Preimplantation factor promotes first trimester trophoblast invasion

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OBJECTIVE: Preimplantation factor is a novel embryo-derived peptide that influences key processes in early pregnancy implantation, including immunity, adhesion, remodeling, and apoptosis. Herein, we explore the effects of synthetic preimplantation factor on trophoblast invasion.

STUDY DESIGN: Invasion patterns of immortalized cultured HTR-8 trophoblast cells were analyzed through Matrigel extracellular matrix ± synthetic preimplantation factor (25-100 nM) in a transwell assay. Effects were compared with epidermal growth factor 10 μg/mL, scrambled aminoacid sequence of preimplantation factor, or media alone as controls.

RESULTS: Synthetic preimplantation factor enhances trophoblast invasion at physiologic doses (at 50 nM, 260%; 95% confidence interval [CI], 174–346%; $P = .05$; 100 nM ,178%; 95% CI, 170–184%; $P < .02$), compared with scrambled amnioacid sequence preimplantation factor or control media. Epidermal growth factor added to synthetic preimplantation factor does not further enhance trophoblast invasion (synthetic preimplantation factor 50 nM + epidermal growth factor, 238%; 95% CI, 237–239%; $P < .03$; synthetic preimplantation factor 100 nM + epidermal growth factor 269%; 95% CI, 265–273%; $P < .04$).

CONCLUSION: Preimplantation factor should be further investigated as it shows a potential preventative or therapeutic role for pregnancy complications associated with inadequate trophoblast invasion.

Key words: first trimester trophoblast, preimplantation factor, trophoblast invasion

Cite this article as: Duzyj CM, Barnea ER, Li M, et al. Preimplantation factor promotes first trimester trophoblast invasion. Am J Obstet Gynecol 2010;203:402.e1-4.

Embryo implantation into maternal endometrium and placental formation with trophoblast invasion require a complex interplay of embryo-derived cell signaling for establishing maternal immune receptivity.[1,2] This interplay was variously described over time by in vivo and in vitro models, and appears to involve initial polarization of the blastocyst, followed by cytokine signaling by IL-6, vascular endothelial growth factor (VEGF), and epidermal growth factor (EGF) among others.[3-6] From the maternal side, immune acceptance and endometrial remodeling is characterized by recruitment of immune cells including natural killer cells, T regulatory cells, dendritic cells and macrophages, as well as uterine stromal cell decidualization.[7] Implantation quality and depth of placental invasion has been positively correlated with overall pregnancy well being, and inversely correlated with adverse outcomes including intrauterine fetal growth restriction, preeclampsia and miscarriage.[4] Ideally, a compound that could promote or "rescue" placental invasion would represent a significant advance in reproductive technology.

We have identified a novel embryo-secreted peptide, preimplantation factor (PIF), (MVRIKPGSANKPSDD) secreted only by viable embryos, and absent in nonviable ones. PIF is detected early on and throughout pregnancy in the circulation of several species of pregnant mammals and is expressed in the placenta.[8,9] Synthetic PIF (sPIF), structurally identical to native PIF, replicates PIF action, modulates peripheral immune cells and creates a favorable immune environment shortly after fertilization. We recently reported that sPIF displays essential multitargeted effects promoting implantation.[10] In human decidual cultures, sPIF regulates immunity, promotes embryo-decidual adhesion, and controls adaptive apoptotic processes.

In this study, we explore the ability of sPIF to promote trophoblast invasion, a critical step in successful mammalian reproduction. We hypothesized that sPIF would exert a positive autocrine effect on trophoblast invasion in addition to its previously demonstrated paracrine effect on the maternal decidua, further supporting placental development. We

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Presented as a poster at the 30th Annual Meeting of the Society for Maternal-Fetal Medicine, Chicago, IL, Feb. 1-6, 2010.

Received Feb. 25, 2010; revised May 7, 2010; accepted June 24, 2010.

