# Oxidative stress and antioxidants for idiopathic oligoasthenoteratospermia: is it justified ?


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# Introduction

- **Infertility**: failure to achieve a pregnancy within one year of regular (at least 3 times / month) unprotected intercourse
  - Affects 15% of sexually active couples
  - Causative **male factor**: 40%

# Male factor

- Alteration in sperm conc. ± motility ± morphology in at least one sample of two sperm analysis (WHO 1999 guidelines)
- Oligo-astheno-teratozoospermia: low sperm count with a high percentage of slow-moving and abnormal sperm
  - idiopathic: 30%

- Tx for idiopathic oligoasthenoteratozoospermia (iOAT) can be problematic.
  - Drugs and dietary supplement available → many prescribed without rationale or evidence
  - ART proposed as a possible solution for male factor infertility in general but **expensive, not universally available, and with limited success**

- Oxidative stress contributes to defective spermatogenesis and the poor quality of sperm associated with idiopathic male factor infertility.
- **Aim**: to review the current literature on the effects of various types of antioxidant supplements in patients to improve fertilization and pregnancy rates in idiopathic oligoasthenoteratozoospermia (iOAT)
  - **Pubmed and the Cochrane database**

# Outlines

- Cause of iOAT
- Role of oxidative stress in iOAT
- Pharmacotherapy for iOAT

# idiopathic oligoasthenoteratozoospermia (I)

- Defective spermatogenesis
- Origin unknown
- Characteristics: (semen analysis)
  - Necrosis and apoptosis of gametes (oligoteratospermia)
  - ↓ percentage of normal sperm forms
  - Impairment of sperm motility (asthenospermia)

# idiopathic oligoasthenoteratozoospermia (II)

- Normal PE
- Histology: mixed atrophy of testicles

| Classification | Sperm conc. |
|---|---|
| Isolated astheno ± teratospermia | No alteration |
| Moderate | < 20x $10^6$/ml and > 5x $10^6$/ml |
| severe | < 5x $10^6$/ml |

# idiopathic oligoasthenoteratozoospermia (III)

- Normal hormone profile but sometimes with lower T and inhibin and higher estradiol, FSH, LH → may be age-related
- Non-inflammatory functional alternation in post-testicular organs (ex. Epididymis) may be implicated in iOAT by alterating DNA methylation of gametes
  - Methylation mediates transcriptional repression by recruiting histone deacetylase
  - Gene→ demethylation for gene transcription

# idiopathic oligoasthenoteratozoospermia (IV)

- Asymptomatic infections without leukospermia: herpes virus, adeno-associated virus and Chlamydia trachomatis (CT)
- Familial occurrence, esp. among brothers and maternal uncles
  - Autosomal recessive
  - Genetic etiology

