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Abnormal expression of matrix metalloproteinase-2 and -9 in interspecific pregnancy of rat embryos in mouse recipients

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Abstract

The high failure rate of interspecific pregnancy is a major obstacle to the successful interspecific cloning of mammals. Embryo transfer between rats and mice provides a unique model for studying the causes of such failures. Previous research has shown that the upper time limit for the survival of rat embryos in mouse uteri was the seventh day of pregnancy (Day 7). To study the reasons for the failure of interspecific pregnancy between rats and mice, we transferred rat blastocysts into mouse uteri on the third day of pseudopregnancy. Unexpectedly, intact rat embryos could still be observed in mouse uteri on Day 9 and the implantation rate was as high as 30.6%. However, compared with mouse embryos, the further development of transferred rat embryos in mouse uteri was retarded. On Day 10, transferred rat embryos shrank with much blood. From Day 11 on, they lost their intact structure and the recipient uteri developed dropsy. On Day 12, the embryos shrank further and completely separated from the mouse uteri. By Day 13, they had been absorbed without any remains. In an in vitro co-culture (CT) system, the attachment rate of rat embryos on a monolayer of mouse uterine epithelial cells was similar to that of mouse embryos, but the outgrowth rate of rat embryos was significantly lower. Further investigation by gelatin zymography showed that matrix metalloproteinase-2 (MMP-2) and metalloproteinase-9 (MMP-9) activities in transferred rat embryos was significantly less than in mouse embryos. The same result was obtained in the in vitro CT assay. These results suggest that rat embryos can complete adhesion but not the invasion when transferred into mouse uteri. The reduced invasive ability, and especially, the associated reduction of MMP-2 and -9 activity, is one of the reasons for the failure of interspecific pregnancy.

Keywords: Interspecific pregnancy; MMP-2; MMP-9; Rat; Mouse

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1. Introduction

Examples of successful interspecific pregnancy are rare [1]. This high failure rate is a major obstacle to a successful interspecific cloning. The upper time limit for the survival of rat blastocysts transferred into mouse uteri is the seventh day of pregnancy [2]. This suggests that the transferred rat embryos can initiate but not complete implantation. Therefore, embryo transfer between rats and mice provides a good model for studying the reasons for the failure of interspecific pregnancy. Previous studies have shown that although rat embryos could adhere closely to the epithelium of mouse uteri, they could not establish normal contact with it [2]. However, these studies did not determine whether the adhesion of rat blastocysts to the mouse endometrial luminal epithelium was completed, or if subsequent invasion of this tissue was limited.

Matrix metalloproteinases (MMPs) act as an important signal molecule in maternal–fetal communication during embryo implantation. Direct interaction between embryo and uterus is initiated by recognition of the hatched, free embryo, followed by membrane apposition, and adhesion between the trophectoderm–trophoblast and endometrial luminal epithelium. Finally the embryo passes through the luminal epithelium and epithelial basement membrane and implants in the endometrial stroma. Key molecules involved in the invasion are MMPs and plasminogen activators (PAs), which degrade the extracellular matrix (ECM) [3]. MMPs are a family of zinc- and calcium-dependent proteolytic enzymes that possess the ability to degrade ECM and basement membrane components [4,5]. MMP-2 (gelatinase A) and MMP-9 (gelatinase B), produced by the trophoblast, are the two most important members of this family, and degrade type IV collagen and penetrate the basement membranes [6–9]. Librach et al. reported that metalloproteinase inhibitors and the MMP-9 antibody completely inhibited cytotrophoblast invasion in mice [10]. Dubois et al. observed that the number of mice born per breeding pair was significantly lower in gelatinase B-deficient than in wild-type mice, in addition, individual litters in knockout mice were smaller, and the percentage of infertile breeding pairs was higher in gelatinase B-deficient mice [11]. Angiogenesis in gelatinase A-deficient mice was significantly less than in wild-type littermates [12]. Consequently, MMP-2 and -9 are regarded as invasion markers during embryo implantation.

Our objective in this study was to determine whether the adhesion of rat blastocysts in mouse uteri is complete and whether their subsequent invasive ability is limited. In addition, we also examined changes in MMP-2 and -9 expression. We transferred the rat blastocysts into the mouse uteri to observe the morphological and histological changes and also performed an in vitro study. The activity of MMP-2 and -9 in both in vivo and in vitro models was measured using gelatin zymography.

