SUCCESSFUL CULTURE IN VITRO OF SHEEP AND CATTLE OVA

H. R. TERVIT, D. G. WHITTINGHAM* AND L. E. A. ROWSON

Agricultural Research Council, Unit of Reproductive Physiology and Biochemistry,
307 Huntington Road, Cambridge CB3 0JQ, and
*Physiological Laboratory, University of Cambridge

(Received 15th April 1972, accepted 9th May 1972)

Fertilized sheep and cattle ova have not been reported to develop readily during culture in vitro. Up to 60% of sheep morulae develop normally during culture (Moor & Cragle, 1971) but earlier cleavage stages undergo limited development (Hancock, 1963; Kraemer, 1966; Tervit & McDonald, 1969; Moore, 1970) and it has been suggested that there is a block to development in vitro at the eight- to twelve-cell stage (Wintenberger, Dauzier & Thibault, 1953). Only the early cleavage stages of cattle ova have been cultured and these have not been reported to develop beyond the twenty-four-cell stage in vitro (Thibault, 1966; Brinster, 1968; Sreenan, 1968; Sreenan, Scanlon & Gordon, 1968). This communication describes the successful culture of one-cell to eight-cell sheep ova and one-cell and eight-cell cattle ova to the morula and blastocyst stages and reports a high embryo survival after transfer of cultured ova to recipient animals.

Welsh mountain ewes were induced to superovulate and were mated at the ensuing oestrus. They were subjected to laparotomy 48 to 72 hr after the onset of oestrus and the embryos were flushed from the reproductive tract in a modified Dulbecco's phosphate-buffered salt solution (PBS) supplemented with 0.33 mm-sodium pyruvate, 5.56 mm-glucose, 4 mg/ml bovine serum albumin and 100 U/ml penicillin (sodium salt). This medium was similar to that used successfully for the freeze-preservation of mouse embryos (Whittingham, 1971a) and had an osmolarity of 290 mosmol and a pH of 7.2. The ova were held at 37°C in this medium until they were placed in culture. Cattle ova were flushed from the reproductive tracts of superovulated donors with TCM-199 by the technique described by Rowson, Moor & Lawson (1969) and were held at 37°C in this medium before being placed in culture.

Preliminary experiments examined the development of eight-cell sheep ova in a series of biological and chemically defined media. The embryos were cultured in droplets of medium under light-weight liquid paraffin in an atmosphere of 5% CO₂ in air (Brinster, 1963). The results showed that ova cleaved most readily in a medium with a composition based on the biochemical analysis of sheep oviduct fluid (Restall & Wales, 1966). In this medium, which will be referred to as synthetic oviduct fluid (SOF), eight-cell ova cleaved to an average of twenty-four cells as measured by the number of nuclei in each embryo after 3 days in culture. The medium contained 107.70 mm-NaCl, 7.16 mm-KCl,
1.19 mM-KH$_2$PO$_4$, 1.71 mM-CaCl$_2$, 0.49 mM-MgCl$_2$, 25.07 mM-NaHCO$_3$, 3.30 mM-Na lactate, 0.33 mM-Na pyruvate, 1.50 mM-glucose, 32 mg bovine serum albumin (1 ml), 100 units penicillin (sodium salt)/ml and 50 µg streptomycin/ml. The osmolarity was 270 mosmol and the pH 7.2 to 7.4. The medium was prepared in 13 ml vols from stock solutions using the routine described by Whittingham (1971b). After millipore (0.45 µm) filtration, it was stored in air-tight test-tubes at 5° C.

Since oxygen concentration has been shown to be an important factor for the normal development of cultured mouse embryos (Whitten, 1971), sheep embryos were cultured in SOF in the presence of varying oxygen concentrations in an attempt to further their development in vitro. The embryos were cultured in 6-ml glass test-tubes using the method described by Biggers, Whitten & Whittingham (1971). One ml of SOF was equilibrated, in the tubes, by gassing for approximately 1 min with one of the following gas mixtures: 5% CO$_2$/95% N$_2$; 5% CO$_2$/5% O$_2$/90% N$_2$; 5% CO$_2$/10% O$_2$/85% N$_2$; or 5% CO$_2$ in air, and the gassed tubes were sealed and placed in an incubator at 37° C overnight. The sheep ova collected in modified PBS were washed in 2 ml SOF, previously gassed with 5% CO$_2$ in 95% N$_2$ and were then placed into equilibrated test-tubes which were regassed with the appropriate gas mixture, corked and incubated at 37° C. Every 2nd day, the cultures were regassed. As a control, ova were cultured in droplets of medium under liquid paraffin in an atmosphere of 5% CO$_2$ in air. After culture, the embryos were either fixed, stained and examined for the number of nuclei present or held in the supplemented PBS until transfer to recipient animals.

