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Angionesis and the inception of pregnancy

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Citation

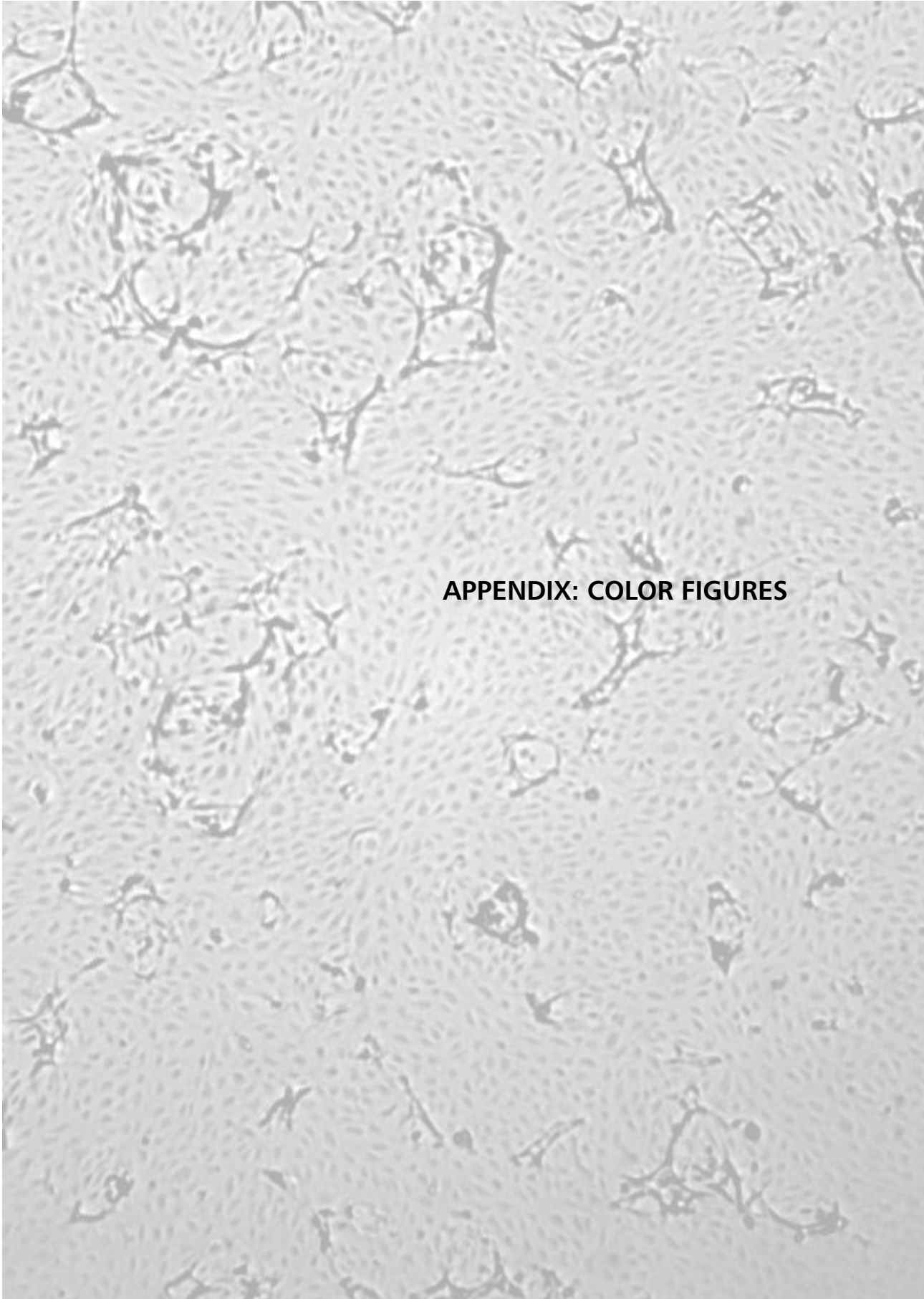
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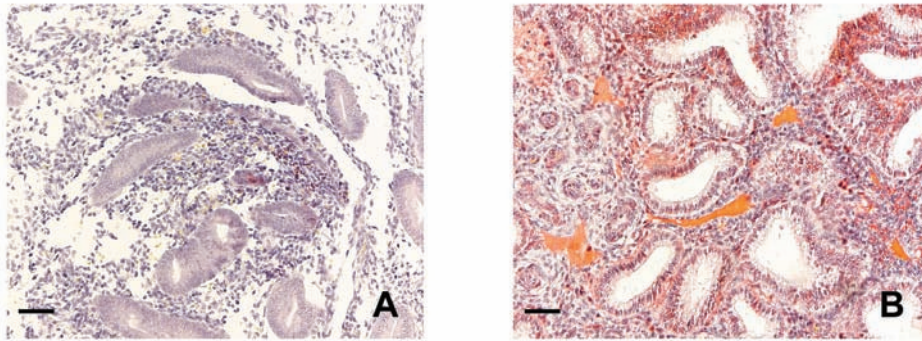
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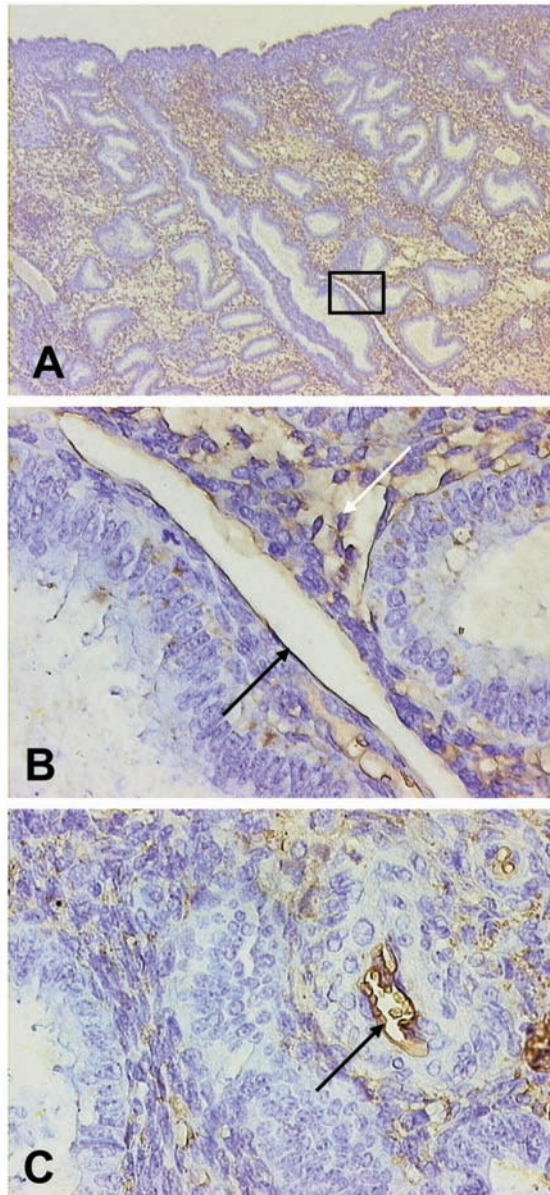
APPENDIX: COLOR FIGURES



