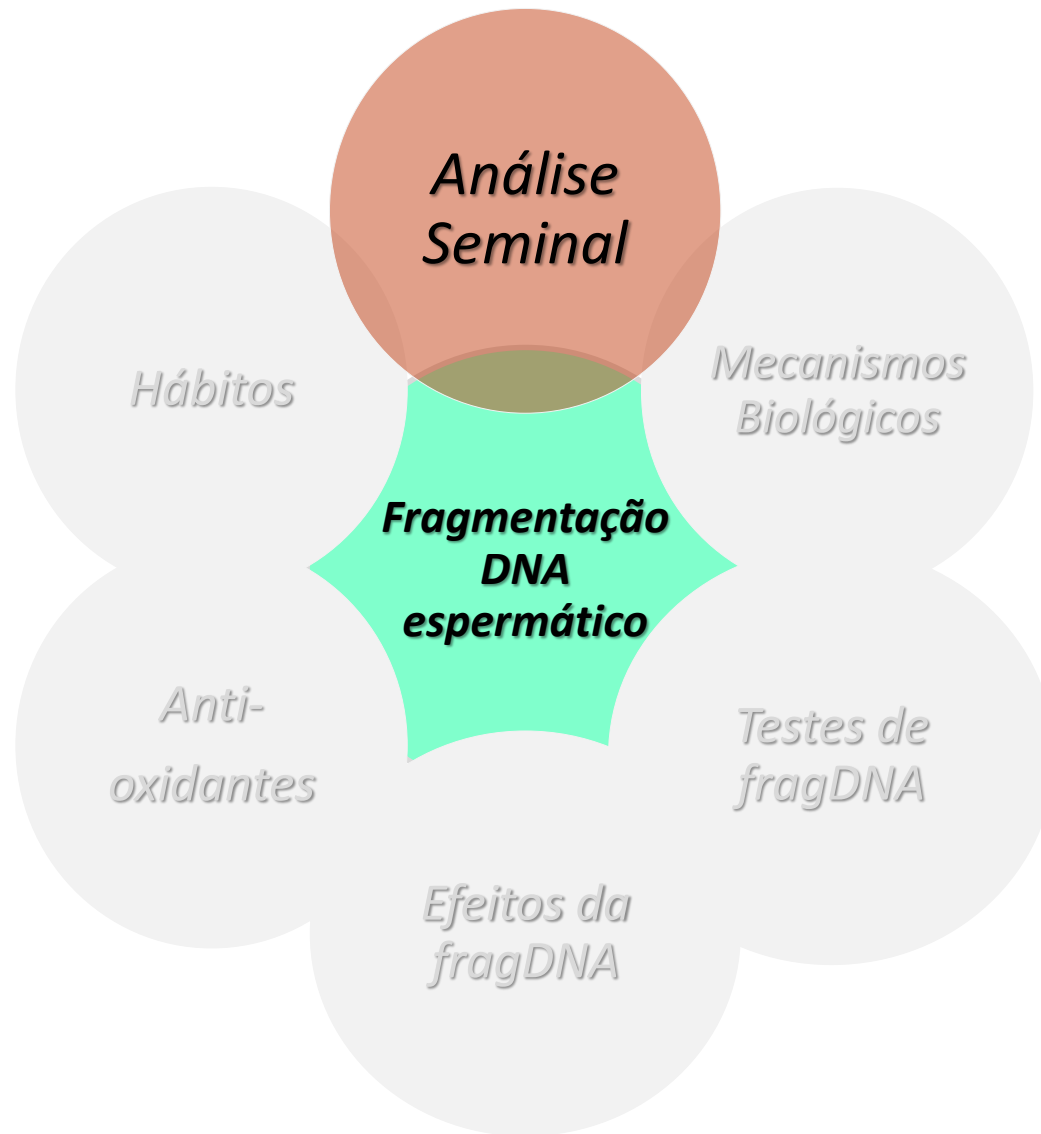




Fragmentação do DNA espermático

Edson Borges Jr.

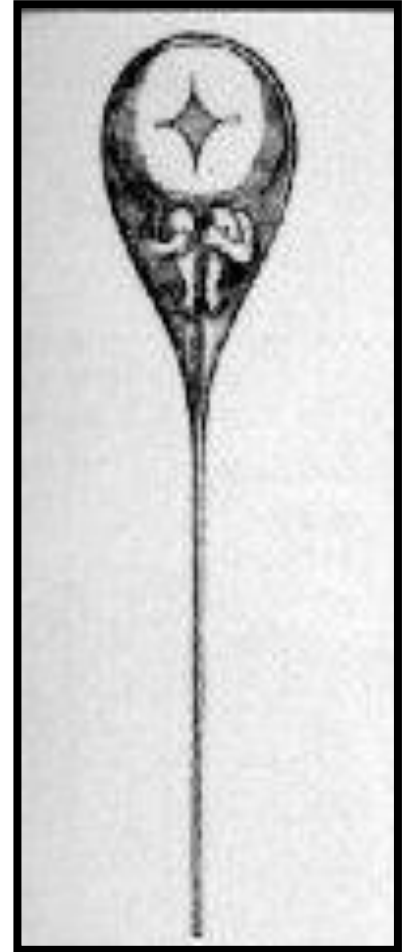




FERTILITY

Análise Seminal

- ❖ *Análise Macroscópica*
cor, viscosidade, pH, volume, liquefação
 - ❖ *Análise Microscópica*
concentração, motilidade, morfologia
-

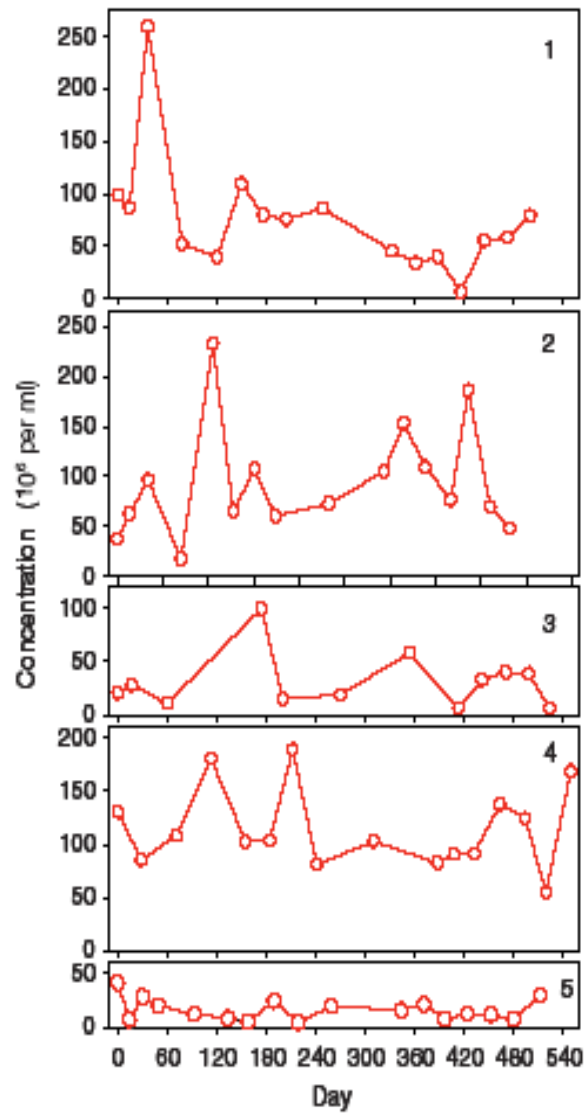
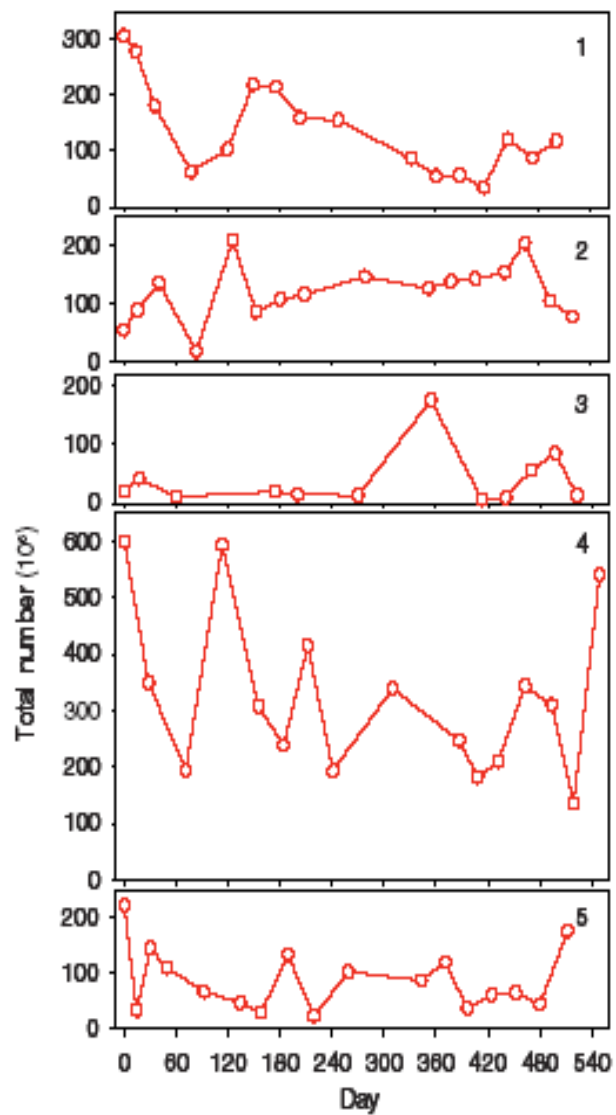


Análise Seminal

Não é um teste de fertilidade!



