Causas do Aumento da Infertilidade Masculina no Século XXI

Vitor Oliveira

Serviço de Urologia do Centro Hospitalar de Vila Nova de Gaia/Espinho

Causas do Aumento da Infertilidade Masculina no Século XXI

- Disruptores Endócrinos, Stress Oxidativo, Fragmentação do DNA, Protaminas
- Obesidade
- Tabaco
- Idade Avançada
- Varicocele
- Fraldas Descartáveis?

Table 19-6. Distribution of Patients by Diagnostic Category after Full Evaluation

Category	No. Patients	%
Varicocele	806	38
Idiopathic	482	23
Obstruction	271	13
Normal	197	9
Cryptorchidism	73	3
Testicular failure	54	3
Antisperm antibodies	42	2
Ejaculatory dysfunction	49	2
Gonadotoxin*	43	2
Endocrinopathy	25	1
Pyospermia	22	1
Genetic/chromosomal†	11	0.5
Torsion	11	0.5
Erectile dysfunction	8	0.4
Testis cancer	9	0.4
Ultrastructural	7	0.3
Viral orchitis	7	0.3
Systemic illness	4	0.2
Hypospadias	1	0.05

*Includes exposure to chemotherapeutic agents and radiation, heat, drugs, etc.

†Includes chromosomal abnormalities such as Klinefelter's syndrome and genetic abnormalities such as CFTR mutations.

O Que Sabemos

2001

Campbell-Walsh Urology, 9th Ed

Causas de Disfunção Espermática

- Disruptores Endócrinos
- Stress Oxidativo
- Fragmentação do DNA
- Protaminas

DES na Grávida:

- Diminuição da qualidade dos Ez
- Maior incidência de malformações genitais
- Criptorquidia
- Hipospadias
- Carcinoma do testículo

SÍNDROME DE DISGENESIA TESTICULAR

N Damgaard et al, 2002; Strohsnitter et al, 2001

	Insult to sperm nuclear content	Exposure of testis and fetus
Causes	• Oxidative stress (due to varicoceles,leukocytospermia, urinary tract infections) • Hormonal deficiencies • Microdeletions in Y-chromosome • Protamine deficiency	• Endocrine disruptors • Air pollutants • Life-style
Effects	Alterations in DNA/RNA (qualitative/quantitative) 	• Increased sperm DNA damage • AlteredSertoli cell function • Altered methylation patterns • Altered RNA profile • Disruption of apoptotic pathway • Increased susceptibility to environmental factors
Outcome	• Poor seminal parameters (concentration, motility, morphology) • Poor fertilization rates / infertility • Epigenetic transfer onto offspring	• Testicular dysgenesis syndrome • Abnormal sperm count/function • Poor fertilization rates / infertility • Epigenetic transfer to future generations

Vulnerabilidade do Sistema Reprodutor Masculino: possíveis causas, efeitos e resultados

AC Varghese *et al*, RBM, 2008

Alvarez, 2003

- Aumento da temperatura
- Factores pró-inflamatórios
- Hábitos sociais
- Drogas
- RT
- Xenobióticos

Pesticidas

- Agricultores Mexicanos (Organofosfatos):
  - Taxas de aneuploidia nos Ez (75% vs 12%)
- China (Carbaryl):
  - Aumento da aneuploidia, Fragmentação DNA
- Agricultores EUA:
  - Menor concentração Ez e motilidade

Recio et al 2001, Sanchez-Pena et al, 2004; Xia et al, 2005; Swan, 2006

CHUMBO

- Diminui volume Ez
- Diminui concentração
- Diminui nº de Ez Normais
- Diminui Vitalidade

