Co-culture of 1-cell outbred mouse embryos on bovine kidney epithelial cells: effect on development, glycolytic activity, inner cell mass:trophectoderm ratios and viability

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In an attempt to enhance embryo development, we have co-cultured 1-cell OF1 mouse embryos on bovine kidney epithelial (Madine–Darby bovine kidney; MDBK) cells in a complex medium called complex mouse tubal fluid (cMTF; based on the energy substrate levels found in the mouse oviduct, containing non-essential amino acids, glutamine and EDTA). To determine the quality of the blastocysts obtained, we examined several parameters: morphology, total cell numbers, inner cell mass (ICM):trophectoderm (TE) ratio, glycolytic activity and viability after transfer. A significantly lower number of blastocysts developed on MDBK cells compared with cMTF medium. cMTF blastocysts had a significantly higher glycolytic activity and a lower blastocyst cell number than those grown in co-culture, while both in-vitro groups had higher ICM:TE ratios compared with in vivo. Blastocysts grown on MDBK cells displayed an elevated ICM number compared with those grown in cMTF medium alone. However, the percentage of fetuses after transfer remained drastically low in both culture groups compared with in-vivo blastocysts. In conclusion, co-culture did not increase the number of zygotes reaching the blastocyst stage. Although co-culture blastocysts show some similarities to in-vivo embryos in cell number and glycolytic activity, no enhancement in viability was observed.

Key words: co-culture/embryo culture/inner cell mass/metabolism/trophectoderm

Introduction

Over the past 20 years considerable progress has been made in culturing preimplantation embryos. However, many problems remain, including retarded development in vitro compared with in vivo, a decrease in viability (Harlow and Quinn, 1982; Sakkas et al., 1989) and species-specific blocks at the time of embryonic genomic activation (Goddard and Pratt, 1983; Gandolfi and Moor, 1987; Lawitts and Biggers, 1991a). A main challenge of embryo culture studies is therefore to enhance embryo development by finding conditions as close as possible to those in vivo. Since the initial embryo culture studies (Whittingham, 1971; Quinn et al., 1982), several successful media modifications have been reported. For example, Chatot et al. (1989) successfully cultured mouse embryos from a blocking strain by elevating the lactate pyruvate ratio and by adding glutamine and EDTA to a medium without glucose, while Gardner and Leese (1990) obtained a glycolytic rate closer to that in vivo by using a culture medium based around the carbohydrate composition of mouse tubal fluid (MTF). Modifications to this medium were made by Gardner and Sakkas (1993), who showed that components such as amino acids, vitamins and growth factors increased embryo development and cell number. Embryo development in medium containing amino acids has also been shown to support a pattern of gene expression similar to that in embryos developed in vivo (Ho et al., 1995). Gardner and Lane (1993) have also examined the role of specific groups of amino acids and found that blastocyst formation and cell number were higher when non-essential amino acids and glutamine were added to the medium. Interestingly, the presence of amino acids in the culture medium was also responsible for an inhibition of embryo development, as amino acids spontaneously break down at 37°C to produce embryotoxic ammonium ions (Gardner and Lane, 1993; Lane and Gardner, 1994).

Whilst the formulation of a defined medium has led to improvements in embryo development, another approach has been the use of co-culture or medium which was conditioned by cells. The increased interest in this technique followed a report by Gandolfi and Moor (1987), who successfully used co-culture with sheep oviduct epithelial cells to improve the culture and viability of sheep embryos. Similar studies were subsequently conducted by Eyestone and First (1989) on cattle, Ouhibi et al. (1990), Sakkas and Trounson (1990) and Lai et al. (1992) in mice and Sakkas et al. (1989) in goats. Several authors (Gandolfi and Moor, 1987; Ellington et al., 1990; Mermillod et al., 1993) have since suggested the presence of embryotrophic factors but have failed to identify them specifically. More conclusive evidence exists for the presence of growth factor receptors such as insulin-like growth factor (IGF)-I, IGF-II, insulin, epidermal growth factor (EGF) and platelet-derived growth factor (Adamson, 1993; Harvey et al., 1995) on early embryos. Therefore if cells are secreting growth factors, these could enhance embryo development.

