Effect of Glucose in a Semi-Defined Culture Medium on Development of Mouse 1-Cell Embryos

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Abstract: Mouse 1-cell embryos from C57BL/6J were cultured in CZB medium containing lactate, pyruvate, glutamine, EDTA and BSA. When examined 24, 36 and 48 h after culture, embryos developed to the 2-cell (86%), 4- to 7-cell (59%) and ≥8-cell (54%) stages, respectively, but hardly to the morula stage 72 h after culture. When 5.55 mM-glucose was added to the medium 24 or 36 h after culture, significantly higher proportions (71-75%) of blastocyst-stage embryos were obtained 96 h after culture compared with when glucose was added 0 (43%) and 48 (26%) h after culture. Exposure of embryos to glucose for only 12 h between 36 and 48 h after culture could support their development to the blastocyst stage (64%). If only glucose was present during this period, the additional exposure of embryos to glucose between 24 and 36 h and/or 48 and 96 h after culture neither inhibits nor promotes development of embryos to the blastocyst stage. Key words: Mouse, One-cell embryo, In vitro development, Glucose requirement (Received 19 January 1994, Accepted 8 February 1994)

Introduction

It is well known that development of 1-cell mouse embryos in vitro is blocked at the 2-cell stage with an exception of some inbred and F1 strains. Although it has been reported that a high lactate/pyruvate ratio and the addition of ethylene diamine-tetraacetic acid (EDTA) or superoxide dismutase to culture medium is beneficial for culture of 1-cell embryos derived from some blocking strains of mice, there is considerable variation in the proportion of development beyond the 2-cell stage in different media. To maintain whole development in vitro of 1-cell mouse embryos to the blastocyst stage it is important to clarify the factors which affect the blastocyst formation after overcoming a 2-cell block.

Recently, Chatot et al. found that 1-cell CF-1 embryos, which usually exhibit the 2-cell block to development in vitro, developed beyond the 2-cell stage but not to...
the blastocyst stage when they were cultured continuously in a medium (CZB medium) lacking glucose, but that exposure of embryos to glucose from the 3- to 4-cell stage (48 h after culture) through the early morula stage (72 h after culture) is essential to maintain development of embryos to the blastocyst stage. However, in some nonblocking strains, the addition of glucose to media at the start of culture does not induce the 2-cell block and supports development to the blastocyst stage. Although 1-cell stage embryos of the C57BL/6J strain do not exhibit the 2-cell block, the proportion of embryos developed to the blastocyst stage in media with glucose greatly differs by different laboratories.

In our laboratory, when cultured in CZB medium which is lacking glucose, C57BL/6J 1-cell embryos could develop beyond the 2-cell stage but not to the blastocyst stage (unpublished data). Therefore, the present study was undertaken to examine the effects of glucose on development of C57BL/6J 1-cell embryos in CZB medium.

**Materials and Methods**

**Medium:** The medium used for manipulating and culture of embryos was CZB medium formulated by Chatot et al. This medium consisted of 81.62 mM-NaCl, 4.83 mM-KCl, 1.18 mM-KH₂PO₄, 1.18 mM-MgSO₄·7H₂O, 25.12 mM-NaHCO₃, 1.7 mM-CaCl₂·2H₂O, 31.3 mM-sodium lactate, 0.27 mM-sodium pyruvate, 1 mM-glutamine, 5 mg globulin-free bovine serum albumin (BSA; No.A-7638, Sigma Chemical Co., St. Louis, MO, USA)/ml, 0.11 mM-EDTA, 0.7 mg streptomycin sulfate/ml and 100 U sodium penicillin G/ml.

