Implantation-Dependent Expression of Trophinin by Maternal Fallopian Tube Epithelia during Tubal Pregnancies

Possible Role of Human Chorionic Gonadotrophin on Ectopic Pregnancy

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**Trophinin, tastin, and bystin have been identified as
molecules potentially involved in human embryo im-
plantation. Both trophoblasts and endometrial epi-
thelial cells express trophinin, which mediates apical
cell adhesion through homophilic trophinin-trophi-
nin binding. We hypothesized that trophinin's func-
tion in embryo implantation is unique to humans and
investigated the expression of trophinin, tastin, and
bystin in ectopic pregnancy, a condition unique to
humans. In tubal pregnancies, high levels of all three
were found in both trophoblasts and fallopian tubal
epithelia. Trophinin expression in maternal cells was
particularly high in the area adjacent to the tropho-
blasts, whereas trophinin was barely detectable in
intact fallopian tubes from women with *in utero* preg-
nancies or without pregnancies. When explants of
intact fallopian tube were incubated with the human
chorionic gonadotrophin (hCG), trophinin expres-
sion was enhanced in epithelial cells. Since the tro-
phectoderm of the human blastocyst secretes hCG
before and after implantation, these results suggest
that hCG from the human embryo induces trophinin
expression by maternal cells. As both β-subunit of**
**hCG and trophinin genes have diverged in mammals,
the present study suggests a unique role of hCG and
trophinin in human embryo implantation, including
the pathogenesis of ectopic pregnancy.   *(Am J Pathol
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Ectopic pregnancy occurs at a rate of 0.25 to 1.4% in all
pregnancies in humans, and over 95% of all ectopic
pregnancies occur in the fallopian tube.[1,2] Extrauterine
implantation does not occur naturally or experimentally in
other animals including non-human primates.[3–5] It is not
known why extrauterine implantation occurs only in hu-
mans.

The human embryo enters the uterine cavity 4 days
after ovulation.[6] Blastocyst implantation takes place
around day 7 after ovulation. During this period, the en-
dometrial luminal epithelium becomes receptive to blas-
tocyst adhesion in response to rising serum progesterone
and peptide hormones from the newly formed corpus
luteum, opening an implantation window.[7,8]

The blastocyst free from the *zona pellucida* is sticky *in
vitro*. Human trophoblastic cells avidly bind to fibronectin,
laminin, and type IV and V collagen,[9] all of which are
present in decidualized endometrial stroma. Therefore,
injuries to the epithelium that expose this underlying ma-
trix can enable trophoblast elements to adhere and grow,
potentially causing ectopic pregnancy. The receptive hu-
man endometrium expresses α_V/β_3 integrin on the apical
surfaces of the luminal epithelium.[8] This integrin type is

Table 1.   Expression of Trophinin, Tastin, and Bystin Proteins in the Fallopian Tube from Patients Unrelated to Tubal Pregnancy

Patient no.	Age	Menstrual cycle or gestational age (day)	Trophinin	Tastin	Bystin
1	24	proliferative phase	+*	+++	−
2	28	98 days pregnancy	−	++	+++
3	42	49 days pregnancy	+	+++	+++
4	19	secretory phase	−	+	−
5	19	secretory phase	−	++	++
6	48	secretory phase	+	++	++
7	47	proliferative phase	+	++	++
8	50	proliferative phase	−	++	+++
9	50	proliferative phase	−	+	++
10	40	44 days pregnancy	−	+++	+
11	37	proliferative phase	−	+	++

*−, +, ++, and +++ show no, minimal, moderate, and maximal immunohistochemical reactivity, respectively.

also found in the normal human fallopian tube epithelium, suggesting the existence of a tubal implantation window.[10] However, given that tubal pregnancy does not occur in animals, it is difficult to explain the mechanism of ectopic pregnancy by the activity of evolutionarily conserved molecules such as integrins.

Previously, we identified and characterized a novel molecular complex, composed of trophinin, tastin and bystin, mediating apical cell adhesion between human trophoblasts and endometrial epithelial cells.[11–17] Trophinin is an integral membrane protein, and part of this polypeptide is exposed on the cell surface. Tastin and bystin are cytoplasmic proteins required for trophinin to exhibit efficient cell adhesion activity. Trophoblastic cells and endometrial epithelial cells expressing these molecules adhere to each other at their respective apical plasma membranes. Human endometrium tightly regulates expression of trophinin, which is expressed only within a restricted region of the apical side of luminal endometrial epithelium at a time coincident with the "implantation window."[11] In the human placenta at early stages of pregnancy, trophinin, tastin and bystin are strongly expressed in the trophoblast of chorionic villi and in the endometrial glandular epithelium, particularly at the utero-placental interface.[15] Studies of mouse trophinin indicate significant differences from human trophinin in expression patterns and roles *in vivo*.[16,17] Trophinin is not found in mouse trophoblastic cells during and after implantation.[16,17] Trophinin null mutant mice show no defects in embryo implantation.[17] The *in vivo* role of human trophinin in embryo implantation remains to be proved.