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Figure 6. Fibrin staining in secretory endometrium.

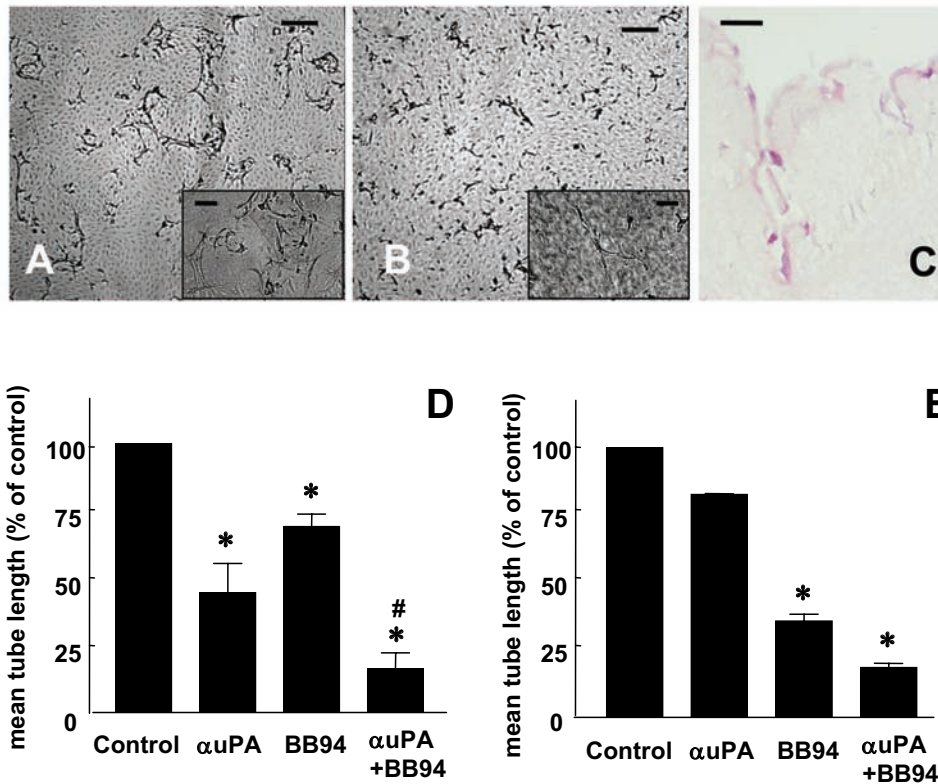
In vivo the ECM of the endometrium consists of a number of proteins, such as laminin, fibrin, collagen type I, II, IV and VI, fibronectin and heparan sulphate proteoglycan. The composition of the ECM varies during the cycle, as is shown here on paraffin sections of late-proliferative (A) and secretory (B) endometrium of two patients with a Martius Scarlet Blue staining. This staining stains fibrin red, collagen blue and erythrocytes yellow. During the secretory phase an increase of fibrin deposition is seen in the ECM of the endometrium. Bar = 100 µM.