The enhancement of embryo development in co-culture systems has been observed in many cases, but how the cells were acting was not determined. One of the perceived problems of co-culture is the use of inadequate culture medium to
support both the embryo and cells (Leppens and Sakkas, 1995). We have shown previously that Ménézo’s B2 medium conditioned by Madine–Darby bovine kidney epithelial (MDBK) cells could support mouse blastocyst formation, while Ménézo’s B2 medium alone was inadequate. In these experiments, a beneficial effect was observed only when poor embryo development occurred in medium alone. This has led to the theory that the cells in co-culture only act by removing embryotoxic factors from poor culture medium (Bavister, 1992). In this study, we have tried to overcome the problem by using cMTF medium, a complex medium which has already been shown to support enhanced mouse embryo development (Gardner and Lane, 1993).

The aim of this study was to show the effect of cMTF medium during co-culture. We have cultured outbred mouse embryos from the 1-cell stage until the blastocyst stage in two conditions: (i) cMTF medium, a medium based on MTF supplemented with non-essential amino acids, glutamine and EDTA (Gardner and Lane, 1993; Lane and Gardner, 1994), and (ii) a co-culture system using MDBK cells, with cMTF as the base medium. From the blastocysts obtained, we have examined numerous parameters to determine their quality. We have compared their morphology on days 3 and 5 of culture, determined the glycolytic activity of blastocysts and their respective cell numbers [trophectoderm (TE) and inner cell mass (ICM)] and tested for blastocyst viability after transfer.

Materials and methods

Culture media

In all experiments, a modified MTF medium was used called cMTF (Gardner and Leese, 1990; Gardner and Lane, 1993; Lane and Gardner, 1994). MTF medium was characterized by a reduced sodium chloride concentration (103.4 mM) compared with the original formulation (114.2 mM). The culture medium cMTF had the following composition: 103.4 mM NaCl, 4.78 mM KCl, 1.19 mM KH2PO4, 1.71 mM CaCl2·2H2O, 0.37 mM sodium pyruvate, 4.79 mM sodium lactate, 20 mM NaHCO3, 3.40 mM glucose and 0.01 g/l phenol red. This cMTF medium (pH 7.4, osmolality 288–292 mosmol/kg) was supplemented with non-essential amino acids used at the concentration present in Eagle’s minimal essential medium (Gibco, Basel, Switzerland), l-glutamine (1 mM) and, where stated, EDTA (100 μM). Embryo collection was performed in a HEPES-buffered modification of cMTF (cMTF buffer; Gardner and Lane, 1993). In this medium, 20 mM NaHCO3 were replaced with 20 mM HEPES, pH 7.4. Antibiotics (100 μl penicillin G, 1000 μg/ml streptomycin sulphate) and 4 mg/ml bovine serum albumin (BSA; Sigma Pharmaceuticals, Buchs, Switzerland) were added to all culture media. After 48 h of culture, the embryos were placed in fresh medium (without EDTA) to overcome the toxic effect of ammonium ions (Gardner and Lane, 1993; Lane and Gardner, 1994).

Embryo culture

Embryos were obtained from 3–4 week old outbred (OFI) mice (BRL, Fullinsdorf, Switzerland) which exhibit the 2-cell block in vitro (Sakkas et al., 1993). The females were superovulated by i.p. injection of 7 IU pregnant mare’s serum (PMS; Folligon; Veterinaria, Zurich, Switzerland) and an additional injection 48 h later of 5 IU human chorionic gonadotrophin (HCG; Chorulon; Veterinaria). They were then placed individually with OFI males of proven fertility. The following morning, the effectiveness of mating was confirmed by the presence of a vaginal plug (day 1). The 1-cell embryos were collected in cMTF buffer at 21–24 h post-HCG. Removal of the cumulus cells was achieved in cMTF buffer containing 1 mg/ml hyaluronidase (Sigma). One-cell embryos were pooled, washed, and cultured in cMTF buffer and then placed randomly into groups of 35–40 in 80 μl of the different test media under light mineral oil (Sigma) at 37°C in 5% CO2 in air.