2. Materials and methods

2.1. Animals

Adult (virginal; 5–6 weeks old) mice of the outbred Kunming white strain and SD rats were purchased from the Experimental Animal Center, Institute of Heredity Science,

Chinese Academy of Sciences, and raised at room temperature (approximately 25 °C) in a constant photoperiod (light:dark cycle, 14:10 h). Food and water were freely available. Females were treated intraperitoneally with 5 IU of pregnant mare's serum gonadotrophin (PMSG) and 48 h later with 5 IU of human chorionic gonadotrophin (hCG). Following hCG injection, each female was caged with a same strain male overnight. The first morning that a vaginal plug was observed was designated Day 1 of pregnancy. The pseudopregnant mice used as recipients for embryo transfer experiments were obtained by mating female mice with surgically sterilized males.

2.2. Embryo collection

Mouse blastocysts were flushed from the uterus with Hank's medium on Day 4 of pregnancy, and rat blastocysts were collected from the uterus on Day 5. Then blastocysts were transferred into droplets of preheated Ham's F-12 containing 2.20 mM calcium lactate, 2.05 mM glutamine, 12.5 mM $NaHCO_3$, and 400 IU/ml gentamycin sulfate, and incubated at 37 °C, 5% CO_2 in a humidified chamber.

2.3. Embryo transfer

Normally developed blastocysts were selected and transferred in 0.1 ml droplets of preheated embryo culture medium after preimplantation embryos were flushed from the uterus. Eight rat blastocysts from each droplet were transferred into a single uterine horn of Day 3 pseudopregnant mice in 1–3 μl total volume. The same number of mouse blastocysts were transferred into each uterine horn of Day 3 pseudopregnant mice as a control. The recipient females were killed on different days of pregnancy and the number of fetuses counted. The implantation rate was defined as the number of fetuses/the number of transferred blastocysts × 100.

2.4. In vitro co-culture

The preparation of a monolayer of uterine epithelial cells and the co-culture (CT) of embryos on uterine epithelial cells were carried out using the method described in Zeng and Cao [13]. In brief, the process was as follows: 30 uteri from Day 4 pregnant mice were split longitudinally to expose the epithelial cell surface and placed into a solution containing 6 mg/ml trypsin in 10 mM phosphate-buffered 0.9% saline (PBS, pH 7.2) without Ca^{2+} and Mg^{2+}. Tissues were incubated at 4 °C for 2 h followed by another 0.5 h at 25–30 °C. After digestion, they were gently shaken to dislodge the epithelium from the endometrial basement membrane. The epithelial cells and fragments were collected by centrifugation at 1500 × *g* for 10 min. Cells were washed three times with Ham's F-12 (Gibco) containing 2.2 mM calcium lactate, 2.05 mM glutamine, 12.5 mM $NaHCO_3$, and 400 IU/ml gentamycin sulfate, and then resuspended in Ham's F-12 with 10% fetal calf serum (FCS, Sigma). A cell suspension adjusted to 1×10^6 cells/ml was placed in 24-well Falcon plates, and incubated at 37 °C, 5% CO_2 in a humidified chamber.

After 18 h, a monolayer of epithelial cells had formed. This monolayer was further identified by immuno-staining for cytokeratin and HE staining, and was found to be

comprised of 92.9 ± 1.0% epithelial cells. The monolayer was rinsed three times in Ham's F-12 medium, after which the CT medium, Ham's F-12 medium supplemented with 0.4% BSA (Sigma), was added. At the same time, high quality hatched blastocysts were selected and transferred into wells (about 30 blastocysts per well). After 24, 48 and 72 h of CT, the attachment or outgrowth status of blastocysts was observed by phase-contrast microscopy (Olympus) and defined according to the following criteria: if the blastocysts were found to stay in the same place after the plate was shaken for 20 s at one rotation per second, they were designated as ''attached''. If not, they were designated as ''non-attached''. After 48 h of CT, blastocysts began to outgrow outwards. When primary giant trophoblast cells were visible around attached blastocysts, the latter were designated as ''outgrowth''.

We used two indices to measure the invasive ability of blastocysts on a monolayer of uterine epithelial cells: (1) the percentage of embryos attaching, which is the ratio of embryos attached to the monolayer of uterine epithelial cells relative to the total number of embryos hatched; (2) the percentage of blastocysts with outgrowths, which is the ratio of embryos with primary giant trophoblast cells relative to the total number of embryos hatched.

2.5. Histology of the maternal–fetal interface

For histological analysis, the uteri and the conceptus were fixed in Bouin medium overnight. Paraffin sections (6 μm) were stained with hematoxylin and eosin.