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Figure 5. Expression of u-PA in blood vessels in human secretory phase endometrial tissue.

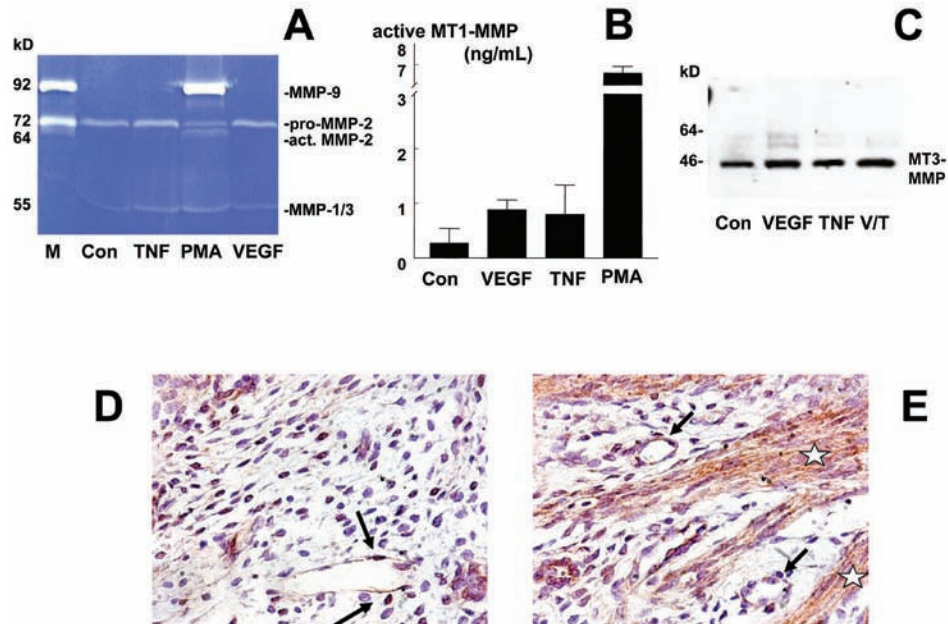
Immunohistochemistry was performed with labeled antibody to u-PA on paraffin sections of human endometrium, as described in *Materials and Methods*. A, Brown staining shows accumulation of u-PA in the stromal endometrium; the surface and glandular epithelium are negative for the u-PA antigen (20x magnification). The *rectangle area* is enlarged in B. B and C, *Black arrows* indicate examples of positive endothelial cells, and a *white arrowhead* indicate positive stromal cells (200x magnification). The same results were obtained using proliferative phase endometrium.



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Figure 1. Capillary-like tube formation by hEMVEC in a fibrin or collagen matrix depends on u-PA and MMP activities.