Avaliação do *status* funcional do testículo no momento da coleta



FERTILITY

Approaches to improve the diagnosis and management of infertility

human
reproduction
update

P. Devroey^{1,4}, B.C.J.M. Fauser² and K. Diedrich³ on behalf of the
Evian Annual Reproduction (EVAR) Workshop Group 2008[†]

Human Reproduction Update, Vol.15, No.4 pp. 391–408, 2009

- ***AS adequada para alterações seminais graves;
para alterações moderadas/leves: inadequada***

Evaluation of sperm damage: beyond the World Health Organization criteria

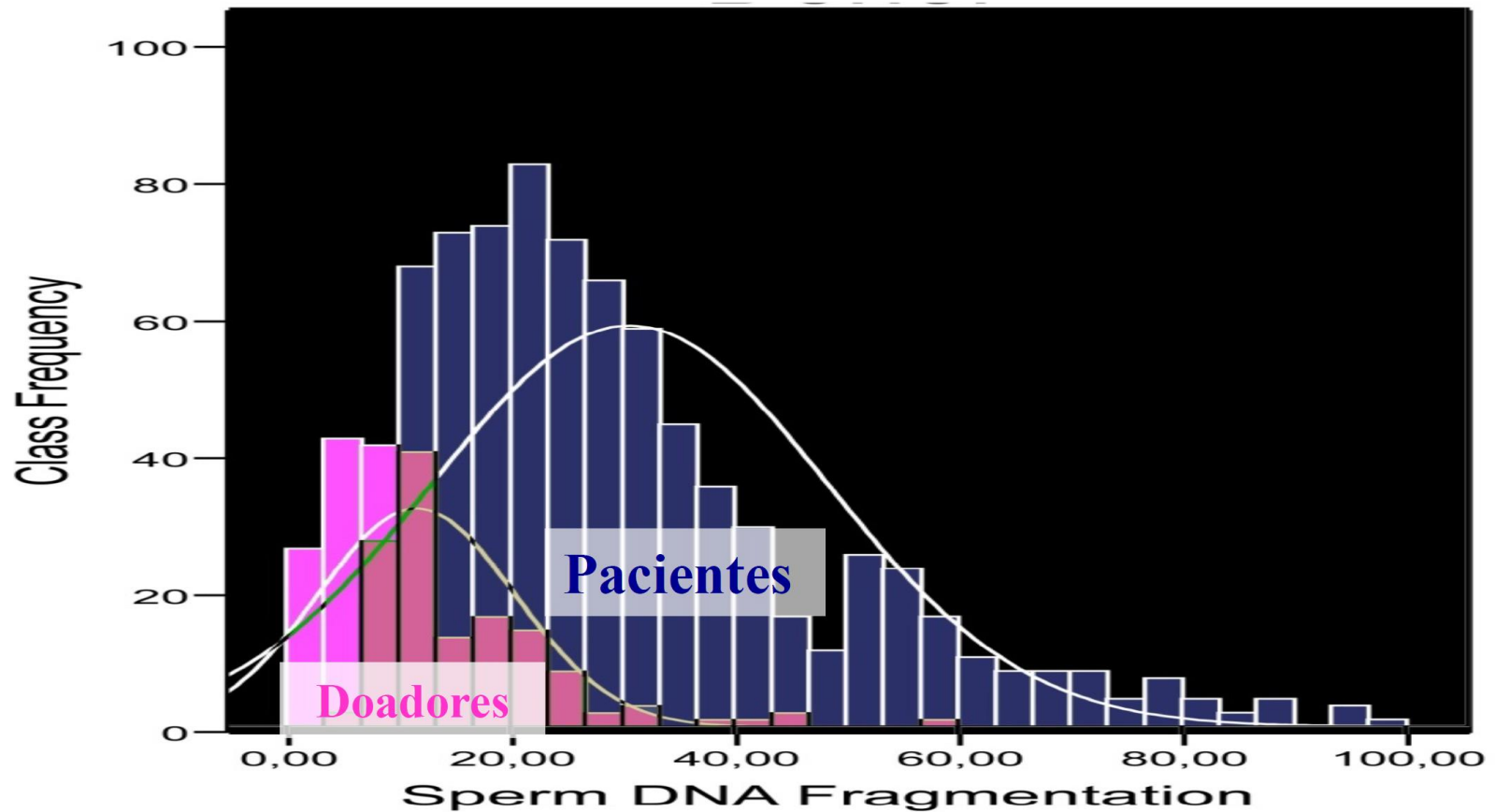
Nabil Aziz, M.R.C.O.G., M.D.,^a and Ashok Agarwal, Ph.D., H.C.L.D.^b

Fertility and Sterility® Vol. 90, No. 3, September 2008

- ✓ *grande flutuação na concentração, motilidade e morfologia*
- ✓ *variação intra / inter observador*
- ✓ *AS não investiga as propriedades biológicas e subcelulares do sptz*
- ✚ ***necessidade de outros testes funcionais mais específicos***



