Semen evaluation via traditional light microscopy provides useful information about sperm motility and basic morphology but has a limited ability to predict reproductive performance or fertilization ability in an assisted reproduction setting. In-depth analysis carried out quickly and with repeatable precision on a large number of sperm cells is of paramount importance to human assisted reproductive therapies (ART) and farm animal biotechnology. As a result, flow cytometric evaluation using biomarkers to detect specific spermatozoon characteristics is growing in popularity in both andrology laboratories and agricultural studs. Here we investigate the application of two biomarkers, the sperm chromatin structure assay (SCSA) and the post-acrosomal ww-domain binding protein (PAWP) to human assisted reproductive therapy and an agricultural bull stud, respectively. A retrospective analysis of the result of SCSA, infertility treatment outcomes, and the occurrence of spontaneous abortions (SAB) and multiple births was conducted in 233 couples attending an infertility clinic. A statistically significant correlation was found between SCSA parameters and SAB, and lower levels of chromatin defects were found in men from couples with triplet pregnancies. In a second trial, semen samples from 162 sires in artificial insemination (AI) service were analyzed using flow cytometry after labeling with an anti-PAWP antibody. Measurements yielded statistically significant correlations between PAWP intensities and several semen quality/fertility parameters used by the AI industry. Based on these two studies, we conclude that biomarkers such as SCSA and PAWP are useful for the evaluation of spermatozoa and may be predictive of the outcome of assisted reproduction.