Attachment, Outgrowth, Invasion and Formation of the Egg Cylinder in Mouse Half Embryos *In Vitro*

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ABSTRACT. To investigate the effect of the decrease in the number of embryonic cells on the development and morphogenesis of mouse embryos at the peri-implantation stage, half embryos which developed from bisected morulae were morphologically classified into eu- blastocysts (eu-blasts), pseudo-blastocysts (pseudo-blasts), trophoectodermal vesicles (TVs) and non-integrated forms (NIFs) and then cultured on plastic substratum, uterine-epithelial-cell monolayer and type I collagen gel. When half embryos were cultured on plastic substratum and cell monolayer, the rates of attachment and trophoblast outgrowth in the eu- and pseudo-blasts were not significantly different from those of the controls. The TVs and NIFs showed significantly lower rates of outgrowth than the controls (P<0.01). When half embryos were cultured on type I collagen gel, no significant difference was observed in the rate of primitive endoderm formation between the eu-blasts and controls after 36 hr of culture. In the eu-blasts, however, the developmental rate to the egg cylinder at stage 8 was significantly lower than in the controls after 72 hr (P<0.05). The pseudo-blasts revealed significantly lower rates of endoderm formation and development to the egg cylinder than the controls after 36 and 72 hr, respectively (P<0.05). In the TVs and NIFs, the rates of outgrowth were significantly lower than those of the controls (P<0.05) and no egg cylinder was observed. The invasion of type I collagen gel by the cytoplasmic protrusions of trophoblast cells was observed regardless of the type and developmental stage of the embryos. The results show that a decrease in the number of embryonic cells affects the formation of the primitive endoderm and the development to the egg cylinder in vitro. — KEY WORDS: embryo, implantation, in vitro culture, micromanipulation, mouse.


The bisection of embryos has been used for the production of genetically identical mice [14, 18, 19, 34]. Bisection of embryos is a simple technique but decreasing the number of embryonic cells affects the subsequent development of embryos.

Mouse half embryos produced by the destruction of a blastomere at the 2-cell stage have revealed retardation of development on day 5.5 to 8 and subsequent compensatory growth by day 10.5 to 11 of pregnancy [22, 30, 31]. The implantation rates of half embryos which developed from the morula stage have been reported to be lower than those of whole embryos [19, 34]. In the preliminary experiments, a severe decrease in the number of embryonic cells affected the decidual cell reaction in the uterus [12]. However, the relationship between the timing of implantation and morphology of the half embryos is not clear. It is difficult to obtain information about interactions between the trophoblast cells and endometrium during the implantation from intact mice. Therefore, we used the *in vitro* system as a model of implantation.

The purpose of this study was to clarify the effect of the decrease in the number of embryonic cells on the development and morphogenesis of mouse embryos at the peri-implantation stage. Half embryos with various morphologies were cultured *in vitro* on plastic substratum, uterine-epithelial-cell monolayer and type I collagen which is a component of the interstitial connective tissue stroma. The attachment of the embryos, trophoblast outgrowth, formation of the primitive endoderm and development to the egg cylinder stage were examined. The invasiveness of the trophoblast cells to the type I collagen gel was also examined by electron microscopy.

MATERIALS AND METHODS

*Mice and embryos:* Six-week-old Slc:ddY female mice were superovulated by an intraperitoneal (i.p.) injection of 7.5 IU pregnant mare serum gonadotropin (Serotropin; Teikoku Zoki, Tokyo, Japan) followed 48 hr later by an i.p. injection of 7.5 IU human chorionic gonadotropin (hCG, Gonatropin; Teikoku Zoki). After the hCG injection, females were housed overnight with Slc:ddY males and examined for the presence of a vaginal plug on the following morning. Females were killed by cervical dislocation at 78 hr post hCG injection. Compacted morulae (16–22 cells) were collected from the oviducts and uterus by flushing with Dulbecco’s phosphate-buffered saline (PBS) containing 10% (v/v) heat-inactivated calf serum (CS, Gibco Laboratories, Grand Island, NY, U.S.A.).