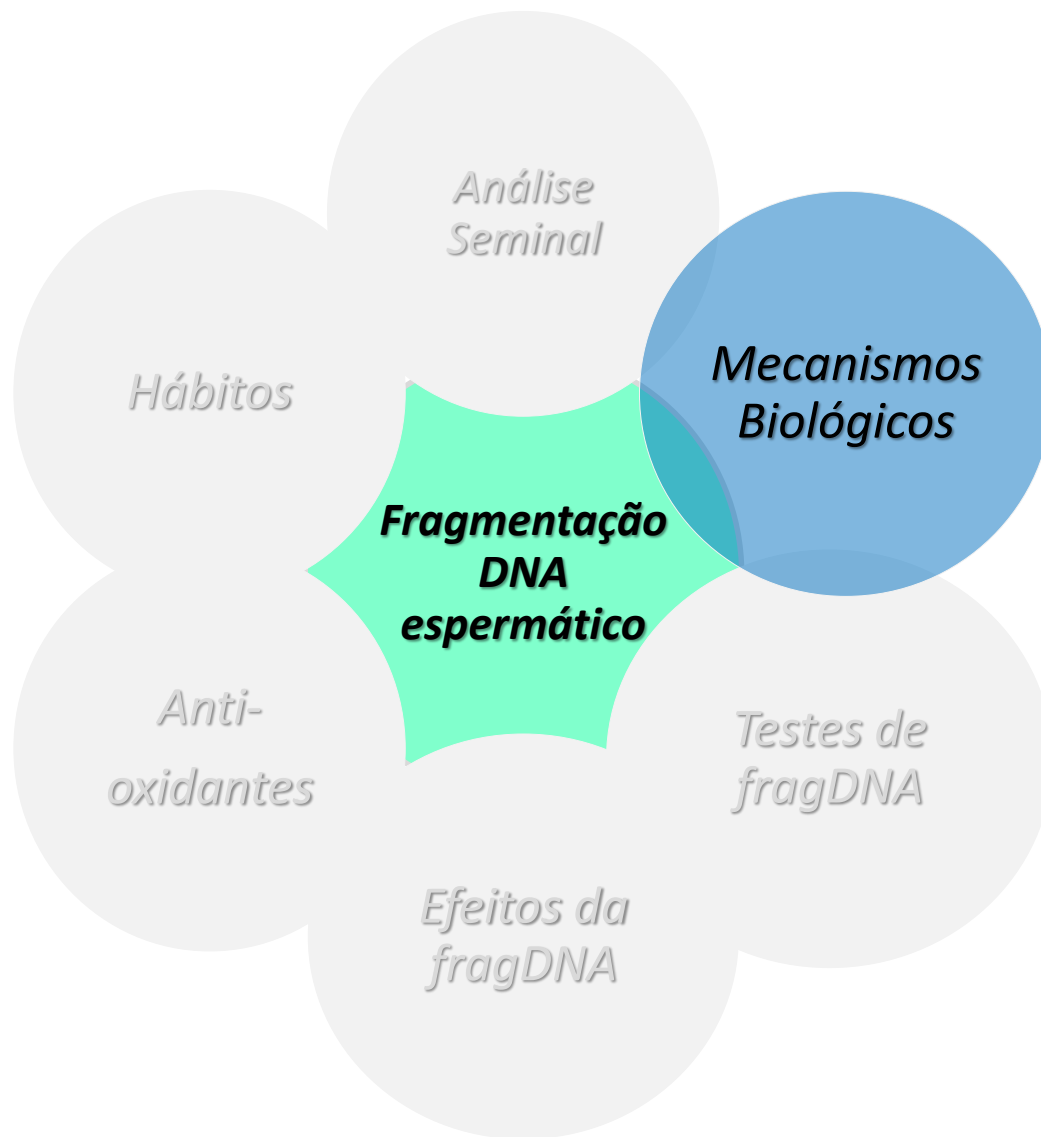
Taxa de Fragmentação do DNA espermático



Gosalvez et al. J Reprod Biotechnol Fertil. 2015



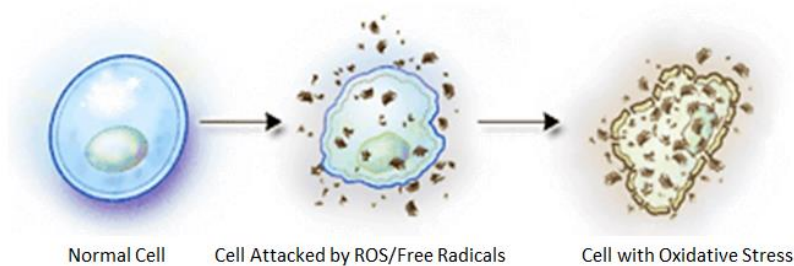
FERTILITY



FERTILITY

Mecanismos biológicos da Fragmentação do Espermatozoide

- ***ALTERAÇÃO DA PROTAMINA***
- ***APOPTOSE ABORTIVA***
- ***ESTRESSE OXIDATIVO***



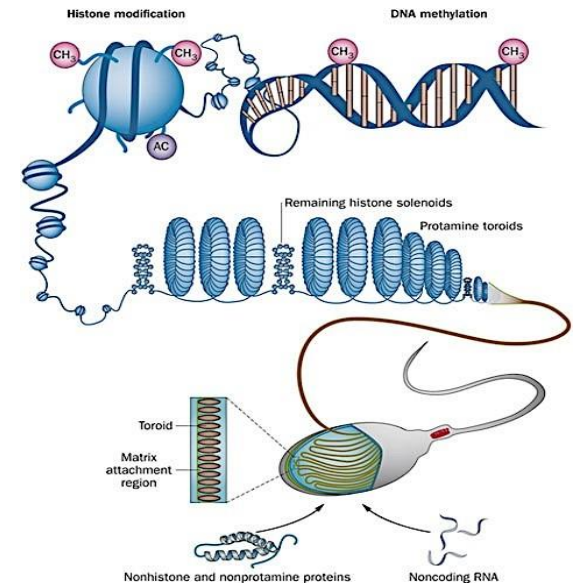
▪ **ALTERAÇÃO DA PROTAMINA**

- ✓ Substituição de histona/protamina durante a espermiogênese

Condensação da cromatina

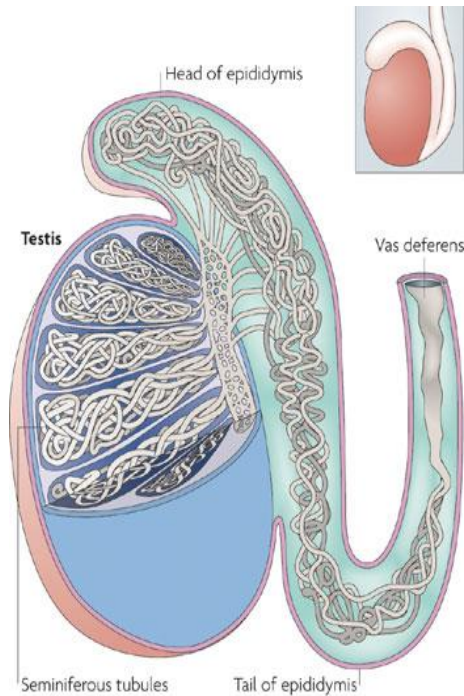
(Histonas → Protaminas)

- 1 - compactação do núcleo do espermatozoide
- 2 - proteção do DNA durante a passagem no trato genital
- 3 - estabilidade da cromatina (fragmentação do DNA e apoptose)



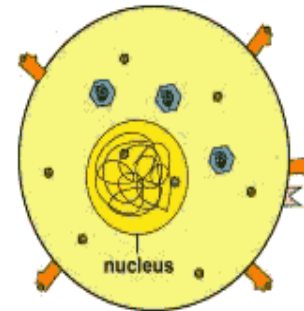
■ **APOPTOSE ABORTIVA**

- ✓ Durante a maturação espermática (testículo e epidídimo)



Nature Reviews | Genetics

APOPTOSIS



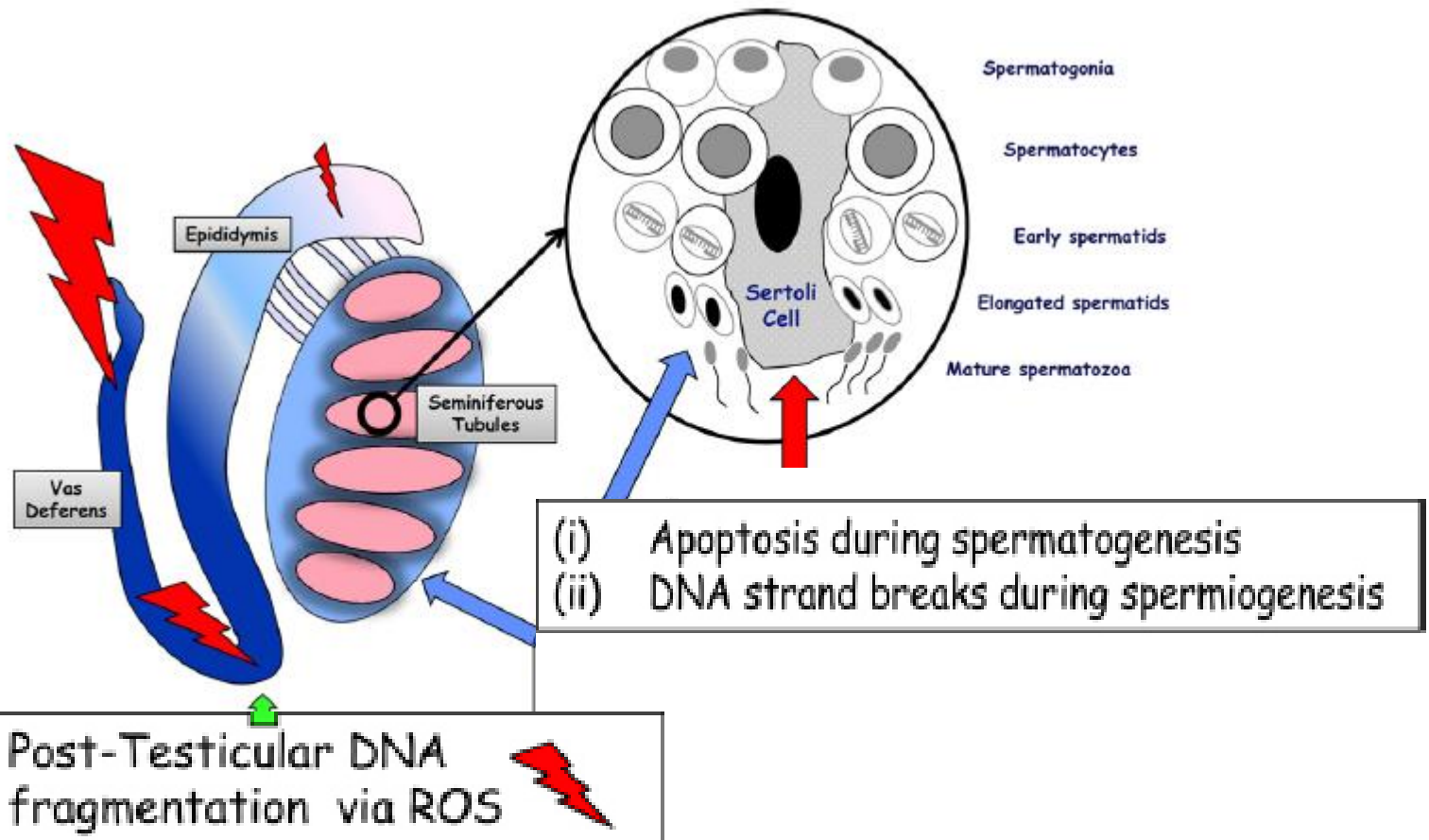
FERTILITY

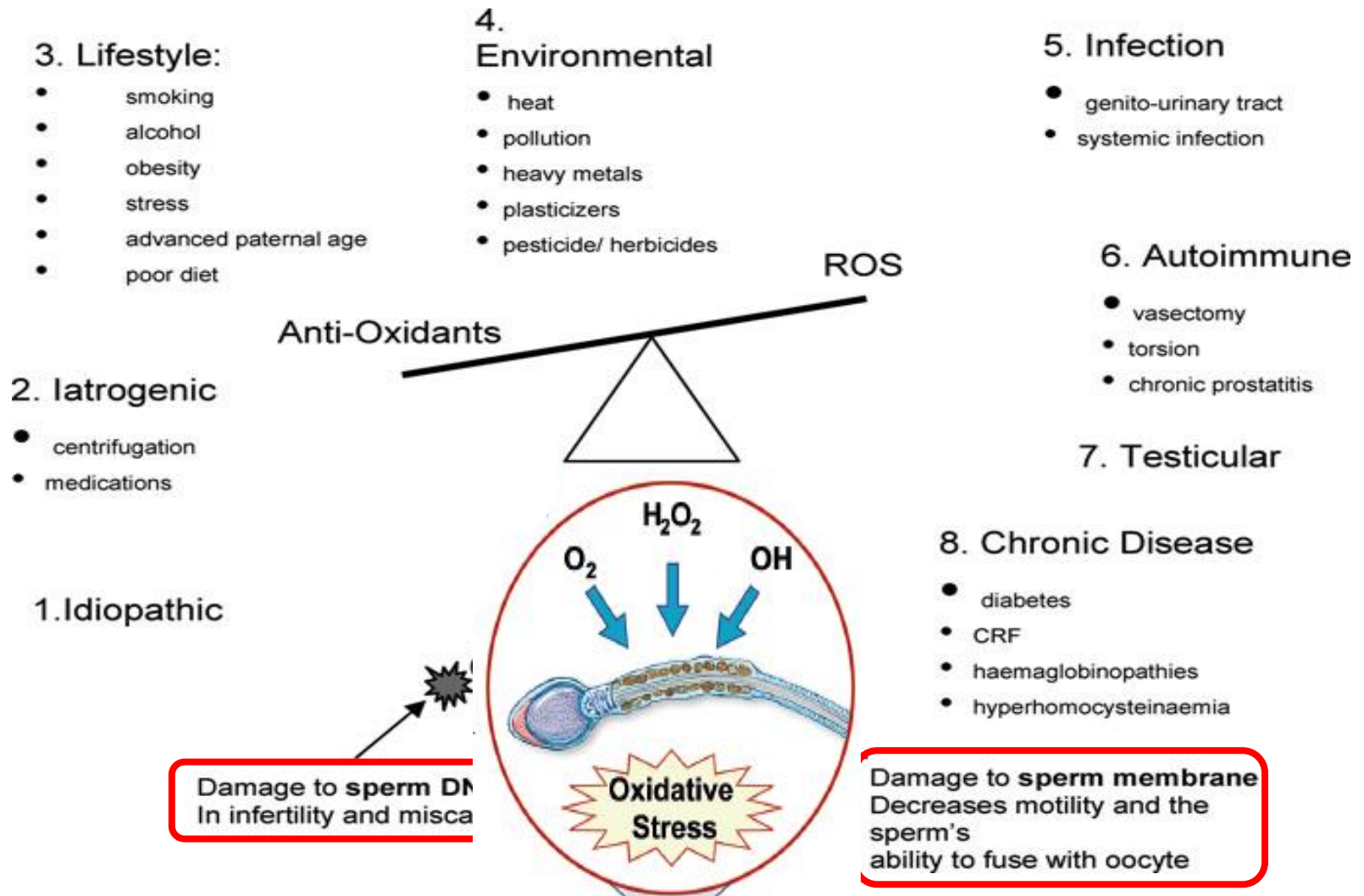
▪ **ESTRESSE OXIDATIVO**

✓ **Trânsito no testículo e epidídimo**

✓ **Pós-ejaculação:**

- . leucócitos, espermatozoides imaturos, níveis anormais de antioxidantes no plasma seminal



