IN VITRO DEVELOPMENT OF iSCNT PRODUCED LONG TAILED MONKEY (Macaca fascicularis) EMBRYOS

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Abstract: Monkey is a wild life animal which has very close genetic evolution with that of a human. SCNT in monkey is difficult and expensive due to low number of oocyte obtained and high cost of superstimulation hormones. Hence, bovine enucleated oocyte had been used as recipient cytoplast. This work was undertaken to investigate the optimum procedure to produce monkey iSCNT. The result found that monkey iSCNT embryo could develop to 16 cell stage but did not successfully develop to blastocyst. Surprisingly, the iSCNT embryos showed early blastocyst like embryo at day 5 to day 7 but only with 16 nuclei.

Keywords: monkey, embryo, cloning, in vitro development, bovine

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Introduction: Somatic cell nuclear transfer (SCNT) is a technique used for production of identical animal embryo or live offspring by using somatic cell nucleus from donor animal injected into enucleated oocyte. The SCNT can be used for production of many kinds of animals including primates. However, a monkey oocyte is not easily obtained and very few oocyte per head. Moreover, exogenous hormones for superstimulation have high cost. Therefore, alternative sources of recipient cytoplast for SCNT should be considered. Bovine oocyte cytoplasm has been proved as a universal recipient cytoplast for various mammalian species including endangered animal such as gaur (Bos gaurus) (Lanza et al., 2000) and other wild animals including monkey (Dominko et al., 1999). This technique is called interspecies SCNT (iSCNT). However, the developmental rate of iSCNT monkey embryo using bovine enucleated oocyte as recipient cytoplast has been proved as a universal recipient cytoplast for various mammalian species. The activated oocytes were separated into two groups for bovine and monkey suitable culture media/condition, Group I: SOF media at 38.5°C, 5% CO2, 5% O2, 90% N2 and Group II: HECM-9 at 37°C, 5% CO2, 5% O2, 90% N2. Half of the culture media was changed and embryo development was observed daily. Embryonic cell number was counted by 2 µg/ml DAPI staining. Parthenogenetic activation (PA) were also done with matured bovine oocytes and cultured along with the two treatments as control group.

Methods: A small piece of monkey (Macaca fascicularis) skin tissue from biopsied was taken and cultured in vitro for fibroblasts grow out. The fibroblasts were then used as donor cell nuclei. Bovine (Bos taurus) ovaries were obtained from abattoir. The oocytes were aspirated and cultured in in vitro maturation media for 22h. The nuclear genetic material of the bovine was removed and replaced with single fibroblast cell of monkey. The couples were incubated in fusion medium and fused together by electric pulses using fusion electrodes. The successfully fused oocytes were activated with 7% ethanol for 5 min and incubated in 1.25 µg/ml cytochalasin D + 10 µg/ml cycloheximide for 5h. The activated oocytes were separated into 2 groups for bovine and monkey suitable culture media/condition, Group I: SOF media at 38.5°C, 5% CO2, 5% O2, 90% N2 and Group II: HECM-9 at 37°C, 5% CO2, 5% O2, 90% N2. Half of the culture media was changed and embryo development was observed daily. Embryonic cell number was counted by 2 µg/ml DAPI staining. Parthenogenetic activation (PA) were also done with matured bovine oocytes and cultured along with the two treatments as control group.

Results: Monkey fibroblasts were outgrown from tissue at about day 5-6 after in vitro culture. The iSCNT embryos from both 2 culture systems showed similar cleavage as that of PA embryos (Table 1). The iSCNT embryos cultured in HECM-9 media showed lower development to 8 cell stage...
than cultured in SOF but similar morula developmental rate in every treatment. Some of the embryonic cells of iSCNT embryo extrude from the zonapellucida at day 5 as showed in Fig 1C. However, the iSCNT embryos showed early blastocyst like morphology with unclear ICM and TE while bovine PA embryos were very clear ICM and TE and it could develop to expand to hatching blastocyst stage as usual (Fig. 1).

Table 1. iSCNT and parthenote embryos development.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No. Cleavage (%)</th>
<th>8C (%)</th>
<th>Mor (%)</th>
<th>Blast (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>iSCNT SOF</td>
<td>57 (93.0)a</td>
<td>53</td>
<td>46</td>
<td>23</td>
</tr>
<tr>
<td>iSCNT HECM</td>
<td>58 (98.3)a</td>
<td>57</td>
<td>31</td>
<td>26</td>
</tr>
<tr>
<td>PA SOF</td>
<td>60 (91.7)a</td>
<td>55</td>
<td>45</td>
<td>23</td>
</tr>
<tr>
<td>PA HECM</td>
<td>60 (90.0)a</td>
<td>54</td>
<td>43</td>
<td>30</td>
</tr>
</tbody>
</table>

* The early blastocysts like embryos a,b different superscripts within column indicate significant differences (P<0.01)

The result also showed that HECM-9 better support bovine PA embryos development under the non co-culture system when compared with SOF media.

When the iSCNT embryos at early blastocyst like embryo were stained with 2 µg/ml DAPI for 10 min and observed for embryonic cell number under UV light, it was found that the early blastocysts like embryos have only 13.7 ± 1.3 and 15.3 ± 2.2 nuclei (n = 4) from iSCNT in SOF and iSCNT in HECM-9, respectively, whereas PA embryos have 115 ± 9.1 and 110 ± 8.8 nuclei (n = 4) from PA in SOF and PA in HECM-9, respectively, (Fig. 2).

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References: