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Summary

The regulation of human implantation is not fully understood. HCG as one of the earliest embryonal signals may be a major regulator in the parakrine embryo-endometrial communication. The expression of full-length hCG/LH-receptor mRNA could be demonstrated in human endometrium throughout the follicular and secretory phase of the menstrual cycle. In contrast, in early pregnancy decidua only truncated variants could be detected. To investigate direct effects of hCG on the human endometrium, an intrauterine microdialysis device was developed to measure parakrine mediators within the uterine cavity in vivo. Using this system, hCG was applied in the secretory phase and the endometrial response was evaluated. The administration of hCG (500 IU/ml) provoked a significant inhibition of intrauterine IGFBP-1 and M-CSF, while LIF, VEGF and MMP-9 were significantly stimulated. Taken together there appear to be multiple direct effects of hCG on the endometrium that precede the classical endocrine role of the hormone.

Introduction

Human implantation is one of the final frontiers in reproductive medicine. While there are sophisticated stimulation protocols to achieve follicular maturation in the majority of patients and the laboratory techniques for fertilisation and early embryo development have improved dramatically over the last decade, little is known about what happens to the embryos after the transfer into the uterine cavity. Since up to 75 % of embryos transfered in IVF/ET programs do not implant in the endometrium, the further improvement of pregnancy rates requires more insight into the molecular mechanisms governing the endometrial preparation for implantation and into the implantation process itself.

Our laboratory has recently developed an intrauterine microdialysis device (IUMD) that allows the measurement of parakrine endometrial mediators in the human female in vivo and to investigate the effects of embryonic products (e.g. human chorionic gonadotropin, hCG) as well as of pharmacological interventions on endometrial differentiation and function (Licht et al. 1998; Licht et al. 2001)

Using this system, we have investigated the effects of hCG on several parakrine parameters relevant in the endometrium. As hCG is one of the first embryonal signals sent to the maternal endometrium, it is of great interest to investigate possible effects of this hormone on the endometrial milieu. The results of these “in vivo”-data were subsequently confirmed on a molecular level in endometrial cell culture models.

Human endometrium and implantation

The preparation of human endometrium for implantation requires an appropriate secretory transformation driven by the sequential actions of estradiol and progesterone. From artificial cycles applied in oocyte donation programs we know, that probably no other factors are essential for the crude endometrial preparation.

Implantation itself can only take place during a narrow time frame in the secretory phase that is usually referred to as “implantation window”. While there is still no general consensus about the duration of this window of receptivity, most researchers agree that it is located somewhere between days 5 and 10 after the beginning of the LH-peak. In epidemiological studies, Wilcox and coworkers (Wilcox et al. 1999) have shown, that implantations before day 5 do not occur, while delayed implantations after day 10 are possible, but lead to an exponential increase of early abortions. Therefore, the mechanisms that control the opening and especially the closing of this window of receptivity appear to be of great importance.

The embryo-endometrial dialogue

The “fine-tuning” of endometrial receptivity appears to be modulated by an embryo-endometrial “cross-talk” via parakrine factors. After fertilization in the fallopian tubes, the embryo arrives in the uterine cavity on days 5-6 as a blastocyst ready to hatch from its zona pellucida. The incoming blastocyst is already capable of secreting several parakrine signals (e.g. human chorionic gonadotropin (hCG), interleukin (IL)-1, and insulin-like growth factor (IGF)-2) that may modulate endometrial differentiation and function at the implantation site (Simon et al. 1996; Tabibzadeh et al. 1990; Tabibzadeh and Babaknia 1995). On the other hand, the endometrium is known to produce several cytokines and growth factors (e.g. leukemia inhibitory factor (LIF), macrophage-colony stimulating factor (M-CSF), epidermal growth factor (EGF)), that are known to modulate trophoblast differentiation and embryonic development (Benveniste et al. 1978; Garcia-Lloret et al. 1994; Hoshina et al. 1985; Sawai et al. 1995). Therefore it is highly conceivable, that a successful implantation will only take place when a good embryo giving the appropriate signals meets a responsive endometrium.

