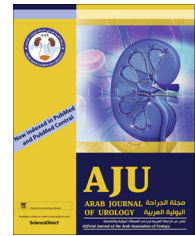




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Laboratory assessment of oxidative stress in semen

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KEYWORDS

Semen, male infertility;
Oxidative stress;
Chemiluminescence;
Total antioxidant
capacity;
Oxidation-reduction
potential

ABBREVIATIONS

ORP, oxidation reduction
potential;
OS, oxidative stress;
ROS, reactive oxygen
species;
TAC, total antioxidant
capacity;
TUNEL, terminal
deoxynucleotidyl
transferase-mediated
dUTP nick-end
labelling

Abstract Objectives: To evaluate different laboratory assessments of oxidative stress (OS) in semen and identify a cost-efficient and highly sensitive instrument capable of providing a comprehensive measure of OS in a clinical setting, as early intervention and an accurate diagnostic test are important because they help maintain a balance of free radicals and antioxidants; otherwise, excessive OS could lead to sperm damage and result in male infertility.

Materials and methods: A systematic literature search was performed through a MedLine database search using the keywords ‘semen’ AND ‘oxygen reduction potential’. We also reviewed the references of retrieved articles to search for other potentially relevant research articles and additional book chapters discussing laboratory assessments for OS, ranging from 1994 to 2017. A total of 29 articles and book chapters involving OS-related laboratory assays were included. We excluded animal studies and articles written in languages other than English.

Results: Direct laboratory techniques include: chemiluminescence, nitro blue tetrazolium, cytochrome C reduction test, fluorescein probe, electron spin resonance and oxidation–reduction potential (ORP). Indirect laboratory techniques include: measurement of Endtz test, lipid peroxidation, chemokines, antioxidants/micronutrients/vitamins, ascorbate, total antioxidant capacity, or DNA damage. Each of these laboratory techniques has its advantages and disadvantages.

Conclusion: Traditional OS laboratory assessments have their limitations. Amongst the prevalent laboratory techniques, ORP is novel and better option as

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it can be easily used in a clinical setting to provide a comprehensive review of OS. However, more studies are needed to evaluate its reproducibility across various laboratory centres.

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Introduction

Oxidative stress (OS) is the result of an imbalance between the formation of reactive oxygen species (ROS) and the inability of the available antioxidants to neutralise the excessive production of ROS. OS results in lipid peroxidation, protein changes and DNA damage and sperm death [1]. OS is one of the most common aetiologies affecting 30–80% of men with infertility [2,3]. Studies have shown that OS is one of the major causes of male infertility, especially for those who have unexplained and idiopathic male infertility [1]. OS can not only affect spermatozoa but also can potentially have consequences on a systematic level such as decreasing the amount of testosterone or LH [4]. Increased OS also directly damages sperm DNA, thus compromising the paternal genomic contribution to the embryo [5]. Due to these characteristics, OS in male infertility can lead to serious health risks if not carefully controlled over time. There are several laboratory tests available to measure OS; however, many of these are either expensive or cumbersome to carry out as routine diagnostic tests. Based on the current literature, there is a greater need to review available tests and identify the need for the development of a novel approach to reliably measure OS.

The present paper will aim to: (i) review traditional laboratory assessments including direct and indirect techniques relevant to OS in male infertility, along with advantages and disadvantages of each technique, (ii) provide detailed description on the most prevalent laboratory techniques currently used in the laboratories, and (iii) provide insight for future directions to see how laboratory assessments for measuring OS could be more effective in the clinical setting.

Relationship between OS and principles of laboratory techniques

The rationale for various laboratory techniques used to measure OS are a result of direct measurement of ROS or indirect measurements through oxidised products of ROS production. Direct laboratory techniques measure OS or free radicals such as ROS and reactive nitrogen species, whereas indirect laboratory techniques measure lipid peroxidation, antioxidants, cofactors, or other end products secondary to ROS production. More specifi-

cally, indirect measurements could be an accumulative result of oxidised products resulting from sources of ROS such as the oxidised form of nicotinamide adenine dinucleotide (NADPH)-oxidase in the sperm, the reduced form of NAD (NADH)-dependent oxidoreductase in mitochondria, or leucocytospermia [6].

Available laboratory techniques

There are a number of laboratory techniques for measuring OS. Traditional OS laboratory techniques include direct and indirect assessment of OS. Direct laboratory techniques include chemiluminescence, nitro blue tetrazolium (NBT), cytochrome C reduction test, fluorescein probe, electron spin resonance and oxidation reduction potential (ORP). Indirect laboratory techniques include measurement of Endtz test, lipid peroxidation, chemokines, antioxidants/ micronutrients/vitamins, ascorbate, total antioxidant capacity (TAC), or DNA damage. Each of these laboratory techniques has its advantages and disadvantages, as shown in [Tables 1 and 2](#).

Limitations of traditional laboratory measurement of OS

More than one study has indicated that there has been misclassification of fertility due to lower reference values set by the WHO fifth edition 2010 guidelines [20]. Other contributing factors could result from limitations of routine semen analysis [8]. Thus, an accurate, cost-efficient, highly sensitive, and comprehensive OS laboratory assessment is crucial for a male infertility routine clinical laboratory test.

Common laboratory techniques available for OS measurement

Although there is no general cure for OS-induced male infertility, preventative actions such as having a cost-efficient and comprehensive laboratory test can be carried out for early intervention. These tests can potentially lower OS related symptoms such as increased lipid peroxidation, DNA fragmentation, and poor antioxidant reserves as observed in varicocele, idiopathic/unexplained infertility and asthenozoospermia. Amongst those laboratory assessments discussed above, some of the prevalent ones include the chemiluminescence assay, TAC assay, and ORP test.

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