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mRNA and protein expression of FGF-1, FGF-2 and their receptors in the porcine umbilical cord during pregnancy

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Abstract: The fibroblast growth factors (FGFs) are multifunctional proteins that, among other roles, regulate structural reorganization of uterine and placental vascular bed during pregnancy. Thus, we analyzed mRNA and protein expression and immunohistochemical localization of FGF-1 and FGF-2, and their receptors (FGFR-1 and FGFR-2) in the developing umbilical cord (UC) on days 40, 60, 75 and 90 of pregnancy and after the physiological delivery in the pig (day 114). qPCR analysis demonstrated an increase in FGF-1 and FGF-2 mRNA levels beginning on day 75 and on day 114 of pregnancy, respectively. In addition, significantly increased FGFR-1IIIc mRNA expression was also found on day 114. On the other hand, no significant changes in FGFR-2IIIb mRNA expression were observed. Western Blot analysis revealed a decrease in FGF-1 and FGFR-2 protein expression after day 40. Beside an increased protein expression of FGF-2 on day 60, no significant changes in FGFR-1 protein expression were detected. Immunohistochemical staining enabled detection of FGF-FGFR system, with different intensity of immunoreaction in endothelial and tunica media cells of the umbilical vessels and in allantoic duct and amniotic epithelium as well as in myofibroblasts. In conclusion, our results show that members of FGF-FGFR system are expressed specifically in UC structures. Furthermore their day of pregnancy-related expression suggest that they may be an important players during UC formation and development.

Key words: FGF, FGFR, umbilical cord, pig, pregnancy

Introduction

The normal umbilical cord (UC) growth is an essential prerequisite for dynamic fetal development during pregnancy. Regular exchange of nutritional substances, respiratory gases and biologically active factors by the umbilical and placental vessels protect the fetus from intrauterine growth retardation and death. UC in several species contains two arteries, one vein and the allantoic duct, which are surrounded by specific embryonic tissue named Wharton's jelly. The whole UC is covered by the amniotic epithelium, which undergoes structural reorganization from single layer at the beginning to a multilayered structure at term [1]. Blood circulated in UC vessels do not only provide transport for nutritional substances and respiratory gases but also is an important source of stem cells which can be used to treat hematopoietic and genetic disorders [2]. The major limitation to a wider use of this source of stem cells is their relative low number after isolation from single unit of cord blood [3]. Collagen, elastin, hyaluronic acid and several sulphated proteolgycans are major extracellular components of Wharton's jelly, which contains low number of cells [4,5]. High amounts of these compounds in the Wharton's jelly have been suggested to be responsible for resistance after stretching or compression evoked by fetal movements and uterine contraction [1,6,7]. Formation of UC structures during early stage of pregnancy and their growth are correlated with size of the fetus [1,8]. Structural dysfunction related especially with the umbilical vessels and Wharton's jelly may result in a decrease in efficiency of the umbilical and placental circulation, which can affect fetus intra-uterine mortality and post-partum survival of piglets. Furthermore, intrapartum stillbirth accounts for 20% of all prenatal mortality in pigs and it is predominantly a result of fetal asphyxiation associated with prema-

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ture rupture of the UC [9-11]. Higher frequency of piglet asphyxiation is usually the consequence of UCs that are too short and too thin in comparison to the distance from the ovarian end of the uterine horn to the vulva, and fetus weight [8].

Mechanisms controlling UC development are still poorly known. However, formation and dynamic growth of UC seem to be controlled by the locally produced growth factors, among others, numerous and still expanding fibroblast growth factor (FGF) family and their receptors. FGFs represent multifunctional heparan-like glycosaminoglycans-binding mitogens that stimulate migration, differentiation and proliferation of cells of mesenchymal and neuroectodermal origin [12,13]. Among the 22 different FGFs family members, FGF-1 and FGF-2 with molecular weight of 18-24 kDa, are well characterized. The mitogenic and angiogenic effect of FGFs observed in target cells is the result of their interactions with receptors of four major known transmembrane tyrosine kinase receptors – FGFR1-4 [14,15]. Alternative splicing resulting in the formation of IIIb or IIIc isoforms of FGFR-1, -2 and -3 specifies the sequence of the Ig domain III [16,17]. The two alternative forms display different ligand-binding characteristic. It has been shown that FGFR-1IIIc isoform is expressed in cells of mesenchymal orgin (vascular endothelium), while FGFR-2IIIb is exclusively expressed in epithelial cells [18].

Participation of FGF-FGFR system in UC formation and development is possible given its stimulatory influence especially on placental vascular adaptation during pregnancy [19,20]. Furthermore, FGF-1 and FGF-2 probably regulate production of large amounts of collagen and glycosaminoglycans by miofibroblasts what affects on UC elasticity and degree of hydration [21,22]. Therefore, in the present study we have compared mRNA and protein expression levels of FGF-1 and FGF-2 as well as two respective receptors FGFR-1IIIc and FGFR-2IIIb and their immunohistochemical localization, in the porcine UCs on days 40, 60, 75, and 90 of pregnancy and on the day of parturition (day 114).