# The role of oxidative stress in iOAT

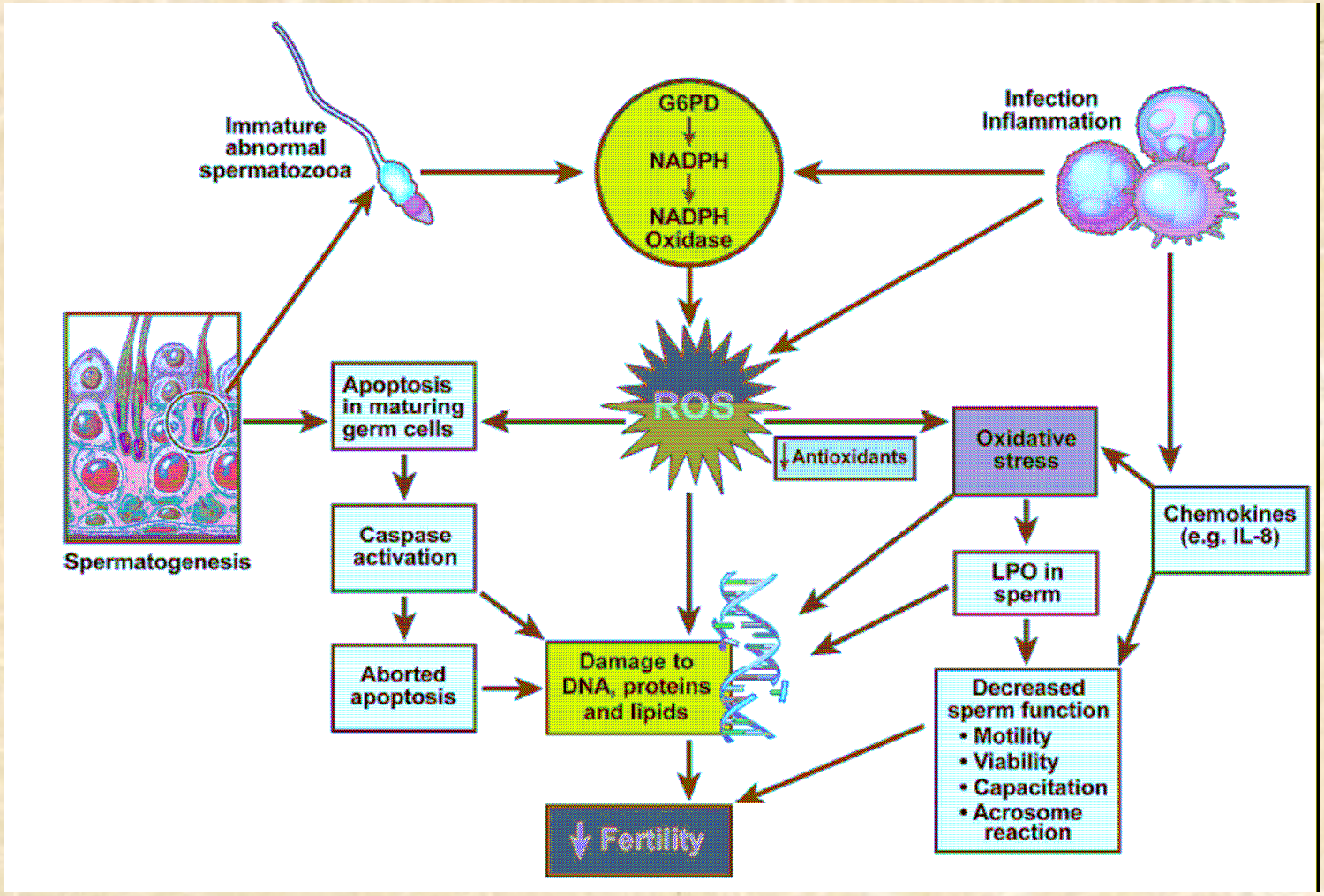
- Reactive oxygen species (ROS) are molecules with at least one unpaired electron → rendering them highly unstable and reactive in the presence of lipids, aa., and nucleic acids
  - At physiological levels → essential for normal reproductive function, acting as metabolic intermediates, regulation of vascular tone, gene regulation and in facilitated sperm capacitation and acrosome reaction
  - **At higher levels** → negative effects

# ROS

- The main source in seminal plasma is leukocytes and immature spermatozoa.
- Spermatids and mature spermatozoa are highly sensitive to ROS. (∵ membrane rich in polyunsaturated lipids)
  - Altering membrane integrity → impair sperm motility and morphology → lead to sperm cell death
- At which time point did damage to spermatozoa occur?
  - Within semen (during the time required for liquefaction)
  - In the epididymis (spermatozoa stored before ejaculation)
  - In the testis

- The seminal plasma had highly conc. of antioxidants.
- total antioxidant capacity of seminal plasma:
  - anti-ROS enzymes: superoxide dismutase (SOD), catalase, glutathione peroxidase (GSH-Px)
  - low molecular weight substances: a-tocopherol, b-carotene, ascorbate, urate
  - transition metal chelators: transferrin, lactoferrin, ceruloplasmin

# Mechanism of oxidative stress in human semen



- The concentration of malondialdehyde (MDA), a marker of lipid peroxidation, was found to be almost twice as **high** in sperm pellet suspensions of asthenospermic and oligoasthenospermic patients compared to normospermic males.
- Kobayashi: *in vitro* addition of exogenous SOD to semen samples led to improved sperm motility and a decrease in MDA concentration.

- **Zinc deficiency** was seen to confer vulnerability to oxidative stress *in vitro*, leading to increased sperm DNA fragmentation and apoptosis.
  - intracellular zinc modulates the levels of pro-apoptotic p53, p21 and bcl 2 family gene expression and caspase-3 activity
  - Bcl-2 has a role in regulating programmed cell death as an antioxidant at the outer membrane of the mitochondria.
  - Omu et al.: Bcl-2 was more highly expressed in normozoospermia than in oligozoospermia and asthenozoospermia men

- Apoptosis and markers of programmed cell death are inversely correlated with sperm motility, morphology, vitality and concentration.
  - ***survivin*** (programmed cell death inhibitor): ↓ expression → correlated with increased severity of iOAT
- ↑ apoptosis implicated in several types of OAT, not pathognomonic for iOAT
  - hormonal infertilities
  - anti-sperm antibody-associated infertility
  - varicocele
  - testicular torsion
  - inflammation

- Mitochondrial DNA oxidative damage has been observed in asthenospermic infertile men and confirmed in experimental models.

