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Modulation of placental vascular endothelial growth factor by leptin and hCG

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Vascular endothelial growth factor (VEGF) has been identified as an endothelium-specific mitogen and inducer of angiogenesis and endothelial cell survival. Leptin and hCG have also been suggested as possible regulators of angiogenesis in various models. In-vivo and in-vitro assays revealed that leptin has an angiogenic activity and that the vascular endothelium is a target for leptin. Thus, we hypothesized that products of cytotrophoblastic cells may play a role in placental angiogenesis and we therefore investigated the effects of leptin and hCG on cytotrophoblast VEGF secretion. We incubated cytotrophoblastic cells (CTB) with recombinant human leptin (rhLept) (0–4 pg/ml) or hCG (0–30 000 IU/ml) for 4 h. rhLept significantly stimulated hCG ($P = 0.0045$) and decreased VEGF release ($P = 0.0008$) by CTB in a concentration-dependent manner. On the other hand, increasing concentrations of hCG (0–30 000 IU/ml), induced a significant inhibition of leptin secretion ($P = 0.0028$) and a marked dose-dependent stimulation of $VEGF_{165}$ secretion ($P < 0.0001$). We observed an increase of >1000-fold in basal trophoblastic VEGF secretion with physiological concentrations of hCG *in vitro*. An inhibitory effect of hCG on trophoblastic leptin secretion was also observed, suggesting that hCG might exert a possible negative feedback on trophoblastic release of leptin. We hypothesize that trophoblastic products such as hCG and leptin are probably involved in the control of VEGF secretion at the maternal–fetal interface.

Key words: angiogenesis/hCG/leptin/trophoblast/VEGF

Introduction

Angiogenesis is generally a quiescent process in the healthy adult male with an extremely low turnover of endothelial cells. However, in the female, angiogenesis is highly regulated and turned on for brief periods of time in selected organs such as the ovary and the endometrium. Examples of such regulated angiogenesis include follicular growth, corpus luteum formation, endometrium differentiation and repair, and embryo implantation and development (Reynolds *et al*., 1992; Reynolds and Redmer, 1995). Among the list of angiogenic factors (Folkman and Klagsbrun, 1987), vascular endothelial growth factor (VEGF) was identified as an endothelium-specific mitogen and inducer of angiogenesis and endothelial cell survival (Plouet *et al*., 1989; Conn *et al*., 1990; Alon *et al*., 1995; Gerber *et al*., 1998).

Analysis of the DNA sequence of a variety of human VEGF clones indicates that VEGF may exist as one of four different isoforms as a result of alternative splicing. $VEGF_{165}$ is the major VEGF isoform (Ferrara, 1999). In human placenta, VEGF expression has been analysed by in-situ hybridization and immunohistochemical studies (Ahmed *et al*., 2000). It is localized to the villous trophoblast, the decidua and macrophages of both fetal and maternal origin (Sharkey *et al*., 1993; Ahmed *et al*., 1995; Vuorela *et al*., 1997). Trophoblast cells also secrete VEGF *in vitro* (Shore *et al*., 1997; Ahmed *et al*., 2000). VEGF appears unique in its role as an angiogenic factor, in that the two known human receptors for VEGF, VEGFR-1 (formerly flt-1) and VEGFR-2 (formerly KDR) are expressed on endothelial cells (de Vries *et al*., 1992; Terman *et al*., 1992). Interestingly, human trophoblast cells, particularly the invasive extravillous cytotrophoblast cells (CTB) also express VEGF receptors (Charnock-Jones *et al*., 1994; Athanassiades *et al*., 1998). Both VEGF receptor forms are present in first trimester placental tissue (Ahmed *et al*., 1995; Vuckovic *et al*., 1996).

Leptin and hCG, both produced by human trophoblasts (Chardonnens *et al*., 1999), have also been suggested as possible regulators of angiogenesis in various models (Rizk *et al*., 1997; Henson and Castracane, 2000). Leptin was found to generate a growth signal via a tyrosine kinase-dependent pathway and promote angiogenic processes via activation of the leptin receptor (Ob-R) in endothelial cells (Park *et al*., 2001). In-vivo and in-vitro assays revealed that leptin has an angiogenic activity and that vascular endothelium is a target for leptin (Sierra-Honigmann *et al*., 1998). In contrast to the leptin-deficient ob/ob mice, where no vascular fenestrations are detected, capillary fenestrations are found in leptin-producing adipose tissue in lean mice. Thus, leptin plays a critical role in the maintenance and the regulation of vascular fenestrations in adipose tissue. Furthermore, leptin and fibroblast growth factor (FGF)-2 and VEGF synergistically stimulate angiogenesis (Cao *et al*., 2001).

Taking these observations together, we hypothesized that products of cytotrophoblastic cells may play a role in placental angiogenesis. We therefore investigated the effects of leptin and hCG on cytotrophoblast VEGF secretion.

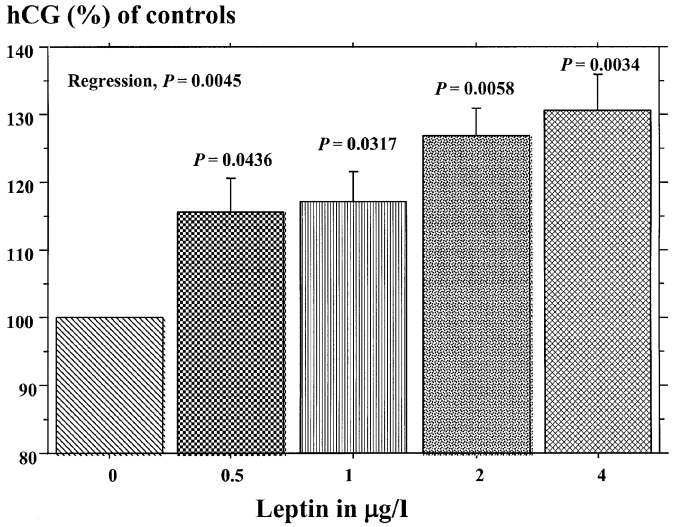

Figure 1. Release of hCG (% of controls) at 4 h from cytotrophoblastic cells incubated for 4 h with a range of concentrations of recombinant human leptin. Statistics were performed by analysis of variance and *P* values refer to differences compared with control (Leptin, 0). Error bars are SEM (n = 4 experiments in duplicate).

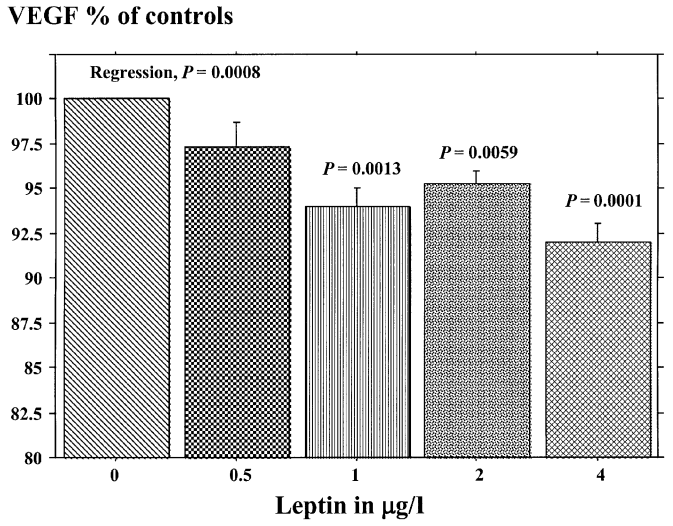

Figure 2. Release of vascular endothelial growth factor (VEGF) (% of controls) at 4 h from cytotrophoblastic cells incubated for 4 h with a range of concentrations of recombinant human leptin. Statistics were performed by analysis of variance and *P* values refer to differences compared with controls (Leptin, 0). Error bars are SEM (n = 4 experiments in duplicate).

Materials and methods

CTB were isolated and purified as previously described (Bischof *et al*., 1991). Briefly, trophoblast tissue was obtained from legal abortions at 8–12 weeks of normal pregnancy (from the last menstrual period). The tissue was digested with trypsin and cytotrophoblastic cells separated from blood cells and syncytia on a discontinuous Percoll gradient and immunopurified by antibody-coated magnetic particles (anti-CD45; Dyna Beads, Switzerland) in order to eliminate contaminating leukocytes. After immunopurification the cell suspension represents 90–97% cytotrophoblastic cells, with a 3–7% contamination by fetal stromal cells. Lymphomyeloid cells are no longer present (Bischof *et al*., 1991). CTB viability was assessed by Trypan Blue (Sigma) exclusion and cells diluted to 10^6 cells/ml with culture medium. Recombinant human leptin (rhLept) was purchased from R&D Systems (Bühlmann Laboratories, Switzerland). hCG (Profasi) was purchased from Ares–Serono (Switzerland).

Culture conditions

CTB (100 µl, 10^6 cells/ml, >90% viability) were incubated in duplicates in the presence or absence of rhLept (0–4 pg/ml) or hCG (0–30 000 IU/ml). Incubation was performed in 12-well tissue culture plates (Costar, USA) under a 5% CO_2 and 95% air atmosphere in a humid incubator at 37°C for 4 h. Culture medium was Dulbecco's modified Eagle's medium (Gibco, Switzerland) containing 2 mmol/l L-glutamine, 4.2 mmol/l magnesium sulphate, 25 mmol/l HEPES, 1% gentamycin, 1% amphotericin B, 100 µg/ml streptomycin and 100 IU/ml penicillin, in the absence of serum. The supernatants were collected after 4 h of incubation, aliquoted and stored at –20°C until assayed. Duplicate wells were run for each treatment condition and the experiments repeated four times with different CTB preparations.

Hormone and protein assays

$VEGF_{165}$ was measured in the supernatants by an enzyme-linked immunosorbent assay (ELISA) (R&D Systems, Germany) with a sensitivity of 5.0 pg/ml and intra- and inter-assay coefficient of variation (CV) <6.5 and <8.5% respectively. HCG was measured in the supernatants by a microparticle enzyme immunoassay (IMX; Abbot, USA) with a sensitivity of 0.1 mIU/ml and intra- and inter-assay coefficients of variation <4%. Leptin was measured in the supernatants by an ELISA (DRG diagnostics, Cis-Medipro, Switzerland) with a sensitivity of 0.2 ng/ml and an intra- and inter-assay CV of 4.3 and 8.6% respectively. According to the manufacturer, the leptin assay does not cross-react with human proteins such as proinsulin, C-peptide, glucagon or insulin-like growth factor-I.

Statistics

In order to normalize the distribution and minimize the inter-experimental variability, all results were expressed as a percentage of their respective controls, CTB in the absence of hCG or rhLept, and presented as mean ± SEM. Statistical evaluations were performed with a personal computer using the Statview Program (Abascus). Tests included one-way analysis of variance with Fisher's test to compare the effects of different concentrations of rhLept or hCG. Regression analysis was performed to determine the dose dependency of rhLept or hCG effects. $P < 0.05$ was considered to be statistically significant.

Results

Effects of leptin on hCG and VEGF secretion in human CTB

RhLept (0–4 µg/l) augmented hCG release by CTB in a concentration-dependent manner (P = 0.0045, Figure 1). Basal hCG release was 118 ± 12 IU/l. When compared to controls, hCG release was 115.7 ± 6.8% (P = 0.0436), 117.3 ± 6% (P = 0.0317), 127 ± 5.7% (P = 0.0058) and 130.5 ± 7.5% (P = 0.0034), after incubation with 0.5, 1, 2 and 4 pg/ml of rhLept respectively.

RhLept (0–4 µg/l) decreased VEGF release by CTB in a concentration-dependent manner (P = 0.0008, Figure 2). Basal VEGF release was 7.0 ± 0.5 pg/ml. When compared with control, VEGF release was 94 ± 1.8% (P = 0.0013), 95.2 ± 1.2% (P = 0.0059) and 92 ± 1.9% (P = 0.0001) after incubation with 1, 2 and 4 pg/ml of rhLept respectively. However, this modest effect was still observed after incubation for 20 h (results not shown).

Effects of hCG on leptin secretion in human CTB

Basal leptin secretion was 1.29 ± 0.17 ng/ml. When incubated for 4 h (n = 4) with increasing concentrations of hCG (0–30 000 IU/ml), a significant inhibition of leptin secretion was observed. When compared with controls, leptin secretion was 92.3 ± 2.9% (P = 0.0095), 91.3 ± 5.3% (P = 0.0042), 90.8 ± 2% (P = 0.0028) and 90.8 ± 5.2% (P = 0.0028) after incubation with 5000, 10 000, 20 000 and 30 000 IU/ml of hCG respectively (Figure 3). This effect was still observed after incubation for 20 h with removal of hCG at 4 h (results not shown).

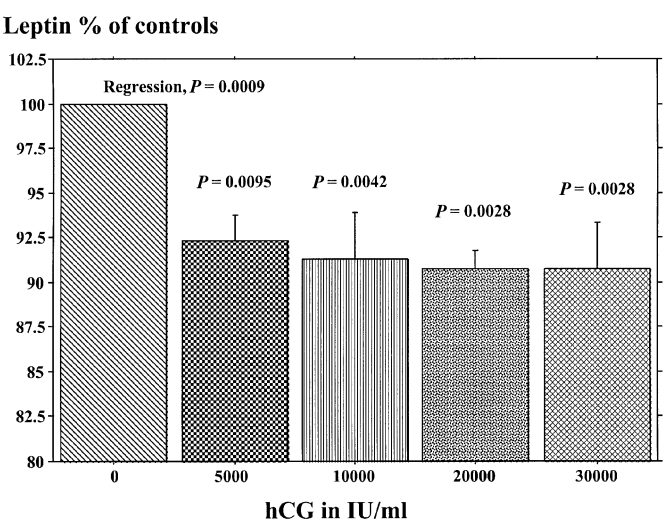

Figure 3. Release of leptin (% of controls) at 4 h from cytotrophoblastic cells incubated for 4 h with a range of concentrations of hCG (Profasi). Statistics were performed by analysis of variance and *P* values refer to differences compared with control (hCG, 0). Error bars are SEM ($n = 4$ experiments in duplicate).

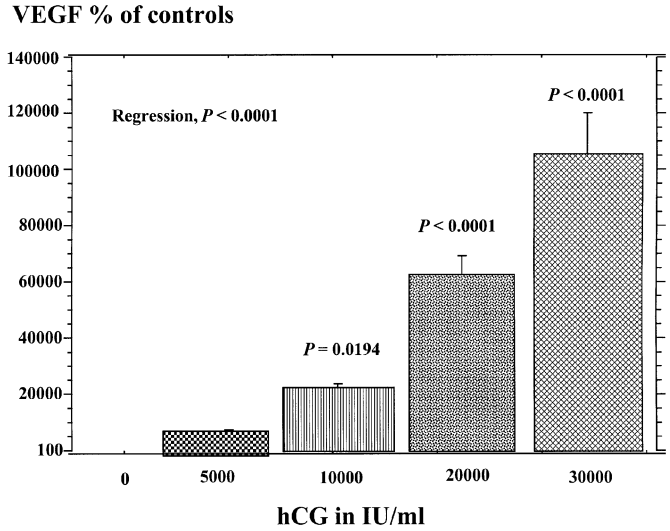

Figure 4. Release of vascular endothelial growth factor (VEGF) (% of controls) at 4 h from cytotrophoblastic cells incubated for 4 h with a range of concentrations of hCG (profasi). Statistics were performed by analysis of variance and *P* values refer to differences compared with controls (hCG, 0). Error bars are SEM ($n = 4$ experiments in duplicate).

Effects of hCG on VEGF secretion in human CTB

CTB were incubated for 4 h with raising concentrations of hCG (0–30 000 IU/ml, $n = 4$ experiments). Basal VEGF secretion was 111.9 ± 0.1 pg/ml. There was a marked dose-dependent increase in $VEGF_{165}$ secretion ($P < 0.0001$) in response to hCG reaching a maximum of 106 000 ± 14 500% of controls ($P < 0.0001$), when incubated with 30 000 IU/ml of hCG (Figure 4). When VEGF secretion was measured at 8 h after incubation with hCG, VEGF secretion was dose-dependently increased 10-fold above controls ($P < 0.0001$). At 24 h after incubation, we did not find any effect of hCG on trophoblastic VEGF secretion (results not shown).

Discussion

An appropriate blood supply is essential for the development of the feto-placental unit. Maternal blood flow in the intervillous space increases 20-fold during pregnancy due to vasomotor changes of the distal intramyometrial portions of uteroplacental arteries and the transformation and dilatation of decidual segments. This results essentially from the invasive behaviour of cytotrophoblastic cells that invade and colonize the endometrial spiral arteries allowing for a loss of their elasticity (Fisher, 2000). The increased blood requirement is also met by intense angiogenic processes taking place in the fetal stroma of the placental villi and the maternal endometrium. Abnormal angiogenesis during early pregnancy may lead to fetal growth retardation and/or pre-eclampsia (Roberts, 1998). Although several angiogenic factors have been identified at the feto-maternal interface, the regulation of this process remains obscure (Folkman and Klagsbrun, 1987; Smith, 2000).

In the present study we observed an increase of >1000-fold in trophoblastic VEGF secretion *in vitro* when CTB were cultured with physiological concentrations of hCG. This observation is consistent with other studies where VEGF expression was induced by LH in the ovary (Geva and Jaffe 2000) and by hCG in human endometrium (Licht *et al*., 2001) and in the ovary (Lee *et al*., 1997). This indicates that hCG probably plays an important role in the control of endometrial and placental vascularization by paracrine (on the endometrium) as well as juxtacrine (on the trophoblast) mechanisms. Since the stimulatory effect of hCG on VEGF secretion is no longer observed at 24 h, it is tempting to speculate that hCG favours VEGF secretion rather than synthesis.

Since leptin stimulates hCG secretion through a GnRH-dependent pathway (Chardonnens *et al*., 1999) and increases the pulsatile release of hCG in first-trimester trophoblastic explants (Islami *et al*., 2003), we wondered if this peptide was able to directly or indirectly (through hCG) modify the expression of VEGF. Despite leptin stimulating hCG, it inhibited the release of VEGF. Although this effect was statistically significant and maintained up to 24 h of culture, it was modest compared with the effect of hCG on VEGF. The physiological significance of an inhibitory effect of leptin and a stimulatory effect of hCG on trophoblastic VEGF secretion is far from being understood particularly since *in vivo* hCG is produced by syncytiotrophoblast and not cytotrophoblast as in in-vitro experiments. Thus our results cannot easily be applied to an in-vivo situation.

It is also interesting to note that, in addition to the stimulatory effect of leptin on hCG secretion, we observed an inhibitory effect of hCG on trophoblastic leptin secretion. This effect seems to be maximal at 5000 IU/ml of hCG, possibly because of saturation of hCG receptors. This new observation suggests that hCG might exert a possible negative feedback on the trophoblastic release of leptin.

Our in-vitro observations are somewhat difficult to reconcile with in-vivo observations in patients with pre-eclampsia. Indeed, it has been reported that this pathology increases circulating VEGF (Sharkey *et al*., 1996) as well as leptin (Mise *et al*., 1998). Both of these increases can be mimicked *in vitro* by culturing trophoblastic cells under hypoxic conditions (Taylor at al., 1997; Grosfeld *et al*., 2001). In contrast, hCG is decreased under such hypoxic culture conditions (Esterman *et al*., 1996). Thus, if these in-vitro results are also true *in vivo*, then in pre-eclamptic pregnancies the stimulation of VEGF by hCG would be reduced. One possible explanation for this discrepancy is that hypoxia-induced VEGF release can be regarded as a compensatory mechanism (angiogenesis) that employs a different pathway from normoxic conditions. However, these in-vitro experiments were carried out with term placentas, where the interactions between hypoxia, hCG and VEGF could be different from first trimester trophoblast. During first trimester pregnancy, we would

speculate that hypoxia induces hCG secretion, which stimulates trophoblastic VEGF release. Clearly the physiological interaction between hCG, leptin and VEGF under hypoxic conditions is a complex one and remains to be studied in an appropriate model. Our data support the idea that trophoblastic products such as hCG and leptin are probably involved in the control of VEGF secretion at the maternal–fetal interface.

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References

Ahmed, A., Li, X.F., Dunk, C., Whittle, M.J., Rushton, D.I. and Rollason, T. (1995) Colocalisation of vascular endothelial growth factor and its Flt-1 receptor in human placentae. *Growth Factors*, **12**, 235–243.

Ahmed, A., Dunk, C., Ahmad, S. and Khaliq, A. (2000) Regulation of placental vascular endothelial growth factor (VEGF) and placenta growth factor (PIGF) and soluble Flt-1 by oxygen. *Placenta*, **21**, S16–S24.

Alon, T., Hemo, I., Itin, A., Peer, J., Stone, J. and Keshet, E. (1995) Vascular endothelial growth factor acts as a survival factor for newly formed retinal vessels and has implications for retinopathy of prematurity. *Nat. Med.*, **1**, 1024–1028.

Athanassiades, A., Hamilton, G.S. and Lala, P.K. (1998) Vascular endothelial growth factor stimulates proliferation but not migration or invasiveness in human extravillous trophoblast. *Biol. Reprod.*, **59**, 643–654.

Bischof, P., Friedli, E., Martelli, M. and Campana, A. (1991) Expression of extracellular matrix-degrading metalloproteinases by cultured human cytotrophoblast cells: effects of cell adhesion and immunopurification. *Am. J. Obstet. Gynecol.*, **165**, 1791–1801.

Cao, R., Brakenhielm, E., Wahlestedt, C., Thyberg, J. and Cao, Y. (2001) Leptin induces vascular permeability and synergistically stimulates angiogenesis with FGF-2 and VEGF. *Proc. Natl. Acad. Sci. USA*, **98**, 6390–6395.

Chardonnens, D., Cameo, P., Aubert, M.L., Pralong, F.P., Islami, D., Campana, A. and Bischof, P. (1999) Modulation of human cytotrophoblastic leptin secretion by interleukin-1α and 17β-oestradiol and its effect on hCG secretion. *Mol. Hum. Reprod.*, **5**, 1077–1082.

Charnock-Jones, D.S., Sharkey, A.M., Boocock, C.A., Ahmed, A., Plevin, R., Ferrara, N. and Smith, S.K. (1994) Vascular endothelial growth factor receptor localisation and activation in human trophoblast and choriocarcinoma cells. *Biol. Reprod.*, **51**, 524–530.

Conn, G., Bayne, M.L., Soderman, D.D., Kwok, P.W., Sullivan, K.A., Palisi, T.M., Hope, D.A. and Thomas, K.A. (1990) Amino acid and cDNA sequences of a vascular endothelial cell mitogen that is homologuos to platelet-derived growth factor. *Proc. Natl Acad. Sci. USA*, **87**, 2628–2632.

deVries, C., Escobedo, J.A., Ueno, H., Houck, K., Ferrara, N. and Williams, L.T. (1992) The fms-like tyrokinase, a receptor for vascular endothelial growth factor. *Science*, **255**, 989–991.

Esterman, A, Finlay, T.H. and Dancis, J. (1996) The effect of hypoxia on term trophoblast: hormone synthesis and release. *Placenta*, **17**, 217–222.

Ferrara, N. (1999) Molecular and biological properties of vascular endothelial growth factor. *J. Mol. Med.*, **77**, 527–543.

Fisher, S.J. (2000) The placenta dilemma. *Semin. Reprod. Med.*, **18**, 321–326.

Folkman, J. and Klagsbrun, M. (1987) Angiogenic factors. *Science*, **235**, 442–447.

Gerber, H.P., Dixit, V. and Ferrara, N. (1998) Vascular endothelail growth factor induces expression of the antiapoptotic proteins Bcl-2 and A1 in vascular endothelail cells. *J. Biol. Chem.*, **273**, 13313–13316.

Geva, E. and Jaffe, R.B. (2000) Role of vascular endothelial growth factor in ovarian physiology and pathology. *Fertil. Steril.*, **74**, 429–438.

Grosfeld, A., Turban, S., Andre, J., Causac, M., Challier, J.C., Haugel-de-Mouzon, S. and Guerre-Millo, M. (2001) Transcriptional effect of hypoxia on placental leptin. *FEBS Lett.*, **502**, 122–126.

Henson, M.C. and Castracane, V.D. (2000) Leptin in pregnancy. *Biol. Reprod.*, **63**, 1219–1228.

Islami, D., Bischof, P. and Chardonnens, D. (2003) Possible interactions between leptin, gonadotrophin releasing hormone (GnRH I and GnRH II) and human chorionic gonadotrophin (hCG). *Eur. J. Obstet. Gynecol. Reprod. Biol.*, in press.

Lee, A., Christenson, L.K., Patton, P.E., Burry, K.A. and Stouffer, R.L. (1997) Vascular endothelial growth factor production by human luteinised granulosa cells in vitro. *Hum. Reprod.*, **12**, 2756–2761.

Licht, P., Russu, V. and Wildt, L. (2001) On the role of human chorionic gonadotropin (hCG) in the embryo endometrial microenvironment: implications for differentiation and implantation. *Semin. Reprod. Med.*, **19**, 37–47.

