Placenta (2006), **27**, 758–770
doi:10.1016/j.placenta.2005.06.007

Fibroblast Growth Factor (FGF)-1, FGF2, FGF7 and FGF Receptors are Uniformly Expressed in Trophoblast Giant Cells During Restricted Trophoblast Invasion in Cows☆

C. Pfarrer[a,*], S. Weise[b], B. Berisha[c], D. Schams[c], R. Leiser[a], B. Hoffmann[b] and G. Schuler[b]

[a] Institute for Veterinary Anatomy, Histology and Embryology, Justus-Liebig-University, D-35392 Giessen, Germany; [b] Clinic for Obstetrics, Gynecology and Andrology of Large and Small Animals, Justus-Liebig-University, D-35392 Giessen, Germany; [c] Institute of Physiology, Technical University of Munich-Weihenstephan, D-85350 Freising, Germany

Paper accepted 16 June 2005

The bovine placenta is characterized by a limited invasion of trophoblast giant cells (TGC). In contrast to mononuclear trophoblast cells (MTC), TGC are non-polarized cells, which migrate and fuse with single uterine epithelial cells throughout gestation. Fibroblast growth factors (FGF) were shown to be associated with the migratory activity of cells, cell differentiation and angiogenesis, and due to its localization in trophoblast cells were proposed as important regulating factors in hemochorial placentae of rodents and humans, and the (syn)epitheliochorial placenta of pig and sheep. Since migrating bovine TGC are of epithelial origin, but exhibit similarities to mesenchymal cells we hypothesize that the restricted trophoblast invasion in cattle is characterized by a specific FGF expression pattern. Therefore, the spatiotemporal expression of specific FGF factor:receptor pairs, either acting on cells of mesenchymal origin or on epithelial cells was examined in bovine placental tissues throughout gestation and prepartum by immunohistochemistry, semiquantitative RT-PCR and in situ hybridization. FGF1 protein was found in trophoblast, caruncular epithelium (CE) and stroma (CS), stroma of chorionic villi (SCV), and in fetal and maternal blood vessels. FGF2 signals dominated in maternal vascular endothelia (VE), immature TGC, and MTC, whereas staining in other cell types was clearly weaker. FGF7 protein was detected in fetal and maternal blood vessel as well as in immature TGC and MTC predominantly at the chorionic plate. FGFR immunoreaction was localized in immature TGC, MTC, and to a clearly lesser extent in CS, CE and fetal and maternal blood vessels. Mature TGC stained negatively for all examined factors and FGFR. The corresponding mRNAs specific for FGF1, -2, -7, total FGFR, and FGFR2 isoforms IIIb and IIIc were colocalized in immature TGC, whereas hybridization was substantially lower in CE and absent in CS, SCV and mature TGC throughout gestation, but switched to CS and VE immediately prepartum. Semiquantitative RT-PCR revealed higher mRNA levels for FGF1, FGFR, and FGFR2IIIc in cotyledons compared to caruncles ($p < 0.05$), whereas it was the opposite with FGF2 ($p < 0.001$). FGF7 and FGFR2IIIb mRNA levels did not differ between caruncles and cotyledons. Significant changes ($p < 0.05$) of mRNA levels related to gestational age were found for FGF1 and FGFR2IIIc, but not for FGF2, -7, total FGFR, and FGFR2IIIb. The specific localization of all examined FGF family members in TGC suggests that TGC, apart from their classical function as producers of hormonal products, play other important roles in the regulation of bovine placentomal growth, differentiation and angiogenesis.
Placenta (2006), **27**, 758–770

Keywords: Placenta; Bovine; Fibroblast growth factors; Fibroblast growth factor receptors; Trophoblast giant cells

INTRODUCTION

Throughout its life span the placenta has to adapt itself to steadily changing demands of the growing fetus by permanent growth and differentiation. A specific phenomenon in synepitheliochorial placentomes of the cow is the continuous migration of trophoblast giant cells (TGC) into the maternal epithelium and the subsequent fusion with single caruncular epithelial cells [1]. This migration does not proceed beyond the maternal basement membrane, and, in contrast to placentation

☆ Supported by the German Research Foundation (DFG) grant SCHU 1195/1-1 and the Ewald und Hilde Berge-Stiftung.
* Corresponding author. Department of Obstetrics and Gynecology, Justus-Liebig-University Giessen, Klinikstr. 32, D-35392 Giessen, Germany. Tel.: +49 641 99 45282; fax: +49 641 99 45109.
E-mail address: christiane.pfarrer@gyn.med.uni-giessen.de (C. Pfarrer).

0143–4004/$–see front matter

in the sheep, generally results in the formation of trinucleated feto-maternal hybrid cells; therefore it is considered as restricted trophoblast invasion [2]. Up to date TGC have been associated with the production of hormones, like progesterone, prostacyclins, prostaglandins, and placental lactogen and pregnancy-specific (glyco)proteins [3–5]. However, the fact that TGC are of epithelial origin, but are not polarized and thus programmed differently led us to suspect that they may indeed have additional functions. The control of the limited invasion of TGC is still poorly understood, however, likely candidates to be involved may be FGF7 and FGF10, which are expressed by cells of mesenchymal origin [6] but specifically promote the proliferation of epithelial cells [7]. In sheep uterus and placenta, FGF7 and FGF10 mRNAs are localized in different tissue compartments of mesodermal origin, while the common receptor is found exclusively in epithelial cells, suggesting independent roles for both factors [8]. In contrast, during early pregnancy in the pig, FGF7 and its receptor are both expressed in the endometrial epithelia, and the receptor additionally occurs in conceptus trophectoderm suggesting paracrine epithelial–epithelial interactions [9].

