Chapter 1
Oxidative Stress and Toxicity in Reproductive Biology and Medicine: Historical Perspectives and Future Horizons in Male Fertility

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Abstract Since the discovery by John MacLeod in 1943 that spermatozoa produce small amounts of hydrogen peroxide, a member of the so-called reactive oxygen species (ROS), the importance and functions of these highly reactive oxygen derivatives in physiology and pathology are a subject of numerous studies. It has been shown that they play essential roles, not only in causing oxidative stress if their concentration is excessively high, but also in triggering crucial cellular functions if their concentration is low. On the other hand, antioxidants counterbalance the action of ROS to maintain a fine balance between oxidation and reduction as an excessive amount of antioxidants leads to a condition called reductive stress and is as harmful as oxidative stress. This book *"Oxidative Stress and Toxicity in Reproductive Biology and Medicine – A Comprehensive Update on Male Infertility"* authoritatively summarizes the current knowledge of various causes of oxidative stress including various andrological conditions and environmental pollution as well as the physiological effects of ROS. Moreover, this book expands into the treatment of oxidative stress with antioxidants and phytomedicine, a rapidly developing area. As a first of its kind, this book also sheds light on the effects of the redox potential during the fertilization process and thus highlights the importance of the correct balance of oxidants and antioxidants, even in the culture medium in assisted reproduction. The editors have brought together an impressive group of renowned

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experts to share their knowledge on the topic of oxidative stress and its clinical management in andrology and assisted reproduction.

Keywords Oxidative stress · Reductive stress · Antioxidants · Sperm functions · Fertilization

1.1 Introduction

It is almost 80 years ago that John MacLeod [45] made the pioneering discovery that the primary role of oxygen in spermatozoa is not for metabolism and motility as they cannot oxidize glucose, lactate, and pyruvate. MacLeod's observation went even further by indicating that high partial pressure of oxygen is not only inhibiting motility but also producing small amounts of hydrogen peroxide (H_2O_2), a highly oxidizing chemical compound that is now counted among the so-called reactive oxygen species (ROS). Since that time, scientists conducted a lot of research to understand not only the impact of extrinsic oxygen and highly reactive oxygen derivatives such as ROS but also cells' own intrinsic ROS production on cellular and organismal physiology and development and also their pathological impact on disease. By now, we have learnt much about the chemistry and biochemistry of oxygen [12] and its highly reactive derivatives with reaction times in the nano- to millisecond range [29], as well as the metabolic role of H_2O_2 in cellular signaling cascades and oxidative stress [53]. It is clear now that for normal physiologic function of cells, including reproduction in general and spermatozoa in specific, ROS at very low concentrations are essential because these molecules do not only trigger essential sperm functions such as capacitation, acrosome reaction, and spermatozoa-oocyte binding [10, 17, 49, 50] but also cause oxidative stress at higher concentrations [11].

Oxidative stress is a concept that was formulated by Helmut Sies in 1985 [52]. Groundbreaking work by John Aitken [9] and Ashok Agarwal [3] lead to the recognition of oxidative stress as major cause of male [2, 51] and female infertility [4, 5, 44] leading to miscarriage or birth defects [56]. Later it became clear that due to the dual effect of ROS on sperm function [18, 19] there must be a very fine balance between ROS essential for proper physiological activity and antioxidant protection from cellular oxidative damage. Hence, not only the ROS but also the antioxidants have to be regulated to counterbalance oxidative stress. Too high concentrations of antioxidants have also been found to be detrimental, as this condition can cause reductive stress [28, 34, 43] and is as harmful as oxidative stress [13]. In light of the recent, rapid developments in both the fields, redox-biology and human reproduction, it is time for a comprehensive update.

Oxidative Stress and Toxicity in Reproductive Biology and Medicine – A Comprehensive Update on Male Infertility provides such an update at the right time

highlighting the important topics. This book covers human fertility, including assisted reproduction, in general, the special emphasis is on male reproduction and provides a unique collection of chapters spanning from the description of various sources of ROS in the reproductive system and their physiological roles in the generation of ROS to their pathological influence on fertility in a variety of diseases. Since not only diseases or aging but also environmental pollution can cause increased oxidative stress, the book also describes a number of environmental toxicants as well as radiation and their effects on fertility. This is followed by a section dedicated to the treatment of oxidative stress with herbal medicine and antioxidants. The topics covered are then rounded off with chapters on the impact of male oxidative stress on recurrent pregnancy loss, the treatment of oxidative stress in embryo culture as well as the impact of reductive stress on male fertility.

The sources of ROS causing oxidative stress in the reproductive system are manifold and include physiological production of ROS in all aerobic living cells. These, small amounts of ROS have been shown to trigger essential physiological functions such as capacitation [17]. On the other hand, excessive production or exposure of cells to excessive amounts of ROS caused by adverse lifestyle [22, 26, 47] and related conditions such as obesity [42], diabetes mellitus [1, 24], or poor nutrition negatively [54, 62] affect fertility in men and women and modification of the lifestyle conditions can have positive effects on fertility [62]. In addition, genital tract infections/inflammations [33, 35], scrotal hyperthermia due to tight underwear [38, 48], sedentary position [25, 55], varicocele [37] or cancer [57] and its treatment with radiation [8, 15] and chemotherapy [20, 36] all have significant negative effects on fertility. On the other hand, infertility is also regarded as a surrogate marker for cancer development [40].