**Embryo Collection and Culture of Embryos:** Mature (4-6 weeks old) random-bred C57BL/6J female mice which were originally purchased from Clea Japan Inc. and maintained under controlled light (14 h light: 10 h darkness; light on at 6:00) were superovulated with intraperitoneal injection of 5 IU PMSG (Teikoku-Zoki Co., Tokyo) followed by 5 IU hCG (Sankyo Co., Tokyo) 48 h later and naturally mated with males of the same strain. On the following morning (24-25 h after hCG), 1-cell embryos were flushed from the excised oviducts in about 0.1 ml CZB medium supplemented with 0.1% hyaluronidase (No. H-3506, Sigma Chemical Co.). After cumulus cells were completely dispersed, embryos were washed three times with the medium without hyaluronidase. Nine to 20 embryos were then placed in a 50-μl drop of the same medium under paraffin oil (No. 261-17, Nacalai Tesque Inc., Kyoto) in a 35 × 10 mm falcon polystyrene culture dish (No.1008, Becton and Dickinson, NJ, USA) and cultured in a CO₂ incubator (5% CO₂ in air at 37°C).
Experimental Studies: To clarify the process of early cleavage of embryos cultured in CZB medium, embryos were examined at a 12-h interval from 24 to 48 h after culture in Experiment 1. To examine the effect of glucose on development of embryos at different stages, embryos cultured in CZB medium were transferred to newly prepared medium supplemented with 5.55 mM-glucose 0, 24, 36, 48 and 96 h after culture in Experiment 2. In Experiment 3, to examine the effect of the presence of glucose during various culture periods on development of embryos, those cultured in CZB medium were washed three times and transferred to newly prepared medium with 5.55 mM-glucose 24 or 36 h after culture and again washed three times and transferred to glucose-free CZB medium 12-36 h later. In Experiments 2 and 3, embryos were examined 48, 72 and 96 h after culture for their developmental stages under a phase-contrast microscope.

Statistical analysis: Each experiment was replicated 3 (Experiments 1 and 3) or 4 (Experiment 2) times. In Experiments 2 and 3, the proportions of embryos developing to each stage were subjected to an arc-sine transformation and the transformed values were assigned for one-way analysis of variance (ANOVA). When ANOVA revealed a significant treatment effect, the treatments were compared by Duncan’s multiple range test.

Results

Early development of embryos in CZB medium (Experiment 1)

As shown in Table 1, almost all embryos (86%) completed the first cleavage 24 h after culture. The embryos could develop beyond the 2-cell stage reaching to the ≥4-cell (59%) and ≥8-cell (54%) stages 36 and 48 h after culture, respectively.

Development of embryos in CZB medium supplemented with glucose at various times after culture (Experiment 2)

As shown in Table 2, the proportion of embryos developed to the ≥4-cell stage 48 h after culture was significantly (P<0.05) lower in the medium with glucose from the start of culture (69%) than in the medium without glucose until 24 (91%), 36 (92%) and 48 (86-93 %) h after culture. High proportions of ≥morula (88 %) and blastocyst (75 %) stage embryos were obtained 72 and 96 h after culture, respectively, when glucose was not added to the medium until 36 h after the start of culture. These values were not different from those of embryos that had been cultured without glucose until 24 h after the start of culture (83% for
moorulae and 71% for blastocysts) but significantly (P<0.05) higher than those (10-66% for morulae and 0-43% for blastocysts) of all other groups.

<table>
<thead>
<tr>
<th>Time of observation (h)</th>
<th>No. and % of embryos developed to 1-cell</th>
<th>2-cell</th>
<th>3-cell</th>
<th>4-7-cell</th>
<th>≥8-cell</th>
</tr>
</thead>
<tbody>
<tr>
<td>24</td>
<td>4(4)</td>
<td>96(86)</td>
<td>11(10)</td>
<td>0(0)</td>
<td>0(0)</td>
</tr>
<tr>
<td>36</td>
<td>4(4)</td>
<td>26(23)</td>
<td>5(5)</td>
<td>65(59)</td>
<td>2(2)</td>
</tr>
<tr>
<td>48</td>
<td>4(4)</td>
<td>8(7)</td>
<td>2(2)</td>
<td>24(22)</td>
<td>60(54)</td>
</tr>
</tbody>
</table>

* A total of 111 embryos was cultured in 3 replicates.

