The relationship between semen quality and mineral composition of semen in various ram breeds

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Abstract

Semen was collected during the breeding season from rams by electroejaculation from two native (13 Najdi and 8 Naemi) and three imported (6 Merino, 7 Somalian and 9 Sudanese) sheep breeds. There was a marked reduction in sperm concentration and the percentage live spermatozoa (p<0.01) produced by the Somalian (Barbari) and Sudanese (Sawakni) breeds. On the other hand, these two breeds showed the highest percentage of spermatozoal individual motility (p<0.05) and the highest concentrations of sodium (Na) chloride (Cl) and inorganic phosphorus (P) in the whole semen, seminal plasma and spermatozoa. Superior sperm density, percentage live spermatozoa and seminal concentrations of potassium (K) and calcium (Ca) were evidenced in the Najdi, Naemi and to a lesser extent, in the Merino rams. The biological effects of these inorganic constituents, together with magnesium (Mg), in the semen on semen quality should be considered in the interpretation of the results obtained in the fertility evaluation of the various ram breeds. © 2000 Published by Elsevier Science B.V.

Keywords: Minerals; Spermatozoa; Breeds; Ram

1. Introduction

Genetic improvements of farm animals rely on the intensive use of a few superior males either for natural mating, or in artificial insemination programs. The production of meat and milk may be increased through selective breeding of ewes with rams exhibiting desirable genetic combinations. However, sperm density, viability and activity as well as other sexual characteristics of the different breeds may limit the extent to which rams can be used for breeding. The influence of the major biologically active inorganic components on these sperm parameters has been extensively studied in various breeds of rams (Scott et al., 1963; Dott and White, 1964; Wallace and Wales, 1964; Quinn et al., 1965; Quinn and White, 1966; Stechell, 1974; Hamamah and Gatti, 1998). However, these investigations were confined to one specific breed and came to no conclusion regarding the effect of breed on the interaction between the chemical composition of semen and semen quality.

The present attempt was, therefore, conducted to identify the breed-related variations in inorganic elements including Na, Cl, K, Ca, P and Mg of the different seminal fractions and their concentration effects on semen quality of native and imported sheep breeds.

2. Materials and methods

Twenty one native (13 Najdi and 8 Naemi), 6 Australian (Merino), 7 Somalian (Barbari) and 9 Sudanese (Sawakni) rams were selected at the
expected time of greatest output of spermatozoa (October to February 1998) from the farm of the Faculty of Agriculture and Veterinary Medicine, King Saud University, together with a private farm nearby to Bureidah. Animals were clinically healthy and ranging in age from 2 to 5 years. When the five breeds are combined, they produce an age profile (2.9±0.3 years) typical of a single breed.

Semen collection by electroejaculation from the 43 rams was undertaken using a bipolar electrode with a 4-s stimulation period and an 8-s rest period. Immediately after collection, each sample was placed into a portable, temperature controlled waterbath (37±8°C) to prevent cold shock and only samples of good initial motility (not <70%) were evaluated. Each ejaculate was evaluated for the following criteria:

1. Individual spermatozoa motility percent, semen was diluted with 2.9% sodium citrate dihydrate solution was recorded on a subjective scale of (0–100%) to the nearest 5% after viewing several microscopic fields.
2. Sperm cell concentration was quantified by direct cell count using the improved Neubauer haemocytometer (Sorenson, 1979).
3. Percentage live sperm was recorded as described by Campbell et al. (1956). Duplicate smears from each freshly collected ejaculate were stained with eosin-nigrosin stain. A total of 200 spermatozoa were examined randomly (100 in each of the two smears).

Following evaluation, approximately two-thirds of the collected sample was processed as follows:

1. Semen was drawn off into a capillary blood tube and centrifuged at 4000×g for 5 min to obtain the packed sperm volume (PSV) according to the equation:

$$PSV(\%) = \frac{\text{Length of packed sperm column (mm)}}{\text{Length of total semen column (mm)}} \times 100$$

2. 50µl of semen was drawn into a tube containing 1 ml deionized water (dilution rate=1:21) to provide a lysed sperm sample (LSS)
3. The remaining semen sample was centrifuged as previously mentioned for harvesting of the seminal plasma sample (SPS).

Concentrations of the electrolyte in spermatozoa were determined according to the formula reported by Mulei and Daniel (1988) with certain modifications:

Inter-spermatic mineral content

$$= \frac{100 \times 21 \times A - (100 - PSV) \times B}{PSV}$$

where A and B are concentrations (mg/100 ml) of the mineral in LSS and SPS, respectively.

Na and K were determined in the whole semen, seminal plasma and spermatozoa by emission flame photometry, Ca by atomic absorption spectrophotometry, whereas Cl, P and Mg were assessed using commercial diagnostic kits (bio-Merieux, France).

The statistical significance of the results was evaluated by the least-squares analysis of variance using the general linear models (GLM) procedures of the Statistical Analysis System (SAS, 1996).

3. Results

As shown in Table 1, the overall mean values (±SE) of sperm count (1943.4±132.3×10⁶/ml), percentage of motile spermatozoa (80.5±6.4%) and percentage of live spermatozoa (75.2±5.8%) were significantly affected by breed. Spermatozoa of the native (p<0.01) and Merino (p<0.05) rams were higher in concentrations and percentage live than Barbari and Sawakni rams (Table 1) displaying the highest (p<0.01) percentage of motile spermatozoa than the former breeds.

In all of the breeds studied, concentrations of Na, Cl and P in the seminal plasma always exceeded those in the spermatozoa, while concentrations of K, Ca and Mg was greater in the spermatozoa. Despite this general agreement between breeds, the Barbari and Sawakni rams showed the highest (p<0.01) concentrations of Na, Cl, and P whereas the other breeds displayed the highest (p<0.05) concentrations of K and Ca in the whole semen, seminal plasma and spermatozoa. There was no definite trend in the distribution of Mg in the different semen fractions amongst breeds.

Correlation coefficients of spermatozoal counts, the percentage of motile spermatozoa and the percentage live sperm with mineral levels of the ram semen; seminal plasma and spermatozoa are set out in Table 2.

As might be expected from the distribution of chemical components between the sperm and plasma,
Overall, Sawakni (n=9) and Naemi (n=8) showed a significant decrease in the percentage of live spermatozoa (%Lc) and an increase in the percentage of motile spermatozoa (%M) compared to Nazadi (n=13). The percentage of sperm constituent (S.C.) also increased in Naemi and Sawakni compared to Nazadi.

Table 2
Correlation between major inorganic constituents of ram semen and semen quality

<table>
<thead>
<tr>
<th>Na</th>
<th>Cl</th>
<th>K</th>
<th>Ca</th>
<th>P</th>
<th>Mg</th>
</tr>
</thead>
<tbody>
<tr>
<td>S.C.: semen constituent</td>
<td>−0.581**</td>
<td>−0.611**</td>
<td>+0.736**</td>
<td>+0.290*</td>
<td>−0.301*</td>
</tr>
<tr>
<td>S.C.: plasma constituent</td>
<td>−0.663**</td>
<td>−0.725**</td>
<td>+0.169</td>
<td>+0.283*</td>
<td>−0.425**</td>
</tr>
<tr>
<td>S.C.: sperm constituent</td>
<td>−0.355*</td>
<td>−0.299*</td>
<td>+0.386*</td>
<td>+0.316*</td>
<td>−0.511**</td>
</tr>
<tr>
<td>%M: semen constituent</td>
<td>+0.215</td>
<td>+0.191</td>
<td>−0.224</td>
<td>−0.096</td>
<td>+0.242</td>
</tr>
<tr>
<td>%M: plasma constituent</td>
<td>+0.421**</td>
<td>+0.285*</td>
<td>−0.236</td>
<td>−0.133</td>
<td>+0.327*</td>
</tr>
<tr>
<td>%M: sperm constituent</td>
<td>+0.379**</td>
<td>+0.314*</td>
<td>−0.574**</td>
<td>−0.306*</td>
<td>+0.284*</td>
</tr>
<tr>
<td>%L: semen constituent</td>
<td>−0.268</td>
<td>−0.139</td>
<td>+0.363*</td>
<td>+0.294*</td>
<td>−0.292*</td>
</tr>
<tr>
<td>%L: plasma constituent</td>
<td>−0.236</td>
<td>−0.088</td>
<td>+0.446**</td>
<td>+0.325*</td>
<td>−0.308*</td>
</tr>
<tr>
<td>%L: sperm constituent</td>
<td>−0.270</td>
<td>−0.104</td>
<td>+0.618**</td>
<td>+0.303*</td>
<td>−0.276*</td>
</tr>
</tbody>
</table>

