EFFECT OF PRE-FREEZE PERCOLL WASH AND ARTIFICIAL MOTILITY STIMULATION ON CRYOPRESERVED HUMAN SPERMATOZOA. R. K. Sharma, S. Kohn*, A. J. Thomas Jr. and A. Agarwal, Andrology Research & Clinical Laboratories, Department of Urology, Cleveland Clinic Foundation, Cleveland, OH 44195.

Sperm preparation methods and artificial stimulants improve sperm motility and other motion parameters in fresh ejaculate. Cryopreservation results in poor sperm motility, which correlates with poor fertilization rates. Sperm motility must be maintained for a minimum period for intrauterine insemination and other assisted reproductive procedures. The present study compares the data for improvement in sperm motion parameters after sperm preparation alone and with artificial stimulation. Semen samples from normal healthy volunteers were divided in two groups. Group I samples were frozen without further treatment. Samples in group II was processed by density-gradient separation. The freezing medium was TES:Yolk-buffer and immersed in liquid nitrogen (-196°C) before use. Sperm motility and sperm kinematics (curvilinear velocity [VCL], straight line velocity [VSL], average path velocity [VAP], amplitude of lateral head displacement [ALH], and linearity [LIN]) were analyzed on a motion analyzer. Sperm viability was evaluated by eosin-nigrosin staining. Membrane integrity was determined with the hypoosmotic swelling test. After thawing, samples from both groups were incubated in modified human tubal fluid (5% human serum albumin) at 37°C for a period of 24 h. Aliquots from each group were removed at 1 h (short period), 6 h, and 24 h after incubation (extended period). All parameters were analyzed at these time intervals and compared with 0 h (base line). Pentoxifylline at a concentration of 2.5 mM and 5 mM and 2-deoxyadenosine at a concentration of 2.5 mM dissolved in modified human tubal fluid (5% human serum albumin) was added to the sperm suspension. Semen analysis was done before the addition of the stimulants and after 60 minutes of incubation. Results were compared between specimen without stimulants versus stimulants as well as between the different concentrations of pentoxifylline and 2-deoxyadenosine. For short periods (less than 1 h) of incubation, both untreated and Percoll-wash results were comparable. Motility loss for longer periods is reduced in Percoll wash samples. Pentoxifylline increases only motility, whereas 2-deoxyadenosine improves motility and all other motion parameters except linearity. Motility increases significantly differ for both stimulants at both incubation times (0 and 60 minutes), compared to no stimulant at 0 minute's incubation. Compared with the untreated samples, Percoll-wash samples had no advantage in regard to artificial stimulation. Motility loss is less over an extended time in Percoll-treated cryopreserved spermatozoa. Motility is also improved by incubation with pentoxifylline and 2-deoxyadenosine and can be maintained for 60 minutes. This stimulation of motility coupled with a reduced loss over time may be useful in improving fertilization outcome.