Co-culture system

During these experiments, MDBK cells were used for co-culture. These cells have been used previously in co-culture and in conditioned medium studies (Ouhibi et al., 1990; Menezo et al., 1993). They have been reported to improve embryo development (Ouhibi et al., 1990) and share the same embryonic origin (mesoderm) as oviduct cells. In addition, the use of cell lines instead of oviduct cells is preferable in the human as these cells can be checked for contamination, e.g. by viruses. The cells were maintained in Dulbecco’s modified Eagle medium (DMEM; Gibco) supplemented with 10% fetal calf serum (FCS) and antibiotics. Before each experiment, a passage of the MDBK cells was performed (at the same time as PMS injection) and 7×106 cells/ml were seeded in a 96-well plate (Costar; Integra Biosciences, Argovie, Switzerland). The confluenacy of the cells was examined before the experiment, and DMEM with 10% FCS medium was removed. The cell monolayers were then washed with cMTF medium, and 80 μl of equilibrated cMTF medium containing EDTA were placed on the cells. This was then covered with light mineral oil. Cell morphology was retained during the culture period in cMTF medium and there was no evidence of cell death. During the experiments, embryos were grown in cMTF medium with EDTA during the first 48 h, after which embryos were placed in cMTF medium without EDTA. The presence of EDTA in the medium has been shown to have a beneficial effect only during the early cleavage stages (Abramczuk et al., 1977; Metha and Kessling, 1990). Embryos grown in cMTF medium alone were cultured using the same protocol.

Assessment of morphology

Embryo morphology on day 3 of culture (number of embryos ≥4-cells at 69 h post-HCG) was assessed during the passage from medium with EDTA to medium without EDTA. On day 5, the number of blastocysts at 120 h post-HCG was recorded.

Assessment of glycolytic activity

The glycolytic activity was determined in day 5 blastocysts following the procedure reported by O’Fallon and Wright (1986) and Rieger and Guay (1988). Briefly, the labelled substrate D-5-[3H]glucose (Amersham International, Amersham, UK) was dried under nitrogen and resuspended in cMTF buffer to give a nominal concentration of 0.25 μCi/μl and a final specific activity of 13 Ci/μl. Individual blastocysts were taken up in 3 μl (Soccorex; Millian, Geneva, Switzerland) buffer containing 0.019 mM radioactive-labelled glucose and 5.6 mM cold glucose placed previously on the detached lid of a 2 ml polypolypropylene microcentrifuge vial (Milian). The lid was fitted to the vial which contained 1.5 ml 0.1 N NaOH to act as a water reservoir for the exchange of [3H2]O. Vials were then incubated for 3 h at 37°C. The efficiency of recovery of radiolabelled products was estimated at intervals from 0 to 180 min by placing amounts of [3H2]O in 3 μl microdroplets of culture medium and culturing them exactly as for the embryo. The recovery efficiency was observed after 3 h of incubation at 37°C. Three sham preparations, containing all reagents but no embryos, were incubated for each experiment to serve as a control for all non-specific measurements of activity due to machine background or spontaneous breakdown of the labelled substrate. Three other samples were prepared by adding 3 μl directly
of the radioactive cMTF buffer into the 1.5 ml NaOH solution; these samples served to determine the total concentration of the radioactively labelled substance at the moment of the experiment. At the end of the incubation period, 1 ml of the NaOH trap was taken and placed into a 20 ml plastic scintillation vial containing 10 ml organic counting solution (Lumac; Lumagel, Bäle, Switzerland) and counted using a Beta spectrophotometer (Packard, Zurich, Switzerland). The amount of substrate metabolized by each embryo was estimated, as described by Rieger and Guay (1988).

