Lipid peroxidation in fresh and cryopreserved normozoospermic specimen. Y. Wang, R.K. Sharma, and A. Agarwal. Andrology Research & Clinical Laboratories, Department of Urology, Cleveland Clinic Foundation, Cleveland, OH.

Objectives: Poor fertilization and pregnancy rates are seen with cryopreserved semen. Freeze-thaw procedures induce sublethal damage to sperm plasma membrane resulting in poor motion and functional characteristics. The purpose of this study was to determine: if sperm membrane damage that occurs during freeze-thaw process is attributed to an increase in lipid peroxidation (LPO) levels; the effect of sperm concentration on LPO generation in normal men, and the relationship between LPO levels and sperm motility, viability, and membrane integrity.

Design: Prospective study to evaluate level of LPO in fresh and freeze-thaw normal donor specimens.

Materials and Methods: Semen specimens were obtained from normal healthy volunteers (n = 10) after 2 to 3 days of sexual abstinence. Sperm concentration and motility was evaluated after liquefaction. Semen specimens that tested negative for the presence of white blood cells (<1 X 10⁶/mL) were included in this study. Each ejaculate was divided into two parts. LPO was measured using malonaldehyde (MDA) production in the presence of ferrous ion promoter by thiobarbituric acid method and expressed as nM MDA/h/10⁶ sperm. One aliquot was analyzed for LPO levels without any treatment (fresh) and the second part was frozen by the vapor freezing technique using Test yolk buffer-glycerol as freezing medium. Samples were thawed for 20 minutes at 37°C in an incubator. Sperm concentrations of 5, 10, 20 and 40 X 10⁶/mL were used to study the effect of concentration on MDA levels in frozen specimens. MDA levels in fresh and frozen specimens were compared at a sperm concentration of 20 X 10⁶/mL. Percentage motility, viability, and membrane integrity were examined in thawed specimen after removal of cryoprotective medium.

Results: MDA levels showed no significant difference between fresh (17.94 ± 4.04 nM MDA/h/10⁶ sperm) and cryopreserved specimens (17.85 ± 4.07 nM MDA/h/10⁶ sperm). Increasing the sperm concentration results in an increase in MDA levels. MDA levels increased from 0.84 ± 0.17 nM MDA/h at 5 X 10⁶ sperm/mL to 1.24 ± 0.38 nM MDA/h at 10 X 10⁶ sperm/mL (32% increase, P=.0017), 1.92 ± 0.77 nM MDA/h at 20 X 10⁶ sperm/mL (35% increase, P=.03), and 3.03 ± 1.5 nM MDA/h at 40 X 10⁶ sperm/mL (37% increase, P=.104). There was no correlation between percentage motility, viability, and membrane integrity with MDA levels in frozen-thawed specimens either before or after the removal of cryoprotective medium.

Conclusions: MDA level is not different in fresh and frozen specimens. Cryopreservation induced membrane damage seen in normal semen specimens is not related to lipid peroxidation but may be associated with stress induced during freezing. Levels of MDA show an increase with an increase in the sperm concentration. There is no relationship between MDA levels and semen characteristics. A sperm concentration 20 X 10⁶/mL can be used to measure the MDA levels in studies using computer-assisted semen analyzer.