Table 2. Development of C57BL/6J 1-cell embryos in CZB medium supplemented with glucose (5.55 mM) at various times after the start of culture

<table>
<thead>
<tr>
<th>Time of glucose addition (h)</th>
<th>No. of embryos cultured</th>
<th>No. and % of embryos developed to 2-4-cell [48]</th>
<th>2-8-cell [72]</th>
<th>≥8-cell [96]</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>58</td>
<td>40(69)</td>
<td>38(66)</td>
<td>25(43)</td>
</tr>
<tr>
<td>24</td>
<td>58</td>
<td>53(91)</td>
<td>48(83)</td>
<td>41(71)</td>
</tr>
<tr>
<td>36</td>
<td>60</td>
<td>55(92)</td>
<td>53(88)</td>
<td>45(75)</td>
</tr>
<tr>
<td>48</td>
<td>58</td>
<td>54(93)</td>
<td>28(48)</td>
<td>15(26)</td>
</tr>
<tr>
<td>96 (no addition)</td>
<td>58</td>
<td>50(86)</td>
<td>6(10)</td>
<td>0(0)</td>
</tr>
</tbody>
</table>

*Percentage of the number of embryos cultured.

Development of embryos exposed to glucose in CZB medium for different periods (Experiment 3)

As shown in Table 3, development of embryos to the ≥4-cell stage was not affected by the different periods (12-36 h) of the exposure to glucose from 24 or 36 h after culture. When embryos were exposed to glucose for 12 h from 24 h after culture, the proportion (48%) of ≥morula-stage embryos was significantly (P<0.01) lower than in those exposed to glucose for 24 (81%) and 36 (87%) h but not different from the values in those exposed to glucose for 12 (72%) and 24 (76%) h from 36 h after culture. However, blastocyst development (64-70%) was greatly promoted (P<0.01) when embryos were exposed to glucose for 24-36 and 12-24 h from 24 and 36 h after culture, respectively, compared with those exposed to glucose only between 24 and 36 h after culture.
Table 3. Effect of the presence of glucose (5.55 mM) in CZB medium during various culture periods on the development of C57BL/6J 1-cell embryos

<table>
<thead>
<tr>
<th>Presence of glucose during (h)</th>
<th>No. of embryos cultured</th>
<th>≥4-cell</th>
<th>≥Morula</th>
<th>Blastocyst</th>
</tr>
</thead>
<tbody>
<tr>
<td>24-36</td>
<td>50</td>
<td>41(82)</td>
<td>24(68)</td>
<td>11(22)</td>
</tr>
<tr>
<td>24-48</td>
<td>54</td>
<td>47(87)</td>
<td>44(81)</td>
<td>36(67)</td>
</tr>
<tr>
<td>24-60</td>
<td>54</td>
<td>47(87)</td>
<td>47(87)</td>
<td>38(70)</td>
</tr>
<tr>
<td>36-48</td>
<td>50</td>
<td>45(90)</td>
<td>36(72)</td>
<td>32(64)</td>
</tr>
<tr>
<td>36-60</td>
<td>50</td>
<td>44(88)</td>
<td>38(76)</td>
<td>33(66)</td>
</tr>
</tbody>
</table>

*a Percentage of the number of embryos cultured.

*b Numbers in parenthesis indicate the time of examination (h after culture).

*c Values with each different superscripts are significantly different within each column, P<0.01 at least.

Discussion

The results of the present study indicate that C57BL/6J 1-cell embryos can develop beyond the 2-cell stage but hardly to the morula and blastocyst stages when they were cultured in CZB medium which contains lactate, pyruvate, glutamine, EDTA and BSA but no glucose. To maintain blastocyst formation, the addition of glucose to the medium was essential. However, the presence of glucose for the first 24 h of culture rather inhibited blastocyst formation. Exposure of embryos to glucose for only 12 h from 36 to 48 h after culture could support their development to the blastocyst stage. If only glucose was present during this period, the additional exposure of embryos to glucose between 24 and 36 h and/or 48 and 96 h after culture neither inhibits nor promotes development of embryos to the blastocyst stage. This requirement of glucose was also applicable to development of embryos to the morula stage with less strictness compared with for blastocyst formation.