# Antioxidant treatment of iOAT

- Medical treatment for male infertility → frustrating
  - Multifactorial disorder
- Pharmacotherapy: hormones + antioxidants
  - to improve and maintain semen parameters
- Sperm conc. ↑ associated with disproportionately higher fecundability
- Benefit from therapy depends on initial semen parameters

# Antioxidants

- Efficacy: not to be well established
- Safety: high dose of vit A may have embryotoxic and teratogenic effects
- It is unknown whether ROS production can be a criterion to select men for antioxidant therapy.
  - No reliable, predictive, and inexpensive tests to determine the extent of ROS exposure or antioxidant capacity

# Empirical therapies

- sparse scientifically acceptable evidence
- based on rationale and lacking significant side-effects
- GRADE scores reflecting the quality of evidence: high, moderate, low, very low

Table 1: Scoring and evaluation of the quality of evidence regarding antioxidant therapy in idiopathic oligoasthenoteratozoospermia

| Antioxidant | GRADE score | Quality of evidence |
|---|---|---|
| Tamoxifen | 2 | Low |
| FSH | 2 | Low |
| Selenium | 2 | Low |
| Vitamin E | 2 | Low |
| Vitamin E and C | 3 | Moderate |
| Carnitine | 4 | High |
| Zinc | 3 | Moderate |
| Tranilast | 2 | Low |

GRADE: Grading of recommendations, assessment, development and evaluations

# Hormone therapy

- Reproductive tract microenvironment of men: androgen-dependent
- **Tamoxifen** was first introduced as an empirical tx
  - acts on hypothalamic receptor to stimulate gonadotropin release
  - affects Leydig cell function to increase tubular and epididymal 5a-dihydrotestosterone

- Willis et a.: Tamoxifen 10 mg daily for 6 months → fail to improve sperm count
- Comhaire et al.: tamoxifen led to the greatest increase in pregnancy rates in the groups where the pre-treatment pregnancy rate was the lowest
- tamoxifen + Testosterone promote pituitary and Leydig cell activity in men with idiopathic oligozoospermia

- Testosterone undecanoate administration exerts its actions mainly on epididymal function, improving sperm motility, morphology and fertilization capacity.
- FSH has been shown to stimulate spermatogenesis in an animal model.
  - High dose improving disturbed sperm structure
  - ↑ sperm parameters and pregnancy outcome: **Controversial**
  - Testicular volume and DNA condensation ↑
- iOAT → **highly heterogeneous group** → not all pts likely to respond to hormone therapy

# Carnitines*

- water-soluble antioxidant
- mostly derived form diet
- Play a role in **sperm energy metabolism** → providing primary fuel for sperm motility via post-testicular effect
  - Spermatozoa exhibit ↑ L-carnitine & L-acetyl carnitine during epididymal passage (concurrent with acquisition of motility)
  - Carnitines accumulate in the epididymis in both free and acetylated forms

- Carnitines enhance cellular energetics in mitochondria by facilitating the entry and utilization of free fatty acids within the mitochondria and also restore the phospholipid composition of mitochondrial membranes by decreasing fatty acid oxidation.
- Carnitine protects sperm DNA and cell membranes from free radical-induced damage and apoptosis

  → correlated with sperm conc. and motility

  → higher fecundity

- The initiation of sperm motility is thought to occur in the epididymal lumen.
  - The degree of acetylation of carnitine was shown to be greater in motile than in immotile spermatozoa.
  - defective sperm motion parameters → ↓ L-acetyl-carnitine/L-carnitine ratio

- Oral carnitine has a favorable effect on sperm motion parameter in iOAT.
  - Carnitine 2~4g daily for 2~4 months significantly improves sperm motility from baseline level
  - Carnitine's action may differ depending on pretreatment parameters (most significantly improvement in lower baseline motility)
  - No improvement in morphology (post-testicular effect)
  - No further improvement at 3 and 6 months (effects are stable)

# NSAID

- The tubuloseminal plasma of OAT patients has been shown to exhibit elevated levels of prostaglandin.
  - low-dose NSAIDs → improve sperm quality and fertility in an animal model
  - carnitine administration was reported to promote the accumulation of PGE2 in seminal fluid.
  - NSAIDs + carnitines: effective

- a certain level of prostaglandins and oxidative stress is required for physiologic sperm functioning
  - *(in vitro)* higher dosage of NSAIDs may inhibit sperm motility
- Suppository route → more pronounced and direct effect on seminal plasma due to rectal-prostatic lymphatic pathways
- Cinnoxicam 30 mg supp. + oral L-carnitine and acetyl-L-carnitine significantly increased sperm concentration, motility and morphology and pregnancy rates

# Vit E

- a-tocopherol,  lipid-soluble antioxidant
- residing mainly in the cell membranes
- breaking pathological ROS-induced chain reactions and enhancing the activity of various antioxidants
  - Not influencing ROS production
- Used extensively in a variety of diseases
- *(in vitro)* protect spermatozoa from oxidative damage and enhance sperm performance in hamster egg penetration assay
- ↓ MDA conc. to normospermic level

# Vit C

- ascorbic acid, water-soluble ROS scavenger with high potency
- Conc.: 10X higher in seminal plasma then in serum
- Protect spermatozoa against endogenous oxidative DNA damage
  - Seminal plasma ascorbic acid conc. positively correlated with percentage of morphologically normal spermatozoa

- **Vit C+ Vit E**: effective ? ?

# Selenium

- Selenium (Se) is an essential element for normal testicular development, spermatogenesis, and spermatozoa motility and function.
- Se may protect against oxidative DNA damage in human sperm cells: mechanism controversial
  - Selenoenzymes
  - Selenoproteins: at least 25 selenoproteins in human body to maintain sperm structure integrity

- **Se deficiency**
  - important loss of motility
  - breakage at the midpiece level
  - increased incidence of sperm-shape abnormalities, mostly of the sperm head
- **Selenium + Vit E**: improved sperm motility and lipid peroxidation markers

# N-acetyl cysteine

- NAC, derivative of naturally occurring amino acid L-cysteine
- Precursor of glutathione (GSH)
  - ↑ endogenous reducing agent → directly ↓ OS by scavenging free radicals
- ↑ sperm conc. and acrosome reaction; ↓ ROS level; no effect on morphology

- **NAC + Selenium**

# Zinc

- Zinc therapy may reduce iOAT by preventing oxidative stress, apoptosis and sperm DNA fragmentation.
  - seminal plasma zinc conc.
- Zinc therapy yields various benefits.
  - Semen parameters
  - ↓ MDA, TNF, DNA fragmentation
  - ↑ antioxidant capacity, anti-inflammtory cytokine IL-4

- **Zinc + folic acid**: both essential for transfer RNA and DNA synthesis
  - *(in vivo, in vitro)* zinc deficiency alters the absorption and metabolism of dietary folate
  - Zinc sulfate 66 mg + folic acid 5 mg
  - Larger RCTs required before widely use

# Mast cell stabilizers

- Maseki et al., first reported **mastocytosis** in the testes of infertile males → indicating relationship between mast cell proliferation and testicular dysfunction
- Testicular biopsy samples show that mast cell counts are higher in men with idiopathic infertility than in normospermic men.

- The administration of a mast cell blocking agent was reported to have positive effects on the semen parameters of infertile men.

- Mechanism: inhibit the production or release of histamine, lipid mediators and cytokines from inflammatory cells and macrophages
- Tranilast for 1 year: idiopathic azoospermia → appearance of spermatozoa in the seminal fluid
- Yamamoto et al.: tranilast 200 mg daily for 3 months
  - ↑ pregnancy rate in idiopathic severe oligozoospermia
- improvement not sustained after therapy discontinued

# Lycopene

- a naturally synthesized carotenoid found in fruits and vegetables
- an important component of the human redox defense mechanism against free radicals
- Levels tend to be lower in men suffering from infertility.
- Gupta and Kumar: oral lycopene 200 mg twice a day for 3 months → good response in higher baseline conc.
- Further RCTs required

# Pentoxifylline

- a competitive nonselective phosphodiesterase inhibitor that ↑ intracellular cAMP and ↓ inflammation by inhibiting TNF-a and leukotriene synthesis
- reported to decrease ROS production → to preserve sperm motility and improve semen parameters
  - Tesarik et al.: pentoxifylline improved sperm motion characteristics in unselected asthenospermic patients, but not ↑ % of motile spermatozoa
- Oral pentoxifylline
  - no effect at a low dosage
  - at high dosage: to increase sperm motility and some motion parameters without altering sperm fertilizing ability

# Adrenergic antagonists

- No clear pathophysiological basis
- Two placebo-controlled trials demonstrated an improvement in sperm conc. but not of ejaculate volume, morphology, motility or pregnancy rates in the treatment group.

# Conclusions

- ART is not universally available, is expensive and has limited success.
- Pharmacotherapy
  - lower cost and satisfactory effectiveness should be considered as 1st line treatment in iOAT
  - yet to be standardized
  - Seasonal, regional, and racial difference in sperm count and quality make it difficult.
  - Available forms → mostly only marginally satisfactory response

- iOAT is a syndrome having different etiologies and only some subsets will respond to tx.
- Conflicting results and conclusions in published studies arise from differences in methodology of study design and semen analysis, and demographic characteristics of study population.
  - Scoring system to rank quality of evidence
  - Further RCTs required

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GRADE: Grading of recommendations, assessment, development and evaluations

# Thank you !!