Materials and methods

The porcine umbilical cords. The UCs were obtained from 60 piglets (parents: Large White x Polish Landrace) classified into five groups according to gestation age: days 40 (n=12), 60 (n=12), 75 (n=12), 90 (n=12) of pregnancy and one postnatal group – 114, arbitrary day of pregnancy (n=12). The UCs from gilts in 40, 60, 75 and 90 day of pregnancy were obtained just after slaughter at a local slaughterhouse. However, the UCs from animals classified into postnatal group were collected directly from piglets during natural parturition. The gilts from this group were continuously monitored until the end of pregnancy. Additionally, the UCs from animals on days 60, 75, 90 of pregnancy and from postnatal group were divided into three parts: periplacental, middle and perifetal. The selected fragments of UCs for Western Blot and qPCR analyses were snap frozen in liquid nitrogen, before storage at -80°C. For immunohistochemical staining, the tissues were fixed in 4% paraformaldehyde in 0.1M phosphate buffer (PB, pH 7.4) for 4 h at 4°C, washed in 0.1M PB and stored in 30% sucrose for cryoprotection.

All animals were housed and used according to the animal care guidelines. All experimental procedures had been previously submitted to and approved by the Local Research Committee and conducted according to the national guidelines for agricultural animal care.

Total RNA isolation and reverse transcription. The frozen UCs placed in Lysing Matrix D tubs (MP Biomedicals Inc. Solon, OH, USA) with Fenozol (A&A Biotechnology, Gdańsk, Poland) were mechanically homogenized in FastPrep®-24 homogenizer (MP Biomedicals Inc. Solon, OH, USA). Next, total RNA was extracted from tissues homogenates by Total RNA Kit (A&A Biotechnology, Gdańsk, Poland) according to the manufacturer's protocol. The quality of total RNA was determined by both the ratio of A260/A280 and RNA gel electrophoresis.

Before the reverse transcription reaction, constant amount of 1μg of total RNA were treated with DNase I (Sigma Aldrich, Munich, Germany). The reverse transcription reaction was performed with using Reverse Transcription System kit (Promega, Madison, WI, USA) according to the manufacturer's protocol.

qPCR. Primers for qPCR were designed by Primer 3 software (http://frodo.wi.mit.edu/; FGF-1, FGF-2) and Primer Express 2.0 (Applied Biosystems, Foster City, CA, USA; FGFR-IIIc, FGFR-2IIIb) using cDNA sequences from Gene-Bank. Primers for β-actin were used according to the literature [23]. Forward and reverse primer sequences used to amplify mRNA for FGF-1, FGF-2, FGFR-1IIIc, FGFR-2IIIb and β-actin genes and expected product size were placed in Table 1. All qPCR analysis was performed with a ABI Prism 7300 sequence detection system using SYBR Green PCR master mix (Applied Biosystems). Serial dilutions of the appropriate cDNA were used as standard curves for gene quantification. The cycling conditions were as follows: initial denaturation 95°C for 10 min, followed by 40 amplification cycles at denaturation in 95°C for 15 s, annealing 59°C (for FGFR-1IIIc) and 60°C at 45 s (for FGF-1, FGF-2, FGFR-2IIIb and β-actin) followed by extension at 72°C for 45 s. After each PCR reaction, melting curves were obtained by stepwise increases in the temperature from 60 to 95°C to ensure single product amplification. The specificity of RT-PCR products was confirmed by gel electrophoresis and sequencing. Expression level for investigated factors was normalized to the expression of housekeeping gene *β-actin* to obtain arbitrary units of relative amount of the PCR product.

Western Blot. The cytosol and membrane fractions for Western Blot analysis were obtained from 60 UC segments (twelve per group). Tissue were placed in the homogenization buffer (50mM Tris-HCl, pH 7.4; 10mM EDTA, 150mM NaCl, 1% Triton X-100, 1μM pepstatin A, 5μg/ml leupeptin, 5μg/ml aprotinin, 100mM PMSF) and mechanically homogenized on ice. Homogenates were centrifuged at 2500 x g for 10 min at 4°C and the collected supernatant was centrifuged additionally at 105 000 × g for 1 h at 8°C in order to separate cytosol and membrane fractions, which were kept frozen at -80°C to future analyses. The protein level was spectrophotometrically determined by Bradford's method [24]. An equal amount (30μg) of cytosol (FGF-1, FGF-2, β-actin); and membrane (FGFR-1, FGFR-2) fractions were separated using SDS-PAGE gel and transferred onto a 0.2μm nitrocellulose membrane (Sigma Aldrich). After transfer, non-specific protein binding was blocked by incubation of nitrocellulose membranes with 5% non-fat dry milk in TBS-T buffer (50 mM Tris-HCl, pH 7.4; 150 mM NaCl, 0.1% Tween-20) for 1 h at room temperature. The membranes were then incubated with rabbit polyclonal antibody raised against FGF-1 (sc-7910; 1:400), FGF-2 (sc-79; 1:400), FGFR-1 (sc-121; 1:350), FGFR-2 (sc

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Table 1. Summary of qPCR primers sequences, products size and GeneBank Accession Numbers and/or references for investigated factors and receptors.

