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EFFECT OF COENZYME Q10 IN ALLEVIATING THE OXIDATIVE STRESS ON FROZEN BOVINE SPERMATOZOA

Veterinary Science

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ABSTRACT

Oxidative stress induced by reactive oxygen species (ROS) has a direct effect on male infertility as it causes sperm dysfunction by way of lipid peroxidation of sperm cell membrane and sperm DNA damage. A simple and reliable procedure to overcome the adverse effects of higher ROS levels is important in the treatment of infertility. Incubation of spermatozoa with Coenzyme Q10 (Co-Q10) can improve spermatozoa membrane stability together with increased integrity and functionality of the mitochondrial membrane, thus an increase in sperm survival. In this study, one of the common environmental contaminants, phthalate was used to induce oxidative stress upon spermatozoa and Co-Q10 was used in the experiment to relieve the ROS-induced oxidative stress. It was found that phthalate adversely affected the integrity of acrosomes and caused the denaturation to sperm DNA. Supplementation of Co-Q10 was found to relieve the phthalate-induced toxicity on the acrosomal membrane. It was also found that Co-Q10 helped protect the sperm's DNA against the denaturation caused by phthalate. The study concluded that Co-Q10 can be a possible protectant for spermatozoa during assisted reproductive technologies.

KEYWORDS

Oxidative stress, male infertility, endocrine disruptors

INTRODUCTION

Among the various environmental contaminants that contribute to the development of male infertility, phthalates invite much attention. Phthalates are one of the classes of environmental contaminants that are used as plasticizers for polyvinyl chloride plastics to increase their flexibility, durability, and longevity. Animal and human populations are potentially exposed to these chemicals due to the widespread application of plastic products in daily life, further making it a common environmental contaminant (Ge et al., 2007).

Growing evidence from research points out that phthalates adversely affect male reproductive functioning. Phthalates were found in semen at statistically higher amounts in infertile men than in fertile males (Pant et al., 2008). Researchers point out the role of phthalates in testicular disorders due to their estrogenic properties. Deleterious effects induced by phthalates have an intrinsic relation with oxidative stress.

Oxidative stress (OS) has been identified as one among many mediators of male infertility which results in sperm dysfunction (Sharma and Agarwal, 1996). OS leads to increased cellular damage triggered by oxygen and oxygen-derived free radicals called reactive oxygen species (ROS). Researches provide ample evidence of phthalate-induced oxidative stress on spermatozoa. Decreased motility, abnormal acrosome reaction, altered mitochondrial function, reduced fertilization rate, and embryo development observed in phthalate-exposed spermatozoa were all found to be linked to elevated level of ROS. An excessive level of ROS can adversely affect the DNA, protein, and lipid structure in the sperm, leading to infertility (Buhler and Miranda, 2000).

Antioxidants are the molecules that can counteract oxidative stress by preventing the overproduction of ROS in cells and tissues. Coenzyme Q_{10} (Co-Q_{10}) is a lipid molecule in mammalian cells, involved in energy generation through the mitochondrial electron transport chain. It has an antioxidant effect that indicates possible involvement in male infertility as these factors are directly related to sperm viability, and a spermatozoon without energy or in oxidative stress (Balercia et al., 2009).

MATERIALS AND METHODS

Three treatment groups and one control group would be utilised for performing each technique.

i. Frozen semen sample- Control group
ii. Frozen semen sample treated with phthalates (100µM)
iii. Frozen semen sample treated with phthalates (100µM) and Coenzyme Q_{10}(10µM)

1. Microscopic Examination For Sperm Viability And Morphology: Proportion of dead, morphologically abnormal, and normal spermatozoa were recorded for each sample using the Giemsa staining method. The staining procedure followed the protocol of Watson, 1975. The counting of intact, partially damaged, and fully damaged acrosomes was carried out under a compound microscope at 100x magnification.

2. Sperm DNA integrity analysis by Acridine Orange (AO) test: The AO assay measures the ability of sperm nuclear DNA to denature by acid which forms a metachromatic shift of AO fluorescence from green (native DNA) to orange-red (denatured DNA). Samples were rapidly thawed in a 37°C water bath, placed on ice and diluted with TNE buffer (0.015 N NaCl, 0.01 M Tris, and 0.001 M EDTA, pH = 6.8) to bring sperm concentration to 1.5×10^6 per ml. 200 µl of diluted samples were treated with 400 µl acid detergent solution (0.08 N HCl, 0.1% Triton-X 100, pH = 1.2) for exactly 30 s to induce DNA denaturation. After 30 seconds, 1.2 ml AO staining solution was added (citric acid 0.1 M, Na_2HPO_4 0.2 M, EDTA 0.001 M, NaCl 0.15 M, and AO stock 6 □g/mL in distilled water, pH 6), and each sample was analyzed by fluorescent imaging.

