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## Seasonal changes in the protein content and composition of ram seminal plasma

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### ABSTRACT

Seasonal changes in ram semen composition are accompanied by changes in fertility of frozen semen. These changes in fertility are not all easily explained simply in terms of the changes seen in sperm numbers per ejaculate. The possibility that changes in the composition of seminal plasma may be involved in the seasonal changes in ram fertility needs to be considered.

An experiment that examined the protein content and composition of seminal plasma was conducted. Semen samples were collected from 10 rams (5 each from the BV -ve and BV +ve selection lines for early lambing) and the seminal plasma separated by centrifugation. Samples were collected with AV in the first week of each month for 12 months (after a 4-day period of abstinence). The protein concentration was determined and the samples were subjected to electrophoresis by SDS-PAGE using mini gels. Seminal plasma samples from all rams for each month were analysed on the same gel for each of the 12 months. In addition samples for all 12 months for each ram were also analysed on the same gel. The seasonal changes in seminal plasma protein profiles were recorded.

There was a seasonal change in the total protein concentration of seminal plasma with higher levels (36.9 vs 23.0 mg/ml,  $P < 0.001$ ) observed during the breeding season (January to June). In the rams selected for out-of -season breeding (BV-ve) protein levels were also elevated in December and again in June and July.

Several protein bands that were present in seminal plasma from all rams during the breeding season were absent from a number of rams during the non-breeding season (August to December). Their absence was confirmed on larger format 1D SDS-PAGE gels. The majority of these proteins fell between 20 and 70 kDa molecular weight. The absence of these proteins from seminal plasma coincides with the time of the year when we observed poor maintenance of motility in fresh semen and a reduction in the velocity of frozen/thawed sperm from semen collected from the same rams.

Further work is required to identify and characterise these proteins and to elucidate their function and role in ram fertility.

**Key words:** Season; seminal plasma; proteins.

### INTRODUCTION

Seasonal patterns of semen production and sperm quality from rams of different breeds have been reported (Colas, 1979; Amir and Volcani, 1965). These changes in ram semen composition are accompanied by changes in the fertility of frozen semen which cannot all be readily explained simply in terms of the changes seen in sperm numbers per ejaculate. Smith, *et al.*, (1997) reported seasonal changes in the maintenance of sperm motility as well as in sperm numbers. However, the possibility that changes in the composition of seminal plasma may be involved in the seasonal changes in ram fertility needs to be considered. Differences in field fertility after insemination with frozen bull semen have been linked to concentrations of specific seminal plasma proteins (Killian, *et al.*, 1993). This raises the possibility that certain seminal plasma proteins could be influencing the ability of sperm to retain their fertility after cryopreservation. The present experiment was conducted to examine the seasonal changes in protein content and composition of ram seminal plasma.

### METHODS

Ten rams (5 BV-ve - early lambing and 5 BV+ve - normal lambing) were selected on the basis of breeding value (BV) for date of lambing from the "Ruakura early-lambing" selection flocks (Smith, *et al.*, 1992). The trial began in August 1994 and continued for 12 months to July 1995. Once a month (after a 4-day rest period) two to three

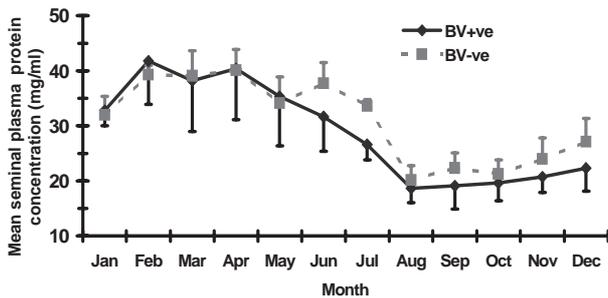
ejaculates were collected by artificial vagina from each ram evaluated for volume and density and processed. After collection semen samples were kept on ice for all subsequent procedures. Proteolytic inhibitors (benzamine and PMSF) were added to a final concentration of 1 mM each. The seminal plasma was separated by centrifugation (2x 10 min) in an Eppendorf centrifuge (11,600xg). Equal volume aliquots of seminal plasma from each collection for an individual ram, on the same day in a month, were pooled and stored at -20°C until assay. Protein content of the seminal plasma was determined using a modified Lowry method (Upreti, *et al.*, 1988).

Protein composition of the seminal plasma samples was analysed by electrophoresis in 12.5 % denaturing acrylamide gels (Laemmli, 1970). Monthly samples for each ram were analyzed together on individual gels. In addition, samples from all 10 rams for each month were analysed together on individual gels. This allowed comparison of protein banding pattern across the 12 months for each ram, as well as for variations between individual rams for each of the 12 months. Seminal plasma samples were diluted 1:5 with 10 mM Tris-Cl, pH 8.0, containing 1 mM EDTA and 10 µl was loaded on each gel lane. After electrophoresis, the gels were stained with Coomassie-blue and the protein banding patterns visually appraised. When seminal plasma samples were compared across the 12 months for each of the selected rams, the protein loading was normalised across all the lanes because of the variation in protein concentration between the samples.

