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IMMUNOHISTOCHEMICAL DISTRIBUTION OF IGF-1, bFGF AND THEIR RECEPTORS IN DECIDUAL, EMBRYONIC AND TUBAL HUMAN PREGNANCY TISSUE

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ABSTRACT

Embryo implantation is a complicated process involving mother and *conceptus* cells differentiation, proliferation and invasion that are essential for successful pregnancy. Almost every cell in the human body is affected by IGF-1 that is one of the most potent natural activators of cell growth and multiplication, and also a potent inhibitor of the programmed cell death. bFGF, acting via its receptor FGFR1, is one of the factors involved in mediating the angiogenesis, proteolysis and apoptosis during the implantation. OBJECTIVE: To establish pregnancy inducted difference of appearance of bFGF, IGF-1 and their receptors in the embryonic, decidual and tubal tissue. STUDY DESIGN: In this study 14 tubal pregnancy and 10 decidual tissue samples were evaluated immunohistochemically in order to define the distribution of bFGF, FGFR1, IGF-1, IGF-1R. RESULTS: The Mann-Whitney U test was used as appropriate for the evaluation of significant differences. FGFR1 appearance dominated on bFGF in the decidual ($z=2.539$, $p=0.01$), tubal ($z=2.539$, $p=0.01$) and embryonic ($z=2.539$, $p=0.01$) tissue. IGF-1 and IGF-1R appearance in the decidual, tubal and embryonic tissue was not statistically different. It was the same as IGF-1 and IGF-1R expression in gravid endometrium, but in the ectopic implantation site IGF-1R was particularly absent ($z=1.935$, $p=0.05$), only mesothelium and some epithelial cells stained. CONCLUSION: IGF-1, IGF-1R, bFGF and FGFR1 are widely appearing growth factors in actively developing and

differentiating of the human embryonic tissue during the first trimester. Both endometrial and fallopian tube tissues express more FGFR1 than bFGF that testify the stimulation of compensatory adaptation of the organ during pregnancy. IGF-1 and IGF-1R richly appear in gravid endometrium. IGF-1 is widely distributed in both the mother and the embryo tissues but only some of them are IGF-1R marked in a case of ectopic pregnancy. The deficit of IGF-1R in the fallopian tube might be a result of cell growth restriction and the impaired process of trophoblast invasion.

Key words: *growth factors, implantation, tubal pregnancy, immunohistochemistry.*

INTRODUCTION

Embryo implantation is a complicated process involving mother and *conceptus* cells differentiation, proliferation and invasion that are essential for successful pregnancy. Only 25 to 30% of conceptions result in a live birth [22]. A significant proportion of pregnancy loss is caused by embryo chromosomal disorders, so more than 50% of the first trimester spontaneous aborts are aneuploid [6, 13, 18]. Embryo implantation failure due to impaired uterine receptivity took place in up to 30% of early pregnancy loss [7]. However, 1–2% of embryo implantations in the world are ectopic [9, 27]. The research of the abnormally implanted embryo had shown no karyotype changes [8, 10], so molecular signalling at the time of blastocyst nidation could probably be the key to the explanation of normal and abnormal implantation [1].

The insulin like growth factor (IGF-1) and the basic fibroblast growth factor (bFGF) are found during pre-implantation and implantation in uterus [3] in addition, these factors and their receptors regulate cellular events of the early embryonic period. As growth factors orchestrate cell growth, differentiation and proliferation during embryogenesis, the aim of our study was to define the appearance of IGF-1, bFGF and their receptors in uterine pregnancy, tubal pregnancy and embryonic tissues.

MATERIAL AND METHODS

The study was performed with the permission of the Ethics Committee of Riga Stradins University (18.12.2007). Human oviduct parts were obtained from 14 patients of Riga 1st Hospital, who had undergone salpingoectomy for tubal pregnancy with informed consent. The human embryo and the gravid endometrium tissue were obtained from 10 patients who had unplanned pregnancy termination in the Riga Medical Centre "Elite" with informed consent. Tissue samples were taken from January 2007 to January 2008. Age, parity, the contraception method, pelvic inflammatory and sexually transmitted diseases episodes, the partner count had been carried out for all the patients.