RESULTS AND DISCUSSION

1. Microscopic examination for sperm viability and morphology by Giemsa Staining

The integrity of acrosome was tested by Giemsa's staining methods for control and treatment groups (Figure 1a and 1b). Data was statistically analyzed by paired 't' test using NCSS software. The data were found to be normally distributed by the Shapiro-Wilk test. The mean values for the control, treatment 1 (spermatozoa treated with phthalate), and treatment 2 (spermatozoa treated with phthalate and Co-Q_{10}) were 62.66667±0.8027 S.E, 42.66667±0.9545 S.E and 52.16667±1.1948 S.E respectively. The t-value calculated for control and treatment 1 (paired sample t-test) was 2.5706 at 5% level level of significance. So, there was a significant difference from the mean and it is interpreted that phthalates caused significant toxicity to spermatozoa. The calculated t-value for treatments 1 and 2 was 2.5706 at 5% level of significance, thus there is a significant difference between the mean values of treatment groups i.e., the phthalate toxicity is relieved considerably upon the addition of Co-Q_{10}.

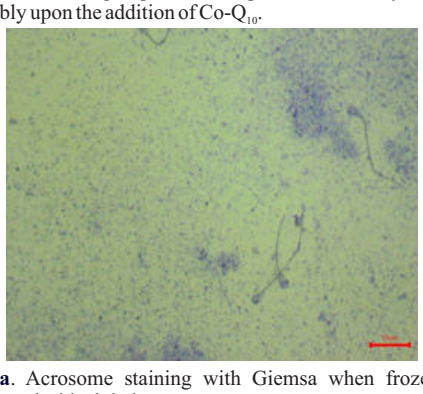

Figure 1a. Acrosome staining with Giemsa when frozen semen sample treated with phthalates

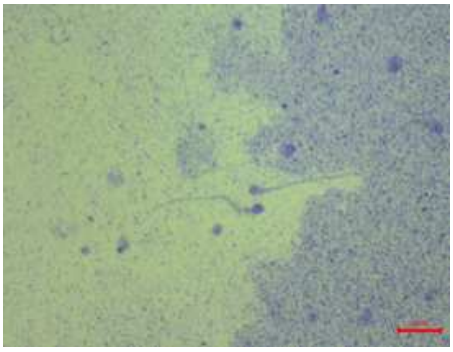

Figure 1b. Acrosome staining with Giemsa when frozen semen sample treated with phthalates and Co-Q_{10}

The study showed that the phthalates badly affected the integrity of acrosomes. At the same time, Co-Q_{10} played a positive role in protecting the acrosomal membrane from phthalate-induced toxicity. The findings in the study were supported by earlier reports that exposure to phthalates impairs normal spermatogenesis and steroidogenesis due to oxidative stress in the male reproductive organs, particularly in the testes and epididymis. By causing oxidative stress in germ cells or apoptosis in target Sertoli cells, they interfere with the spermatogenic process. Phthalates also adversely affect the Leydig cells' ability to produce steroidogenic enzymes by increasing ROS (Sedha et al., 2015). The determination of the ability of spermatozoa to activate the acrosome reaction is supposed to be a useful parameter in evaluating infertility. The difference in the acrosome-intact values correlates with the function of the membrane to protect spermatozoa acrosome (Boccia et al., 2007).

2. Sperm DNA integrity analysis by Acridine Orange (AO) test:

Upon staining with Acridine Orange, evaluation by fluorescent microscopy has shown that phthalate toxicity made DNA denature at a higher rate showing orange/red fluorescence for the spermatozoa (Figure 2a). Supplementation of Co-Q_{10} contributed significantly to protecting the DNA from denaturation caused by ROS, evidenced by the presence of more intact double-stranded DNA showing apple green-colored fluorescence under a fluorescent microscope (Figure 2b).

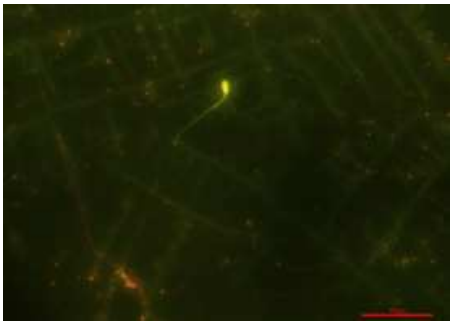

Figure 2a: Staining with acridine orange when frozen semen sample treated with phthalates

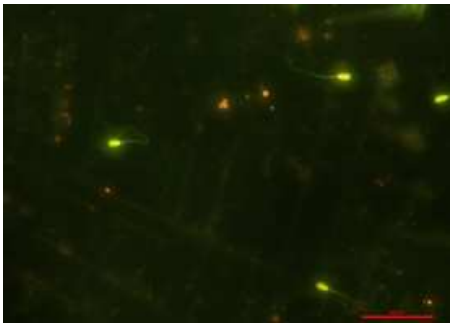

Figure 2b: Staining with acridine orange when frozen semen sample treated with phthalate and Co-Q_{10}

This observation is in agreement with the reports of several previous studies all of which reported an increase in the percentage of AO red cells upon oxidative damage. Earlier studies confirmed the higher abundance of AO-red cells in spermatozoa of infertile/sub-fertile males (Ajina et al., 2017; Talebi et al., 2008).

CONCLUSION

In conclusion, our study evaluated the beneficial effects of Co-Q_{10} during sperm preparation for assisted reproductive technologies. We highlighted the viability and acrosomal integrity improvement of spermatozoa when Co-Q_{10} was utilised during sperm preparation. Further investigation into spermatozoan DNA integrity also supported the favourable role of Co-Q_{10} in protecting against oxidative stress produced by environmental contaminants like phthalates.

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