a Represents spermatozoal count ×10^7/ml.
b Percentage of motile spermatozoa.
c Percentage of live spermatozoa.
* p<0.05; ** p<0.01.

The Na, Cl and P concentrations of the whole semen decrease and the K, Ca and Mg increases as the ejaculate becomes more dense. The percentage of motile spermatozoa decreases as the K and Ca levels increase and the Na, Cl, P and Mg decrease. The percentage live (unstained) spermatozoa were correlated positively with K and Ca and negatively with P in the different semen fractions (Table 2).
4. Discussion

Extensive studies on evaluating semen parameters were conducted in rams of different breeds. The higher sperm density and percentage live sperm shown by the native breeds (Najdi and Naemi) agreed with those reported for the British (Suffolk) rams (Jennings, 1976; Jennings and McWeeney, 1976) and the Egyptian (Awassi and Ossimi) rams (Amir, 1966; Abdel-Hakeam et al., 1978; Abou-Ahmed et al., 1986; Seida et al., 1986). While no data are available in the literature concerning results of semen characteristics in the Barbari and Sawakni rams, results of sperm density and viability estimates reported by Salamon (1964), Dott and Foster (1972) and Lino and Braden (1972) in Merino rams at Australia so far exceeded our results. The discrepancy might be related, in part, to environmental factors such as high ambient temperature and low humidity in the relevancy current study, which has been shown to affect semen characteristics in the ram (El-Wishy et al., 1976; Loubser and Van Niekerk, 1983; Abou-Ahmed et al., 1986).

In this study, the overall mean (Table 1) of ion concentrations found in the whole semen is in agreement with that recorded in the ram by Scott et al. (1963), Wallace and Wales (1964) and Stechell (1974), but Na, Cl and K values were about twice the level reported by Quinn et al. (1965) and Quinn and White (1966).

The correlations set out in Table 2 might explain the encountered variations in semen quality of the various ram breeds studied. As might be expected from the distribution of the ions between the sperm and plasma, higher concentrations of K, Ca and Mg in the whole semen of the native breeds may be due to the more dense ejaculates. The positive correlation (Table 2) between all, except K and Ca, ions in plasma and spermatozoa and the percentage motility could explain the higher motility evidenced by the Barbari and Sawakni spermatozoa. Moreover, the higher spermatozoal K and Ca levels notably caused some reduction in spermatozoal activity in the native and Merino breeds. These results support the reports of earlier work that K was detrimental to metabolism at high concentrations (Cragle and Salisbury, 1959) for bull spermatozoa and that Ca depressed the metabolism of bull and ram spermatozoa and exaggerated the detrimental effects of K (Blackshaw, 1953). The data suggest a reciprocal relationship between intracellular contents of K, Ca and P with respect to percentage live sperm (unstained); a high percentage of live cells being associated with high K and Ca and low P levels. These results were true for the native (Najdi and Naemi breed) and Merino spermatozoa and this meant that the K, Ca and P are concerned with the permeability of ram spermatozoa as reported by Mikulecky and Tobias (1964).

In conclusion, the distribution of major ions studied between sperm fraction and seminal plasma could provide the basis for variation of semen quality between ram breeds and should be considered in the interpretation of the results obtained in the evaluation of the fertility of rams.

References