BRIEF COMMUNICATION

RESULTS OF INSEMINATIONS WITH RAM SPERMATOZOA IN DIFFERENT MEDIA

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(Received 29th April 1964)

Previous studies have shown that stored ram semen (ram spermatozoa kept in contact with seminal plasma for 4½ hr at 30° C) is less likely to cause conception when inseminated into a ewe than fresh semen with a lower impedance change frequency (Dott, 1961). In the same paper it was shown that epididymal semen could be stored at 30° C and ejaculated semen at 20° C for 4½ hr without causing a serious drop in the conception rate obtained with them. It could be concluded from these experiments that some factor in the seminal plasma adversely affected the fertilizing ability of ram spermatozoa. This effect is dependent on time and temperature but it is not known whether it is dependent on the continued presence of seminal plasma. Ewes have been inseminated with ejaculated ram spermatozoa separated from the seminal plasma, as the first in a series of experiments to test this.

In these preliminary experiments the spermatozoa were freed of seminal plasma by a washing procedure, the first stage of which was a 1:1 dilution and the final theoretical dilution of the constituents of the seminal plasma was 1:2592. This was brought about in four identical cycles of dilution and centrifugation at about 300 g. In practice the theoretical dilution is not achieved for constituents of the seminal plasma (Dott & Walton, 1960). The sperm density of the final suspension was the same as in the ejaculated semen.

The effects of four washing fluids were studied, two phosphate buffers and two bicarbonate buffers. The composition of the phosphate buffers was (i) sodium phosphate: 0·128 m-NaCl, 0·0204 m-Na₂HPO₄.12H₂O, 0·0048m-NaH₂PO₄.2H₂O; (ii) sodium potassium phosphate: 0·124 m-NaCl, 0·005 m-KCl, 0·0204 m-Na₂HPO₄.12H₂O, 0·0048 m-NaH₂PO₄.2H₂O and the bicarbonate buffers contained (iii) sodium bicarbonate: 0·128 m-NaCl, 0·026 m-NaHCO₃; (iv) sodium potassium bicarbonate: 0·124 m-NaCl, 0·005 m-KCl, 0·026 m-NaHCO₃. All were adjusted to pH 7·3.

After washing in sodium potassium bicarbonate, the spermatozoa were packed into a pellet which was more difficult to disperse than the loose aggregations obtained in the other three solutions.

The results of insemination are given in Table 1 and an overall χ² analysis showed that the spermatozoa washed in phosphate buffer produced a higher

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conception rate than the spermatozoa washed in bicarbonate buffer (significant at 1% level). However it is of interest that thirty-three out of the total of fifty-two inseminations resulted in conceptions (63.5%) and a conception rate of 60 to 70% is reasonable for inseminations with fresh, untreated ram semen (Dott, 1961).

### Table 1

**RESULTS OF INSEMINATIONS WITH RAM SPERMATOZOA WASHED IN VARIOUS BUFFERS**

<table>
<thead>
<tr>
<th></th>
<th><strong>Phosphate buffers</strong></th>
<th></th>
<th><strong>Bicarbonate buffers</strong></th>
<th></th>
<th><strong>Total</strong></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. inseminations</td>
<td>No. inseminations resulting in pregnancy</td>
<td>No. inseminations</td>
<td>No. inseminations resulting in pregnancy</td>
<td>No. inseminations</td>
<td>No. inseminations resulting in pregnancy</td>
</tr>
<tr>
<td>Sodium</td>
<td>11</td>
<td>10</td>
<td>10</td>
<td>8</td>
<td>21</td>
<td>16 (76%)</td>
</tr>
<tr>
<td>Sodium-potassium</td>
<td>10</td>
<td>8</td>
<td>21</td>
<td>6</td>
<td>31</td>
<td>17 (55%)</td>
</tr>
<tr>
<td>Total</td>
<td>21</td>
<td>18 (84%)</td>
<td>21</td>
<td>9</td>
<td>52</td>
<td>33 (63.5%)</td>
</tr>
</tbody>
</table>

Results for phosphate and bicarbonate buffers differ significantly at 1% level.

Nearly all the reports on artificial insemination of ram semen have concluded that a dilution of about 1:6 is the maximum consistent with a good conception rate; it would seem that this is not because of the dilution of some essential substance or the loss of material from the spermatozoa, since a dilution of 1:2500 of the seminal plasma did not affect the conception rate of washed ram spermatozoa. It is probable that the critical factor in dilution is the sperm density at the external os of the cervix.

The other fact with which most reports on ram semen agree is that the spermatozoa are unable to maintain their fertility after storage. This may be because the seminal plasma had not been sufficiently diluted, thus allowing the 'seminal factor', the presence of which may be deduced from the work described by Dott (1961), to act on the spermatozoa. If this is so then insemination with stored, washed ram spermatozoa should be successful unless seminal plasma exerts its effect on the spermatozoa quickly and irreversibly.

### REFERENCES
