Staining Protocol for Spermatids and Sperm Cells using Anti-protamine Antibodies

[Adapted from: Aoki, VW, Emery, BR, Liu, L, Carrell, DT. Protamine levels vary between individual sperm cells of infertile human males and correlate with viability and DNA integrity. J Androl 27: 890 (2006).]

1. The semen sample (or suspension of isolated spermatids) is washed in Phosphate Buffered Saline (PBS), pH 7.4, and the sperm/spermatids are pelleted by centrifugation.
2. The pellets are resuspended in a small volume of PBS and a drop is smeared onto a pre-cleaned glass slide and examined to evaluate the density of sperm/spermatids spread across the slide. The suspension may need to be diluted to yield smears with appropriate sperm/spermatid densities on the slide.
3. The slide is allowed to air dry at ambient temperature for a minimum of 1 hour.
4. To ensure antibody accessibility to the nuclear proteins, the sperm/spermatid nuclei are decondensed slightly by incubating the slides in 10 mM DTT, 0.1M Tris buffer pH 8, followed by a 2-hour incubation in a solution containing 10 mM lithium diiodo-salicylate (LIS) and 1 mM DTT in 0.1 M Tris buffer.
5. The slide containing the sperm/spermatid cells is then fixed in 4% paraformaldehyde in PBS, pH 7.4 for 1 hour.
6. Following fixation, the slide is rinsed in PBS and the cells are permeabilized by treatment with 2% Triton X-100 and 0.1% bovine serum albumin in PBS for 15 minutes.
7. The slide is incubated overnight with a 1/500 dilution of the primary antibody (Hup1M, Hup1N or Hup2B).
8. The slide is then washed in PBS containing 2% Triton X-100 (15 min)
9. The slide is subsequently incubated with a secondary antibody (e.g. goat antimouse antibody diluted 1/1000) tagged with a fluorochrome or other tag at ambient temperature overnight.
10. If the secondary antibody is biotinylated, an additional step is required to treat the slide with the desired dye-tagged form of streptavidin as per instructions from the manufacturer.

As a final step, the slide is rinsed to remove unbound secondary antibody and then viewed under the microscope.