Theriogenology 81 (2014) 210–219

Contents lists available at ScienceDirect

Theriogenology

journal homepage: www.theriojournal.com

The effect of dietary *n*-3 polyunsaturated fatty acids supplementation of rams on semen quality and subsequent quality of liquid stored semen

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ARTICLE INFO

Article history:
Received 26 June 2013
Received in revised form 26 August 2013
Accepted 2 September 2013

Keywords:
Fertility
Sheep
Sperm
PUFA
Lipid peroxidation
Mucus penetration

ABSTRACT

The objective of this study was to examine the effect of dietary *n*-3 polyunsaturated fatty acid (PUFA) supplementation of rams on semen quality and subsequent sperm function of liquid stored semen. Mature rams of proven fertility were individually housed and were blocked according to breed, body weight, and body condition score and randomly allocated within block to one of two dietary treatments (N = 7 per treatment). Rams were offered a base diet of hay and concentrate, with the concentrate enriched with either: (1) saturated palmitic acid (CON) or (2) high *n*-3 PUFA fish oil (FO) supplements. Both lipid supplements were added at 2% (wt/wt) of the total diet as fed and both were partially rumen-protected. The animals were fed their respective diets for a total of 9 weeks and blood samples were collected on weeks 0 (pre-experimental), 4, and 9, relative to initial allocation of diet (week 0), for measurement of plasma concentration of fatty acids, metabolites, insulin like growth factor 1 (IGF-1) and insulin. Semen was collected from each ram (on 1 day in each week) in weeks 4, 5, 7, 8, and 9, and each ejaculate was assessed for volume, wave motion, and concentration of sperm, after which it was diluted in a skim milk-based extender and stored at 4 °C. A second ejaculate was collected on weeks 4, 7, and 9, centrifuged, and the sperm frozen for subsequent lipid analysis. A sample of semen from each ram was assessed at 24, 48, and 72 hours after collection for sperm progressive linear motion, ability to penetrate artificial mucus, and the ability to resist lipid peroxidation (at 24 and 48 hours only) using the thiobarbituric acid reactive substances assay. There was no effect of diet on plasma insulin concentrations or on any of the metabolites measured, however, there was a diet by week interaction for plasma IGF-1 concentration ($P < 0.05$). This was manifested as the FO supplemented rams having higher IGF-1 concentrations on week 9 compared with the control treatment ($P < 0.05$), but not at the earlier sampling dates. Compared with the pre-experimental values, supplementation with FO increased plasma concentrations of total *n*-3 PUFAs by 3.1-fold and decreased *n*-6 PUFA concentrations by 1.84-fold. Consequently, the ratio of *n*-6 to *n*-3 PUFA was decreased in the FO-supplemented rams ($P < 0.001$). Dietary supplementation with FO increased the concentration of eicosapentaenoic acid in sperm from week 4 to 9 by 2.7-fold ($P < 0.05$) leading to a 1.5-fold increase in total *n*-3 PUFA in the same period. Ejaculates collected from rams supplemented with FO yielded a higher semen concentration ($P < 0.05$), however, there was no difference between diets on any of the other semen quality parameters including semen volume, wave motion, progressive linear motion, ability to penetrate artificial mucus, or ability to resist lipid peroxidation. In conclusion, dietary supplementation of rams with *n*-3 PUFA

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0093-691X/$ – see front matter
http://dx.doi.org/10.1016/j.theriogenology.2013.09.002

successfully increased the n-3 PUFA content of plasma and sperm but has limited effects on the quality of liquid stored semen.

1. Introduction

It is well established that dietary supplementation of lipid can increase the dietary energy density (reviewed by Staples et al. [1]) but recently the inclusion of specific polyunsaturated fatty acids (PUFAs) has been shown to have a number of positive benefits for the reproductive performance of female ruminants including enhanced steroidogenesis [2–5], greater ovarian follicle and luteal size [3,6], increased embryo survival [7], and gene expression [2,8]. PUFAs of the *n*-3 and *n*-6 series are essential fatty acids, because they cannot be synthesized *de novo* in mammals and therefore, must be provided by the diet. Childs et al. [9] demonstrated that supplementation of heifers with a semirumen protected high *n*-3 PUFA supplement significantly enhanced the concentration of *n*-3 PUFAs in a number of reproductive tissues compared with unsupplemented control animals, and others using the same model [2,8,10] observed differential expression of a number of genes critical to $PGF_{2\alpha}$ in the bovine endometrium.