**Differential labelling of the TE and ICM**

Each individual blastocyst used in the above glycolysis test was removed from the lid of the centrifuge vial and used to determine the cell number. The numbers of TE and ICM cells of each individual blastocyst were counted by the differential labelling technique using two polynucleotide-specific fluorochromes (propidium iodide and bisbenzimide; Hoechst 33342, Sigma), as described previously by Handside and Hunter (1986). Briefly, blastocysts were incubated in pronase 0.5% (Sigma) at 37°C for 5–10 min to remove the zona pellucidae. They were then washed in buffer and incubated in rabbit anti-mouse spleen cell serum (ICN, Rudolf Streuli AG, Zurich, Switzerland) for 10–15 min at room temperature, washed in buffer and incubated for a further 8 min in guinea pig complement (ICN) diluted to 1:1 in propidium iodide (20 μg/ml) (Sigma). Next the embryos were washed in phosphate buffered saline (PBS) and placed overnight at 4°C in bisbenzimide diluted in ethanol (25 μg/ml). The following day, embryos were fixed on a slide with glycerol. Counting was performed using a Zeiss fluorescence microscope.

**Assessment of embryo viability**

The viability of day 5 embryos was assessed by transferring blastocysts in groups of four to six (depending on the number available at the time of transfer) to the uteri of day 3 pseudopregnant recipients. Transfers were performed between 15:00 and 17:00 h. The same number of blastocysts from cMTF medium or co-culture, or blastocysts generated in vivo and recovered at 96 h after HCG administration, were randomly transferred to opposite uterine horns. Recipient females were examined on day 15 of pregnancy for the number of implantation sites, number of fetuses, normality of fetuses and their relative weight.

**Statistical analysis**

The χ² test was used to compare the percentages of embryos which reached different morphological stages, i.e. cleavage, compacted and blastocyst. Differences between the number of cells and the glycolytic activity in the embryo at the blastocyst stage were compared using a one-way analysis of variance and Sheffe’s F-test for multiple comparisons.

**Results**

**Comparison of embryo development between cMTF medium and co-culture on MDBK cells**

Embryo development was assessed on day 3 (the day of changing medium from cMTF with EDTA to a cMTF medium without EDTA) and again on day 5 (Table I). On day 3, the number of embryos with four or more cells was assessed. A significantly (P < 0.001) higher percentage of embryos had progressed past this stage in cMTF medium (75.7%) compared with co-culture (36.2%). On day 5, embryo development remained more advanced in cMTF medium alone. A significantly (P < 0.001) higher number of blastocysts developed in cMTF medium (37.6%) compared with co-culture (9.0%).

**Comparison of cell numbers in blastocysts**

For each blastocyst in which the glycolytic activity was previously determined, the cell number was also determined. Total cell numbers were higher in co-culture (26.7 ± 8.8) compared with cMTF medium (21.7 ± 8.7) (Table II). Embryos grown in cMTF medium had significantly lower cell numbers than in-vivo blastocysts (30.2 ± 8.0) (P < 0.0001) taken at

<table>
<thead>
<tr>
<th>Culture conditions</th>
<th>69 h post-HCG (day 3)</th>
<th>120 h post-HCG (day 5)</th>
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<tbody>
<tr>
<td>No. of embryos with</td>
<td>No. of blastocysts (%)</td>
<td></td>
</tr>
<tr>
<td>4-cells</td>
<td></td>
<td></td>
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<tr>
<td>Control cMTF</td>
<td>210/412* (58.8)</td>
<td>170/329* (52.6)</td>
</tr>
<tr>
<td>Co-culture MDBK</td>
<td>150/399* (37.6)</td>
<td>120/339* (35.2)</td>
</tr>
</tbody>
</table>

Embryos were grown in cMTF medium ± 0.1 mM EDTA until 69 h after human chorionic gonadotrophin (HCG) administration and then transferred to cMTF without EDTA. The same media protocol was used for co-culture on MD(BK) cells. Values were obtained from 11 experiments and are given relative to the total number of embryos assessed.