2.6. Gelatin zymography

Protein extraction from uteri and embryos was performed according to the Trizol reagent protocol (Cat. No. 15596; Gibco). The protein extract (30 μg) or the medium of CT at 24, 48 and 72 h, was mixed with 4× sample buffer (8% SDS, w/v; 0.04% bromophenol blue, w/v; 40% glycerol, v/v; 0.25 M Tris), then subjected to electrophoresis in a 10% polyacrylamine gel containing 0.5 mg/ml gelatin (Sigma). The gel was washed in 2.5% Triton X-100, 50 mM Tris–HCl, at pH 7.5 for 1 h to remove SDS and incubated for 18 h in calcium assay buffer (50 mM Tris, 200 mM NaCl, 10 mM $CaCl_2$, 1 μM $ZnCl_2$, 1% Triton X-100, pH 7.5) at 37 °C. After staining with 0.2% Coomassie brilliant blue R-250 in 50% methanol and 10% acetic acid, the gel was destained with 10% acetic acid. Protein extract from the uterus of pseudopregnant mice or rats and the medium of only embryo or only epithelial cell culture were used as a control. Activity of MMP-2 and -9 was detected by gelatin zymography and quantified by computer-aided densitometry.

2.7. Statistical analysis

All results are shown as mean ± S.E. (standard error). Interspecific or intraspecific data within the same time point were analyzed using Student's *t*-test. A value of $P < 0.05$ was considered significant.

Table 1
Implantation rate of rat or mouse blastocysts transferred into mouse uteri on the ninth day of pregnancy

Embryo transfer model	Number of embryos transferred	Number of implantation sites	Number of alive or still discernible embryos	Implantation rate (%)	*P* value
Mouse blastocysts	424	265	223	62.5	<0.01
Rat blastocysts	408	125	97	30.6	<0.01

3. Results

3.1. Embryo implantation

A total of eight blastocysts from a mouse or a rat were transferred into a single uterine horn of each recipient mouse on Day 3 of pseudopregnancy. The implantation rate was checked on Day 9 of pregnancy. The implantation rate of rat blastocysts in mouse uteri was 30.6%, significantly lower than that of mouse blastocysts in mouse uteri (62.4%; $P < 0.01$, Table 1).

3.2. Development of rat embryos in mouse uteri

By Day 8, the transferred rat embryos were developing normally (Fig. 1a). On Day 9, rat embryos with an ectoplacental cone (EPC) could be observed in mouse uteri, but their development was retarded (Fig. 1b and g). On Day 10, the embryos began to shrink (Fig. 1c and h); On Day 11, the embryos lost their intact structure and the uteri had developed dropsy (Fig. 1d); on Day 12, the embryos shrank further, and had completely separated from the uterine wall (Fig. 1e). By Day 13, rat embryos had been absorbed without any remains (Fig. 1f).

3.3. Morphological and histological changes of rat embryos transferred into mouse uteri

Hematoxylin–eosine staining was done to compare the morphology. Compared to mouse embryos, the transferred rat embryos had intact histological structure on Day 9 (Fig. 2a and b), but the nearby deciduas became porotic (Fig. 2c and d); on Day 10, much blood appeared in the deciduas and the deciduas were more porotic (Fig. 2e and f).

3.4. Embryo and epithelial cell co-culture

An in vitro CT system was used to test differences in the degree of adhesion and outgrowth between rat or mouse embryos on a monolayer of mouse uterine epithelial cells (Fig. 3a and b). Results were observed at different times (24, 48 and 72 h) after the hatched embryos were transferred onto the monolayer. There was no significant difference in the adhesion rate of rat and mouse embryos at 24, 48 and 72 h of CT. However, at 24 h of CT, the outgrowth rate of rat embryos was significantly less than that of mouse embryos ($P < 0.05$). After 48 h of CT, the outgrowth rate of rat embryos was still significantly less than that of mouse embryos ($P < 0.001$, Fig. 4a and b).

