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Dietary flax seed oil and/or Vitamin E improve sperm parameters of cloned goats following freezing-thawing

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ABSTRACT

Semen cryopreservation is affected by individual differences and use of clones animal from the same source is the main tool to eliminate genetic variation. Among many nutrients that are necessary for fertility, essential fatty acids and antioxidants are vital for production of healthy sperm by improving sperm membrane integrity and protecting sperm from oxidative stress. The goal of the current study was to investigate whether a flax seed oil or/and Vitamin E dietary supplementation could improve semen quality of cloned bucks following semen cryopreservation. Accordingly, eight adult cloned Bakhtiari bucks were divided randomly into four groups. Bucks were offered a base diet of hay and concentrate. The concentrate was enriched with flax seed oil, 30 gr/kg body weight/day (OIL), Vitamin E (VIT), 3 gr/kg body weight/day, or combined flax seed oil and the vitamin E (OIL-VIT). The concentrate with no supplements was considered as control group (CONT). Both flax seed oil and Vitamin E supplements were added to the total diet. The bucks were fed with their corresponding diets for a total of 9 weeks while sperm collection was carried out within 10–14 weeks. Ejaculates were diluted with Andromed® and were frozen in liquid nitrogen. Sperm parameters and reactive oxygen species (ROS) contents were evaluated following freezing/thawing. According to the results of our study, dietary supplementation with flax seed oil, or/and Vitamin E can improve sperm motility, vitality and number of sperm with intact plasma membrane following freezing-thawing. But the degree of improvement in these parameters was significantly higher when Flax seed oil and vitamin E were co-supplemented.

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1. Introduction

Intra-class variances due to differences in genetic and environmental factors are unavoidable nuisance factors that can substantially affect results of any experiment on animals. Even though this problem has been theoretically solved by providing a large homogenous sample size, this intra-class discrepancy has remained the main problem for evaluation of independent factors in animal science and medical experiments. Use of identical twins or high number of clones from the same sources was suggested as the main tool to eliminate the genetic source of individual differences [4].

Artificial insemination (AI) in goat is a powerful tool that can allow breeders to use superior bucks and subsequently to improve rate of genetic gain, decreasing risk of sexually transmitted diseases and accuracy of time of kidding [5,6]. Although a doe can be inseminated with fresh semen, success of AI mainly depends on sperm cryopreservation technique [7]. A variety of injuries occur at cellular and molecular levels during semen cryopreservation which may impair sperm function and fertilization potential when compared to fresh semen. Reduction in sperm fertilization potential during semen cryopreservation can be partly accounted by excessive production of reactive oxygen species (ROS) which plays a central role in induction of cryo-injury through oxidative stress [8–10]. Like other cells, sperm plasma membrane is made of a phospholipid bilayer which contains large amounts of polyunsaturated fatty acids (PUFA). This lipid composition naturally

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undergoes a series of modifications in sperm head during capacitation and acrosome reaction [11,12]. This provides sperm membrane fluidity which is needed for committing membrane fusion event during fertilization process [13]. However, the sperm PUFAs are extremely vulnerable to oxidative damages which are mainly generated by ROS [14]. To overcome this problem, antioxidants are added in semen extenders during semen cryopreservation of most of farm animal species [15–17]. On the other hand, semen quality depends on season, breed, genetic value of buck, buck health, age, and most importantly the diet. It seems that feeding specific supplements can improve semen quality by increasing sperm quantity, motility, viability as well as antioxidants capacity in cellular and seminal plasma. Among many nutrients that are necessary for fertility, essential fatty acids are vital for production of healthy sperm by improving sperm membrane structure and protecting sperm from oxidative stress [18–20].

With regard to effect of individual variations on sperm freezing-thawing outcome, we have a great opportunity of having nine cloned bucks, which were produced by somatic cell nuclear transfer (SCNT) from the same cell line, to test different hypothesis while eliminating genetic source of variation [20]. The aim of the present study was to evaluate the effect of dietary supplementation of flax seed oil as a PUFA, or/and Vitamin E as an antioxidant, on sperm parameters of cloned bucks following freezing-thawing.

2. Material and methods

2.1. Chemicals

All chemicals used in this study were purchased from Sigma Chemical CO. (St. Louis, MO, USA) unless stated otherwise. Andromed® as semen extender was obtained from Minitube, Germany.

2.2. Animals

This experiment was conducted at the animal farm of Reproductive Biotechnology research center at Royan Institute (Isfahan, Iran latitude 32°39′N). Eight adult cloned Bakhtiari bucks were randomly selected from nine cloned bucks aged 2–3 years, and then were divided randomly into four groups (two per group).