Genes	Primers (5'–3')	Product size (bp)	EMBL/references
FGF-1	F: GGCTGAAGGCGAAATCACAA R: TATACACCTCCCCCACGCTTT	209	X60317
FGF-2	F: GAAGAGCGACCCTCACATCAA R: CAGTGCCACATACCAACTGGA	219	AJ577089
FGFR-1IIIc	F: CGACAAAGAGATGGAGGTGCT R: CGCGTCCTCAAAGGAGACAT	52	AJ577088
FGFR-2IIIb	F: AGTCGCTAGAGTTGCGCTGC R: TTAGTCCAACTGATCACGGCG	54	NM001099924
β-actin	F: ACATCAAGGAGAAGCTCTGCTACG R: AGGGGCGATGATCTTGATCTTCA	366	U07786 [21]

-122; 1:350) or β-actin (sc-47778; 1:1500) used as a reference protein (Santa Cruz Biotechnology, Santa Cruz, CA, USA), in TBS-T buffer overnight at 4°C. After several washings with TBS-T the membranes were incubated for 1 h with goat anti-rabbit IgG biotin-labeled antibody in dilution 1/700 (FGF-1, FGF-2), 1/600 (FGFR-1, FGFR-2) and 1/3000 (β-actin), (Vector Laboratories Inc. Burlingame, CA, USA). Additionally, to test the specificity of the FGF-2 and FGFR-1 and FGFR-2 antisera, primary antibodies (1 μg) were incubated with specific blocking peptides (6 μg) (Santa Cruz Biotechnology) in TBS-T buffer for 2 h at room temperature with gentle shaking. Immunoreactive bands were detected using the avidin-biotin-peroxidase method (ABC Reagent, Vectastain® Kit, Vector Laboratories Inc.) in combination with 3,3'-diaminobenzidine (Sigma Aldrich). The intensity of the Western Blot bands were analysed with the Kodak 1D software v. 3.5 visual quantitative system (Eastman Kodak, Rochester, NY, USA).

Immunohistochemistry. Cryostat sections (10 μm) from different parts of UC (periplacental, middle and perifetal) were mounted on chromogelatin-precoated Superfrost plus® slides (Menzel-Galaser, Braunschweig, Germany). Endogenous peroxidase activity was blocked by incubating tissue sections in methanol with 3% H_2O_2 for 30 min at room temperature. Then tissue sections were incubated with 10% normal goat serum for 1 h at room temperature (NGS, Vector Laboratories Inc.) to reduce non-specific background staining. Afterwards, they were incubated overnight at room temperature with the same antibodies used for Western Blot diluted as follows: 1/200 FGF-1, FGF-2 and 1/150

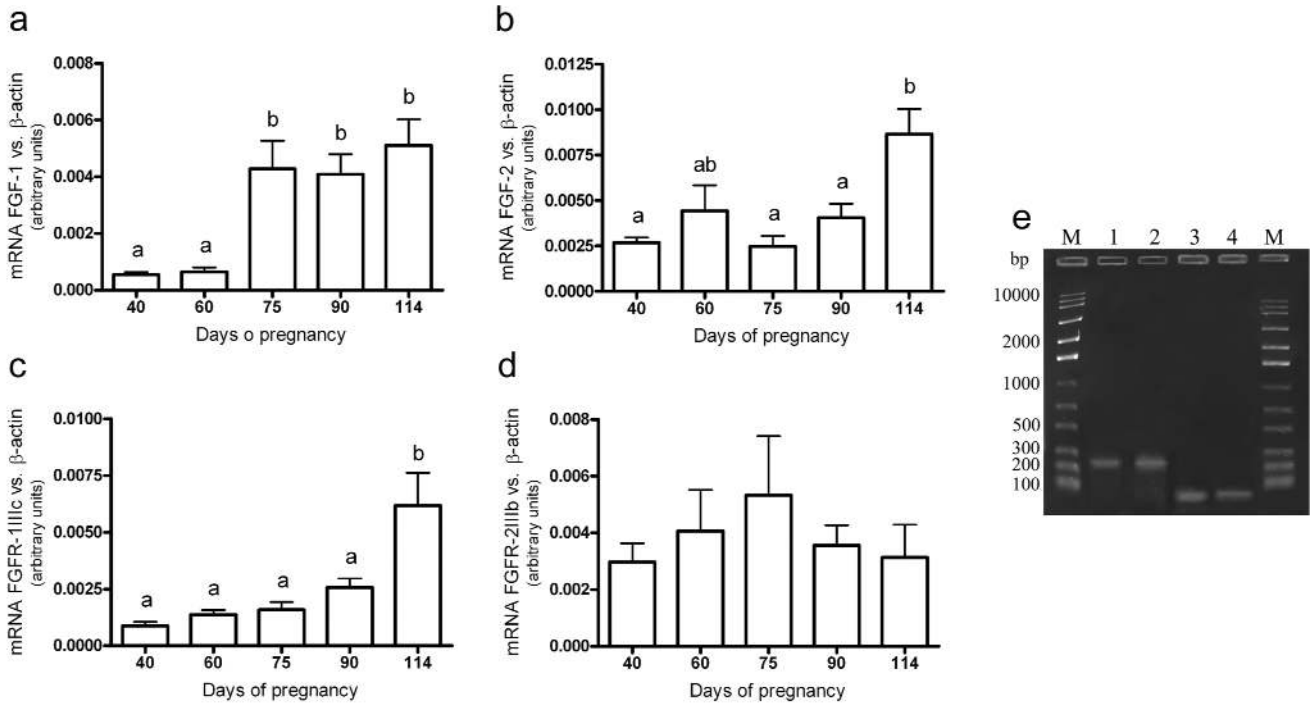

Fig. 1. qPCR analysis of mRNA FGF-1 (**a**), FGF-2 (**b**), FGFR-1IIIc (**c**) and FGFR-2IIIb (**d**) expression in porcine umbilical cord on days 40, 60, 75, 90 and 114 of pregnancy. Data are presented as mean ± SEM relative to b-actin gene expression (n=12). Different small letters indicate significant differences ($P < 0.05$). (**e**) Representative photography of the agarose electrophoresis gel of the qPCR amplification products for: line M – molecular mass standard, line 1 – FGF-1 (209 bp), line 2 – FGF-2 (219 bp), line 3 – FGFR-1IIIc (52 bp), line 4 – FGFR-2IIIb (54 bp) resolved by 2% agarose gel.