The pregnancy-related expression of FGFs in the utero-placental unit has been suggested to be species-specific and was associated with the modulation of uterine–conceptus interactions [10]. However, the presence of a conceptus may be responsible for the differential control of FGF expression [10]. Proliferative activity and angiogenesis in the placenta and/or endometrium are attributed to the presence of FGF1 and/or FGF2 in the human, rat, mouse, pig and sheep, representing hemochorial and (syn)epitheliochorial placental types. Embryo implantation in mice seems to be supported by the action of FGF1 and/or FGF2 [11]. In pigs, pregnancy-specific expression of FGF1 and FGF2 in early gestation indicates roles for both factors during non-invasive embryo implantation of this species [10]. However, the localization of FGF1 and FGF2 in different tissue layers suggests that different ways of action are utilized [10]. In the synepitheliochorial placenta of the sheep, which resembles a slightly more invasive type than the pig, the expression of FGF2 is associated with angiogenesis and stimulation of proliferation and differentiation of trophoblast cells [12].

FGFs predominantly exert their actions through binding of low molecular weight isoforms to specific membrane receptors (FGFRs) which are subject to alternative splicing [7,13–15]. Binding studies revealed that FGF1 can activate all FGF receptor splice variants, while FGF2 preferentially activates the c splice forms, and FGF7 almost exclusively activates the b splice form of FGFR2 [13]. However, high molecular weight isoforms may be responsible for auto/intracrine signaling pathways [16].

In order to gain more information on the control of the restricted trophoblast invasion of the bovine placenta, and the function and programming of TGC, and consequently the impact on placentomal growth and angiogenesis we evaluated the expression of specific factor:receptor pairs (FGF1 and total FGFR, FGF2 and FGFR2IIIc, and FGF7 and FGFR2IIIb) in bovine placentomes from different stages of gestation and immediately prepartum on protein and mRNA level.

MATERIALS AND METHODS

Sample collection and fixation

For immunohistochemistry, placentomes from 150, 220, 240 and 270 days of pregnancy and from parturient cows forming five observational groups each consisting of three animals were collected, perfusion-fixed in 10% neutral phosphate buffered formalin and paraffin embedded. The three prepartal cows were defined by declining serum progesterone levels (<1.5 ng/ml) detected by automated chemiluminescence system (ACS180) using a PGRE kit (Bayer Vital GmbH, Fernwald, Germany). Serum levels were tested in 8-h intervals during the last 2 weeks of gestation and caesarean sections were done immediately after progesterone had declined when no signs of active labor were visible. The collection of samples from living animals was approved by the local authority (Regierungspräsidium Giessen, protocol II25.3-19c20/15cGI18/14).

In order to extend the study to mRNA level, additional placentomes from 52 generally healthy cows were collected at a slaughterhouse, perfusion-fixed with Bouins solution and paraffin embedded for in situ hybridization. In parallel, placentomes from the same cows were snap-frozen in liquid nitrogen for RT-PCR. The animals were assigned to the following groups: early gestation (≤ 3 months, $n = 6$ and 4th month, $n = 9$) and late gestation (5th month, $n = 5$; 6th month, $n = 6$; 7–8th month, $n = 5$, and >8 months, $n = 5$). Additionally, placentomes from eight cows were separated into maternal caruncle and fetal cotyledon in order to detect differences in the expression between fetal and maternal components of the placentome in early gestation (≤ 4 months, $n = 4$) versus late gestation (≥ 5 months, $n = 4$). The gestational age was assessed according to fetal crown-rump-length [17].

Immunohistochemistry

Antibodies (ABs) against FGF1 (rabbit-anti-bovine-FGF1 antiserum, rαbFGF1, catalog Nr. 06-101, UBI/Biomol, Hamburg, Germany), FGF2 (1. murine monoclonal anti-bovine-FGF2, clone bFM-2, catalog Nr. 05-118, UBI/Biomol; 2. rabbit-anti-bovine-FGF2, rαbFGF2) [18], FGF7 (rabbit anti-FGF7, Acris Antibodies, Hiddenhausen, Germany) and FGFR (monoclonal murine IgM, clone VBS1, recognizing FGFR1 [*flg* gene] and to a lesser extent FGFR2 [*bek* gene], Chemicon International, Hofheim, Germany) were used for indirect immunohistochemistry (ABC method, Vectastain-Universal-ELITE-ABC-Kit®, Vector Laboratories, Burlingame, CA, USA). As secondary ABs, biotinylated goat-anti-rabbit-IgG (BA-1000), horse-anti-mouse-IgG (BA-2000), and goat-anti-mouse-IgM (BA-2020, all Vector Laboratories) were used. Negative controls were set up by replacing rαbFGF1, -2 and -7 with heat inactivated normal rabbit