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LETTER TO THE EDITOR

Oxidative stress and the etiology of male infertility

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One of the few insights we have gained into the etiology of male infertility over the past 20 years has been the realization that these cells, and the precious cargos they carry, are extremely vulnerable to oxidative stress [1]. Furthermore, the fact that antioxidant therapy seems to have a beneficial impact on semen quality suggests that such stress does not just correlate with defective sperm function but is causally involved in its genesis [2, 3]. However, one of the fundamental questions that remains is the actual mechanism by which reactive oxygen species (ROS) are generated in cases male infertility.

A recent publication in the Journal of Assisted Reproduction and Genetics by Jiang et al. [4] represents a potentially important contribution to this debate by identifying significant associations between seminal cytokine profile, ROS generation, and the efficiency of sperm chromatin remodeling. However, the interpretation of the data presented by these authors is complicated by a number of factors including the following:

1. The use of chemiluminescence to detect ROS in unfractionated semen is confounded by the presence of leukocytes (particularly neutrophils) that contaminate every human ejaculate. These cells are much more active generators of ROS than spermatozoa, and it has already been established that they make a major contribution to the chemiluminescence signals generated in unfractionated human semen samples [5]. Unfortunately, excluding specimens that are leukocytospermic does not eliminate the confounding influence of leukocyte contamination on the results obtained by Jiang et al. [4]
2. The use of chemiluminescent signal intensity as a threshold for categorizing patients into high and low ROS generators is also problematical. One of the major limitations of chemiluminesescence is that the read-out of this procedure cannot be standardized because the signal generated by any given luminometer is dependent on the individual response characteristics of the system's photomultiplier(s). For this reason, thresholds of normality have to be established for each individual instrument using samples from normal fertile controls. It is not possible to categorize patients using chemiluminesence thresholds established in independent laboratories. This fundamental point has been made previously [6] and yet J Assist Reprod Genet continues to publish data citing reference values for seminal chemiluminescence [7], despite the inherent inaccuracy of this approach.
3. The observed relationship between oxidative stress and the efficiency of chromatin remodeling in the male line is in keeping with previous publications demonstrating that strong relationships exist between the efficiency of chromatin protamination, as measured by chromomycin 3 (CMA3) fluorescence, and both ROS generation and oxidative DNA damage—the authors of this paper cannot claim to be the first to make this link [8, 9].
4. The finding that certain seminal cytokines are positively correlated with ROS generation suggests that inflammation is a key factor in the etiology of male infertility. Since previous studies have found positive correlations between male genital tract infection, ROS generation and seminal

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cytokine levels [10] analysis of male secondary sex gland infection in this cohort of patients would have been extremely informative [10, 11].

Notwithstanding these reservations, the results reported by Jiang et al. [4] raise a number of interesting and exciting possibilities that could form the basis of future research in this area.

Firstly, is it possible that the inflammatory reaction, including the generation of pro-inflammatory cytokines, is an immunological response to the presence of defective spermatozoa? The normal differentiation of these cells could have been perturbed during spermiogenesis leading to the observed impairment in chromatin remodeling. In addition, these defective spermatozoa might have been characterized by the generation of high levels of ROS, as a result of disrupted electron flow through the mitochondrial electron transport chain [12] or the induction of excessive sperm NADPH oxidase activity [13].

Alternatively, defective spermatozoa suffering from impaired chromatin remodeling might have triggered an inflammatory reaction within the male reproductive tract resulting in the generation of ROS and cytokines by infiltrating leukocytes, particularly neutrophils and macrophages. In this context, it is important to recognize that male infertility has previously been associated with low level leukocytic infiltration independent of infection [11, 14].

Clearly, in future studies, it will be important to account for male secondary gland infection in patients whose infertility stems from the induction of oxidative stress and to use flow cytometric methods to determine the precise source of the ROS responsible for impairing the fertilizing potential of the spermatozoa.

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