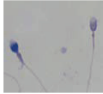
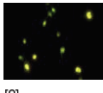
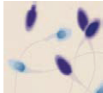
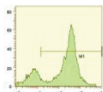
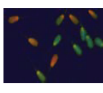
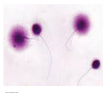
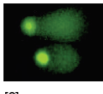


Kelton Tremellen; Human Reproduction Update, Vol.14, No.3 pp. 243–258, 2008



FERTILITY

Table 1 Sperm DNA fragmentation (SDF) testing methods

	Test	Principle	Advantage	Disadvantage
 [1]	AO test	Metachromatic shift in fluorescence of AO when bound to single strand (ss)DNA. Uses fluorescent microscopy	Rapid, simple and inexpensive	Inter-laboratory variations and lack of reproducibility
 [2]	AB staining	Increased affinity of AB dye to loose chromatin of sperm nucleus. Uses optical microscopy	Rapid, simple and inexpensive	Inter-laboratory variations and lack of reproducibility
 [3]	CMA3 staining	CMA3 competitively binds to DNA indirectly visualizing protamine deficient DNA. Uses fluorescent microscopy	Yields reliable results as it is strongly correlated with other assays	Inter-observer variability
 [4]	TB staining	Increased affinity of TB to sperm DNA phosphate residues. Uses optical microscopy	Rapid, simple and inexpensive	Inter-observer variability
 [5]	TUNEL	Quantifies the enzymatic incorporation of dUTP into DNA breaks. Can be done using both optical microscopy and fluorescent microscopy. Uses optical microscopy, fluorescent microscopy and flow cytometry	Sensitive, reliable with minimal inter-observer variability. Can be performed on few sperm	Requires standardization between laboratories
 [6]	SCSA	Measures the susceptibility of sperm DNA to denaturation. The cytometric version of AO test. Uses flow cytometry	Reliable estimate of the percentage of DNA-damaged sperm	Requires the presence of expensive instrumentation (flow cytometer) and highly skilled technicians
 [7]	SCD or Halo test	Assess dispersion of DNA fragments after denaturation. Uses optical or fluorescent microscopy	Simple test	Inter-observer variability
 [8]	SCGE or comet assay	Electrophoretic assessment of DNA fragments of lysed DNA. Uses fluorescent microscopy	Can be done in very low sperm count. It is sensitive and reproducible	Requires an experienced observer. Inter-observer variability

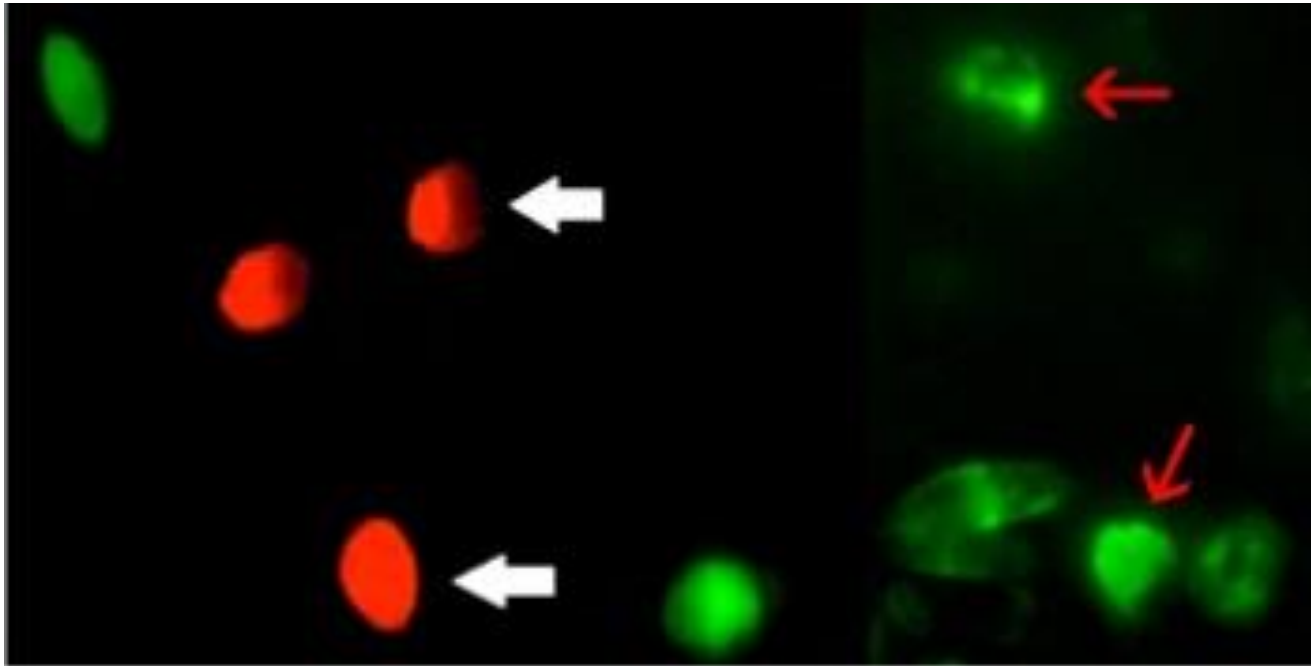
[1] Acridine orange (AO) stains normal DNA fluoresces green; whereas denatured DNA fluoresces orange-red. [2] Aniline blue (AB) staining showing sperm with fragmented DNA and normal sperm. [3] Chromomycin A3 (CMA3) staining: protamine deficient spermatozoa appear bright yellow; spermatozoa with normal protamine appear yellowish green. [4] Toulidine blue (TB) staining: normal sperm appear light blue and sperm with DNA fragmentation appear violet. [5] Terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) assay fluorescent activated cell sorting histogram showing percentage of SDF. [6] Sperm chromatin structure assay (SCSA): flow cytometric version of AO staining. [7] Sperm chromatin dispersion (SCD) test: spermatozoa with different patterns of DNA dispersion; large-sized halo; medium-sized halo [2]; very small- sized halo. [8] Comet images showing various levels of DNA damage.

Testes de Fragmentação do DNA espermático

- TUNEL (*TdT-mediated dUTP nick end-labeling*)
- SCSA (*sperm chromatin structure assay*)
- ENSAIO COMETA
- TESTE DE DISPERSÃO DA CROMATINA
(SCD – halosperm)

TUNEL (TdT-mediated dUTP nick end-labeling)

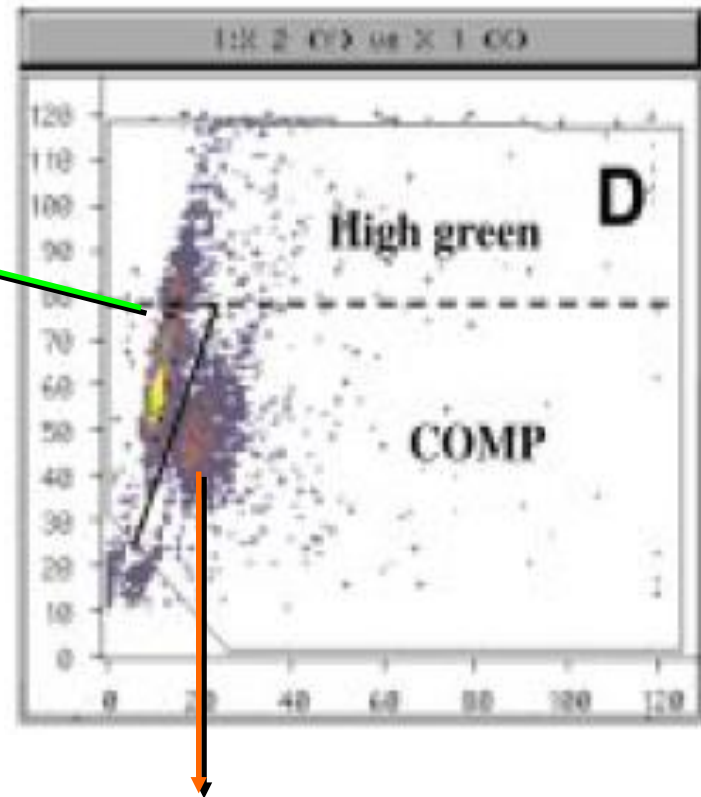
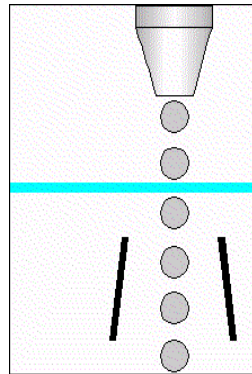
- Célula marcada visualizada diretamente por microscopia de fluorescência ou quantificada por citometria de fluxo.