The tissue samples were fixed in 2% formaldehyde and 0.2% picric acid mixture with 0.1 M phosphate buffer (ph 7.2). Then the samples were rinsed in the thyroid buffer containing 10% sucrose and embedded in paraffin. The tissues were cut into 6-μm-thick sections and were dewaxed with toluene and rehydrated through a graded ethanol series. The sections were stained with haematoxylin and eosin (H&E) using standard procedures to obtain a review picture of the slide.

We used the biotin–streptavidin method for the determination of the basic fibroblast growth factor (FGF basic rabbit polyclonal to bFGF (ab16828), dilution 1:200, *Abcam,* UK); the fibroblast growth factor receptor 1 (FGFR1 rabbit polyclonal to FGFR1 (ab10646), dilution 1:100, *Abcam* UK); insulin-like growth factor 1 (IGF-I goat polyclonal to IGF-1 (MAB291), dilution 1:100, *R&D systems*, Germany); the insulin-like growth factor 1 receptor (IGF-IR mouse monoclonal to IGF-1R (AF-305-NA), dilution 1:100, *RnD Systems*, Germany.

At least five microscopic fields (X200) were analyzed using the microscope Leica DM RB (Leica Microsystems, Germany).

The distribution of these factors was detected semi-quantitatively (0/– occasional positive structure in the visual field, + few positive structure in the visual field, ++ moderate number of positive structure in the visual field, +++ numerous positive structure in the visual field. The data were analyzed by the nonparametric rank analysis with SPSS Statistic 17 software. A Mann-Whitney U test was used as appropriate for the evaluation of significant differences. A p-value <0.05 was considered as statistically significant.

RESULTS

The average patient's age in the tubal pregnancy group was 29.6 years (23–43). This pregnancy was the first for 3 of them (25%). n=6 patients (50%) had a previously documented pelvic inflammatory disease (PID) episode and n=1 had undergone right sided salpingectomy due to the previous ectopic pregnancy. Only one of them used the intrauterine device (IUD) for contraception. The number of partners and the legal abortion count were not significantly different. The average patient's age in the uterine pregnancy termination group was 30.9 years (23–43). This pregnancy was current for all the patients. None of the patients had any documented PID episode or tubal surgery. None of the patients used contraception.

Routine haematoxylin and eosin slides showed tubal mucosal edema. It was typical to see the proliferation of epitheliocytes, the infiltration of lymphocytes and leukocytes as well as capillary stasis. Only the embryonic structure found in any case of tubal pregnancy were chorionic villi binding to the fallopian tube structures or to germ membranes (yolk sac as well). No specific finding, despite decidual endometrium and trophoblast tissue, was seen in the routine uterine pregnancy slides.

The immunohistochemical analysis of tubal and pregnant endometrium tissues resulted in the different appearance of the recurred growth factors (Figure 1).

IGF-1 was widely distributed in the fallopian tube epithelium (Figure 2) but IGF-1R focally stained the apical surfaces of tubal epitheliocytes (Figure3). Despite, epithelium IGR-1R stained only mesothelium and was absent in chorionic structures. Cytotrophoblast, sincytiotrophoblast and extraembryonic mesenchymal cells contained IGF-1 (Figure 4). Peripheral trophoblast focally contained IGF-1 positive cells. IGF-1 as well stained macrophages and neutrophils. Endometrium showed numerous IGF-1R cells, but IGF appeared in epithelium moderately. Trophoblasts contained moderate numbers of IGF-1 cells in a case of uterine pregnancy (Figure 5). Peripheral trophoblast widely expressed both IGF-1 and IGF-1R. Separate connective tissue cells in endometrium demonstrated IGF-1 and IGF-1R immunoreactivity. IGF-1 was widely distributed in both mother and embryo tissues but IGF-1R only in some of them. IGF-1 and IGF-1R appeared in embryonic respiratory, duodenal and mesonephric epithelia (Figure 6, 7). Embryonic *hepar*

contained focally a moderate number of IGF-1 and IGF-1R positive cells. Mesenchymal cells around the future skeleton and *perichondrium* moderatly demonstrated IGF-1 (Figure 8) and IGF-1R positive cells.

