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Effects of high dietary energy level on the cryotolerance of ram semen

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Abstract

In general ram semen does not cryopreserve acceptably in terms of post-thawed survival and fertilization. In South Africa it is common to feed high energy diets to breeding rams in performance testing schemes and in preparation for shows and sales, as conditioned (fat) rams often realize better prices. Anecdotal evidence suggests that high energy diets and the over-conditioning of rams reduce sperm cryotolerance. This over-conditioning/obesity of rams is often blamed for the poor quality and freezing ability of their semen. Thus a study to evaluate the effects of high dietary energy levels on the cryotolerance of ram semen was conducted during the natural breeding season. Twenty four, 11 - 12 mo old Dorper rams were randomly allocated to two groups (12 rams each) and fed *ad libitum* at two energy levels: A low energy (LE; 6.52 MJ ME/kg DM) and a high energy (HE; 9.39 MJ ME/kg DM) diet for a period of 127 days. The rams in the HE group recorded a higher body weight gain, grew faster (average daily gain of 229 *vs*. 112 g/d, respectively) and were heavier (body weight of 71 *vs*. 56.5 kg, respectively) at the end of the trial. At the end of the trial period, semen was collected from all rams with the aid of an artificial vagina and cryopreserved using a one-step dilution (1+4) technique. The fresh and frozen (post-thawed) semen samples were evaluated using standard laboratory techniques for quality (overall and progressive sperm motility, percentage live and abnormal sperm) and were compared between the two diet groups. The fresh semen quality results provided the baseline reference or control values. As expected, the cryopreserved semen (post-thawing) recorded a lower percentage live and motile sperm than the fresh semen samples. Overall motility (57 *vs*. 60%), forward sperm progression (1.7 *vs*. 1.8) and percentage live sperm (50 *vs*. 60%) of cryopreserved semen (post thawing) were lower in the HE group compared to the LE group. Results also indicated that, although rams fed HE diet recorded lower semen quality values than those on the LE diet, for the qualitative semen parameters considered (both fresh and post-thawed semen), these differences were not significant. It could be concluded that the conditioning of yearling rams for a period of four months with a diet containing up to 9.39 MJ ME/kg DM does not seem to have any detrimental effect on semen cryotolerance. Further research on the effect of HE diets (at higher concentrations and for longer periods) on the cryotolerance of ram semen is warranted to allow more conclusive recommendations regarding the use of such diets to condition rams intended for semen collection and artificial insemination.

Keywords: Feeding, energy level, semen cryopreservation, cryotolerance, sperm, Dorper, rams
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Introduction

In general ram semen cryopreserves poorly compared to bull and even human semen, with a 40 to 50% sperm mortality during the freeze-thawing process. There is thus a need for more research to improve the efficiency of cryoperservation of ram semen (Watson, 2000).

Anecdotal evidence in South Africa seems to suggest that high energy diets and the over-conditioning of rams reduce the cryotolerance of sperm. The over-conditioning/obesity of rams is often blamed for the poor quality and freezing ability of their semen. It is also common practice to feed high energy diets to rams in performance testing schemes and in the preparation for shows and sales, because conditioned (fat) rams generally realize better prices (Fourie *et al*., 2004). However, very little is known about the effects of such practices on the subsequent fertility of rams. Overfeeding of sires (boars, bulls and rams) has been reported to have a possible detrimental effect on reproductive capacity (Brown, 1994). Similarly, Coulter *et al.* (1997) and Labuschagné *et al*. (2002) demonstrated the detrimental effects of high energy diets on the fertility of young bull in the form of lower semen quantity and quality. Similar findings have been reported by Fourie *et al.* (2004) in young Dorper rams. However, nothing has been reported in the literature regarding the effects

of high energy diets on the resistance/tolerance of ram semen to cryopreservation. The aim of this study was to evaluate the effects of feeding high energy diets to rams on the cryotolerance of their semen.

Materials and Methods

Twenty four 11 - 12 mo old Dorper rams previously raised on natural pastures, were used in the study. The rams were trained for semen collection using an artificial vagina (AV) and were randomly allocated to two groups of 12 rams each (average body weight of 42.4 kg). All rams were housed in individual metabolism cages in a well-ventilated building. For a period of 127 days (during the natural breeding season - autumn) one group received a low energy (LE, maintenance level of 6.52 MJ ME/kg DM) diet and the other group a high energy (HE, fattening level of 9.39 MJ ME/kg DM) diet. Both diets were balanced on an equivalent basis with regard to crude (136 g/kg) and degradable protein (85 g/kg), and Ca (6.5 g/kg) and P (2 g/kg) using NRC (1985) feeding standards. The rams in each group received the experimental diets and water *ad libitum.*

At the end of the experimental feeding period, semen was collected from all rams, (7:30 to 9:30) with the aid of an AV filled with water (54 ºC) and an ewe restrained in a neck clamp. The volume of the ejaculates collected was recorded directly from the calibrated collection tube before being placed in a water bath (32 ºC). Semen was diluted with a pre-warmed (32 ºC) TL-Hepes solution (990 µL TL-Hepes and 10 µL semen) and evaluated within two minutes using standard procedures. Forward sperm progression and overall motility were evaluated on a scale of 0 (no sperm movement) to 5 (very fast forward sperm movement) by recording 100 individual sperm and the concentration of the semen sample, determined with the aid of a Newbawer haemocytometer. A thin eosin/nigrosin (60 µL eosin/nigrosin and 6 µL semen) semen smear was made from each sample on a pre-warmed (35 ºC) glass slide. For morphology evaluation 100 individual sperm cells were observed microscopically (Loskutoff & Crichton, 2001).