DDT - Pesticida

- Diminui motilidade
- Diminui volume
- Aumenta Teratozoospermia

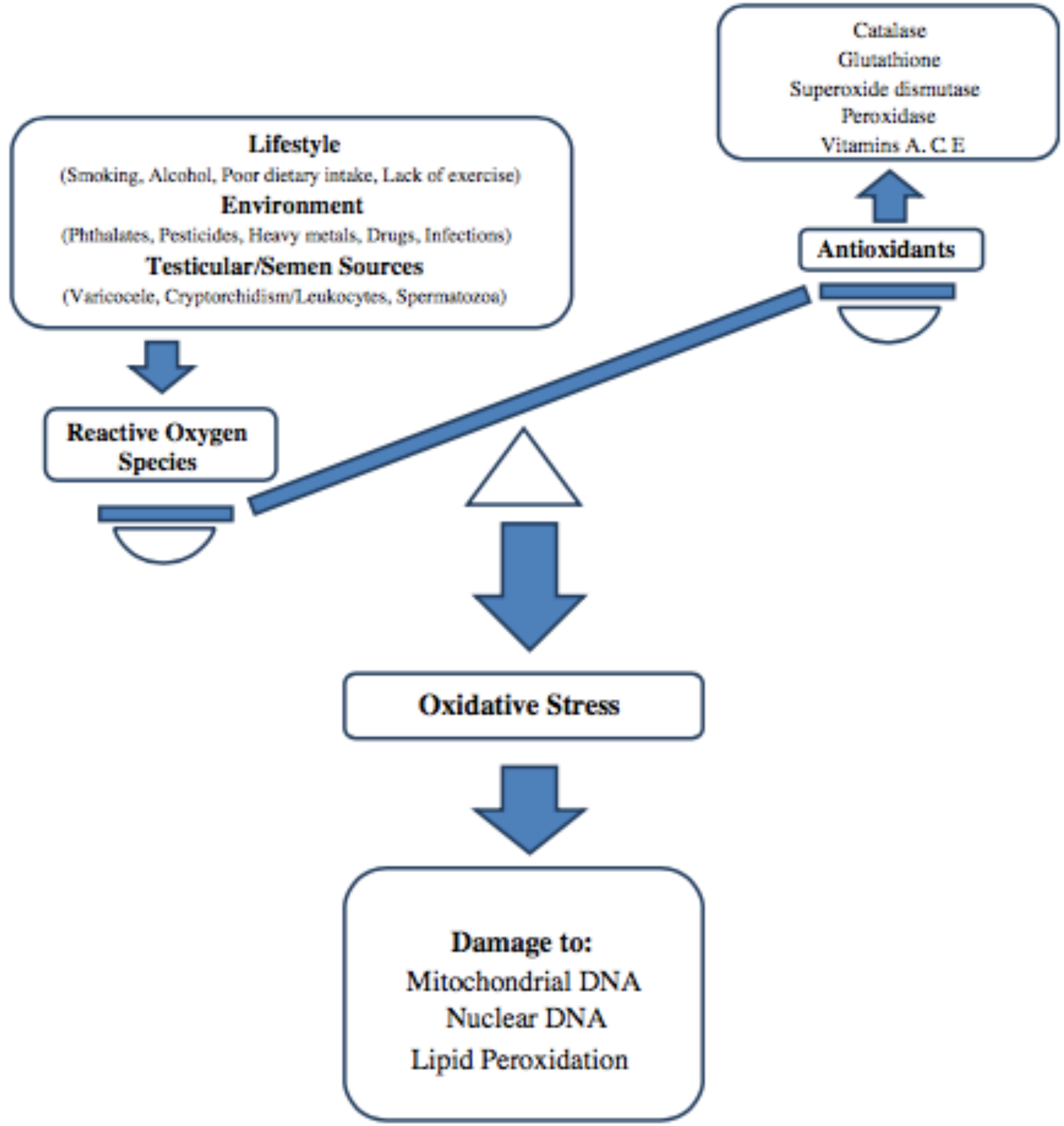


Figure 2 Model of the build up of oxidative stress in the semen. The model highlights the imbalance caused by accumulating ROS and depleting antioxidant, which brings about a state of oxidative stress. Various lifestyle and environmental factors along with testicular and seminal sources cause the generation of ROS. Antioxidants comprise both enzymatic and non-enzymatic types.

A Agarwal et al, 2014

Table 9 Candidate protein biomarkers that may be indicative of oxidative stress-induced male infertility.