We hypothesized that trophinin's function in embryo implantation is unique to humans. The present study was undertaken to evaluate this hypothesis by determining the expression pattern of trophinin in ectopic pregnancy, a condition unique to humans. Here we show that, in tubal pregnancies, trophinin is strongly expressed by both the embryonic trophoblasts and maternal fallopian tube epithelia. The expression pattern of trophinin suggests that a factor derived from the implanting embryo induces trophinin expression by maternal cells. We found that the human chorionic gonadotrophin (hCG) induces trophinin expression by epithelial cells in the fallopian tube, which was determined by quantitative RT-PCR for trophinin transcripts and immunohistochemistry for trophinin protein. Thus the results suggest strongly that trophinin and hCG together play unique roles in human embryo implantation, including the pathogenesis of ectopic pregnancies.

Materials and Methods

Tissue Collection

Twenty-three tissue blocks of fallopian tubes resected from 23 patients with ectopic tubal pregnancy were retrieved from the archives of Shinshu University Hospital and Keio University Hospital, Japan. In addition, paraffin blocks of intact fallopian tubes removed from 11 patients with benign uterine or ovarian diseases were also retrieved from the same archives. Three of these 11 patients were found to be associated with *in utero* pregnancy. All archival samples were obtained with informed consent. Age, gestational age, or menstrual cycle of the patients are listed in Tables 1 and 2. These specimens were fixed for 48 hours in 20% buffered formalin (pH 7.4), embedded in paraffin, and sectioned serially at 3-μm thickness for immunohistochemistry or at 7-μm thickness for *in situ* hybridization.

In parallel, four tissue specimens of fresh intact fallopian tubes were obtained from the patients who underwent hysterectomy for uterine diseases after informed consent was obtained at Keio University Hospital. All of the patients were cyclic women, and their ages ranged from 26 to 52 years. The fallopian tube specimens from two patients (26- and 43-years-old) were cut into small pieces and cultured at 37°C in a 5% CO_2 incubator in Ham's F-12 medium (Nissui Pharmaceutical Co, Tokyo, Japan) containing 10% fetal calf serum in the presence of 10 IU/ml or 100 IU/ml hCG (from pregnancy urine, Teikoku Hormone Mfg, Tokyo, Japan). As a control, hCG was omitted from the culture medium. On culturing for 6 and 24 hours, the explants were subjected to quantitative analysis of trophinin mRNA. On the other hand, the fallopian tube specimens from two patients (41- and 52-years-old) were cultured for 24 hours in the presence or absence of hCG in the similar manner as described above, and tissues were analyzed for trophinin protein by immunohistochemistry.

Table 2. Expression of Trophinin, Tastin, and Bystin Proteins in the Fallopian Tubes during Tubal Pregnancy

Patient no.	Age	Gestational age (day)	Trophinin	Tastin	Bystin
1	30	41	+++*	+++	+++
2	36	61	+++	++	+++
3	24	122	+++	+++	+++
4	37	73	+++	+++	+++
5	35	49	++	+++	++
6	29	56	+++	+++	+++
7	26	44	+++	+++	+++
8	35	50	++	++	++
9	37	51	+++	++	+++
10	25	50	+++	+++	+++
11	40	58	++	+++	++
12	30	48	+	+	++
13	36	55	+++	+++	+++
14	36	58	++	++	++
15	34	49	+++	++	++
16	29	56	+++	++	+
17	37	49	++	++	+++
18	22	47	+++	++	+++
19	31	52	++	+++	+++
20	34	43	++	+	+++
21	24	57	+++	+++	+++
22	29	71	+++	+++	+++
23	22	47	++	+++	++

*−, +, ++, and +++ show no, minimal, moderate, and maximal immunohistochemical reactivity, respectively.

Immunohistochemistry

The tissue specimens were fixed for 48 hours in 20% buffered formalin, embedded in paraffin, and sectioned serially at 3-μm thickness. Immunohistochemistry was performed by an indirect immunoperoxidase method using monoclonal antibodies specific for trophinin, bystin, and tastin, as described.[15] Briefly, tissue sections were immersed in absolute methanol containing 0.3% H_2O_2 for 30 minutes and then washed with PBS. For primary antibodies, monoclonal anti-trophinin (clone 3–11, mouse IgM), anti-bystin (clone 19, mouse IgM), or anti-tastin (clone 38, mouse IgM) antibodies were used, and for the secondary antibody, goat anti-mouse Ig conjugated with horseradish peroxidase (DAKO, Carpinteria, CA) was used. Peroxidase activity was visualized with a diamino-benzidine-hydrogen peroxide solution. Similarly, immunohistochemistry for hCG was performed using polyclonal anti-hCG antibodies (DAKO) and anti-rabbit Ig conjugated with horseradish peroxidase (DAKO) as a secondary antibody. Control experiments were carried out either by omitting the primary antibodies or using an irrelevant isotype-matched primary antibody; ie, HIK1083 (Kanto Kagaku, Tokyo, Japan) specific for GlcNAcα1→4Galβ→R.[18] No specific signal was noted in these control experiments.