Like all mammalian cells, sperm have a plasma membrane that is made up of a phospholipid bilayer and this contains large amounts of PUFAs. PUFAs are involved in physicochemical modifications of the sperm head during capacitation [11] and gives the sperm membrane the fluidity it needs to participate in the membrane fusion events associated with fertilization [12]. However, these PUFAs are extremely vulnerable to oxidative damage generated by reactive oxygen species (ROS) [13–15]. Although ROS are involved in cell signaling at low levels, excessive production of ROS can lead to oxidative stress, lipid peroxidation, DNA damage, and associated impairment of sperm function [16]. In addition, sperm have a relatively small amount of cytoplasm which contains low concentrations of antioxidants [17]. Therefore, there is a critical balance between lipids, ROS, and the antioxidant system in the environment surrounding the sperm, which is required to ensure their efficient functionality. It is perhaps this complex interaction that has led to conflicting reports in the scientific literature on the relationship between the fatty acid profile of sperm and subsequent fertility. There are two main approaches to date which have been used to investigate the role of fatty acids in sperm cell function, namely: the comparison of the fatty acid profile of high and low fertility males, and dietary supplementation with PUFAs to alter the milieu in which spermatogenesis occurs and therefore, the composition of the sperm membrane [18].

Sperm from infertile men has been reported to have higher concentrations of saturated fatty acids [19] and lower levels of *n*-3 fatty acids [20]. Similarly, Am-in et al. [21] compared the sperm fatty acid profiles from boars having normal or low sperm motility, and reported that the level of saturated fatty acids and the ratio of *n*-6:*n*-3 PUFAs was negatively correlated with sperm motility, viability, morphology, and plasma membrane integrity.

Although dietary supplementation with a wide range of PUFA supplements has been shown to alter the sperm fatty acid profile, there are conflicting results on the effect of this on fresh and frozen-thawed sperm quality. Studies with rams [22], bulls [23], fowl [24,25], and boars [26–29], have suggested benefits after dietary supplementation of *n*-3 fatty acids on male reproductive parameters, whereas, other studies in rams [30], humans [31], turkeys [32], chickens [33], rabbits [18], and boars [34,35] failed to show any significant effect on semen quality. Despite this, and to the best of our knowledge, there is no study investigating the effect of dietary *n*-3 PUFA supplementation of rams on the subsequent quality of liquid stored semen.

Thus, the objective of this study was to examine the effect of dietary *n*-3 PUFA supplementation of rams on the incorporation of PUFAs into ejaculated sperm and subsequently evaluate its effect in stored liquid semen through assessments of progressive linear motion (PLM), ability to penetrate artificial mucus, and ability to resist lipid peroxidation.

2. Materials and methods

2.1. Experimental design

The study was carried at the Animal Production Research Centre, Teagasc, Athenry, Galway, Ireland. Mature (11–36-month-old) Belclare (N = 6), Suffolk (N = 5), and Texel (N = 3) rams of proven fertility and with a mean ± SEM live weight of 67.0 ± 4.43 kg and body condition score (BCS) of 3.6 ± 0.09 units were used for the experiment. Rams were individually housed and were blocked by breed, body weight, and BCS, and randomly allocated within block to one of two treatments (N = 7 per treatment): control (CON; 2% [wt/wt] saturated fat; palmitic acid) and fish oil (FO; 2% [wt/wt] FO). Both lipid supplements were added at 2% (wt/wt) of the total diet as fed and rams were fed their respective diets for a total of 64 days (9 weeks). Blood samples were collected on week 0 (pre-experiment), 4, and 9 (Fig. 1) for measurement of plasma concentration of fatty acids and a number of blood metabolites. Semen was collected from each ram (on 1 day

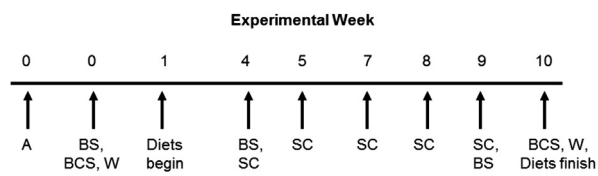

Fig. 1. Schedule of tasks undertaken for the experiment. A, acclimatization; BCS, body condition score; BS, blood sample; SC, semen collection; W, weigh.