COMPARISON OF MONOPERCOLL SPERM SEPARATION WITH TWO-LAYER PERCOLL METHOD. K. Seifarth*, D. Garlak*, L. Cordek*, C. Fitzhugh*, A.J. Thomas, Jr. and A. Agarwal, Andrology Research & Clinical Laboratories, Department of Urology, Cleveland Clinic Foundation, Cleveland, OH 44195

Previous studies have shown that sperm recovery by MonoPercoll procedure is significantly better than the swim-up technique for male factor patients and in patients with normal sperm characteristics. The cost and labor of conducting a two-layer Percoll procedure vs single layer sperm washing procedure are significantly more. Therefore, the purpose of this study was to compare the effect of MonoPercoll procedure and the two-layer Percoll on sperm characteristics of normozoospermic men. Semen specimens from 10 normal donors were allowed to liquefy prior to processing. Semen specimens were divided into two equal aliquots. One aliquot was processed by layering 1 mL of 80% Percoll (MonoPercoll), and the other on a two-layer Percoll (Perwash) gradient (lower phase 90% and 47% upper phase). Both aliquots were centrifuged at 1600 rpm for 20 min at room temperature. The supernatant was removed and pellet resuspended in 2 mL of sperm washing medium (human tubal fluid, HTF) and centrifuged for 7 min at 1600 rpm. The final sperm pellet was resuspended in 1 mL of HTF. Routine computer-assisted semen analysis and sperm function tests were done to examine the following parameters: total motile sperm, percentage recovery of motile cells, motility (MOT), curvilinear velocity (VCL), linearity (LIN), amplitude of lateral head displacement (ALH), sperm motility after 60 minutes of separation, hypo-osmotic swelling (HOS) test, bovine cervical mucus penetration test, percentage viability by eosin-nigrosin staining test and sperm morphology differentiation by WHO and Kruger’s method. MOT, LIN, ALH, percentage recovery, HOS and viability were significantly better in specimens processed by Perwash than MonoPercoll (P <0.05). Results of sperm morphology by WHO and Kruger’s criteria were similar between the two methods. In summary, discontinuous two-layer Percoll procedure provides significantly better semen characteristics than MonoPercoll sperm separation technique in normozoospermic men.