In Situ *Hybridization*

To detect trophinin, tastin, and bystin transcripts, *in situ* hybridization was carried out using RNA probes specific for these molecules, as described previously.[15,19] Briefly, digoxigenin-labeled anti-sense and sense probes were prepared by *in vitro* transcription from pGEM-3Zf(+) plasmids (Promega, Madison, WI) containing specific DNA fragments of trophinin (nucleotides −3 to +149; the first nucleotide of the initiation codon is defined as +1), tastin (−26 to +124), and bystin (+627 to +796). The deparaffinized tissue sections from tubal pregnancy and intact fallopian tubes were immersed in 0.2 mol/L HCl for 20 minutes, digested with 100 μg/ml proteinase K at 37°C for 20 minutes, and post-fixed with 4% paraformaldehyde. The slides were rinsed with 2 mg/ml glycine, acetylated for 10 minutes in 0.25% acetic anhydride in 0.1 mol/L triethanolamine (pH 8.0), defatted with chloroform, and air-dried. After prehybridization with 50% deionized formamide/2X SSC for 1 hour at 45°C, the slides were hybridized with 0.5 mg/ml of anti-sense or sense probe in 50% deionized formamide, 2.5 mmol/L EDTA (pH 8.0), 300 mmol/L NaCl, 1X Denhardt's solution, 10% dextran sulfate, and 1 mg/ml brewer's yeast tRNA at 45°C for 48 hours. After hybridization, the slides were washed in 50% formamide/2X SSC for 1 hour at 45°C and digested with 10 mg/ml RNase A at 37°C for 30 minutes. After sequential washing with 2X SSC/50% formamide at 45°C for 1 hour and 1X SSC/50% formamide at 45°C for 1 hour, the sections were subjected to immunohistochemistry for detection of hybridized probes using an alkaline phosphatase-conjugated anti-digoxigenin antibody (Roche Molecular Biochemicals, Alameda, CA). Digoxigenin was detected by alkaline phosphatase by incubating with 5-bromo-4-chloro-3-indolyl phosphate and nitroblue tetrazolium in the presence of 0.2 mmol/L levamisole and 10% polyvinylalcohol for 30 minutes at room temperature. No specific signals were detected in the control experiments using sense probes.

Quantitative Analysis of Trophinin mRNA

The intact fallopian tubes obtained from two patients (26- and 43-years-old) on hysterectomy were cultured in the presence or absence of hCG for 6 hours and for 24 hours as described above. Total RNA was isolated from the tissue explants using Isogen (Nippon Gene, Tokyo, Japan). After digestion with DNase I, reverse transcription of the total RNA was performed in a final volume of 20 μl containing 4 μl of 5X first strand buffer, 2 μl of 2 mmol/L dNTPs, 0.2 μl of 0.1 mol/L DTT, 1 μl of RNase inhibitor, 0.5 μl of M-MLV reverse transcriptase, and 1 μl of oligo dT primer at 42°C for 60 minutes.

Quantitative analysis of trophinin mRNA expressed in the fallopian tubes was carried out using an ABI PRISM 7700 Sequence Detection System (PE Applied Biosystems, Foster City, CA). Specific primer pair and probe were designed with the aid of the Primer Express program (PE Applied Biosystems). Forward and reverse primers for human trophinin were 5′-AGGGAAGAGT-TAGGCGATGATTC-3′ (nucleotides +22 to +44), and 5′-TTGGGCTCTGGCCTCAATT-3′ (nucleotides +72 to + 90), respectively. The TaqMan probe, 5′-CAAAT-GAAAATCTGCTCCAGGCCTG-3′ (nucleotides +46 to +70), which carries 5′-FAM (6-carboxyfluorescein) reporter label and 3′-TAMURA (6-carboxy-N, N, N′,
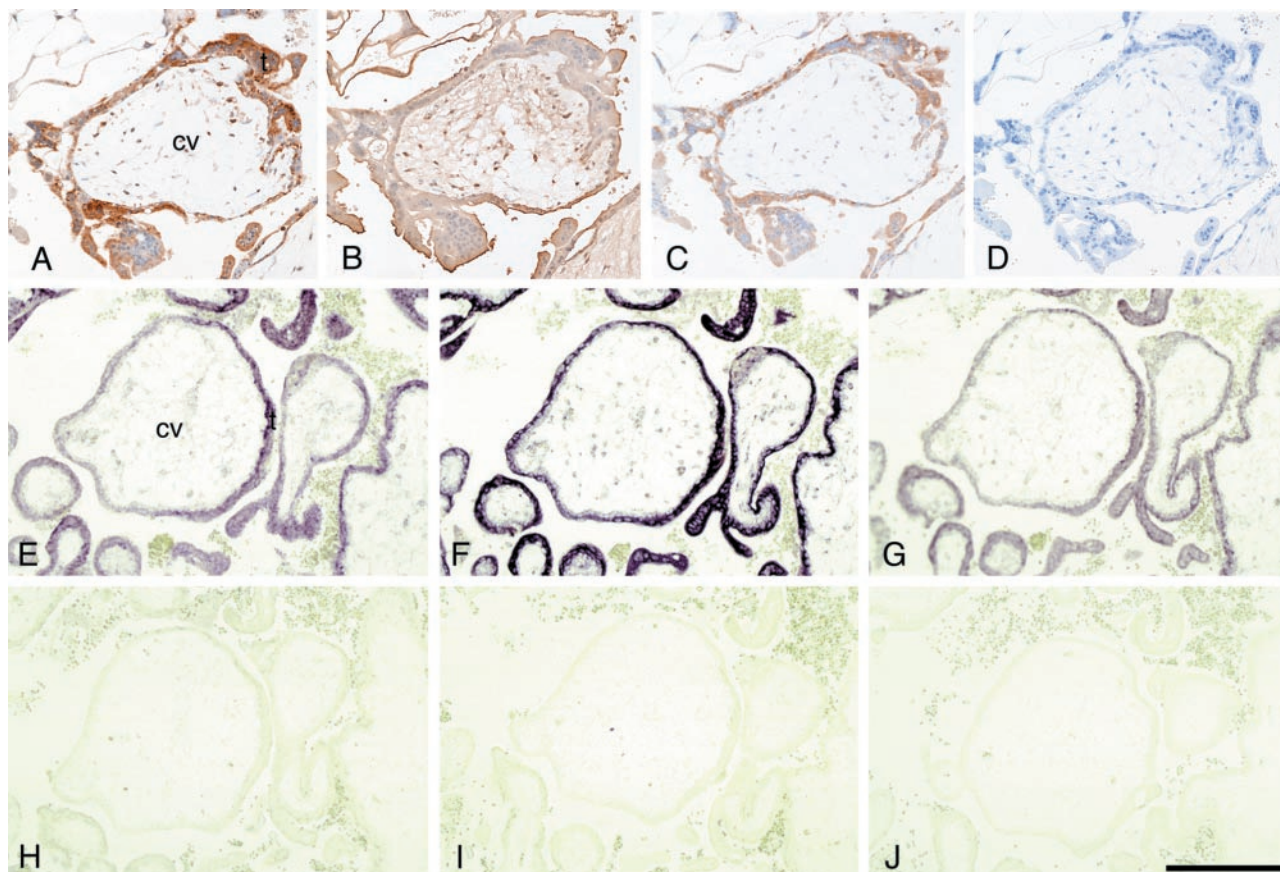
Figure 1. Expression of trophinin, tastin, and bystin proteins and their transcripts in the chorionic villi from tubal pregnancy. Immunohistochemistry for trophinin (**A**), tastin (**B**), bystin (**C**), second antibody alone (**D**), and *in situ* hybridization for trophinin (**E**, antisense; **H**, sense), tastin (**F**, antisense; **I**, sense), and bystin (**G**, antisense; **J**, sense) using digoxigenin-labeled RNA probes. All photographs presented are in the same magnification, and the **bar** in (**J**) indicates 200 μm. (cv, chorionic villi; t, trophoblasts).

