei disperse chromatin around, forming a low stained peripheral halo, which is distinguishable under low magnification microscopy. These images contrast sharply with the intensely stained nuclei of the spermatozoa which ocuppies a centered position. On the contrary, those spermatozoa containing unfragmented DNA develop a very small dispersion halo, and appears as a very thin crown around the core. **SPERM HALOMAX ®** allows the quick assessment of the proportion of spermatozoa with fragmented DNA.

MATERIAL AND EQUIPMENT REQUIRED BUT NOT PROVIDED

Fluorescence microscope, refrigerator at 4ºC, water bath at 37ºC and 90-100ºC, latex gloves, glass coverslips (18x18 mm, 22x22mm, 24X60 mm), micropipettes, plastic tank for horizontal incubations, distilled water, 70%, 90% and 100% ethanol, staining solutions.

RECOMMENDED STAINING SOLUTIONS

For fluorescence microscopy: any DNA-specific binding fluorochrome could be used (DAPI, propidium iodide, ethidium bromide or synergy brand related fluorochromes). Antifading solution.

INSTRUCTIONS FOR USE

Including the sperm sample in agarose microgel

Set the lysing buffer at room temperature (22ºC)

1) Dilute the sperm sample in culture medium or Phosphate Buffered Saline (PBS), to give a final concentration of 5 – 10 million spermatozoa per millilitre. Either fresh or liquid nitrogen frozen samples can be used.

2) In order to melt the agarose, place vial with low melting agarose onto a float and both into a water bath at 90-100ºC for 5 minutes.

3) Transfer vial together with the float to a thermostatic water bath at 37ºC, and leave it for 5 min to equilibrate agarose temperature.

4) Once agarose is at 37ºC, add 25 µl of semen solution to the vial and mix thoroughly.

5) Place a drop of the cell suspension in vial onto the treated face of the slide (marked surface) and cover with a glass coverslip. Avoid making air bubbles by gently pressing. We recommend a drop of 15, 25 or 50 microlitres to coverslips of 18x18 mm, 22x22 mm or 24x60 mm, respectively. Slides need to be always placed in horizontal position.

6) Place the slide on the cooled plate into the fridge and leave the sample to solidify for 5 minutes.

Processing the sperm sample

7) Remove the coverslip smoothly, and set the slide (in horizontal position) in 10 ml of the lysing solution. This is the volume needed for one slide. Scale the volume depending on the number of slides to be processed. Incubate for 5 min at room temperature (22ºC). **Work under fume hood or cover the tray during incubation!**

8) Transfer the slide to another plastic tray with distilled water. Wash it for 5 min.

9) Dehydrate in sequential 70, 90 and 100% ethanol baths (2 min each) and air dry. Remember to always keep the slide in horizontal position.

10) Once the slides are dried, they can be stored for several months at room temperature in a dry place.

Staining the sample

Proceed to stain the slide just before visualization and analysis. For fluorescence microscopy any DNA-specific binding fluorochrome could be used (DAPI, Propidium Iodide. Ethidium Bromide or Synergy Brand related fluorochromes).

Concentrations of use are identical to those for routine fluorescence microscopy. (i.e. propidium yodide 2,5 µg/ml, and mounted in Vectashield antifading).

Once obtained the concentration, mix the fluorochrome with an antifading solution in proportion 1:1. A final volume of 5 µl is recommended for staining a 25x 25 coverslip.

UNDER THE MICROSCOPE AND CLASIFYING THE NUCLEOIDS

Any magnification can be used, from 10x to 100x. 20x, 40x or 60x dry objetives are recommended. The nucleoid corresponding to the intensely protein depleted nuclei is made up of two parts: the *core*, which appears at central position, and the peripheral *halo* corresponding to the chromatin/DNA dispersion ringlets. The analysis of a minimum number of 500 espermatozoids per sample is recommended. The criteria for classification are as follows:

SPERMATOZOA WITH FRAGMENTED DNA: Spermatozoa with a large and spotty halo of chromatin dispersion.

SPERMATOZOA WITH UNFRAGMENTED DNA: Spermatozoa with a small and compact halo of chromatin dispersion.

