

# Impact of Sperm DNA Integrity on In Vitro Cycles

Dennis Marchesi, MS; Wanshu Di, BA; Avner Hershlag, MD

## Abstract

As more people turn to assisted reproduction techniques, there is a greater need for a better predictor of outcomes. Semen analysis testing remains the gold standard; however, results are inconsistently correlated with sperm's fertilization capacity. DNA fragmentation rates have been proposed as another measure of sperm quality and good correlations to IVF/ICSI outcomes have been shown. In this study, the toluidine blue assay was used as a measure for sperm DNA fragmentation, in an attempt to assess the predictive value of fragmentation rates with respect to IVF/ICSI success, including oocyte fertilization, embryo quality and pregnancy. This will be useful clinically to help determine the best treatments for patients.

## Background

The popularity of assisted reproductive techniques has increased in recent years, and so the need for better outcome predictors has become relevant. Semen analysis is currently the gold standard for evaluating the sub-fertile male. However, the insights offered by the analysis are limited and cannot provide the full scope of the fertilization capacity of sperm. In vitro fertilization (IVF) success rates have shown a direct association between poor semen parameters and fertilization outcomes. Thus, there is a call for better assessment of sperm quality so that better care and increased success can be provided to the IVF patients. Similarly, a better assessment of sperm quality can help predict the optimal insemination method, thus increasing the success rates of intra cytoplasmic sperm injections (ICSI) and conventional IVF.

DNA fragmentation rates have been proposed as a possible measure of sperm quality. Tests that measure fragmentation include sperm chromatin structure assay (SCSA) (Everson et al, 1980), terminal dUTP nick-end labeling assay (TUNEL) (Gavrieli et al, 1992), and toluidine blue (TB) test (Mello, 1982). The TB test has been proposed as a cheaper and more practical method of measuring sperm chromatin integrity.

Recent papers have found a positive correlation between high DNA fragmentation and poor IVF/ICSI results, including fertilization, cleavage and implantation rates (Simon et al, 2010; Speyer et al, 2010). These reports used various methods in determining fragmentation rates such as SCSA (Speyer et al, 2010), Comet assay (Simon et al, 2010), and TUNEL assay (Sharma et al, 2010). However, TB test has not been used to correlate between sperm DNA fragmentation and IVF/ICSI outcomes. This study intends to use the TB test to predict IVF success rates, including oocyte fertilization, embryo quality and pregnancy.

## Materials & Methods

### *Semen Specimen*

- Samples are collected in sterile specimen cups from all males
- Samples are analyzed following liquefaction 30-60 minutes following ejaculation
- Roughly 400 participant samples will be analyzed over a one year period
- Minimal aliquot (approximately 50 uL) of sample will be removed for study (typical specimen volume is 2-6 mL (2,000-6,000 uL) during routine IVF screening approximately 4-6 weeks prior to oocyte retrieval

### *Toluidine Blue Stain*

- Isolated specimen are smeared on three separate microscope slides and air dried
- Slides were fixed in methanol at 4 degrees C for 30 minutes
- Slides were then dipped 5 times in 0.1% nigrosin background for 1 second per each dip
- Slides were dried on a slide warmer for 15 minutes, then hydrolyzed in 0.1 N HCl at 4 degrees C for 5 minutes
- Slides were then rinsed 3x in distilled water for 2 minutes each
- The smears were stained with 0.05% TB for 15 minutes (stain consists of 50% citrate phosphate (1:9 buffer), pH3.5)
- Slides were rinsed briefly in distilled water and lightly blotted with filter paper
- Lastly, slides were dehydrated twice with tertiary butanol at 37 degrees C for 3 minutes each time
- One of the slides will be mounted in DPX overnight, the other will be read without coverslip

### *Scoring Results*

- Slides are scored under light microscope for both morphology and TB staining
- At least 200 sperms from each slide were scored
- Sperm cell heads with good chromatin integrity will appear light blue (orthochromatically dyed, see arrowhead labeled A in Figure 1.)
- Sperm cell heads with diminished integrity will appear deep violet (metachromatic, see arrowhead labeled B in Figure 1.)

### *Controls*

- Positive controls were created by treating random samples with dithiothreitol (DTT), creating nuclear DNA with reduced disulfide cross-linking (Andreetta et al, 1995) as well as DNase I to induce double-stranded breaks in DNA (Erenpreisa et al, 2003); both DTT and DNase I will cause metachromatic binding of dye to yield darkly stained heads
- Negative controls will be treated with PBS along with DNase I; PBS will not grant access of DNase to the genome

### *IVF correlations*

- IVF/ICSI patients will be treated with standard IVF stimulation
- Following oocyte retrieval, patients were divided into three groups: conventional IVF, IVF/ICSI-split, and ICSI
- In all cases where the IVF screening was normal (WHO, 2010), the eggs were split between conventional IVF and ICSI to prevent fertilization failure
- In cases of an abnormal screening, ICSI was the preferred fertilization technique
- In addition to TB staining results, data for IVF/ICSI cases included fertilization rates, embryo quality (cell number and grading), as well as clinical and viable pregnancies
- Sperm DNA integrity (DNA fragmentation) expressed as TB score will be correlated to all other fertility variables

### Tentative Hypothesis

In the current study, we hope to find DNA fragmentation, as measured by toluidine blue assay, to be predictive of fertilization outcomes.

### Current status

The study remains open to enrollment

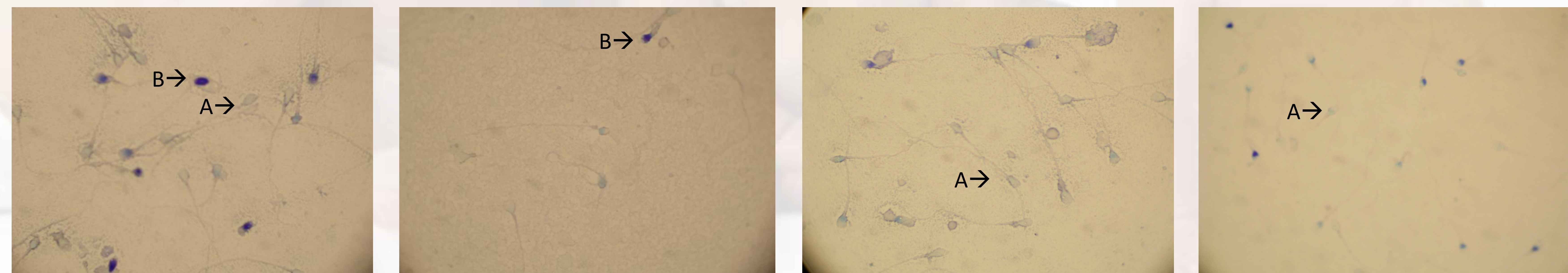


Figure 1. Panel of light microscopy views of toluidine blue stained sperm. (A) arrowheads indicate sperm with good chromatin integrity; (B) arrowheads indicate sperm with diminished integrity.