

maturity results could be evaluated with usefulness of sperm preparation techniques⁽¹⁷⁾. In addition, Sills *et al.*⁽¹⁸⁾ mentioned that the selection of sperm preparation methods depend on the quality of the ejaculates. The ejaculates with ROS production by spermatozoa and leukocytes should not be separated by centrifugation method due to severely spermatozoa damage.

It was noticed that the problem caused by ROS can resolve by performed directly from liquefied semen underneath an overlay of culture medium and aspirate directly from the interface region with total number of spermatozoa recovered⁽¹⁹⁾. However, Aitken and Clarkson⁽²⁰⁾ suggested that poor IUI outcome may be related to improper preparation techniques with release of harmful ROS as well as the separation of motile and active sperm from the rest of the semen can significantly improve pregnancy rates⁽²¹⁾. Furthermore, it was reported that common laboratory factors like centrifugation, washing, temperature fluctuation, and processing delay harmfully affect semen quality both positively and negatively due to direct influence of laboratory interventions on the cytoskeletal assemblies of sperm⁽²²⁾.

Many studies focus on isolating the population of infertile men who are most likely to benefit from vitamin E supplementation. Potential populations could include men with increased ROS levels, increased DNA fragmentation or asthenozoospermia. The supplementation of sperm preparation medium with vitamins C and E may reduce free radical production and decrease ROS induced DNA damage in patients with poor sperm quality. This in turn may provide a greater chance of successful fertilization, as there is an inverse correlation between percentage of sperm with DNA

fragmentation and fertilization rates in vitro with both IVF⁽²³⁾ and ICSI⁽²⁴⁾. There is also possibility that oral administration of ascorbate may facilitate a reduction in induced DNA damage, although this is an area that requires further investigation before any firm conclusions can be drawn. As a result, vitamin E enhanced has the potential to help numerous couples that suffer from male infertility.

The dosage and duration of vitamin E supplementation also needs to be explored and optimized. While, vitamin C act as a scavenger of a wide range of ROS⁽²⁵⁾, which explains its ability to successfully counteract the effects of free radicals both in terms of induced DNA damage and ROS production. It has previously been shown to be the major antioxidant in seminal plasma of fertile men, contributing up to 65% of the total chain breaking antioxidant capacity⁽²⁶⁾. The concentration of this antioxidant in seminal plasma is 10 times greater than the concentration found in blood plasma. The study by Moilanen and Hovatta suggested that vitamin E is less possible to have a protective role given that its seminal plasma concentrations were below the beneficial levels. The study found that this vitamin concentration in the spermatozoal membrane rather than in the seminal plasma is positively correlated with improved sperm parameters⁽²⁷⁾.

The combination of vitamins could substantially reduce ROS levels and impair its normal physiologic function. The current study outlines the beneficial effects of antioxidant supplementation on induced DNA damage. Previous studies have shown that vitamin E affords sperm cells some protection from oxidative attack both in vivo⁽²⁸⁾ and in vitro⁽²⁹⁾ studies. The oral administration of vitamin E has also been shown to lead