SAFETY WARNINGS AND ENVIRONMENTAL PRECAUTIONS

☐☐Biological samples have to be handled as potentially infectious.
☐☐Care should be taken to avoid contact with skin or eyes, and to prevent inhalation. Gloves should be worn to handle the products. Lysing solution contains β-mercaptoethanol. Work under air removal environment and please follow the manufacturer's Material Safety Data Sheet regarding safe handling.
☐☐Do not dispose waste products into the environment.
☐☐Please follow the specific safety regulation of your Faculty or Research Center with respect to chemicals storage and toxic products disposal.

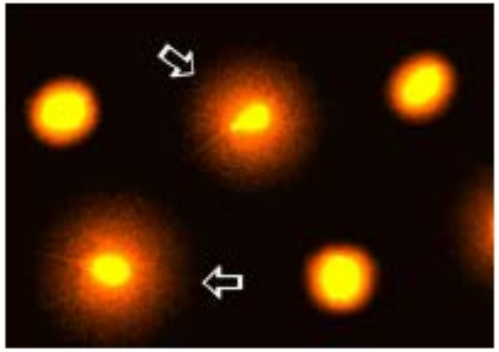
REAGENTS STABILITY AND STORAGE

The components of the kit are stable at room temperature; always keep in a dry and light protected place. Expiration: the reagents supplied are stable for a minimum period of one year. Keep fluorochrome at 4ºC.

TROUBLESHOOTING THE PROBLEM –IT'S COMMON CAUSE – OUR SUGGESTED APPROACH

1) Weakly stained halos – Too short colouring exposure time –Extend time exposure to dye.
2) Failure to differentiate halo and core - Too long colouring exposure time – Discolour by intense buffer washing and colour again but reducing time exposure to dye.
3) The halos appear displaced from the core in one-way direction – Slides were not kept in horizontal position during sample processing.

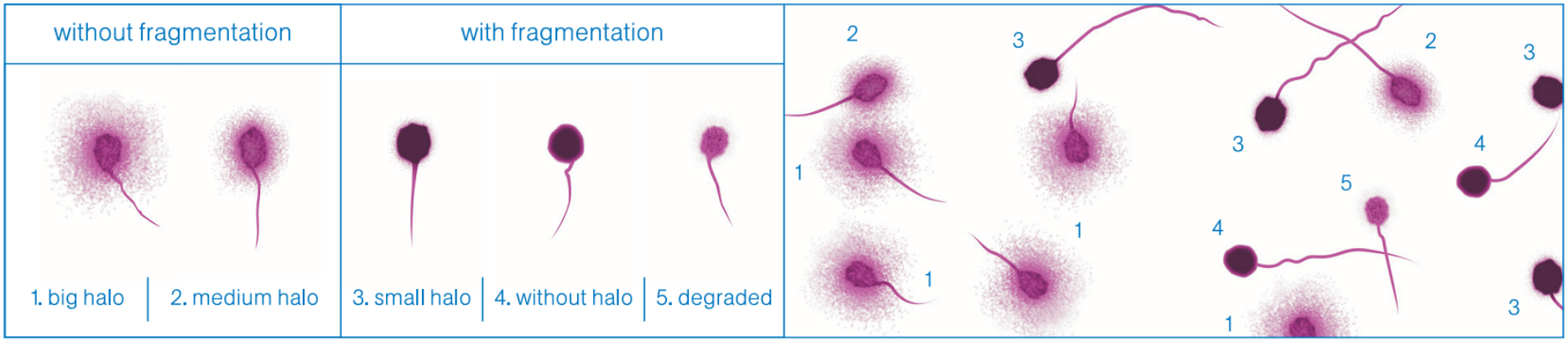
Figure: Arrows show those with fragmented DNA



sperm classification

Removal of sperm nuclear proteins results in nucleoids with a central core and a peripheral halo of dispersed DNA loops. The sperm tails remain preserved. For sperm classification we must take into account that sperm DNA fragmentation is a continuous process which produces a series of different halo sizes. Initially, the study of a minimum of 500 spermatozoa per sample is recommended, adopting the criteria of Fernández et al. (Fertil Steril 84: 833-842, 2005). Avoid scoring cells close to the edge of the microgel. Classification:

- **Spermatozoa without dna fragmentation:**
  - **spermatozoa with big halo:** those whose halo width is similar or higher than the minor diameter of the core (Figure 1).
  - **spermatozoa with medium-sized halo:** their halo size is between those with large and with very small halo (Figure 2).
- **Spermatozoa with fragmented dna:**
  - **spermatozoa with small halo:** the halo width is similar or smaller than 1/3 of the minor diameter of the core (Figure 3).
  - **spermatozoa without halo:** (Figure 4).
  - **spermatozoa without halo and degraded:** those that show no halo and present a core irregularly or weakly stained (Figure 5).
  - **"others":** cell nuclei which do not correspond to spermatozoa. One of the morphological characteristics which distinguish them is the absence of tail.