*Values with the same superscript were significantly different: P < 0.001.
Table II. Total cell numbers, inner cell mass (ICM) and trophectodermal cell numbers of day 5 blastocysts cultured in control medium (cMTF) or co-culture (MDBK cells) compared with in-vivo day 4 blastocysts

<table>
<thead>
<tr>
<th>Assessment</th>
<th>No. of embryos</th>
<th>Control cMTF</th>
<th>Co-culture MDBK</th>
<th>No. of embryos</th>
<th>In vivo</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total cell number per embryo (±SD)</td>
<td>64</td>
<td>21.7 ± 8.7&lt;sup&gt;b&lt;/sup&gt;</td>
<td>24</td>
<td>26.7 ± 8.3&lt;sup&gt;b&lt;/sup&gt;</td>
<td>38</td>
</tr>
<tr>
<td>Trophoderm (±SD)</td>
<td>64</td>
<td>13.4 ± 6.7&lt;sup&gt;c&lt;/sup&gt;</td>
<td>24</td>
<td>14.2 ± 5.9&lt;sup&gt;d&lt;/sup&gt;</td>
<td>38</td>
</tr>
<tr>
<td>ICM (±SD)</td>
<td>64</td>
<td>8.3 ± 4.9&lt;sup&gt;f&lt;/sup&gt;</td>
<td>24</td>
<td>12.5 ± 6.6&lt;sup&gt;e&lt;/sup&gt;</td>
<td>38</td>
</tr>
<tr>
<td>ICM:trophectoderm (±SD)</td>
<td>64</td>
<td>0.8 ± 0.6&lt;sup&gt;g&lt;/sup&gt;</td>
<td>24</td>
<td>1.0 ± 0.7&lt;sup&gt;h&lt;/sup&gt;</td>
<td>38</td>
</tr>
</tbody>
</table>

For abbreviations, see Table I.

<sup>a</sup>-<sup>h</sup>Values with the same superscript were significantly different: <sup>a</sup>,<sup>c</sup>,<sup>e</sup><sub>P < 0.001</sub>; <sup>b</sup><sub>P < 0.002</sub>; <sup>d</sup><sub>P < 0.005</sub>. The ICM:trophectoderm ratio was calculated for each embryo. Mean and SD values were then calculated.

96 h post-HCG, while the total number of cells in co-culture embryos did not differ statistically. The difference in cell numbers of co-culture blastocysts was caused by a higher number of ICM cells (12.5 ± 6.6) when compared with cMTF medium (8.3 ± 4.9) (P < 0.001) (Table II). In contrast to the differences observed with ICM cells, the number of TE cells in the blastocysts did not differ between co-culture (14.2 ± 5.0) and cMTF medium (13.4 ± 6.7). Both culture protocols gave rise to blastocysts with significantly lower numbers of TE cells compared with in-vivo blastocysts (18.8 ± 5.8, P < 0.002) even though they were 24 h older (Table II). By comparing the ICM:TE ratio, we found that there were no statistical differences between co-culture (1.0 ± 0.7) and cMTF medium (0.8 ± 0.6) embryos. When compared with in-vivo blastocysts (0.6 ± 0.3), we observed the ratio to be significantly higher in both groups (Table II) (P < 0.001).