The role of hCG

Being one of the earliest embryonic products, hCG is one of the prime candidates for such an embryo-endometrial signal. The hCG-subunits are already transcribed in 8-cell embryos (Bonduelle et al. 1988) and the blastocyst has been shown to secrete relatively high concentrations of bioactive hormone (Lopata and Hay 1989). Since hCG is produced by syncytiotrophoblasts after the fusion of cytotrophoblasts as a function of trophoblast differentiation (Hoshina et al. 1985), and several endometrial cytokines considered essential for implantation, such as LIF (Sawai et al. 1995) and M-CSF (Garcia-Lloret et al. 1994) have been shown to modulate hCG-biosynthesis – probably by interfering with trophoblast differentiation - hCG could well be a key regulator, triggering whether or not the embryo will implant.

If this hypothesis is true, such a direct “juxtacrine” or “parakrine” effect of hCG on the endometrium would precede the classical endocrine role of the hormone rescuing the corpus luteum after having gained access to the maternal vascular system. A direct effect of hCG within the uterine cavity would imply the presence of functional receptors for the hormone in the endometrium.

HCG/LH-receptors in the endometrium

hCG and LH bind to a common cell surface receptor that is a member of the G-protein-coupled receptor-family (Segaloff and Ascoli 1993). The gene for the hCG/LH-receptor is located on chromosome 2 p 21 and covers an area of about 70 kbp. It consists of 11 exons divided by 10 intervening introns. The major part of the extracellular domain is encoded by exons 1-10 while exon 11 alone encodes a small part of the extracellular domain plus the entire transmembrane- and intracellular receptor region. In addition to the full-length receptor, truncated receptor isoforms are frequently found in normal and malignant tissues. They seem to represent either the

entire extracellular domain of the hCG/LH-receptor (exons 1-10) or parts of it and are not incorporated into cell membrane nor secreted, but accumulate inside the endoplasmatic reticulum or the golgi apparatus. These truncated receptors have been shown to bind hCG but not LH with high affinity but are not "functional" in the classical sense due to their inaccessibility for the ligand and their lack of coupling to the second messenger systems. Their physiological role – if any - is unknown at present.

Besides the classical target tissues of hCG (ovary, testis), hCG/LH-receptors have been described in a variety of tissues within the female and male reproductive system. Several groups have demonstrated the presence of hCG/LH-binding sites and receptor-like immunoreactivity in the porcine, rabbit, rat, mouse, bovine and human endometrium (Bernardini et al. 1995; Bhattacharya et al. 1993; Bonnamy et al. 1993; Fazleabas et al. 1999; Freidman et al. 1995; Jensen and Odell 1988; Lin et al. 1994; Reshef et al. 1990; Ziecik et al. 1986) . Receptor expression seems to be cycle dependent with secretory endometria expressing higher levels as compared to proliferative or postmenopausal samples. In human endometrium and decidua both glandular and stromal elements appear to be receptor-positive. However, there has been a debate about the physiological relevance of these extragonadal receptors due to their low level of expression, especially since Stewart et al. (Stewart et al. 1999) have been unable to amplify receptor mRNA using RT-PCR.

Our laboratory has recently shown by nested RT-PCR that endometrium from the proliferative phase as well as from the secretory phase does indeed express full-length hCG/LH-receptor mRNA (Licht et al. 2003b). In early pregnancy decidua, however, only truncated receptor mRNA could be amplified suggesting, that the expression of functional receptors may be regulated by changes in the alternative splicing pattern. It is unclear to date when exactly during early pregnancy the

functional hCG/LH-receptors disappear from the decidua and whether or not they reappear during the remainder of pregnancy. During the implantation window, however, full-length receptors appear to be expressed in human endometrium.

Intrauterine microdialysis

A major problem in the assessment of endometrial parakrine function in humans has been the unavailability of appropriate means for sampling and measurement of the cytokines and growth factors of interest. The majority of data available today is therefore derived from experiments with animals and may not apply completely to the human system. To overcome these problems, our laboratory has recently developed an intrauterine microdialysis device (IUMD) for the dynamic assessment of endometrial parakrine mediators in the human female in vivo (Licht et al. 1998; Licht et al. 2001). Using this system, we have been able to measure the intrauterine concentrations of several mediators of interest as well as to simulate the parakrine milieu of a very early pregnancy in the uterine cavity by the application of low concentrations of urinary hCG via the IUMD and measuring the response of the endometrium.