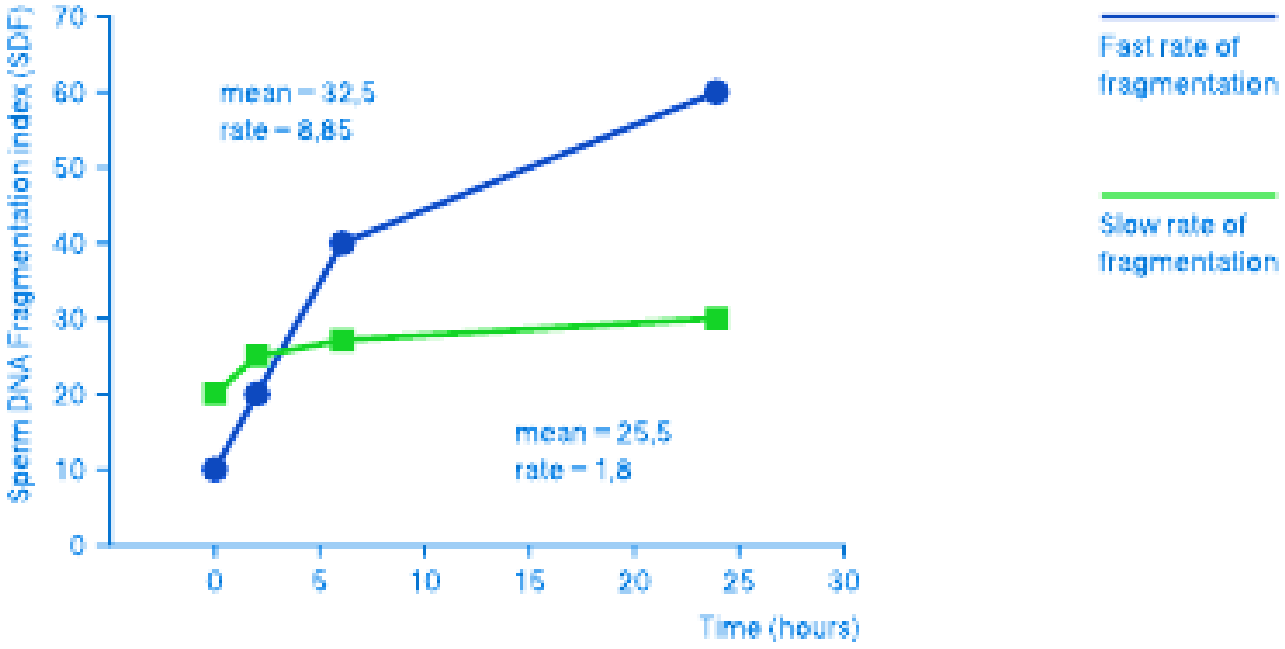


Dyn-Halosperm® distinguishes between fragmented and non-fragmented DNA in sperm cells and allows the calculation of the Sperm DNA Fragmentation Index (SDF), i.e. the percentage of spermatozoa with fragmented DNA. In addition, using the Dyn-Halosperm® kit it is possible to study sperm DNA fragmentation over four different time points.

Sperm DNA fragmentation is related to fertilization rate[1], embryo quality[2], embryo development and male pathologies (such as varicocele[3] or infections by *Chlamydia trachomatis*[4]).

Each individual has a unique dynamic profile of sperm DNA fragmentation[4].

