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Human chorionic gonadotropin: A hormone with immunological and angiogenic properties

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ABSTRACT

The success of implantation depends on a receptive endometrium, a normal blastocyst and synchronized cross-talk at the maternal–fetal interface. The progression of pregnancy then requires immunological tolerance which allows conceptus survival. A cascade of cytokines mediates this dialogue and is crucial in the cross-talk between the immune and endocrine systems. The first known human embryo-derived signal is chorionic gonadotropin (hCG) by which the embryo profoundly influences immunological tolerance and angiogenesis at the maternal–fetal interface. hCG levels coincide with the development of trophoblast tolerance. Indeed, it increases the number of uterine natural killer cells that play a key role in the establishment of pregnancy. hCG also intervenes in the development of local immune tolerance through the cellular system of apoptosis via Fas/Fas-Ligand. It modulates the Th1/Th2 balance and acts on complement C3 and C4A/B factors modulating decidual immunity. The transient tolerance evident during gestation is at least partially achieved via the presence of regulatory T cells which are attracted by hCG at the fetal–maternal interface. Finally, hCG treatment of activated dendritic cells results in an up-regulation of MHC class II, IL-10 and IDO expression, reducing the ability to stimulate T cell proliferation. Successful implantation requires an extensive endometrial angiogenesis in the implantation site. Recent data demonstrate angiogenic effects of hCG via its interaction with endometrial and endothelial LH/hCG receptors. Our review focuses on these functions of hCG, giving new insight into the endocrine–immune dialogue that exists between the conceptus and immune cells within the receptive endometrium at the time of implantation.

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Abbreviations: FSH, follicle-stimulating hormone; hCG, human chorionic gonadotropin; IL, interleukine; LH, luteinizing hormone; IDO, indoleamine 2,3-dioxygenase; IGF, insulin-like growth factor; IGF-BP, insulin-like growth factor binding protein; LH-R, LH-receptor; M-CSF, macrophage-colony stimulating factor; MMP, matrix metalloproteinase; Th, T helper lymphocyte; TIMP, tissue inhibitor of metalloproteinase; TSH, thyroid stimulating hormone; uNK, uterine natural killer; VEGF, vascular endothelium growth factor.

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1. Introduction

Implantation is a very complex and tightly coordinated process whereby the trophoblast establishes intimate contact with its mother's specialized tissue, the endometrium. Normal implantation is decisive for a successful pregnancy. The embryo apposes then adheres to the maternal endometrium and initiates a close dialogue with it. Successful pregnancy requires two actors: the receptive endometrium and the functionally normal blastocyst, both cross-talking in a paracrine mode at the maternal–fetal interface. At the maternal level, the steroid hormones, estrogens and progesterone, prepare the endometrium to

achieve an adequate receptive status, called the “implantation window” (Psychoyos, 1986), which is the optimal period for embryo implantation; while a network of redundant and interconnected molecules are the local mediators of the dialogue at the maternal–embryonic interface (Fazleabas et al., 2004; Minas et al., 2005; Perrier d’Hauterive, 2004). Among the numerous mediators of this cross-talk of maternal and embryonic origin, we will focus on the specific and very early embryonic signal: human chorionic gonadotropin hormone (hCG) and its role in pregnancy immunology and angiogenesis. This hormone is the specific mediator that announces the embryo’s presence to the maternal organism.

2. hCG and embryo implantation

hCG is a heterodimeric placental hormone that belongs to the glycoprotein hormone family, such as LH, FSH and TSH. These hormones are composed of two subunits linked in a non-covalent way. The alpha subunit is identical amongst all members of the family and is coded on chromosome 6. The beta subunit, which is different and unique for each hormone, is encoded by different genes located on chromosome 19 (LH, hCG and TSH) or on chromosome 11 (FSH). The hCG beta subunit is encoded by six different but very similar genes located in a gene cluster on chromosome 19 together with the beta-luteinizing hormone gene (Policastro et al., 1986). The beta hCG is the biggest beta subunit with a large glycosylated domain. This confers upon hCG a higher stability and facilitates its rapid secretion. The beta subunits of LH and hCG have 96% homology that allow them to share the same receptor, the LH/hCG-Receptor (LH-R). hCG binds to this receptor with an affinity 4–5-fold higher compared to LH. Unlike the other glycoproteins which are synthesized in the anterior lobe of the pituitary gland, hCG is principally produced by trophoblasts (and particularly the syncytium), but also by some malignant tumors. hCG is involved in different pregnancy-promoting processes: maternal pregnancy recognition, corpus luteum survival, stimulation of progesterone production, enhancement of embryo implantation, control of trophoblast differentiation, stimulation of angiogenesis and finally regulation of the maternal–fetal immune relationship (Ticconi et al., 2007).

