EVIDENCE FOR LOSS OF LIPID FROM BOVINE SPERMATOZOA DUE TO FREEZING

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SUMMARY

The 1.26 mg/ml of lipid extracted from seminal plasma separated from spermatozoa prior to freezing (prefreeze) was lower (P < 0.01) than the 1.92 mg/ml obtained from seminal plasma separated following freezing of the whole raw semen. There was also a significant difference (P ~ 0.05) between the 2.34 and 1.63 mg/ml extracted from the pre- and post-freeze spermatozoa. Freezing of whole raw semen prior to separation apparently results in an alteration in the quantity of lipid found in the cells and seminal plasma. Possible alteration of lipid classes due to freezing was also observed.

When ram spermatozoa are cold-shocked there is decreased metabolic activity, rapid breakdown of ATP, increased cellular permeability, and loss of certain cellular proteins and lipoproteins as well as other phosphorus containing compounds (2, 15, 16). In cold-shocked bovine spermatozoa Blackshaw and Salisbury (3) found a significant loss of potassium and lipid phosphorus and an uptake of sodium and calcium, with a subsequent reduction in aerobic glycolysis and respiration upon incubation at 37 C.

Hartree and Mann (5) reported that considerable plasmalogen, based on a palmitaldehyde standard, was lost from ram spermatozoa following cold-shock and that similar losses resulted from freezing and thawing from -15 C.

The observations herein represent primarily the lipid concentration of bovine spermatozoa before and after freezing and thawing and preliminary data on its composition.

MATERIALS AND METHODS

Semen was obtained with an artificial vagina from bulls, principally Holstein, in routine use at the New England Selective Breeding Association (NESBA), Woodbridge, Connecticut, and from bulls used in the University of Connecticut dairy herds (UCONN). Sperm cell concentration was determined photometrically on each ejaculate immediately after collection. The quantity of semen collected in approximately 30 min was pooled and a 5-ml aliquot removed for dry weight determinations and the remainder centrifuged at 25,000 × g for 30 min. The seminal plasma was drawn off and the fractions, seminal plasma and spermatozoa, placed in crushed dry ice for transport to the University laboratory. Semen collected at the University dairy barns was treated in the same manner, except that storage was at -20 C.

Semen was also obtained from the New York Artificial Breeders Cooperative (NYABC), Ithaca, New York. After collection and determination of sperm concentration, each ejaculate was poured directly into bottles and stored at approximately -20 C. This procedure was repeated until approximately 100 ml of semen had been accumulated per bottle, then it was shipped in crushed dry ice to the University of Connecticut, where it was stored at -20 C until needed. Prior to separation by centrifugation and extraction of the lipid, the semen was thawed in warm water and a 5-ml aliquot taken for dry weight determinations.

Dry weight determinations, cellular disruption, lipid extraction, and identification of the various lipid classes were as previously outlined (6, 7).

Data reported herein on seminal plasma and spermatozoa separated before freezing and storage at -20 or -79 C have been designated prefreeze, whereas those separated after freezing and storage have been labeled post-freeze.

Portions of these data were subjected to “t” test, taking into consideration, wherever applicable, unequal numbers and different variances obtained from different populations (4).
RESULTS

During the course of previous investigations with bovine semen, lipids were extracted from twelve prefreeze and four post-freeze samples of spermatozoa from 2,138 ml of semen. The per cent lipid from the pre- and post-freeze samples averaged 13.6 and 7.8, respectively; whereas, lipid content of 3,138 ml seminal plasma separated from nine prefreeze and six post-freeze composite samples averaged 1.23 and 2.20%, respectively. It became evident almost immediately that there was a considerable difference between the two types of samples in quantity of lipid obtained, which was surprising since the samples were composed of numerous ejaculates. Thus, it was suspected that the differences were due to the method of treating the semen prior to centrifugation, i.e., freezing and storage.

Due to the objectives of the earlier studies (6, 7), no attempt was made to identify the plasma with the spermatozoa from which it was separated. Consequently, no comparisons could be made to definitely account for the differences in quantity of lipid extracted. Hereafter, the seminal plasma was identified with the cell fraction from which it was separated, regardless of treatment. In Table 1 it can be clearly seen that there was a larger quantity of lipid in the post-freeze seminal plasma (P < 0.01), accompanied by a lower amount of lipid in the sperm fraction (P < 0.05) as compared to the corresponding prefreeze fractions; whereas, there was no difference in total lipid extracted. Thus, it appears evident that the differences in lipid quantity of pre- and post-freeze seminal plasma and spermatozoa are due to treatment rather than semen source.

The major lipid classes in bovine spermatozoa and seminal plasma from semen separated before freezing have been identified and their relative percentages determined (7). Due to the differences observed in total lipid quantity in the various fractions (Table 1), an investigation of lipid classes in semen separated and analyzed after freezing (post-freeze) might show some alteration in the lipid classes. Results of such a preliminary study of composite ejaculates from three bulls are presented in Table 2.

The differences in lipid quantity of the seminal plasma and cells are not as clearly demonstrated as in Table 1, but the trend is the same, i.e., the concentration of lipid in the seminal plasma is higher and that in the spermatozoa lower than previously observed for prefreeze semen (Table 1). The per cent phospholipids were lower in the spermatozoa and higher in the seminal plasma than found previously (7). In addition, there were some slight alterations of some of the other lipid classes, but the numbers are too small to properly evaluate these trends. Therefore, additional studies are planned to extend these studies, using appropriate controls.