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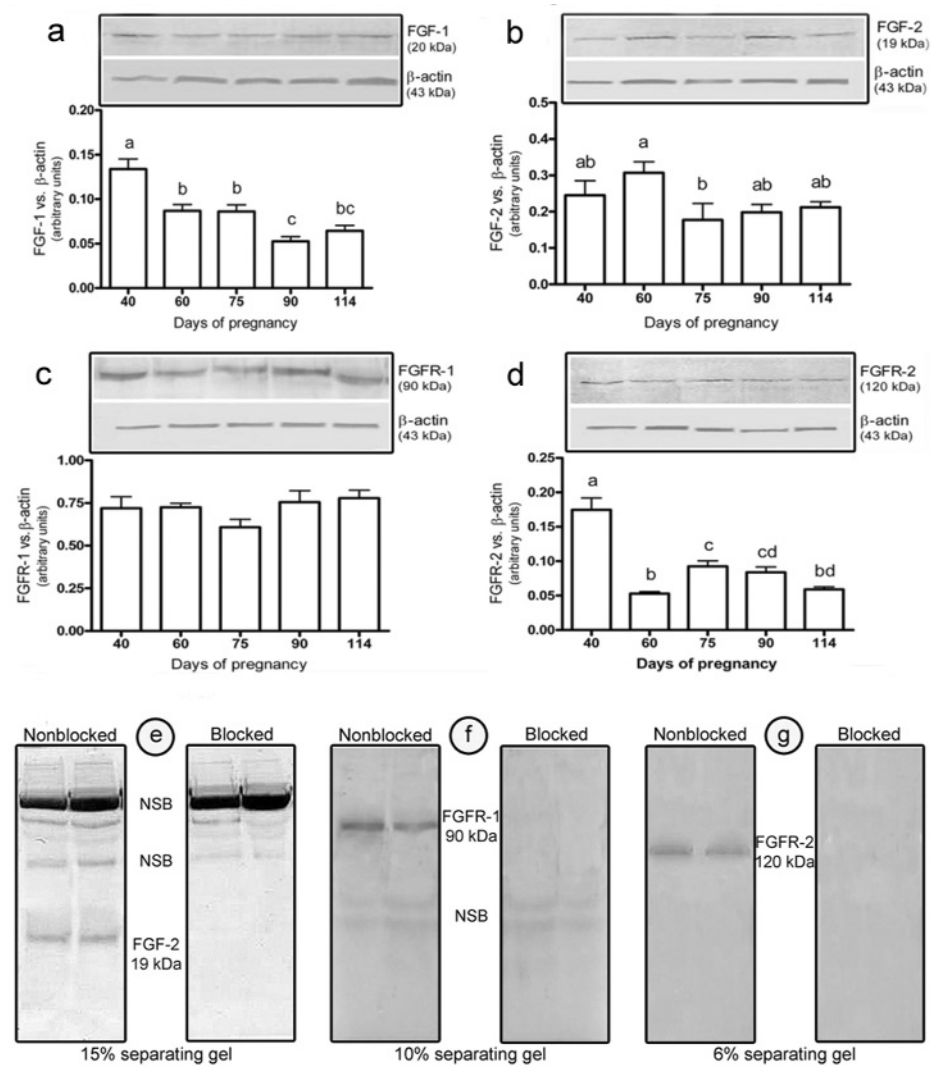

Fig. 2. (**a-d**) Western Blot analysis of FGF-1 (a), FGF-2 (b), FGFR-1 (c) and FGFR-2 (d) in the porcine umbilical cord on days 40, 60, 75, 90 and 114 of pregnancy. Upper panels: representative blots of tissue homogenates (30 µg) resolved by 15% (FGF-1, FGF-2) and 10% (FGFR-1), and 6% (FGFR-2) SDS-PAGE and immunoblotted with FGF, FGFR and β-actin as a reference protein antibody. Lower panels: results of densitometric quantification of bands (KODAK ID v. 3.5; Eastman). All values are the mean ± SEM of the protein level in the UC (n=12). Protein expression level for investigated factors and receptors were normalized to the reference protein β-actin. Different small letters indicate significant differences ($P < 0.05$). (**e-g**) Western blot analysis of FGF-2 (e), FGFR-1 (f) and FGFR-2 (g) antibody specificity in the umbilical cord tissue homogenates (30 µg) form day 75 and 90 of pregnancy in absence (nonblocked) or presence of blocking peptides (blocked). NSB – nonspecific binding.

FGFR-1, FGFR-2. Primary antibodies of FGFs and FGFRs were detected with goat anti-rabbit IgG biotin conjugate secondary antibody (Vector Laboratories Inc.) in dilution 1/500 for FGF and 1/400 for FGFR for 1 h at room temperature. Next, sections were rinsed in phosphate buffer saline (PBS) and incubated in strepavidin-biotin-peroxidase complex solution (ABC Reagent, Vectastain® Kit, Vector Laboratories Inc.). The reaction product was visualized using 3,3-diaminobenzidine (DAB, Sigma Aldrich). Two types of controls were performed to determine the specificity of immunohistochemical staining: (1) the primary antibody was omitted during the immunostainig procedure; (2) the primary antibody was substituted with nonspecific IgG. The sections were cover-slipped with DPX mounting medium (Park Scientific Ltd., Northampton, UK). Twelve sections from each group which contained all analyzed structures (umbilical vein, arteries, amniotic epithelium, allantoic duct and Wharton's jelly) were selected. Finally, the sections were photographed and visible color products of FGF-1, FGF-2, FGFR-1, and FGFR-2 immunoreactions were examined according to the standard 4 point scale for intensity: (-) negative, (+) week reaction, (++) strong reaction and (+++) very strong reaction [25].

