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Effect of argan oil on liquid storage of ram semen in Tris or skim milk based extenders☆

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ABSTRACT

Due to its high antioxidant content, the argan oil could play a beneficial role in liquid storage of ram semen. The aim of this study was to investigate effects of different concentration of argan oil (ARO) on spermatologic parameters, lipid peroxidation and DNA fragmentation during liquid storage of ram semen until 48 h. Also effects of extenders and temperature on same parameters were assessed. For these aims, semen samples were collected from Boujaâd rams, extended with Tris egg yolk or skim milk extenders without (control) or supplemented with different concentrations of ARO (1%, 2%, 5% and 10% v/v) at a final concentration of 0.8×10^9 sperm/mL and stored until 48 h at 5 °C or 15 °C. The sperm quality assessments were performed at different intervals during storage (0, 8, 24 and 48 h). Sperm progressive motility started to decrease after 8 h of storage in all temperatures – extenders combinations and dropped steadily during the 8–48 h interval. However, sperm viability, progressive motility and membrane integrity were markedly higher in ARO groups (especially in 1% in Tris and 5% in skim milk) until 24 h and 48 h storage at both temperatures compared to controls. The argan oil also decreased the level of spontaneous and induced malondialdehyde (MDA) and the sperm DNA fragmentation until 48 h storage. In conclusion, it was determined that addition of argan oil to conventional extenders may improve the quality of ram semen during liquid storage in different temperatures.

1. Introduction

Artificial insemination (AI) in sheep is an efficient tool for genetic improvement and also management of reproduction. One of the critical steps of AI is the ram semen preservation, liquid storage or freezing. The freezing–thawing of ram semen results in stronger damages to spermatozoa when compared to liquid storage (Evans and Maxwell, 1987). Therefore, intrauterine

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insemination may be needed to increase success of AI with frozen–thawed semen while only cervical insemination with liquid storage semen provides satisfactory results.

Successful liquid storage of ram spermatozoa is dependent on the reversible reduction of motility and metabolic activity of the spermatozoa, thereby prolonging their fertile life. This is achieved by storing semen at low temperatures (5 °C and 15 °C) and in diluents including compounds that help to delay the production of reactive oxygen species. Many extenders have been used for ram semen preservation but Tris egg yolk and skim milk are the most used and also skim milk based extenders has given satisfactory results in storage of ram semen, compared to Tris based extenders (Ari et al., 2011). The effective components of egg yolk are the low density lipoprotein and the phospholipids such as the phosphatidycholine which maintains and prevents the sperm membrane against the cold shock (Bergeron et al., 2004; Bergeron and Manjunath, 2006; Forouzanfar et al., 2010; Kulaksız et al., 2010). In skim milk, the casein micelles can protect ram spermatozoa during storage at low temperature (Martin, 1966), reduce sperm lipid loss, while maintaining sperm motility and viability (Bergeron et al., 2007). Insemination with fresh semen should be performed within 10 h after collection to obtain a maximum fertility. This limits the spreading of the artificial insemination method in farms located far from the semen production places such as artificial insemination centers (Druart et al., 2009).

One mechanism which is responsible for the negative biochemical and physiological changes during sperm storage can be the production by the spermatozoa of reactive oxygen species (ROS) induced by the degradation of high level of polyunsaturated fatty acids content in spermatozoa membranes (Hong et al., 2010; Lamirande et al., 1997; Maxwell and Salamon, 1993; Vishwanath and Shannon, 2000). ROS can attack a variety of biological macromolecules such as proteins and lipids, causing oxidative damages (Kelly et al., 1998) and affect acrosome integrity and DNA fragmentation (Gosalvez et al., 2007; López-Fernández et al., 2008; Mammoto et al., 1996). The ram seminal plasma contains appreciable amount of endogenous antioxidants such as superoxide dismutase and much lower concentrations of glutathione peroxidase and Catalase (Abu-Erreish et al., 1978; Mann and Lutwak-Mann, 1981). However, their concentrations may be considerably reduced by the semen dilution while the anti-oxidant supplementation of the extenders has been shown to improve the motility and reduce the cell degree damages (Jones and Mann, 1976; Krzyzosiak et al., 2000; Maxwell and Stojanov, 1996; Ollero et al., 1996; Sánchez-Partida et al., 1997; Sarlós et al., 2002; Upreti et al., 1997; Yousefian et al., 2014). For instance it has been observed that catalase and superoxide dismutase can act as an antioxidant to protect the ram sperm from oxidative stress (Câmara et al., 2011; Maxwell and Stojanov, 1996). Incorporating antioxidants in the extenders could be a promising solution to prevent detrimental membrane changes during the chilling, freezing and thawing process (Maxwell and Watson, 1996) and then extend the operational life of spermatozoa which could facilitate large-scale insemination.

Argania spinosa (L.) tree is endemic to Morocco and is known worldwide for its oil, which is extremely rich in unsaturated fatty acids and used in the food and cosmetic industries. This tree allows to harvest Argan seed oil documented (Harhar et al., 2011; Hilali et al., 2005; Khallouki et al., 2003; Matthäus et al., 2010) to be rich in tocopherols (alpha, beta, gamma and delta) which are considered to have high biological natural antioxidant activity. It is believed that the main biochemical function of tocopherols is to protect polyunsaturated fatty acids from peroxidation (Beringer and Dampert, 1976; Drissi et al., 2004; Kamal-Eldin and Andersson, 1997). In addition, other components of argan oil that can act as an antioxidant are the phenolic acids (vanillic acid, ferulic acid and syringic acid) (Khallouki et al., 2003). Thus, the aim of this study was to investigate effects of argan oil during liquid storage on ram semen parameters.

2. Materials and methods

2.1. Chemicals

Unless otherwise specified, all chemicals used in this study were purchased from Sigma (St. Louis, MO, USA) and Merck (Merck Schuchardt OHG, Germany).

2.2. Argan oil composition

The cosmetic argan oil used in this work originated from Essaouira area located at the southwest part of Morocco. It was extracted by a cold pressing and commercially available at Zit Sidi Yassine Ltd. (a Swiss-Moroccan company).

2.3. Animals and samples collection

Five adult fertile Boujaâd rams (3–4 years of age) were used in this study. They were housed at INRA-Morocco, Regional Center of Settat, Laboratory of Reproduction Biology, with a proper balanced diet and a free access to water. A total of 50 ejaculates (10 ejaculates from each ram) were collected using an artificial vagina (40–42 °C) during the breeding season. Immediately after collection, all ejaculates were placed in a water-bath at 37 °C. Sperm concentration was evaluated using a calibrated spectrophotometer. The ejaculates were evaluated and pooled if the following criteria were obtained: concentration > 2×10^9 sperm/mL, wave motion > 3, and individual motility > 70%.

2.4. Extender preparation

A skim milk (11 g skim milk in 100 mL distilled water) was heated 10 min at 95 °C (Colas et al., 1968) and Tris egg yolk (2.666 g Tris, 0.44 g fructose, 1.398 g citric acid, egg yolk 12% in 100 mL distilled water, pH 6.8) were prepared to be used as extenders. Penicillin and streptomycin (0.5 mg/mL) were added to the extenders. Then they were supplemented with different concentrations of argan oil (0%, 1%, 2%, 5% and 10%). The extenders without oil addition were considered as a control. Spermatozoa were diluted to a final concentration of 0.8×10^9 spermatozoa/ml and then