hCG is one of the earliest molecules produced by the embryo. Indeed, its mRNA is transcribed as early as the 8-cell stage (Jurisicova et al., 1999) and the blastocyst produces the protein before its implantation (Bonduelle et al., 1988; Lopata and Hay, 1989). hCG is increasingly produced after implantation by the syncytiotrophoblast (Hoshina et al., 1985). Significant levels of hCG can already be measured in the maternal blood 10 days after ovulation. The peak of hCG production by the placenta is reached between the 10th and the 11th week of gestation, then the production decreases at the 12th week to remain at low levels for the remainder of pregnancy. hCG affects the corpus luteum to prevent luteolysis and favour stimulation of progesterone production. These actions represent the major and first described roles of this molecule in early pregnancy (Keay et al., 2004). But more recently, significant paracrine

roles of hCG in the modulation of the uterine environment and in preparation for implantation were described. Modulation of endometrial cell activities (epithelial, stromal, myometrial, endothelial or immunological cells) occurs prior to and in preparation for blastocyst implantation has been shown (Berndt et al., 2006, 2009; Cole, 2009; Perrier d’Hauterive et al., 2004, 2005).

During embryo implantation, endometrial epithelial cells acquire high expression of trophinin, a mediator of cell adhesion by homophilic binding at the apical surface of trophoctoderm and epithelium. The capacity for apical cell adhesion with trophinin-expressing human trophoblast cells is increased in presence of hCG associated with IL-1 beta (Sugihara et al., 2008).

In myometrial smooth muscle cells, hCG induces not only the proliferation of the cells (Horiuchi et al., 2000; Kornyei et al., 1993; Lee et al., 2004) but also a reduction of their contractibility via the regulation of the gap junctions between smooth muscle cells and intracellular calcium, as well as an increase of progesterone receptor expression, which together allow the implantation of the blastocyst.

Finally, blastocyst hCG also plays important roles in immune tolerance of the fetal allograft and also increases endometrial angiogenesis, so that it participates actively in placental development (Berndt et al., 2006; Zygmunt et al., 2002).

Our review focuses on these functions of hCG, giving new insight into the endocrine–immune dialogue that exists between the conceptus and immune cells within the receptive endometrium at the time of implantation.

3. The LH/hCG receptor (LH/hCG-R)

The LH/hCG-R is a member of the rhodopsin family cluster within the large G-protein coupled receptor superfamily that contains some 800–900 genes in the human genome (Puett et al., 2007). LH/hCG-R forms one of the three classes in this cluster and is encoded on chromosome 2. This unique gene is composed of 10 introns and 11 exons, and span approximately 80 kb. Its cDNA encodes a glycoprotein of 675 amino acids. This receptor associates two functional units: a large ectodomain containing leucine-rich repeats which permit the recognition and the specific binding of hCG (or LH) coupled to 7 transmembrane domains and an intracellular segment bound to G protein. This segment allows the signal transduction generated by hormone binding to the extracellular domain (Ascoli and Segaloff, 1989; Dufau, 1998). This G-protein coupled receptor activates mainly the cAMP/PKA pathway (Srisuparp et al., 2003). Both hCG and LH activities are mediated by the same LH/hCG-R, but some hCG properties have been described to be mediated via the mannose receptor (CD206) rather than by the classical LH/hCG-R (Kane et al., 2009). The expression of LH/hCG-R was previously thought to be restricted to gonadal tissues but recent studies have shown its presence in many other tissues throughout the reproductive organs. Moreover, luminal and glandular epithelial cells contain more LH/hCG-R compared to stromal, myometrial, and vascular smooth muscle cells (Reshef et al., 1990).