FGFR1 appeared both in tubal and decidual tissue. Numerous positive structures were seen in tubal and endometrial epithelia (Figure 9). *Citolemmae* of muscle cells, endotheliocytes, nerve fibers and peripheral trophoblast cells demonstrated immunoreactivity for FGFR1 in both implantation sites. A moderate number of FGFR1 positive structures in extraembrional mesenchyma, citotrophoblast and syncitiotrophoblast appeared (Figure 10). Tubal and endometrial epithelium contained moderately bFGF (Figure 11). A few bFGF positive cells were seen in tubal and endometrial stroma (Figure 12)

The numerous distribution of FGFR1 had been established in sklerogenic mesenchyma, perichondrium, in the proliferating cartilage area and in degenerating *chorda dorsalis* of human embryo. The largest part of the muscle, nerve fibers's plasmolemmae and endotheliocytes contained FGFR1. FGFR1 immunoreactivity concentrated in the spinal ganglions. The epitheliums of skin and its appendages tongue and salivary glands have been positive for FGFR1. The mesothelium of pleura and pericardium has contained FGFR1. Myocardium has been weakly positive FGFR1. bFGF have been shown the same distribution in embryonic tissues as FGFR1 but moderately.

The Mann-Whitney U test was used as appropriate for the evaluation of significant differences. FGFR1 appearance dominated on bFGF in the decidual ($z=2.539$, $p=0.01$), the tubal ($z=2.539$, $p=0.01$) and the embryonic ($z=2.539$, $p=0.01$ tissue. IGF-1 and IGF-1R appearance in the decidual, the tubal and the embryonic tissue was not statistically different. It was the same as IGF-1 and IGF-1R expression in gravid endometrium, but in the ectopic implantation site IGF-1R was particularly absent ($z=1.935$, $p=0.05$), stained only mesothelium and some epithelial cells.

Table 1. The distribution of bFGF, FGFR1, IGF-1 and IGF-1R in tissue

Structure/ Factor	***FGFR***	***bFGF***	***IGF1R***	***IGF-1***	***FGFR***	***bFGF***	***IGF1R***	***IGF-1***
	Tubal pregnancy tissue				Uterine pregnancy tissue			
Epithelium	+++	+	+	+++	+++	++	+++	+++
Myocytes	+++	+	0/–	0/–	+++	+	0/–	0/–
Endo-theliocytes	++	+	0/–	0/–	++	+	0/	0/–
Nerve fibers	++	+	0/–	0/–	++	+	0/	0/–
Mesothelium	++	+	++	++	none	none	none	none
Extra-embryonic mesenchyma	+++	0/–	0/–	0/–	+++	0/–	0/	0/–
Cytotro-phoblast	++	0/–	0/–	+++	++	0/–	0/	+
Sincytiotro-phoblasts	+++	0/–	0/–	+++	++	0/–	0/	++
Peripheral trophoblast	+	+	0/–	++	+	+	+++	++
Macrophages	0/–	+	0/–	+	+	+	++	+
Neutrophils	0/–	+	0/–	+	+	+	++	+

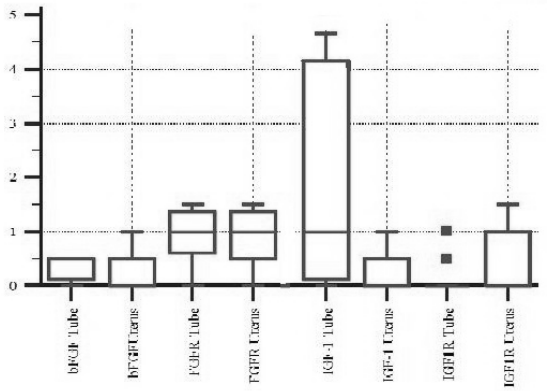

Figure 1. Immunohistochemical distribution of bFGF, FGFR1, IGF-1, IGF-1R in tubal and pregnant endometrium tissues.