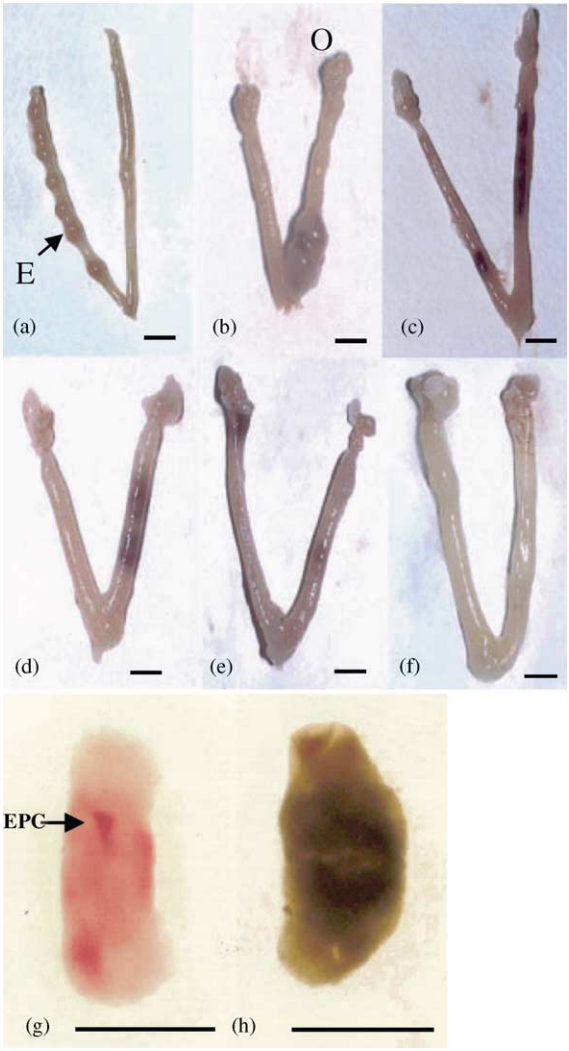

Fig. 1. The development of rat embryos transferred into a mouse uterus. (a) Rat embryos in mouse uterus on Day 8 of pregnancy, (b) rat embryos in mouse uterus on Day 9 of pregnancy, (c) rat embryos in mouse uterus on Day 10 of pregnancy, (d) rat embryos in mouse uterus on Day 11 of pregnancy, (e) rat embryos in mouse uterus on Day 12 of pregnancy, (f) rat embryos in mouse uterus on Day 13 of pregnancy, (g) embryo and deciduas with EPC on Day 9 of interspecific pregnancy, and (h) embryo and deciduas on Day 10 of interspecific pregnancy. O, ovary; E, embryo; and EPC, ectoplacental cone. Scale bars for all panels represent 0.3 cm.

3.5. Gelatin zymography

We extracted proteins from uteri and embryos on different days after rat or mouse embryos were transferred into mouse uteri. The extracts, or the mediums of CT were subjected to gelatin zymographic assay in order to evaluate the activity of MMP-2 and -9.

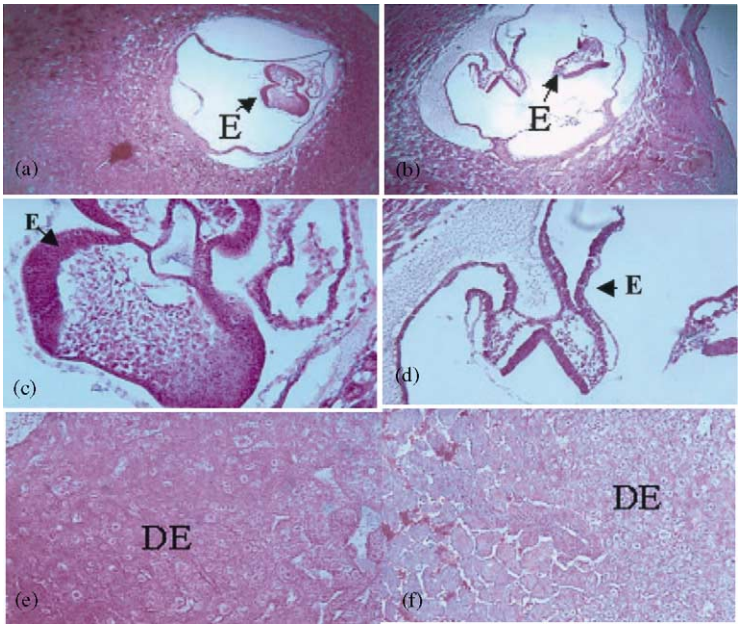

Fig. 2. Histological changes of rat or mouse embryos in mouse uteri. (a) Maternal–fetal interface of mouse embryos in mouse uteri on Day 9 of pregnancy × 100, (b) the maternal–fetal interface of rat embryos in mouse uteri on Day 9 of pregnancy × 100, (c) mouse embryos in mouse uteri on Day 9 of pregnancy × 200, (d) rat embryos in mouse uteri on Day 9 of pregnancy × 200, (e) decidua on Day 10 of mouse intraspecific pregnancy × 200, and (f) decidua on Day 10 of interspecific pregnancy of rat embryos in mouse uteri × 200. E, embryo and DE, decidual cells.

Compared to mouse embryo transfers, MMR-2 activity in rat embryo transfers decreased significantly from Day 5 to Day 10. The MMP-9 activity pattern was similar to that of MMP-2, however, on Day 7, the activity of MMP-9 was the reverse of that observed for MMP-2 (Fig. 5). From Day 9 on, MMP-2 activity in the uteri of pseudopregnant rats was

