In spite of advances in assisted reproduction, the survival and fertilizing ability of thawed spermatozoa is poor. Sperm processing is a fundamental step in assisted reproduction. The purpose of this study was to evaluate if sperm processing by swim-up technique before freezing reduced post-thaw membrane damage and spontaneous acrosome reaction. Samples from 15 normal donors were divided into two aliquots. The first aliquot was frozen without any treatment (unprocessed), and the second after processing by the swim-up method. The samples were evaluated for sperm motion characteristics with a computer-assisted semen analyzer. Viability and membrane integrity were assessed with Hoechst-33258 staining and the hypoosmotic swelling (HOS) test. Acrosomal status was evaluated by fluorescent isothiocyanate-conjugated peanut agglutinin assay before freezing and after thawing. Compared with the unprocessed samples, samples processed by the swim-up method had better motion characteristics, viability, and membrane integrity before freezing (P<0.005). After thawing, the above measures decreased significantly (P<0.005) in both types of samples. Spontaneous acrosome reaction was significantly increased in viable spermatozoa in both types of samples (P<0.005) after thawing. Although the swim-up technique allows selection of a highly motile, viable, and acrosome-intact sperm population in fresh specimen, this improvement is not seen after cryopreservation. Acrosome damage in viable cells after cryopreservation is independent of prior treatment. The decrease in sperm motion and functional parameters after cryopreservation can explain the poor fertilizing ability of human spermatozoa especially in samples with poor pre-freeze semen characteristics as in oligozoospermic and cancer patients.