4. hCG effects on endometrium and immunity

The process of acceptance of the foreign blastocyst by the maternal endometrium is complex and requires the dialogue between many systems. In parallel with its direct action on endometrium (epithelium and stroma), hCG also contributes to maternal tolerance of the embryo. This function, which exemplifies the intimate inter-relationship between the immune and endocrine systems, is mediated through different mechanisms shown by several studies.

In the immune system, lymphocytes acquire the ability to discriminate between self and non-self both centrally in neonatal thymus and peripherally. The embryo/trophoblast unit, with distinctive immunologic and genetic characteristics from the maternal and paternal gametes, is a semi-allograft that needs to be tolerated and not rejected by the maternal immune response (Fraccaroli et al., 2009). This has to be achieved as soon as the blastocyst hatches from the immunologically inactive zona pellucida. T cells represent around 20–30% of the leukocyte population present in human endometrium and they are involved in adaptive/specific immunity responsible for tolerance or rejection (as Th2 and Th1 responses) of the fetal allograft. During pregnancy hCG down-regulates Th1 cells, CD8⁺ T cells and macrophages, up-regulates Th2 cells and increases the ratio of CD4⁺CD25⁺/CD4⁺ T cells in the spleen and pancreatic lymph nodes.

To investigate the direct effects of hCG on the human endometrium, an intrauterine microdialysis device was developed by Licht et al. (Licht et al., 1998) to measure paracrine mediators within the uterine cavity *in vivo*. Using this system, hCG was administered in the secretory phase and the endometrial response was evaluated. The administration of hCG (500 IU/ml) provoked a significant inhibition of intrauterine IGF-binding protein-1 (IGF-BP-1) (a marker of decidualization) and M-CSF, while LIF (a cytokine required for embryo implantation), VEGF (a pro-angiogenic growth factor) and MMP-9 (a regulator of tissue remodelling) were significantly stimulated. These effects demonstrate important paracrine effects of hCG on decidualization, tissue remodelling, implantation and on vascularization and angiogenesis, suggesting that the embryo increases the duration of the implantation window. Moreover, hCG as the first hormonal signal of the embryo selectively reduces IGF-I and IGF-BP-1 and thus postpones the decidual reaction. It may therefore contribute to the modulation of endometrial receptivity and differentiation during early implantation (Fluhr et al., 2008b). Finally, by increasing trophoblastic MMP-9 secretion and by reducing endometrial TIMP-1, -2 and -3 expression, hCG might be an important tool for the invading embryo to regulate the depth of its implantation (Fluhr et al., 2008a).

4.1. hCG and Th1/Th2 balance

During pregnancy, the balance of Th1 (cell-mediated immunity) and Th2 (humoral immunity) cytokines is characterized by an initial prevalence (but not an exclusivity) of Th2 cytokines, followed by a progressive shift towards

Th1 predominance late in gestation. When this balance is abnormal, it may initiate and intensify the cascade of inflammatory cytokine production involved in adverse pregnancy outcomes (Challis et al., 2009). In a more general way, Khan et al. showed that the administration of hCG to nonobese diabetic mice (NOD) before the beginning of clinical symptoms of diabetes reduced the increase in glycaemia, reversed establishment of insulinitis, and inhibited the development of Th1 autoimmune diabetes (Khan et al., 2001).

A recent large proteomic study demonstrated the influence of several molecules produced by the trophoblast that regulate the mother's immune tolerance. Among these molecules, hCG is implicated in inhibiting T lymphocytes (Dong et al., 2008).

4.2. hCG and regulatory T cells

The transient tolerance during gestation is thought to be achieved partially via the presence of CD4⁺CD25⁺Foxp3⁺ regulatory T cells (Treg) (Schumacher et al., 2009). hCG increases the pool of these cells in the periphery during pregnancy (Zenclussen et al., 2006). Moreover, according to Schumacher et al., Treg cells may be attracted to the maternal–fetal interface by a mechanism involving high blastocyst and trophoblast hCG production.

4.3. hCG and uNK

Kane et al. has recently described hCG as a new regulator of uterine natural killer (uNK) cell proliferation, mediated via the mannose receptor (CD206), a member of the c-type lectin receptor family that binds glycoproteins with N-linked carbohydrate side chains such as those present on pituitary hormones (Simpson et al., 1999), rather than by the classical LH/hCG-R that was not expressed. These novel observations provide new interesting insight into the endocrine–immune dialogue that exists between the conceptus and immune cells within the receptive endometrium, and have implications for the role of uNK cell–trophoblast interactions and pregnancy outcome (Kane et al., 2009).