In addition to the aforementioned production of high ROS levels due to medical reasons, in the past century, environmental pollution has become a significant cause of infertility, of which parts of the causes are oxidative stress-driven where the pollution is directly or indirectly triggering high ROS production. The resultant oxidative stress does not only have a direct negative effect on the gametes [21, 60] but also detrimental effects on the testes [32, 46]. Furthermore, the detrimental effects are not only direct but also indirect if pregnant women are exposed to environmental toxicants. Among other problems, this can lead to disturbances in the sex determination.

About 80% of the global population is dependent on traditional and herbal medicine for their primary health care [27] including reproductive issues. This book addresses this globally important issue by including four chapters on the role of herbs and phytomedicine. Many plants are used not only due to their contents of pharmacologically active compounds such as atropine, triterpene saponins, and lactones, or flavonoids and tannins, but also due to the high concentrations of antioxidants such as lycopene and various vitamins. Interestingly, about 25% of modern prescription medicines contain bioactive plant compounds. In Western medicine, still little is known about herbal remedies to treat diseases [14]. Therefore, it is important to bring these aspects of the usage of herbal medicine as primary or supplementary therapy option to the attention of the readers [16].

Since ROS play such important physiological roles in all bodily functions, shedding light on redox imbalances in reproductive events as a whole is important, including embryo development [31, 59]. The oxygen concentration in the embryo surrounding environment is only about 8% in the fallopian tube, about 5% or less in the uterus [58] and is becoming hypoxic or anoxic during early implantation [41]. This situation makes a metabolic shift from oxidative phosphorylation to glycolysis necessary [31, 59]. Hence, keeping the redox balance between oxidants and antioxidants is of utmost importance for normal embryonic development as too high concentrations of either ROS or antioxidants detrimental because essential transcription factors such as Nrf2, NFκB, or AP1 are redox-sensitive and if not properly triggered can interfere in signaling pathways [23, 59] and lead to embryonic death and teratogenesis [30, 61]. Therefore, this book, as the first of its kind in the field of human reproduction, throws more light not only on the pathology of male factors leading to recurrent pregnancy loss but also on the essential functions of the finely balanced redox systems needed for successful fertilization with its implications even in embryo culture systems.

Last, but not least, this book on oxidative stress and its toxicity in reproductive biology also addresses the use of antioxidants, which are not only freely available over the counter but also often prescribed by clinicians to treat oxidative stress-related conditions of infertility. In most cases, this is done without having properly diagnosed the patient for oxidative stress. Oral antioxidants provide an excellent safety and are cost-effective [6, 7, 39]. On the other hand, in respect to their effects, the bivalent actions of both ROS and antioxidants to trigger essential physiological reactions and to cause harm are widely unknown. Whereas for ROS the detrimental effects are generally known, the detrimental effects of antioxidants are either not known or are ignored. However, as ROS have beneficial effects in triggering e.g. sperm capacitation, antioxidants have detrimental effects, particularly if they are available at too high concentrations when they are causing reductive stress [34], a condition which is as harmful as oxidative stress [13].

Since the discovery of ROS and the recognition of oxidative stress as major contributing factor to numerous diseases including infertility, we have learnt a lot about oxidative stress and generally associate negative events with it. Opposed to this, antioxidants are thought to be ‘good’ and ‘healthy’ and neutralize the “bad” effects of oxidative stress. With the increase of knowledge, however, we began to realize that such “black and white painting” is incorrect. It is now clear that ROS are also crucial triggers for essential physiological functions. On the other hand, for antioxidants, we are just at the beginning of understanding that they can also have serious detrimental effects. This includes not only their functions but also the realization of reductive stress as cause of disease and infertility. The lesson that we have to learn is that only a finely regulated balance between oxidation and reduction provides a healthy environment for cellular function. Therefore, it is essential to properly diagnose the redox status and the causes of a patient’s infertility before starting with a treatment which could worsen the fertility status. Hence, a personalized diagnosis with subsequent adjusted treatment is of utmost importance. The problem that we are facing at this stage is that we do not know what is “normal,” not only for male

infertility but also for female infertility. For embryo development, there are even no data available yet.

To come to this point has been a long journey, and with this limited information available for human infertility treatment, this book provides the reader with an authoritative overview of redox stress in human reproduction. The comprehensive knowledge provided in this book will assist not only andrologists and urologists in better comprehension of male infertility but is also a source of information for gynecologists, embryologists, and basic scientists to further their understanding of fertilization as a highly complex process. With all the up-to-date evidence provided, this book will be an invaluable source of information to improve patient management and laboratory procedures.

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