The microdialysis technique was initially used by neuroendocrinologists in order to measure the concentrations of neurotransmitters in certain areas of the central nervous system (Hamani et al. 1997). In the meantime this technique has been adapted to several non-neural tissues including the reproductive system (Robinson 1995). Especially corpus luteum function has been extensively studied using microdialysis techniques and valuable information about ovarian endocrine function has been obtained (Miyamoto and Schams 1991). Concerning the uterus, there is one report in literature measuring the influence of progesterone on the sodium and potassium concentrations in the rat uterine fluid by microdialysis (Nordenvall et al.

1989). Edwards et al. (Edwards et al. 1968) adopted a similar approach inserting diffusion chambers into the uterus to study the effects of endometrial secretions on spermatozoa. The uterus, from many points of view, is an ideal organ for microdialysis. As compared to other tissues, the microdialysis device can be inserted with little invasive measures and membranes with a large surface can be introduced. Therefore, recovery rates between 5% and 20% of the actual concentrations in utero can be achieved depending on the molecular weight of the substance of interest (Licht et al. 1998; Licht et al. 2001). These recovery rates are significantly higher than those reported from other microdialysis systems probably because of the larger surface and allow a fairly accurate estimation of actual intrauterine concentrations. A major advantage of intrauterine microdialysis as compared to endometrial biopsies or uterine flushing techniques, that assess endometrial morphology or secretion at a single point of time, is that microdialysis allows a dynamic monitoring of the response of the tissue to a stimulus. This feature of the technique was taken use of in our studies.

The intrauterine microdialysis device (IUMD) used (figure 1) was custom made. It consists of two 5 gauche double-lumen catheters connected by plasmapheresis tubing with a very high molecular weight cut-off of 2000 kDa that allow the penetration of all cytokines and growth factors of interest. The system was transcervically inserted into the uterus in form of a loop, gently blocked and continuously perfused with sterile saline at a flow rate of 30 μl/min by means of a high precision peristaltic pump. Intrauterine mediators diffuse into the perfusate along a concentration gradient. The concentrations measured in the uterine effluent (recovery) were dependent on the flow-rate, the surface of the microdialysis membranes and the molecular weight of the cytokines and growth factors of interest. The recovery rates for the various mediators were constant and varied between 5%

and 20% of the original concentrations in utero. The effluent was collected in hourly intervals (1.8 ml) and frozen immediately at -80°C. The samples were analyzed by commercially available immunoassays (Licht et al. 1998; Licht et al. 2001).

HCG and endometrial receptivity

IGFBP-1 is a well established marker protein for decidualization. Within the uterus it is selectively secreted by decidualized endometrial stromal cells. Applying the intrauterine microdialysis technique in 92 women at various time-points during the secretory phase, we could show, that intrauterine IGFBP-1 levels were low in all women before day 10 after the beginning of the LH-peak and increased significantly thereafter ($p<0.001$) (Licht et al. 2002) (figure 2). This dramatic increase in intrauterine IGFBP-1 concentrations following day 10, coincides exactly with the closing of the implantation window and the restriction of endometrial receptivity. A possible functional role of IGFBP-1 in restricting endometrial receptivity is suggested by two lines of evidence. First, IGFBP-1 has been shown to bind to $\alpha_5\beta_1$-integrins on the cell-surface of invading trophoblasts and to inhibit the implantation process (Irwin and Giudice 1998). On the other hand, IGF-II – another major product of the trophoblasts – is a physiological target for IGFBP-1. IGF-II has recently been shown to act as an angiogenic factor (Herr et al. 2003). High intrauterine levels of IGFBP-1 may therefore in addition inhibit angiogenesis that is essential for appropriate implantation and placentation.