The endometrium contains a population of CD56bright CD16dim uterine natural killer (uNK) cells that are distinct from circulating peripheral CD56dimCD16bright NK cells (Kalkunte et al., 2008). Natural killer cells, implicated in innate immunity in the gravid uterus, represent around 70% of the endometrial leukocyte population. The number of these endometrial uNK cells increases at the onset of decidualization during the secretory phase of the menstrual cycle with a further increase in the number of endometrial uNK cells when the conceptus implants and during the early stages of placentation. As a result uNK cells become the predominant lymphocyte subtype in the decidua during the first trimester of pregnancy. It is clearly demonstrated that uNK cells play a key role in the establishment, maintenance, and regulation of early pregnancy in particular in the remodelling of uterine spiral arteries, required for adequate vascular supply to the placenta (Moffett and Loke, 2006).

4.4. hCG and Fas/Fas-L system

Kayisli et al. suggested that hCG could be a key placental factor for the development of local immune tolerance through another mechanism, via the cellular system of programmed death mediated by Fas/Fas-Ligand (Kayisli et al., 2003). hCG up-regulates FasL in the human endometrium allowing trophoblastic invasion into the endometrium but also inducing apoptosis in T cells and endometrial cells. Thus hCG contributes to both the controlled trophoblastic invasion and immune tolerance of trophoblast.

4.5. hCG and complement system

Sherwin et al. demonstrated the up-regulation by hCG of the expression of C3 and C4A/B gene expression in the baboon endometrium, suggesting a role for hCG in modulation of the peri-implantation and decidual immune environment. C3 is integral to the activation of complement via the classical, alternative, and lectin activation pathways, with C4 being part of the classical activation pathway. The multiple actions of C3 allow it to promote phagocytosis, support local inflammatory responses to pathogens, and also to select appropriate antigens for the humoral response. C3 has been localized to stromal and glandular compartments in the secretory phase of the menstrual cycle although studies of endometrial complement expression are limited. The biological role of glycodefin, which is the most abundant secreted protein in early human pregnancy, is unclear, although *in vitro* immunosuppressive activity has been demonstrated. Taken together, the up-regulation of complement C3, C4, and glycodefin expression suggests that hCG may help orchestrate the endometrial immune adaptation to pregnancy (Sherwin et al., 2007).

4.6. hCG effect on IDO

hCG treatment of activated dendritic cells induces up-regulation of MHC class II expression, and increases IL-10 and IDO expression, which together lead to a decreased ability to stimulate T cell proliferation (Wan et al., 2008). hCG also up-regulates IDO in placenta and this accounts for the ability of hCG to suppress autoimmune diabetes in NOD mice (Ueno et al., 2007). This modulating influence of hCG on dendritic cell differentiation and function may have an important contribution to maternal–fetal tolerance as well as the remission of several autoimmune diseases during pregnancy (Wan et al., 2008).

4.7. hCG and macrophages

hCG can promote innate functions of macrophages, such as the clearance of apoptotic cells and the resolution of inflammation, which are highly relevant for the maintenance of pregnancy (Wan et al., 2007). Results of another study demonstrated that high doses of hCG induced IL-8 production by monocytes. Its signal is not mediated by the classical LH/hCG-R but probably via primitive systems such as C-type lectins (Kosaka et al., 2002).

5. hCG and angiogenesis

Angiogenesis is a fundamental process by which new capillary blood vessels form from pre-existing ones, regulated by vascular endothelial cell-specific growth factors and inhibitors. The female reproductive system undergoes physiological angiogenesis during the menstrual cycle, folliculogenesis, ovulation and corpus luteum formation and implantation, in particular during placenta formation (Gutman et al., 2008). Among angiogenic factors, VEGF is viewed as a prime regulatory factor of blood vessel growth during angiogenesis since it is a highly specific mitogen for endothelial cells and it induces angiogenesis and increases permeability of blood vessels. Successful implantation, placentation and subsequent gestation require coordinated and finely regulated vascular development and adaptations on both sides of the maternal–fetal interface. Due to high demand for increased blood supply, the vasculature of the uterus and endometrium undergoes three main adaptative changes: vasodilatation, increased permeability and development and maturation of new vessels (Torry et al., 2007). Disturbance in uterine blood supply or vascular remodelling is associated with higher fetal morbidity and mortality due to miscarriages, pre-eclampsia or intrauterine growth restriction. The physiological changes in uterine vascular remodelling are regulated by growth factors such as VEGF, as well as by hormonal factors from maternal or trophoblast origin.

