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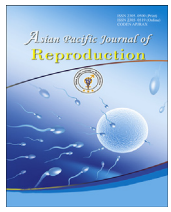

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Effect of selenium on human sperm parameters after freezing and thawing procedures

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ABSTRACT

Objective: To evaluate the effects of pre-freezing treatment of human semen samples with selenium on semen parameters after thawing procedure.

Methods: Each sample was divided into four groups: two groups were washed and other two remained unwashed. 5 μg/mL of selenium was added to one of the washed and unwashed group.

Results: The results showed that sperm motility in unwashed selenium-treated samples was higher compared to washed untreated samples ($P < 0.05$). DNA damage percentage in unwashed treated samples compared to both untreated samples showed no difference ($P > 0.05$) but in washed sperm samples, DNA damaged percentage decreased in treated samples compared to untreated ones ($P < 0.05$). Moreover, the effect of selenium on morphology of washed treated sperms was higher than unwashed untreated samples after thawing procedure. Normal morphology percentage in unwashed treated samples was significantly higher than both unwashed samples after thawing ($P < 0.001$). In the washed treated samples a higher number of sperms with normal morphology compared to washed untreated samples ($P < 0.001$) were observed.

Conclusions: Based on the results, we recommend 5 μg/mL of selenium should be used in infertility clinics to increase sperm quality after freezing and thawing procedures.

1. Introduction

Causes of infertility may include male, female or both and other unexplained factors. About 50% of infertility is believed that result from male factors associated with abnormal semen parameters [1]. Some forms of abnormalities that may be observed in these men include oligospermia, asthenospermia, teratospermia (oligoasthenozoospermia) and azoospermia. Human sperm freezing is used extensively in assisted reproductive technology especially before chemotherapy, radiotherapy and some surgical procedures [2]. Oxidative stress and reactive oxygen radicals (ROS) can be produced in semen, and it has been shown to have some harmful effects on spermatozoa [3]. In semen abnormal spermatozoa, germinal cell precursors and leukocytes have potential to produce ROS [4]. On the other hand, ROS generated by spermatozoa plays an important role in normal physiological processes such as sperm capacitation, acrosome reaction and maintenance of fertilization ability [5]. Due to high susceptibility of spermatozoa to oxidative stress and ROS, several damages on spermatozoa such as lipid peroxidation, DNA damage, apoptosis and sperm motility reduction can be induced [6].

Antioxidants are molecules that have ability to inhibit or reduce oxidative process in other molecules by scavenging released free radicals [7]. Antioxidants are divided into two groups, enzymatic and non-enzymatic. Enzymatic antioxidants include superoxide dismutase, glutathione peroxidase, catalase and glutathione reductase, whereas non-enzymatic antioxidants include vitamins A, C, E, pyruvate, glutathione, and coenzyme Q. Antioxidant function of selenium (Se) is mediated through glutathione peroxidase enzyme activity. There are several anti-oxidants in semen that are known to improve sperm quality such as vitamin E and C, as well as Se and Zn which are components of antioxidant systems. However, presumably these antioxidant agents are insufficient in preventing lipid peroxidation and

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sperm plasma membrane injury during the freezing-thawing process because the protective antioxidant systems in sperms originate from cytoplasm and sperms discard their huge part of cytoplasm as residual body during final stages of differentiation. Freezing and thawing procedures cause significant reduction in motility and metabolic activity in sperms, however, vitamin E and Se have been found to improve freezing induced damages and reduced sperm motility [8]. During cryopreservation, semen is exposed to cold shock and atmospheric oxygen which in turn increases the susceptibility to lipid peroxidation due to higher production of ROS. Sperm plasma membrane is one of the important structures affected by cryopreservation which then can affect the viability and fertility of the sperm cells [9].