Using the microdialysis system, we have applied 500 IU hCG to the endometrium of 45 women in the secretory phase and investigated the response of the endometrium. The concentration of hCG was high enough to result in significant intrauterine hCG-concentrations, but low enough, that no hCG could be detected in the peripheral circulation (<2 mIU/ml) and the serum levels of estradiol and progesterone as the

endocrine parameters of corpus luteum function were not affected by the intrauterine application. While there was no effect of hCG on the low endogenous IGFBP-1 concentrations during the implantation window (days 5-9), intrauterine IGFBP-1 levels were significantly reduced by the hCG application thereafter (days 10-12, $p<0.001$). This effect was equally significant, when each woman served as her own control and the fractions before and after the onset of the hCG-application were compared as well as when untreated controls were compared with hCG-treated women (Licht et al. 2002) (figure 3).

To further investigate this observation on a molecular level, decidualized human endometrial stromal cells were treated with various concentrations of recombinant hCG for either 24 or 48 hours. The results obtained clearly confirmed the microdialysis data, showing a highly-significant inhibition of both IGFBP-1 mRNA levels as measured by real-time RT-PCR ($p<0.001$) as well as of IGFBP-1 protein levels. The effect was both time- and dose-dependent with maximum inhibition after 48 hours and in the highest hCG-concentration applied (100 IU/ml) (unpublished observations).

It is unclear today, how long the inhibitory effect of hCG on IGFBP-1 biosynthesis lasts. On the long run, decidual IGFBP-1 concentrations have been shown to increase significantly during early pregnancy. A possible explanation for this paradoxical phenomenon would be a down-regulation of hCG/LH-receptors in early decidua. This may occur in part by changes in the alternative splicing pattern of the receptor mRNA resulting in truncated receptor isoforms (Licht et al. 2003b).

Taken together, there is evidence in vitro as well as in vivo, that hCG significantly inhibits endometrial IGFBP-1 biosynthesis. By this mechanism, good blastocysts producing high local hCG concentrations may be able to increase their individual

window of receptivity and have a better chance to achieve an appropriate implantation.

HCG and the regulation of angiogenesis

Vascular endothelial growth factor (VEGF) is a key regulator of both neoangiogenesis and vascular function. VEGF is present in uterine cavity in high quantities and can be measured using the microdialysis system (Licht et al. 2003a). As for IGFBP-1 the intrauterine VEGF-levels increase towards the end of the secretory phase and correlation analyses indicate that this increase in intrauterine VEGF-levels may be a function of the decidualization process. The application of hCG led to a significant increase of intrauterine VEGF levels ($p<0.01$), suggesting an involvement of hCG in the initiation of neoangiogenesis, a process essential for the formation of a functional placenta (figure 3).

In addition to its effects on VEGF concentrations, hCG has been shown to be a weak angiogenic factor itself (Zygmunt et al. 2002). The hCG-mediated increase in functional IGF-II – another independent angiogenic factor – caused by the inhibition of IGFBP-1 concentration, is a third mechanism by which hCG may support angiogenesis during implantation (Licht et al. 2002).

Through different pathways hCG seems to be able to support an appropriate endometrial angiogenesis during implantation and placentation.

HCG and parakrine parameters of implantation

It is entirely unclear at the moment, which of the many mediators present in the embryo-maternal interface is really important for human implantation. In laboratory rodents the situation is much clearer. Here, a large body of evidence has accumulated outlining the importance of several endometrial cytokines and growth

factors for the implantation process. Targeted disruption of the maternal expression of the gene for LIF in knock-out mice, for example, produces a selective implantation defect in the affected animals that can be overcome by substitution of recombinant cytokine or by transferal of the embryos to mated animals (Stewart et al. 1992). LIF is a pleiotropic cytokine with roles in endometrial as well as in embryo differentiation. A similar phenomenon is observed in naturally occurring op/op mice, that have an inborn defect in the 5'-region of the gene for M-CSF (Pollard et al. 1991). There is evidence, that both parameters may also be important - although probably not essential - for human implantation.

Using the microdialysis system, both factors could be detected in the uterine fluid. While M-CSF displayed a significant increase towards the end of the secretory phase, LIF was secreted at low levels throughout the observation period. The application of hCG provoked a clear inhibition of M-CSF ($p<0.05$) while LIF was highly significantly stimulated by hCG ($p<0.001$) (2) (figure 3). This observation in vivo is in accordance with published data obtained in vitro, indicating that hCG was able to stimulate LIF biosynthesis in cell cultures (Perrier et al. 2004).

