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Programme & Abstracts

44-PL3. Image spectral cytometry for sperm chromatin testing: new approaches

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Importance of sperm DNA integrity for predicting of fertilization outcome, both *in vivo* and *in vitro* has been proved in many studies, and assays for its determination are recommended for the infertility workup. However, sperm chromatin maturity and stability may also be an important parameter of sperm quality, probably no less than DNA integrity, but no easily applicable test for estimation of sperm chromatin packaging exists so far.

Recently, we have elaborated the cationic dye Toluidine Blue (TB) test (at pH 3.5) as an alternative to existing methods for sperm DNA integrity evaluation. In this study, we developed two new methodical approaches for evaluation of sperm chromatin stability and structure using modification of this TB test.

The first approach, based on pH shift, has been tried on the group of 58 patients, comparing staining results at pH 4.5 with that at pH 3.5. In this way, ionic binding between DNA and sperm chromatin proteins can be evaluated. We have found that all sperm samples can be divided into two groups - those with stable and unstable chromatin. In the first case, no increase in the number of TB darkly stained sperm cells - is caused by elevation of TB pH, while this was the case for the second group. Sperm chromatin stability was weakly associated with background DNA integrity status. We also found no significant correlation between sperm chromatin stability and standard sperm parameters with the only exception for motility in samples with low level of DNA damage. Therefore sperm chromatin stability, detected by TB pH shift assay, seems to possess its own value and should be further estimated for its prognostic power in fertilization outcome - both *in vivo* and *in vitro*.

Second approach is based on the data obtained earlier by Erenpreisa et al. (1992) on somatic cells stained by TB, which showed that impairment of DNA integrity (in early apoptosis) causes increase of TB absorption in the blue-green (metachromatic) part of spectrum, while additional disorder of the chromatin packaging (in late apoptosis), induces additional increase of absorption in the red (batochromic) part of spectrum. Therefore we used the ratio of red/green optical density of TB-test (pH 3.5) stained sperm cell nuclei in order to discriminate of the cells with disordered chromatin structure. 120 sperm samples from infertile and fertile patients have been analysed in this way. The obtained data expressed as the 3 grade polynom and its mathematical analysis allow to discriminate the proportion of these cells. This particular group should be further studied as an object of clinical interest.