MAJOR CATIONS IN THE SEMEN OF ANGONI (SHORT-HORN ZEBU) BULLS

G. IGBOELI AND A. M. RAKHA
Agricultural Research, National Council for Scientific Research, P.O. Box 49, Chilanga, Lusaka

(Received 2nd May 1970)

Summary. Whole semen, seminal plasma and the pre-ejaculate fraction collected from ten Angoni bulls four times a week for 6 weeks were analysed for sodium (Na+) and potassium (K+) concentrations using a flame photometer and for calcium (Ca++) and magnesium (Mg++) using an atomic absorption spectrophotometer.

Na+ concentrations (mg/100 ml) in whole semen, seminal plasma and the pre-ejaculate fraction were 320 ± 9.9, 347 ± 8.4 and 335 ± 47.1, respectively. Corresponding values for K+ were 69.4 ± 3.1, 71.4 ± 4.5 and 152 ± 37.8; for Ca++ were 34.0 ± 1.4, 35.3 ± 1.1 and 4.1 ± 1.4 and for Mg++ were 8.8 ± 0.06, 8.3 ± 0.3 and 5.7 ± 1.7.

These concentrations were compared to the cation composition in blood. Correlation coefficients were calculated.

INTRODUCTION

The importance of the ionic and osmotic environment on sperm motility and metabolic activity is well established (Sorenson & Anderson, 1956; Salisbury, 1962; Quinn, White & Wirrick, 1966). However, no data are available on the cation composition of the semen of indigenous African bulls. It is of interest to compare the chemical composition of the semen of these tropical breeds with that of their counterparts from temperate regions.

Since the local breeds will presumably be used extensively in artificial insemination, information on the cation composition of the ejaculates of local bulls is vital as a prelude to a study aimed at developing suitable storage media for their semen, should the need arise.

MATERIALS AND METHODS

Climatic considerations

The Angoni cattle are the predominant short-horn Zebu type, found in the Eastern Province of Zambia and in Malawi (Mason & Maule, 1960). In Zambia, the altitude varies from 3000 to 5000 ft. There are three recognizable seasons—a rainy season (November to April, with a mean rainfall of 28 to 70 in.), followed by a cold season from May to July (mean temp. 48° F) and a hot
dry season from August to November (mean temp. 83°F). The present study was conducted during the rains, between February and March.

Experimental

Ejaculates were collected from ten Angoni bulls four times a week (two each on Mondays and Thursdays) during a 6-week period using the artificial vagina (Igboeli & Rakha, 1971). Soon after evaluation for motility and sperm concentration, half of each ejaculate was taken for this study. Half of this was retained for whole semen analysis, while the other half was centrifuged at 15 to 20°C, and 8000 g for 30 min using centrifuge tubes of narrow bore and the supernatant was removed and stored separately (Quinn, White & Wirrick, 1965). Care was taken to prevent cold shock before centrifugation. All samples were stored frozen at −20°C until analysed.

During the process of sexual preparation preceding each first ejaculate, the pre-ejaculate fraction was collected using a funnel mounted on a long handle. Care was taken to avoid contamination by urine.

In order to meet the volume requirements for analysis of whole semen, seminal plasma, and pre-ejaculate fraction, it was found necessary to pool samples in each case. Apart from bull differences, it was of interest to determine differences between samples collected on Mondays (4 days' rest) and Thursdays (3 days' rest) as well as differences between first and second ejaculates and their interactions. Samples were therefore pooled regardless of the week of collection.

Sodium and potassium determinations were carried out with a flame photometer on samples suitably diluted with distilled de-ionized water. For calcium and magnesium determinations, the samples were diluted in a special swamping solution and estimated by means of an Atomic Absorption spectrophotometer (Willis, 1960a, b; Quinn et al., 1965). Whole blood and plasma collected from all ten bulls were similarly processed and analysed.

The statistical significance of these results was assessed by the analysis of variance procedure. Correlation coefficients were also calculated.