Bucks were offered a base diet according to small ruminant nutrition system(SRNS) Cornell University (Version:1,9,4468). Treatments groups were contained flax seed oil, 30 gr/kg body weight/day (OIL), Vitamin E (VIT), 3 gr/kg body weight/day, or combined flax seed oil and vitamin E (OIL-VIT). In addition, the concentrate with no supplements was considered as the control group (CONT). Both flax seed oil and Vitamin E supplements were added to basic diet. Diets were formulated as percent of Dry Matter (100 ± 5). Control group received a basic diet (Alfalfa; 22.4, Straw; 44.9, Barley; 7.67, Corn; 22.7, Salt; 1.1, Mineral supplements; 0.55, Vitamin supplements; 0.55, Mono Calcium Phosphate; 0.14,, Calcium; 0.42 and potassium 0.21% of dry matter containing 7.07% Crud protein and 1.74 mcal metabolism energy), whereas, VIT group received basic diet supplemented with vitamin E, 3 gr/kg body weight/day contain 1.74 mcal metabolism energy, OIL group received basic diet supplemented with flax seed oil, 30 gr/kg body weight/day, containing 1.91 mcal metabolism energy and OIL-VIT group received basic diet supplemented with both vitamin E and flax seed oil, 3 and 30 gr/kg body weight/day, respectively containing 1.91 mcal metabolism energy.

The bucks were fed with their corresponding diets for a total of 9 weeks while the sperm collection was carried out within 10–14 weeks.

2.3. Semen collection, processing, and freezing-thawing

Semen collection and processing were carried out according to Forouzanfar et al. [17]. In brief, totally 10 ejaculates were obtained by artificial vagina from the bucks twice a week, started from week-10 to week-14, which was corresponded to the breeding season (April/May, 2015). The collected samples of raw semen from each cloned bucks were kept separate and transported at 35 °C for approximately 30 min to the laboratory for microscopic evaluations. A total of 6 from 10 ejaculates which showed at least 70% motile, 80% morphologically normal sperm and 1–2 ml volume in each treatments and control groups were mixed and used for further steps. For freezing, all of the treatments were repeated for at least 6 times with the mixed semen samples from the two bucks in each group. Each semen sample was diluted to final concentration of 400 × 106 spermatozoa/ml (200 × 106 spermatozoa per straw) with Andromed® extender, cooled to 4 °C over a period of 2 h, drawn into 0.5 ml French straws (Biovet, L'Agile France), and heat-sealed. Then the straws were kept at 4 °C for one hour followed by exposure to liquid nitrogen (LN2) vapor for 12 min, plunged into LN2, and stored in LN2 until being thawed, and used for evaluation of sperm parameters. Thawing step was carried out by plunging the straws in a 37 °C water bath for 30 s. The thawed samples were kept at room temperature and individually evaluated by a single trained individual.

2.4. Measurement of sperm motility after freezing-thawing process

Measurement of sperm motility was carried out according to Shafiei [14]. After thawing, 4–5 straws from each replicate were diluted with fertilization medium (Tyrode's albumin lactate pyruvate medium- Fert-TALP) to final concentration of 1×10^6spermatozoa/ml. The percentages of rapid progressive that swim fast in a straight line (class A), slow progressive that move forward but tend to travel in a curved line (class B), and non-progressive that do not move forward despite that they move their tails (class C), as well as the total motility, which refers to the population of sperm that display any type of movement were assessed using a computer-assisted sperm analysis (CASA) system (Video Test, ltd: version Sperm 2.1© 1990-2004, Russia) [21]. For each sample, 5 μl of sperm suspension was loaded on a pre-warmed slide and then covered by an 18 × 18 mm coverslip, a minimum of 500 sperm per sample were analyzed in at least three different microscopic fields.

2.5. Assessment of sperm plasma membrane integrity

Sperm plasma membrane integrity was estimated by the hypo-osmotic swelling test (HOST) based on curled and swollen tails [22]. Following 30 min incubation of 25 μl sperm suspension and 200 μl of HOST solution (100 mOsm/l, 57.6 mM fructose and 19.2 mM sodium citrate) at room temperature, 5 μl of homogenized mixture was mounted and directly investigated using an inverted microscope (Olympus, CKX41 - Japan). The percentage of intact-membrane spermatozoa was estimated by recording of at least 200 spermatozoa in more than five different microscopic fields.

2.6. Assessment of sperm viability

Sperm viability was evaluated using the eosin-nigrosin staining following the thawing process as described previously [15]. In brief, 50 μl of diluted semen was mixed with 100 μl of eosin (1%) in a test tube for 30 s and stained with nigrosin (10%) for 30 s. One drop of stained semen was loaded on a slide and a semen smear prepared. The smears were air dried and examined directly. At least 200

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