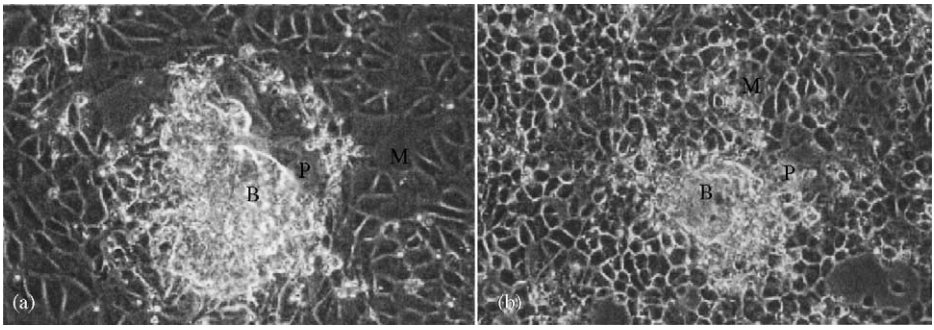

Fig. 3. Outgrowth of rat and mouse blastocysts on a monolayer of mouse uterine epithelial cells. (a) Outgrowth of mouse blastocyst × 200 and (b) outgrowth of rat blastocyst × 200. B, blastocyst; M, monolayer of uterine epithelial cells; and P, primary trophoblastocyst giant cells.

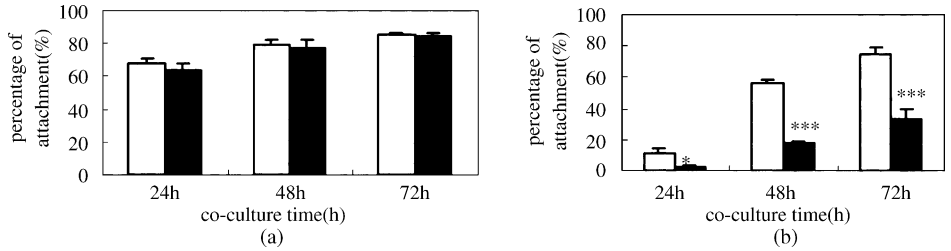

Fig. 4. Percentage of attachment (a) or outgrowth (b) of rat and mouse blastocysts on a monolayer of mouse uterine epithelial cells. Percentage of attachment or outgrowth of rat and mouse blastocysts was determined by comparing the total number of hatched blastocysts with the total numbers of attached or outgrowth blastocysts. Results are expressed as mean ± S.E. of four replicates ($n = 40$ blastocysts per well). The asterisks ($^{*,\ ***}$) indicate significant differences between attachment or outgrowth of rat blastocysts and mouse blastocysts, respectively at the same time point ($P < 0.05$, $P < 0.001$, respectively). (□) mouse, (■) rat.

more than that in pseudopregnant mice but MMP-9 activity was the opposite (Fig. 6). In the CT system, the activity of MMP-2 and -9 was not detected in mouse uterine epithelial cells (Fig. 7B). When only rat or mouse blastocysts were cultured, MMP-9 activity in rat blastocysts was similar to that in the same number of mouse blastocysts; but MMP-2