RESULTS

Potassium concentration in whole semen collected on Mondays averaged 72.6 mg/100 ml and was significantly higher (P < 0.05) than the mean (66.1 mg/100 ml) for semen collected on Thursdays. Similarly, magnesium concentration in first ejaculates (9.0 mg/100 ml) was significantly higher (P < 0.05) than in second ejaculates (8.6 mg/100 ml) collected on the same day. In the seminal plasma, the sodium concentration in ejaculates collected on Mondays (359 mg/100 ml) was significantly higher (P < 0.05) than in those collected on Thursdays (335 mg/100 ml). Apart from these findings, no other significant differences were found, and accordingly only the overall mean values are presented in Table 1.

The concentration of sodium ranged from 347 mg% in seminal plasma to 320 mg% for whole semen. These high values were also found in blood plasma (373 mg%) and in whole blood (324 mg%). By contrast, potassium concentra-
Major cations in semen of Angoni bulls

Concentrations were generally low both in the whole semen and the seminal plasma, and were approximately half those concentrations found in the pre-ejaculate fraction.

The sodium:potassium ratio was 2:1 in the pre-ejaculate fraction but was much higher (5:1) in both the seminal plasma and the whole semen.

**Table 1**
CONCENTRATIONS OF MAJOR CATIONS IN THE SEMEN AND BLOOD OF ANGONI BULLS

<table>
<thead>
<tr>
<th></th>
<th>Sodium</th>
<th>Potassium</th>
<th>Calcium</th>
<th>Magnesium</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-ejaculate fraction</td>
<td>335 ± 47-1</td>
<td>152 ± 37-8</td>
<td>4-1 ± 1-4</td>
<td>5-7 ± 1-70</td>
</tr>
<tr>
<td>Seminal plasma</td>
<td>347 ± 8-4</td>
<td>71-4 ± 4-5</td>
<td>35-3 ± 1-1</td>
<td>8-3 ± 0-30</td>
</tr>
<tr>
<td>(225 ± 12-9)</td>
<td>(155 ± 6-4)</td>
<td>(39-6 ± 1-7)</td>
<td>(8-1 ± 0-25)</td>
<td></td>
</tr>
<tr>
<td>Whole semen</td>
<td>320 ± 9-9</td>
<td>69-4 ± 3-1</td>
<td>34-0 ± 1-4</td>
<td>8-8 ± 0-06</td>
</tr>
<tr>
<td>(235 ± 15-7)</td>
<td>(142 ± 12-0)</td>
<td>(44-1 ± 2-6)</td>
<td>(8-8 ± 0-18)</td>
<td></td>
</tr>
<tr>
<td>Blood plasma</td>
<td>373 ± 29-1</td>
<td>17-3 ± 3-4</td>
<td>9-9 ± 0-06</td>
<td>3-3 ± 0-05</td>
</tr>
<tr>
<td>Whole blood</td>
<td>324 ± 17-9</td>
<td>40-0 ± 3-1</td>
<td>5-9 ± 0-04</td>
<td>3-0 ± 0-07</td>
</tr>
</tbody>
</table>

Mean values (mg/100 ml) ± S.E. Values in parentheses, taken from Quinn et al. (1965), are shown for comparison.

No differences were found between the levels of calcium and magnesium in both seminal plasma and whole semen, but these values were much higher than in whole blood and blood plasma. The lowest concentration of calcium was found in the pre-ejaculate fraction, which had a calcium:magnesium ratio of 2:3.

In the seminal plasma, apart from the significant correlations between sodium and potassium (-0-516), and between calcium and magnesium, no other correlation coefficients were significant. No significant correlations were found between any cation in the pre-ejaculate fraction versus those in whole semen or seminal plasma. The correlation coefficients between the various cations in whole semen are shown in Table 2.