hCG acts on several molecules implicated in angiogenesis such as VEGF and both its receptors VEGFR-1 and -2, but also on the angiopoietins and their receptor Tie-2, basic fibroblast growth factor or placental-derived growth factor (Reisinger et al., 2007).

The presence of LH/hCG-R on endothelial cells of the uterine vessels has been described (Toth et al., 1994). Toth et al. showed that the *in vivo* administration of hCG reduces vascular resistance in the human uterus and reduces *in vitro* the vasoconstrictor eicosanoids of the vascular wall (Toth et al., 2001). hCG is now proposed as a new angiogenic factor (Zygmunt et al., 2002). In a 3D model of *in vitro* angiogenesis, hCG promotes angiogenesis by supporting the migration and the formation of capillary structures by the uterine endothelial cells. Berndt et al. demonstrated an increase in vessel sprouting with hCG deposited on endothelial endometrial cells (in aortic ring and matrigel plug assays) and mediated via the LH/hCG-R. An indirect effect was also showed via the increase of VEGF in presence of hCG (Berndt et al., 2006). Finally, Herr et al. showed that hCG stimulates proliferation of human placental microvascular endothelial cells (HPMVEC) in a dose-dependent manner and stimulated sprout formation when compared to controls in a spheroid angiogenesis assay (Herr et al., 2007).

6. Summary

The mechanisms underlying human implantation and particularly immune tolerance of pregnancy still remain to be defined in detail. hCG is the prime mediator by which the embryo announces its presence to the maternal organism since it is produced even before implantation. Among the

wide range of mediators present at the implantation site, a role is becoming evident for hCG as specific blastocyst signal involved in orchestrating the implantation cascade. hCG is implicated in several actions that promote tolerance and angiogenesis and thus has physiological important implications for successful pregnancy.

