Posttranslational modifications of nuclear and nonnuclear proteins in spermatozoa

Summary

The number of couples who need the help of assisted reproductive technology (ART) has increased over the years. Approximately half of the cases are caused by male infertility, which is often diagnosed as idiopathic infertility. Therefore, the search for male fertility markers will improve male infertility diagnosis, thereby facilitating advanced sperm treatment and selection *via* ART. Posttranslational modifications (PTMs) of sperm nuclear and nonnuclear proteins are suitable candidates for such markers. The PTMs of protamines and histones reflect sperm chromatin maturity and its readiness for fertilization, and accordingly, they can predict the outcome of ART. However, the PTMs of nonnuclear proteins, including cytoplasmic, cytoskeletal, and membrane proteins, reflect the ability of sperm to undergo hyperactivation, capacitation, or acrosome reactions, which are processes essential for fertilization. We hypothesize that the PTMs of nuclear and nonnuclear proteins can reflect sperm quality and, thus, serve as a valuable marker in ART. Additionally, we suggest that the *in vitro* addition of hydrogen sulfide into the sperm-manipulating media improves sperm motility and viability *via* persulfidation. We used Western blot detection in combination with protein identification by mass spectrometry to reach our goals. Furthermore, we used immunocytochemistry and flow cytometry to localize and quantify our targets throughout the whole sperm population. Our results show that the dimethylation of histone H3 at lysine K4 (H3K4me2) is a suitable quality marker of sperm chromatin since an association of H3K4me2 and chromatin condensation of human sperm was found. In addition, we demonstrated that environmental pollutants are able to modulate the well-known PTMs of sperm proteins using mice as an experimental model. This study shows an alteration of the sperm-wide acetylome and phosphorylome in mouse spermatozoa, demonstrating that acetylation and phosphorylation of nonnuclear proteins are suitable candidates for revealing the cause of idiopathic infertility. Finally, we address the presence of hydrogen sulfide and persulfidation, hydrogen sulfide-derived PTM, in male reproduction. In contrast to the aforementioned PTMs, hydrogen sulfide provides a unique possibility of sperm persulfidation *via* exogenous hydrogen sulfide donors, leading to an improvement of sperm parameters. Altogether, this work demonstrated the importance of PTMs of nuclear and nonnuclear proteins for spermatozoa functionality and suggested their usage as sperm quality markers in ART. Moreover, PTMs of sperm proteins can be modulated during *in*

vitro manipulation, and we suggest this technique as a method by which to improve *in vitro* conditions for spermatozoa manipulation.

Key words: (ART), H3K4me2, hydrogen sulfide, PTMs, persulfidation, male infertility