Suggested protein biomarker	*UniProt ID*	*Function*	*Expression with pathological ROS or oxidative stress*	*Expression with physiological ROS or without oxidative stress*	*Reference*
Seminal plasma					
DJ-1	Q99497	Protects against oxidative stress and apoptosis, eliminates H_2O_2, positive regulator of androgen receptor-dependent transcription	Decreased	Identified	Wang et al. (2009)
Tubulin-folding cofactor B (TBCB)	Q99426	Aids in the regulation of tubulin heterodimer dissociation	Increased	Not stated	Herwig et al. (2013)
Alpha-1-antichymotrypsin (AACT)	P01011	Physiological role undefined, may be involved in controlling oxidative damage, highly anti-inflammatory, significant role in defence mechanism against pathological processes	Increased	Identified	Herwig et al. (2013)
Aldose reductase (ALDR)	P15121	Catalyses the reduction of aldehydes and carbonyls	Increased	Identified	Herwig et al. (2013)
Diacylglycerol kinase (DGK) eta	Q86XP1	Upstream regulator of oxidative stress-induced activation of PKD signalling pathway	Increased	Not identified	Herwig et al. (2013)
Prolactin-inducible protein (PIP)	P12273	Involved in immunoregulation, antimicrobial activity, apoptosis and tumour progression	Increased	Identified (very low amount)	Sharma et al. (2013a)
Spermatozoa					
Lactotransferrin-2	P02788	Antioxidant Chelates iron (an essential catalyst for ROS production)	Not identified	Increased	Hamada et al. (2013)
Peroxiredoxin-1 (PRDX1)	Q06830	Detoxifies H_2O_2	Decreased	Increased	Hamada et al. (2013)
Histone cluster 1, H2ba (HIST1H2BA)	Q96A08	Core component of nucleosome which wrap and compact DNA into chromatin	Increased	Identified	Sharma et al. (2013b)
Malate dehydrogenase 2, NAD (mitochondrial) (MDH2) precursor	P40926	Catalyses the reversible oxidation of malate to oxaloacetate (citric acid cycle)	Increased	Not identified	Sharma et al. (2013b)
Transglutaminase 4 (TGM4)	P49221	Catalyses post-translational cross-linking of proteins, part of a calcium-dependent enzyme	Increased	Identified	Sharma et al. (2013b)
Glutathione peroxidase 4 (GPX4) isoform A precursor	P36969	Catalyses the reduction of hydrogen peroxide, protects against oxidative damage	Increased	Identified	Sharma et al. (2013b)
Glutamate-ammonia ligase (GLUL)	P15104	Catalyses the synthesis of glutamine (from glutamate and ammonia), which plays a role in inhibition of apoptosis, cell proliferation and signalling, aids in control of body pH	Increased	Identified	Sharma et al. (2013b)
Heat shock protein 70 kDa protein 5 (HSPA5)	Q24JP5	Plays a role in the folding and assembly of proteins in the endoplasmic reticulum; possible important role in monitoring protein transport through the cell	Increased	Not identified	Sharma et al. (2013b)

