Human semen samples may show sample to sample (inter-sample) variability of sperm parameters due to multi-factorial nature of sperm production and transport through male reproductive tract. We reviewed charts of 109 men referred to the Sperm Bank at Cleveland Clinic Foundation. We compared inter-sample variability of pre-freeze and post-thaw (24 hours after freezing) sperm motility and motion kinetics [average path velocity (VAP), straight-line velocity (VSL), curvilinear velocity (VCL), linearity (LIN) & amplitude of lateral head displacement (ALH)] of cryopreserved semen collected at 2-3 days intervals. Patients were classified into 5 groups: infertile men (n =19), testicular cancer (n =22), Hodgkin’s disease (n =15), lymphoma (n =18), and other cancers (n =19). Semen samples collected from 16 fertile men, prior to vasectomy, served as controls. Two to 4 samples were collected from each individual. The post-thaw variability of sperm motility, VSL, VAP, LIN & ALH were significantly higher than pre-freeze variability (P<0.0001, 0.02, 0.006, 0.009 & 0.0001; respectively). The increase in post-thaw variability was equally observed in fertile and infertile men, and in men with different testicular pathologies. Post-thaw variability was not correlated with individual's age, length of disease, history of smoking, or alcohol intake. In conclusion, semen samples collected for cryopreservation may show a high degree of post-thaw variability of sperm motility & motion kinetics despite low degree of pre-freeze variability. Further research is required to explain the exact mechanism(s) of such phenomenon, which may help select ideal samples for cryopreservation.