Statistical analysis. The Real Time PCR and Western Blot numerical data are expressed as mean ±SEM. Significant differences were analyzed using one-way ANOVA followed by the Bonferroni multiple-comparison test (GraphPad PRISM v.4.0, GraphPad Software, Inc., San Diego, CA). The differences were considered to be significant at $p<0.05$.

Results

mRNA expression of FGF-1, FGF-2, FGFR-1IIIc and FGFR-2IIIb

Preliminary analysis of mRNA expression of examined members of FGF-FGFR system did not show significant differences between periplacental, middle and perifetal parts of UCs. Therefore, numerical data obtained from mentioned fragments of UCs were analyzed together. Gel electrophoresis of the qPCR amplification products demonstrated single transcripts for the two isoforms of FGF (FGF-1 – 209 bp, FGF-2 – 219 bp) and FGF receptors (FGFR-1IIIc – 52 bp, FGFR-2IIIb – 54 bp) (Fig. 1e). FGF-1 mRNA increased ($p<0.01$) from days 40 and 60 to day 75 of pregnancy, but did not change between days 75, 90 and 114 of pregnancy (Fig. 1a) whereas the abundance of

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FGF-2 mRNA was greatest ($p<0.05$) on days 60 and 114 of pregnancy (Fig. 1b). FGFR-1IIIc mRNA in UC was most abundance on day 114 ($p<0.01$; Fig. 1c) whereas there was no effect of day of pregnancy on values for FGFR-2IIIb (Fig. 1d).

Protein expression of FGF-1, FGF-2, FGFR-1 and FGFR-2

Protein expression of FGF-FGFR system members, similarly to mRNA expression did not show significant differences between periplacental, middle and perifetal parts of UCs. Numerical data obtained from mentioned above fragments of UCs were pooled and analyzed together. The FGF-1 protein was detected at approximately 20 kDa (Fig. 2a). The greatest expression of FGF-1 was on day 40 ($p<0.001$; 40 vs. 90; $p<0.01$; 40 vs. 60, 75 and 114). Furthermore, a decrease in FGF-1 protein was detected on day 90 as compared to days 60 and 75 of pregnancy ($p<0.01$). The antibody to FGF-2 detected a protein at approximately 19 kDa (Fig. 2e). Expression of FGF-2 increased only on day 60 as compared to day 75 of pregnancy ($p<0.05$) (Fig. 2b). The antibody FGFR-1 recognized a protein of 90 kDa as expected (Fig. 2f), but expression of this protein was no affected by day of pregnancy (Fig. 2c). The antibody to FGFR-2 detected a protein of 120 kDa, as expected (Fig. 2g) and this protein was most abundant on day 40 of pregnancy ($p<0.001$), but was also more abundant on day 75 and 90 than on days 60 and 114 (Fig. 2d).

Immunohistochemical localization of FGF-1, FGF-2, FGFR-1 and FGFR-2

Tissue sections of UCs stained for FGF-1 (Fig. 3a-h), FGF-2 (Fig. 3i-p) along with FGFR-1 (Fig. 4a-h) and FGFR-2 (Fig. 4i-p) demonstrated positive reaction in the endothelium and tunica media of the UC vein (Fig. 3/4a,e,i,m) and artery (Fig. 3/4b,f,j,n) in the amniotic epithelium (Fig. 3/4c,g,k,o) and allantoic duct (Fig. 3/4d,h,l,p), and in myofibroblasts of Wharton's jelly (Fig. 3/4c,g,k,o). No staining for studied factors and receptors was observed in negative control slides (data not shown).

Strong intensity of immunohistochemical reaction for FGF-1 was detected in the endothelium of umbilical vein and arteries, and in the amniotic epithelium, and allantoic duct (Table 2). On the other side, weaker but still strong reaction for FGF-1 was observed in tunica media of umbilical vessels and in myofibriblasts of Wharton's jelly. Immunoreactivity of the FGF-2 was very strong in all the analyzed structures for all the days of pregnancy observed (Table 2). Similar to the FGF-1, FGFR-1 immunoreaction was more intensive in the endothelium of vein and arteries as well as in amniotic epithelium and allantoic duct when compared with the tunica media of the umbilical vessels (Table 2). As for FGFR-2, the highest intensity of immunoreaction was observed in endothelium of umbilical vessels, in the amniotic epithelium, allantoic duct and in myofibroblasts of Wharton's jelly, too. Immunoreactivity for FGFR-2 in tunica media of vein and arteries was weaker in comparison with the above mentioned structures (Table 2).

Table 2. Schematic presentation of FGF-1, FGF-2, FGFR-1 and FGFR-2 immunoreactivity changes on day 40, 60, 75, 90 and 114 of pregnancy in structures of porcine umbilical cord. Immunostaining score: (-) negative, (+) weak reaction, (++) strong reaction, (+++) very strong reaction.