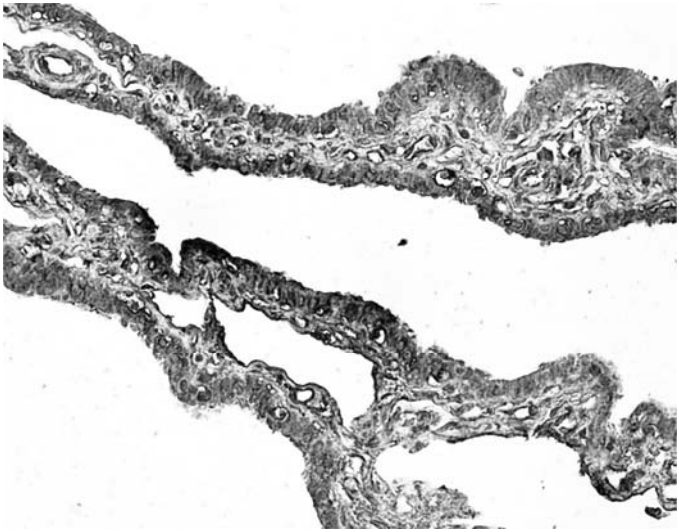

Figure 2. IGF-1 was widely distributed in fallopian tube epithelium. IMH IGF-1 250X.

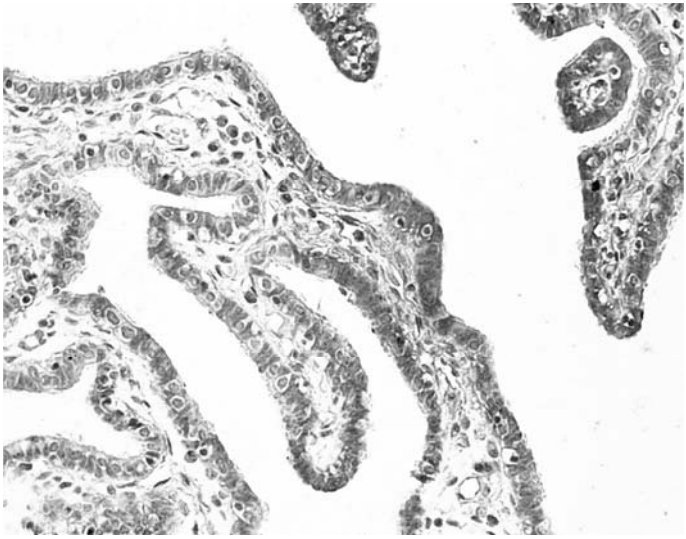

Figure 3. IGF-1R focally stained the apical surfaces of tubal epitheliocytes. IMH IGF-1R 250X.