**RESULTS**

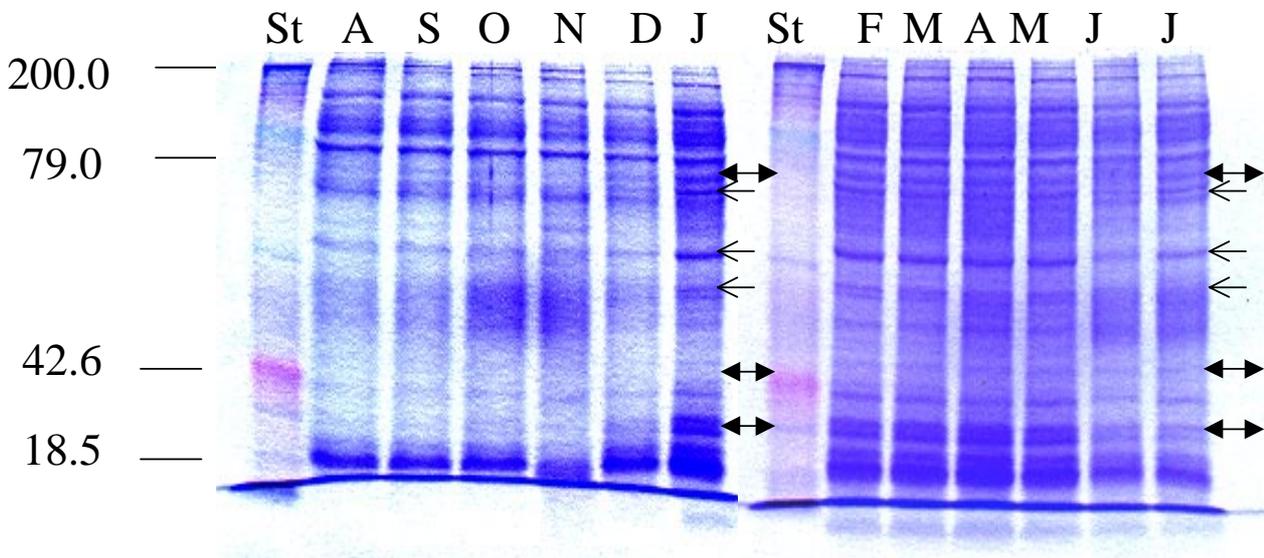
There was a seasonal change in the total protein concentration of seminal plasma with higher levels (36.9 vs 23.0 mg/ml;  $P < 0.001$ ) observed during the breeding season (January to June) compared to the non-breeding season (August to November). In the rams selected for out-of-season breeding (BV-ve) protein levels were also elevated in December and in June and July (Figure 1).

**FIGURE 1:** Effect of breeding values (BV's), for date of lambing, on the seasonal pattern of total protein concentration (mg/ml) in ram seminal plasma (mean  $\pm$  se).



Several protein bands that were present in samples from all rams during the breeding season were absent from the samples of a number of rams during the non-breeding season (Figure 2, -see double arrowheads). The absence of these bands was confirmed on larger format 1D SDS-PAGE gels (results not shown) and the majority of these proteins fell between 20 and 70-kDa molecular weight. There were also proteins which were present in both the breeding and non-breeding season samples, but which were more abundant in the samples collected within the breeding season (Figure 2, -see single arrows).

**FIGURE 2:** Mini gels for each month for ram # 1229(BV +ve). The double arrows indicate bands that are present in the breeding season (Jan. to July) but absent in the non-breeding season (Aug. to Dec.) while the single arrow represent bands present in both seasons but whose intensity is reduced in the non-breeding season. St = molecular weight standards.



**DISCUSSION**

The seasonal changes in protein content and composition of the seminal plasma parallels that observed for both changes in semen volume and sperm concentration (Smith, et al. 1997) reported for these same animals. Lower total protein concentrations and the absence of specific protein bands from seminal plasma, coincides with the time of the year when maintenance of sperm motility in fresh semen and a reduction in the velocity of frozen/thawed sperm was observed. Colas and Brice (1976) have shown that ram semen collected and frozen in this period (non-breeding season) is of lower fertility than semen collected and frozen in the autumn. Ram seminal plasma proteins adsorbed to the sperm surface have been reported to have a beneficial effect on the viability of ram sperm after cold-shock treatment (Garcia-Lopez, et al., 1996).

Further work is required to identify and characterise these proteins and to elucidate their function and role in the seasonal differences in fertility of ram semen. Until the proteins and their site of secretion are identified the seasonal control mechanisms can only be speculated upon. Seasonal changes in plasma gonadotrophin levels is well known (Xu, et al., 1991) and associated changes in the number of LH and FSH receptors in the testis has been reported (Barenton and Pelletier 1983). This is reflected in changes in testicular steroid secretion, which may very likely influence the composition of the secretory products of the seminal vesicles and the epididymis.

It is of particular interest that the rams with different BV's for date-of-lambing showed significant differences in the patterns of seminal plasma protein content. Rams from the flock with the earlier breeding season (BV-ve) showed an earlier increase and latter decrease in seminal plasma protein concentration. This coincides with the earlier onset

in reproductive activity of the ewes from this same selection flock (Smith, *et al.*, 1992) and with the differences in the patterns of LH pulse frequency under oestradiol negative feedback seen in ovariectomised ewes from these flocks (Smith, *et al.*, 1995). Brewer, *et al.*, (1995) reported a higher LH response to GnRH treatment in these BV-ve rams during November and December which tends to coincide with the differences in total seminal plasma proteins between the two genotypes. Identification of the specific proteins and their site of production as well as knowledge on the regulatory mechanisms may enable the manipulation of the protein content of seminal plasma. This could result in the minimisation of seasonal differences in fertility of frozen ram semen.

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