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# Expression of insulin-like growth factor in the placenta of intrauterine growth-retarded human fetuses

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## Summary

Many cases of intrauterine growth retardation (IUGR) are the result of placental and fetal tissue insufficiency. Insulin-like growth factor-I (IGF-I) is known to play a role in placental and fetal growth. An immunocytochemical study was performed to localize IGF-I peptides in human placenta and umbilical cords of normal (n = 3) and IUGR (n = 3) fetuses. The peripartum fetal conditions were evaluated as well. Immunoreactive IGF-I was detected in the cytotrophoblast, syncytiotrophoblast, amnion, endothelial cells of fetal capillaries and in the decidua in both normal and IUGR placental tissue. A more robust immunostaining and increased numbers of positively stained cells were found in the decidua of IUGR placenta ($p < 0.001$). Intense immunostaining was also found in endothelial cells, smooth muscle cells and fibroblasts of the umbilical vein. IGF-I immunoreactivity was also present in stroma (Hofbauer cells and/or fibroblasts) of IUGR villi. Our results indicate that expression of IGF-I is high in specific sites in placenta and umbilical cords, which indicates a paracrine and/or endocrine function. The increased expression of IGF-I in placenta of IUGR fetuses indicates its involvement in restoring normal growth by means of a positive feed-back mechanism.

*Key words:* insulin-like growth factor – intrauterine growth retardation – human placenta

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## Introduction

Insulin and structurally related IGF-I are mitogenic peptides which have been implicated in the embryonic development of the rat (Rotwin et al., 1987; Cowley and Pratten, 1996). In addition to factors produced by the developing embryo itself, it is likely that maternally-derived growth factors also play an important role with their postulated initial site of action being the extra-embryonic membranes, which surround the embryo throughout gestation.

IGF-I and IGF-II are synthesized within the placenta and are believed to play important roles in the regulation of placental growth and endocrine function (Hill et al., 1993; Hild-Petito et al., 1994; Bauer et al., 1998). Bioavailability of IGFs is determined at a cellular level by several specific binding proteins (IGFBPs), which are widely but selectively distributed in all developing tissues. Since IGFs and IGFBPs are paracrine factors, identification of sites of synthesis of IGFs and their binding proteins indicates potential sites of biological action (Coulter and Han, 1996; Han et al., 1999). In these studies, immunocytochemistry was used to localize IGF-I and IGF-II peptides, and IGFBP-1, -2 and -3 in human placentae. Strong immunostaining of IGFBP-1 was observed in decidual cells. The human placenta produces IGF-I and contains receptors for IGF-I (Grizzard et al., 1984; Wang et al., 1988; Chastant et al., 1994). Receptors have been detected as early as the sixth week of gestation in human placental tissues (Grizzard et al., 1984), and an increase in IGF-I mRNA has been detected in first and second trimester human placentae (Wang et al., 1988). IGF-I receptors were present in the placenta at day 20 of pregnancy (Chastant et al., 1994).

IGF-I has an important role in embryonic growth retardation, suggesting that IGF-I is important in prenatal growth (Liu et al., 1993; Baker et al., 1993). Intrauterine growth retardation (IUGR) is of major clinical concern, and the factors and mechanisms involved in growth retardation are still poorly understood. IUGR infants are born with low levels of circulating IGF-I (Lassare et al., 1991; Giudice et al., 1995), and their growth rate during the first year of life is related to levels of circulating IGF-I (Thieriot-Prevost et al., 1988).

The aim of the present immunocytochemical study was to evaluate the presence and distribution of immunoreactive IGF-I in human placentae and to examine the relationship between IUGR and tissue levels of IGF-I.

## Material and methods

*Subjects.* Three women without any obstetrical or medical problems with estimated fetal weight within normal percentiles of corresponding gestational weeks (control group) and 3 women with IUGR were included in the study. Antenatal evaluations of the pregnant women included fetal surveillance tests such as Doppler ultrasonography (US), cardiotocography, fetal biometric measurements using obstetrical US and the evaluation of IUGR risk factors. For the exact determination of gestational ages, first trimester US crown rump

length (CRL) measurements were performed. The type of delivery, fetal weight, placental weight and diameter, umblical arterial acidemia, Apgar scores and hospitalization in a neonatal intensive care unit were all evaluated.

### *Clinical analysis*

*Diagnosis of fetal growth restriction.* The last menstrual date of the mothers, risk factors associated with IUGR, uterine fundal height and first trimester obstetrical US for the determination of gestational age were checked. Last trimester estimated fetal weights of fetuses were determined using US abdominal circumference measurements. An US abdominal circumference less than the 10th percentile for gestational age was taken as an indication of growth retardation.

*Cardiotocographic evaluation.* Cardiotocographic evaluations (Oxford-Sonicaid-Teamcare®, Abingdon, England) were performed after 30 weeks of gestation to determine fetal well-being. The test involves an electronic recording of fetal heart rate obtained through an US transducer placed on the maternal abdomen.

*Colour Doppler US.* Blood flow in the umbilical cord and middle cerebral artery (MCA) was determined by Doppler velocimetry (3.75 Mhz, convex probe, colour pulsed wave Doppler; Ecocee; Toshiba, Tokyo, Japan). Umbilical artery velocimetry was used as a potential indicator of fetal acidosis and placental vascular resistance.

*Blood gas analysis.* Umbilical arterial blood sampling was obtained immediately after clamping the cord. Umbilical artery blood pH was analysed using a blood gas analyser (Corning-855-Biobak®; Ciba, Halstead, England). Acidemia during labor was defined as a pH lower than 7.15 in the umblical artery, or 7.27 in cases of cesarean section.

*Sources of material.* Human placental tissue and umbilical cords were obtained from normal full-term spontaneous deliveries and cesarean section deliveries (34–38 weeks gestation; from a control group and from mothers that delivered IUGR babies, at the Kocaeli University, Faculty of Medicine). Small pieces of fetal placental tissue and umbilical cord were fixed for 24–48 h in neutral buffered 4% paraformaldehyde, dehydrated in increasing alcohol series (70, 80, 90 and 100%) and xylene, prior to embedding in paraffin wax. Sections (5 μm thick) were obtained using a sliding microtome. Polyclonal anti-human IGF-I antibodies (UBZ-495) were kindly provided by Dr. A. F. Parlow (National Hormone and Pituitary Program, Torrance CA, USA). A secondary antibody kit (Histostain plus) was purchased from Zymed (San Francisco CA, USA).

*Immunocytochemistry.* Human placenta and umbilical cord sections were deparaffinized and rehydrated and immunocytochemistry was performed for human IGF-I using the avidin-biotin-peroxidase method. The rehydrated sections were pretreated with 3% hydrogen peroxide for 10 min to eliminate endogenous peroxidase activity. Sections were then washed in PBS-Triton X 100 (Tx). To eliminate the nonspecific binding, sections were pretreated with normal rabbit serum. Sections were incubated with 1 : 1 800 diluted anti-human IGF-I primary antibodies for 24 h at 4 °C in a humidified chamber. Following washing in PBS-Tx, biotinylated anti-rabbit antibodies were applied for 15 min at room temp. Following washing in PBS-Tx, streptavidin-peroxidase conjugate was applied to the sections for 15 min at room temp. Following washing in Tris, 0.6% hydrogen peroxide and 0.02% diaminobenzidine (DAB) was applied for approx 5 min at room temp. As control, the primary antibody was omitted and replaced with nonimmune serum. Immunoreactivity was examined by light microscopy (BX50F-3; Olympus, Tokyo, Japan).

*Histology.* Histology of placental tissue and characterization of cells were evaluated on the basis of Welsch (1994), Eroschenko (1996) and Lewis and Benirschke (1997).

*Histoplanimetrical analysis.* In each immunostained section, quantification of IGF-I-positive cells was performed in 0.10 $mm^2$ fields of decidual tissues with an ×40 objective using an ocular micrometer system (Olympus). Data are presented as means with standard

deviations (SD). For statistical analysis, each mean was compared using the Mann-Whitney U-Wilcoxon rank sum W-test and the level of significance was taken as $p = 0.05$.

## Results

*Clinical evaluation.* The mean age of the mothers was 27.5 in the control group and 26.7 in the IUGR group. Gestational ages were 38.7 and 34 weeks in the control and IUGR group, respectively. In the control group, all deliveries were normal and spontaneous via the vagina, whereas all deliveries in the IUGR group were by cesarean section, due to persistant variable or late decelerations as determined by cardiotocographic evaluation. Although none of the newborns in the control group needed resucitation or intensive care and Apgar scores were normal, one of the newborns of the IUGR group was lost just prior to delivery, and 2 were hospitalized in a neonatal intensive care unit. Umbilical artery colour Doppler US evaluation revealed that, the mean umbilical arterial resistance (systole/diastole) was 4.72 in the IUGR group and 2.42 in the control group (Table 1). These values indicated uteroplacental insufficiency in the IUGR group. Mean Doppler US values of the MCA were 2.85 and 3.97 in the IUGR and control group, respectively (Table 1). Since the uteroplacental vascular bed showed increased resistance in the IUGR group, dilatation of fetal cerebral arteries due to brain sparing effects resulted in decreased MCA values. The mean placental weight was 327.5 g and 577.5 g in the IUGR and the control group, respectively (Table 1). Intrapartum umblical arterial pH values were 7.0 and 7.16 in the IUGR and the control group, re-

**Table 1.** Peripartum evaluation of fetal conditions.

| Placenta (number) | Umblical artery pH | Doppler US | | Placental weight (g) | Diameter of placenta (cm) | Fetal weight at birth (g) | Cardio-toco-graphy | Type of delivery |
|---|---|---|---|---|---|---|---|---|
| | | UA(s/d) | MCA(s/d) | | | | | |
| IUGR group | | | | | | | | |
| 1 | 7.21 | 4.1 | 3.0 | 350 | 14 | 1680 | VD | C/S |
| 2 | 7.10 | 4.2 | 2.9 | 330 | 13 | 1650 | LD | C/S |
| 3 | 7.14 | 4.8 | 3.1 | 330 | 13 | 1730 | LD | C/S |
| Control group | | | | | | | | |
| 1 | 7.27 | 2.4 | 4.4 | 600 | 17 | 4700 | Normal | NSD |
| 2 | 7.13 | 4.1 | 2.5 | 530 | 18 | 3350 | Normal | NSD |
| 3 | 7.20 | 2.7 | 4.0 | 580 | 17 | 3700 | Normal | NSD |

Abbreviations:
UA, umblical artery; MCA, middle cerebral artery; S/D, systole/diastole; C/S, cesarean section; NSD, normal spontaneous delivery; VD, variable deceleration; LD, late deceleration

spectively (cut off values for umblical artery pH to demonstrate acidosis is 7.15 in normal labor and 7.27 in cesarean section; Table 1). pH values indicated the presence of acidemia in the umblical artery in the IUGR group.

*Immunocytochemistry.* Immunoreactive IGF-I was found in many areas of

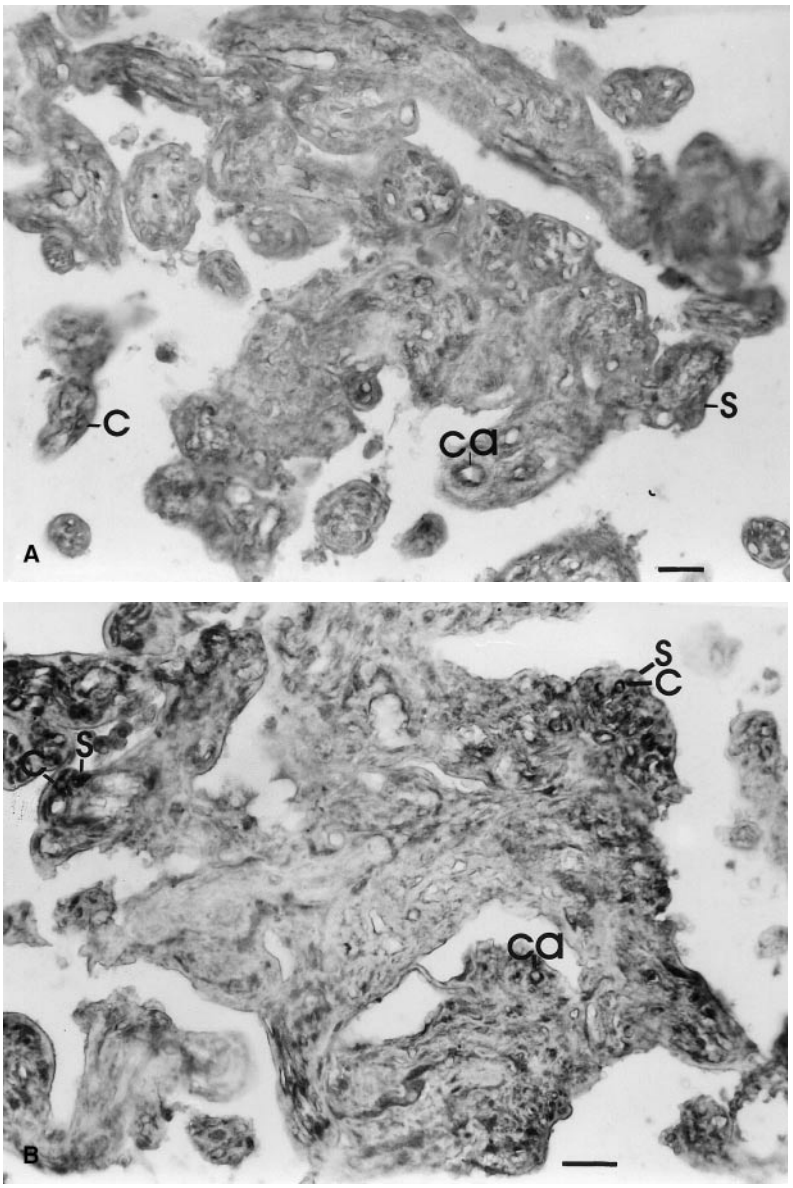


**Fig. 1.** Immunocytochemical localization of IGF-I in control (**A**) and IUGR (**B**) human placental villi. Immunostaining was observed in cytotrophoblast (c), syncytiotrophoblast (s), and endothelial cells of capillaries (ca). The mesenchyme of fetal stem villi was weakly stained. Bars, 100 μm.

the placentae and umbilical cords, both in the control and IUGR group. Immunocytochemical localization of IGF-I was obtained in relatively few cells of the cytotrophoblast, syncytiotrophoblast, and endothelial cells of fetal capillaries in both groups (Figs. 1 and 2). IGF-I immunoreactive stromal cells (Hofbauer cells and/or fibroblasts) were located only in some areas of villi in the IUGR group (Fig. 2). The distribution and intensity of IGF-I immunoreactivity was very similar in trophoblastic cells and vascular endothelial cells in both groups and in fetal villi in the IUGR group (Figs. 1 and 2). Immunostaining was present in decidualized cells (epitheloid shaped cells), extravillous

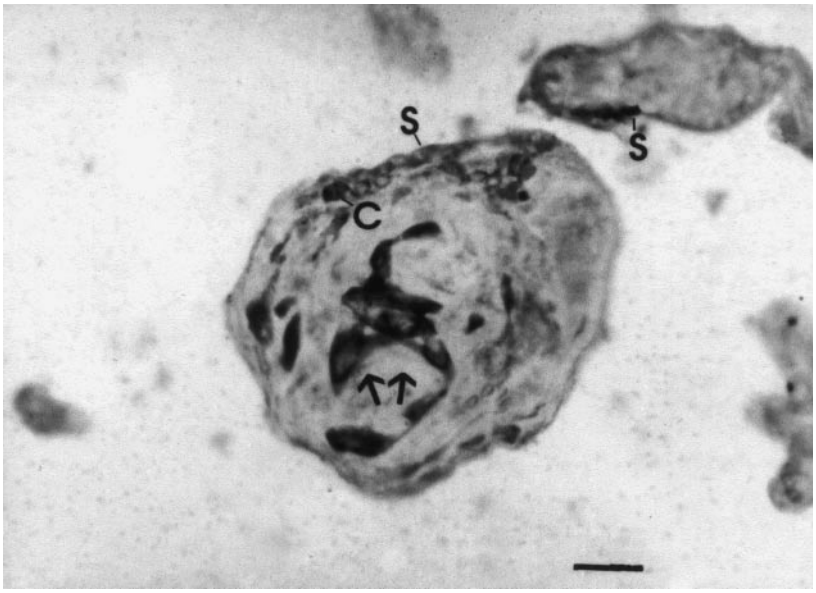


**Fig. 2.** Immunostaining of IGF-I in IUGR human placental stem villi. Relatively few cells of the cytotrophoblast (c) and syncytiotrophoblast (s) were IGF-I-positive. IGF-I immunoreactivity was also present in Hofbauer cells and/or fibroblasts (arrows). Bar, 100 μm.

**Table 2.** Number of IGF-I-positive cells in decidual tissue. IGF-I-containing cells were counted in 0.10 $mm^2$ fields of decidual tissue using an ocular micrometer system.

| Placenta (number) | Mean ± SD |
|---|---|
| IUGR 1 | 30.2 ± 7.9* |
| IUGR 2 | 37.4 ± 9.5* |
| IUGR 3 | 41.0 ± 8.8* |
| Control 1 | 10.0 ± 3.9 |
| Control 2 | 10.1 ± 4.6 |
| Control 3 | 9.9 ± 4.4 |

* Significantly different from control value, $p < 0.001$

trophoblasts (round shaped cells) and in small numbers of fibroblastic cells (spindle-shaped cells) of the decidua (Fig. 3 A, B). Intensity and distribution of immunostaining of IGF-I were low (Fig. 3 A), whereas the number of IGF-I-

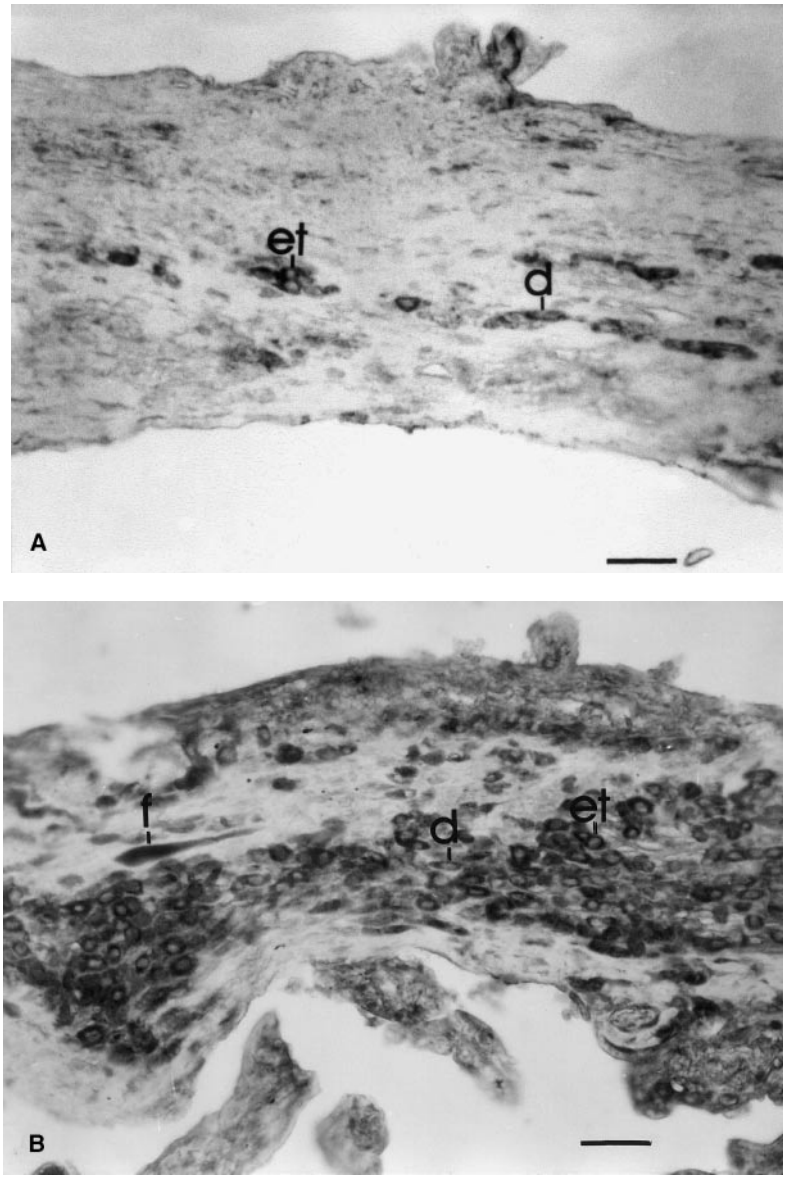


**Fig. 3.** Immunocytochemical localization of IGF-I in control (**A**) and IUGR (**B**) human placenta. Note the increased intensity and abundance of IGF-I-positive cells in the decidua; d, (epitheloid) extravillous trophoblast; et, rounded fibroblasts; f, spindle-shaped fibroblasts. Bar, 100 μm.

positive cells were significantly increased in the decidua in the IUGR group (Fig. 3 B; Table 2). In the umbilical cord, immunoreactivity of IGF-I in smooth muscle cells and endothelial cells, both in the artery and vein, was very weak in the control group (Fig. 4 A). However, intensity of immunostain-

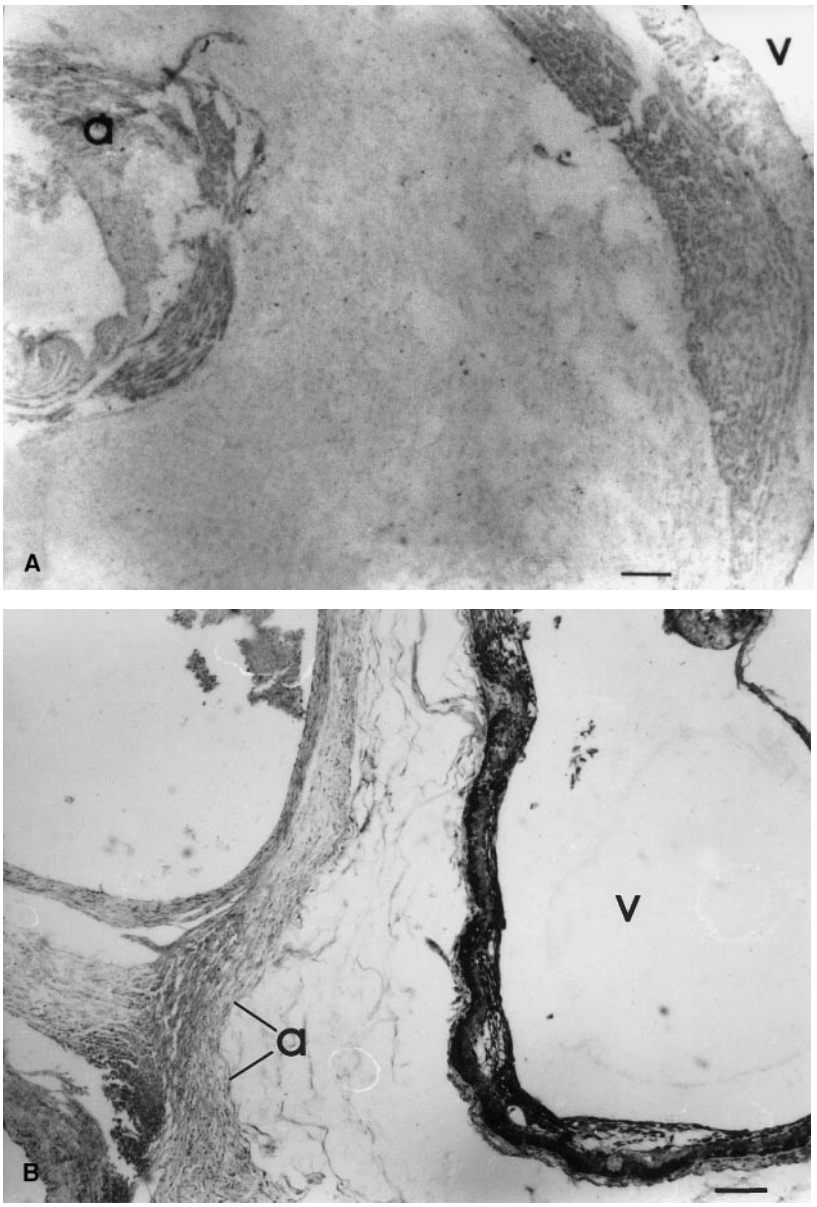


**Fig. 4.** Immunocytochemical localization of IGF-I in control (**A**) and IUGR (**B**) human umbilical cord. Note the increased levels of IGF-I positivity in endothelial cells and smooth muscle cells of the umbilical vein (v) as compared to the umbilical artery (a). Bar, 200 μm.

ing was increased in smooth muscle cells and endothelial cells of the umbilical vein in the IUGR group (Fig. 4 B). IGF-I immunostaining was similar in epithelial cells of the amnionic membrane and fibroblasts in both groups (Fig. 5).

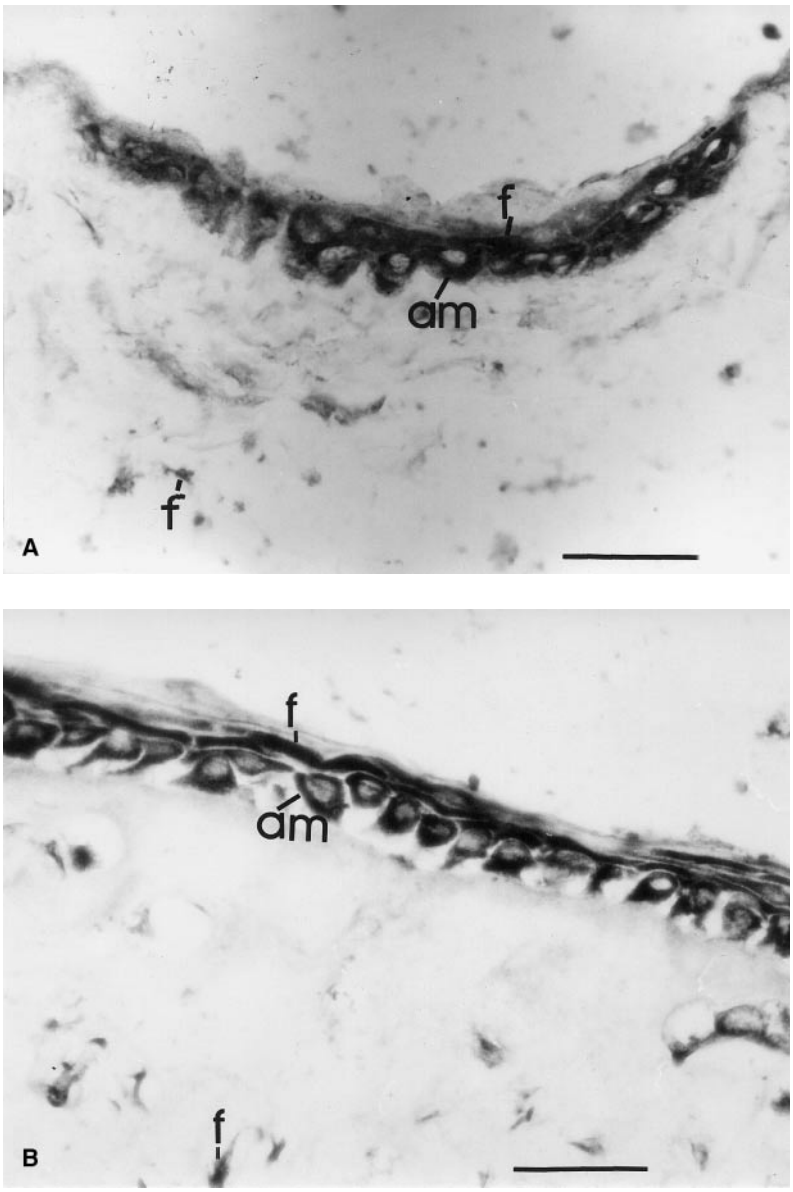


**Fig. 5.** Immunostaining of IGF-I in the amnion membrane that surrounds the human umbilical cord in the IUGR group. Note the IGF-I-positive amniotic epithelial cells (am), and fibroblasts (f) in Wharton's jelly. Bar, 100 μm.

## Discussion

Recent studies have demonstrated the presence and distribution patterns of immunoreactive IGF-I in the placenta and umbilical cord. In the present study, we compared the distribution and intensity of IGF-I immunoreactivity and peripartum fetal conditions of normal term placentae with those of IUGR placentae. IUGR fetuses showed uteroplacental insufficiency and increased cerebral arterial blood flow as compared to normal fetuses. In the IUGR specimens, both fetal and placenta size and weight were reduced as compared to normals. Normally, resistance in the placental bed decreases with gestational age. When placental perfusion is reduced, pH values in the umbilical vein and artery decrease (Gordon and Johnson, 1985). All of the IUGR fetuses in the present study underwent cesarean section due to abnormal cardiotocographic findings. In addition, Doppler measurements in the MCA were decreased (brain-sparing effects) whereas, in umbilical arteries these were increased. The present data demonstrate the compensatory redistribution of the vasculature in response to uteroplacental insufficiency in IUGR placentae.

The presence of immunoreactive IGF-I in human placenta and umblical cord was clearly shown in the present study. Immunostaining was associated with relatively few cells of the syncytiotrophoblast, cytotrophoblast, stroma of fetal villi (Hofbauer cells and/or fibroblasts), the decidua and umblical cord. The presence and distribution of IGF-I immunoreactivity is in line with previous studies (Han et al., 1988; Hill et al., 1989, 1993). An immunocytochemical study has been performed previously on placentae of fetuses showing normal growth and growth restriction (Holmes et al., 1999). The same study indicated that the distribution and intensity of IGF-I receptor in villous endothelium, stroma, trophoblast and decidua in normal and growth-restricted fetuses were similar. The study of Sorem and Siler-Khodr (1998) showed that IGF-I production and release in IUGR were aberrant. It was demonstrated that 3 of 9 IUGR placentae showed different patterns, one showing elevated IGF-I production and release, and in the other 2, a decrease was observed. It was concluded that abnormal regulation and production of IGF-I by the placenta was a possible factor affecting pregnancies complicated by IUGR. In the PCR study of Abu-Amero et al. (1998), significant differences in the levels of transcripts for IGF-I were not observed. However, IGF-II and IGF-I receptor expression was observed to be significantly higher in the IUGR term placentae. It was concluded that the increase in IGF-II and IGF-I receptor expression in IUGR placentae may represent a counter-regulatory mechanism in response to growth retardation.

In the present study, IGF-I immunoreactive cells were distinctly abundant and increased intensity of IGF-I-positivity was found in the decidua ($p < 0.001$). Furthermore, increased immunostaining was detected in the umblical vein and some stromal villi (Hofbauer cells and/or fibroblasts) in IUGR placentae as compared to normal term placentae. However, the amounts of

IGF-I immunoreactive cells in other areas of the placentae were similar in both groups. Our results seems to be partly in contrast with the results of Sorem and Siler-Khodr (1998). Although our results are also partly different from the results of Abu-Amero et al. (1998), it is important to realize that transcription and peptide synthesis may not be correlated. In addition, placental size and fetal growth rate are correlated, but the mechanisms underlying growth restriction are still not understood properly. Furthermore, the higher levels of IGF-I in IUGR placental tissues may not be related with concentrations of IGF-I in serum, because the increased numbers of IGF-I-positive cells in the decidua may well be a locally regulated increase and may not reflect the overall condition of IUGR placentae. However, it has been demonstrated that IGF-I, IGF-II and IGFBP-1 levels in the amniotic fluid do not correlate with birth and placental weight (Verhaeghe et al., 1999).

Interestingly, fetal IGF-I levels are not under the control of pituitary growth hormone during pregnancy, and it is possible that other hormones, such as human placental lactogen and prolactin, are the crucial effectors (Pilistine et al., 1984; Hill et al., 1988). Some studies have shown that placental growth factor (PlGF) mRNA was increased by 2–3 fold and PlGF protein levels were also increased in IUGR as compared to normal placentae (Khalig et al., 1999). These authors claim that increased levels of PlGF are likely due to increased oxygen concentrations (hyperoxia). It was hypothesized that at the early onset of IUGR, "placental hyperoxia" downregulates trophoblast PlGF levels, and that PlGF expression is increased in IUGR. The results of our study may be in line with those of Khalig et al. (1999), although PlGF expression and IGF-I expression may not be similar. Other sources of IGF-I in fetal plasma may be the umbilical vein and amniotic cells, since the umbilical vein but not umbilical arteries showed overexpression of IGF-I in IUGR fetuses. This result indicates that the decreased serum levels of IGF-I in IUGR fetuses, as demonstrated by other authors, may have upregulated synthesis of IGF-I in the umbilical vein. It is possible that the locally increased numbers of IGF-I-immunoreactive cells in decidua, umbilical vein, and Hofbauer cells and/or fibroblasts have a paracrine/autocrine function to restore the impaired growth in terms of a positive feedback mechanism in response to the low concentrations of IGF-I in the serum.

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