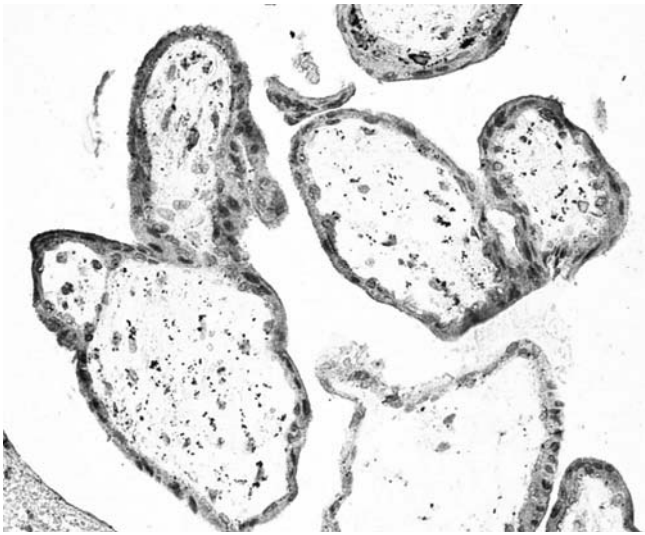

Figure 4. Cytotrophoblast, sincytiotrophoblast and extraembryonic mesenchymal cells moderately contained IGF-1. IMH IGF-1 400X.

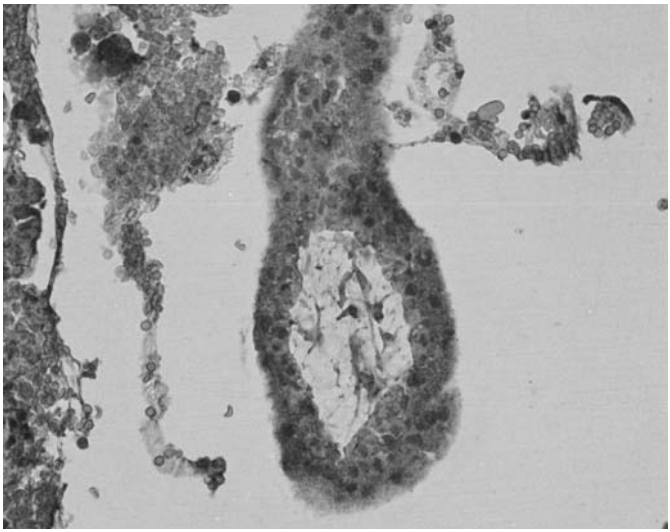

Figure 5. Trophoblasts contained moderate numbers of IGF-1 cells in a case of uterine pregnancy. IMH IGF-1 400X.

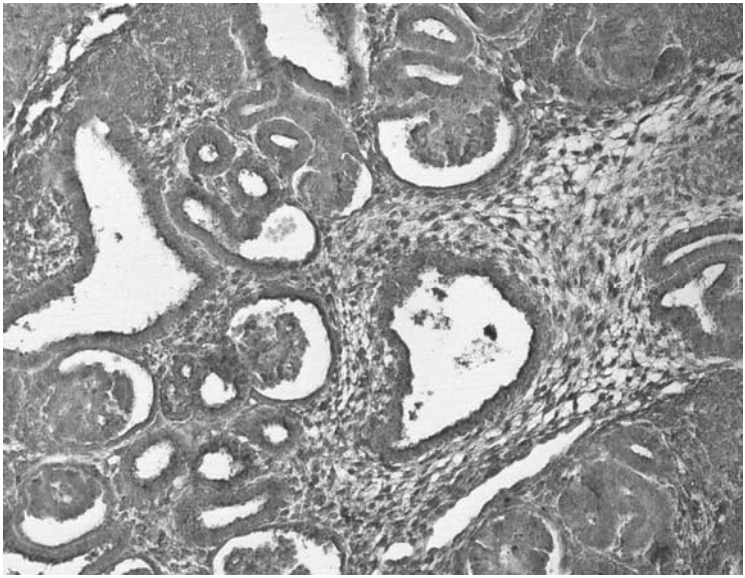

Figure 6. IGF-1 in embryonic kidney. IMH IGF-1 400X.