N′-tetramethylrhodamine) quencher group was synthesized by PE Applied Biosystems. A relative standard curve representing tenfold dilutions of a trophinin cDNA (pcDNAI-trophinin[11]) ranging from 10^{10} to 10^1 copies/ml was used for linear regression analysis for trophinin mRNA. A standard curve ranging from 10^{10} to 10^1 copies/ml for a housekeeping gene, glyceraldehyde-3-phosphate dehydrogenase (GAPDH), was prepared in the same tube that trophinin cDNA was prepared, and this was used for normalizing the samples.[20]

Multiplex PCR was carried out in 50 μl of reaction mixture containing 3 μl of cDNA sample, 1X Universal PCR Master Mix (PE Applied Biosystems), 800 nmol/L of the primer pair for trophinin, 800 nmol/L of the primer pair for GAPDH, 200 nmol/L of the TaqMan probe for trophinin, and 200 nmol/L of the TaqMan probe for GAPDH that carries 5′-VIC reporter label and 3′-TAMURA quencher group (PE Applied Biosystems). These reaction tubes were placed in the ABI PRISM 7700 Sequence Analyzer, and preheated at 95°C for 10 minutes, followed by 50 cycles of denaturation at 95°C for 15 seconds and annealing/extension at 60°C for 1 minute. The quantity of trophinin mRNA was determined using the standard curve of trophinin cDNA on normalizing the sample size by GAPDH. The quantity of trophinin mRNA was ex-

pressed as the relative amount of GAPDH mRNA, thus the ratio of trophinin mRNA to GAPDH multiplied by 100. The assays were performed in duplicate, and the mean values of the two experiments were indicated.

Results

Expression of Trophinin, Tastin, and Bystin by Trophoblasts of Chorionic Villi during Tubal Pregnancy

Immunohistochemistry of the fallopian tube from ectopic pregnancy patients using anti-hCG antibodies showed expression of hCG, a trophoblast marker, in the chorionic villous trophoblasts but not in the tubal epithelia (data not shown). Immunohistochemistry showed strong expression of trophinin, tastin, and bystin proteins in trophoblasts of the chorionic villi (Figure 1, A to C). These results were confirmed by *in situ* hybridization, demonstrating that transcripts of these molecules are strongly expressed in trophoblastic cells (Figure 1, E to G). The expression patterns of these proteins and transcripts are similar to those observed previously in the human placenta in normal pregnancy.[15]
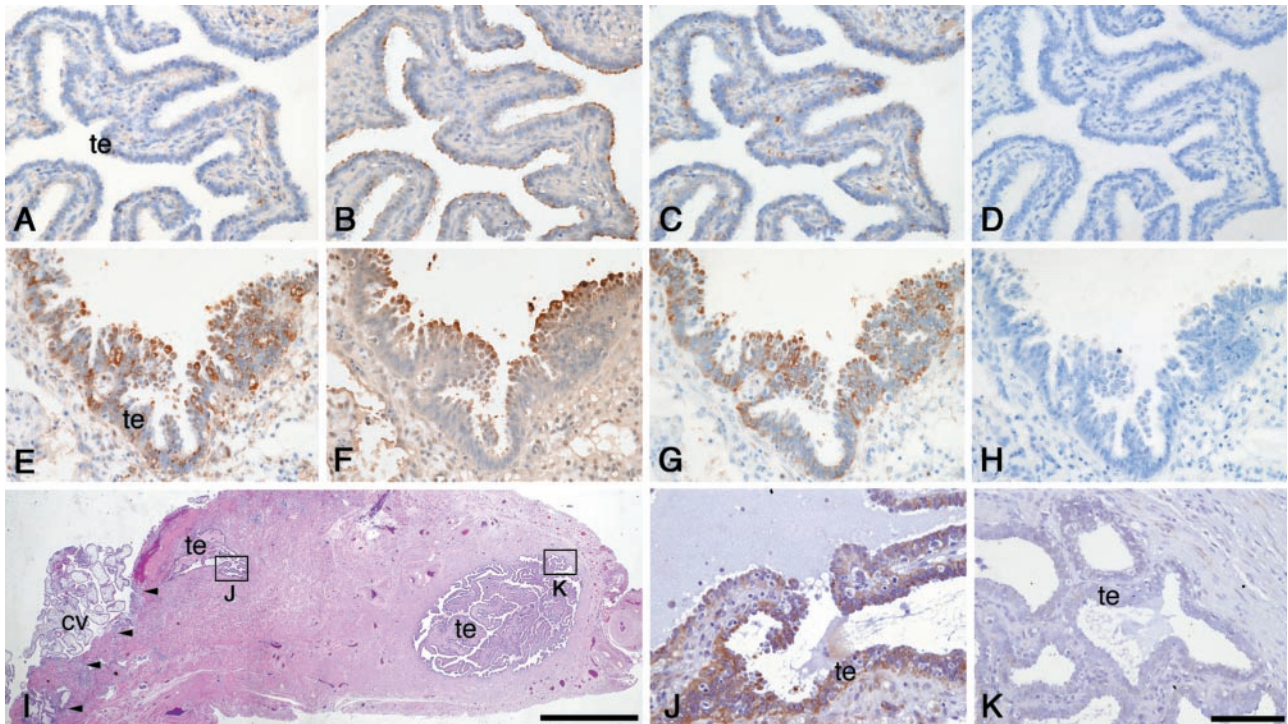
Figure 2. Expression of trophinin, tastin, and bystin proteins in intact fallopian tube and in fallopian tube with tubal pregnancy. Immunohistochemistry of an intact fallopian tube (**A–D**) and a fallopian tube with tubal pregnancy (**E–K**) for trophinin (**A**, **E**, **J**, **K**), tastin (**B**, **F**), and bystin (**C**, **G**). Hematoxylin and eosin-stained specimen from a tubal pregnancy (**I**), and high magnification of insets showing immunohistochemistry for trophinin in the area close to (**J**) or distant from (**K**) the chorionic villi. The implantation site in (**I**) is marked by **arrowheads**. All photographs except (**I**) are in the same magnification, and the **bar** in (**K**) indicates 100 μm. **Bar** in (**I**) indicates 2.0 mm. (cv, chorionic villi; te, tubal epithelia).

Overexpression of Trophinin Protein in Maternal Epithelial Cells in Tubal Pregnancy

Immunohistochemistry of intact fallopian tubes from non-pregnant and *in utero* pregnant women showed barely detectable levels of trophinin protein in tubal epithelial cells (Figure 2A, Table 1). On the other hand, various amounts of tastin and bystin proteins were detected in the cilia and the cytoplasm of the intact fallopian tube epithelia, respectively, regardless of non-pregnancy or *in utero* pregnancy (Figure 2, B and C, Table 1).

The signals for trophinin protein in tubal epithelia adjacent to the implantation site of tubal pregnancies were remarkably higher (Figure 2E) than those in intact fallopian tubes (Figure 2A, Table 2). Trophinin proteins were found in the cytoplasm of tubal epithelia with large apical protrusions or pinopodes (Figure 2E). Strong signals for tastin and bystin proteins were also seen in tubal epithelia with tubal pregnancies (Figure 2, F and G, Table 2). The levels of tastin and bystin appear to be elevated in association with ectopic pregnancy compared to those seen in intact fallopian tubes (Tables 1 and 2).

In addition, we also found that within a specimen of tubal pregnancy (Figure 2I), maternal epithelia close to the chorionic villi showed a stronger signal for trophinin (Figure 2J) than epithelial cells distant from the chorionic villi (Figure 2K). Similar observations that strong signals for trophinin were detected in the maternal endometrial glandular epithelial cells adjacent to the chorionic villi were made previously in human placenta from early pregnancy.[15] These results suggest the possibility that an embryonic factor functions locally to induce trophinin expression in maternal cells.

