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RESEARCH HIGHLIGHT

Altered histone retention and epigenetic modifications in the sperm of infertile men

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It is well know that the nuclei of spermatogenic cells experience one of the most extremely marked chromatin changes known in cells. At the end of spermatogenesis, the histones are removed in many species and the DNA is condensed by the highly positively charged protamines forming highly compact nucleoprotamine complexes.[1–3] In the human mature sperm cell, the nucleoprotamine complexes condense most of the DNA, while 5%–15% of the DNA remains associated to nucleosomes.[4,5] For many years, it was thought that the only function of the sperm cell was to transmit the paternal genomic DNA into the next generation.[1] This idea started to change with the discovery of sex-specific imprinting of genes mediated through DNA methylation differences set during gametogenesis, and epigenetically transmitted to the next generation.[6] But DNA methylation is not the only type of epigenetic information that the sperm cell may deliver to the oocyte. There was already evidence since two decades ago that the distribution of the genes in the protamine- and nucleosome-associated regions in sperm was not random.[4,7,8] More recently, the nucleosome-associated regions have been dissected using nucleases and the resulting fractions analyzed using microarrays or deep genome sequencing demonstrating that the sperm cell delivers to the oocyte a potential wealth of epigenetic information, set by the genome-wide differential distribution of the genes in the histone versus protamine-associated regions in sperm.[9,10] In addition, it was also demonstrated that activating (H3K4me2) and repressive histone modifications (H3K27me3) localized to promoters of genes encoding transcription factors important for embryo development and morphogenesis.[10] The presence of this bivalent (activating and repressing) histone marks is also observed in the embryonic stem cells where it is thought to help poise genes either for activation or repression later in development. The presence of repressive and active histone methylation marks on distinct promoters has been detected both in human and mouse sperm.[10,11] Of relevance, a similar epigenetic profile has also been found in zebrafish, a fish species which does not employ protamines and instead, uses increased amounts of linker histone H1 and depletion of chromatin-decondensing modifications (such as H4K16ac) to accomplish sperm chromatin condensation.[12,13] In zebrafish sperm chromatin, it has been found that there is an apparent historical record of genes activated during spermatogenesis, and that genes important for embryo development are packaged in blocks of multivalent chromatin.[12]

However, an important question remaining to be answered was whether this epigenetic profile of the sperm chromatin could be altered in the sperm cells of infertile patients. It was already known that some infertile patients had anomalies in the protamine content and DNA methylation.[2,3,5,14] But the question remained on whether the sperm-specific poising of genes in the nucleosome-associated regions was also altered. In a recent report, Hammoud *et al.*[15] demonstrated the presence of changes in histone retention and epigenetic modifications at developmental and imprinted gene loci in the sperm of infertile men. The authors analyzed seven patients with reproductive dysfunction, three with poor embryogenesis after *in vitro* fertilization and four infertile patients with abnormal protamination. Genome-wide analysis of the location of histones and histone modifications was then analyzed by isolation and purification of DNA bound to histones and protamines using a combination of high-throughput sequencing on the histone-bound fraction of DNA and hybridization to Agilent arrays on the protamine-bound fraction.[15] DNA methylation of the sperm DNA fractions was also examined using bisulfite sequencing. Of importance, this study demonstrated that five of the seven infertile men had random (non-programmatic) histone retention genome-wide. This situation is clearly in contrast to that present in normal fertile men where histone retention was previously shown to be programmatic or non-random.[15] In addition, this study also found that the location of activating (H3K4me2) and repressive (H3K27me3) histone marks was highly similar to that present in fertile men, but that there was a reduction in developmental transcription factors and certain imprinted genes. They also found an altered DNA methylation status of candidate developmental promoters and imprinted loci. One of the hypotheses to explain the detected incomplete removal of nucleosomes could be the presence of problems with the chromatin remodeling during the histone to protamine exchange.[5,14,15]

The findings could have several clinical implications, but they are at a preliminary stage, since the basic mechanisms and function of the normal sperm epigenome are not yet understood in detail. For example, it is unclear whether the resulting improper non-programmatic histone retention may affect the embryo, since the sperm histones and the protamine are replaced following fertilization and therefore, any sperm chromatin abnormalities could be reset to normal. However, if the physiological genome-wide reprogramming (including DNA methylation) following fertilization requires proper programmatic histone retention, then the altered non-programmatic histone retention may result in abnormal reprogramming of the paternal pronucleus.[15] The clinical implications of the

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moderate altered bivalent histone marks present in some of infertile men (H3K4me2/H3K27me3) are not yet known either, but could have an effect if it is considered that in some imprinting disorders, a moderate reduction of methylated CpGs may result in clinical expression. Therefore, the detected moderate changes in the epigenetic marking in the sperm of infertile men may have a cumulative effect on fecundity or result in increased epigenetic risks to the offspring.[15]

In subsequent studies, it will be interesting to extend the observations to a larger number of patients and also analyze additional types of infertile patients. Another interesting issue will be to determine whether there is heterogeneity in the nucleosome poising and epigenetic marking in the different sperm cells from a single sperm sample. It is known that a normal sperm sample contains a very substantial fraction of morphologically abnormal sperm cells, a small fraction of immature sperm cells and a variable proportion of somatic cells. The proportion of abnormal sperm cells and contaminating cells may be substantially increased in infertile patients. While the sample preparation method uses somatic cell lysis buffer to eliminate the potentially contaminating somatic cells, the contribution of the potentially variable proportion and heterogeneity of abnormal spermatozoa present in infertile patients is unclear. Other interesting related issues to investigate in the future will be to measure other types of epigenetic information potentially delivered by the sperm cell, in addition to the nucleosome poising, DNA methylation and histone modifications. Recent work targeting the sperm nuclear proteome has already identified several chromatin-related proteins such as DNA-binding proteins, histone variants, zinc fingers and transcription factors indicating that we may be considering only a small fraction of all potential epigenetic information delivered by the sperm cell.[16,17] Thus, it will be interesting to look at the genomic distribution and function of these additional sperm chromatin-associated proteins, also potentially relevant for epigenetic marking, proper fertilization and embryo development and of their potential alterations in infertile patients.

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