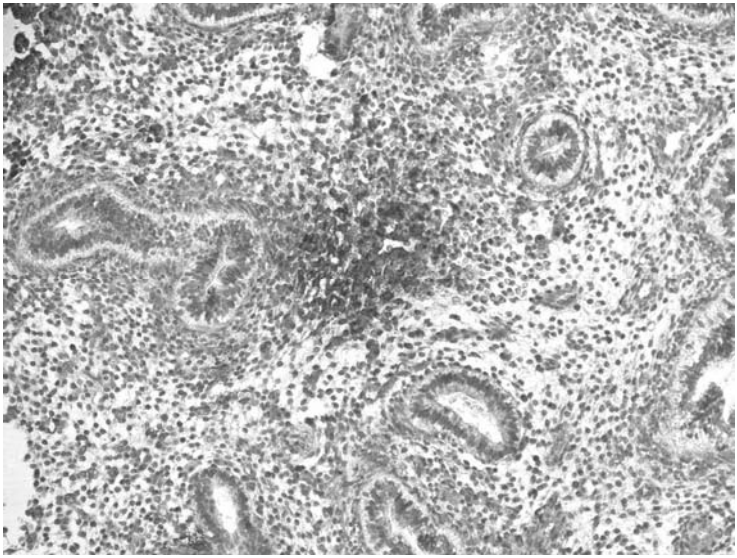

Figure 7. IGF-1 in embryonic lungs. IMH IGF-1 400X.

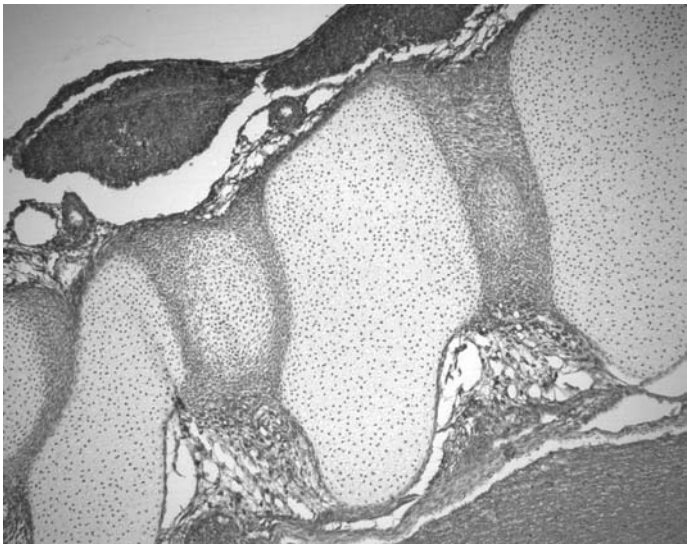

Figure 8. Mesenchymal cells around the future skeleton and *perichondrium* moderatly demonstrated IGF-1. IMH IGF-1 400X.

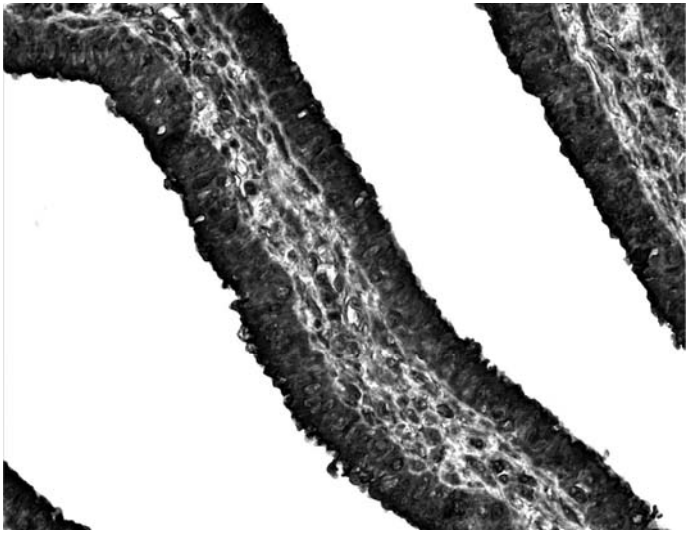

Figure 9. Numerous FGFR-1 positive structures were seen in tubal epithelium. FGFR1 IMH 400X.

