Placenta (2006), **27**, 291–306
doi:10.1016/j.placenta.2005.02.014

Extracellular Matrix Components and Matrix Degrading Enzymes in the Feline Placenta during Gestation

I. Walter[a,*] **and S. Schönkypl**[b]

[a] Department of Pathobiology, Institute of Histology and Embryology, Veterinärplatz 1, 1210 Vienna, Austria; [b] Animal Shelter Arche Noah, 8041 Graz, Austria

Paper accepted 17 February 2005

In the endotheliochorial placenta of the cat, the maternal surface epithelium and parts of the connective tissue have to be removed to bring the fetal blood vessels in close contact to the maternal capillaries. The composition of the extracellular matrix (ECM) in the feline uterus is not known and it is still not clear if and which parts of the maternal ECM persist during gestation in the placental labyrinth. We demonstrated various extracellular matrix components (collagen types I, III, IV, and laminin) and matrix metalloproteinases (MMP-1, -2, -13) using immunohistochemistry and studied the distribution of intermediate filaments (vimentin, cytokeratin) and α-smooth muscle actin (SMA) in the placental girdle on specimens of different stages of gestation. Collagen types I and III were mainly present in the fetal chorionic lamellae whereas diminished in the maternal placental labyrinth part. Collagen IV and laminin were expressed in fetal basement membranes and mesenchyme. Maternal endothelial cells and stromal cells showed a positive immunoreaction for anti-collagen type IV and laminin. MMP-2 was identified in the maternal stroma, including decidual cells. Endothelia of maternal blood vessels within the labyrinth contained MMP-1, -2 and -13, probably associated with angiogenesis. In the trophoblast MMP-1 and -13 were demonstrated. Maternal stem vessels were accompanied by a thick layer of syncytiotrophoblast. Around these vessels, collagen type I and SMA were present in a periendothelial region between the endothelium and the trophoblast. These findings indicate that a strictly regulated balance between ECM deposition and ECM degradation in the feline placental labyrinth is necessary for proper placental development and function.

Placenta (2006), **27**, 291–306

Keywords: Cat; Placenta; Extracellular matrix proteins; Matrix metalloproteinases

INTRODUCTION

In the endotheliochorial placenta of the cat, a significant part of the maternal connective tissue has to be degraded by the invasive trophoblast to bring the fetal membranes and blood vessels in close contact to the maternal capillaries. Therefore, the placenta is a model for a strictly regulated process of progressive growth and invasion [1]. In the present investigation we studied the organisation and fate of the ECM components and ECM degrading enzymes (matrix metalloproteinases) during the progression of feline placentation. In the cat, the chorio-allantoic placenta forms a zonary girdle after implantation which separates two paraplacental cupules [2]. The placental girdle consists of a lamellar zone, characterized by elongated parallel fetal and maternal lamellae, a junctional zone, where fetal and maternal tissue face each other, and a zone of remaining endometrial glands [2,3]. In the paraplacenta, the uterine epithelium becomes highly proliferative. This hyperplastic surface and the adjacent connective tissue become symplasmic, degenerated and eroded, eventually resulting in maternal vascular breakdown [4]. In contrast to this haematomal zone, the invasion process of the fetal tissue is limited in the lamellar zone, where the maternal endothelium stays intact. However, it is not known for sure which parts of the maternal ECM proteins of the endometrium persist during fetal invasion. Leiser and Koob [2] stated that some maternal connective tissue cells, including enlarged decidua cells (giant cells) and a thickened basal lamina are persistent in the feline placenta. No studies specifically demonstrating ECM components in the feline endometrium are available. Therefore, we analyzed the composition and fate of ECM proteins in the feline placental labyrinth. To identify ECM proteins we labelled collagens (types I, III, IV) and laminin by fluorescence immunohistochemistry in specimens of first, second and third trimester of pregnancy. Antibodies for cytokeratin and vimentin were used to enable the

* Corresponding author. Tel.: +43 1 25077 3406; fax: +43 1 25077 3490.
E-mail address: ingrid.walter@vu-wien.ac.at (I. Walter).

0143–4004/$–see front matter

identification of fetal and maternal epithelial and stromal cells within the labyrinthine and junctional zone, respectively. An antibody against α-smooth muscle actin (SMA) was used to demonstrate blood vessels within the placental labyrinth and to identify stromal cells with contractile potential. A further aim of the present study was to determine the presence and distribution of matrix degrading enzymes which are supposed to play a pivotal role during implantation and placental development. Matrix metalloproteinases are enzymes capable of degrading gelatin, collagens, laminin, fibronectin and other matrix proteins [5]. They are produced in a latent form intracellularly and are then transported to the intercellular space. Here, they are activated through cleavage by other proteolytic enzymes losing a 10 kDa peptide upon activation. Distribution of MMP-1 (interstitial or fibroblast collagenase; degrading capability: collagens types I, II and III) MMP-2 (gelatinase A; degrading capability: gelatin), MMP-9 (gelatinase B; degrading capability: gelatin), and MMP-13 (collagenase 3, degrading capability: Collagen I, II, III, VI, gelatin, fibronectin, aggrecan) was examined by immunohistochemical methods.