A Agarwal et al, 2014

OBESIDADE

- Diminui:
    - TT
    - SHBG
    - Inibina B

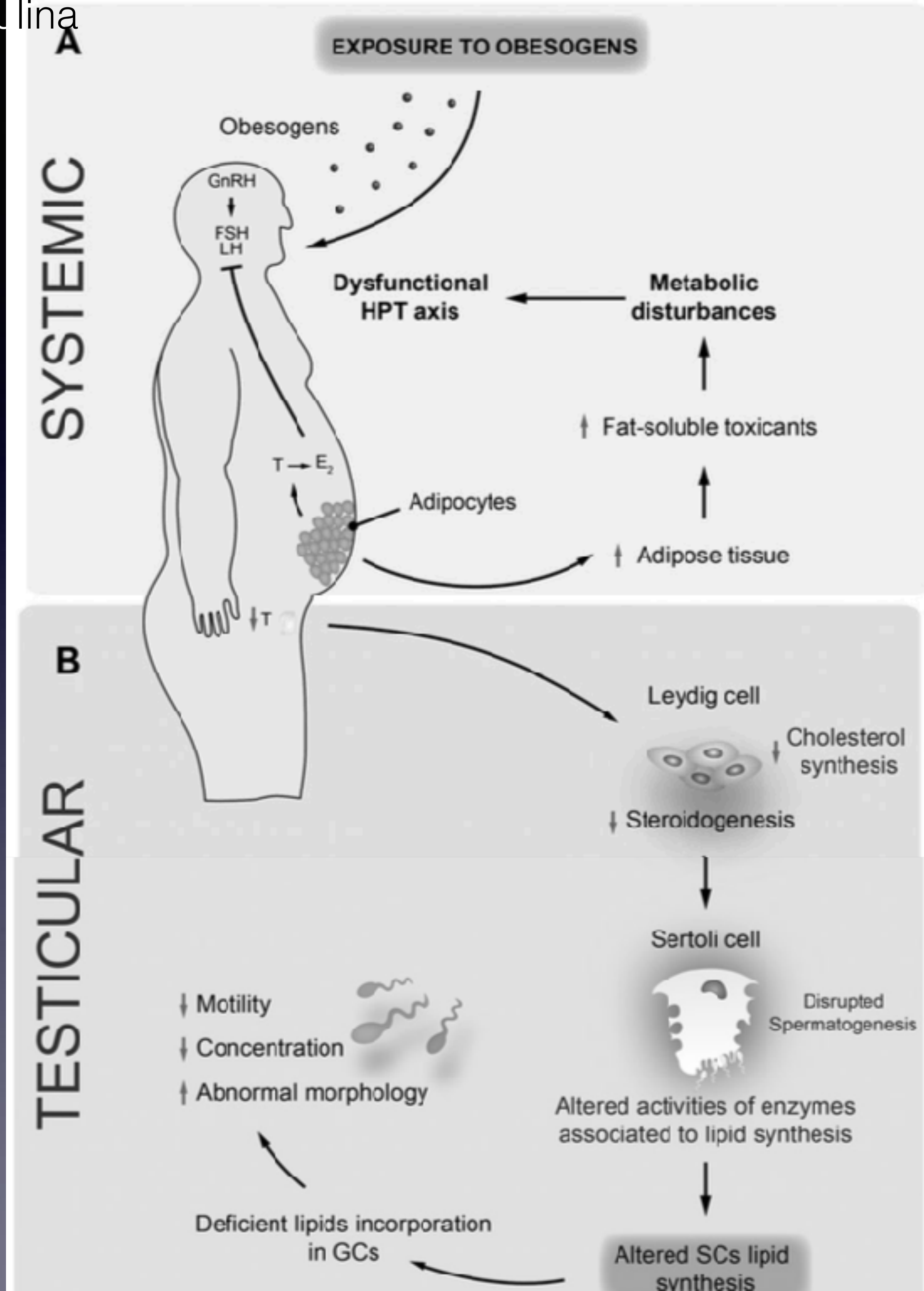
OBESOGENS

- Compostos ambientais que favorecem o ganho de peso (Ubiquitários)
    - Modificam o Eixo HHT (Disruptores Endócrinos)

>> Modificações Epigenéticas

>> Gametas Masculinos

>> Descendência (3 gerações)



AM Cardoso et al, 2017

Table 1 Summary of the proposed effects of obesogens in both glucose and lipid metabolism and the toxic effects in reproductive system

Obesogen	Glycolytic metabolism effects	Lipid metabolism effects	Reproductive toxic effects
2,4-D	↓GLUT3, PFK1 and LDH mRNA, ↓Lactate production (4)	↑MDA, ↑LDL/HDL index, ↓PUFAs ratio, ↑Lipid peroxidation (5)	↑ Spermatogenic disorders, ↑Sterility (6), Delayed preputial separation (7)
B[α]P	↑Lactate production (8), ↓LDH (9)	↓Serum LPC, ↑Serum SM (10)	↓DSP, ↓Reproductive parameters, ↓Testicular steroidogenic enzymes activity (11)
BPA	↓IRS-1, ↓GLUT2 (12) ↓HEX, ↓PFK (13)	↑ACC and FAS mRNA, ↑LPL (14)	↓Sperm count and motility (15), Erectile dysfunction (16)
CPYF	↑LDH (17)	↑Lipid peroxidation, ↑MDA, ↑4-HNE (18)	↑Abnormal spermatozoa, ↓Sperm motility and count (19)
DES	↑LDH activity (20)	↑Lipid deposition (21)	Penis morphological abnormalities (21)
GES	↓Glucose uptake (22)	↑MCAD mRNA, ↓MDA, ↑GSH (19)	↓Sperm counts, ↑Sperm motility, LCs hyperplasia (20)
Lead	↑Lactate production (23)	↑Lipid peroxidation, ↑CAT activity, ↑GSH, ↓SOD activity (23)	↓Semen volume, ↓Sperm concentration and viability (24)
MSG	↑Glucose, ↑Insulin (25)	↑Triglycerides, ↑Free fatty acids (26)	↓LCs number (27)
NIC	↓Glycolysis, ↓PFK1 activity (27)	↑Phospholipids, ↑VLDL, ↑Triglycerides, ↓HDL, ↑Cholesterol, ↑LDL (28)	↓Libido, ↓Sperm motility and count (29)
PARTH	↓LDH (30)	↓Adiponectin, ↑TNFα, ↓TBARS (29)	↓Weight of reproductive organs, ↓Sperm count, ↓Fertility (31)
PBDEs	↓Glucose/insulin ratio (32)	↑Cholesterol, ↑Lipolysis (33)	↓Sperm counts (34)
PCBs	↑Lactate production (31)	↓Fatty acids, ↑Fatty acid degradation related genes (35)	↓Sperm quality and integrity, ↓Reproductive hormones (36)
PFOA	↓PI3K-AKT, ↑Insulin sensitivity, ↑Glucose tolerance (37)	↑Fatty acid oxidation, ↑Adipose tissue atrophy, ↑Cholesterol (38,39)	↓Sperm count and concentration
PIO	↑Glycolysis, ↓Insulin secretion (40)	↓Free fatty acids, ↑HDL, ↓Triglycerides, (34)	↑Sperm motility and viability (41)
PTLs	↑Pyruvate production, ↑Lactate production (42)	↓ACC, ↑LCAD, ↑TP-beta (43)	Affected male maturity, ↓Sperm motility and concentration (44)
SUs	↑Glycolysis, ↑L-lactate (35)	↓VLDL, ↓LDL, ↑HDL/LDL ratio (36)	↓T levels (44)
TBT	↓G6P, ↓F6P, ↓GLUT1 (45)	↑Lipid accumulation (46)	↑Apoptotic germ cells, ↓T production (47)