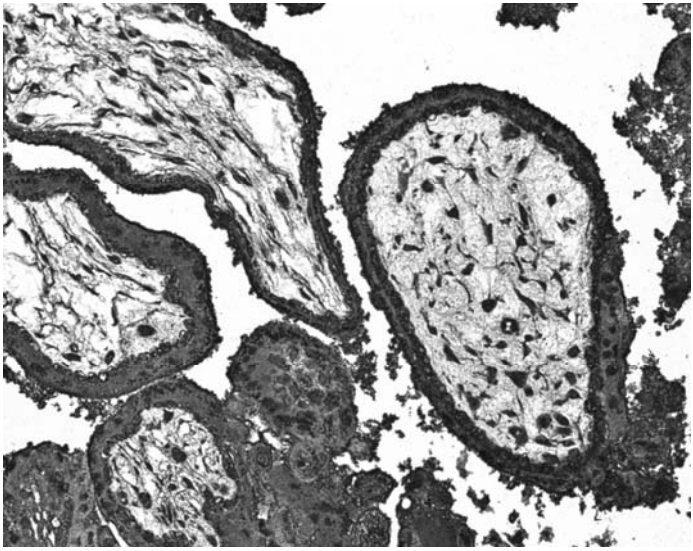

Figure 10. FGFR1 in extraembrional mesenchyma, citotrophoblast and syncitiotrophoblast. FGFR1 IMH 400X.

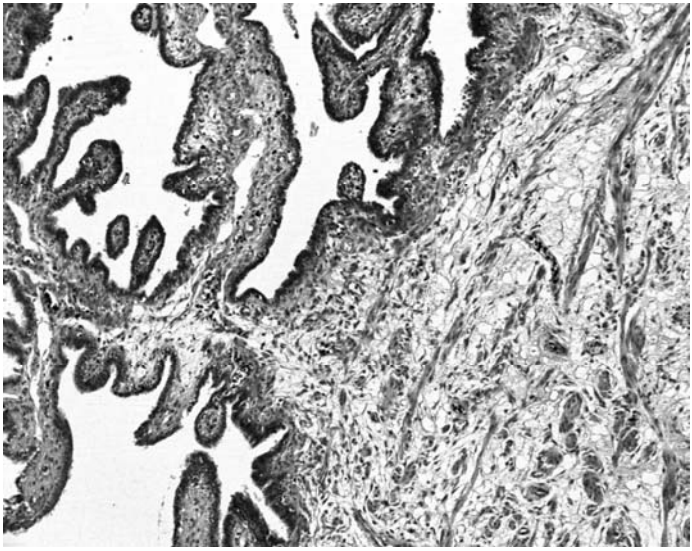

Figure 11. Tubal epithelium moderately contained bFGF. bFGF IMH 250X.

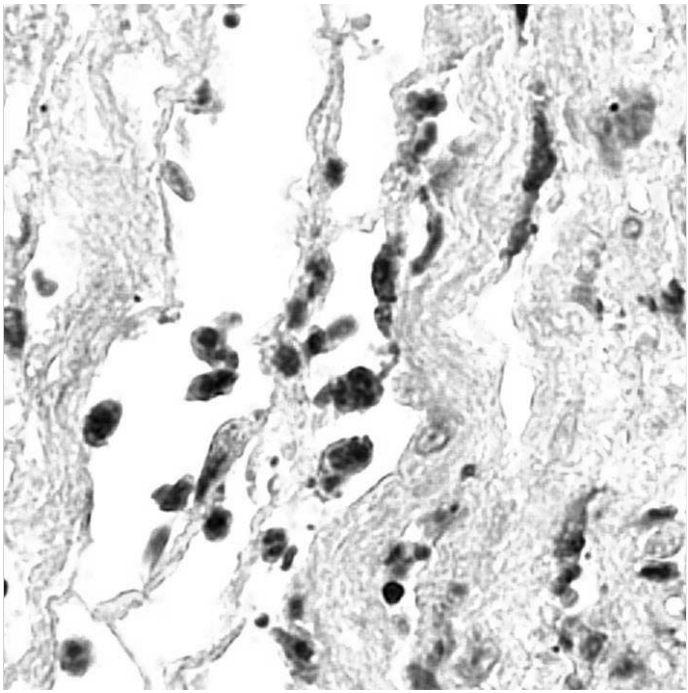

Figure 12. A few bFGF positive cells were seen in tubal stroma. bFGF IMH 400X.