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References

- Ascoli, M., Segaloff, D.L., 1989. On the structure of the luteinizing hormone/chorionic gonadotropin receptor. *Endocr. Rev.* 10, 27–44.
- Berndt, S., Blacher, S., Perrier d'Hauterive, S., Thiry, M., Tsampalas, M., Cruz, A., Péqueux, C., Lorquet, S., Munaut, C., Noël, A., Foidart, J.M., 2009. Chorionic gonadotropin stimulation of angiogenesis and pericyte recruitment. *J. Clin. Endocrinol. Metab.* 94 (11), 4567–4574, Epub 2009 Oct 16.
- Berndt, S., Perrier d'Hauterive, S., Blacher, S., Pequeux, C., Lorquet, S., Munaut, C., Applanat, M., Herve, M.A., Lamande, N., Corvol, P., van den Brule, F., Frankenne, F., Poutanen, M., Huhtaniemi, I., Geenen, V., Noel, A., Foidart, J.M., 2006. Angiogenic activity of human chorionic gonadotropin through LH receptor activation on endothelial and epithelial cells of the endometrium. *FASEB J.* 20, 2630–2632.
- Bonduelle, M.L., Dodd, R., Liebaers, I., Van Steirteghem, A., Williamson, R., Akhurst, R., 1988. Chorionic gonadotrophin-beta mRNA, a trophoblast marker, is expressed in human 8-cell embryos derived from triprounulate zygotes. *Hum. Reprod.* 3, 909–914.
- Challis, J.R., Lockwood, C.J., Myatt, L., Norman, J.E., Strauss 3rd, J.F., Petraglia, F., 2009. Inflammation and pregnancy. *Reprod. Sci.* 16, 206–215.
- Cole, L.A., 2009. New discoveries on the biology and detection of human chorionic gonadotropin. *Reprod. Biol. Endocrinol.* 7, 8.
- Dong, M., Ding, G., Zhou, J., Wang, H., Zhao, Y., Huang, H., 2008. The effect of trophoblasts on T lymphocytes: possible regulatory effector molecules—a proteomic analysis. *Cell Physiol. Biochem.* 21, 463–472.
- Dufau, M.L., 1998. The luteinizing hormone receptor. *Annu. Rev. Physiol.* 60, 461–496.
- Fazleabas, A.T., Kim, J.J., Strakova, Z., 2004. Implantation: embryonic signals and the modulation of the uterine environment—a review. *Placenta* 25 (Suppl. A), S26–31.
- Fluhr, H., Bischof-Islami, D., Krenzer, S., Licht, P., Bischof, P., Zygumunt, M., 2008a. Human chorionic gonadotropin stimulates matrix metalloproteinases-2 and -9 in cytotrophoblastic cells and decreases tissue inhibitor of metalloproteinases-1, -2, and -3 in decidualized endometrial stromal cells. *Fertil. Steril.* 90, 1390–1395.
- Fluhr, H., Carli, S., Deperschmidt, M., Wallwiener, D., Zygumunt, M., Licht, P., 2008b. Differential effects of human chorionic gonadotropin and decidualization on insulin-like growth factors-I and -II in human endometrial stromal cells. *Fertil. Steril.* 90, 1384–1389.
- Fraccaroli, L., Alfieri, J., Leiros, C.P., Ramhorst, R., 2009. Immunomodulatory effects of chemokines during the early implantation window. *Front. Biosci. (Elite Ed.)* 1, 288–298.
- Gutman, G., Barak, V., Maslovitz, S., Amit, A., Lessing, J.B., Geva, E., 2008. Regulation of vascular endothelial growth factor-A and its soluble receptor sFlt-1 by luteinizing hormone in vivo: implication for ovarian follicle angiogenesis. *Fertil. Steril.* 89, 922–926.
- Herr, F., Baal, N., Reisinger, K., Lorenz, A., McKinnon, T., Preissner, K.T., Zygumunt, M., 2007. HCG in the regulation of placental angiogenesis. Results of an in vitro study. *Placenta* 28 (Suppl. A), S85–S93.
- Horiuchi, A., Nikaido, T., Yoshizawa, T., Itoh, K., Kobayashi, Y., Toki, T., Konishi, I., Fujii, S., 2000. HCG promotes proliferation of uterine leiomyoma cells more strongly than that of myometrial smooth muscle cells in vitro. *Mol. Hum. Reprod.* 6, 523–528.
- Hoshina, M., Boothby, M., Hussa, R., Pattillo, R., Camel, H.M., Boime, I., 1985. Linkage of human chorionic gonadotrophin and placental lactogen biosynthesis to trophoblast differentiation and tumorigenesis. *Placenta* 6, 163–172.
- Juriscova, A., Antenos, M., Kapasi, K., Meriano, J., Casper, R.F., 1999. Variability in the expression of trophoblastic markers beta-human chorionic gonadotrophin, human leukocyte antigen-G and pregnancy specific beta-1 glycoprotein by the human blastocyst. *Hum. Reprod.* 14, 1852–1858.
- Kalkunte, S., Chichester, C.O., Gotsch, F., Sentman, C.L., Romero, R., Sharma, S., 2008. Evolution of non-cytotoxic uterine natural killer cells. *Am. J. Reprod. Immunol.* 59, 425–432.
