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Effect of dietary fish oil supplementation on ram semen freeze ability and fertility using soybean lecithin– and egg yolk–based extenders

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ABSTRACT

Ram semen cryopreservation is not efficient for artificial insemination in commercial herds. Beneficial effects of dietary fish oil have been evaluated for cryopreservation of ram semen in soybean lecithin (SL) and egg yolk (EY)–based extenders. A factorial study (two diets × two extenders) was used to analyze the effects of two diets supplemented with fish oil (n-3 fatty acid) or palm oil (saturated fatty acids; [SFAs]) to freeze ram semen in two extenders containing SL or EY. Motility characteristics, membrane integrity, abnormal morphology, mitochondria activity, acrosome integrity, apoptotic status, and fertilizing ability were assessed after freeze-thawing. Although diet had significant ($P \leq 0.05$) effects on the quality parameters of frozen-thawed sperm, effects of extenders on these traits were not significant ($P > 0.05$). The higher significant ($P \leq 0.05$) percentage of total motility and progressive motility were observed in n-3/SL (44.83 ± 1.56 and 28.33 ± 1.4) and n-3/EY (43.33 ± 1.56 and 28.50 ± 1.4) than SFA/SL (32.16 ± 1.56 and 14.00 ± 1.4) and SFA/EY (31.66 ± 1.56 and 12.66 ± 1.4) groups. Moreover, n-3/SL and n-3/EY produced the higher significant ($P \leq 0.05$) percentage of membrane integrity of sperm (39.83 ± 1.4 and 37.33 ± 1.4) than SFA/SL and SFA/EY (29.83 ± 1.4 and 28.5 ± 1.4). For viability results, the higher significant percentage of live sperm was observed in n-3/SL and n-3/EY (43.16 ± 1.38 and 45.66 ± 1.38) than SFA/SL and SFA/EY (28.66 ± 1.38 and 27.5 ± 1.38). For fertility trials, n-3–based diets (n-3/SL and n-3/EY) improved significantly ($P \leq 0.05$) pregnancy rate (44% and 46%), parturition rate (42% and 42%), and lambing rate (46% and 44%) compared with the SFA-based diets (SFA/SL and SFA/EY). No interaction effects have been found between diets and extenders ($P > 0.05$). It seems that dietary fish oil can improve the semen performance after freezing–thawing process and artificial insemination aside from type of extenders.

1. Introduction

Polyunsaturated fatty acids (PUFAs) in sperm cells play a crucial role for metabolism energy [1], plasma membrane fluidity [2], and many related function to fertilization events [3]. Ram sperm cells contain high amount of omega-3 polyunsaturated fatty acids specially docosahexaenoic

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acid (DHA) and eicosapentaenoic acid (EPA), which are synthesized in the body from short-chain fatty acids (alpha-linolenic acid) [4]. Docosahexaenoic acid and EPA are provided for body using their precursors (alpha-linolenic acid) in the diet [5] or direct inclusion to the diet [2,4]. Efficacy of dietary fish oil as a common source of omega-3 fatty acids has been evaluated to improve sperm quality as well as fertility in human [2,6,7], bull [8], boar [9–12], ram [13,14], goat [15], and avian species [16,17]. These beneficial effects are mainly related to their impacts on the sperm plasma membrane, which leads to the protection of sperm against environmental damages [14]. Sperm plasma membrane is strongly affected by various biochemical and anatomical damages in freezing procedure [18]. Therefore, supplementation of diet with sources of DHA and EPA can be an efficient strategy for protection of sperm against cryoinjuries. Moreover, we used a new plant-based extender for cryopreservation of ram semen containing soybean lecithin (SL), which was applied for freezing of ram [19], goat [20], and human spermatozoa [21]. Soybean lecithin considerably increased the percentage of motility, viability, and cellular parameters of frozen-thawed sperm via formation of a layer around sperm against reactive oxygen species [19]. Up to now, no study has been conducted for evaluation of frozen-thawed sperm quality and fertility obtained from ram fed diets supplemented with omega-3 and cryopreserved with SL-based extender. Therefore, we performed this experiment to assess several frozen-thawed sperm parameters such as motion characteristic, membrane integrity, morphology, mitochondria activity, and acrosome integrity and viability. Finally, artificial insemination was performed to evaluate the fertility potential of thawed sperm.