Factor/ receptor	Day	Umbilical cord structures						
		VE	AE	TMUV	TMUA	AE	ADE	M
FGF-1	40	+++	+++	++	++	+++	+++	++
	60	+++	+++	++	++	+++	+++	++
	75	+++	+++	++	++	+++	+++	++
	90	+++	+++	++	++	+++	+++	++
	114	+++	+++	++	++	+++	+++	++
FGF-2	40	+++	+++	+++	+++	+++	+++	+++
	60	+++	+++	+++	+++	+++	+++	+++
	75	+++	+++	+++	+++	+++	+++	+++
	90	+++	+++	+++	+++	+++	+++	+++
	114	+++	+++	+++	+++	+++	+++	+++
FGFR-1	40	+++	+++	++	++	+++	+++	++
	60	+++	+++	++	++	+++	+++	++
	75	+++	+++	++	++	+++	+++	++
	90	+++	+++	++	++	+++	+++	++
	114	+++	+++	++	++	+++	+++	++
FGFR-2	40	+++	+++	++	++	+++	+++	+++
	60	+++	+++	++	++	+++	+++	+++
	75	+++	+++	++	++	+++	+++	+++
	90	+++	+++	++	++	+++	+++	+++
	114	+++	+++	++	++	+++	+++	+++

VE – venous endothelium, AE – arterial endothelium, TMUV – tunica media of umbilical vein, TMUA – tunica media of umbilical artery, AE – amniotic epithelium, ADE – allantoic duct epithelium, M – myofibroblasts.

Discussion

Results of this study are the first to describe changes in expression of FGF-1, FGF-2, FGFR-1IIIc and FGFR-2IIIb with respect to cellular localization of mRNAs and proteins in tissues of the UC of the pigs during pregnancy and on the day of parturition. Earlier reports indicated expression of FGFs and FGFRs in various cells of human and ovine placenta [19,20,26,27] which suggest that they may act as important regulators of UC function, especially functions related to blood circulation in the UC. High level of FGF-1 and FGFR-1 protein expression on day 40 of pregnancy and modest increase in expres-

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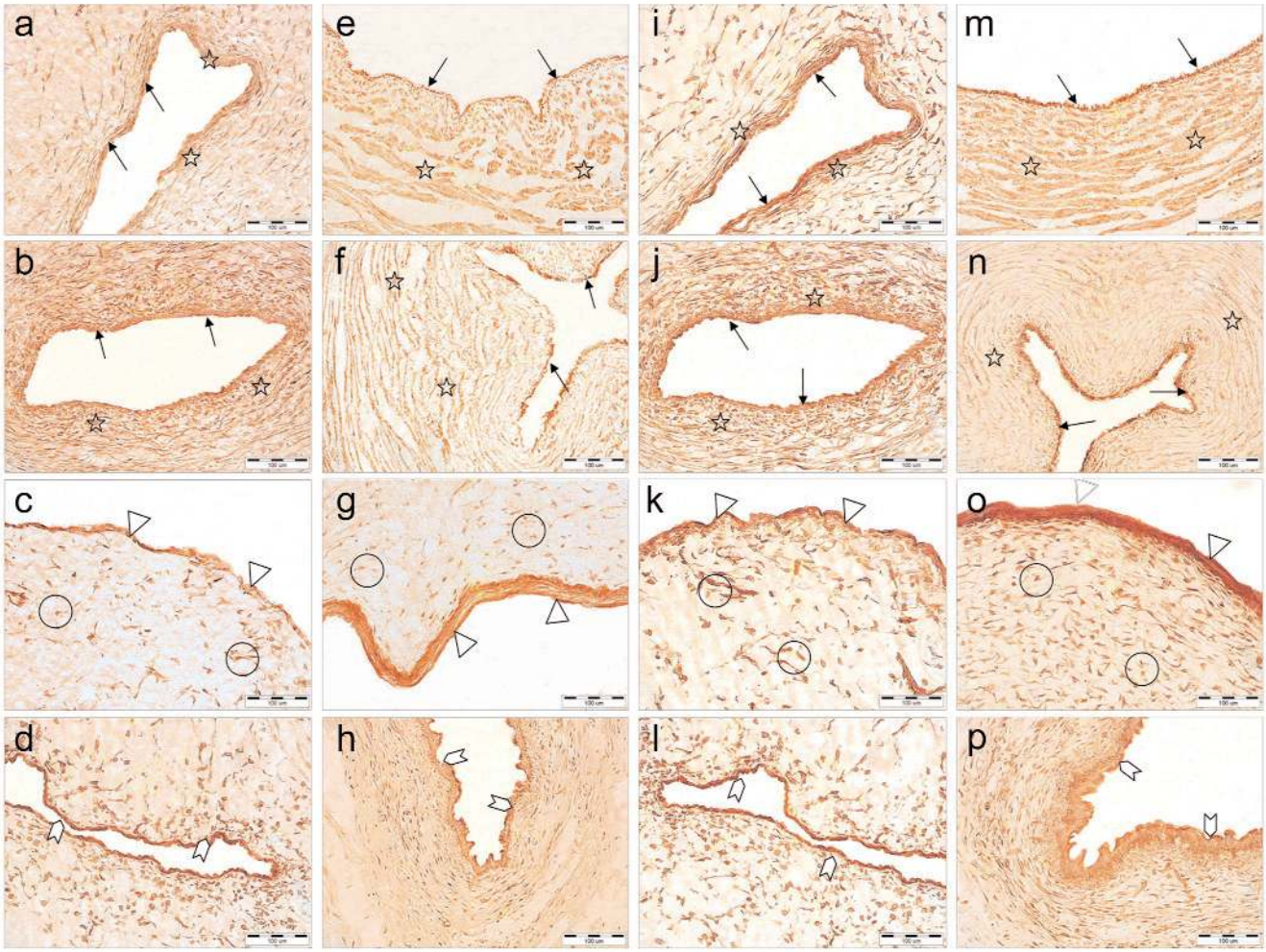

