Effects of TEST-Yolk Buffer and Glycerol Cryopreservation on Human Spermatozoa Morphology and Function, J. Hallak, R.S. Sidhu, A.J. Thomas Jr., and A. Agarwal; Andrology Research and Clinical Laboratories, Dept. of Urology, Cleveland Clinic Foundation, Cleveland, OH.

Objective: Cryoprotective medium is important to the survival of human sperm during long-term freezing in liquid nitrogen. Glycerol and Test-yolk buffer (TYB) are two commonly used cryopreservative media. We compared the effects of these two media to determine which better preserves sperm function and morphology.

Design: A prospective clinical study.

Materials and Methods: Semen was obtained by masturbation from 10 healthy donors after 2 to 3 days of sexual abstinence. After liquefaction, each sample was divided in two aliquots. Each aliquot was cryopreserved by either mixing glycerol only (6% v/v with the ejaculate) or TYB, which contains glycerol and other buffers (1:1 with semen, final glycerol content 6% v/v). Prefreeze and post-thaw total sperm count, percent motility, morphology (Kruger's strict criteria and WHO method) and the hypo-osmotic swelling, bovine cervical mucus penetration, and viability tests were evaluated. Motility was analyzed at 0, 60, 120, and 180 min after thawing and washing with human tubal fluid medium.

Results: Percent motility decreased significantly in postthaw samples compared to prefreeze values in both media. Postthaw motility was greater in TYB specimens compared to glycerol (17.2 ± 18.7; 10.2 ± 15.5; P < 0.004). TYB aliquots also had higher motility for up to 180 min compared to glycerol aliquots (P = 0.05). Percent viability was significantly greater with TYB than glycerol (8.7 ± 8.6; 5.7 ± 5.9; P < 0.02). Sperm morphology scored by Kruger's and WHO criteria declined significantly in post-thaw specimens frozen with either medium (P < 0.003). Comparing prefreeze and postthaw morphology by WHO method showed TYB better preserved morphology (P < 0.005). The postthaw bovine cervical mucus test value was significantly lower in glycerol preserved specimens (P < 0.005). Postthaw hypo-osmotic swelling did not significantly differ from prefreeze results nor between the two media.

Conclusions: Sperm motility decreased less over time in specimens cryopreserved in TYB compared to glycerol. The TYB specimens had significantly better functional and morphological characteristics compared to the glycerol-only specimens. This may be due to the addition of egg yolk and zwitter ion buffers to glycerol in the TYB. We, recommend using Test-yolk buffer for long-term storage of human spermatozoa.