2. Materials and methods

Chemicals used in this study were obtained from Sigma Co. (St. Louis, MO, USA) and Merck (Darmstadt, Germany) unless otherwise indicated. Approval for the present study was given by the Research Ethics Committees of Royan Institute.

2.1. Animals and diets

Twenty mature fat-tailed Zandi rams with proven fertility (3 to 4 year old and 75 ± 2.5 kg body weight) were randomly assigned into two groups in the breeding season as follows: (1) control group received a diet (Agriculture and Food Research Council [AFRC], 1995) supplemented with palm oil (3% dry matter [DM]) and (2) fish oil–based diet group received a diet (AFRC, 1995) supplemented with fish oil (3% DM, Khazar Fish Powder Company, Iran). Both diets were set isocaloric and isonitrogenous (metabolizable energy: 2.24 Mcal/kg in DM and crude protein: 12% in DM, Table 1). After 60 days feeding of rams with diets, semen collection was begun, and the semen collected from each group was divided into two aliquots for cryopreservation in two extenders (egg yolk (EY)–based extender and SL-based extender). Therefore, this experiment was performed in a factorial-based design (two diets × two extenders) with six replicates.

Table 1
Components of diets.

Components	Control (palm oil)	Treatment (fish oil)
Alfalfa (% DM)	41.96	41.96
Corn silage (% DM)	28	28
Straw (% DM)	9.5	9.5
Barley (% DM)	6.04	6.04
Wheat bran (% DM)	10.18	10.18
Palm oil (% DM)	3	0
Fish oil (% DM)	0	3
$CaCO_3$ (% DM)	0.5	0.5
NaCl (% DM)	0.32	0.32
Vitamin E (% DM)	0.5	0.5
Metabolizable energy (Mcal/kg DM)	2.24	2.24
Crude protein (% in DM)	12	12
Ether extract (% in DM)	5.28	5.28
Neutral detergent fiber (% in DM)	48.6	48.6
Calcium (% in DM)	0.79	0.79
Phosphorus (% in DM)	0.31	0.31

2.2. Semen collection and cryopreservation

Semen samples were collected from each ram using artificial vagina twice a week during 3 weeks (six replicates). Ejaculation samples were accepted for experiment if the following parameters were observed: volume ranging between 1 and 2 mL; sperm concentration of 3×10^9 sperm/mL; and sperm total motility higher than 70 percent. To eliminate individual differences, semen obtained from each group was pooled and then divided into two groups to be diluted with two extenders (extender supplemented with 1% SL vs. extender supplemented with 20% EY). The component of the basic extender was according to our previous study [22]. After dilution of semen with extenders, semen was loaded into 0.25 mL French straws reach up to final concentration of 100×10^6 sperm/mL and sealed with polyvinyl alcohol powder and held at 4 °C for 120 minutes. Then, straws were placed 7 cm above the liquid nitrogen for 12 minutes and finally plunged into the liquid nitrogen for storage. For evaluation of frozen semen, straws were melted at water bath (37 °C, 40 seconds).

2.3. Sperm evaluation after cryopreservation

2.3.1. Motion characteristics

Sperm class analyzer (Animal version 12.3 CEROS; Hamilton-Thorne Biosciences, Beverly, MA, USA) was used to evaluate motion characteristics. Evaluated parameters consisted of total motility (%); progressive motility (%); average path velocity (m/s); straight-line velocity (m/s); curvilinear velocity (m/s); amplitude of lateral head displacement (m); beat/cross frequency (Hz); straightness (%); and linearity (%) [23].

2.3.2. Membrane integrity and morphology

Hypo-osmotic swelling test was performed according to Revell and Mrode [24] with slight corrections to evaluate membrane integrity. Twenty microliters of semen was added to 200 μL of the hypo-osmotic solution (9.0-g