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Influence of sperm concentration on the motility, morphology, membrane and DNA integrity along with oxidative stress parameters of ram sperm during liquid storage

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ABSTRACT

The aim of this study was to determine the influences of two different concentrations in terms of motility, morphology, membrane integrity (viability and HOST response: HE-test; modified hypoosmotic swelling test (HOST) associated with supravital eosin staining test), DNA integrity (COMET assay) and oxidative stress parameters (MDA, malondialdehyde; AOA, total antioxidant activity; GSH, reduced glutathione; NOx, nitric oxide) of liquid stored ram sperm for 5 days. Two different concentrations suitable for laparoscopic and cervical inseminations were employed. A total of 5 Pirlak rams (Daglic × Kivircik, local breed) with satisfactory breeding potential were selected. Semen samples were collected by artificial vagina. Ejaculates were extended to 25 and 100 million sperm per ml with Tris-based egg-yolk (T) extender at room temperature and stored at 4 °C. The concentration significantly had effects on motility, morphology, membrane and DNA integrity along with oxidative stress parameters ($P < 0.05$). Overall changes in the motility, morphology, membrane and DNA integrity along with oxidative stress parameters of ram sperm diluted with T in the 100×10^6 ml^{-1} concentration and preserved at 4 °C for a short term was found to be better preservation than that of diluted with T in the 25×10^6 ml^{-1} concentration. Significant concentration × days of storage interactions were found for all parameters except the NOx. Further studies are required to elucidate the oxidative stress changes on sperm quality parameters in different concentrations during the liquid storage of ram semen.

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1. Introduction

Depending on the time required for the transportation of the semen doses from the central station to the farms, such a short storage time makes it difficult to inseminate the ewes at an optimal time in the heat (Evans, 1988). Thus, storage of diluted semen has been widely used in artificial insemination (AI) programs. Diluted and cooled ram semen is an alternative to frozen semen when the insemination is done within a short period of time after collection. Comparing to the fresh semen, cooled ram semen suffers from a decrease in motility and morphological integrity, accompanied by a decline in the survival ability in the female reproductive tract, reduction in fertility and increased embryonic loss. These damages are less pronounced in diluted and chilled semen than in frozen–thawed ram semen (Aisen et al., 2002; Fiser and Fairfull, 1984; Gil et al., 2003). If the semen is diluted and stored, the practical use of liquid semen under farm conditions may be facilitated. Ram semen has been widely extended with Tris plus egg-yolk designed to protect and maintain spermatozoa during the processing and storage of the semen (El-Alamy and Foote, 2001; Gundogan, 2009;

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Maxwell and Stojanov, 1996; Paulenz et al., 2003; Simpson and White, 1986).