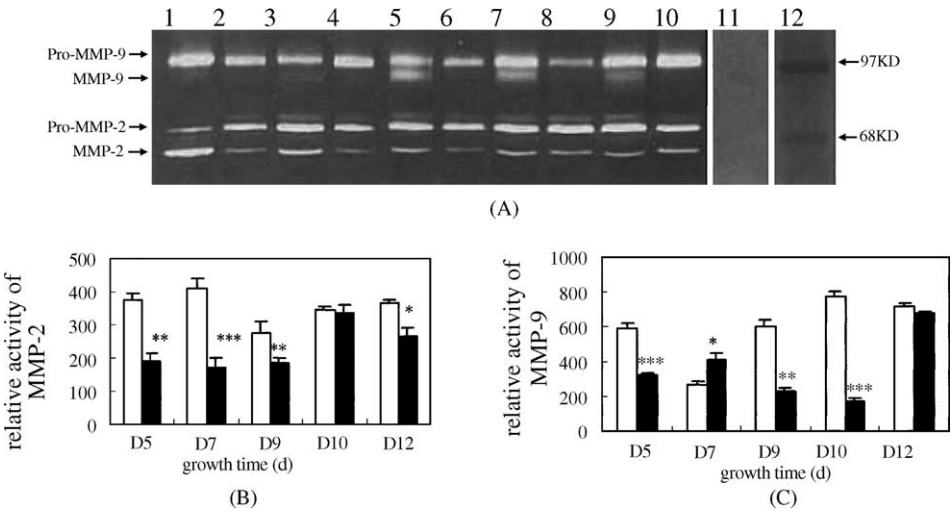

Fig. 5. Activity of MMP-2 and -9 secreted by fetus and uteri on different days after mouse or rat embryos were transferred into mouse uteri. (A) Samples (20 μg) from fetus and uteri on different days after mouse or rat embryos were transferred into mouse uteri were analysed for gelatinase activity in the presence of 5 mmol/l Ca^{2+} and Ca^{2+} plus 50 mmol/l EDTA. Bands which correspond to the relative molecular masses of pro-MMP-9, MMP-9, pro-MMP-2 and MMP-2. Lanes 1, 3, 5, 7, and 9: mouse samples Day 5, Day 7, Day 9, Day 10, and Day l2, respectively. Lanes 2, 4, 6, 8, and 10: rat samples Day 5, Day 7, Day 9, Day 10, and Day 12, respectively. Lane 11: samples hatched with EDTA. Lane 12: standard protein molecular weight. (B) Total MMP-2 and (C) MMP-9 activity detected by gelatin zymography was quantified by computer-aided densitometry. Results are expressed as mean ± S.E. of four replicates. The asterisks ($^{*,\ **,\ ***}$) indicate significant differences in MMP-2 or -9 activity at the same time point between interspecific and intraspecific embryo transfer ($P < 0.05$, $P < 0.01$, $P < 0.001$, respectively). (□) mouse, (■) rat.

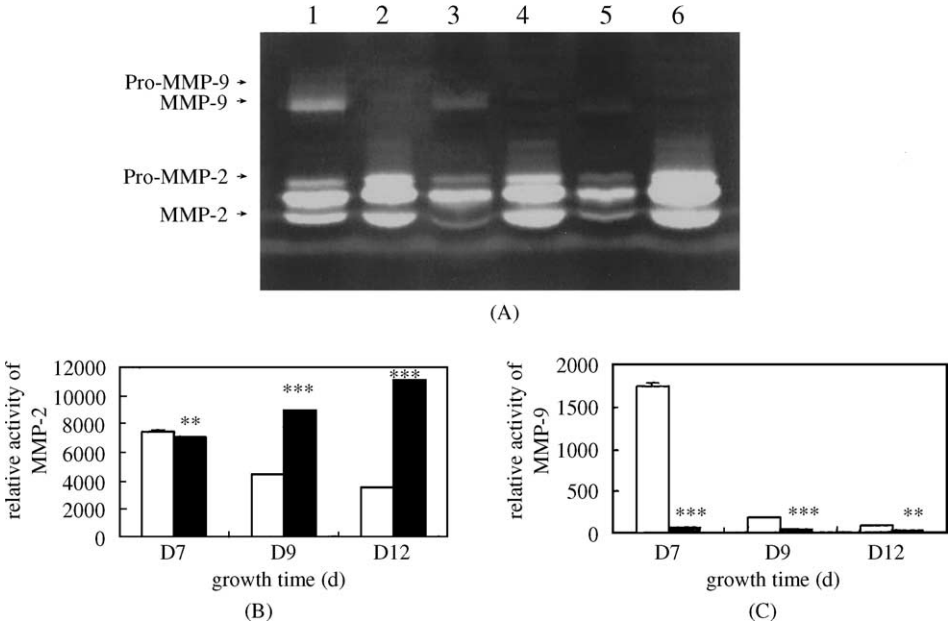

Fig. 6. MMP-2 and -9 secreted by uteri of pseudopregnant mice or rats. (A) Samples (20 μg) from pseudopregnant mouse or rat uteri on different days were analysed for gelatinase activity in the presence of 5 mmol/l Ca^{2+}. Bands which correspond to the relative molecular masses of pro-MMP-9, MMP-9, pro-MMP-2 and MMP-2. Lanes 1, 3, and 5: pseudopregnant mouse samples Day 7, Day 9, and Day 12, respectively. Lanes 2, 4, and 6: pseudopregnant rat samples Day 7, Day 9, and Day 12, respectively. (B) Total MMP-2 and (C) MMP-9 activity detected by gelatin zymography was quantified by computer-aided densitometry. Results are expressed as mean ± S.E. of four replicates. The asterisks (**, ***) indicate significant differences in MMP-2 or -9 activity at the same time point between pseudopregnant mice and rats ($P < 0.01$, $P < 0.001$, respectively). (□) mouse, (■) rat.

activity in rat blastocysts was higher than that in mouse blastocysts (Fig. 7A). However, after 48 and 72 h of CT, MMP-2 and -9 activity in rat embryos on a monolayer of mouse uterine epithelial cells was significantly lower than that in mouse embryos (Fig. 8).

4. Discussion

Reducing the failure rate of interspecific pregnancy is important to successful interspecific cloning. The reasons for the failure of interspecific pregnancy are varied and include immunological rejection, genomic incompatibility, the different types of placenta and gestation periods and so on. To our knowledge, this is the first report wherein the upper time limit for the survival of rat blastocysts in mouse uteri was the ninth day of pregnancy and the activity of MMP-2 and -9 significantly decreased in interspecific pregnancy of rat embryos in mouse recipients. Briones and Beatty found no development after 'rat to mouse' transfers [14]. Later on, Tarkowski [2] found that the upper time limit for the survival of rat blastocysts in mouse uteri was the seventh day of pregnancy. The difference

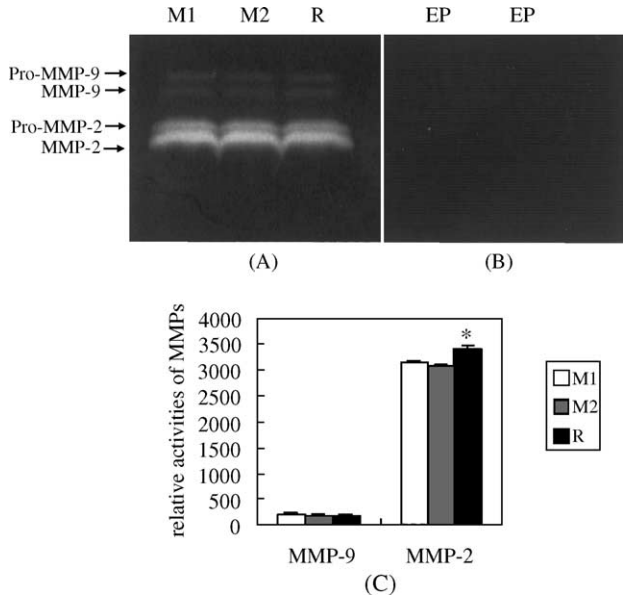

Fig. 7. MMP-2 and -9 secreted by blastocysts or uterine epithelial cells. (A) Samples (20 μg) from blastocysts or uteri epithelial cells (B) were analysed for gelatinase activity in the presence of 5 mmol/l Ca^{2+}. Bands which correspond to the relative molecular masses of pro-MMP-9, MMP-9, pro-MMP-2 and MMP-2. Lanes M1, M2, and R: mouse blastocysts, mouse blastocysts, and rat blastocysts, respectively. Lanes EP: mouse uterine epithelial cells. (C) Total activities of MMP-2 and -9 detected by gelatin zymography were quantified by computer-aided densitometry. Results are expressed as mean ± S.E. of four replicates. The asterisk (*) indicates significant differences in MMP-2 activity at the same time point between mouse and rat blastocysts ($P < 0.05$).

between our results and these previous studies may be due to different experimental techniques, culture medium or lineages of rats and mice. We observed the EPC development on Day 9 of interspecific pregnancy. In normal pregnancy EPC development occurs after embryos have attached themselves to the endometrial epithelium. This suggests that the transferred rat embryos were able to complete attachment. Furthermore, the attachment percentage of rat embryos on a monolayer of mouse uterine epithelial cells was similar to that of mouse embryos, which provides additional evidence that rat embryos can complete the attachment in mouse uteri.

In the present study, the development of the transferred rat embryos was retarded on Day 9 and MMP-2 and -9 activity significantly decreased. This suggested that the invasiveness of the transferred rat embryos was reduced. Embryo implantation is a complex process. Immunologically, it consists of a heterologous graft in which the uterine immune system and the embryo's antigenicity collaborate to make possible both implantation and the maintenance of the pregnancy. Biologically, several different mechanisms must be successively implemented for these two epithelia to fuse and then for one to allow invasion by the other [15]. The complex process requires the involvement of cytokines and MMPs. MMPs play an important role in embryo implantation because they can directly degrade ECM components and allow the embryo to invade the stroma. Intraspecific embryo implantation is a natural model of allograft tolerance. The embryo, an allogenic

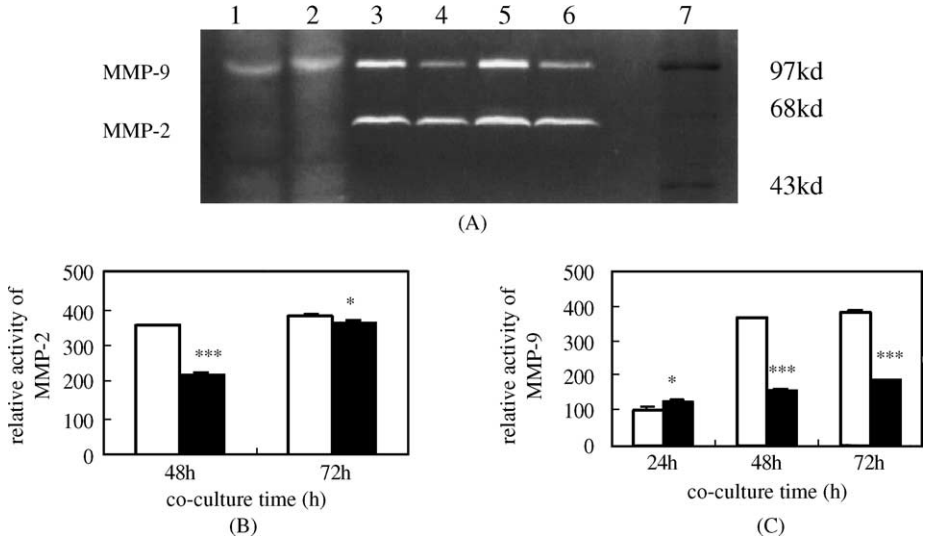

Fig. 8. Activity of MMP-2 and -9 at different time of co-culture of mouse or rat blastocysts on a monolayer of mouse uterine epithelial cells. (A) Samples (20 μg) from the medium of co-culture were analysed for gelatinase activity. Bands which correspond to the relative molecular masses of MMP-9 and -2. Lanes 1, 3, and 5: samples from the medium of mouse embryos on a monolayer of mouse uterine epithelial cells at 24, 48, and 72 h, respectively. Lanes 2, 4, and 6: samples from the medium of rat embryos on a monolayer of mouse uterine epithelial cells at 24, 48, and 72 h, respectively. Lane 7: standard protein molecular weight bands. (B) Total activities of MMP-2 and (C) MMP-9 detected by gelatin zymography were quantified by computer-aided densitometry. Results are expressed as mean ± S.E. of four replicates. The asterisks ($^{*,\ ***}$) indicate significant differences in MMP-2 or -9 activity at the same time point between mouse and rat embryos on a monolayer of mouse uterine epithelial cells ($P < 0.05$, $P < 0.001$, respectively). (□) mouse, (■) rat.

graft, cannot be rejected by the uterine immune system [16]. Rat embryos are heterogeneous antigens with respect to mouse uteri, and consequently induce an acute immunological and biological reaction that, indirectly or directly, affects the expression of MMP-2 and -9. Using an interspecific pregnancy model in the mouse, Clark et al. suggested that failed pregnancies were due to lack of trophoblastic stimulation at the decidua with subsequent fetal death to the result of failure to suppress maternal cytotoxic lymphocyte activation [17]. In immunological rejection, some cytokines secreted by macrophages and lymphocytes modulate the expression of MMP-2 and -9. For example, soluble IL-4 enhanced T cell-induced MMP-1 and inhibited MMP-9 production both at the protein and mRNA levels in monocytes cultured for 7 days in the presence of GM-CSF [18]. Decidual NK cells and macrophage cells enhanced the transcription of TNFα and TGFβ1, and the latter two can suppress the secretion of MMPs.

During preimplantation, maternal–embryo communication is mediated by the trophectoderm [19]. Rats and mice belong to different genera; some of their genes are heterogenous, even incompatible, which means that signals from the trophectoderm of rat embryos cannot be recognized by the mouse uterine endometrium. There are differences between rats and mice in the carbohydrate epitopes expressed by the endometrial epithelium [20], and carbohydrate epitopes can regulate the expression of MMPs. Thus,

on one hand, the inability of transferred rat embryos to properly interpret signals from the mouse uteri causes a decrease in their MMP-2 and -9 activity, thus reducing their ability to invade the uterine endometrium. On the other hand, transferred rat embryos suffer retarded development because they cannot obtain sufficient nutrition from mouse uteri.

The in vitro study showed that the activity of MMP-2 and -9 in heterogeneous CTs was lower than in homogeneous CTs after 24 h, while the activity of MMP-2 and -9 in rat blastocysts was no less than that in the same number of mouse blastocysts, and uterine epithelial cells did not secrete MMP-2 and -9. These results indicated that in heterogeneous CTs, mouse uterine epithelial cells suppressed MMP-2 and -9 activity in rat blastocysts. In addition, the outgrowth rate of rat blastocysts on a monolayer of mouse uteri epithelial cells was significantly less than that of mouse blastocysts. This further suggested that the ability of rat blastocysts to invade mouse uteri was much less than that of mouse blastocysts. Gene incompatibility may be an important reason for the inhibition of MMP-2 and -9 activity in rat blastocysts in CT, but the actual mechanism needs further investigation. On Day 7, the activity of MMP-9 in transferred rat embryos was higher than in transferred mouse embryos. There may exist some kind of compensation mechanism.

The excessive expression of MMPs will lead to unrestricted invasion by trophoblast cells and cancers, however, the suppression of MMPs expression will reduce the invasiveness of embryos and cause the failure of placental development and pregnancy. In conclusion, rat embryos can complete adhesion but not the invasion when transferred into mouse uteri. This decreased invasiveness, especially, the reduction of MMP-2 and -9 activity, is one reason for the failure of interspecific pregnancy.

Acknowledgements

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