The specific role(s) of glucose during culture of 1-cell embryos obtained from various strains of mice that do not generally exhibit the 2-cell block has been examined in detail. However, it seems that the pattern of glucose requirement for development to the blastocyst stage is slightly different according to the different strains. Chatot et al. have reported that some 1-cell embryos obtained from B6D2F1/J (C57BL/6J × DBA/2J) or CD1 females mated to B6SJLF1/J (C57BL/6J × SJL/J) males could develop to the blastocyst stage in CZB medium but that the addition of glucose to the medium from the start of culture optimally maintained blastocyst development; the beneficial effect of the addition of glucose 48 h after culture as observed in blocking strain was not observed. Although
Chatot et al. suggested that this was the result of an intrinsic difference in the ability of the blocking and nonblocking strains to metabolize glucose, this hypothesis was not necessarily supported by Du and Wales who recently observed that both strains responded similarly to the presence of glucose. On the other hand, Brown and Whittingham have reported that no 1-cell embryos from B6CBF1 (C57BL/6 × CBA/ca) mice mated to the same strain males developed to the blastocyst stage when glucose was omitted from bicarbonate-buffered mouse embryo culture medium, M16, which contains lactate and pyruvate but that an exposure of embryos to glucose for a 22-24h period at any stage during the first 72 h of culture supported development to the morula and blastocyst stages. These findings may suggest that there is some differences in the requirement of glucose among different nonblocking strains. In the present study, exposure of C57BL/6J embryos to glucose was only necessary from 36 through 48 h after culture, at which time of culture embryos developed to the 4-to 8-cell stage, for development to the morula to blastocyst stage in CZB medium. The requirement of glucose during this specific period was quite similar to that observed in 1-cell embryos from blocking strain, CF-1 females mated to B6SJLF1 males. Although it has been reported that uptake and incorporation of glucose by mouse embryos increase at the 4-to 8-cell stage, the precise nature of the specific timing and of the strain difference of the requirement of glucose remains unclear. It seems that glucose is required for the synthesis of some stage-specific embryonic component(s) involved in development to the morula and blastocyst stages.

The requirement of glucose for development of embryos in vitro is different according to animal species. In the cattle, the presence of glucose throughout culture is detrimental for development of 1-cell embryos to the blastocyst stage but blastocyst formation from the 8- to 16-cell stage is promoted by the presence of glucose. Participation of glucose in in vitro developmental block of embryos at specific stage is also reported in the sheep, hamster, and pig. However, glucose is an essential component in chemically defined medium for development of rat 1-cell embryos to the blastocyst stage.

In conclusion, development of C57BL/6J 1-cell embryos to the morula and blastocyst stages is inhibited when they were cultured in CZB medium with glucose for the first 24 h or without glucose throughout culture but greatly promoted when they were exposed to glucose for 12 h between 36 and 48 h after culture.
References


マウス1細胞期胚の体外発生におよぼすグルコースの影響

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C57BL/6J系マウス1細胞期胚をCZB培地を用いて体外培養した結果，胚はそれぞれ24，36および48時間後に，2~(86%)，4~7~(59%)および≥8~(54%)細胞期に到達したが，さらに培養を継続しても桑実期以降への発生は困難であった。培養開始後24または36時間で胚をグルコース（5.55 mM）添加CZB培地に移した場合，培養開始後96時間の胚盤胞形成率（71~75%）は，培養開始時（43%）あるいは培養開始後48時間（26%）からグルコースの存在下で培養された胚と比較して有意（P<0.05）に高かった。グルコースの存在下で培養する時期を培養開始後36（4-細胞期）から48（8-細胞期）時間までの12時間に限定することによって，64%の胚は胚盤胞まで発生したが，この時期以外にさらに培養開始後24から36時間までの12時間および48から96時間までの48時間のいずれか一方または両者の期間グルコースの存在下で培養しても胚盤胞形成率（67~75%）は抑制も促進もされなかった。以上の結果から，CZB培地においてC57BL/6J系1-細胞期胚の胚盤胞への発生を維持するためには4~8-細胞期の段階でグルコースを添加する必要のあることが示唆された。