The target in the animal reproduction is to predict sperm fertilizing ability. Thus, farms usually assess the quality of seminal doses before used for AI. From time to time, the standard in vitro semen assay methods often do not provide reliable prediction of fertility in terms of ability to identify sub-fertile samples. However, other tests have also been developed based on sperm function and resistance of spermatozoa to osmotic changes of media (hypoosmotic swelling and osmotic resistance tests) (Avdatek et al., 2010). In addition, attempts have also been made to find molecules in seminal plasma that can be used as markers of spermatozoal fertility (e.g. heparin-binding proteins, clusterin, acrosin, reactive oxygen species or ATP) (Braundmeier and Miller, 2001). Sperm DNA damages have been associated with poor embryonic development and genetic abnormalities in the offspring (Kasimanickam et al., 2007; Loft et al., 2003). It is apparent that intact functional sperm membranes are important to achieve fertilization and DNA integrity is essential for embryonic development. Reactive oxygen species (ROS) in semen have a potential role in normal fertilization (Agarwal et al., 2006; Dacheux et al., 1981), but high levels of ROS in semen damage the spermatozoa, which results in infertility (Aitken, 1994; Upreti et al., 1997; Yeni et al., 2010). Levels of malondialdehyde (MDA), total antioxidant activity (AOA), reduced glutathione (GSH) and nitric oxide (NOx) indicate damage of lipids and proteins respectively by ROS. MDA is one of the products of lipid peroxidation and commonly used as a parameter to indicate oxidative stress (Kamal et al., 1990). It is important to ensure that any measurement of AOA is accurate and reliable and yet easy to use as a diagnostic tool in the evaluation and follow-up of male infertility (Donnelly et al., 1999; Fingerova et al., 2007; Kampa et al., 2002; Kolettis et al., 1999). GSH is the major non-protein thiol in mammalian cells and known to have numerous biological functions (Luberda, 2005). The scavenging action of GSH helps to counteract the effects of oxidative stress in sperm cells, which may result in irreversible loss of motility, leakage of intracellular enzymes and damage of chromatin. The protective action of GSH against ROS is facilitated by the interaction with its associated enzymes, such as glutathione peroxidase. NOx is known to involve in diverse physiological and pathophysiological processes in various organ systems, including the male reproductive tracts, hormonal aspects of testicular and epididymal function as well as in spermatogenesis and germ cell degeneration (Lue et al., 2003; O'Bryan et al., 2000; Thippeswamy et al., 2006). Hence, it would be interesting to evaluate the changes in the structural, functional and oxidative stress parameters of sperm during the cooled storage period in rams. Studies have been carried out about the storage of ram semen in liquid form and the effects of extenders on quality of ram semen stored at 4–5 °C (Gundogan et al., 2003; Gundogan, 2009; Kasimanickam et al., 2007; Lopez et al., 1999; Lopez-Sáez et al., 2000). But these studies mainly reported about the changes in the motility during cooled storage, and to our knowledge there are no studies about determining the changes in motility, abnormal sperm rate, HE-test and DNA integrity along with the oxidative stress parameters in diluted ram semen within two different concentrations and stored at 4 °C. A dose of $10–25 \times 10^6$ sperm is recommended for laparoscopic intrauterine insemination and $50–100 \times 10^6$ sperm is recommended for transcervical insemination in ewes (Gourley and Riese, 1990; O'Hara et al., 2010; Paulenz et al., 2003). The objective of the present study was to describe the changes in daily motility, morphology, HE-test, DNA integrity and oxidative stress parameters in Pirlak ram sperm that was diluted with Tris-based egg-yolk extender in two different concentrations and stored at 4 °C over a period of 5 days. However, under the prevailing conditions in Turkey, the effect of concentrations on the evaluated parameters as a clear in order to assess the changes during the storage the upper limit of the recommended doses as 25×10^6 and 100×10^6 spermatozoa ml^{-1} were selected in the present study.

2. Materials and methods

2.1. Semen collection and processing

Five sexually mature Pirlak rams (Daglic × Kivircik, local breed) (3 years of age) maintained at Afyon Kocatepe University, Research and Manipulation Farm of the Faculty of Veterinary Medicine in Afyonkarahisar, Turkey, were used in the study. From each ram 5 ejaculates were collected every other day using an artificial vagina. A total number of 25 ejaculates were included in the study. Immediately after collection, the ejaculates were immersed in a warm water bath at 37 °C until evaluation in the laboratory. Semen assessment was performed within 10 min after collection. Chemicals used in this study were purchased from Sigma Chemical Co. (St. Louis, MO, USA). The volume of each ejaculate was measured in a conical tube graduated at 0.1 ml intervals. Subjective sperm motility was assessed after collecting semen samples using a phase contrast microscope (Olympus CX31, Olympus Optical Co., Ltd., Japan) at 400× magnification equipped with a heated stage adjusted to 37 °C. Motility estimations of each sample were performed in five different fields by the same person throughout the study and the mean value averaged from five successive estimations was used as the final motility score. Sperm concentration for per milliliter of semen was haemocytometrically determined as described by Ax et al. (2000) using a Thoma counting chamber after dilution with distilled water at 1:200 (v/v). All sperm parameters in the ejaculate were within what is considered to be a normal range for ram semen (semen volume ≥0.7 ml; sperm motility >70%; sperm concentration $>3.0 \times 10^9$ ml^{-1}). Semen suspensions were extended to the concentrations of 25 and 100 million sperm per ml with Tris-based egg-yolk extender (T: containing Tris (3.63 g), fructose (0.5 g); citric acid (1.99 g) and distilled water 100 ml, and mixed with 15% (v/v) of egg yolk) at room temperature and then diluted semen samples were stored at 4 °C in water bath. Prior to storage at 4 °C, estimations of the first parameters were released and findings were saved as initial parameters (Day 0).