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A Plausible Remodel of Sperm Chromatin Stability and Assessment for Forensic Casework Applications

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Review

The unique sperm chromatin structure

The spermatozoon is the only type of cell designed to transfer from one individual to another. Its unique purpose is to transport a haploid genome unharmed to the egg cell. Thus, several important adaptations of cell structure and organization must occur for the spermatozoon to survive such a transition. This is accomplished by a specialized sperm chromatin structure which is completely different from that of somatic cells. The packaging of the sperm chromatin protects the DNA from damage during storage and transport to the oocyte, and then enable a rapid and complete unpacking of the intact paternal genome in the ooplasm. It has been postulated by Björndahl et al. [1,2] that zinc has a pivotal role in maintaining the structural stability and in enabling a rapid decondensation at the appropriate time. An alternative model has been proposed by them to challenge the current concepts on sperm chromatin stability. Their observations

(i) That zinc depletion at ejaculation allows a rapid and total sperm chromatin decondensation without the addition of exogenous disulfide cleaving agents and

(ii) That the human sperm chromatin contains one zinc for every protamine for every turn of the DNA helix suggests an alternative zinc dependent model may be plausible.

A remodeling of the sperm chromatin was proposed where zinc bridges rather than disulfide bridges form the basis for sperm chromatin stability. The sperm chromatin structural stability is illustrated in Figure 1 & 2.

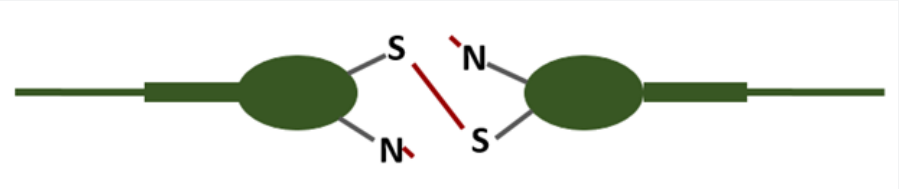

Figure 1: A diagrammatic representation (current concept) of a disulfide bridge-dependent sperm chromatin stability.

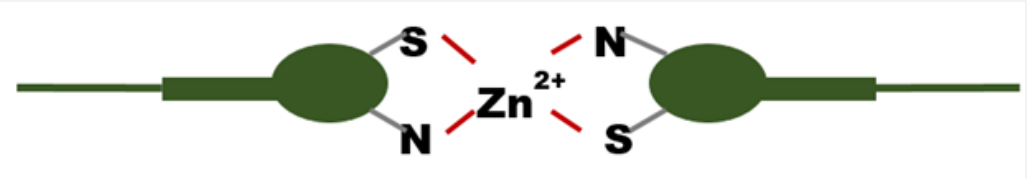

Figure 2: A diagrammatic representation of a zinc-stabilized sperm chromatin stability.

Sperm chromatin structural stability *in vitro*

In vitro, the inherent, zinc-dependent stabilization of the chromatin is rapidly lost and replaced by another type of stability [3]. The chromatin of human spermatozoa can be rapidly decondensed by simple zinc removal with EDTA, which chelates cations like Zn^{2+} [4,5], simultaneously with exposure to the anionic detergent sodium dodecyl sulphate (SDS), which removes membranes and forces a repulsion of chromatin fibers [6]. Both EDTA and SDS are chemicals commonly employed in buffers for DNA extraction and thus, are potentially able to achieve decondensation of sperm chromatin without the need for disulfide breaking agents like dithiothreitol (DTT).

It has also to be acknowledged that the zinc content of the sperm ejaculate is affected by the prostatic fluid and the seminal vesicular fluid present in the seminal plasma [2,7]. The prostatic fluid has a high biological availability of Zn^{2+} that is likely to prevent a loss of chromatin zinc whereas the seminal vesicular fluid contains citrate and zinc ligands which have a high affinity for zinc, resulting in less free zinc. Thus, Björndahl et al. [1,2] have concluded that at ejaculation or in vitro, the human sperm chromatin has a zinc-dependent chromatin stability.