Expression of Trophinin Transcripts by Maternal Cells in Tubal Pregnancy

To confirm the results obtained by immunohistochemistry, we compared levels of trophinin, tastin, and bystin transcripts in non-pregnancy, *in utero* pregnancy and tubal pregnancy by *in situ* hybridization. Thus we examined three intact fallopian tubes from patients unrelated to tubal pregnancy and three fallopian tubes from tubal pregnancies. The results showed that levels of trophinin transcripts in the fallopian tube epithelia from tubal pregnancies were higher than those seen in intact fallopian tubes from *in utero* pregnancies or non-pregnancies (Figure 3A and G). We also observed that signals for trophinin transcripts are strong in the maternal epithelia adjacent to the chorionic villi, whereas epithelia far from the chorionic villi barely express trophinin transcripts (data not shown). These observations suggest that trophinin expression by maternal cells is induced by tubal pregnancy. By contrast, although the expression levels of tastin and bystin mRNAs were slightly increased in tubal pregnancy (Figure 3, B, C, H, and I), these differences in tastin and bystin transcripts between intact fallopian tubes and the fallopian tubes from tubal pregnancies were not as significant as those observed for trophinin.
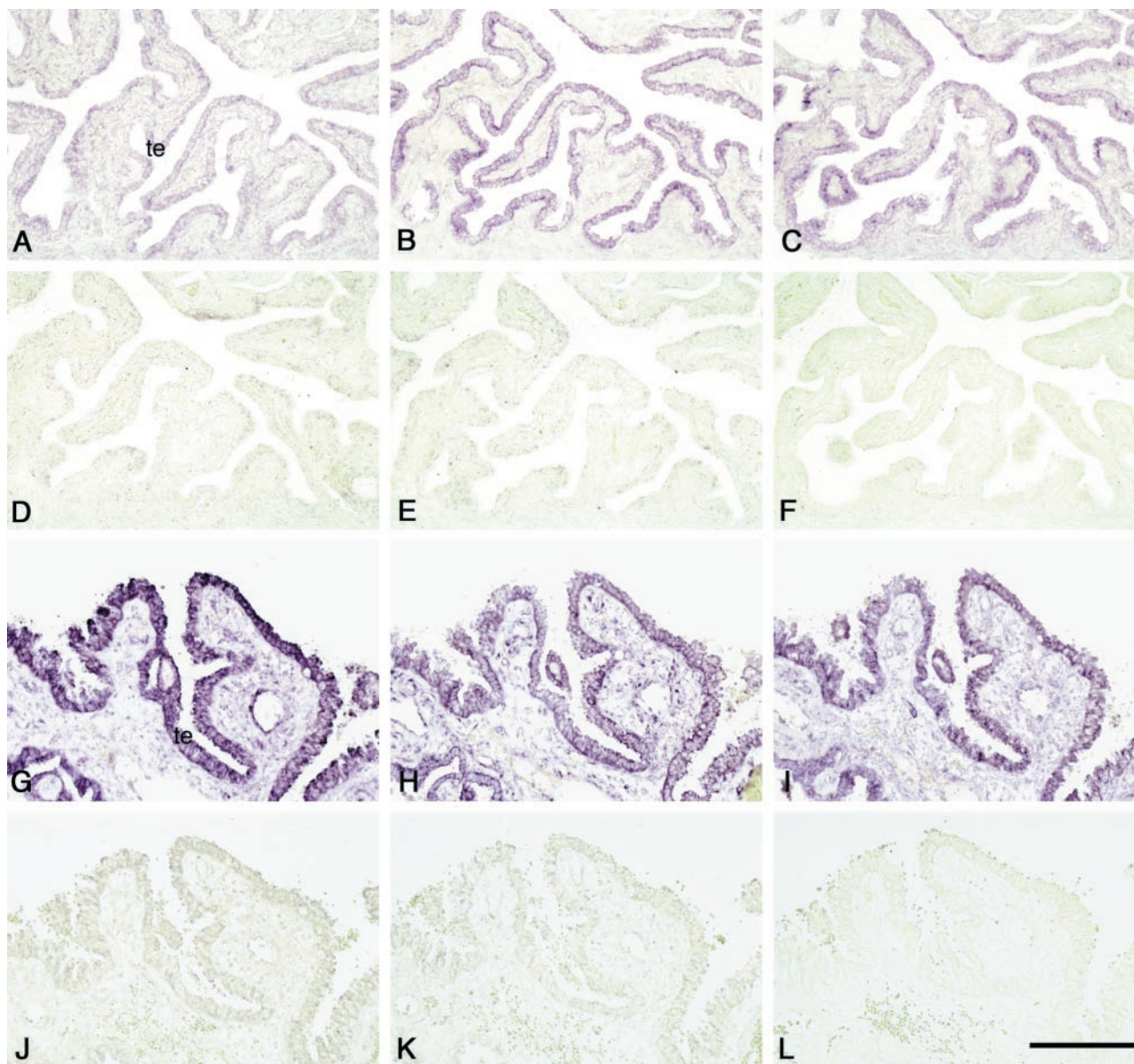
Figure 3. Expression of trophinin, tastin, and bystin transcripts in intact fallopian tube and in that with tubal pregnancy. *In situ* hybridization of an intact fallopian tube (**A–F**), and a fallopian tube with tubal pregnancy (**G–L**) for trophinin (**A** and **G**, antisense; **D** and **J**, sense), tastin (**B** and **H**, antisense; **E** and **K**, sense), and bystin (**C** and **I**, antisense; **F** and **L**, sense) using digoxigenin-labeled RNA probes. All photographs presented are in the same magnification, and the **bar** in (**L**) indicates 200 μm. (te, tubal epithelia).