*Micromanipulation of embryos:* The embryos were bisected according to the method by Nagashima et al. [18]. In brief, the zona pellucida of the morula was removed by incubation in 0.5% pronase (Actinase E; Kaken Pharmaceutical Co., Inc., Tokyo, Japan) dissolved in PBS for 2 min at 37°C. The zona-free morulae were then decomposed by incubation in Ca²⁺-Mg²⁺-free PBS supplemented with 6 mg/ml bovine serum albumin (Sigma Chemical Co., St. Louis, MO, U.S.A.) for 15 min at 37°C. The decomposed morulae were placed in PBS containing 10% CS under paraffin oil and then bisected using a fine
glass needle attached to a micromanipulation unit (MO-202; Narishige, Tokyo, Japan) under an inverted microscope (Diaphot-TMD; Nikon, Tokyo, Japan).

**Embryo culture and morphological classification:** Four pairs of half embryos were cultured in a 30 μl drop of BMOC-3 [4] covered with paraffin oil in tissue culture dishes (Nuncelon; Nunc, Kamstrup, Denmark) for 36 hr at 37°C in an atmosphere of 5% CO₂ in air. The zona-free decompacted morulae which were not bisected were cultured as control.

After *in vitro* culture in BMOC-3, the half embryos were morphologically classified into the following 4 groups according to the classification by Tarkowski and Wróblewska [32] and Nagashima *et al.* [18]: eu-blastocysts (eu-blasts) containing a distinct inner cell mass (ICM) and well-developed trophoderm; pseudo-blastocysts (pseudo-blasts) containing a poorly developed ICM enclosed by the trophoderm; trophodermal vesicles (TVs) with no enclosed cells and non-integrated forms (NIFs) with disorganized cluster of small trophodermal vesicles and cells, some of which were vacuolated. In the preliminary experiments, when a total of 460 morulae were bisected and cultured in BMOC-3, 280 (60.9%) morulae yielded monozygotic pairs of eu-blasts. The percentages of 920 half embryos classified into the eu-blasts, pseudo-blasts, TVs, NIFs and degenerated embryos were 79.1, 12.0, 3.3, 3.2 and 2.5%, respectively. The cell numbers of the eu-blasts, pseudo-blasts, TVs and NIFs were 51, 25, 18 and 10% of the controls which developed to the blastocyst stage, respectively.

Half and control embryos were further cultured in CMRL-1066 medium (Gibco) supplemented with 20% heat-inactivated fetal calf serum (FCS, Gibco), 1 mM glutamine (Sigma), 1 mM sodium pyruvate (Sigma), 100 U/ml penicillin and 100 μg/ml streptomycin in 4-well multidishes (Nunc) for 72 hr at 37°C in 5% CO₂ in air. A total of 1 ml CMRL-1066 medium including the endometrial cells (Experiment 1) or type I collagen gel (Experiment 2) was placed in a well. One to 10 half and control embryos were cultured in a well. The medium was changed every other day.

**Preparation of uterine epithelial cells:** Endometrial tissues were obtained from donor mice after embryo collection. The uterine epithelial cells were isolated according to the method by Sherman [26] with modifications. Uteri were trimmed in Ca²⁺-Mg²⁺-free PBS to remove tissue fat and slit along their length with fine scissors. The uteri were then incubated in a solution of 0.5% trypsin (1:250; Difco Laboratories, Detroit, U.S.A.) and 2.5% pancreatin (Kanto Chemical Co., Inc., Tokyo, Japan) dissolved in Ca²⁺-Mg²⁺-free PBS for 2 hr at 4°C. They were then washed in Ca²⁺-Mg²⁺-free PBS. The uterine epithelia were freed from the stromal tissue as transparent sheets in relatively large pieces by fine forceps under a stereomicroscope. The epithelial sheets were placed in a solution of 0.05% trypsin and 0.02% EDTA dissolved in Ca²⁺-Mg²⁺-free PBS and incubated for 5 min at 37°C. The cells were suspended in the CMRL-1066 medium and sedimented by centrifugation at 100 × g for 5 min. The cells were incubated in tissue culture dishes for 2 hr at 37°C in 5% CO₂ in air to remove the stromal cells which quickly attached to the plastic dish. The epithelial cells floating in the medium were collected, placed in the wells at 2–4 × 10⁶ cells/ml and further cultured for 32 hr. The medium was changed before co-culture with the embryos to remove the dead cells and cellular debris.

**Preparation of extracellular matrices:** Cellmatrix type I-A was purchased from Nitta Gelatin Co., Inc. (Osaka, Japan). It consisted of 3.0 mg/ml type I collagen derived from porcine tendons. The following components were added to a sterile 50-ml centrifuge tube at 4°C: 4.0 ml type I collagen, 0.5 ml 10 × concentrated Eagle’s minimum essential medium (Nissui Pharmaceutical Co., Inc., Tokyo, Japan) and 0.5 ml reconstitution buffer which consisted of 0.05 N NaOH, 0.26 M NaHCO₃ and 0.2 M Heps. The mixture was then diluted 1:1 (v:v) with FCS-free CMRL-1066 medium to adjust the final concentration of the type I collagen to 1.2 mg/ml. For polymerization, 500 μl mixture was placed in a well and incubated for 30 min at 37°C in 5% CO₂ in air. The gels were rinsed with the CMRL-1066 medium containing 20% FCS and were left to equilibrate overnight before the addition of the embryos.

**Light and transmission electron microscopy:** The embryos were fixed in 2.5% glutaraldehyde in 0.1 M phosphate buffer at pH 7.3, post-fixed in 1.0% osmium tetroxide in the same buffer, dehydrated in a graded series of ethanol, treated with propylene oxide, embedded in epoxy resin and sectioned. The semi-thin sections were stained with 0.5% toluidine blue at pH 7.5 and observed under a light microscope. The ultra-thin sections were contrasted with ethanolic uranyl acetate and lead citrate, and observed under an electron microscope (JEM-1210; JEOL, Tokyo, Japan).

**Experiment 1:** A total of 191 eu-blasts, 69 pseudo-blasts, 37 TVs, 52 NIFs and 198 control embryos were cultured in the wells with or without the uterine-epithelial-cell monolayer. The attachment of embryos to the plastic substratum or the cell monolayer was examined after 6, 24, 36, 48 and 72 hr of culture by gently shaking the multidishes under the stereomicroscope. The trophoblast outgrowth was simultaneously observed under the inverted microscope.

**Experiment 2:** The half and control embryos were cultured on type I collagen gel in the wells. The presence of the primitive endoderm which is characteristic of the peri-implantation embryos at stage 6 in the staging system of Theiler [33] was examined in the embryos which attached to the gel after 36 hr of culture by observation of the semi-thin sections. The developmental rates to the egg cylinder with the primitive ectoderm and endoderm (stage 7) and with the primitive ectoderm and endoderm and the ectoplacental cone (stage 8) were examined after 72 hr under the stereomicroscope. The trophoblast invasion of type I collagen gel was examined after 72 hr under the light and electron microscopes.

**Statistical analysis:** Data were obtained from 3–10 replicates of the experiments. Chi-square analysis with Yates’ correction was performed to determine differences in the percentages of attachment, trophoblast outgrowth and
primitive endoderm formation of the embryos and the developmental rates to the egg cylinder between the half and control embryos. Values of P<0.05 were considered significant.

RESULTS

Experiment 1: The half and control embryos cultured in CMRL-1066 medium attached to the plastic substratum and the uterine-epithelial-cell monolayer. The embryos then showed collapse of the blastocoele and outgrowth or spreading of the trophoblast cells as a monolayer on the plastic substratum. However, the trophoblast cells did not overgrow on the cell monolayer. They displaced the uterine epithelial cells in the monolayer and outgrew on the plastic substratum (Fig. 1) as reported by Salomon and Sherman [24].

When the half and control embryos were cultured on the plastic substratum, the eu-blasts and pseudo-blasts showed maximum rates of attachment without significant difference from the controls after 36 hr of culture (Fig. 2A). The TVs and NIFs revealed significantly lower rates of attachment than the controls after 36 hr (P<0.01), but they exhibited

![Fig. 1. Phase contrast microscopy of a control embryo cultured on the monolayer of the uterine epithelial cells for 72 hr. Asterisk indicates the trophoblast outgrowth. Arrowheads indicate the border between the trophoblast outgrowth and epithelial cells. × 250.](image)

![Fig. 2. The attachment of the embryos to the plastic (A) and the uterine-epithelial-cell monolayer (B), and the trophoblast outgrowth of the embryos cultured without (C) and with (D) the cell monolayer. Asterisks indicate significant difference from the controls (P<0.01). ○: control, •: eu-blastocyst, □: pseudo-blastocyst, ■: trophodermal vesicle, △: non-integrated form.](image)
maximum levels without significant difference from the controls after 48 hr. When the half and control embryos were cultured on the cell monolayer, all embryos showed maximum rates of attachment without significant difference after 48 hr (Fig. 2B). The half and control embryos cultured on the plastic substrate and cell monolayer showed maximum rates of trophoblast outgrowth after 48 hr (Fig. 2C, D). There was no significant difference in the rates of outgrowth among the eu-blasts, pseudo-blasts and controls. However, the rates of outgrowth in the TVs and NIFs were significantly lower than those in the controls even after 72 hr (P<0.01). The TVs and NIFs which attached but did not show the outgrowth resulted in degeneration and/or detachment from the substrata.

**Experiment 2:** The half and control embryos cultured on the type I collagen gel attached to the surface of the gel and showed the trophoblast outgrowth as in Experiment 1. At the beginning of culture, the primitive endoderm was observed on the surface of ICM facing the blastocoele in less than 10% of the eu-blasts and controls (Table 1). After 36 hr of culture, 89.8% of the controls attached to the gel. There was no significant difference in the rate of attachment to the gel among the eu-blasts, pseudo-blasts and controls. The primitive endoderm was detected in 89.7% of the eu-blasts without significant difference from the controls (Fig. 3). However, the pseudo-blasts revealed a significantly lower rate of primitive endoderm formation than the controls (P<0.01). The TVs and NIFs showed significantly lower rates of attachment than the controls (P<0.05). No primitive endoderm was observed in the TVs and NIFs.

After 72 hr of culture, the trophoblast outgrowth was observed in all of the control embryos and eu-blasts (Table 2). The egg cylinder (stages 7 and 8 in the staging system of Thelar [33]) was formed in 98.8% of the controls and 90.0% of the eu-blasts (Fig. 4A). In the eu-blasts, the developmental rate to the egg cylinder at stage 8 was significantly lower than in the controls (P<0.05). In the pseudo-blasts, the trophoblast outgrowth was observed in 94.4% but the ectoplacental cone was formed in only 5.6%. Spreading of the ICM cells on the trophoblasts occurred in the embryos which did not develop to the egg cylinder stage. The spreading was observed in 33.3% (12/36) of the pseudo-blasts. The TVs and NIFs showed no significant difference in the rates of attachment to the gel from the controls. However, the rates of outgrowth were significantly lower than those of the controls (P<0.05). They formed no cell mass on the spreading cells (Fig. 4B).

The trophoblast outgrowth seemed to be located on the surface of the gel under the inverted microscope. In the semi-thin sections of the egg cylinder, the trophoblast cells spread along the surface of the gel but their basal membrane was located under the surface of the gel. The ultrastructure of the primitive ectoderm and endoderm and the ectoplacental cone in the egg cylinders which developed from the eu-blasts, pseudo-blasts and controls was similar to those in previous reports [10, 36]. The trophoblast cells in the outgrowth showed a distinct polarity in the surface structure. They possessed the microrillae on the apical surface toward the medium and a large number of short cytoplasmic protrusions invading the gel on the basal surface (Fig. 5) as reported by Wordinger et al. [37]. A large nucleus was observed at the basal site of the cells. Abundant mitochondria, rough endoplasmic reticula and lysosomes were distributed throughout the cytoplasm. The collagen

Fig. 3. Semi-thin section of the eu-blastocyst cultured on the type I collagen gel (C) for 36 hr. Arrowhead indicates the primitive endoderm. Line indicates the surface of the gel. x 800.

<table>
<thead>
<tr>
<th>Type of embryo</th>
<th>No. of embryos examined</th>
<th>No. (%) of embryos with primitive endoderm</th>
<th>No. of embryos cultured</th>
<th>No. (%) of embryos attached</th>
<th>No. of embryos examined</th>
<th>No. (%) of embryos with primitive endoderm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eu-blast</td>
<td>46</td>
<td>3 (6.5)</td>
<td>62</td>
<td>59 (95.2)</td>
<td>39</td>
<td>35 (89.7)</td>
</tr>
<tr>
<td>Pseudo-blast</td>
<td>15</td>
<td>0 (0)</td>
<td>33</td>
<td>28 (84.8)</td>
<td>12</td>
<td>9 (75.0)</td>
</tr>
<tr>
<td>TV</td>
<td>15</td>
<td>0 (0)</td>
<td>20</td>
<td>11 (55.0)</td>
<td>10</td>
<td>0 (0)</td>
</tr>
<tr>
<td>NIF</td>
<td>15</td>
<td>0 (0)</td>
<td>21</td>
<td>10 (47.6)</td>
<td>10</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Control</td>
<td>42</td>
<td>4 (9.5)</td>
<td>49</td>
<td>44 (89.8)</td>
<td>41</td>
<td>41 (100)</td>
</tr>
</tbody>
</table>

a) At the beginning of culture on type I collagen gel in CMRL-1066 medium containing 20% FCS.
b) Values in the same column with different superscripts are significantly different (b, c: P<0.05; d, e, f: P<0.01).
Table 2. Formation of the egg cylinder in half embryos cultured in vitro

<table>
<thead>
<tr>
<th>Type of embryo</th>
<th>No. of embryos cultured</th>
<th>No. (%) of embryos attached</th>
<th>No. (%) of embryos with trophoblast outgrowth</th>
<th>No. (%) of egg cylinders</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Stage 7&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Stage 8&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Eu-blast</td>
<td>90</td>
<td>90 (100)</td>
<td>90 (100)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>62 (68.9)&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Pseudo-blast</td>
<td>36</td>
<td>36 (100)</td>
<td>34 (94.4)&lt;sup&gt;d&lt;/sup&gt;</td>
<td>20 (58.8)&lt;sup&gt;df&lt;/sup&gt;</td>
</tr>
<tr>
<td>TV</td>
<td>18</td>
<td>17 (94.4)</td>
<td>12 (66.7)&lt;sup&gt;e&lt;/sup&gt;</td>
<td>0 (0)&lt;sup&gt;f&lt;/sup&gt;</td>
</tr>
<tr>
<td>NIF</td>
<td>17</td>
<td>16 (94.1)</td>
<td>10 (58.8)&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0 (0)&lt;sup&gt;f&lt;/sup&gt;</td>
</tr>
<tr>
<td>Control</td>
<td>84</td>
<td>84 (100)</td>
<td>84 (100)&lt;sup&gt;c&lt;/sup&gt;</td>
<td>40 (47.6)&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup> The embryos were cultured on type I collagen gel in CMRL-1066 medium containing 20% FCS for 72 hr.
<sup>b</sup> Morphological classification by the staging system of Theiler (1989).
<sup>c</sup>-<sup>f</sup> Values in the same column with different superscripts are significantly different (P<0.05).

Fig. 4. Embryos cultured on the type I collagen gel for 72 hr. A) An egg cylinder at stage 8 which developed from the control embryo showing ectoplacental cone (E) and trophoblast outgrowth (asterisk). B) Outgrowth of the trophectodermal vesicle without embryonic cell mass. A: ×120. B: ×140.

Fig. 5. Ultra-thin section of the trophoblast outgrowth in the eu-blastocyst cultured on the type I collagen gel (C) for 72 hr. Cytoplasmic protrusions (arrowheads) invade the gel. Areas of the gel entrapped by the protrusions can be seen (asterisks). Many vacuoles are present in the cytoplasm and protrusions. ×7,500.

**DISCUSSION**

The present study describes the attachment and outgrowth of the trophoblast and the morphological differentiation of the ICM in mouse half embryos with various morphologies in vitro. The morphological classification of the half embryos reflects the effect of the decrease in the number of embryonic cells and the presence or absence of the ICM.

Trophoblast cells of the mouse blastocysts adhere to various kinds of substrata during in vitro culture [1, 5, 6, 9, 11, 13, 23, 27, 35-37]. The expression of the integrin in the trophoblast was regulated at the peri-implantation stage...
In the initial attachment of the trophoblast cells to the uterine epithelial cells, a number of molecules have been reported as mediators [6, 7, 15, 16, 25]. In the present experiments, the embryos attached to the plastic substratum, the monolayer of the uterine epithelial cells and the type I collagen gel. No difference was observed in the rates of attachment among the eu-blasts, pseudo-blasts and controls. Therefore, this result shows that the trophoblast cells in these embryos adhere to the substrata without time lag in vitro. In the TVs and NIFs, however, the percentages of attachment to the plastic substratum and the collagen gel were significantly lower than those of the controls after 36 hr of culture (P<0.05), but not different after 72 hr. The mechanism of this delay in the attachment is not clear. The TVs produced by 3H-thymidine treatment of 8-cell embryos were reported to secrete attachment-associated proteins similar to the blastocysts [20]. Further study should be carried out to examine whether the delay in the attachment relates with the expression of the adhesion molecules on the surface of the embryonic cells.

The trophoblast outgrowth on the plastic substratum and the type I collagen gel was observed in almost all of the eu-blasts, pseudo-blasts and controls. However, in the TVs and NIFs, the outgrowth was detected in only 50–70% even after 72 hr of culture. Low percentages of the outgrowth in the TVs and NIFs which attached to the cells in monolayer may reflect a failure in the displacement of uterine epithelial cells by the embryonic cells. When the mouse blastocysts were cultured on the uterine-epithelium monolayer, the uterine cells which formed intercellular junctions between the trophoblast cells moved away to form a “halo” around the embryos and the region was soon occupied by the trophoblast [3, 9]. The spreading and mobility of the trophoblast cells might be regulated by the ICM.

The formation of primitive endoderm is first observed in the morphological differentiation of ICM at the peri-implantation stage [17, 21, 33]. In the eu-blasts, although the rate of primitive endoderm formation was not different from that of the controls after 36 hr of culture, the developmental rate to the egg cylinder at stage 8 was lower than in the controls after 72 hr. Therefore, it is suggested that the decrease in the number of embryonic cells causes the retardation or failure of development after formation of the primitive endoderm. This finding may reflect the growth retardation which has been reported in the post-implantation development in mouse half embryos [22, 31]. In the pseudo-blasts, the rate of primitive endoderm formation was lower than in the controls after 36 hr, and the spreading of ICM was frequently observed after 72 hr. The severe decrease in the number of embryonic cells may inhibit formation of the primitive endoderm in the ICM, while the trophoblast cells gain the property of adhesion. The spreading of ICM may be enhanced by in vitro culture. The blastocoele was ruptured, and the mural trophoblast did not envelop the embryonic tissue but spread away in the present culture system.

The trophoblast cells in the egg cylinders revealed the polarity in the surface structure and projected abundant short cytoplasmic protrusions into the gel. Wordinger et al. [37] reported that mouse blastocysts developed extensive trophoblast cell processes into the type I collagen pads after 48 to 72 hr of culture. However, the relationship between the invasiveness of the trophoblast and the developmental stage of embryos was not known. In the present study, cytoplasmic protrusions invading the type I collagen gel were observed in the trophoblast cells regardless of the developmental stage of the embryos after 72 hr of culture. Furthermore, the spreading cells in the TVs and NIFs also showed cytoplasmic protrusions invading the gel. Therefore, it is demonstrated that the ability of the trophoblast cells to invade the type I collagen gel by the cytoplasmic protrusions does not depend on the presence and differentiation of the ICM.

Mouse trophoblast outgrowth has been reported to degrade the extracellular matrices composed of glycoproteins, elastin and types I and III collagen in vitro [8]. In the interstitial collagen model, the trophoblast cells altered the collagen matrix in the vicinity of cytoplasmic protrusions [37]. The degradation of extracellular matrix by the trophoblast cells was mediated by the metalloproteinases and inhibited by the tissue inhibitor of metalloproteinases [2]. Therefore, the trophoblast invasion of the extracellular matrix is thought to relate with the proteolytic activity of trophoblast cells [2, 37]. In the present study, the trophoblast cells in the egg cylinders showed their basal membrane under the surface of the type I collagen gel, indicating the invasion of the gel. However, the degradation of the collagen gel was not clear because the collagen fibrils were concentrated beneath the trophoblast cells. The trophoblast cells may press the gel
during the invasion by the cytoplasmic protrusions.

In conclusion, the present in vitro model of implantation shows that the trophoblast cells of mouse half embryos attach to the substrata, subsequently outgrow and invade the type I collagen gel. However, the rates of outgrowth decreased in the TVs and NIFs. It is demonstrated that a decrease in the number of embryonic cells affects the formation of the primitive endoderm and the development to the egg cylinder in vitro. Further study should be conducted to examine the development and morphogenesis of half embryos and the interaction between the trophoblast cells and uterine tissue using a more suitable culture system containing the uterine epithelial cells on the basal lamina and the stromal or decidual cells within the matrix.

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