Se is an essential metal-like trace element in mammalian diet and its importance has been well established in humans [10]. Foods such as sea foods, liver, grain, egg yolk, milk, water and soil are major natural sources of Se with its levels generally depending on soil [11]. Typical dietary intake of Se in the US is 80–120 μg/d, and the recommended daily allowance is 70 μg in men and 50 μg in women [12]. Se is a component of the glutathione peroxidase enzyme which is known as an important antioxidant and a marker of oxidative stress [13]. Experimental evidences confirmed the positive effects of Se on human health [14]: reduction of disease symptoms and improvement of some disorders such as infertility, viral infections, cancers, cardiovascular disease and so on [15]. Also Se is an essential element in spermatogenesis and male fertility as well [16]. In a study that was performed by Moslemi *et al.* [17]. Effect of Se–vitamin E supplementation on different parameters of 690 infertile men and pregnancy rate was evaluated. Their results showed that supplemental Se and vitamin E may improve semen quality and pregnancy rate in idiopathic male infertility diagnosed with asthenoteratospermia or asthenospermia in semen analysis, and also have beneficial and protective effects especially on sperm motility [17]. Other studies have also shown that administration of Se to subfertile patients induced a statistically significant rise in sperm motility [18]. Various other studies have revealed the effects of cryopreservation on human sperms function and fertility whereas those conducted on animal model using Se as the most potent component of antioxidant system improved sperm quality and quantity [19]. For example, Sermak *et al.* demonstrated that addition of Se in the quantity of 1 μg/mL to cryomedium of ram semen improved survival rate following freezing-thawing process [20]. Unfortunately, there are few or no literature publications on the effects of post treatment of human semen with Se on semen parameters. As a first study, this research aimed to evaluate the effect of pre-freezing treatment of human semen with Se on different parameters of human sperm after thawing process.

2. Materials and methods

This study was approved by the Ethical Committee of the Faculty of Basic Sciences of Payame Noor University (Tehran, Iran). This experimental study was performed on 42 semen samples referred to Shariati infertility center in Tehran during February and March 2013, because of infertility problems of their wives but did not show any indications of infertility. All the 42 donors who participated had an informed written consent and the mean age of these individuals was (32.1 ± 3.9) years. In all participants, after 3 d of sexual abstinence, semen samples were collected by masturbation in sterile containers and were transferred to laboratory immediately after ejaculation. The semen samples were allowed to complete liquefaction at 37 °C for 30 min. After liquefaction, a small aliquot was removed from each specimen and the sperm parameters were determined according to World Health Organization (WHO) guidelines [21]. Each sample was then divided into two groups: one group unwashed and the other washed. Washed samples were processed by swim-up technique. In swim-up technique, semen be overlaid directly with culture medium and the sperm allowed to swim from the seminal plasma into the culture medium. Washed and unwashed samples were also divided into two subgroups: one without Se treatment as control and the other with 5 μg/mL of sodium selenite (Na_2SeO_3). Control and treated specimens were then cryopreserved by liquid nitrogen vapor method [22].

An aliquot of the freezing medium equaling 25% of the original specimen volume was added to each of the samples. This process was repeated until the ratio of freezing medium to ejaculum (semen) became 1:1. Cryovials were loaded with 1 mL freezing medium/semen mixture and kept at −20 °C for 8 min. The samples were then exposed to liquid nitrogen vapor at −79 °C for 2 h before plunging into liquid nitrogen for storage at −196 °C. After 2 wk interval, the samples were thawed at room temperature for 5 min and then transferred to 37 °C water bath for 20 min. To remove the freezing medium, the thawed samples were diluted with Hams F_{10}(Biochrom.K.G) medium at a ratio of 1 volume of freezing medium/semen to 3 volumes of freezing medium and then washed by centrifugation at 300*g* for 7 min. The supernatant was removed and the pellet resuspended in 0.7 mL Hams F_{10} medium. A small aliquot was removed from each sample for assessment of different parameters.

Light microscope (Nikon, Tokyo, Japan) with 40× magnification was used for motility evaluation. Sperms morphology was evaluated using Papanicolaou staining method [21]. In this method the staining was as follows; anterior area of acrosome stained light blue, posterior area dark blue, sperm neck slightly red, tail blue and excess residual cytoplasm pink.

Eosin-nigrosin staining was used for vitality evaluation. Stained sperms indicated permeability of sperm to dye and membrane damage whereas unstained sperms had healthy membrane which did not permit the dye to enter into the sperm cells. If sperm head stains red or dark pink it was considered dead whilst those whose head stained light pink or white was considered alive.

In order to evaluate sperm motility, we used light microscope with 40× magnification and based on WHO protocol, sperm motility was classified into three classes: progressive, non-progressive and immotile.

Ultimately, DNA damage was analyzed by toluidine blue staining based on WHO protocol. Toluidine blue staining is a metachromatic staining procedure used for chromatin structure evaluation. The basis of this procedure is the highly incorporation of Toluidine blue dye into damaged and compacted chromatins. When this dye is incorporated into histone and arginine-rich chromatin, the nucleus stains dark purple whereas its incorporation into protamine-rich chromatin stains the nucleus light blue [23]. To determine the significant differences between the studied groups, statistical analysis including mean ± SD, correlation coefficients and two way Anova tests were performed in three independent experiments using Prism (version 5) software. All tests were performed at a confidence level of 95%.

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