Sperm DNA Fragmentation Index (SDF):



Information provided by Dyn-Halosperm®

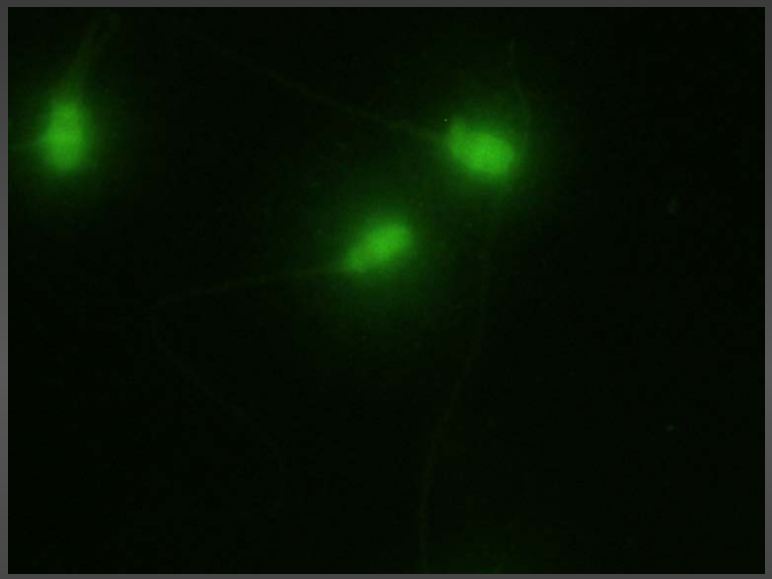
The Dyn-Halosperm® protocol provides the following information about DNA fragmentation for a given semen sample:

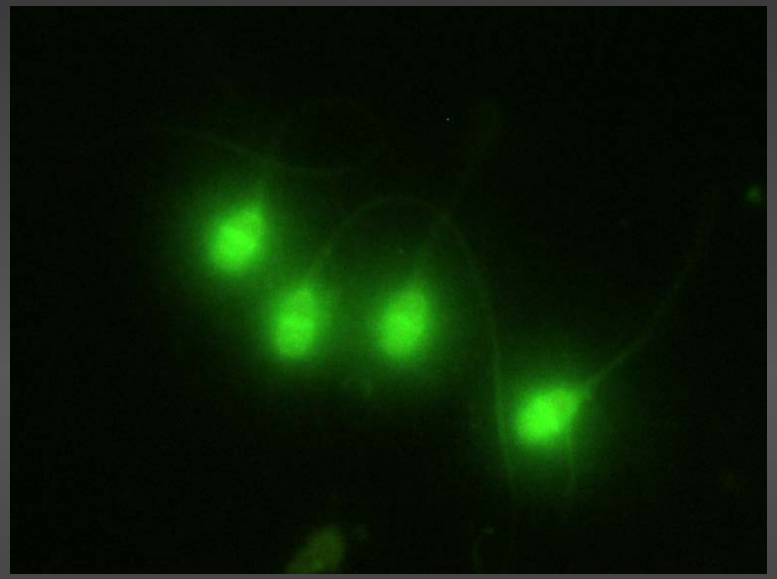
1. Basal fragmentation level.
2. Rate of change.
3. Mean.

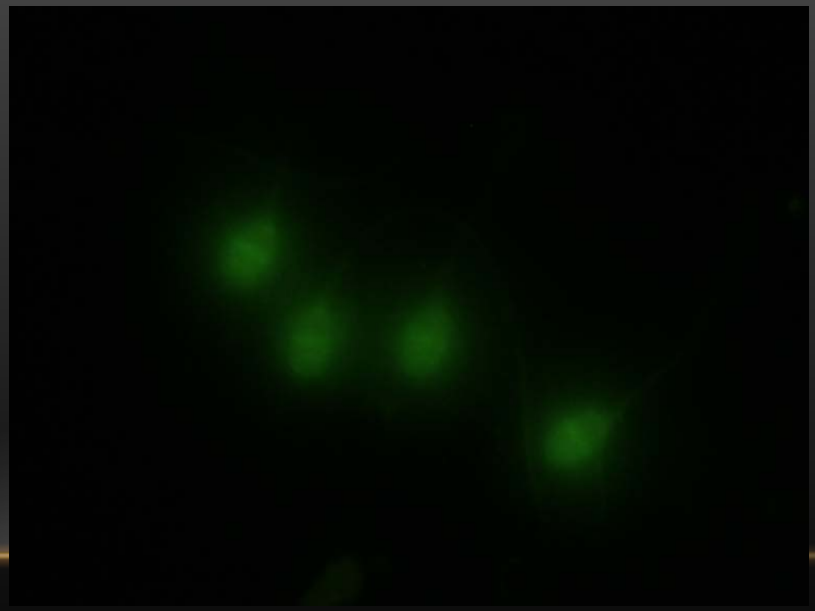
Clinical applications based on basal fragmentation and rate of change