Semen was cryopreserved using a one-step dilution (1+4) technique and the Salamon's semen extender medium containing 5% glycerol (Table 1).

Table 1 Composition of the Salamon's semen extender used for one-step dilution and cryoperservation of ram semen

Ingredients	Amount/100 mL
Tris (hydroxymethyl) amino-methane (g)	3.634
Glucose (g)	0.500
Citric acid (monohydrate) (g)	1.990
Egg yolk (mL)	15
Glycerol (mL)	5
Penicillin (IU)	100,000
Streptomycin (mg)	100

Source: Evans & Maxwell (1987)

Within 10 min of collection, 0.5 mL semen from each ram was added slowly to 2 mL of a pre-warmed semen extender (in a 5 mL plastic screw top test tube, placed in a water bath at 32 °C). The 5 mL tubes containing the extended semen (2.5 mL) were gently agitated, tightly closed and placed into 50 mL plastic screw top tubes containing water at 32 ºC. These tubes were then placed in a coolbox (4 - 5 ºC) for equilibration. After collecting semen samples from all rams, the coolbox containing the extended semen was transferred to a walk-in refrigerator (4 – 5 ºC).

After a total equilibration period of 4 h (in the coolbox and at the walk-in refrigerator) the extended semen was packed into pre-labelled 0.25 mL plastic straws (six per animal) and sealed with PVP straw sealing powder. The filled straws were then placed in liquid nitrogen (LN^2) vapour at a level of *ca*. 4 cm

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above the LN^2 level (-70 ºC) for 10 min before being plunged into the LN^2 (-196 ºC) (Loskutoff & Crichton, 2001). The frozen straws were then transferred in goblets and stored in a LN^2 flask for a week and then thawed in water at 38 ºC for 30 sec and evaluated for sperm quality, using the same methods described above for fresh semen (Salamon & Maxwell, 2000). The semen quality of the cryopreserved semen in both groups was analysed statistically using the GLM procedures of SAS (1995), and means were declared significantly different at a level of $P < 0.05$.

Results and Discussion

The most important semen quality parameters (mean ± s.e.) in both fresh and cryopreserved Dorper ram semen between the two experimental groups are summarised in Table 2.

Table 2 The mean (± s.e.) characteristics (indicators of semen quality) of fresh and post-thawed cryopreserved semen of yearling Dorper rams fed a low- or high-energy diet for 127 days

Semen parameters	Dietary energy level			
	Fresh semen		Cryopreserved–thawed semen	
	LE	HE	LE	HE
Overall motility (%)	84 [a] ± 2	77 [a] ± 3	60 [b] ± 3	57 [b] ± 5
Forward progression (0-5)	2.8 [a] ± 0.1	2.7 [a] ± 0.2	1.8 [b] ± 0.1	1.7 [b] ± 0.2
Sperm concentration ($x10^6$/mL)	1851 [a] ± 121	1834 [a] ± 169	370 [b] ± 40	367 [b] ± 51
Live sperm (%)	70 [a] ± 2	64 [a] ± 3	60 [b] ± 2	52 [b] ± 3

[a,b] Means within rows different superscripts differ significantly at $P < 0.05$

LE - Low energy (6.52 MJ ME/kg DM); HE - High energy (9.39 MJ ME/kg DM)

In general the semen quality results in Table 1 are acceptable for Dorper ram semen collected by means of an AV (Matthews *et al.*, 2003). As expected, the cryopreservation process to which the semen samples were subjected significantly reduced all semen parameters considered except for sperm morphology. This paper focused on the cryopreserved (post-thawed) ram semen, while the fresh semen results were mainly presented to provide baseline reference values.

Although the HE group in general recorded lower means for the semen parameters considered, no significant differences were recorded in either the fresh or post-thawed cryopreserved semen between the two feeding groups (LE and HE). In addition, no significant differences were recorded in the fresh semen (prior to cryopreservation) between the two feeding groups at the end of the 127 d trial period. This fact may partly explain the lack of significant differences in the cryopreserved semen (post-thawing). Although the HE level used in this trial (9.39 MJ ME/kg DM) resulted in a higher ADG (229 *vs*. 112 g/day) during the trial and heavier final body weights (71 *vs*. 56.5 kg) at the end of the trial, no detrimental effects were recorded on semen quality and sperm cryotolerance. However, in order to condition rams for shows and sales many farmers in South Africa often feed their rams for shorter periods of time on diets containing much higher energy levels than that (HE diet) used in this trial (Fourie *et al*., 2004). Rams commercially available are consequently often in a much fatter body condition than that achieved in this study. Other ram breeders, again, feed rams on HE diets for a much longer period, starting at a much younger age. Sometimes rams are fed HE diets continuously from weaning to the time of sale. In order to study the effect of high energy diets on the cryotolerance of ram semen, more extreme dietary energy levels need to be considered. The variance within groups for most of the semen parameters studied was also relatively high. This suggests the need for the use of larger experimental groups in order to increase the chances of recording significant differences between the different feeding regimens.

Conclusions

It could be concluded that the conditioning of yearling Dorper rams for a period of four months with a diet containing up to 9.39 MJ ME/kg DM does not seem to have any detrimental effect on their semen quantity, quality and freezing ability. However, further research on the effect of HE diets on ram fertility is warranted to evaluate the use of such diets to condition rams intended for breeding or for semen collection, cryopreservation and artificial insemination.

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