MATERIAL AND METHODS

Histology

Specimens of cat placentae ($n = 24$) were obtained from patients that were presented by patient owners for ovariohysterectomy at the animal shelter, Graz. Animals were anaesthetized with Ketamin (Ketasol®) and Xylazin (Rompun®). Ovariohysterectomy was performed as abdominal surgery from the linea alba according to standard methods. Samples were taken from the placental girdle and immediately immersed in 4% buffered formaldehyde. After fixation, specimens were embedded in paraffin and serial sections (3 μm) were cut and mounted on TESPA (3-aminopropyldiethoxysilane, Sigma Chemicals, Vienna, Austria) coated slides. Specimens were classified within three stages of gestation by determining the age of the embryos (fetuses) by length and developmental stage according to Rüsse [6]: early gestation (<20 days post coitum, dpc, $n = 9$), midgestation (21–40 dpc, $n = 8$) and late gestation (>40 dpc, $n = 7$).

One section of each specimen was stained haematoxylin and eosin (H&E) for general histological examination.

Immunohistochemistry

Paraffin sections were rehydrated and peroxidase activity was removed by 0.3% H_2O_2 in methanol. After 5 min washing in tap water, slides were treated with 1.5% normal goat serum for 20 min at room temperature before incubation with the primary antibody overnight at 4 °C. Next day, the slides were washed in PBS and incubated with EnVision+™ (anti-rabbit or anti-mouse immunoglobulins conjugated to peroxidase labelled dextran polymer, DAKO, Glostrup, Denmark) for 30 min at room temperature, finally they were washed with PBS and peroxidase activity was detected with DAB substratum (10 mg 3′,3-diaminobenzidine in 50 ml 0.1 M Tris buffer, pH 7.4) and 0.03% H_2O_2 for 10 min at room temperature (RT). Thereafter, sections were counterstained with hemalum, dehydrated and mounted with DPX (Fluka, Buchs, Switzerland). Antibodies used and their final dilutions in PBS are given in Table 1. For collagen types I and III immunostaining, pretreatment with 0.1% pepsin (porcine, Sigma) in 0.5 M acetic acid for 2 h at 37 °C was necessary. In case of collagen type IV and laminin detection, protease (*Streptomyces griseus*, Sigma) pretreatment (0.1% in 0.05 M Tris buffer) was performed for 20 min at room temperature. Vimentin, cytokeratin, MMP-1 and MMP-13 immunohistochemistry needed microwave antigen retrieval (3×5 min boiling in citrate buffer, pH 6.0). Immunostaining for MMP-2 and MMP-9 was performed without pretreatment. In case of fluorescent stainings, anti-mouse alexa 488 (Molecular Probes, Eugene, OR, USA; dilution 1:100), anti-rabbit FITC (Vector Laboratories, Burlingame, CA, USA, dilution 1:50), or anti-goat FITC (Vector Laboratories, dilution 1:50) was used as secondary antibody, respectively. Slides were counterstained with propidium iodide (2 μl per 50 ml distilled water, 5 min RT), washed and were mounted with Mowiol 4-88 (Polysciences Inc., Warrington, PA, USA). Evaluation of fluorescent stainings was performed in a confocal laser scanning microscope (TCS-NT, Leica, Heidelberg, Germany). Control sections were treated with PBS instead of primary antibody or with unspecific mouse or rabbit IgG from normal serum applied in the same protein concentration as the primary antibody.

SDS-PAGE – immunoblotting

Immunoblotting was performed to verify cross-reactivity of the used MMP-antibodies with feline tissue. Four placental

Table 1. Primary antibodies used in immunohistochemistry

Antibody	Clone/ Cat No.	Type of antibody	Dilution	Source
Anti-collagen type I	1310-01	Polyclonal	1/30	Southern Biotech
Anti-collagen type III	031210301	Monoclonal	1/100	Quartett
Anti-collagen type IV	CIV22	Monoclonal	1/50	Quartett
Anti-laminin	Z0097	Polyclonal	1/500	Dako
Anti-cytokeratin	K8.13	Monoclonal	1/100	Sigma
Anti-vimentin	V9	Monoclonal	1/100	Dako
Anti-MMP-9	Ab9	Polyclonal	1/200	NeoMarkers
Anti-MMP-2	Ab4	Monoclonal	1/100	NeoMarkers
Anti-MMP-1	Ab6	Polyclonal	1/100	NeoMarkers
Anti-MMP-13	VIIIA2	Monoclonal	1/50	NeoMarkers
Anti-α-smooth muscle actin	1A4	Monoclonal	1/400	Dako