Fig. 3. Immunohistochemical localization of FGF-1 (**a-h**) and FGF-2 (**i-p**) in structures of the porcine umbilical cord (UC) on days 40 (a-d/i-l) and 114 of pregnancy (e-h/m-p). Positive staining for FGF-1 as well as FGF-2 was observed in the endothelium (arrows) and muscular layer (asterisks) of the UC vein (a, e, i, m) and artery (b, f, j, n), in the amniotic epithelium (arrowheads; c, g, k, o), allantoic duct (triangle frames; d, h, l, p) and myofibroblasts of Wharton's jelly (circles; c, g, k, o).

sion of FGF2 suggest that they play important role in UC formation. On the other hand, higher expression of FGF-1, FGF-2 and FGFRIIIc on mRNA level within second half of pregnancy or only on day 114 suggests that we should also observe increase of protein expression for these factors and receptor. Given standard models wherein protein level should be correlate with mRNA expression the discrepancy between mRNA and protein expression occurring in our study disturbs the results. However, protein and RNA represent different steps of the multi-stepped cellular genetic information flow process, in witch they are dynamically produced and degraded. Wang [28] based partially on results of Le Roch *et al*. [29], Griffin *et al*. [30] and Tian *et al*. [31] described four scenarios of differential changes in mRNA and protein levels: steady RNA, lower protein; steady RNA, higher protein; lower RNA, steady protein; higher RNA, steady protein. Based on these findings and given that there is no similar study, the discrepancy between protein and mRNA abundance which we have observed is most likely a result of the biology of gene expression rather than the measurement errors. Because posttranscriptional, translational, or post-translational regulations are not well understood further studies are needed in order to clarify the molecular mechanisms underlying FGF-FGFR system expression during umbilical cord development.

Besides low number of myofibroblasts, Wharton's jelly produces and accumulates significant amounts of different growth factors (FGF-2, EGF, TGF-β, PDGF, IGF-I) in comparison with the umbilical arteries. Concentration among the mentioned structures of the umbilical cord was not different for FGF-1 [21]. The high amounts of growth factors may strongly stimulate cells of Wharton's jelly to biosynthesize collagen, hyaluronate and sulphated proteoglycans [32-34]. Previous study of Sobolewski and coworkers [4] showed that Wharton's jelly contains about four times more collagen and twice as much glycosaminoglycan com-

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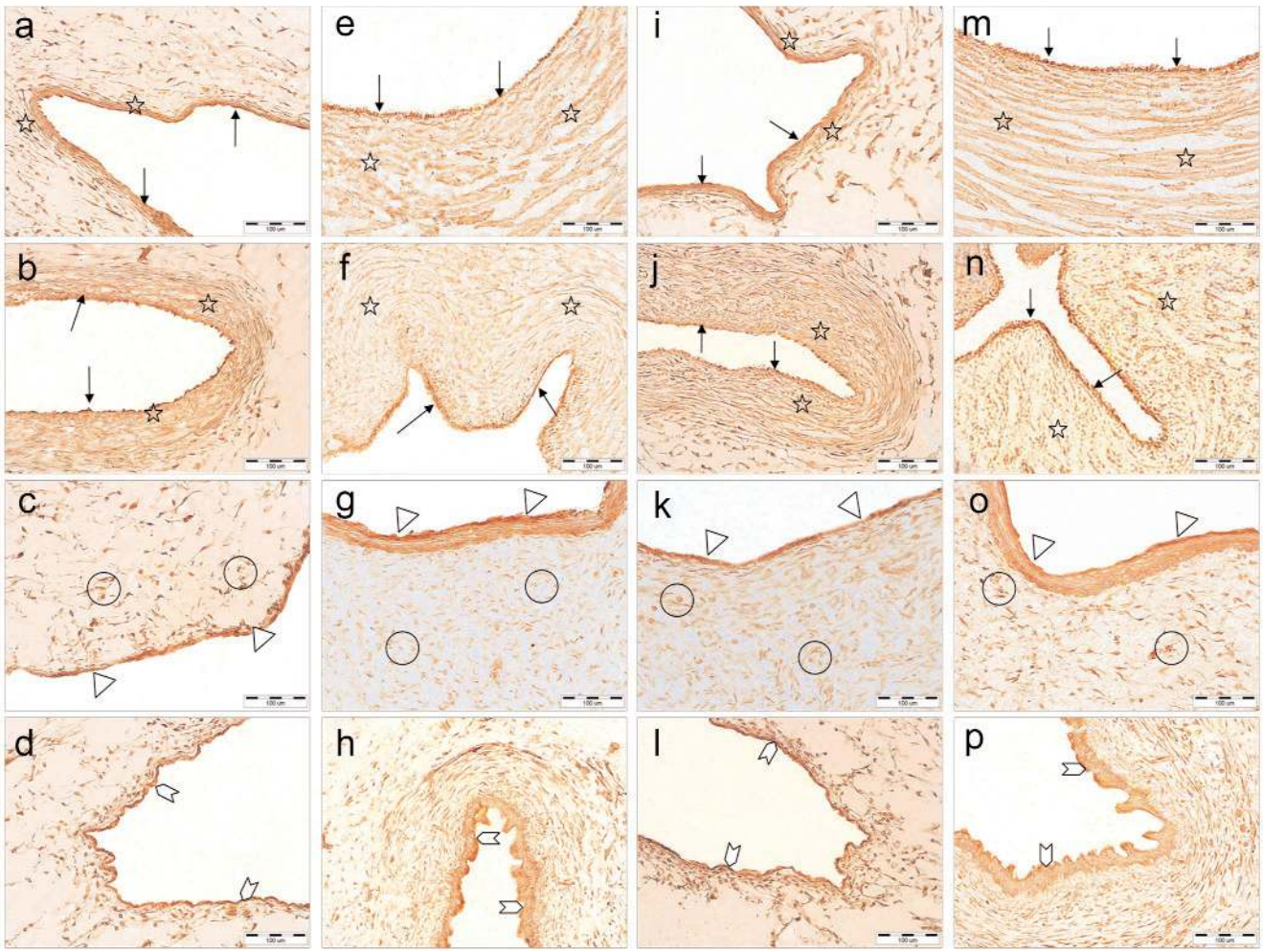

