Viability of Blastocysts and Morulae Cultured from 1-cell Stage and Transferred to Recipients in the Mouse

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F1 hybrid eggs (C.57 BL/6 × CBA) at 1-cell stage were cultured for 3 days to morulae or 4 days to blastocysts before transfer into the oviducts or uteri of recipients on the first or third day of pseudopregnancy. The proportion of pregnant animals, number of implantations and number of live fetuses on day 18 of gestation were not significantly different among the four groups tested. The proportions of live fetuses after transfer of morulae and blastocysts were 26% and 31% in the oviduct, and 37% and 36% in the uterus, respectively.

The interests on parthenogenesis, cloning via homozygosity and nuclear transfer or gene transfer in mammals are now expanding. The eggs treated under the various conditions should be transferred into recipients for the final evaluation of the viability. Embryo transfer in mammals has been used in numerous studies. However, there are few reports on the viability of embryos cultured from 1-cell stage after transfer to recipients, even in the mouse in which methods for in vitro culture of early cleavage stages are well established1-3. HOPPE and PITTS (1973)4, and KASAI et al. (1979)5 obtained offspring in the mouse after transfer of embryos fertilized in vitro and cultured to morula or blastocyst stage. Recently, HOPPE & COMAN (1983)6 reported that significantly fewer live fetuses were produced in the mouse when blastocysts rather than morulae (34.5%), developed from 1-cell stage, were transferred into the uteri of recipients.

The present study demonstrates that the proportion of live fetuses on day 18 of gestation is not significantly different among the embryos transferred at morula or blastocyst stage, into the oviducts or uteri of recipients.

Materials and Methods

Adult hybrid F1 female mice (C.57 BL/6 N[×]× CBA) were superovulated with 5 iu PMSG and 5 iu hCG at 48 h interval, mated with C.57 BL males. Mated females...
were killed 17-18 h after hCG injection, and eggs with cumulus cells were released by tearing the parts of the ampulla under the liquid paraffin (BDH, U.K.) in the plastic dish. The egg clots were moved to 0.1 ml of culture medium, M 167) within the same dish and preincubated for 5 to 6 hrs in the atmosphere of 95% air, 5% CO₂ at 37°C. After preincubation, eggs with cumulus cells were transferred to 0.05% hyaluronidase in M 24) to remove the cumulus cells, washed three times and then eggs with two pronuclei were cultured for 3 days in vitro to morula or for 4 days to blastocyst stage.

Recipient mice were CD-1 strain females mated with sterility-proven vasectomized males of the same strain. The occurrence of mating was detected by the presence of vaginal plug on the following morning (day 1). The embryos developed to morulae or blastocysts were transferred into either oviducts or uteri of females on the first (day 1) or third day (day 3) of pseudopregnancy. Most of the recipients were killed on day 18 of gestation, and the number of implantations, resorptions, live fetuses, and weight of fetuses were recorded.

The statistical significance of the results was determined by χ² analysis.

Results

Of the 250 1-cell eggs cultured for 3 days in vitro, 232 (93%) were developed to morulae. Of the 265 eggs cultured for 4 days, 214 (81%) were developed to blastocysts.

Table 1 summarizes the viability of embryos, which were cultured in vitro for different periods, following the transfer to the oviducts or uteri of recipients at different pseudopregnant stages. The proportions of pregnant females and implantations were slightly high, but not significant, when blastocysts were transferred into the uteri of day 3 pseudopregnant recipients compared with those obtained in the other groups. The proportion of live fetuses after transfer of morulae into the oviducts of day 1 recipients was slightly low compared with those obtained in the other groups, although there was no significant difference. The average fetal weight after transfer of morulae to uteri of day 3 recipients (1.18 g) was significantly (P<0.001) higher than those obtained in the other 3 groups (0.91~0.97 g).

<table>
<thead>
<tr>
<th>Embryo stage</th>
<th>Transfer site</th>
<th>No. of embryos transferred</th>
<th>No. pregnant/ no. of recipient (%)</th>
<th>No. of implantations (%)</th>
<th>No. of fetuses live (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Morula</td>
<td>Day 1 oviduct</td>
<td>94</td>
<td>9/15 (60)</td>
<td>41 (44)</td>
<td>24 (26)</td>
</tr>
<tr>
<td>Morula</td>
<td>Day 3 uterus</td>
<td>108</td>
<td>10/15 (67)</td>
<td>50 (46)</td>
<td>40 (37)</td>
</tr>
<tr>
<td>Blastocyst</td>
<td>Day 1 oviduct</td>
<td>104</td>
<td>10/15 (67)</td>
<td>41 (39)</td>
<td>32 (31)</td>
</tr>
<tr>
<td>Blastocyst</td>
<td>Day 3 uterus</td>
<td>106</td>
<td>11/15 (73)</td>
<td>57 (54)</td>
<td>38 (36)</td>
</tr>
</tbody>
</table>

* % in pregnant females.
Discussion

The present study did not confirm the report of Hoppe and Coman (1983)\textsuperscript{6}) which showed the proportion of live fetuses after transfer of blastocysts cultured from 1-cell stage (28/303, 9.2\%) was significantly low compared with that obtained after transfer of cultured morulae (98/284, 34.5\%). They considered that low viability of cultured blastocysts was due to increased embryonic mortality after implantation, since the proportion of implantations from transferred morulae and blastocysts was similar. The discrepancy between the report of Hoppe and Coman (1983)\textsuperscript{6}) and the present study may be mainly due to the difference of culture system of embryos or to the different strains of mice used. The fact that we found that blastocysts did at least as well as morulae suggests that our culture system is better than theirs. This is because in general, blastocysts are more successful than morulae in embryo transfer (McLaren, 1970)\textsuperscript{9}), but, on the other hand, the extra day in culture required to produce blastocysts may reduce the viability of the embryos if the culture system is suboptimal, thus more than counteracting the expected superiority of the blastocysts. In the present study, the body weight of live fetuses on day 18 of pregnancy after transfer of cultured morulae into the uterus was significantly high compared with that obtained after transfer of blastocysts, although we have no good explanation for that.

The present study also demonstrated that the proportion of live fetuses after transfer of cultured embryos to oviducts of recipients on the first day of pseudopregnancy was not significantly different from that obtained after transfer to the uteri on the third day. This is in agreement with the reports of Bronson and McLaren (1970)\textsuperscript{10}) and Tsunoda and McLaren (1983)\textsuperscript{11}) which showed that the oviduct of the recently mated pseudopregnant mouse was suitable transplantation sites for embryos.

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References