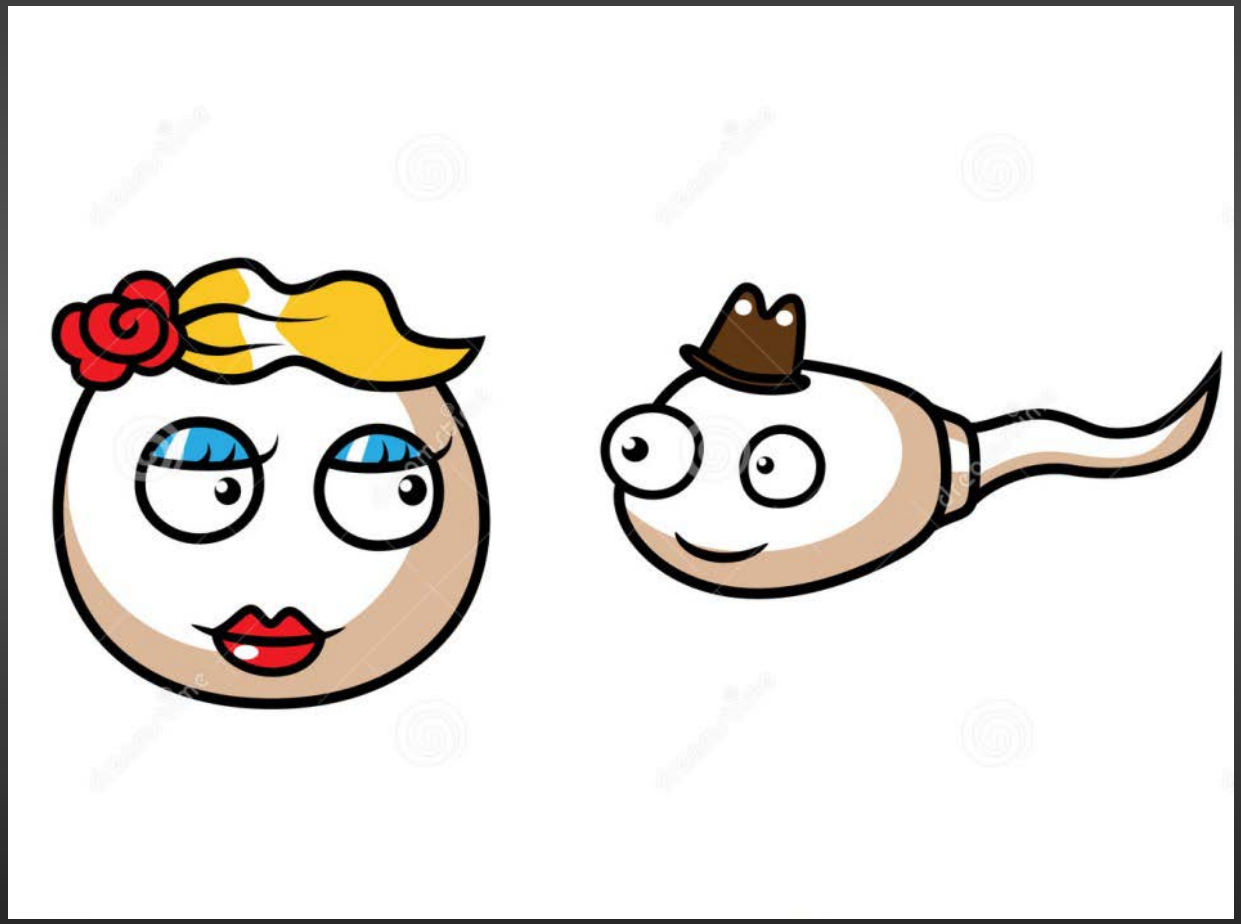
Knowing the evolution of sperm DNA fragmentation over time allows clinicians to:

1. Select the optimum moment to carry out an ART cycle.
2. Select the type of assisted reproduction technique: if the rate of DNA fragmentation is high then it is advisable to use techniques that assure a quick fertilization of the oocyte.
3. Assess the quality of semen samples or donors for suitability.
4. Provide answers to cases of unexplained infertility, ART failure or repeated abortions.









Dziękuję za uwagę