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A STUDY ON THE USE OF UNFROZEN, DILUTED SEMEN FOR THE IN VITRO FERTILISATION OF BOVINE OOCYTES MATURED *IN VITRO*

ANGELA DAWN SEATON

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A STUDY ON THE USE OF UNFROZEN, DILUTED SEMEN FOR THE IN VITRO FERTILISATION OF BOVINE OOCYTES MATURED IN VITRO

A thesis presented in partial fulfilment of the requirements for the degree of Master of Agriculture Science in Animal Science at Massey University

ANGELA DAWN SEATON

1991
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ABSTRACT

The study investigated the use of unfrozen, diluted semen for in vitro fertilisation of bovine oocytes matured in vitro. In experiment 1, semen from each of two bulls was used on two consecutive days ("day-old" and "two-day-old" sperm) to explore the effect of sperm concentration on oocyte penetration rates. The sperm concentrations used were 0.125, 0.25, 0.5, 1.0, 2.0 and 4.0x10⁶/ml. Penetration rates were uniformly high when day-old sperm was used, but low penetration rates were obtained below 1x10⁶/ml with two-day-old sperm. Unfrozen sperm appeared to give better penetration rates than frozen-thawed sperm at concentrations of 0.5-2.0x10⁶/ml. There was no relationship between sperm concentration and incidence of polyspermy. In experiment 2, sperm from the same two bulls were used to investigate the presence of Caprogen extender in the fertilisation medium. Caprogen inhibited penetration when present in concentrations greater than 10ml/litre. Experiments 3 and 4 studied the effect of heparin on penetration rates. In experiment 3, sperm from one bull was used to inseminate oocytes in medium containing 0, 1, 5, 10, 20, 30 or 50μg/ml heparin. There was no relationship between penetration rates and heparin concentrations in the medium, and average penetration rates were high for all concentrations. In experiment 4, five bulls were used to investigate penetration rates at heparin levels of 0μg/ml (frozen-thawed and unfrozen sperm) and 10μg/ml (unfrozen sperm). The results obtained in experiment 3 with sperm from one bull were confirmed; penetration was obtained in the absence of heparin with all five bulls.

Good penetration can be obtained in vitro with unfrozen sperm, and its greater longevity and viability make it a useful alternative to frozen semen for both commercial and research in vitro fertilisation programmes.
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<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>AR</td>
<td>acrosome reaction</td>
</tr>
<tr>
<td>BO</td>
<td>Brackett's-Oliphant's medium</td>
</tr>
<tr>
<td>BSA</td>
<td>bovine serum albumin</td>
</tr>
<tr>
<td>cAMP</td>
<td>cyclic adenosine monophosphate</td>
</tr>
<tr>
<td>COC</td>
<td>cumulus-oocyte complex</td>
</tr>
<tr>
<td>DNA</td>
<td>deoxyribonucleic acid</td>
</tr>
<tr>
<td>FCS</td>
<td>foetal calf serum</td>
</tr>
<tr>
<td>FSH</td>
<td>follicle stimulating hormone</td>
</tr>
<tr>
<td>GAG</td>
<td>glycosaminoglycan</td>
</tr>
<tr>
<td>hCG</td>
<td>human chorionic gonadotropin</td>
</tr>
<tr>
<td>HIS</td>
<td>high ionic strength (medium)</td>
</tr>
<tr>
<td>IVC</td>
<td>in vitro culture</td>
</tr>
<tr>
<td>IVF</td>
<td>in vitro fertilisation</td>
</tr>
<tr>
<td>IVM</td>
<td>in vitro maturation</td>
</tr>
<tr>
<td>LIC</td>
<td>Livestock Improvement Corporation</td>
</tr>
<tr>
<td>LH</td>
<td>luteinising hormone</td>
</tr>
<tr>
<td>m199</td>
<td>medium 199</td>
</tr>
<tr>
<td>OCS</td>
<td>oestrous cow serum</td>
</tr>
<tr>
<td>PBS</td>
<td>phosphate-buffered saline solution</td>
</tr>
<tr>
<td>PHE</td>
<td>penicillamine, hypotaurine, epinephrine</td>
</tr>
<tr>
<td>SAS</td>
<td>Statistical Analysis Systems</td>
</tr>
<tr>
<td>TALP</td>
<td>Tyrode's medium with acetate, lactate, pyruvate</td>
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