To examine whether a relationship existed between cell number and glycolytic activity in individual blastocysts, we compared the data obtained from the three different protocols. Surprisingly, the glycolytic activity and the number of cells determined for each blastocyst did not show any correlation (Figure 2). A plot of glycolytic activity compared with total cell number shows widely scattered values in embryos cultured in cMTF medium alone; however, co-culture embryo results were restricted to a smaller area, similar to that observed for in-vivo blastocysts. The glycolytic activity of in-vivo blastocysts was more homogeneous, while that of co-culture blastocysts was less so. Blastocysts developed in cMTF medium alone showed the most scattered levels of glycolytic activity (Figure 2). The glycolytic activity of embryos measured with the radiolabelling technique did not give an overall indication of viability.

**Embryo viability**

Blastocyst transfers to day 3 pseudopregnant females (Table III) showed a high level of implantation in both co-culture (48.5%) and cMTF medium (47.0%). However, the number of fetuses obtained was very low: only 12.1% were obtained in co-culture and 5.9% in cMTF medium. Control transfers of in-vivo blastocysts showed a high level of fetal development (75%). Both in-vitro treatments failed to enhance embryo viability significantly.

**Discussion**

To obtain an enhancement in blastocyst formation and embryo viability, a large number of groups have tested a variety of medium and co-culture systems. Numerous authors (Whittingham, 1971; Chatot et al., 1989; Gardner and Leese, 1990; Lawitts and Biggers, 1991b; Gardner and Sakkas, 1993) have attempted to formulate improved defined media which enable a high level of mouse blastocyst formation. On the other hand, some groups (Ouhibi et al., 1990; Lai et al., 1992) have shown an enhancement in mouse embryo development using co-culture on different cell types. However, as shown by Ouhibi et al. (1990), certain cell lines are not always beneficial for embryo development. The main problem during co-culture...
Table III. Embryo viability after the transfer of day 5 blastocysts grown in complex mouse tubal fluid (cMTF) or co-culture and day 4 in-vivo blastocysts to day 3 pseudopregnant females

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No. of implantations (%)</th>
<th>No. of fetuses (%)</th>
<th>Mean weight (g) (±SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control cMTF</td>
<td>16/34 (47.0)</td>
<td>2/34 (5.9)</td>
<td>0.26 ± 0.038</td>
</tr>
<tr>
<td>Co-culture MDBK</td>
<td>16/33 (48.5)</td>
<td>4/33 (12.1)</td>
<td>0.27 ± 0.034</td>
</tr>
<tr>
<td>In vivo</td>
<td>15/20 (75.0)</td>
<td>15/20 (75.0)</td>
<td>0.30 ± 0.037</td>
</tr>
</tbody>
</table>

For abbreviations, see Table 1. The numbers of implantations and fetuses were assessed on day 15.

Even though the quantity of co-culture blastocysts was low, their quality may have been improved. Therefore, during this study we have investigated a number of parameters on cultured embryos to determine their quality. Gardner and Sakkas (1993) suggested that the more parameters which are used to examine the efficiency of embryo culture, the greater the understanding gained. Assessments of the development using end-points such as the percentage of embryos which reach the morula or blastocyst stage are inadequate, and must be combined with the use of other parameters to determine the cellular effects and suitability of a particular medium. In fact, the majority of studies (Heyman et al., 1987; Ellington et al., 1990; Ouhibi et al., 1990; Lai et al., 1992; Minami et al., 1992) have assessed the effects of co-culture on embryo development using data gathered from very few tests. In these studies only embryo development and cell number were assessed, and in a few cases viability by transferring embryos was also evaluated. However, in our study we have performed multiple tests, including development, glycolytic activity, TE:ICM ratio and viability.