- Kane, N., Kelly, R., Saunders, P.T., Critchley, H.O., 2009. Proliferation of uterine natural killer cells is induced by human chorionic gonadotropin and mediated via the mannose receptor. *Endocrinology* 150, 2882–2888.
- Kayisli, U.A., Selam, B., Guzeloglu-Kayisli, O., Demir, R., Arici, A., 2003. Human chorionic gonadotropin contributes to maternal immunotolerance and endometrial apoptosis by regulating Fas-Fas ligand system. *J. Immunol.* 171, 2305–2313.
- Keay, S.D., Vatis, M., Karteris, E., Hillhouse, E.W., Randevara, H.S., 2004. The role of hCG in reproductive medicine. *BJOG* 111, 1218–1228.
- Khan, N.A., Khan, A., Savelkoul, H.F., Benner, R., 2001. Inhibition of diabetes in NOD mice by human pregnancy factor. *Hum. Immunol.* 62, 1315–1323.
- Kornyeyi, J.L., Lei, Z.M., Rao, C.V., 1993. Human myometrial smooth muscle cells are novel targets of direct regulation by human chorionic gonadotropin. *Biol. Reprod.* 49, 1149–1157.
- Kosaka, K., Fujiwara, H., Tatsumi, K., Yoshioka, S., Sato, Y., Egawa, H., Higuchi, T., Nakayama, T., Ueda, M., Maeda, M., Fujii, S., 2002. Human chorionic gonadotropin (HCG) activates monocytes to produce interleukin-8 via a different pathway from luteinizing hormone/HCG receptor system. *J. Clin. Endocrinol. Metab.* 87, 5199–5208.
- Lee, T.K., Kim, D.I., Song, Y.L., Lee, Y.C., Kim, H.M., Kim, C.H., 2004. Differential inhibition of *Scutellaria barbata* D. Don (Lamiaceae) on HCG-promoted proliferation of cultured uterine leiomyoma and myometrial smooth muscle cells. *Immunopharmacol. Immunotoxicol.* 26, 329–342.
- Licht, P., Losch, A., Dittrich, R., Neuwinger, J., Siebzehrubel, E., Wildt, L., 1998. Novel insights into human endometrial paracrinology and embryo-maternal communication by intrauterine microdialysis. *Hum. Reprod. Update* 4, 532–538.
- Lopata, A., Hay, D.L., 1989. The potential of early human embryos to form blastocysts, hatch from their zona and secrete HCG in culture. *Hum. Reprod.* 4, 87–94.
- Minas, V., Loutradis, D., Makrigiannakis, A., 2005. Factors controlling blastocyst implantation. *Reprod. Biomed. Online* 10, 205–216.
- Moffett, A., Loke, C., 2006. Immunology of placentation in eutherian mammals. *Nat. Rev. Immunol.* 6, 584–594.
- Perrier d'Hauterive, S., 2004. Implantation: the first maternal-embryo crosstalk. *J. Gynecol. Obstet. Biol. Reprod. (Paris)* 33, S5–8.
- Perrier d'Hauterive, S., Charlet-Renard, C., Berndt, S., Dubois, M., Munaut, C., Goffin, F., Hagelstein, M.T., Noel, A., Hazout, A., Foidart, J.M., Geenen, V., 2004. Human chorionic gonadotropin and growth factors at the embryonic-endometrial interface control leukemia inhibitory factor (LIF) and interleukin 6 (IL-6) secretion by human endometrial epithelium. *Hum. Reprod.* 19, 2633–2643.
- Perrier d'Hauterive, S., Charlet-Renard, C., Dubois, M., Berndt, S., Goffin, F., Foidart, J.M., Geenen, V., 2005. Human endometrial leukemia inhibitory factor and interleukin-6: control of secretion by transforming growth factor-beta-related members. *Neuroimmunomodulation* 12, 157–163.
- Policastro, P.F., Daniels-McQueen, S., Carle, G., Boime, I., 1986. A map of the hCG beta-LH beta gene cluster. *J. Biol. Chem.* 261, 5907–5916.
- Psychoyos, A., 1986. Uterine receptivity for nidation. *Ann. N.Y. Acad. Sci.* 476, 36–42.
- Puett, D., Li, Y., DeMars, G., Angelova, K., Fanelli, F., 2007. A functional transmembrane complex: the luteinizing hormone receptor with bound ligand and G protein. *Mol. Cell Endocrinol.* 260–262, 126–136.
- Reisinger, K., Baal, N., McKinnon, T., Munstedt, K., Zygumunt, M., 2007. The gonadotropins: tissue-specific angiogenic factors? *Mol. Cell Endocrinol.* 269, 65–80.
- Reshef, E., Lei, Z.M., Rao, C.V., Pridham, D.D., Chegini, N., Luborsky, J.L., 1990. The presence of gonadotropin receptors in nonpregnant human uterus, human placenta, fetal membranes, and decidua. *J. Clin. Endocrinol. Metab.* 70, 421–430.
- Schumacher, A., Brachwitz, N., Sohr, S., Engeland, K., Langwisch, S., Dolapchieva, M., Alexander, T., Taran, A., Malferteiner, S.F., Costa, S.D., Zimmermann, G., Nitschke, C., Volk, H.D., Alexander, H., Gunzer, M., Zenclussen, A.C., 2009. Human chorionic gonadotropin attracts regulatory T cells into the fetal-maternal interface during early human pregnancy. *J. Immunol.* 182, 5488–5497.