Mise, H., Sagawa, N., Matsumoto, T., Yura, S., Nanno, H., Itoh, H., Mori, T., Masuzaki, H., Hosoda, K., Ogawa, Y. and Nakano, K. (1998) Augmented placental production of leptin in pre-eclampsia: possible involvement of placental hypoxia. *J. Clin. Endocrinol. Metab.*, **83**, 3225–3229.

Park, H.Y., Kwon, H.M., Lim, H.J., Hong, B.K., Lee, J.Y., Park, B.E., Jang, Y., Cho, S.Y. and Kim, H.S. (2001) Potential role of leptin in angiogenesis: leptin induces endothelial cell proliferation and expression of matrix metalloproteinases in vivo and in vitro. *Exp. Mol. Med.*, **33**, 95–102.

Plouet, J., Schilling, J. and Gospodarowicz, D. (1989) Isolation and characterisation of a newly identified endothelail cell mitogen produced by AtT20 cells. *EMBO J.*, **8**, 3801–3808.

Reynolds, L.P. and Redmer, D.A. (1995) Utero-placental vascular development and placental function. *J. Anim. Sci.*, **73**, 1839–1851.

Reynolds, L.P., Killilea, S.D. and Redmer, D.A. (1992) Angiogenesis in the female reproductive system. *FASEB J.*, **6**, 886–892.

Rizk, B., Aboulghar, M., Smitz, J. and Ron-El, R. (1997) The role of vascular endothelial growth factor and interleukins in the pathogenesis of severe ovarian hyperstimulation syndrome. *Hum. Reprod. Update*, **3**, 255–266.

Roberts, J.M. (1998) Endothelial dysfunction in pre-eclampsia. *Semin. Reprod. Endocrinol.*, **16**, 5–15.

Sharkey, A.M., Charnock-Jones, D.S., Boocock, C.A., Brown, K.D. and Smith, S.K. (1993) Expression of mRNA for vascular endothelial growth factor in human placenta. *J. Reprod. Fertil.*, **99**, 609–615.

Sharkey, A.M., Cooper, J.C., Balmforth, J.R., McLean, J., Clark, D.E. and Charnock-Jones, D.S. (1996) Maternal plasma levels of endothelial growth factor in normotensive pregnancies and in pregnancies complicated by preeclampsia. *Eur. J. Clin. Invest.*, **26**, 1182–1185.

Shore, V.H., Wang, T.H., Wng, C.L., Torry, R.J., Caudle, M.R. and Torry, D.S. (1997) Vascular endothelial growth factor, placenta growth factor adn their receptors in isolated human trophoblast. *Placenta*, **18**, 657–665.

Sierra-Honigmann, M.R., Nath, A.K., Murakami, C., Garcia-Cardena, G., Papapetropoulos, A., Sessa, W.C., Madge, L.A., Schechner, J.S., Savwabb, M.B., Polveriui, P.J. and Flores-Riveros, J.R. (1998) Biological action of leptin as an angiogenic factor. *Science*, **281**, 1683–1686.

Smith, S.K. (2000) Angiogenesis and implantation. *Hum. Reprod.*, **15** (Suppl. 6), 59–66.

Taylor, C.M., Stevens, H., Anthony, F.W. and Wheeler, T. (1997) Influence of hypoxia on vascular endothelial growth factor and chorionic gonadotropin production in the trophoblast derived cell lines: Jeg, Jar, and BeWo. *Placenta*, **18**, 451–458.

Terman, B.I., Dougher-Vermazen, M., Carrion, M.E., Dimitrov, D., Armellino, D.C., Gospodorowicz, D. and Bohlen, P. (1992) Identification of the KDR tyrosine kinase as a receptor for vascular endothelial cell growth factor. *Biochem. Biophys. Res. Commun.*, **187**, 1579–1586.

Vuckovic, M., Ponting, J., Terman, B.I., Niketic, V., Seif, V. and Kumar, S. (1996) Expression of the vascular endothelial growth factor receptor, KDR, in human placenta. *J. Anat.*, **188**, 361–366.

Vuorela, P., Hatva, E., Lymboussaki, A., Kaipainen, A., Joukov, V., Persico, M.G., Alitalo, K. and Halmesmaki, E. (1997) Expression of vascular endothelial growth factor and placenta growth factor in human placenta. *Biol. Reprod.*, **56**, 489–494.

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