OBESOGENS

- Períodos Críticos:
    - Embrionário
    - Nascimento (leite materno)
    - Puberdade
- Período de Latência: - Anos a Décadas
- Tempo e Dose Dependente

ANTIOXIDANTES

- 20 artigos
    - Eficazes na redução do stress oxidativo e no aumento da motilidade
    - Não claro se isso se traduz em aumento das taxas de gravidez de termo
    - Necessário estudos bem desenhados, multicêntricos, randomizados, duplamente cegos

Gharagozloo and Aitken, 2011

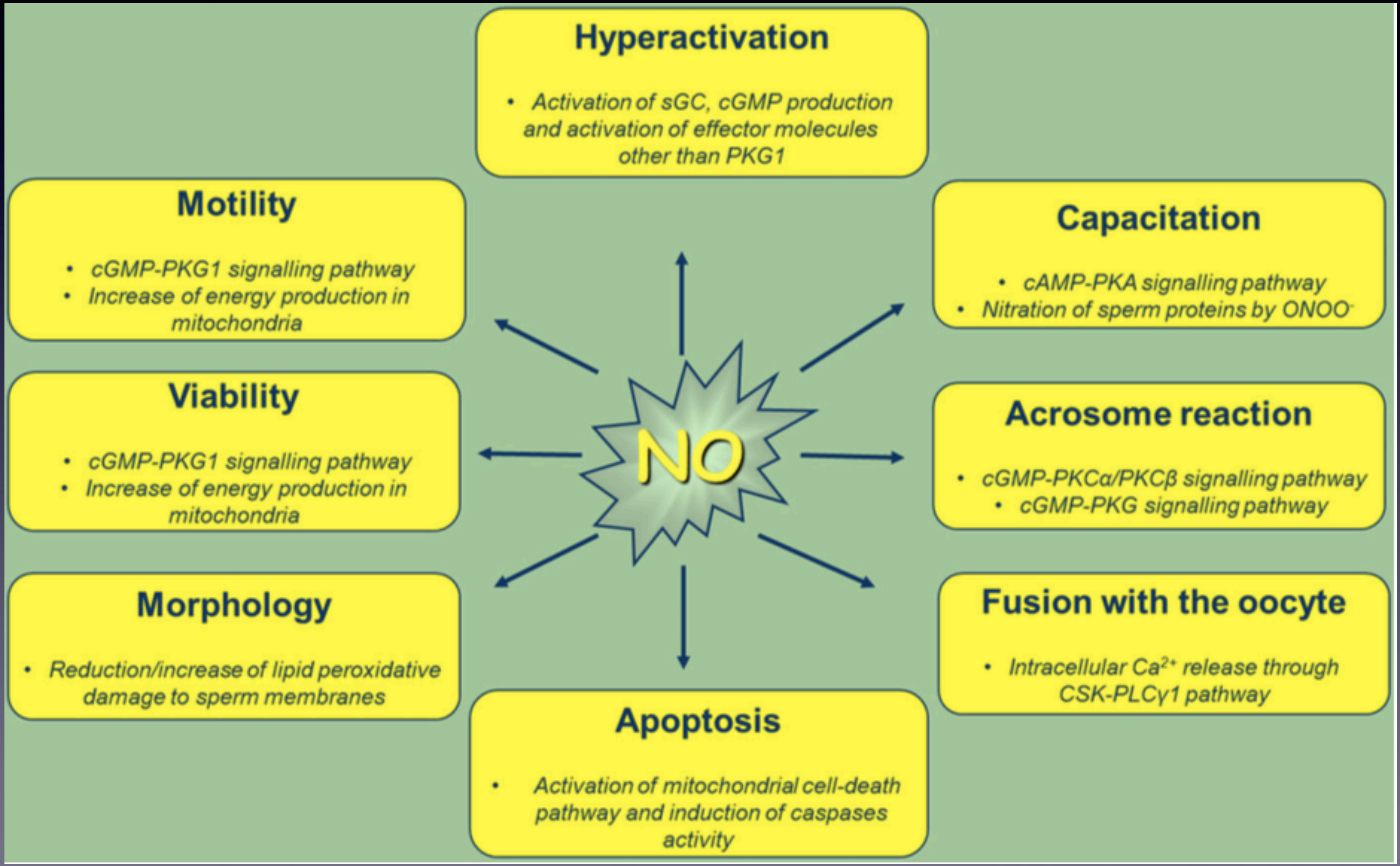
Table I Study characteristics and the effect of oral antioxidants on semen parameters and 2° outcomes.

