Title: Evaluation of fertility potential by Toluidine blue test and the sperm chromatin structure assay

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Objective: Sperm chromatin integrity is important for mammalian fertilization. The sperm chromatin structure assay (SCSA) using flow cytometry is frequently used for the assessment of fragmented sperm DNA. On the other hand, Toluidine blue (TB) assay is a simple alternate test for sperm chromatin assessment. The objective of our study was to evaluate the Toluidine blue test and SCSA for the assessment of sperm chromatin damage in proven and unproven donors.

Design: Prospective-controlled study.

Materials and Methods: Semen samples were collected from 10 unproven donors and 8 proven fertile donors who had initiated a successful pregnancy in the last 2 years. Following liquefaction, seminal ejaculates were evaluated for chromatin abnormalities using Toluidine blue test. In this assay, spermatozoa with abnormal chromatin conformation/ DNA integrity) stain dark violet and those with normal chromatin stain light blue. An aliquot was also examined by the SCSA assay for percentage of spermatozoa with immature nuclear development (high DNA stainability index, %HDS).

Results: Proven fertile males showed lower but non significant incidence of spermatozoa with abnormal DNA compared to unproven fertile men by Toluidine blue method (mean ±SE; 20.9 ±4.4 vs. 27.2 ±4.9; P = 0.09). Similarly, the proven fertile men showed higher incidence of spermatozoa with normal DNA compared to the samples from unproven donor (65.9 ±5.2 vs. 52.7 ±6.3; P = 0.4). In unproven fertile men, %HDS showed a significant positive correlation with the number of spermatozoa staining dark violet (r = 0.85, P = 0.002). %HDS was also negatively correlated with the number of light blue sperm (r = -0.79, P = 0.002).

Conclusions: Assessment of sperm immaturity is important in the evaluation of fertility potential. While SCSA and Toluidine blue are equally effective tests for sperm immaturity measurement in fertile men, Toluidine blue has an added advantage in identifying nuclear immaturity as well as abnormal chromatin in unproven donors.

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