SCSA (SPERM CHROMATIN STRUCTURE ASSAY)

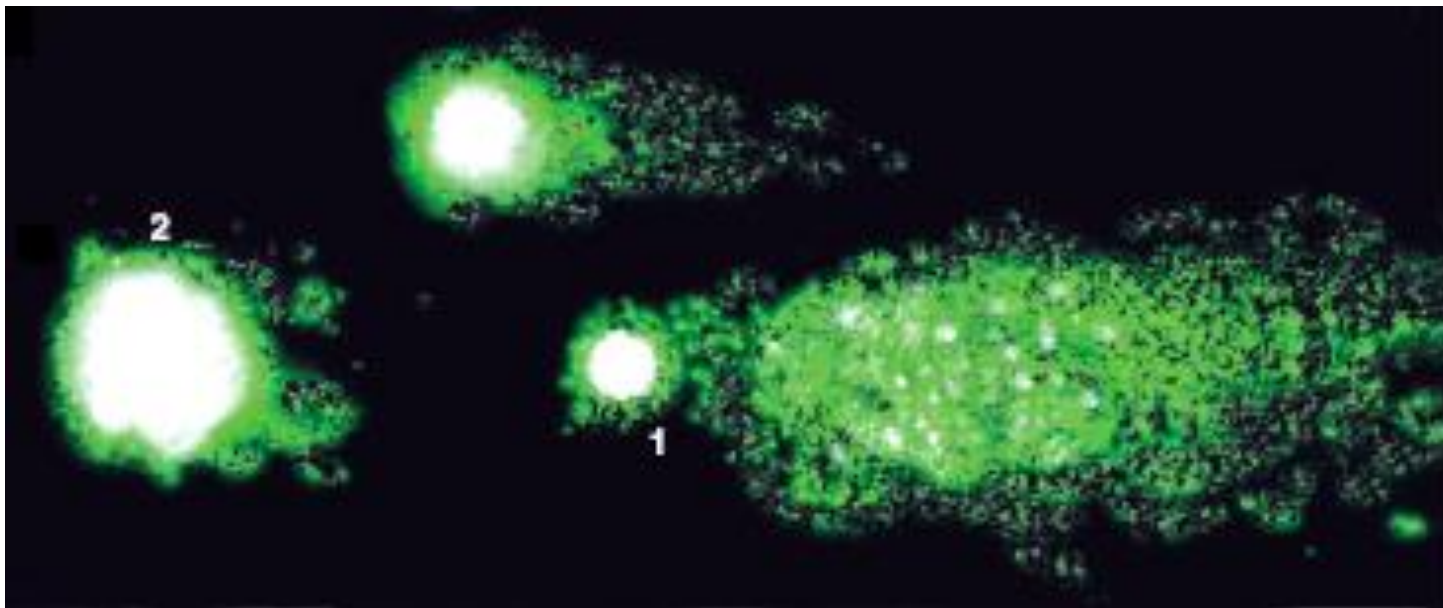
- Leitura em citômetro de fluxo

Cromatina íntegra



Cromatina frágil

ENSAIO COMETA



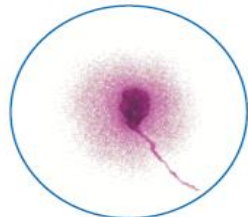
1- espermatozóide com DNA intacto ,
2- espermatozóide com DNA fragmentado

TESTE DE DISPERSÃO DA CROMATINA - halosperm

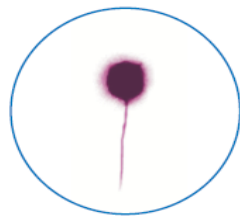
- Microscopia óptica

without fragmentation

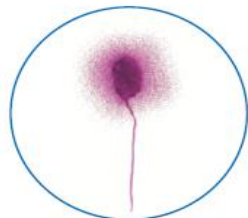
with fragmentation



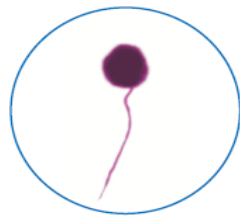
1. big halo



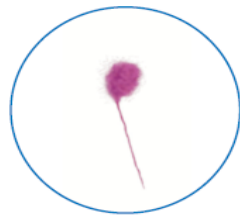
3. small halo



2. medium halo

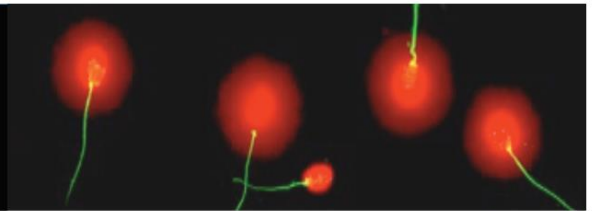


4. without halo

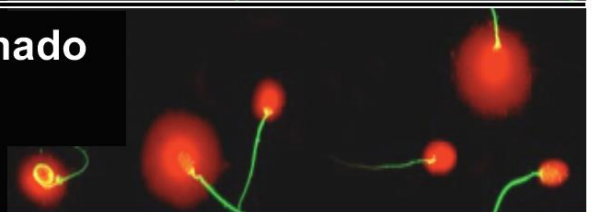


5. degraded

Normal
<20%



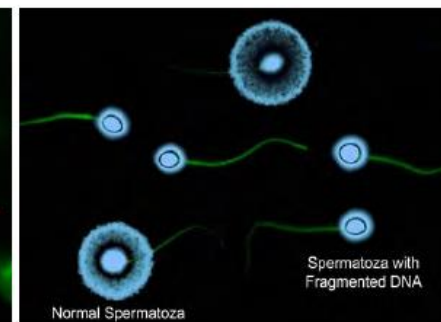
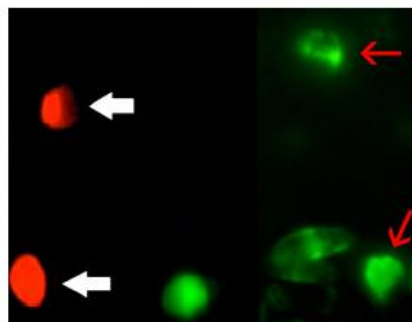
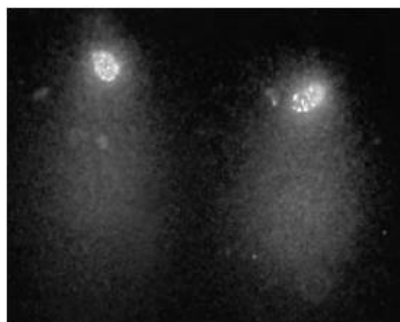
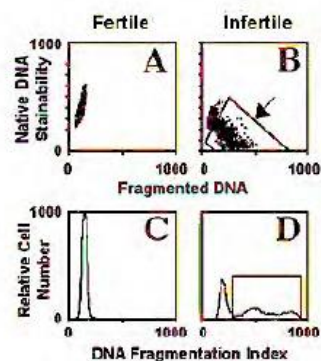
Indeterminado
20-29%

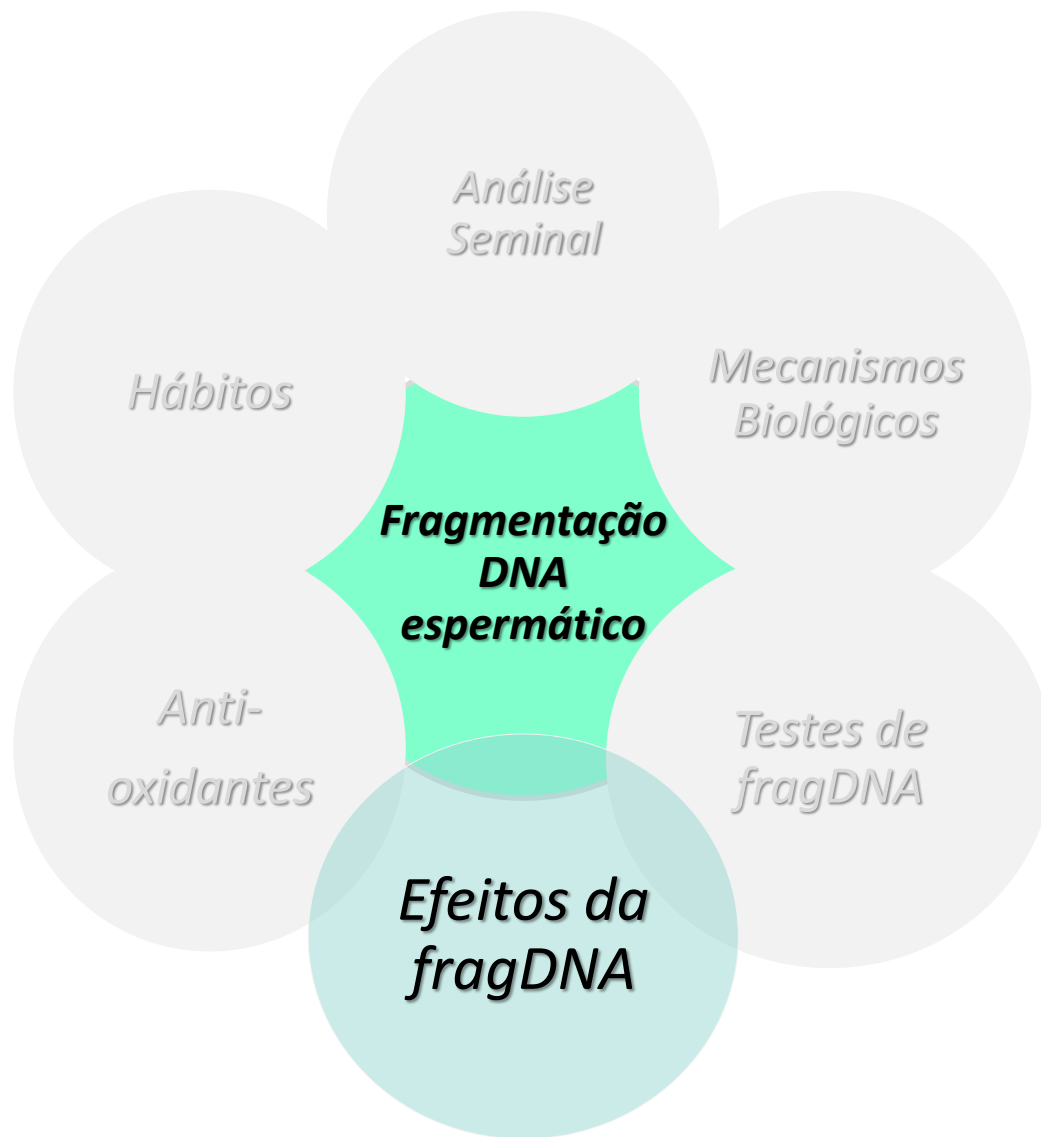


Elevado
≥ 30%



	Labor-intensive	Expensive equipment	Analysis Subjectivity	Validation & Standardization
TUNEL	++++	+++	++	++
SCSA	++	++++	+	++++
Comet	++++	+++	+++	+
SCD	+	+	++	+++