Biological outcomes for sperm chromatin *in vitro*

Extraction of zinc e.g. experimentally by EDTA or due to laboratory routines makes possible two biologically totally different outcomes. There would be immediate decondensation if repulsive forces separate the chromatin fibers (repulsion e.g. experimentally by SDS). Removal of zinc without repulsion of chromatin fibers would allow freed thiols which remain very close to each other to become oxidized into disulfide bridges creating a super stabilized chromatin [1].

The impact of change to the zinc-dependent human sperm chromatin *in vitro* would have to be considered for DNA typing of forensically relevant semen stains. The two biological outcomes described above for sperm chromatin *in vitro* could result in sperm heads with decondensed chromatin or intact sperm heads with super stabilized chromatin.

Forensic relevance of sperm chromatin changes *in vitro*

Depending on the availability of zinc, semen stains collected for forensic investigation potentially, could contain some amounts of sperm heads with decondensed chromatin. There is further loss of zinc during the laboratory routines using EDTA and SDS-based extraction buffers causing more sperm heads to decondense.

The differential extraction procedure is a process employed for semen stains in the forensic laboratory by which the DNA from epithelial cells and sperm cells can be separated into different fractions, namely the non-sperm and sperm extracts respectively. The sperm fraction separation utilizes disulfide breaking agents like dithiothreitol to decondense the disulfide bridges of the sperm chromatin.

However, DNA from decondensed sperm heads can be extracted without the addition of disulfide breaking agents like dithiothreitol. In the conventional differential extraction technique for semen stains, this implies that DNA from sperm heads can be extracted into the non-sperm extract and consequently its cell origin be erroneously inferred.

Differential extraction of sperm mixtures

A study was carried out to determine the separation patterns of sperm mixtures. Semen from multiple different sources were dried on cloth and FTA cards to mimic casework stains. Differential extraction was carried out on imbalanced mixtures of sperm with an excess of DNA from one source, purportedly the major contributor. The differential profiles were analyzed to determine the separation patterns. Control experiments were also carried out with major-minor mixtures of blood and buccal cells collected and dried in similar manner. As reported by Seah et al. [8,9], the experiments demonstrated the DNA of the major sperm contributor persisted in the sperm extract while the DNA of the minor contributor is commonly detected only in the non-sperm extract. There appears to be preferential extraction of the minor sperm contributor in the non-sperm extract. A direct interpretation by the classical disulfide bridge model would infer the DNA of the minor sperm contributor in the non-sperm extract to be from non-sperm origin. The observed disruptive change in the differential extraction behavior for sperm DNA cannot be adequately explained by the currently accepted disulfide bridge-dependent model (Figure 1). The alternative model proposed by Björndahl and Kvist of a zinc bridge-dependent chromatin stability provided a more plausible explanation.

With the blood and buccal mixtures [8], mixed profiles were observed in both non-sperm and sperm extracts. The major contributor predominates in the non-sperm extracts but the sperm extracts exhibited drop-outs due to loss of DNA. Clearly, the separation patterns observed for sperm mixtures are peculiar only to semen and its unique sperm chromatin stability.

Extended studies were carried out where the major-minor sperm mixtures were differentially extracted simultaneously using normal EDTA-containing extraction buffer and EDTA-free extraction buffer. The differential profiles for both the non-sperm and sperm extracts were unaffected by the type of extraction buffer used. In the light of the observations from this extended study, the extraction process appears to introduce no alteration in the sperm chromatin stability for dried semen.

Varying sperm chromatin decondensation for major-minor sperm mixtures

In the context of the major-minor sperm mixtures, it is postulated the extent of sperm chromatin decondensation is greater when sperm cells are sparse, promoting repulsion of chromatin fibers for the minor sperm contributor. By comparison, for the major sperm contributor, the high density of sperm cells remains very close to each other to become oxidized into disulfide bridges creating a super stabilized chromatin, thus requiring disulfide breaking agents like dithiothreitol for decondensation.

Forensic importance of a remodel of sperm chromatin

There is necessity for a remodel of sperm chromatin stability for forensic casework applications. In the investigation of complex sexual assault cases like in a gang-rape situation, there are potentially multiple perpetrators and invariably, semen stains on clothing or bedding items could consist of sperm mixtures. The elucidation of the perpetrator(s) requires a correct perspective of the sperm chromatin change for the semen stains.

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