By modulating several cytokines considered important for the process of implantation, hCG may be able to directly interfere with the implantation process.

HCG, invasion and tissue remodelling

After attachment of the hatched blastocyst on the endometrial surface epithelium, the trophoblasts begin to spread and invade the endometrium. This is achieved by the activation of matrix-metalloproteinases, leading to a controlled digestion of maternal extracellular matrix. It is self-evident, that a tight control of such invasive properties is extremely important to control an appropriate depth of implantation as well as the

invasion of the maternal blood vessels. Three types of tissue-inhibitors of matrix-metalloproteinases (TIMP-1, -2, and -3) are suspected to be involved in this control.

In our microdialysis experiments, we could demonstrate that hCG is indeed able to stimulate the expression of MMP-9 by the endometrium. In contrast, we could not find any effect of hCG on TIMP-1 concentrations within the 8-hour observation period in vivo (Licht et al. 2001) (figure 3).

However, recent in vitro data from our laboratory suggest, that there appears to be indeed a dose- and time-dependent effect of hCG on TIMP-1, -2, and -3 that becomes evident after 48 hours of treatment and that is more pronounced in the higher concentration range of r-hCG applied (unpublished observations).

These observations indicate, that hCG may be able to control endometrial tissue remodelling in part by modulating the secretion of endometrial matrix-metalloproteinases and by down-regulating the physiological inhibitors of these proteinases.

By inhibiting the physiological inhibitors of tissue digestion, a good embryo may be able to modulate endometrial tissue remodelling, resulting in a more stable implantation.

Autocrine effects of HCG on trophoblasts

In addition to the endometrium, the trophoblasts themselves have been shown to be autocrine targets of hCG. Functional hCG/LH-receptors are expressed on both cyto- and syncytiotrophoblasts (Reshef et al. 1990) and hCG is able to stimulate the differentiation of cytotrophoblasts into syncytiotrophoblasts (Shi et al. 1993). Probably as a result of differentiation, syncytiotrophoblasts do also acquire functional receptors, leading to a self-regulation of hCG-biosynthesis that is present in term placenta (Licht et al. 1993) but not in undifferentiated JAR or JEG-3 trophoblasts

(Licht et al. 1994). Recently, Zygmunt and coworkers (Zygmunt et al. 2005) have shown in addition, that hCG is able to stimulate the migration of extravillous trophoblasts by increasing the binding of IGF-II to its receptor.

Hypothetical model of profertility actions of HCG during human implantation

Taken together, there appears to be a coordinated system, regulating human implantation and early pregnancy development, in which hCG plays a central part (figure 4). HCG-biosynthesis is switched on very early during embryo development and hCG is secreted in relatively high local concentrations by the blastocyst arriving in the uterine cavity. Via endometrial receptors, that have been shown to expressed throughout the endometrial cycle, the hormone seems to profoundly modulate endometrial physiology at the implantation site, long before hCG becomes measurable in the peripheral circulation of the mother. A good blastocyst secreting high local concentrations of hCG may profoundly modulate endometrial receptivity at several levels. First, there may be a prolongation of the window of receptivity by a local down-regulation of IGFBP-1. This effect may facilitate a more stable implantation and may be terminated by the down-regulation of full-length hCG/LH-receptors that are not found anymore in early pregnancy decidua. Second, hCG maybe able to increase endometrial angiogenesis at the implantation site via an increase of local VEGF-levels as well as via its own intrinsic angiogenetic properties. Third, the hormone interferes with the biosynthesis of LIF and M-CSF, two cytokines considered important for the implantation process. The exact function of both cytokines in humans is unknown, but both are involved in the embryo-maternal cross-talk and have been shown to modulate trophoblast differentiation. Fourth, hCG causes an up regulation of MMP-9 as well as a significant inhibition of tissue inhibitors of matrix metalloproteinases (TIMP) as demonstrated in vitro. By these

mechanisms, that appear to depend both on time and on high local concentrations, the embryo may be able to modulate the process of endometrial tissue remodelling. All hCG-effects together may finally lead to a stable implantation.

