Efficacy of Ascorbic Acid in Alleviating Oxidative Stress in-vitro Human Sperm Model

Gulfam Ahmad, PhD.1,2,3, Rakesh Sharma, PhD.1, Shubhadeep Roychoudhury, PhD.1, Sandro C. Esteves, M.D., PhD.4, Ashok Agarwal, PhD.1

1American Center for Reproductive Medicine, Cleveland Clinic, Ohio, USA, 2Department of Physiology, University of Health Sciences, Lahore, Pakistan
3College of Medicine, Prince Sattam Bin Abdulaziz University, KSA, 4ANDROFERT, Campinas, SP, Brazil

Abstract

OBJECTIVE: To measure the efficiency of ascorbic acid (AA) against oxidative stress induced by either heat alone or heat potentiated by hydrogen peroxide in sperm suspensions.

DESIGN: In vitro experimental study

SETTING: Tertiary Hospital, Andrology Research Laboratory

SUBJECTS: Twenty-six normozoospermic men as per the 2010 WHO guidelines

INTERVENTIONS: Two concentrations of ascorbic acid (400 µM/L and 600 µM/L) were tested against heat and heat plus hydrogen peroxide (H2O2) induced oxidative stress in sperm suspensions at 34.5°C, 37°C and 39.5°C after 2 and 4 hours of incubation.

MAIN OUTCOME MEASURES: Sperm motility and oxidation reduction potential (ORP) were measured and a 4 hour incubation period at three different temperatures. Sperm motility was assessed manually and ORP was measured by MiOXSYS system.

RESULTS: A significant decrease in ORP was observed as a function of AA concentration. The mean reduction in ORP for 400 µM AA compared to control was 1.61 mV/106 sperm/mL (p < 0.001), and for 600 µM AA compared to control was 7.31 mV/106 sperm/mL (p < 0.001). Significant decrease in sperm motility ranging from 4.89% to 14.02% were observed both as a function of incubation time and addition of H2O2 (p<0.001). The degree of change in motility with the inclusion of H2O2 was dependent on the incubation temperature, and likewise the change in motility with added heat was dependent on the inclusion of H2O2 (p=0.002 for the interaction between H2O2 and temperature). Increasing the temperature from 34.5°C to 37°C resulted in a significant decrease in motility (4.77%) after inclusion of H2O2 (p<0.001). Increasing the temperature to 39.5°C did not decrease motility further. A significant decrease in motility (-4.89%) was also observed as a function of incubation time. A significant decrease in sORP was observed as a function of AA concentration. The mean reduction in sORP for 400 µM AA compared to control was 6.70 mV/106 sperm/mL (p < 0.001), and 7.31 mV/106 sperm/mL for 600 µM AA compared to control was (p < 0.001). A reduction of 0.61 mV/106 sperm/mL was observed when 600 µM AA was compared to 400 µM AA (p<0.001). However, none of the other factors (incubation time, temperature and H2O2) were significantly associated with mean sORP and no significant interactions were found among the factors in the repeated measures model (Table 3). Therefore, unlike the model for motility, the effects of each variable on sORP as shown in Table 3 were described without subsetting to specific values of the remaining variables.

CONCLUSION(S): Ascorbic acid is efficacious to reduce heat-induced oxidative stress in sperm preparations in vitro. The supplementation of ascorbic acid may be advantageous for sperm preparations in IUI, IVF and ICSI.

Material and Methods

Subjects, semen collection and analysis

Semen samples (n=26) were collected after 2-3 days of ejaculatory abstinence. After liquefaction, an aliquot was reserved for standard semen analysis. Sperm concentration, motility and round cells analysis was performed manually on wet preparation using a MicroCell counting chamber (VitroLife, San Diego, CA). Samples with the presence of round cells >1x106/L were used to test the effect of H2O2 on sperm motility and ORP. The samples were prepared by double density gradient centrifugation. The final sperm concentration was adjusted to 10-15 x 106/sperm/mL and used for exposure to heat control ORP.

Exposure to heat stress

Two experiments were conducted. In the first experiment, ORP was observed by using sperm from three different temperatures: 34.5°C, 37°C and 39.5°C. In the second experiment, in addition to heat as in the first experiment, ORP was observed by using sperm from 34.5°C, 37°C and 39.5°C.

Ascorbic acid supplementation

Two concentrations of ascorbic acid, namely 400 µM/L and 600 µM/L (L-ascorbic acid, Sigma), were used as antioxidants to reduce the heat-induced oxidative stress. The samples were incubated at 34.5°C, 37°C, and 39.5°C at 6% CO2. The temperature under investigation, namely 34.5°C, 37°C, and 39.5°C were analyzed after 2 and 4 hours of incubation.

Table 1. Sperm motility and ORP in heat only and heat + H2O2 after 2 and 4 h of incubation and effects of ascorbic acid (AA) supplementation. Values are means ± SEM

Table 2. Repeated measures model for sperm motility (%) to heat induced oxidative stress

Table 3. Repeated measures model for sperm sORP (mV/106 sperm/mL) to heat induced oxidative stress

Conclusion(s)

1. Ascorbic acid is effective in reducing oxidative stress in vitro.

2. Ascorbic acid supplementation may offer a protective role during semen preparation for IUI, IVF and ICSI.