Brief Report

Comparing Human Sperm Quality Preserved at Two Different Temperatures; Effect of Trolox, Coenzyme Q10 and Extracellular Adenosine Triphosphate

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Abstract

Cooling method was proposed to maintain the sperm quality for several days. Nevertheless, during this procedure, sperm is encountered to "cold shock", and its quality decreases time-dependently. This study was designed to improve the in vitro sperm preservation methods. Thirty normal semen samples were examined in Shiraz, Iran, 2017. Fifteen samples were incubated at 22-27 °C and 15 samples were cooled moderately to 4 °C. Each sample was divided into five subgroups; control, solvent, 200 µM Trolox, 40 µM Coenzyme Q10, and 10 mM ATP. ATP was added only 15 minutes before the analysis. Assessments of motility parameters and sperm viability were done every 24 hours. Statistical analysis was performed using SPSS 16 software. The differences between two main groups and subgroups were compared by *t* test and one-way ANOVA, respectively. The effect of time was analyzed by repeated measurement test. Sperm motility and viability were the same in both groups until 24 hours, except the straight line velocity was greater in the cold group. Even after 48 hours, progressive motility and sperm velocity, but not viability, were still the same. The greatest reduction in progressive motility occurred on the second day; and after 72 hours, sperm quality was better preserved in 22-27 °C. Treatment with Trolox, coenzyme-Q10, and extracellular ATP did not have effect on sperm quality. Cold temperature is recommended for in-vitro sperm preservation up to 24 hours, and 22-27 °C is preferred for longer time storage. The sperm does not need antioxidant therapy for quality maintenance, but the extender media must be supplied with nutrients and antibiotics.

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Keywords ● Semen preservation ● Trolox ● Coenzyme Q10 ● Extracellular ATP

What's Known

- Semen quality decreases time-dependently, and some studies show that sperm of mammals is preserved better in cold condition. However, other studies emphasize sperm damage in low temperatures.
- Treatment with antioxidant and extracellular ATP are useful for animal sperm preservation, but the data available about human sperm preservation are not adequate.

What's New

- Moderate cooling rate does not damage the human sperm, at least for 24 hours. Cold temperature is recommended for in vitro sperm preservation up to 24 hours, and 22-27 °C is preferred for longer time storage. The unstimulated human sperm does not need antioxidant therapy for quality maintenance.

Introduction

Cryopreservation of sperm in liquid nitrogen is the only method for long-term storage, but sperm refrigeration is usually used to preserve animal sperm up to 3-5 days.[1] Lowering temperature to 4-10 °C is enough to restrict cell metabolism and acidification.[2] Theoretically, the decrement of metabolism in lower temperatures can attenuate the accelerated demise in sperm because of "live

fast-die young" fact. However, after refrigeration, sperm motility and viability decrease because of "cold shock". Cold shock also causes disruption of selective membrane permeability and damage to the mitochondrial and acrosomal membranes.[1-3]

Some researchers believed that reactive oxygen species (ROS) generation increases during the cooling of sperm.[4] Therefore, many attempts have been made to attenuate the cold shock damages through the use of antioxidants.[5, 6] Regarding the controversial effects of antioxidants on cooled sperm, and considering the fundamental role of mitochondria in ROS production, we decided to use a mitochondrial antioxidant, coenzyme Q10 (CoQ-10), in comparison to a well-known antioxidant, Trolox. CoQ-10 is a lipid-soluble molecule, with an important role in ATP synthesis. It protects against oxidative stress and improves sperm motility.[7-9] On the other hand, extracellular ATP (ATPe) acts as a physiologic regulator of sperm function,[10] and it increases sperm cryopreserved velocity.[11]

Only a few studies have been done on the refrigerated human sperm,[12, 13] and the present study aimed to find a suitable condition for human sperm preservation and clarify the effects of CoQ-10, Trolox, and ATPe on sperm viability and motility patterns.