DISCUSSION

Saacke and Almqquist (17) have shown with electron microscopy that the cell membrane is altered when undiluted bovine spermatozoa are frozen or freeze-dried in the absence of glycerol. Since current theories on cellular membrane structure and composition suggest that lipids, particularly phospholipids and cholesterol, are among the chief components (8), it is likely that the increased lipid in the seminal plasma of the post-freeze samples (Table 1) reflects cellular destruction during the freezing and thawing process with subsequent incorporation of the lipid into the seminal plasma. Although this is in general agreement with Hartree and Mann (5), the quantity of lipid lost from ram spermatozoa after freezing appeared to be much lower than

<table>
<thead>
<tr>
<th>Lipid</th>
<th>Seminal plasma</th>
<th>Spermatozoa</th>
<th>Whole semen</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prefreeze</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>198</td>
<td>1.26</td>
<td>194</td>
</tr>
<tr>
<td>Range</td>
<td>130–238</td>
<td>1.09–1.52</td>
<td>120–281</td>
</tr>
<tr>
<td>Post-freeze</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>440</td>
<td>1.92</td>
<td>398</td>
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<tr>
<td>Range</td>
<td>295–516</td>
<td>1.68–2.05</td>
<td>318–440</td>
</tr>
</tbody>
</table>

TABLE 1
Comparison of the lipid content of bovine semen, spermatozoa, and seminal plasma separated before (prefreeze) and after (post-freeze) freezing.
Composition of the various lipid classes in bovine spermatozoa and seminal plasma from individual animals separated and analyzed after the semen had been frozen and stored at −20°C (post-freeze).

<table>
<thead>
<tr>
<th>Bull</th>
<th>(ml)</th>
<th>Lipid (mg/ml)</th>
<th>Phospholipids</th>
<th>Cholesterol</th>
<th>Diglycerides</th>
<th>Triglycerides</th>
<th>Wax esters</th>
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<tbody>
<tr>
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<tr>
<td>1</td>
<td>48</td>
<td>1.17</td>
<td>73.1</td>
<td>13.2</td>
<td>4.6</td>
<td>6.0</td>
<td>3.2</td>
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<tr>
<td>2</td>
<td>91</td>
<td>1.16</td>
<td>62.0</td>
<td>14.0</td>
<td>7.0</td>
<td>12.8</td>
<td>6.2</td>
</tr>
<tr>
<td>3</td>
<td>35</td>
<td>1.77</td>
<td>77.8</td>
<td>15.0</td>
<td>5.0</td>
<td>1.5</td>
<td>0.7</td>
</tr>
<tr>
<td>Mean</td>
<td>58</td>
<td>1.37</td>
<td>71.0</td>
<td>14.1</td>
<td>5.5</td>
<td>6.8</td>
<td>3.4</td>
</tr>
<tr>
<td>Seminal plasma</td>
<td></td>
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<td></td>
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</tr>
<tr>
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<td>48</td>
<td>1.23</td>
<td>74.2</td>
<td>14.4</td>
<td>4.6</td>
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<tr>
<td>Mean</td>
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<td>1.36</td>
<td>73.4</td>
<td>15.2</td>
<td>4.1</td>
<td>4.5</td>
<td>2.8</td>
</tr>
</tbody>
</table>

* Per cent of total lipid recovered.

† Spermatozoa contained in.

Both ram spermatozoa (5) and human red blood cells (12) lose lipid as a result of washing. Lovelock (11–13), in addition, has shown that cholesterol, phospholipids and lipoproteins, amounting to 25% of the volume of the human red blood cell and in excess of 40% of the dry weight of the total lipids, are lost when subjected to a variety of treatments. Therefore, it appears probable that method of handling, such as washing procedures, storage temperature, etc., could account for the low lipid concentration in ram semen reported by Lovern et al. (14) and in bovine spermatozoa by Terner and Korsh (18). At this point it would be hazardous to suggest that loss of lipid from the spermatozoa is responsible for the deleterious effects of washing and dilution, particularly in light of recent evidence presented by Lodge et al. (9, 10) on the effect of carbon dioxide on dilution and washing of bovine spermatozoa.

It is possible that the amount of lipid found in the prefreeze seminal plasma (Table 1) may not be completely indicative of the quantity contributed by the accessory sex glands, because three composite samples of seminal plasma totaling 557 ml from a vasectomized Guernsey bull contained only 0.69 mg lipid/ml compared to 1.26 mg/ml shown in Table 1. There appear to be several possibilities to account for these differences, (a) the lipid in the seminal plasma of this individual bull may have been exceedingly low, (b) certain parts of the reproductive tract may have an affinity for cellular lipids, particularly the protoplasmic droplets, and this in some manner contributes to the seminal plasma lipid from freshly collected cells, (c) epididymal fluid could be extremely high in lipid material, (d) there may have been a loss of lipid from the cells into the seminal plasma upon contact and dilution, which was further aided by centrifugation during separation. The latter might agree with Lovelock (12), who suggested that when human red blood cells are suspended in fresh saline, the surface lipids would dissolve or disperse in the medium. Bialy and Smith (1) observed a significant decrease in protoplasmic droplets associated with epididymal bovine spermatozoa upon dilution with seminal vesicular fluid. White and Wales (19) found no loss of droplets from epididymal ram spermatozoa incubated in seminal plasma for 2 hr at 37°C. If protoplasmic droplets are composed of lipids and they are present on epididymal cells and absent from ejaculated spermatozoa, the lipid material must either be absorbed by the cell, lost into the seminal plasma, or taken up by the reproductive tract.

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REFERENCES

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