Fig. 4. Immunohistochemical localization of FGFR-1 (**a-h**) and FGFR-2 (**i-p**) in structures of the porcine umbilical cord (UC) on days 40 (a-d/i-l) and 114 of pregnancy (e-h/m-p). Positive staining for FGFR-1 as well as FGFR-2 was observed in the endothelium (arrows) and muscular layer (asterisks) of the UC vein (a e i, m) and artery (b, f, j, n), in the amniotic epithelium (arrowheads; c, g, k, o), allantoic duct (triangle frames; d, h, l, p) and myofibroblasts of Wharton's jelly (circles; c, g, k, o).

pared to the umbilical cord artery wall. The major role of FGF-1 as well as FGF-2 in umbilical cord could be the stimulation of myofibroblasts and smooth muscle cells to produce extracellular matrix components [33]. Increases in concentration of extracellular matrix components of Wharton's jelly might influence mechanical properties of the UC (resistance to blood flow, elasticity, degree of hydration) that prevent occlusion and rupture of umbilical vessels [21,35].

The umbilical vessels are major structures of UC affecting fetus growth. The hemodynamic changes in feto-placental circulation during pregnancy require structural adaptation of the umbilical vessels [36, 37]. Expression of FGF (FGF-1 and FGF-2) and FGFR-1/FGFR-1IIIc in umbilical vessels is probably associated with structural and functional regulation of endothelial and smooth muscle cells. Earlier studies have proven that mostly FGF-1/-2, through activation FGFR-1, induces proliferation of the endothelial cell and migration in different type of vessels [38,39]. In addition, FGF-1 and FGF-2 represent effective mitogens for smooth muscle cells. Growth of vessels wall is mainly controlled by TGF-β and PDGF [40]. However, Millette and coworkers [41] presented a novel mechanism by which PDGF-BB induces the release of FGF-2 and activation of FGFR-1 followed by the sustained activation of ERK and proliferation of human smooth muscle cells. Moreover, action of FGF-1 and FGF-2 in regulation of the umbilical vessels growth can be also related with the stimulation of endothelial cells for the production of different types of metalloproteinases (MMP-1, MMP-3), which are involved in the breakdown of extracellular matrix during tissue remodeling [42,43]. According to Murakami and coworkers [44], FGFs signaling plays a key role in the maintenance of vascular integrity. Contrary to VEGF-VEGFR system, the inhibition of FGF-FGFR system activity leads to dissociation of the VE-cadherin/p120-catenin complex and dis-

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assembly of adherens tight junctions. Increase in the VE-cadherin/p120-catenin binding, through FGF-1 and FGF-2 – FGFR signaling, induces the decrease in vessels permeability [44,45].

Strong immunoreactivity especially for FGF-1 and FGFR-2, observed in the amniotic epithelium and allantoic duct, as well as expression of FGFR-2IIIb mRNA transcript suggests their role in structural reorganization and function of these structures of UC. Similar to its role in regulation of vascular integrity, the FGF-1-FGFR-2IIIb system might be a key player for maintenance of epithelial cell morphology and permeability. The amniotic epithelium and allantoic duct epithelium of umbilical cord during the pig pregnancy is transformed from a single layer (epithelium simplex cuboideum) at the beginning to a multilayered structure (stratified squamous epithelium and multilayer epithelium with superficial cells, respectively) at term [1]. FGF-1, similar to the FGF-7 (keratinocyte growth factor) may be a potent regulator, which modulates the proliferation, differentiation and stratification of umbilical cord epitheliums by FGFR-2IIIb [46]. In turn, the effect of FGF-FGFR system on UC epithelium permeability, similar to the endothelial barrier, appears to participate in regulation of adherence junction formation determined by interactions between epithelial-type of cadherin (E-cadherin), p120-catenin and β-catenin [47,48].

Cell shape, orientation and alignment, as well as cytoskeleton reorganization, in vivo and in vitro, can be regulated by blood flow, blood pressure and fetus movements which affect the UC tension [49]. Even though the molecular signaling of mechano-transduction is not clearly understood, it is known that endothelial and smooth muscle cells undergoing long-term stretching produce not only different growth factors (TGF-β, PDGF, FGF) but also structural and functional proteins, such as collagen, elastin and integrins [50].

In conclusion, our results demonstrate that both FGF-1 and FGF-2 as well as FGFR-1 and FGFR-2 are biosynthesized by UC structures. Furthermore, FGF-1-FGFR-2 system supported by FGF-2 may be an important stimulator and/or regulator of UC formation. On account of discrepancy between mRNA and protein abundance additional studies will be needed, however, to clarify the molecular mechanisms underlying FGF-FGFR system expression during UC development.

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