FERTILITY



www.sciencedirect.com
www.rbmonline.com



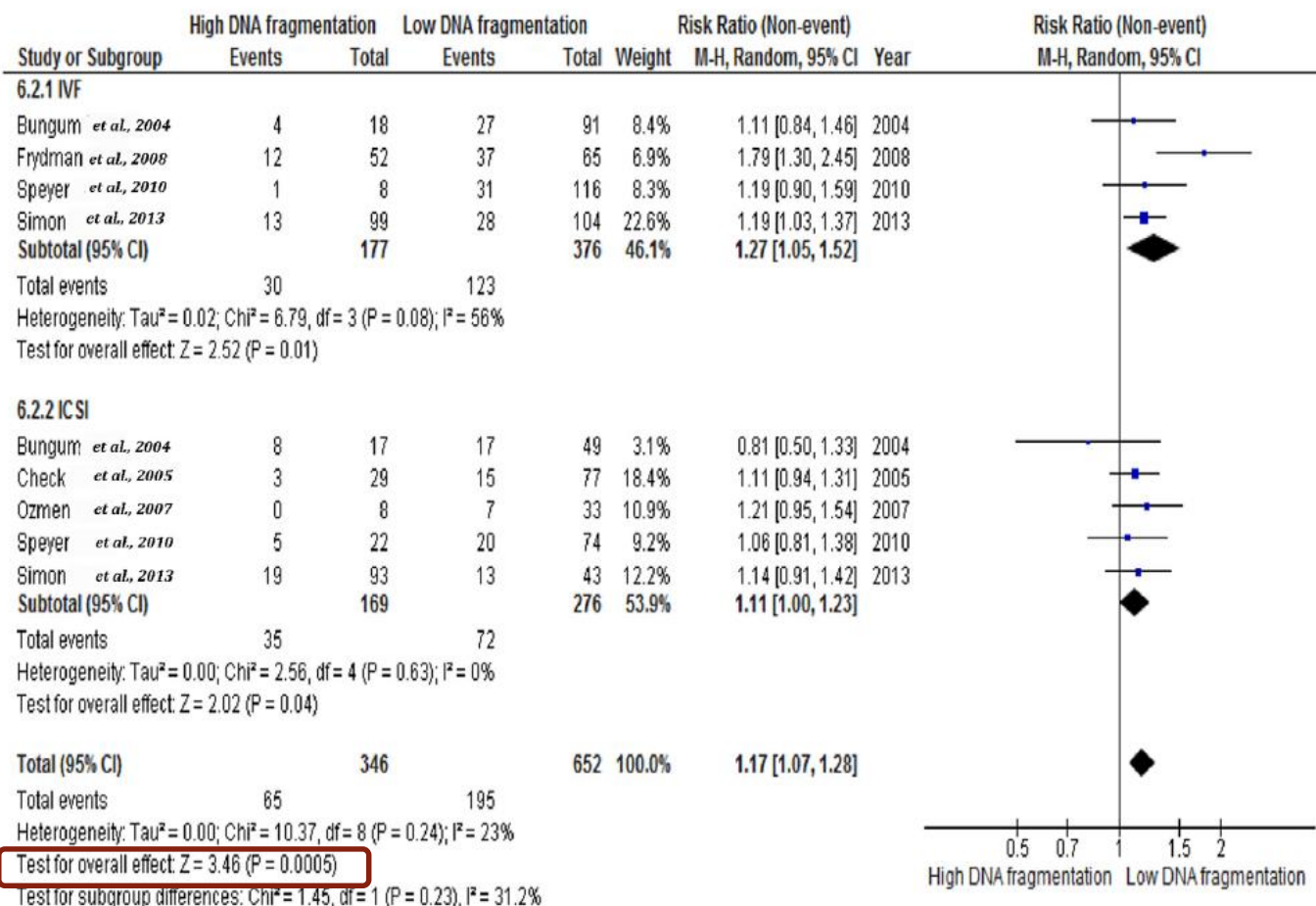
REVIEW

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

Assisted Conception Unit, Guys Hospital, Great Maze Pond, SE1 9RT, UK



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



Open Access

ORIGINAL ARTICLE

Sperm Biology

A systematic review and meta-analysis to determine the effect of sperm DNA damage on *in vitro* fertilization and intracytoplasmic sperm injection outcome

Luke Simon^{1,*}, Armand Zini^{2,*}, Alina Dyachenko², Antonio Ciampi², Douglas T Carrell^{1,3,4}

- 41 articles (with a total of 56 studies)
- 16 IVF studies, 24 ICSI studies, and 16 mixed (IVF + ICSI) studies
- 23 SCSA, 18 TUNEL, 8 SCD, and 7 Comet
- 8068 treatment cycles (3734 IVF, 2282 ICSI and 2052 mixed IVF + ICSI)



FERTILITY

Table 3: Meta-analysis summary: Overall and subgroup odds ratios of studies on sperm DNA damage and pregnancy

<i>Effect</i>	<i>Number of studies</i>	<i>Fixed effects model</i>		<i>Random effects model</i>	
		<i>OR (95% CI)</i>	<i>P</i>	<i>OR (95% CI)</i>	<i>P</i>
Overall effect	56	1.68 (1.49–1.89)	0.0000*	1.84 (1.5–2.27)	<0.0001*
Sperm DNA damage assays					
SCSA	23	1.18 (0.96–1.44)	0.1115	1.22 (0.93–1.61)	0.1522
TUNEL	18	2.18 (1.75–2.72)	0.0000*	2.22 (1.61–3.05)	<0.0001*
Comet	7	3.34 (2.32–4.82)	0.0000*	3.56 (1.78–7.09)	0.0003*
SCD	8	1.51 (1.18–1.92)	0.0011*	1.98 (1.19–3.3)	0.0086*
Types of assisted treatment					
IVF	16	1.65 (1.34–2.04)	0.0000*	1.92 (1.33–2.77)	0.0005*
ICSI	24	1.31 (1.08–1.59)	0.0068*	1.49 (1.11–2.01)	0.0075*
Mixed	16	2.37 (1.89–2.97)	0.0000*	2.32 (1.54–3.5)	0.0001*

Asian Journal of Andrology (2017) 19, 80–90

MSOME *Motile Sperm Organellar Morphology Examination*

IMSI *Intracytoplasmic Morphologically Select Sperm Injection*



FERTILITY

Sperm Organelle Morphologic Abnormalities: Contributing Factors and Effects on Intracytoplasmic Sperm Injection Cycles Outcomes

Daniela Paes de Almeida Ferreira Braga, Amanda S. Setti, Rita C. S. Figueira, Marcillo Nichi, Ciro D. Martinhago, Assumpto Iaconelli, Jr., and Edson Borges, Jr.

Table 1. Multivariate regression analysis of factors contributing to motile sperm organelle morphology defects incidence with variables including sperm DNA fragmentation percentage, patient's age, and sperm aneuploidy frequency

Predictor Variables	Response Variable MSOME	Slope	R ²	P
Percentage of sperm DNA fragmentation	Normal cells	−0.016	0.030	.145
	Abnormal shape	0.010	0.009	.411
	Abnormal size	0.004	0.189	<.001
	Large vacuoles	0.004	0.067	.029
	Small vacuoles	0.006	0.063	.034
Patient's age	Normal cells	−0.012	0.002	.715
	Abnormal shape	−0.001	0.030	.148
	Abnormal size	0.053	0.156	.135
	Large vacuoles	0.065	0.118	<.001
	Small vacuoles	0.198	0.104	<.001
Sperm aneuploidy	Normal cells	0.00291	0.009	.805
	Abnormal shape	0.00115	0.001	.960
	Abnormal size	0.08637	0.006	.528
	Large vacuoles	0.00291	0.009	.805
	Small vacuoles	0.00115	0.001	.960



Title: Sperm DNA fragmentation is correlated with poor embryo development, lower implantation rate and higher miscarriage rate in reproductive cycles of non-male factor infertility

Article Type: Cohort Study

Keywords: Sperm chromatin dispersion; Sperm DNA fragmentation; ICSI; semen analysis; non-male factor infertility

Corresponding Author: Dr. Edson Borges Jr., MD, PhD

Corresponding Author's Institution: Fertility Medical Group

First Author: Edson Borges Jr., MD, PhD



Table 4- Descriptive analysis of seminal parameters according to SDF groups

	<30% SDF (n= 433)	≥30% SDF (n= 42)	p
<i>Paternal age (years)</i>	<i>38.68 ± 5.65</i>	<i>41.19 ± 6.35</i>	<i>0.009</i>
<i>Ejaculatory abstinence (days)</i>	<i>3.92 ± 2.42</i>	<i>5.51 ± 5.46</i>	<i>0.002</i>
<i>Seminal volume (mL)</i>	<i>2.94 ± 0.50</i>	<i>3.79 ± 1.09</i>	<i>0.001</i>
<i>Seminal Concentration (x 10⁶/ml)</i>	<i>77.70 ± 29.83</i>	<i>81.09 ± 33.23</i>	<i>0.677</i>
<i>Total sperm count (x 10⁶)</i>	<i>214.58 ± 72.95</i>	<i>303.71 ± 78.80</i>	<i>0.003</i>
<i>Total sperm motility (%)</i>	<i>63.45 ± 12.75</i>	<i>55.52 ± 17.55</i>	<i><0.001</i>
<i>Progressive sperm motility (%)</i>	<i>54.90 ± 14.27</i>	<i>46.50 ± 16.77</i>	<i><0.001</i>
<i>TMSC</i>	<i>121.11 ± 98.24</i>	<i>146.89 ± 139.09</i>	<i>0.120</i>
<i>SDF (%)</i>	<i>17.48 ± 8.70</i>	<i>37.67 ± 6.39</i>	<i><0.001</i>