Induction of Trophinin by hCG in Fallopian Tubal Epithelial Cells

Trophinin was barely detectable in intact fallopian tubes, regardless of whether they were derived from pregnant or non-pregnant patients (Figure 2A, Table 1). This observation suggests that steroid hormones associated with pregnancy are unlikely to promote strong trophinin expression in the tubal epithelial cells.

Since it is known that the trophectoderm cells of pre-implantation embryo and chorionic villi trophoblasts secrete hCG,[21–24] we determined if hCG induces trophinin expression by maternal cells. On incubation of explants of the intact fallopian tube with hCG at 100 IU/ml for 24 hours, levels of trophinin proteins were significantly in-

creased (Figure 4, A and E). No differences were found in the response to hCG among fallopian tubal explants regardless of whether they were derived from the region close to or far from the uterus. The explants incubated with lower concentrations of hCG (10 IU/ml) also showed elevation of trophinin protein, but at lower levels than at 100 IU/ml (data not shown). On the other hand, significant amounts of tastin and bystin were expressed in intact fallopian tubes (Figure 4, B and C), and their levels were not significantly altered by incubation with hCG (Figure 4, F and G).

To examine whether hCG induces the expression of trophinin at the transcriptional level, we determined the quantity of trophinin mRNA in the explants of intact fallopian tube by a real-time RT-PCR method. Thus the fallo-
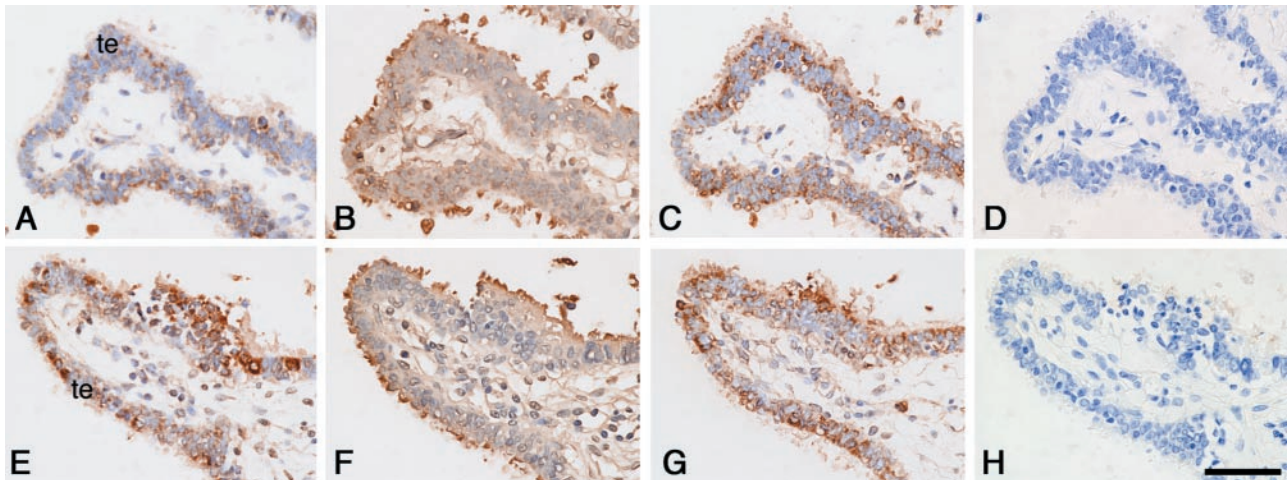
Figure 4. Comparison of trophinin, tastin, and bystin proteins in explants of intact fallopian tube with or without incubation with hCG. Explants were cultured for 24 hours without hCG (**A–D**) or with 100 IU/ml hCG (**E–H**), and were immunostained for trophinin (**A**, **E**), tastin (**B**, **F**), bystin (**C**, **G**), and second antibody alone (**D**, **H**). All photographs presented are in the same magnification, and the **bar** in (**H**) indicates 50 μm. (te, tubal epithelia).

pian tube specimens isolated from two patients unrelated to the ectopic pregnancy were incubated with hCG, and the quantities of trophinin mRNA were compared with those without hCG. In both experiments, trophinin transcripts in the explants increased in a dose-dependent manner of hCG (Table 3). These results strongly suggest that hCG induces the expression of trophinin gene by the fallopian tube epithelia.

Discussion

In this study, we found that trophinin, tastin, and bystin are expressed strongly in the trophoblasts and in maternal epithelia during ectopic tubal pregnancy (Figures 1, 2, and 3, Table 2). Expression patterns of these molecules in tubal pregnancy are similar to those seen in placenta during *in utero* pregnancy.[15] Thus the trophoblasts of chorionic villi and the maternal epithelia adjacent to chorionic villi strongly express trophinin, tastin, and bystin. One intriguing finding in this study is that the intact fallopian tubes isolated from women with *in utero* pregnancy express negligible levels of trophinin (Table 1). This finding indicates that steroid hormones alone cannot induce strong trophinin expression in humans. Furthermore, the results suggest the existence of a locally acting embryonic factor inducing trophinin expression by maternal cells (Figure 2, I to K). Thus trophinin expression by tubal epithelial cells depends on the presence of an embryo. This is in sharp contrast to mouse trophinin,

whose expression is induced by the ovarian hormone, estrogen, independent of the embryo.[16,17]