**Table 2**
CORRELATION COEFFICIENTS BETWEEN MAJOR CATIONS IN WHOLE SEMEN

<table>
<thead>
<tr>
<th></th>
<th>Sodium</th>
<th>Potassium</th>
<th>Calcium</th>
<th>Magnesium</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium</td>
<td>(0-207)†</td>
<td>-0-520*</td>
<td>0-510*</td>
<td>-0-437*</td>
</tr>
<tr>
<td>Potassium</td>
<td>-</td>
<td>(0-650*†)</td>
<td>-0-575*</td>
<td>-0-253</td>
</tr>
<tr>
<td>Calcium</td>
<td></td>
<td>-</td>
<td>(-0-291)†</td>
<td></td>
</tr>
<tr>
<td>Magnesium</td>
<td></td>
<td></td>
<td></td>
<td>(0-794*†)</td>
</tr>
</tbody>
</table>

† Figures in parentheses refer to correlation coefficients between cations in seminal plasma and whole semen.
* P<0-01.

Sodium was correlated positively with calcium but negatively with potassium and magnesium (P<0-01). There was also a significant negative correlation between potassium and calcium.
DISCUSSION

One of the points of particular interest in these results is the very high level of sodium and the low level of potassium in whole semen and seminal plasma. For Hereford bulls, Quinn et al. (1965) reported sodium concentrations of 233 mg/100 g and potassium concentrations of 142 mg/100 g in whole semen; corresponding values for seminal plasma were 225 mg/100 g and 155 mg/100 g. Somewhat higher values than those of Quinn et al. (1965) have been reported for sodium and potassium in whole semen and seminal plasma (Cragle, Salisbury & VanDemark, 1958). In the present results, blood analysis also revealed a much higher sodium concentration than has previously been reported. Whether these concentrations reflect an adaptation to the tropical environment, or are purely genetic, or both is not clear. It is important, however, to determine the effect of the observed increase in sodium and the decrease in potassium concentrations in seminal plasma and whole semen on the total osmolarity of the environment of the ejaculated spermatozoa. It is thought that this could be vital in the formulation of semen extenders suited to these breeds (Steinbach & Foote, 1967). In view of the known effects of potassium on the motility and metabolic activity of bull spermatozoa (Sorensen & Anderson, 1956; Wales & White, 1958; Cragle & Salisbury, 1959; Dott & White, 1964), it would appear that the low concentration of potassium observed in the ejaculates of these bulls, may be of some physiological significance (Gustafsson, 1964). Further work will be required to investigate this possibility.

The negative correlation between sodium and potassium in whole semen and in seminal plasma confirms the reciprocal relationship between these two cations inside and outside the spermatozoa (Rothschild & Barnes, 1954; Cragle et al., 1958; Dott & White, 1964; Quinn et al., 1965). Although spermatozoa as such were not analysed in this study, a high positive correlation found between sperm concentration and potassium on the one hand, and a high negative correlation with sodium on the other (Igboeli & Rakha, unpublished data) supply additional evidence.

Quinn et al. (1965) found that, in the bull, the calcium concentration in seminal plasma not only exceeded that in the spermatozoa but was also much higher than that in the blood. The present results support this finding.

Reports on the cation composition of the pre-ejaculate fraction of bulls are scanty. The present results show that the sodium concentration in this fraction was comparable to that in whole semen and seminal plasma. By contrast, the potassium concentration was almost twice that in whole semen and seminal plasma while the calcium and magnesium concentrations were rather low. If it is assumed that the urethral glands are responsible for the secretion of the pre-ejaculate fraction (Aalbers, 1966) then these glands must have a specialized ability to concentrate potassium. It is thought that the urethral glands contribute a considerable volume to the ejaculate (Seidel, 1968). If this is true, one would expect a significant correlation between potassium concentrations in seminal plasma and the pre-ejaculate fraction. However, the present result does not show this and further work on this breed will be required to investigate the contributions of the major accessory glands to the ejaculate and their various chemical compositions.
ACKNOWLEDGMENTS

The authors are indebted to Mr Dutta-Roy for the statistical analysis, the staff of the Department of Chemistry, Mount Makulu Research Station, for permission to use their Atomic Absorption spectrophotometer, and Mr Y. Kapyanga and Mr J. Nguluwe for technical assistance.

REFERENCES