The analysis of the glycolytic activity in blastocysts, by measuring the conversion of $[^3H]\text{glucose}$ to $[^3H]_2\text{O}$, showed a higher activity when blastocysts were cultured in cMTF medium compared with co-culture. Although a lower percentage of blastocysts was obtained in co-culture, the blastocysts thus obtained had a glycolytic activity closer to that found in vivo. Furthermore, each blastocyst which had its glycolytic rate measured, had its cell number determined. No correlation between glycolytic activity per embryo and total cell number was observed. This was also the same when comparing glycolytic activity with the ICM:TE ratio (data not shown). In fact, the data for individual embryos, when comparing glycolytic activity with total cell number, were completely scattered in cMTF medium alone, while in co-culture they were more homogeneous and more similar to the in-vivo blastocyst pattern.

In addition to differences in the glycolytic activity, co-culture blastocysts had a significantly higher ratio of ICM/TE cells when compared with embryos cultured in cMTF medium alone. It is feasible that MDBK cells may secrete factors that act on the development of the ICM. As shown previously by Harvey and Kaye (1990), insulin positively influences the growth of ICM cells in mouse blastocysts. In contrast, insulin does not act on the glycolytic metabolism (Dufranes et al., 1993). Specific growth factors have been shown to act in certain ways. Harvey and Kaye (1990) and Harvey et al. (1995) showed enhanced blastocyst formation by culturing in the presence of EGF and leukaemia inhibitory factor (LIF), as well as an increased development of TE cells by culturing in the presence of EGF. Rappolee et al. (1992) has also shown a positive effect of IGF-II on the ICM. The inference from these previous studies would be that the secretion of any of these growth factors by feeder cells in a co-culture system may also influence embryo development. As proposed by Ménézo et al. (1993), cell lines such as MDBK continually secrete growth factors. Interestingly, the secretion of LIF has been observed by MDBK cells (Plachot, personal communication). Alternatively, M. Lane and D.K. Gardner (personal communication) have determined that non-essential amino acids and glutamine stimulate TE development, whereas essential amino acids actively stimulate the development of the ICM in mouse blastocysts. Such observations are consistent with the MDBK cells releasing essential amino acids into the culture medium.

The most important and final criterion used in our study was the transfer of day 5 blastocysts to day 3 pseudopregnant females. No differences were observed between culture groups in the numbers of implantation sites or fetuses, or the weights of the fetuses. Fetal development was reduced greatly when compared with the transfer of day 4 in-vivo control blastocysts. In both culture groups, a high rate of resorption after implantation was evident. Successful fetal development depends on the provision of a sufficient number of ICM and TE cells at the time of implantation; an excessive reduction in either lineage diminishes the potential of embryogenesis (Pampfer et al., 1994). In our study both culture groups were well above the ICM:TE ratio of 0.6 observed with in-vivo embryos. This higher ratio reflecting the lower number of TE cells seems to specifically affect post-implantation viability. Reduced fetal development of OF1 strain embryos has also been reported after culture in medium without glucose (Sakkas et al., 1993). The use of outbred strain mice such as OF1 is an interesting model because of their extreme sensitivity to in-vitro culture conditions.

It is clear that certain factors secreted by cells in co-culture can induce differences in embryo development. Therefore some beneficial effect on the quality of co-culture embryos can be observed, even if the number of embryos remains low. Although a number of studies [Gandolfi and Moor (1987) in sheep; Minami et al. (1992) in mice; Mermillod et al. (1993) in cattle] have postulated the existence of a factor that enhances...
embryo development, they have failed to show any direct association between these factors and the embryo.

By analysing a number of parameters on cultured mouse embryos we have a clear idea of the effect of co-culture. Under the current media conditions, co-culture did not increase the number of zygotes reaching the blastocyst stage; however, those blastocysts formed in the presence of somatic cells had an increased ICM and had glycolytic rates closer to in-vivo developed embryos. Therefore further studies are warranted focusing on the mode of action of somatic cells in supporting embryo development.

Acknowledgements
The authors thank Michelle Lane for her assistance and technical advice. This work was supported by the Fonds National Suisse (grant no. 32-39689.93) and The Sir Jules Thorn Charitable Overseas Trust Fund.

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Received on August 10, 1995; accepted on November 4, 1995