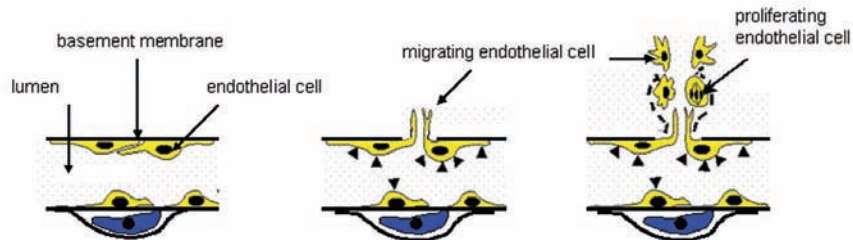
HEMVEC were cultured on top of a three-dimensional fibrin matrix (A,C,D) or 50-50% fibrin/collagen-type-1 matrix (B,E) and stimulated with VEGF-A (10 ng/mL). A and B: Micrographs taken after 4 days of culturing; insets in A and B show details of capillary-like structures. Bar = 300 μ m, Bar insets = 100 μ m. C: Cross section perpendicular to the matrix surface and stained with Hematoxylin-Phloxine-Safran (bar = 50 μ m). D and E: hEMVEC were cultured with 10 ng/mL VEGF-A (control) in the absence or presence of polyclonal anti-u-PA (α uPA, 100 μ g/mL), BB94 (5 μ g/mL) or a combination of BB94 and anti-u-PA. After 3-5 days of culturing, mean tube length was measured by image analysis. The data in panel D are expressed as a percentage of VEGF-A-induced tube formation \pm SEM of 6 independent experiments of duplicate wells performed with 3 different hEMVEC isolations. Panel E represents 3 experiments. *: $p < 0.05$ vs. control, #: $p < 0.05$ vs. α uPA.



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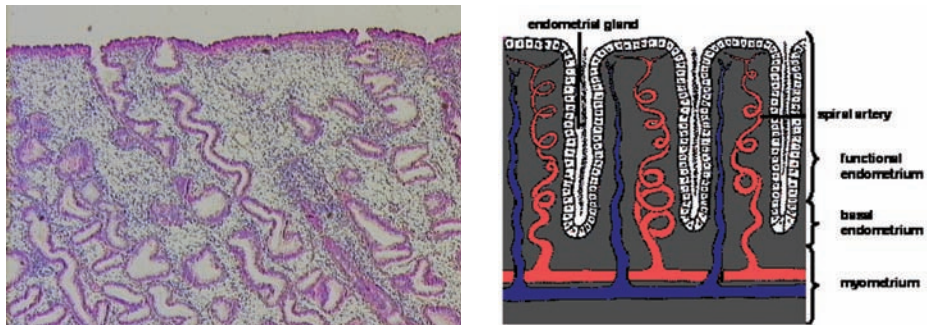
Figure 2. HEMVEC express various MMPs and MT-MMPs.

HEMVEC were cultured for 24 h in M199 supplemented with 0.5% HSA (A) or 20% HS (B,C) and were not stimulated (control) or stimulated with TNF α (2.5 ng/mL), VEGF-A (10 ng/mL) or PMA (10^{-8} M), as indicated. A: Gelatin zymography of 24 h conditioned medium. (M = ladder) B: MT1-MMP activity in cell lysates (mean \pm range of two experiments performed in duplicate wells with two different isolations; detection limit of the assay 0.2 ng/mL). C: Western blot of MT3-MMP in 24 h conditioned medium. D and E: Immunohistochemical analysis of MT3-MMP in endometrial tissue shows the presence of MT3-MMP in endothelial cells (D, *arrows*) and myometrium (E, *stars*). Similar results were obtained in the tissue of three other donors.



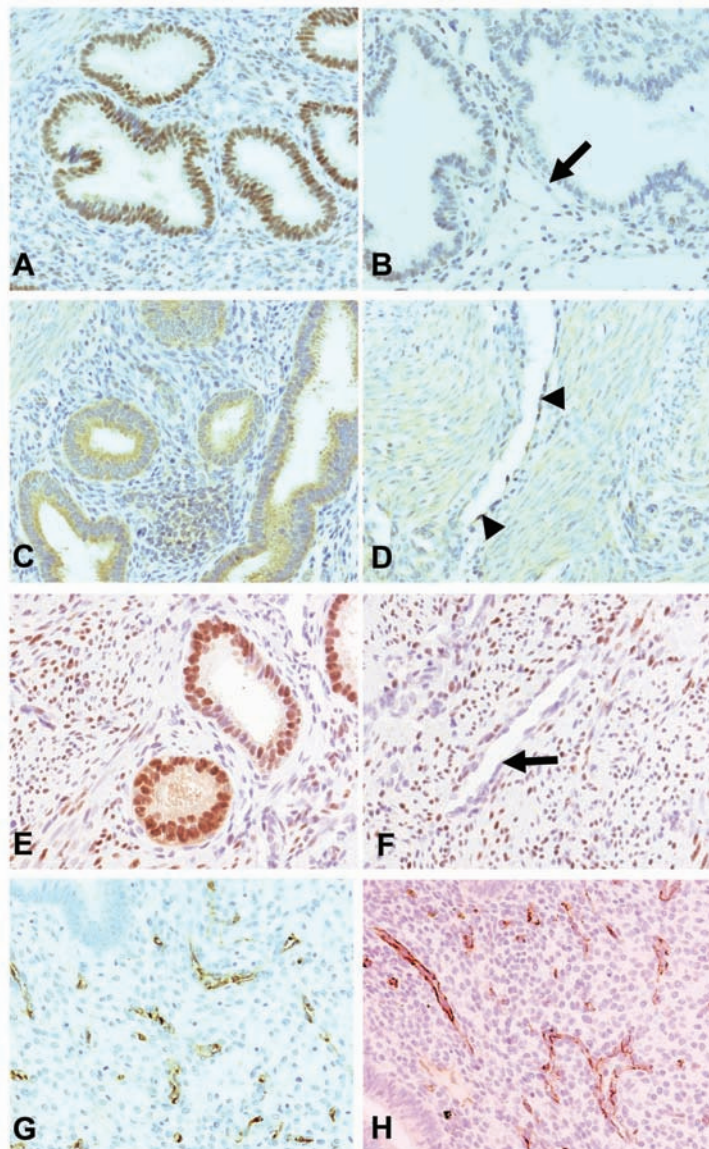
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Figure 1. In mature (non-growing) capillaries the vessel wall is composed of an endothelial cell lining and a basement membrane, in which pericytes (blue) usually are present. Angiogenic factors (▲) bind to endothelial cell receptors and initiate angiogenesis. When the endothelial cells are stimulated by angiogenic growth factors, they secrete proteolytic enzymes like metallo proteinases (MMPs) and enzymes of the plasminogen activator (PA) system, which degrade the basement membrane surrounding the vessel. The junctions between endothelial cells are loosened, the cells migrate through the space created, and the newly formed sprouts migrate and proliferate.]



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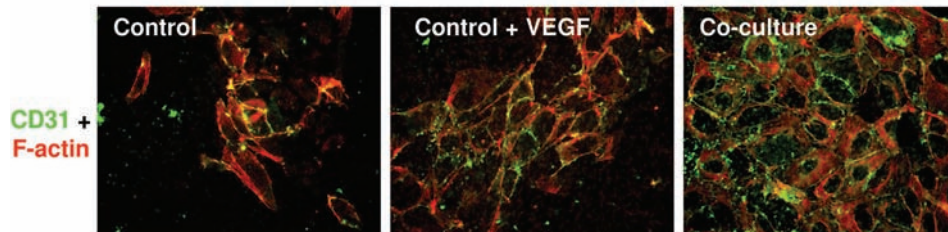
Figure 3. After menstruation from the basal endometrium a new functional endometrium grows. The basal arteries give rise to new blood vessels, which will form spiral arteries and a subepithelial capillary complex. Together with stromal and epithelial growth and differentiation, and increased vascular permeability, an edematous, thick receptive endometrium is prepared for implantation.



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Figure 1. Expression of ER α , ER β and PR in human endometrial tissue.

Immunohistochemistry was performed with labeled antibodies to ER α , ER β and PR on paraffin sections of human endometrium, as described in the methods section. Panel A and B; brown staining shows ER α in the epithelium and in the stromal compartment, the endothelium is negative for the ER α . C and D; endometrial stroma, epithelium and endothelium show positive staining for ER β . E and F; PR staining is seen in the epithelium and in the stroma, the endothelium stains negative for the PR. G and H; von Willebrand and CD31 staining were used to indicate the endothelial cells in the endometrium. Black arrow heads indicate an example of positive endothelial cells, and black arrows indicate negative endothelial cells.



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Figure 7. HESC and VEGF contribute to maintenance of hEMVEC monolayers.

Cultures of hEMVEC and hESC were detached and seeded on the surface of a filter (hEMVEC) or dish (hESC) of a transwell™ system (Costar). The next day the hEMVEC-covered filters were transferred into the wells in which hESC had been grown or wells without cells (control). To half of the control conditions VEGF-A (10 ng/mL) was added. HEMVEC were immunostained for CD 31 (green) and F-actin was visualized by rhodamine-falloidin (red). HEMVEC monolayers remained intact in co-culture, but showed holes in control cells. The addition of VEGF-A improved the quality of the monolayers. [See appendix: color figures]