- Sherwin, J.R., Sharkey, A.M., Cameo, P., Mavrogianis, P.M., Catalano, R.D., Edassery, S., Fazleabas, A.T., 2007. Identification of novel genes regulated by chorionic gonadotropin in baboon endometrium during the window of implantation. *Endocrinology* 148, 618–626.
- Simpson, D.Z., Hitchen, P.G., Elmhirst, E.L., Taylor, M.E., 1999. Multiple interactions between pituitary hormones and the mannose receptor. *Biochem. J.* 343 (Pt 2), 403–411.
- Srisuparp, S., Strakova, Z., Brudney, A., Mukherjee, S., Reierstad, S., Hunzicker-Dunn, M., Fazleabas, A.T., 2003. Signal transduction pathways activated by chorionic gonadotropin in the primate endometrial epithelial cells. *Biol. Reprod.* 68, 457–464.
- Sugihara, K., Kabir-Salmani, M., Byrne, J., Wolf, D.P., Lessey, B., Iwashita, M., Aoki, D., Nakayama, J., Fukuda, M.N., 2008. Induction of trophinin in human endometrial surface epithelia by CGbeta and IL-1beta. *FEBS Lett.* 582, 197–202.
- Ticconi, C., Zicari, A., Belmonte, A., Realacci, M., Rao Ch, V., Piccione, E., 2007. Pregnancy-promoting actions of HCG in human myometrium and fetal membranes. *Placenta* 28 (Suppl. A), S137–143.
- Torry, D.S., Leavenworth, J., Chang, M., Maheshwari, V., Groesch, K., Ball, E.R., Torry, R.J., 2007. Angiogenesis in implantation. *J. Assist. Reprod. Genet.* 24, 303–315.
- Toth, P., Li, X., Rao, C.V., Lincoln, S.R., Sanfilippo, J.S., Spinnato 2nd, J.A., Yussman, M.A., 1994. Expression of functional human chorionic gonadotropin/human luteinizing hormone receptor gene in human uterine arteries. *J. Clin. Endocrinol. Metab.* 79, 307–315.
- Toth, P., Lukacs, H., Gimes, G., Sebestyen, A., Pasztor, N., Paulin, F., Rao, C.V., 2001. Clinical importance of vascular LH/hCG receptors—a review. *Reprod. Biol.* 1, 5–11.
- Ueno, A., Cho, S., Cheng, L., Wang, J., Hou, S., Nakano, H., Santamaria, P., Yang, Y., 2007. Transient upregulation of indoleamine 2,3-dioxygenase in dendritic cells by human chorionic gonadotropin downregulates autoimmune diabetes. *Diabetes* 56, 1686–1693.
- Wan, H., Versnel, M.A., Cheung, W.Y., Leenen, P.J., Khan, N.A., Benner, R., Kiekens, R.C., 2007. Chorionic gonadotropin can enhance innate immunity by stimulating macrophage function. *J. Leukoc. Biol.* 82, 926–933.
- Wan, H., Versnel, M.A., Leijten, L.M., van Helden-Meeuwsen, C.G., Fekkes, D., Leenen, P.J., Khan, N.A., Benner, R., Kiekens, R.C., 2008. Chorionic gonadotropin induces dendritic cells to express a tolerogenic phenotype. *J. Leukoc. Biol.* 83, 894–901.
- Zenclussen, A.C., Gerlof, K., Zenclussen, M.L., Ritschel, S., Zambon Bertoja, A., Fest, S., Hontsu, S., Ueha, S., Matsushima, K., Leber, J., Volk, H.D., 2006. Regulatory T cells induce a privileged tolerant microenvironment at the fetal–maternal interface. *Eur. J. Immunol.* 36, 82–94.
- Zygmunt, M., Herr, F., Keller-Schoenwetter, S., Kunzi-Rapp, K., Munstedt, K., Rao, C.V., Lang, U., Preissner, K.T., 2002. Characterization of human chorionic gonadotropin as a novel angiogenic factor. *J. Clin. Endocrinol. Metab.* 87, 5290–5296.