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REVIEW

Proteomics, oxidative stress and male infertility

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Abstract Oxidative stress has been established as one of the main causes of male infertility and has been implicated in many diseases associated with infertile men. It results from high concentrations of free radicals and suppressed antioxidant potential, which may alter protein expression in seminal plasma and/or spermatozoa. In recent years, proteomic analyses have been performed to characterize the protein profiles of seminal ejaculate from men with different clinical conditions, such as high oxidative stress. The aim of the present review is to summarize current findings on proteomic studies performed in men with high oxidative stress compared with those with physiological concentrations of free radicals, to better understand the aetiology of oxidative stress-induced male infertility. Each of these studies has suggested candidate biomarkers of oxidative stress, among them are DJ-1, PIP, lactotransferrin and peroxiredoxin. Changes in protein concentrations in seminal plasma samples with oxidative stress conditions were related to stress responses and to regulatory pathways, while alterations in sperm proteins were mostly associated to metabolic responses (carbohydrate metabolism) and stress responses. Future studies should include assessment of post-translational modifications in the spermatozoa as well as in seminal plasma proteomes of men diagnosed with idiopathic infertility.

KEYWORDS: biomarkers, male infertility, proteomics, oxidative stress, seminal plasma, spermatozoa

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Introduction

Infertility is defined as the inability to achieve a clinical pregnancy after 12 months or more of regular, unprotected and well-timed intercourse (Practice Committee of American Society for Reproductive Medicine, 2013). Infertility affects around 15% of all couples of reproductive age, with about 50% being associated with abnormalities in the male, called male factor infertility (Sabanegh and Agarwal, 2012). A recent study using the current duration approach to assess the prevalence of infertility estimated that 9 to 14% of American men within reproductive age (i.e. 15 to 44 years old) will probably experience difficulties to conceive (Louis et al., 2013). Male infertility could result from dysfunction at various levels along the hypothalamic-pituitary-gonadal axis: pre-testicular (damage at the hypothalamus or pituitary level), testicular (failure of the testis), post-testicular (normal testicular function but with obstruction or inflammation that leads to infertility) or a combination of these. Causes of male infertility include hypogonadotrophic hypogonadism and Kallmann syndrome, direct trauma, inflammation or infection of the testis, varicocele, cryptorchidism, Y-chromosome microdeletions, testicular cancer and chemotherapy, erectile dysfunction, infrequent or retrograde ejaculation, epididymitis, congenital bilateral absence of the vas deferens, Klinefelter's syndrome (47,XXY), and Sertoli-cell only syndrome (Wiser et al., 2012).

The aetiology of male factor infertility, although multifactorial, remains largely idiopathic (Sabanegh and Agarwal, 2012). Reactive oxygen species (ROS)-induced oxidative stress is well-known to play a major role in male factor infertility (Hamada et al., 2012; Tremellen, 2008). Excess ROS concentrations and oxidative stress in the male reproductive tract are detrimental to spermatozoa (Aziz et al., 2004) and have been associated with negative changes in sperm concentration, motility and morphology, leading to poor semen parameters and eventually to infertility (Khosrowbeygi and Zarghami, 2007). In fact, oxidative stress has been implicated in several male infertility-associated pathologies, including leukocytospermia and varicocele as well as idiopathic infertility (Pasqualotto et al., 2000).

The diagnosis of male infertility routinely begins with a basic semen analysis, which measures various semen parameters including semen volume, colour, pH, liquefaction time, viscosity, sperm count and motility, sperm morphology, concentration of round cells and polymorphonucleocytes, sperm agglutination and sperm viability (if required). Two or more of these basic semen analyses are used to identify abnormalities in: sperm concentration (oligozoospermia or azoospermia), motility (asthenozoospermia) and morphology (teratozoospermia), based on reference values established by the World Health Organization (WHO, 1999, 2010). In addition to the routine evaluation, several advanced tests can be performed to establish the causes(s) of infertility, among them are the assessment of ROS levels, total antioxidant capacity and sperm DNA fragmentation level, DNA compaction and apoptosis, as well as presence and localization of antisperm antibodies and genetic testing (Kovac et al., 2013). However, results of these tests typically either fall within the normal range or do not help determine an exact aetiology of infertility, leading to a classification of 'idiopathic infertility' (Kovac et al., 2013). While assisted reproductive technology (ART) may increase the chances of conception, it does not ensure the genomic integrity of the embryo (Tremellen, 2008). In fact, high ROS concentrations in infertile men have been associated with DNA fragmentation and poor chromatin packing (Tremellen, 2008). Damaged DNA in spermatozoa is indicative of poor cellular health. Sperm DNA damage reduces semen quality and is the cause of infertility in many men (Lewis et al., 2013; Simon et al., 2013). In assisted reproduction, spermatozoa with damaged DNA lower fertilization and pregnancy rates, impair embryo development and quality and increase the risk of spontaneous miscarriage, birth defects and childhood diseases such as cancer (Aitken et al., 2013; Lewis, 2014). The level of DNA damage is suggestive of clinical outcome in assisted reproduction: idiopathically infertile couples with higher levels of sperm DNA fragmentation were found to have lower live-birth rates following IVF (Simon et al., 2013).

Highly specialized techniques such as proteomics allow characterization of the semen profile at a molecular level, proving useful in the assessment of proteins and the understanding of biological pathways that play a key role in male infertility (du Plessis et al., 2011). Advances in this rapidly-evolving field have allowed researchers to better identify seminal plasma and sperm proteins and to determine how their presence or concentration may differ in fertile versus infertile patients (Mitulovic and Mechtler, 2006). Studies looking at the sperm and seminal plasma protein profiles of men with oxidative stress-induced infertility would help in identifying alterations in the protein expression and/or translational modifications that may occur during sperm maturation and functions of proteins involved. Moreover, these studies may be extended to the characterization of other pathologies associated with male infertility at the molecular level.

Despite the established role of oxidative stress in the aetiology of male infertility, there are, as of yet, relatively few studies that have investigated the correlation between ROS-induced oxidative stress and a differential protein expression profile in the human ejaculate using proteomic analysis. Our laboratory has recently published a series of studies on patients diagnosed with primary and secondary infertility and elevated ROS concentrations using proteomic approaches (Hamada et al., 2013; Sharma et al., 2013a, 2013b). Using similar strategies, other laboratories have also studied the proteomic profile of infertile patients with poor semen quality who were also affected with oxidative stress (Herwig et al., 2013; Wang et al., 2009).

Thus, in this review, we aim to summarize and compare the findings of these initial studies that have utilized proteomic analysis to look into the differential expression of proteins in the seminal ejaculate of infertile men with high oxidative stress and fertile men with physiological levels of ROS. In this review, we only included proteomic studies in which the oxidative status of infertile men was measured. Our review begins with an overview of oxidative stress and its impact on male infertility as well as the methodologies and general work flow utilized in proteomic studies, in order to provide some basis to readers less familiar with the field.