Materials and Methods

Sample Collection and Preparation

Thirty normal semen samples were obtained from 20-40 years old healthy men who referred to Shiraz Infertility Treatment Center, Shiraz, Iran (2017). All the participants in this study signed a written informed consent form. The study protocol was approved by the Research Ethics Committee of Shiraz University of Medical Sciences (IR.sums.REC.1395.5807). The semen samples were washed with Ham's F-10 medium (Sigma, N6633), and incubated at 37 °C, 5% CO_2 for one hour. Swim-up sperm was diluted to 10×10^6 million/ml. Fifteen samples were incubated at room temperature between 22-27 °C (RT group), and another 15 samples were incubated at 4 °C (C or cooled group). Each sample was divided into five subgroups containing Ham's F-10 supplemented with 5% BSA and 1% Penstrep (control group), 0.1% dimethyl sulfoxide (DMSO) (solvent group), 200 µM Trolox (Sigma, 238813), and 40 µM CoQ-10 (Sigma, C9538). The last subgroup was incubated in Ham's F-10 during the period of experiment, and 10 mM ATP (Sigma, A3377) was added to the medium only 15 minutes before the analysis. Sperm cooling was done by moderate cooling method.[13] The sperm media were replaced with fresh, adjusted temperature medium every day.

Evaluation of Sperm Motility and Viability

Sperm motility and viability were analyzed on the first day (day 0) and after 24, 48, and 72 hours. Refrigerated sperm was incubated at 25 °C for 60 min before the assessments. Motility parameters, including the percentages of progressive (PM) and non-progressive (N-PM) motile sperm, average path velocity (VAP, µm/s), straight-line velocity (VSL, µm/s), curvilinear velocity (VCL, µm/s), and linearity (LIN, %)(14), were recorded using VT sperm analyzer 3.1.

Eosin staining method was used to evaluate the sperm viability.[14]

Statistical Analysis

Data analyses were done by SPSS software, version 16.0. The differences between RT and C groups and subgroups of each group were compared by *t* test and one-way ANOVA, respectively. The Repeated Measurement test was performed to compare the subgroups after 24, 48, and 72 hours. Results were expressed as Mean±SEM and $P<0.05$ was considered significant.

Results

Sperm PM decreased in RT and C groups by time. The greatest reduction in PM occurred on the second day (Figure 1A). After 48 and 72 hours, total motile sperm (%) was greater when sperm incubated in RT versus 4 °C (P=0.03 and P=0.0001). Treatment with Trolox or CoQ-10 reduced the total motility in the cooled group after 24 hours (Figure 1B).

ATP caused a reduction in fresh sperm PM but did not cause significant changes in motility of preserved sperm (data were not shown).

VCL, VSL, and VAP decreased time-dependently in both groups. However, the VSL of the cooled group was significantly more than RT group after 24 hours. Trolox and CoQ-10 did not have any significant effect (Figure 1).

Mortality rate showed the same reduction in RT and C group until 24 hours. Trolox and CoQ-10 had a protective role in sperm viability after 24 hours of incubation in RT group (Figure 2).

Discussion

Developing techniques that help the short-term preservation of human sperm fertilization potency are very important. Refrigerated storage

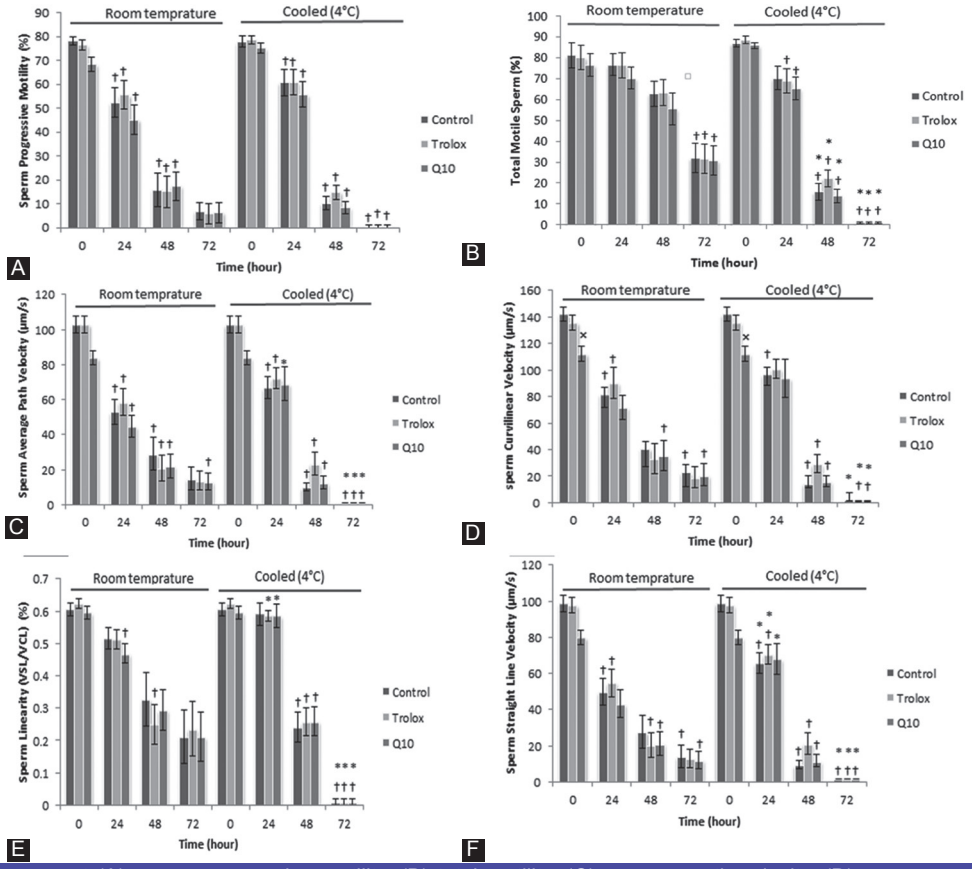

Figure 1: Graphs represent (A) sperm progressive motility, (B) total motility, (C) average path velocity, (D) sperm curvilinear velocity, (E) linearity, and (F) straight line velocity in control, Trolox, and CoQ-10 treated with media incubation at room temperature and cold state. *Significant difference between the related RT and C group at the given time; †Significant difference relative to the last 24 hours; ×Significant difference relative to the control group.

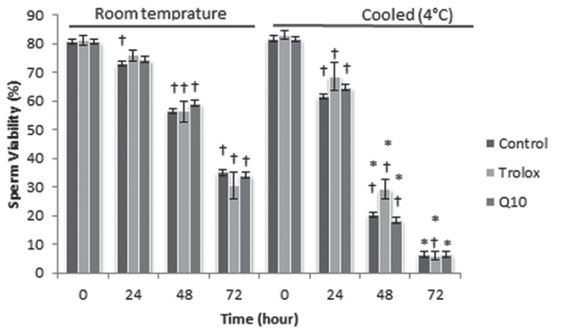

Figure 2: Graphs represent sperm viability in control, Trolox, and CoQ-10 treated with media and incubated at room temperature and cold state. *Significant difference between the related RT and C group at the given time; † Significant difference relative to the last 24 hours.

of sperm is recommended by some animal studies.[1-3] Suitable extenders such as egg yolk, soybean lipids, and bovine serum albumin (BSA) are used to protect sperm from cold shock.[1] In this study, we considered all the known procedures for confronting with cold shock; changed media every 24 hours, controlled the cooling rate, and supplied the extender with BSA and antibiotic. With these considerations, all changes in sperm motility and viability were the same in RT and C groups at 24 hours, except the VSL that was greater in C group. After 48 hours, total motility and viability were lower in the cooled group. Many researchers have found that sperm viability and motility is diminished time-dependently.[12, 15] According to our results, the cooling method can be recommended for human sperm preservation up to 24 hours; and RT preservation is proposed for longer storage.

Some researchers have suggested that an increase in ROS generation is responsible for cold shock damage.[4] CoQ-10 acts by preventing the formation of free radicals while Trolox acts as a radical scavenger. Nevertheless, in our study, these antioxidants could not protect sperm in RT or C groups. We did not measure the rate of ROS generation, but it had previously been reported that incubation in 4 °C is low enough to restrict the metabolism and ROS generation,[2] which is one reason for the ineffectiveness of these antioxidants.

ATPe could not cross the cell membrane, but it improved several motility parameters in asthenozoo sperms and cryopreserved normal human sperm.[10] However, using 10 mM ATPe did not alter sperm motility or viability; and

further studies with different concentration and longer time storage is suggested.

Conclusion

Human sperm quality decreased time-dependently at both room temperature and 4 ºC; however, cold preservation is recommended for 24 hours and preservation at 22-27 °C is preferred for longer time storage. Treatment with antioxidants (Trolox and CoQ-10) or 10 mM ATPe could not improve sperm quality.

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Conflict of Interest: None declared.

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