Author	C	M	Morph	OS/DFI	2° outcomes	Target patient	Active (n)	Placb (n)	Dose/day	Duration	Comment
Vitamin C											
Fraga et al. (1991)	ND	ND	ND	↑, 8-OHdG	ND	Smokers, controlled environment	10	NA	5–250 mg	15 weeks	Depletion/ repletion
Vitamin E											
Suleiman et al. (1996)	ND	40/ 11	ND	↑, MDA	9 versus 0 live birth	Asthenospermic (motility < 40%)	52	35	3 × 100 mg	6 months	
Geva et al. (1996)	ND	ND	NE	↑, MDA	Fert. rate 19.3 → 29.1	Fertile normospermic with low FR	15	NA	200 mg	3 months	Prospective
Zinc											
Omu et al. (2008)	NE	↑	NE	↑, MDA, TAC, DFI	ND	Asthenozoospermia ≥ 40% immotile	11	8	2 × 200 mg	3 months	
L-Carnitine											
Balercia et al. (2005)	NS	↑	ND	↑, TOSC	Two pregnancy versus three placebo	Idiopathic asthenozoospermia	15	15	3 g	6 months	Detailed motility
Acetyl-L-carnitine											
Balercia et al. (2005)	↑	↑	ND	↑, TOSC	Three pregnancy versus three placebo	Idiopathic asthenozoospermia	15	15	3 g	6 months	Detailed motility
Astaxanthine											
Comhaire et al. (2005)	NS	NS	NE	↑, ROS counts	Pregnancy (54.5 versus 10.5%)	Infertile	11	19	16 mg	3 months	IUI
N-acetyl-L-cysteine											
Ciftci et al. (2009)	NE	↑	NE	↑, OSI	ND	Idiopathic with normal sperm parameters	60	60	600 mg	3 months	Also improvement in volume/viscosity
Vitamins C and E											
Greco et al. (2005a)	NS	NE	NE	↑, TUNEL	ND	Idiopathic non smokers, DFI ≥ 15%	32	32	2 × 0.5 g each	2 months	
Greco et al. (2005b)	NE	NE	NE	↑, TUNEL	2/29 versus 14/29 clinical pregnancies	DFI ≥ 15% ICSI patients (1 before and 1 after antioxidant treatment)	38	NA	2 × 0.5 g each	2 months	29 antioxidant responders
Vitamins E and Se											
Keskes Ammar	NE	↑	NE	↑, MDA	ND	Infertile men	12	8 (Vit E	2 × 200 mg and	3 months	Active control
Vitamins E and Zn											
Omu et al. (2008)	NE	↑	NE	↑, MDA, TAC, DFI	ND	Asthenozoospermia ≥40% immotile	12	8	2 × 10 mg, 2 × 200 mg	3 months	
Vitamins C, E and Zn											
Omu et al. (2008)	NE	↑	NE	↑, MDA, TAC, DFI	ND	Asthenozoospermia ≥40% immotile	14	8	2 × 5 mg, 2 × 10 mg, 2 × 200 mg	3 months	
Vitamins C, E, glutathione											
Kodama et al. (1997)	↑	NE	NE	↑, 8-OHdG	ND	Infertile men	14	NA	200 mg, 200 mg, 400 mg	2 months	
Vitamins C, E, Zn and b-carotene											
Ménézo et al. (2007)	ND	ND	ND	↑, SCSA DFI	ND	2 failed IVF/ICSI cycles and DFI > 15%	58	NA	400 mg, 400 mg, 1 µmol, 500 µmol, 18 mg	90 days	Sperm decond. observed
Vitamins C, E, Se, Zn, folic acid and garlic											
Tremellen et al. (2007)	ND	ND	ND	TUNEL	38.5 versus 16% pregnancy rate at 13 weeks gestation	IVF-ICSI patients TUNEL + ve >25% and poor morphology or low motility	36	16	100 mg, 400 IU, 26 µg, 25 mg, 0.5 mg, 5 mg, 1 g	3 months	Patent reports no TUNEL effect [125]
Tunc et al. (2009)	NE	NE	NE	↑, TUNEL ROS	ND	Men exhibiting oxidative stress	50	NA	100 mg, 400 IU, 26 µg, 25 mg, 0.5 mg, 5 mg, 1 g	3 months	
L-carnitine and acetyl-L-carnitine											
Vicari et al. (2001)	NE	↑	NE	↑, ROS	11.2% pregnancy versus 0%	PVE infertile patients 34 normal WBC, 20 abnormal	54	NA	2 × 1 g, 2 × 0.5 g	3 months	
Vicari et al. (2002)	NE	NS	NE	fMLP ROS (NS)	No pregnancy	PVE and abnormal WBC	30	NA	2 × 1 g, 2 × 0.5 g	4 months	Improved viability
Balercia et al. (2005)	↑	↑	ND	↑, TOSC	Five pregnancy versus three placebo	Idiopathic asthenozoospermia	14	15	2 g and 1 g	6 months	Detailed motility

