Cairo University
Faculty of Veterinary Medicine
Theriogenology Department

Influence of some antioxidants supplementation in semen extender on quality of stallion spermatozoa

A Thesis Presented by
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Abstract

The present study was designed to determine the ability of bovine serum albumin (BSA), trehalose (Tr) and zinc (Zn) – supplemented in semen extender – to improve stallion sperm quality parameters during storage at +5ºC and cryopreservation. Semen was collected from four pure Arab stallions (10 to 12 years old) on a regular basis (one ejaculate / week) for three months. After collection, semen was evaluated using conventional methods. Centrifugation of gel free fraction at 600 xg for 15 min for partial removal of seminal plasma was done. Dilution of pelleted spermatozoa using INRA-82 extender containing different concentrations of BSA (0, 10, 15 and 20 mg/ml), trehalose (0, 75, 100 and 150 mM) and zinc sulphate (0, 100, 150 and 200 µM) was performed. Then, diluted semen was cooled at + 5°C and cryopreserved at -196°C. With regard to the studied spermatozoa parameters (progressive motility, live percentage, total abnormalities, viability index and plasma and acrosomal membrane integrities) , the effect of BSA (10 mg/ml) , trehalose (150 mM) and zinc sulphate (200 µM) on cooled-stored (+ 5^0 C) and frozen spermatozoa was significantly ($p < 0.05$) superior to the other concentrations used. In view of results of this study, it can be concluded that cold-storage (+5^0 C) and freeze-thaw processing of spermatozoa of Arab stallion in the presence of BSA 10 mg/ml, trehalose 150 mM and zinc sulphate 200 µM achieved a significant amelioration in the maintenance of ejaculated sperm quality parameters.

Keywords: Stallion semen, bovine serum albumin, trehalose, zinc.

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DEDICATION

To:

My mother and father (My ALLAH bless his soul), from my earliest years they have supported and encouraged me to aim high in my educational achievements.

My Wife for her great support.
My brother and my sisters.

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ATP	**Adenosine triphosphate**
BSA	**Bovine serum albumin**
EDTA	**Ethylenediaminetetraacetic acid**
GEY	**glucose egg yolk extender**
GP_X	**Glutathione peroxidase**
GRD	**Glutathione reductase**
GSH	**Glutathione**
HOST	**Hypo-osmotic swelling test**
LEY	**lactose egg yolk extender**
LPO	**Lipid peroxidation**
mOsm	**Miliosmol**
PUFAs	**Polyunsaturated fatty acids**
ROS	**Reactive oxygen species**
SDS	**sodium dodecyl sulphate**
SOD	**Superoxide dismutase**
TCG	**tris-citric acid-glucose**
TEY	**trehalose egg yolk extender**
Tr	**Trehalose**
Zn	**Zinc**

I-Introduction

The cryopreservation of spermatozoa has allowed specific opportunities for the conservation of genetic resources through sperm banks, the guarantee of a constant commercial supply of semen, and collaboration in in breed improvement programs by means of the artificial insemination (AI) technique **(Holt, 1997)**.

Modern equine reproduction, in most breeds, is based on the use of artificial insemination. The widespread use of AI has accelerated genetic progress by making selected stallions available to breeders outside the country or region where the stallion is located ***(*Pagl**, **Aurich, Müller-Schlösser, Kankofer and Aurich, 2006**). Development in semen technology has become an area of renewed interest for the equine industry due to increase in use of AI using chilled or frozen–thawed semen (**Samper, Estrada and McKinnon, 2007)**. However, the large inter-individual variability in semen quality and characteristics of the equine species, clouds this development (**Macías García, González Fernández, Ortega Ferrusola, Salazar-Sandoval, Morillo Rodríguez, Rodríguez Martinez and Pena, 2011**).

The storage of spermatozoa is associated with a reduction in cell viability and fertilizing capacity. The improvement in semen storage technologies requires an in depth knowledge of gamete physiology and the biochemical processes occurring during semen collection and storage **(Yoshida, 2000).** In many species, including horses, peroxidation of plasma membrane lipids [lipid peroxidation (LPO)] and other cellular components is a major factor involved in sublethal cryodamage to the spermatozoa **(Aitken, Wingate, De Iulius, and Mclaughlin, 2007).** The particular susceptibility of the sperm plasma membrane to peroxidative damage is due to a high cellular content of polyunsaturated fatty acids. The formation of lipid peroxides is one of the most important reasons for the decrease in fertility during storage of semen in the presence of oxygen radicals. The sperm plasma membrane has a very important role in sperm

fertilizing ability and in spermatozoon–oocyte cross-talk (**Lenzi, Picardo, Gandini and Dondero, 1996)**. **Griveau, Dumont, Renard, Callegari and Lannou (1995) and Macias Garcia et al. (2011)** reported that the fluidity of the plasma membrane that the spermatozoon needs to participate in the membrane fusion events associated with fertilization is attributed to its unsaturated fatty acids contents (**Lenzi et al., 1996; Flesch and Gadella, 2000).** However, these molecules of fatty acids are also vulnerable to attack by reactive oxygen species (ROS). Under physiological conditions, excess ROS are neutralized via endogenous antioxidants such as superoxide dismutases. **Storey (1997)** noted that ROS attack to polyunsaturated fatty acids (PUFA) in the sperm cell membrane is followed by initiation of the lipid peroxidation cascade. Excess production of ROS during cryopreservation has been associated with reduce post-thaw motility, viability, membrane integrity, sperm function and fertility (**Uysal and Bucak, 2007**). **Ball, Medina**, **Gravance and Baumber (2001)** reported that the decline in motility and fertility during hypothermic storage of liquid semen results from oxidative damage to spermatozoa. Damage of sperm cells during freezing or morphologically abnormal spermatozoa generates significantly greater amounts of ROS than do live or morphologically normal spermatozoa that may contribute to reduced fertility or problems related to semen preservation (**Ball, Vo and Baumber, 2001)**.

Ball (2008) reported that catalase, superoxide dismutase (SOD) and glutathione peroxidase (GPx) are the primary ROS scavengers described in semen. Reactive oxygen species scavengers in sperm cells are very limited, and seminal plasma is a potent source of ROS scavengers. The total removal of seminal plasma during semen processing may increase the susceptibility of sperm to oxidative stress because of the removal of these enzyme scavengers.

Aitken and fisher (1994) and **Griveau and Lannou (1997)** found that the impact of oxidative stress could be reduced by addition of molecules of antioxidant, and thus improve semen quality after thawing. Antioxidants