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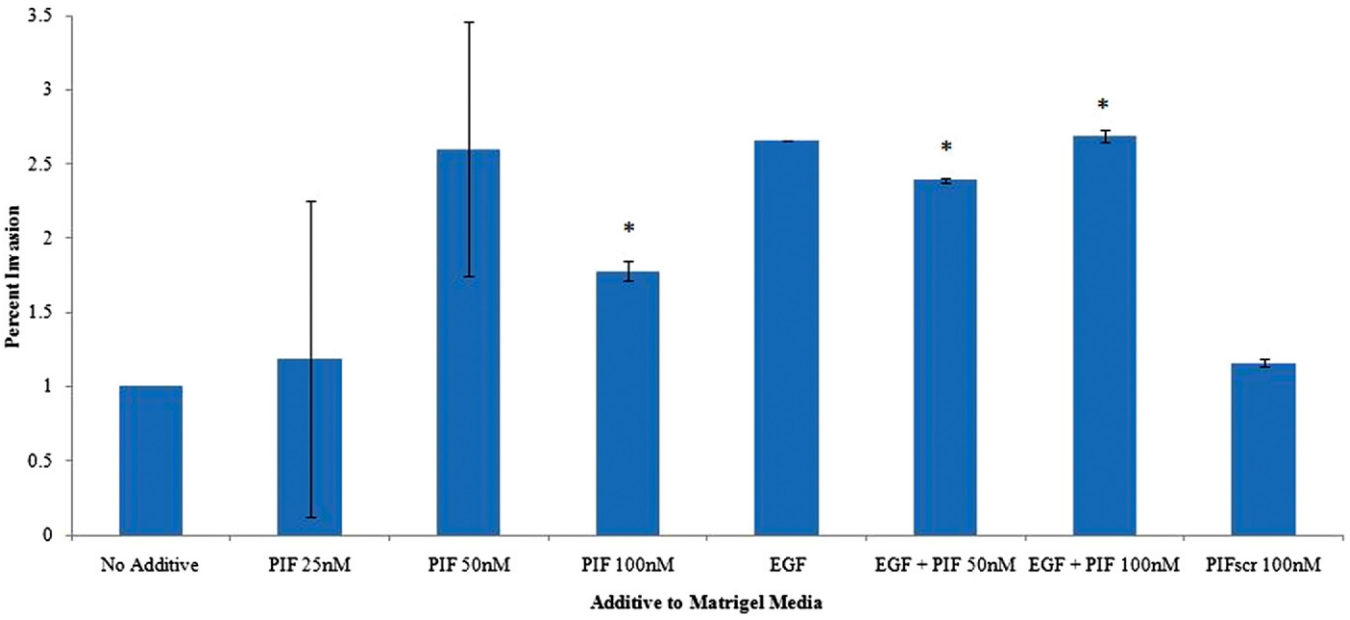
Research conducted at Yale University, New Haven, CT, supported by BioIncept, LLC (M.J.P.), and NIH Grant no. 5R01HD056123-02 (S.J.H.).

0002-9378/$36.00 • © 2010 Mosby, Inc. All rights reserved. • doi: 10.1016/j.ajog.2010.06.060

FIGURE
Trophoblast cells



Percent invasion of trophoblast cells through Matrigel extracellular matrix with associated media additive. Error bar not shown for epidermal growth factor given width of confidence interval would skew visibility of other results.

Duzyj. Preimplantation factor promotes trophoblast invasion. Am J Obstet Gynecol 2010.

planned to compare the effect on trophoblast invasion with EGF, another molecule that has been shown to promote implantation in the decidual and trophoblastic layers.[11,12]

Materials and Methods

Peptide synthesis

Synthetic PIF (MVRIKPGSANKPSDD) and scrambled PIF (PIFscr) (GRVDPSNKSMPKDIA) were produced using solid-phase peptide synthesis (Peptide Synthesizer; Applied Biosystems, Foster City, CA) employing Fmoc (9-fluorenylmethoxycarbonyl) chemistry. Final purification was carried out by reversed-phase high-pressure liquid chromatography (HPLC), and peptide identities were verified by mass spectrometry (Bio-Synthesis, Lewisville, TX).

Trophoblast in vitro transwell invasion assay

Immortalized first-trimester extravillous cytotrophoblast HTR-8/SVneo cells (kindly provided by Dr Charles Graham) were cultured on Matrigel (BD Biosciences, San Jose, CA) coated wells as previously described.[13,14] Trophoblast cells (2×10^5) were incubated for 24 hours with sPIF at 25-100 nM. These concentrations of sPIF were chosen because we found that PIF is present at 50-150 ng/mL (30-100 nM) concentrations in the circulation of pregnant women, and sPIF is effective at modulating several decidual cell functions in the same concentration range.[10] EGF at a concentration of 10 μg/mL, chosen due to its potent chemotactic effect at this dose in previous trials of EGF, was added to wells, including sPIF at all concentrations as a positive control.[11] Results were compared in parallel to exposure with PIFscr or to media alone (both used as negative controls). Cell culture inserts with porous membrane (8 um pore size, 6.5 mm diameter; BD Biosciences) were coated with Matrigel extracellular matrix as per the manufacturer's instructions for 1 hour. The porous inserts were cultured in a 24-well dish containing 600 μL RPMI 1640 complete medium for 24 hours at 37°C in 5% CO_2. The inserts were removed, washed with phosphate buffered saline solution, and the noninvading cells, as well as residual Matrigel were removed from the membranes by aspirating the media as well as using a cotton tip applicator. The membranes were treated with Colorimetric nuclear stain (Chemicon International, Billerica, MA) and washed several times. The membranes were excised from the transwells, and placed on a glass slide with the downside of the filter facing up. For quantification, the cells on the lower surface of the filter were counted under a microscope at $\times$40 magnification. Trophoblast invasion was analyzed from 3 independent replicates. Results were quantified as percent invasion of number of trophoblast through Matrigel in an individual media milieu relative to invasion of trophoblast through Matrigel alone without media additive in the same experiment set. Statistical analysis was performed comparing the means of 3 experiments using multiple paired 2-tailed t tests and analysis of variance (ANOVA), $P < .05$ was considered as statically significant. As no human tissues were used during these experiments, institutional review board approval was not obtained for this project.