In this study, we showed that hCG induces the levels of trophinin transcripts in the fallopian tube (Table 3). This must lead to an elevation of trophinin protein, which was detected by immunohistochemistry (Figure 4). In preimplantation stage human embryos, hCG-β transcripts are detected as early as the two-cell stage,[21] and preimplantation stage blastocysts secrete hCG.[22–24] Thus, it is likely that a human blastocyst secretes hCG before and during implantation. Given that trophinin mediates cell adhesion by homophilic binding,[11] evidence collectively suggests that hCG-β secreted from a blastocyst induces trophinin expression by the maternal epithelium, resulting in trophinin-mediated homophilic binding between the embryo and maternal epithelium.

Using fallopian tubal explants, we found that high concentrations (100 IU/ml) of hCG induce trophinin expression in epithelial cells (Figure 4, Table 3). This concentration of hCG has physiological relevance if local concentrations of hCG are considered. In pregnant women 6 to 12 days after ovulation the first sign of pregnancy is an hCG concentration of greater than 0.325 mIU/ml in the urine on the day of blastocyst implantation.[25] Urine from non-pregnant women shows no detectable levels of hCG, indicating that elevated hCG in urine is derived from an implanting blastocyst. As the concentration of hCG in the urine and plasma is similar and the plasma volume of a 50-kg woman is 1900 ml,[26] the total

Table 3. Quantitative Analysis of Trophinin mRNA in Explants of the Intact Fallopian Tube Incubated with hCG

	Incubation for 6 hours			Incubation for 24 hours		
	0*	10	100	0	10	100
Experiment 1[†]	15.8[‡]	35.4	431.2	1.1	31.2	38.7
Experiment 2	2.3	4.1	7.5	3.4	9.8	27.6

*, Concentration of hCG (IU/ml).
[†], Experiments 1 and 2 were conducted using fallopian tubal explants obtained from each different individual who underwent hysterectomy.
[‡], Trophinin:GAPDH mRNA ratios multiplied by 100 are indicated.

amount of hCG produced by the blastocyst is calculated to be 617.5 mIU (= 0.325 mIU/ml × 1900 ml). Estimating that local volume surrounding the blastocyst in the fallopian tube ranges from 1 μl to 10 μl, the intraluminal concentration of hCG around the blastocyst is estimated to be 617.5 ~ 61.8 IU/ml. Thus the maternal epithelia adjacent to an implanting embryo is likely to be exposed to high concentrations of hCG.

The timing and quantity of hCG produced by the embryo are considered key factors in determining whether a human pregnancy succeeds or fails.[27] Statistics of assisted reproduction show that the rate of gestation increases substantially when several embryos of the two-to-eight-cell stage are transferred into the uterus.[28] The experience of assisted reproduction also suggests that the quality of the oocyte largely determines the success of implantation.[29] In light of present findings, oocyte quality may be closely related to the activity of hCG secretion.

It is well known that tubal pregnancy frequently occurs in chronic salpingitis or salpingitis isthmica nodosa.[30] Since these conditions are associated with inflammation, it is likely that inflammatory destruction of the fallopian tube delays migration of blastocyst within the fallopian tube, thus causing the elevation of intraluminal concentrations of hCG. Such delays will also allow time for the blastocyst to interact with tubal epithelial cells. It is also possible that signal transduction through the luteinizing hormone receptor triggered by hCG binding is coupled to downstream signal transduction of inflammatory cytokines.[31,32] Thus cells undergoing inflammation may be more susceptible to stimulation by hCG.

Evidence suggests that there is cross-talk between the mouse blastocyst and uterus to ensure successful implantation.[33] Thus spaciotemporal actions of steroid hormones, various growth factors, and cytokines are thought to play important roles to prepare the receptive uterus for implantation. However, cellular interactions culminating in implantation and placentation vary significantly among mammalian species.[34,35] We found significant differences in the expression pattern and *in vivo* function of human and mouse trophinins.[16,17] Trophinin is strongly expressed in trophoblasts in the human placenta during early stage of pregnancy.[15] By contrast, trophinin is not expressed in trophoblastic cells during and after implantation in the mouse.[16] Lack of trophinin has no obvious effect on mouse blastocyst implantation.[17]

Genes encoding CG-β are found only in primates.[36,37] Nucleotide sequences of CG-β genes show significant diversion among primate species, and human CG-β diverged further from those found in non-human primates.[37–39] Considering these characteristics of human CG-β and the present findings of the activity of hCG on human trophinin gene expression (Table 3), it is likely that trophinin function in embryo implantation is unique to humans. If this is the case, then the activity of hCG on trophinin gene expression may form the basis of ectopic pregnancy, a condition unique to humans. Recently, possible involvement of hCG in tubal pregnancy was suggested based on the finding of hCG expression by the decidualized endometrium.[40] Further studies are needed to determine the role of hCG on the pathogeneisis of tubal

pregnancy. Nonetheless, information obtained by studies on trophinin and CG-β will provide us with a clue for early detection and prevention of ectopic pregnancy.

Acknowledgments

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