The development of sheep ova in various oxygen concentrations is summarized in Table 1. Initially, ten eight-cell ova were cultured for 3 days at each oxygen level and were stained to examine for the number of nuclei present. Over a period of 3 days, the eight-cell sheep ovum would be expected to develop in vivo to a late morula. Of the stained ova, those cultured in 5% oxygen gave the most comparable development in vitro. Ova cultured in 10% oxygen, although cleaving readily, were less developed after 3 days culture. Before culture, ova were washed in SOF exposed to air, and it is possible that extra oxygen was transferred with the ova to the test-tubes. This addition of oxygen may explain the development of ova during ‘anaerobic’ culture. Alternatively, either the eight-cell ovum may have sufficient energy reserves to support at least one cleavage in the absence of oxygen or some embryos may have been recovered just before mitosis. High concentrations of oxygen inhibited development of ova cultured both in test-tubes and under paraffin oil. This effect is similar to that reported for mouse embryo culture (Whitten, 1971). Under conditions in vivo, the one-cell and two- to four-cell sheep ovum would be expected to develop to a blastocyst over 6 and 5 days, respectively, and attempts were made to culture these early stages in 5% oxygen. After culture for 5 or 6 days, the one-cell ova were slightly retarded and the two- to four-cell ova rather more retarded, with most of the latter developing to morulae.

Cultured sheep ova were transferred to recipients whose oestrous cycles were closely synchronized (+24 hr) with the stage of development of the ova. One uterine horn of each recipient was ligated anterior to the uterine body so that
<table>
<thead>
<tr>
<th>Species of ova</th>
<th>Oxygen concentration</th>
<th>No. of ova cultured and developmental stage at commencement of culture</th>
<th>Length of culture (days)</th>
<th>Developmental stage reached at cessation of culture (no. of ova/stage)</th>
<th>No. of ova transferred to recipient animals</th>
<th>No. of viable embryos recovered from slaughtered recipients $^\ddagger$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sheep</td>
<td>0%</td>
<td>Ten, 8-cell* Two, 8-cell†</td>
<td>3</td>
<td>8-cell 1 2 3 1 2 1</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Two, 1-cell* Four, 1-cell†</td>
<td>6</td>
<td>12-cell 2 3 1</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>5%</td>
<td>Two, 2-to 4-cell* Five, 2-to 4-cell†</td>
<td>6</td>
<td>16-cell 1 1</td>
<td>5</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Four, 2-to 4-cell†</td>
<td>5</td>
<td>Early morulae (approx. 32 cells) 2 3</td>
<td>5</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ten, 8-cell* Six, 8-cell† Three, 8-cell†</td>
<td>6</td>
<td>Morulae (approx. 64 cells)</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>10%</td>
<td>Ten, 8-cell* Two, 8-cell†</td>
<td>3</td>
<td>Later morulae 4 3 1</td>
<td>6</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Three, 8-cell†</td>
<td>4</td>
<td>Early blastocyst 1 4</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>20%</td>
<td>Ten, 8-cell*</td>
<td>3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>Ten, 8-cell*</td>
<td>3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cattle</td>
<td>5%</td>
<td>Ten, 1-cell*</td>
<td>6</td>
<td>8-cell 1 4 2 3</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>One, 8-cell*</td>
<td>4</td>
<td>16-cell 1 1</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Four, 8-cell†</td>
<td>4</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Stained and examined for number of nuclei at cessation of culture.
† Developmental stage visually assessed at cessation of culture. Ova transferred to recipient animals.
‡ Recipient ewes slaughtered on Day 13 or 14 of the oestrus cycle.
§ Recipient cattle palpated per rectum on Day 35 of the oestrous cycle. Pregnant recipients allowed to proceed to term.
ova from different treatments transferred to the two uterine horns could not migrate between horns. The recipients were slaughtered by Day 14 to ensure that embryos in the contralateral horn were not lost through any uterine luteolytic effect due to the death of embryos in the uterine horn ipsilateral to the corpus luteum. After transfer of thirteen cultured eight-cell ova, eleven were recovered as viable conceptuses on Day 13 or 14. The conceptuses were measured for total length and length of embryonic disc and were found to be normal in appearance and to have developed to a stage comparable with non-cultured 13- and 14-day conceptuses (Rowson & Moor, 1966). A lower proportion of cultured one-cell and two- to four-cell ova were recovered as viable embryos and, as expected, ova showing the greatest development during culture survived most readily on retransfer to recipient ewes. The lower survival of cultured one-cell and two- to four-cell ova, relative to eight-cell ova, may be due to the increased length of culture of these ova and not to the cleavage stage at commencement. In a further examination of the viability of the cultured ovum, embryos have been transferred to recipients which have been allowed to proceed to term.

The cattle ova recovered in TCM-199 were washed in 2 ml of pregassed SOF before being placed into test-tubes containing SOF equilibrated with 5% O₂. The development of ova during culture is shown in Table 1. The one-cell cattle ovum would be expected to develop in vivo to a late morula or early blastocyst over 6 days. Most cultured ova were found to be retarded. Eight-cell ova would be expected to develop in vivo to late morulae and blastocysts in 4 days. Cultured ova were found to develop at a similar rate. Four of these cultured eight-cell ova were transferred to synchronized recipients. One ovum was transferred to each uterine horn of two recipients and of these recipients, one exhibited oestrus 17 days after transfer and one was diagnosed as carrying twin embryos when palpated per rectum on Day 35 of the cycle. Again, those ova which achieved the greatest development during culture survived most readily on transfer to the recipient.

The results show that a combination of satisfactory medium and gas phase has allowed sheep and cattle ova to be cultured from the early cleavage stages to viable morulae and blastocysts and has allowed the sheep ovum to develop through the supposed ‘block to development’.

H.R.T. is a recipient of a New Zealand Department of Scientific and Industrial Research Fellowship and D.G.W. a Beit Memorial Fellow.

REFERENCES


Culture of sheep and cattle ova in vitro