DISCUSSION

bFGF is able to accumulate in the nucleolus where it stimulates ribosomal protein transcription [4]. bFGF is the first factor inducting mesodermal differentiation in *vivo* un *in vitro* and is abundant in the cells of mesenchimal and neuroectodermal origin [31]. The embryonic digestive tract, lungs, kidney, salivary and sebaceous glands, striated and smooth muscles express bFGF [11]. bFGF is one of the factors involved in mediating the angiogenesis, proteolysis and apoptosis during the implantation [21, 33]. bFGF appears in the maternal circulation during pregnancy, with peak values late in the 2nd trimester. The levels of bFGF in the maternal serum correlate positively with the fetal size both in the 2nd trimester and at term [14].

The fibroblast growth factor receptor 1 (FGFR1) is the most sensitive bFGF receptor [26]. The widespread distribution of FGFR1 in multiple mature organ systems suggests an important functional role in the normal human adult tissue [16]. It could be found in the membranes of the most anchorage dependent cells also around and in their nuclei [12, 28]. FGFR1 is a widely expressed membrane receptor of developing of human tissues, including neurons, vascular basement membranes, skin, and bone growth plates. Our previous data showed FGFR1 participation in the regulation of the human embryonic tissue formation [20]. Our findings demonstrated that the tubal, decidual and *conceptus* tissue contained more FGFR1 than bFGF. We speculate that the excess of the receptor is due to the compensatory adaptation of the organ to the gestation process.

Almost every cell in the human body is affected by IGF-1 which is one of the most potent natural activators of cell growth and the multiplication and a potent inhibitor of the programmed cell death [32, 5]. In addition to the insulin-like effects, IGF-1 can also regulate cell growth and development, especially in nerve cells, as well as the cellular DNA synthesis [25, 29]. Overexpression of IGF-1R in cancer cells results in increased invasion and *vice versa* [31]. IGF-1 and its receptor (IGF-1R) are essential for embryo growth and survival. Endometrial decidual cells express IGF-1 gene in the implantation site [19], but IGF-1 gene null mice have been shown to be infertile [2]. Maternal IGF-I stimulates fetal growth by activating the placental transport of nutrients to fetus [17]. In a case of fetus's growth retardation, IGF-1 compensatory increasing had been established [23, 15]. Peripheral trophoblast cells of preeclamptic women demonstrated increased immunoreactivity as well [24]. We discovered IGF-1 and IGF-1R in gravid endometrium. The fallopian tube epithelium, trophoblast and connective tissue cells demonstrated a positive reaction for IGF-1 in our study, but IGF-1R in these structures was particularly absent. This finding may indicate the possible restriction of the cell's growth and the restriction of trophoblast invasion in the tissues affected by ectopic pregnancy. Abundant distribution of IGF-1, bFGF and their receptors in the first trimester embryo developing organ systems suggests their participation in human embryogenesis.

CONCLUSIONS

1. IGF-1, IGF-1R, bFGF and FGFR1 are widely appearing growth factors in the actively developing and differentiating human embryonic tissue in the first trimester.
2. Both endometrial and fallopian tube tissues express more FGFR1 than bFGF that testifies the stimulation of the compensatory adaptation of the organ during pregnancy.
3. IGF-1 and IGF-1R richly appears in gravid endometrium.
4. IGF-1 is widely distributed in both mother and embryo tissues but IGF-1R marked only some of them in a case of ectopic pregnancy. The deficit of IGF-1R in the fallopian tube might be a result of cell growth restriction and the impaired process of trophoblast invasion.

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