C, concentration; DFI, DNA fragmentation index; M, motility; MDA, malondialdehyde; Morph, morphology; ND, not determined; NE, no effect; NS, not significant; 2° Outcomes: fertilization rate (FR) or pregnancy; OS, oxidative stress; Placb, Placebo. PVE, prostatovesiculoepididymitis; TAC, total antioxidant capacity; WBC, white blood cell.

Gharagozloo and Aitken, 2011

A Importância do NO



List of redox-active substances in clinical trials or commercially available that ameliorate sperm-fertilizing capacity by (at least partially) modulating the NO-signalling pathway

Compound	Effect	
L-arginine	Increases sperm volume, concentration, motility and morphology by increasing eNOS activity	Scibona *et al.*, 1994; Aydin *et al.*, 1995; Srivastava *et al.*, 2000
Superoxide dismutase mimic, M40403	Improves the functional status of sperm mitochondria, and thus the fertilizing potential of spermatozoa, by increasing NO bioavailability	Otasevic *et al.*, 2013
Sildenafil (Viagra™)	Improves erectile function through inhibition of cGMP-dependent phosphodiesterase	Schwartz and Kloner, 2010
	Increase velocity, capacitation and amplitude of lethal head displacement in spermatozoa (mechanism unclear)	Lefièvre *et al.*, 2000
Tadalafil (Cialis™)	Improves erectile function through inhibition of cGMP-dependent phosphodiesterase	Schwartz and Kloner, 2010
Vardenafil (Levitra™)	Improves erectile function through inhibition of cGMP-dependent phosphodiesterase	Schwartz and Kloner, 2010
Ginsenoside R_e	Improves sperm motility through induction of NOS activity (increase of NO production)	Zhang *et al.*, 2006

B Buzadzic et al, 2015

NO

- Desempenha múltiplos papéis nos Ez
  - Modula o estado de redução oxidativa do Ez (ROS e RNS)
    - Poderá representar uma nova ferramenta para aumentar a fertilidade masculina

Fragmentação do DNA

- Diminui a taxa de gravidez
- Diminui a taxa de parto de termo
- Aumenta a taxa de aborto espontâneo

- Não altera as taxas de gravidez e parto na ICSI:
  - Envio destes casais (DFI>30%) directamente para ICSI

Evenson and Wixon, 2006; Zhang et al, 2008; Zini, 2011; Gil-Villa et al, 2010; Kennedy et al, 2011; Kumar et al, 2012

Tabaco

- Contém altas concentrações de ROS
- Causa dano no DNA
- Diminui a concentração de antioxidantes
- Contém:
    - Cádmio
    - Chumbo
    - Nicotina

Valavanidis et al, 2009; Hengstler et al, 2003; Kiziler et al, 2007; Wong et al, 2000

Tabaco

- NO HOMEM:
  - Taxa de Fragmentação de DNA de 50%
  - Aumenta a Incidência de Cancro nas Crianças

- NA MULHER GRÁVIDA:
  - Diminui qualidade dos Ez
  - Diminui desenvolvimento testicular (Jensen et al, 2004)

