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Influence of supplementing diet with Oleic and Linoleic acid on the freezing ability and sex-sorting parameters of ram semen

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Abstract

In an effort to improve the cryosurvival of both non-sorted and sex-sorted ram spermatozoa the effect of supplementing the ram diet with Oleic and Linoleic acid, in the form of extra virgin olive oil and sunflower oil, respectively, was assessed. Rams ($n=4$/group) were fed either (i) a standard maintenance diet (Control), (ii) maintenance diet + 5% (w/w) sunflower oil (Linoleic), or (iii) maintenance diet + 5% (w/w) extra virgin olive oil (Oleic) for a period of 6 weeks. The effect of these diets on the post-thaw (incubated 37 °C, 6 h) motility characteristics (as measured by CASA) of non-sorted, frozen-thawed ram spermatozoa were assessed every 2 weeks. The sex-sorted, frozen–thawed spermatozoa were assessed at the end of the 6 week trial period in the same manner. Linoleic and Oleic diets had a negative impact ($P<0.05$) on the total sperm motility, viability and acrosome integrity after a 6 week period of dietary supplementation. Furthermore, the average path velocity and straight line velocity of spermatozoa from Oleic-fed rams was less when compared to samples originating from rams fed linoleic acid or the control diets after both 2 and 6 weeks post-diet modification. Curvilinear velocity of oleic spermatozoa 2 weeks post diet modification were inferior for Oleic—($P<0.05$) compared with Linoleic—but not control-fed rams. Spermatozoa from rams fed Oleic diets exhibited lower ($P<0.05$) linearity than spermatozoa from rams fed Linoleic acid (2, 4 and 6 weeks) or the control diets (6 weeks). Diet did not significantly affect any motility characteristic or the viability/acrosome integrity of sex-sorted spermatozoa. Nutritional supplementation with the mono-unsaturated fatty acid, Oleic acid, or the polyunsaturated fatty acid, Linoleic acid, did not improve the cryosurvival of ram spermatozoa — whether or not it had been processed for sex-sorting by flow cytometry. However, these results provide insight into the relationship between nutrition and male reproductive characteristics and further research to elucidate the mechanisms by which diet manipulation affects sperm membranes and subsequent sperm quality is warranted.

Keywords: Cryopreservation; Sex-sorting; Spermatozoa; Sheep; Unsaturated fatty acids; Diet

1. Introduction

Cryopreservation is known to elicit a deleterious effect on the motility, viability and membrane status of spermatozoa (Watson, 1995). These effects are exacerbated when cryopreservation is combined with sex-separation by means of flow cytometry (Hollinshead et al., 2003) — a technique which has produced pre-sexed offspring in a number of species (Garner, 2006). Cryosurvival of sex-sorted spermatozoa is of added importance due to the time taken to sex-sort sufficient spermatozoa for an insemination dose and the inverse relationship between post-thaw sperm survival and total

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sperm dose required for AI. These factors contribute significantly to the price premium associated with sexed spermatozoa (Seidel, 2003). For these reasons, methods to improve the cryopreservation of both non-sorted and sex-sorted spermatozoa have been sought, with reasonable success being achieved (Schenk et al., 1999; Hollinshead et al., 2003). However, further improvement is needed to reduce the total number of sex-sorted spermatozoa required for in vitro fertilisation (IVF) or AI and subsequently improve the commercial potential of sex-preselection technology.

Recently, a greater understanding of the role of polyunsaturated fatty acids, and lipids in general, in sperm function has led to a desire to affect the lipid content of sperm membranes (White, 1993; He et al., 2001). While this usually involves the addition of substances to spermatozoa at some stage during the cryopreservation process, the idea of modifying sperm membranes prior to ejaculation within the male, perhaps via dietary intake, represents an attractive alternative for successful membrane modification (Perez-Pe et al., 2001; Purdy and Graham, 2004). It has long been known that diet plays a significant role in reproductive physiology. In rams, an increased plane of nutrition increases testicular size and sperm production (Oldham et al., 1978), primarily due to heightened digestible energy intake (Murray et al., 1990). Modification of certain dietary components, such as water soluble vitamins, has also improved the rate of spermatogenesis in boars (Audet et al., 2004). An early report in the ram suggested the feeding of soya bean oil, high in Linoleic acid, improved post-thaw motility (Milovanov and Golubj, 1973). This finding is supported by enhanced semen quality in poultry (Kelso et al., 1997; Cerolini et al., 2003) and rabbits (Castellini et al., 2005) fed a diet high in alpha-linolenic acid. Supplementation of boar diets with polyunsaturated fatty acids and anti-oxidants has also aided sperm survival during chilled transport (Strzezek et al., 2004). As for frozen semen, an improvement in post-thaw quality has been reported for spermatozoa from stallions fed a diet high in docosahexaenoic acid (Brinsko et al., 2005), a polyunsaturated fatty acid prevalent in the semen of most species (Parks and Lynch, 1992) and known to readily increase with dietary supplementation (Blesbois et al., 1997; Conquer et al., 2000). Transfer of unsaturated fatty acids from the diet to the semen of ruminants is a somewhat more difficult prospect due to hydrogenation of lipids by rumen microorganisms (Garton, 1969). Further, lipid content of the ruminant diet generally should not exceed 4 to 5% due to the resultant reduction in efficiency of rumen microorganisms in their ability to breakdown cellulose (Brooks et al., 1954; White et al., 1958). Encouragingly, recent research has shown the feeding of extra Linoleic acid to cause a significant increase in the concentration of this fatty acid in the blood plasma of goats, thereby suggesting unsaturated fatty acids to have the capacity to resist biohydrogenation in the rumen (Yeom et al., 2003, 2005). It is therefore feasible that the beneficial effects of Linoleic and Oleic acid on stored ram spermatozoa may be facilitated via manipulation of the diet (Perez-Pe et al., 2001). This has certainly been advocated after anecdotal evidence of improved bull (I. Drummond, pers. comm.) and boar (D. Rath, pers. comm.) semen freezability in animals fed diets high in extra virgin olive oil (Oleic acid), particularly when followed by sex-separation using flow cytometry.

The potential benefit to the quality of frozen–thawed spermatozoa, whether sexed or non-sexed, and the ease with which this may be achieved justifies scientific investigation of the link between dietary oil supplementation and sperm cryopreservation. Thus, the present study aims to determine the effect of supplementing the dietary intake of rams with Oleic and Linoleic acid on the freezing ability and sex-sorting parameters of ram semen.

2. Materials and methods

Procedures herein were approved by The University of Sydney's Animal Ethics Committee.

2.1. Experimental design

Twelve mature, mixed breed rams of European descent were housed in individual pens at The University of Sydney farms (Camden, NSW) and were allocated to one of the three diets: (Treatment 1) a standard ram maintenance diet (Control), (Treatment 2) Control + Linoleic acid in the form of sunflower oil (Linoleic), and (Treatment 3) Control + Oleic acid in the form of extra virgin olive oil (Oleic). Rams were allocated to the treatment groups based on principal component analysis of post-thaw sperm quality ($n=3$ ejaculates), prior to commencement of the trial (while all rams were fed the control diet) in order to balance both high and low quality semen producing rams between treatments. With commencement of the experimental period, ejaculates were collected from each ram once every 2 weeks for a total of 6 weeks and frozen for later post-thaw analysis. At the end of the 6 week experimental period further ejaculates ($n=2$) were collected from each ram, sex-sorted and frozen. All thawed samples were analysed for motility, viability and acrosome integrity during incubation (0, 3, 6 h; 37 °C).