

A Remodel of Sperm Chromatin Stability Corrects an Interpretation Fallacy



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The Unique Sperm Chromatin Structure

The unique purpose of the spermatozoon is to transport a haploid genome unharmed to the egg and it is the only type of cell designed to survive such a transition. This has led to a chromatin structure that is completely different from that of somatic cells. The sperm chromatin is developed with compact sperm-specific packaging for the safe-keeping of the DNA and at the same time have the property to rapidly make the DNA available to the ooplasm. A model was proposed by Björndahl and Kvist [1], to challenge the current concepts on sperm chromatin stability where zinc bridges rather than disulfide bridges can form the basis for this dual biological property.

Differential Extraction and the Sperm Chromatin

Differential extraction of semen stains is intended to separate epithelial DNA from sperm DNA into the non-sperm and sperm extracts respectively. However, the differential profiles of stains containing entirely of sperm mixtures (as usually encountered in gang-rape) revealed sperm DNA even in the non-sperm extracts [2]. The observation that classical differential extraction of sperm DNA mixtures can extract sperm DNA without the addition of disulfide cleaving agent like dithiothreitol debunk the current concept of stabilization of sperm chromatin by disulfide bridges [3], which forms the basis of separation of non-sperm and sperm DNA by differential extraction [4]. The observed disruptive change in differential extraction behavior for sperm DNA which has been inadequately explained by the current disulfide bridge-dependent model (Figure 1), becomes potentially an issue for challenge in court. Of particular importance is the impact on interpretation of sperm mixtures and the associated inference of cell-origin.

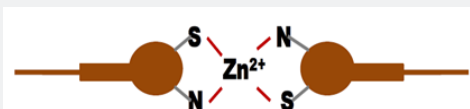


Figure 1: A diagrammatic representation of a zinc-stabilized sperm chromatin stability.

A remodeling of the sperm chromatin structure [1,5,6], proposed a zinc dependent alternative model stabilized by formation of zinc bridges connecting protamine thiols of cysteine and possibly ingroups of histidine (Figure 2), which thus prevents the formation of disulfide bridges in a single mechanism. In the investigation of cases of sexual assault, semen stains on vaginal swabs, clothing and bedding items provide the most incriminating evidence. In a gang-rape scenario, the elucidation of the perpetrators becomes more complex due to the presence of multiple contributors in the semen stains. [7]. Semen stains on bedding or clothing items could invariably consist entirely of sperm DNA from multiple sources. When differential extraction is performed on these stains, the DNA in both epithelial and sperm fractions would logically be of sperm origin.

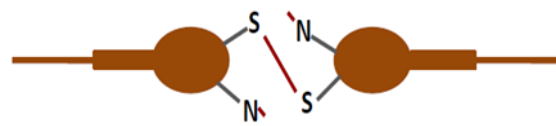


Figure 2: A diagrammatic representation (current concept) of a disulfide bridge-dependent sperm chromatin stability.

The Fallacy and Remodeling Sperm Chromatin Stability

Differential extraction experiments with controlled amounts of major-minor sperm mixtures (two-source and three-source mixtures) from voluntary donors on cloth and FTA cards demonstrated the DNA of the major sperm contributor persisted in the sperm extract and the DNA of the minor sperm contributor(s) is usually detected only in the non-sperm extract [2]. There appears to be preferential extraction of the minor sperm contributor in the non-sperm extract. A direct interpretation by the classical disulfide bridge chromatin model would infer the DNA of the minor sperm contributor in the non-

sperm extract to be from non-sperm cell origin. The observed disruptive change in differential extraction behavior for sperm DNA cannot be adequately explained by the currently accepted disulfide bridge-dependent model (Figure 1). This current concept on sperm chromatin stability has been challenged by Björndahl and Kvist [1], who proposed an alternative model of a zinc dependent chromatin stability with formation of zinc bridges between protamine thiols and potentially imidazole groups of histidine.

The zinc-dependent chromatin stability is rapidly lost during the DNA extraction process for the epithelial fraction where the use of surfactants like SDS (sodium dodecyl sulphate) and EDTA in the extraction buffer provides an effective zinc chelating medium. The loss of zinc causes decondensation of the sperm chromatin [8], and subsequently, rupture of the sperm heads and release of sperm DNA into the non-sperm or epithelial extract. As observed in the study for major-minor sperm mixtures [2], the minor sperm fraction is apparently seen to have a weaker zinc-dependent chromatin stability compared to the more abundant major sperm fraction which arguably would possess a relatively tighter packing of the sperm DNA chromatin fibers. The lesser resilience of the minor sperm source allows easier chromatin decondensation and subsequent release into the non-sperm extract fraction.

Correcting the Fallacy

Interpretation of sperm mixtures commonly encountered in gang-rape thus requires a careful consideration of the separation profiles derived from differential extraction. The detection of the minor sperm contributor in the non-sperm extract impacts on inferring cell-origin of DNA in the non-sperm extract and becomes potentially an issue in court. Correcting

this fallacy requires an assessment of the terms of reference for the two fractions (non-sperm and sperm extracts) in differential extraction with consideration of a plausible zinc bridge chromatin stability. There is a need to revise the current concept on sperm chromatin stability particularly with reference to the implications on forensic casework applications. The zinc-dependent bridge chromatin structure proposed by Björndahl and Kvist has provided a plausible sperm chromatin stability model.

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