Fraga et al, 1996; Ji et al, 1997; Shu et al, 1996

VARICOCELE

- Estado de Stress Oxidativo aumentado
- Aumento da taxa de fragmentação de DNA
- Embora polémico, o tratamento cirúrgico:
    - Melhora os parâmetros seminais
    - Diminui a taxa de fragmentação
    - Diminui os ROS
    - Aumenta os antioxidantes
    - Aumenta o sucesso das TRA
    - Aumenta a incidência de concepção espontânea

Baker et al, 2013; Chen et al, 2008; Hurtado et al, 2007; Leung et al, 2013; Mostafa et al, 2001; Smit et al, 2013

VARICOCELE

- AUA:
    - Grau II e III
    - Parâmetros seminais alterados (OAT)
    - Infertilidade
    - Estudo normal na mulher
- EAU:
    - Testículo diminuído
    - Oligozoospermia, varicocele clínico e Infertilidade Inexplicada (LE 1a)
- UK:
    - Não recomenda o tratamento

Idade Avançada

- Aumento do dano de DNA
    - Aneuploidia
- Diminuição da taxa de apoptose (Singh et al, 2003; Rybar et al, 2011)
- Apenas nos Cromossomas sexuais? (Griffin et al 1995; Martin et al, 1995; Robbins et al, 1995; Kinakin et al, 1997; Shi and Martin, 2000; Das et al, 2013; Hammiche et al, 2011)
- Resultados contraditórios:
    - Muitos estudos não encontraram efeito da idade paterna (Lahdetie et al, 1997; Asada et al, 2000; Sloter et al, 2007)

FRALDAS DESCARTÁVEIS

- n=48
- Aumento de 1ºC na temperatura do escroto dos bebés
    - O impacto no testículo é desconhecido
    - A produção de Ez só começa na puberdade
    - O exemplo da criptorquidia
- É sabido que o aumento da temperatura (1-3ºC) nos adultos é nefasta para a espermatogénese e motilidade dos Ez

DJ Partsch, 2016; B Gilbert and L Lipshultz, 2016

Table 2 Lifestyle factors modifiable without risk.

Lifestyle factor	*Results*	*Recommendations*	*References*
Smoking	Strong correlation with % DFI, DFI markedly higher in infertile smokers	Cessation of smoking	Elshal et al. (2009)
POP/PCB	Positive correlation between exposure and % DFI	PCB accumulate in food chain: avoid fatty fish, particularly farmed	Rignell-Hydbom et al. (2005), Spano et al. (2005), Stronati et al. (2006)
Organophosphorus	Marked increase in % DFI (>30%) in exposed workers	Avoid pesticide exposure	Sanchez-Pena et al. (2004)
Lead	Increase in percentage of spermatozoa with DNA fragmentation	Avoid occupational exposure and smoking or exposure to cigarette smoke	Hsu et al. (2009), Vani et al. (2012)
Bisphenol A	Significant trend of increased DNA damage with increased urinary bisphenol A concentrations	Avoid plastic packaging, tinned foods, heating or storing foods in plastic	Meeker et al. (2010)
Testicular heat	Increase in DNA fragmentation with 2–3°C temperature increase	Avoid cycling with tight pants, avoid sauna use, avoid using laptop on closed legs	Southorn (2002), Ahmad et al. (2012), Sheynkin et al. (2011), Garolla et al. (2013)
Mobile phone radiation	No specific studies on DNA fragmentation, increased ROS and decreased antioxidants	Do not store mobile phone in trouser pocket	Desai et al. (2009)
Obesity	Positive correlation of body mass index and DNA fragmentation, higher incidence in obese males	Weight loss through diet and moderate exercise	Kort et al. (2006), Chavarro et al. (2010), Fariello et al. (2012a), La Vignera et al. (2012), Dupont et al. (2013)

DFI = DNA fragmentation index; PCB = polychlorinated biphenyls; ROS = reactive oxygen species.

C Wright et al, 2014

CONCLUSÕES

- Homens com espermogramas subóptimos devem melhorar o estilo de vida:
    - Devem deixar de fumar
    - Devem perder peso
    - Devem praticar exercício físico moderado
- O estudo do homem deve apurar o impacto das alterações da integridade genómica na fertilidade
- A população, em geral, deve evitar a emissão de químicos tóxicos para o ambiente

CONCLUSÕES

- Alertar a população: Evitar o consumo de alimentos processados

- Como a ICSI pode ultrapassar o problema de infertilidade, é imperativo analisar as causas genéticas