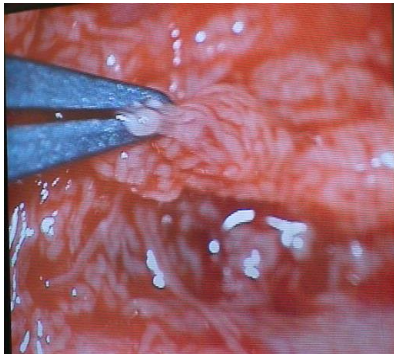
Table 7- Effect of Sperm DNA fragmentation on laboratorial and clinical outcomes

	<30% SDF (n= 433)	≥30% SDF (n= 42)	<i>p</i>
<i>Laboratorial outcomes*</i>			
Fertilization rate	90.10 ± 3.50	85.67 ± 1.03	0.226
Normal cleavage speed rate	72.16 ± 1.30	61.56 ± 4.40	0.021
High quality embryos at day three rate	36.47 ± 1.51	23.89 ± 5.51	0.028
Blastocyst rate	56.25 ± 2.01	39.01 ± 1.40	0.006
Blastocyst quality rate	30.54 ± 2.27	11.32 ± 7.72	<0.001
<i>Clinical outcomes**</i>			
Implantation rate	46.09± 0.55	33.21 ± 1.96	<0.001
Chemical Pregnancy rate	34.99	33.11	0.940
Clinical Pregnancy rate	32.42	30.33	0.774
Miscarriage rate	17.8	39.9	0.021

Use of testicular sperm in nonazoospermic males

Akanksha Mehta, M.D.,^a Sandro C. Esteves, M.D.,^{b,c,d} Peter N. Schlegel, M.D.,^e Craig I. Niederberger, M.D.,^f Mark Sigman, M.D.,^g Armand Zini, M.D.,^h and Robert E. Brannigan, M.D.ⁱ

VOL. 109 NO. 6 / JUNE 2018



PRO: Emerging body of evidence supports use of testicular sperm for nonazoospermic males in several clinical settings



Pro 1. Akanksha Mehta, M.D., Fertile Battle Team Leader

Pro 2. Peter N. Schlegel, M.D.

Elevated Levels of Ejaculated Sperm DNA Damage: The Case for Use of Testicular Sperm

Pro 3. Sandro C. Esteves, M.D.

Recurrent IVF Failure and Recurrent Pregnancy Loss: The Case for Use of Testicular Sperm

CON: First do no harm—more data is needed before adapting use of testicular sperm in nonazoospermic male



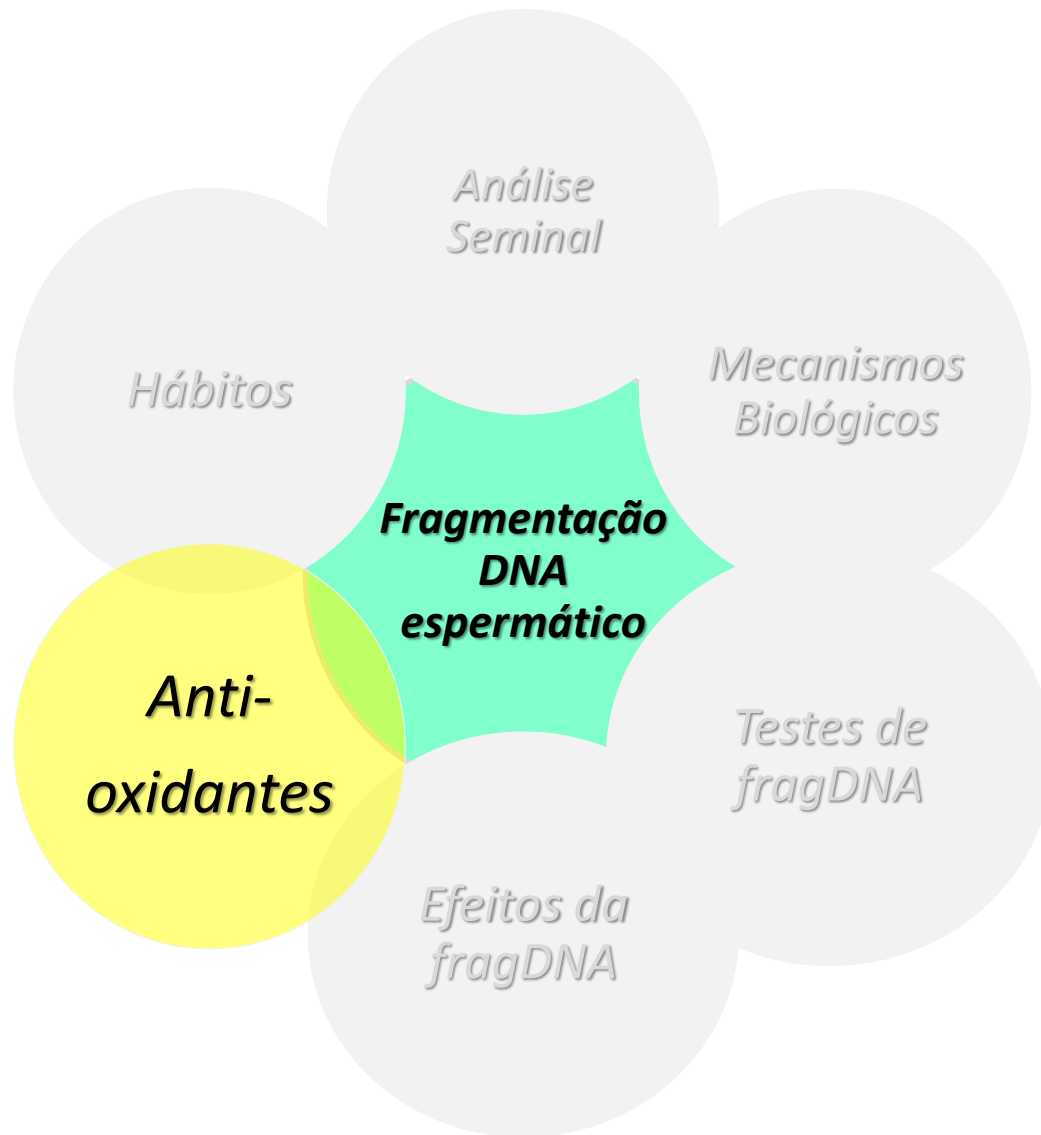
Con 1. Craig I. Niederberger, M.D., Fertile Battle Team Leader

Con 2. Mark Sigman, M.D.

Elevated Levels of Ejaculated Sperm DNA Damage: The Case Against Use of Testicular Sperm

Con 3. Armand Zini, M.D.

Recurrent IVF Failure and Recurrent Pregnancy Loss: The Case Against Use of Testicular Sperm



FERTILITY



Antioxidants for male subfertility

Showell MG, Brown J, Yazdani A, Stankiewicz MT, Hart RJ

Published Online: March 14, 2012

Oxidative stress may cause sperm cell damage. This damage can be reduced by the body's own natural antioxidant defences. Antioxidants can be part of our diet and taken as a supplement. It is believed that in many cases of unexplained subfertility, and also in instances where there may be a sperm-related problem, taking an oral antioxidant supplement may increase a couple's chance of conceiving when undergoing fertility treatment. This [review](#) identified 34 randomised controlled trials involving 2876 couples. Pooled findings from three small trials suggest an increase in live birth rates for the partners of subfertile men taking an antioxidant supplement as part of an assisted reproductive program. However, further well-designed large randomised [placebo](#)-controlled trials are needed to confirm these findings.

- 34 estudos randomizados - 2.876 casais
- Aumento da taxa gestação (OR=4,18)
- Aumento na taxa de nascidos vivos (OR=4,85)



Antioxidants for male subfertility (Review)

Smits RM, Mackenzie-Proctor R, Yazdani A, Stankiewicz MT, Jordan V, Showell MG

- 61 studies with a total population of 6264 subfertile men, aged between 18 and 65 combined 18 different oral antioxidants.
- **Live birth: *OR 1.79***, 95% CI 1.20 to 2.67, $P = 0.005$, 7 RCTs, 750 men.
- **Clinical pregnancy rate: *OR 2.97***, 95% CI 1.91 to 4.63, $P < 0.0001$, 11 RCTs, 786 men.

The effect of sperm DNA fragmentation on miscarriage rates: a systematic review and meta-analysis

Lynne Robinson^{1,*}, Ioannis D. Gallos^{1,2}, Sarah J. Conner^{1,2},
Madhurima Rajkhowa¹, David Miller³, Sheena Lewis⁴,
Jackson Kirkman-Brown^{1,2}, and Arri Coomarasamy^{1,2}

- **16 estudos – 2.969 casais**
- Aumento significativo de *abortamento* em homens com aumento da *fragDNA espermática*: RR = 2,16 (1,54 – 3,03)
- TUNEL: RR = 3,94 (2,45 – 6,32)



Antioxidantes orais – como prescrever

- Vitamina C: 500 mg/dia
- Vitamina E: 400 mg/dia
- Ácido fólico (folato): 2-5 mg/dia
- Zinco: 25 mg/dia
- Selênio: 26 mg/dia
- L-carnitina: 3g/dia



65 dias – tempo da espermatogênese

ORIGINAL ARTICLE

Correspondence:

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Keywords:

Ejaculatory abstinence, ICSI, semen quality,
sperm DNA fragmentation

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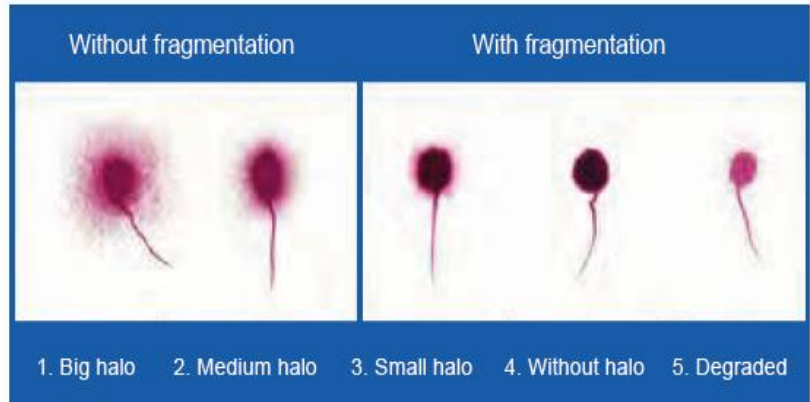
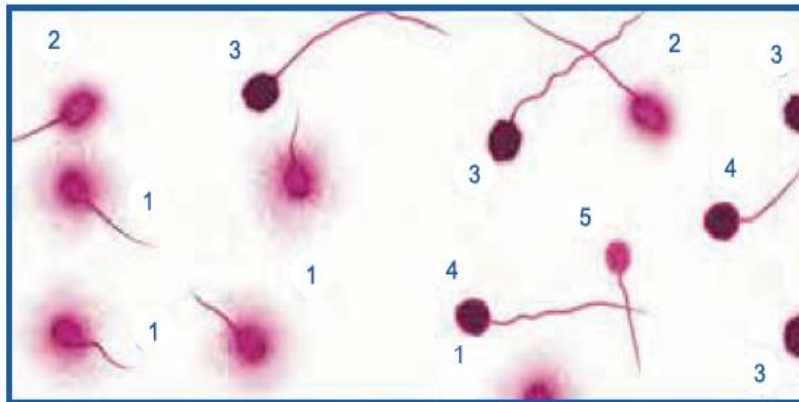
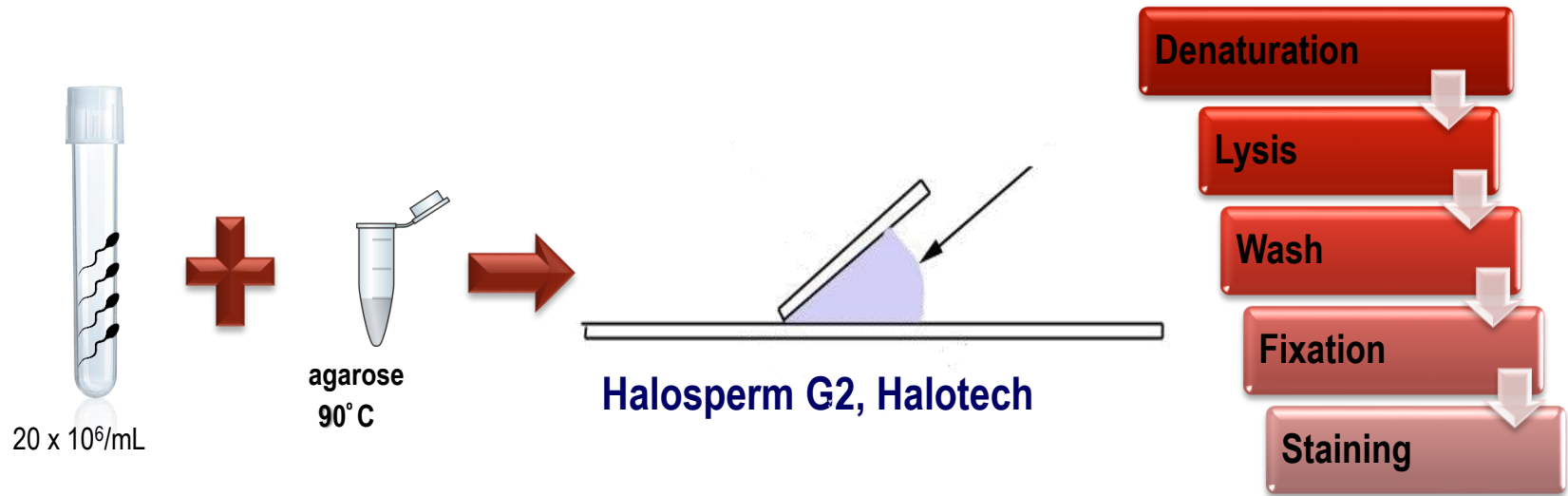
Revisiting the impact of ejaculatory abstinence on semen quality and intracytoplasmic sperm injection outcomes

^{1,2}E. Borges Jr., ^{1,2}D. P. A. F. Braga , ²B. F. Zanetti , ^{1,2}A. Iaconelli Jr.
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SPERM CHROMATIN DISPERSION TEST



**Linear model analysis of the association between sperm parameters and
EA length (n = 818)**

SEMEN PARAMETER	R	SLOPE	R² (%)	P-VALUE
Semen volume (mL)	0.1405	1.62102	5.28	<0.001
Sperm count (x10 ⁶ /mL)	3.1261	52.2206	2.59	<0.001
Total sperm count (x10 ⁶)	18.941	170.650	8.37	<0.001
Total sperm motility (%)	-0.3355	19.0885	0.23	0.212
Progressive sperm motility (%)	-0.1895	19.1802	0.07	0.483
TMSC (x10 ⁶)	9.6396	102.629	6.14	<0.001
Morphology (%)	0.0227	1.29926	0.23	0.215
SDF (%)	0.5355	9.34201	2.57	<0.001

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Table 5 GzLM results for the association between ICSI outcomes and EA length ($n = 483$)

Continuous ICSI outcomes	B	95% CI	<i>p</i> -value
Fertilization rate ^a	−0.983	−1.954 to −0.011	0.047
High-quality embryos rate on day 3 ^a	−0.090	−0.207 to 2.284	0.131
Blastocyst formation rate on day 5 ^a	−2.384	−4.552 to −0.216	0.031
Implantation rate ^{ab}	−3.299	−5.388 to −1.260	0.002
Continuous ICSI outcomes	Exp(B)	95% CI	<i>p</i> -value
Pregnancy rate ^{ab}	0.506	0.290–0.882	0.016
Miscarriage rate ^{ab}	0.736	0.458–1.185	0.207

GzLM: generalized linear model; ICSI: intracytoplasmic sperm injection; EA: ejaculatory abstinence; B: unstandardized regression coefficient; CI: confidence intervals; Exp(B): exponentiation of the B coefficient. ^aAdjusted for maternal and paternal ages, smoking habits and body mass index, seminal parameters, total dose of FSH administered, estradiol levels on the day of hCG administration, number of oocytes and mature oocytes; ^bAdjusted for number of transferred embryos.



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¹Fertility Medical Group, Sao Paulo, Brazil, and ²Sapientiae Institute, Sao Paulo, Brazil

The ejaculatory abstinence ≤ 4 days:

- Significant lower sperm DNA fragmentation index, and higher rates of fertilization, high-quality embryos on day 3, blastocyst development, implantation and pregnancy compared to ejaculatory abstinence > 4 days group.
- The implantation rate was significantly higher and the pregnancy rate tended to be higher with **one day of ejaculatory abstinence**, compared to 2–4 days of ejaculatory abstinence.



FERTILITY



ORIGINAL ARTICLE

WILEY **andrologia**

Paternal lifestyle factors in relation to semen quality and in vitro reproductive outcomes

Edson Borges Jr^{1,2}  | Daniela Paes de Almeida Ferreira Braga^{1,2} |
Rodrigo R. Provenza¹ | Rita de Cassia Savio Figueira¹ | Assumpto Iaconelli Jr^{1,2} |
Amanda Souza Setti^{1,2}

- ❖ Fator masculino isolado
- ❖ 1º ciclo de tratamento
- ❖ Idade mulher < 36 anos
- ❖ 233 ciclos ICSI

1. Quantos cigarros/dia?
2. Consumo semanal de álcool?
3. Frequência de exercícios ?
4. Medicações nos últimos 3 meses? Qual?
5. Exposição a agentes tóxicos, pesticidas, radiação etc..

Linear regression analyses' results for the influence of paternal lifestyle factors on semen quality (n=965)

	Cigarette smoking		Alcohol consumption	
	B	p	B	p
Semen volume	-0.417	0.047	-0.1363	0.592
Sperm count/mL	-7.363	0.014	-12.527	0.040
Total sperm count	-4.43	0.023	-34.91	0.156
Total sperm motility	2.316	0.347	0.342	0.895
Progressive sperm motility	-0.369	0.887	2.547	0.240
TMSC	- 1.38	0.045	-16.33	0.278
Sperm morphology	-0.0563	0.779	0.3751	0.180
SDF	0.014	0.033	5.833	0.002

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Principais indicações para investigação da fragDNA espermático

1. Varicocele palpável e sêmen normal ou limítrofe (espermograma convencional)
2. Infertilidade inexplicada
3. Falha RA (Inseminação intra-uterina, FIV clássica ou ICSI)
4. Aborto de repetição
5. Fatores risco infertilidade (obesidade, tabagismo, álcool, idade, uso gonadotoxinas, infecção, etc..)

Considerações (1)



- ❖ *fragDNA* espermático como nova ferramenta diagnóstica;
- ❖ Teste da dispersão da cromatina – mais adequado e de menor custo;
- ❖ Antioxidantes como forma de tratamento;
- ❖ Modificação do estilo de vida (cigarro, peso);

Considerações (2)



- ❖ Diminuição do tempo de abstinência ejacutória
- ❖ Identificação e tratamento de fatores causais (infecção, varicocele)
- ❖ Utilização MSOME / IMSI
- ❖ Utilização de espermatozoide testicular???

<https://fertility.com.br/producao-cientifica-2019/>

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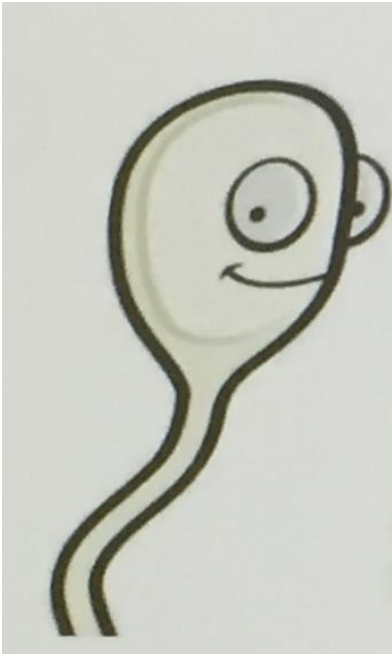
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Aulas Ministradas

Área Médica



Obrigado !



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