In addition to its effects on the endometrium, hCG has been shown to have autocrine effects on the trophoblasts themselves, leading to increased differentiation and invasive potential. Finally, hCG can self-regulate its own biosynthesis, leading to a down-regulation of high hCG-levels at the end of the first trimester.

According to this hypothesis, a good embryo would be characterized not only by an appropriate morphology and developmental potential, but also by a high level of hCG-biosynthesis. HCG biosynthesis, however, has been shown to be coupled to trophoblast differentiation. On the other hand, the endometrium doesn't need a high level of preparation for implantation. Only an appropriate secretory transformation is necessary. The major events governing early implantation would then take place in response to the incoming blastocyst and would only be dependent on the responsiveness of both the endometrium to embryonic signals and of the trophoblasts to endometrial signals. Embryo-maternal parakrine communication and especially hCG would be a central regulator during the establishment of pregnancy.

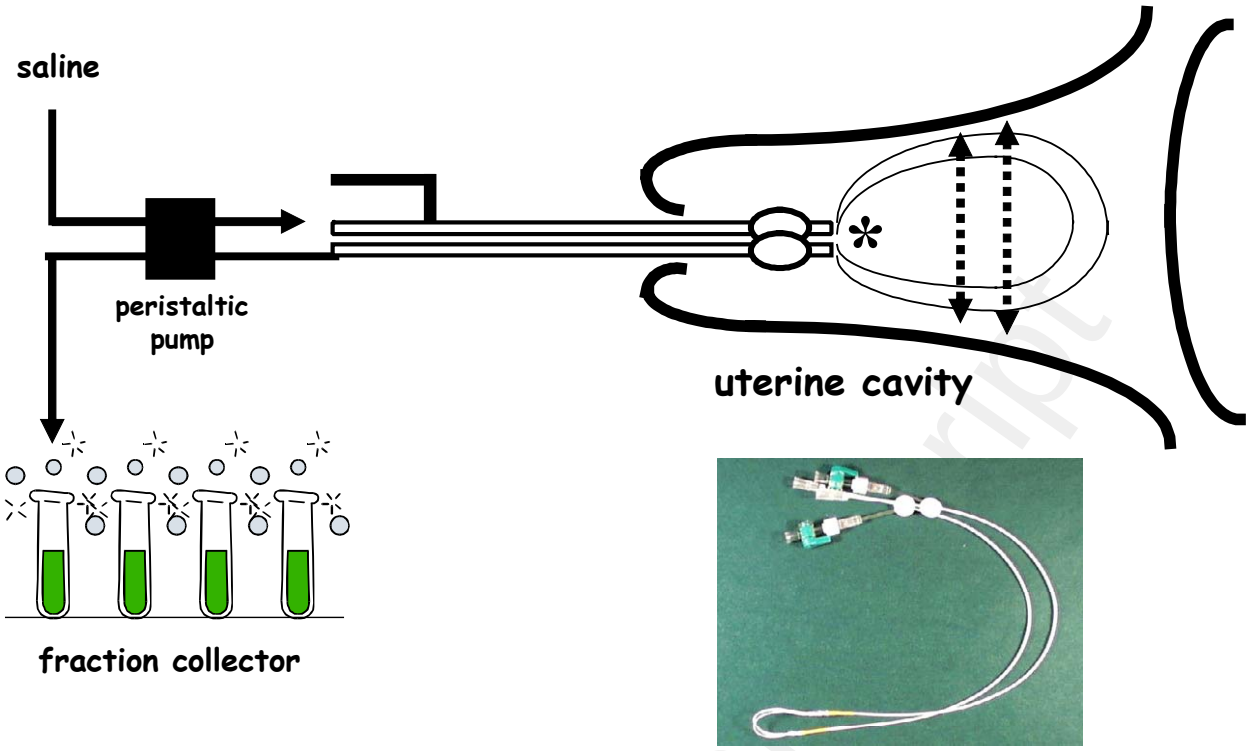

Figure 1

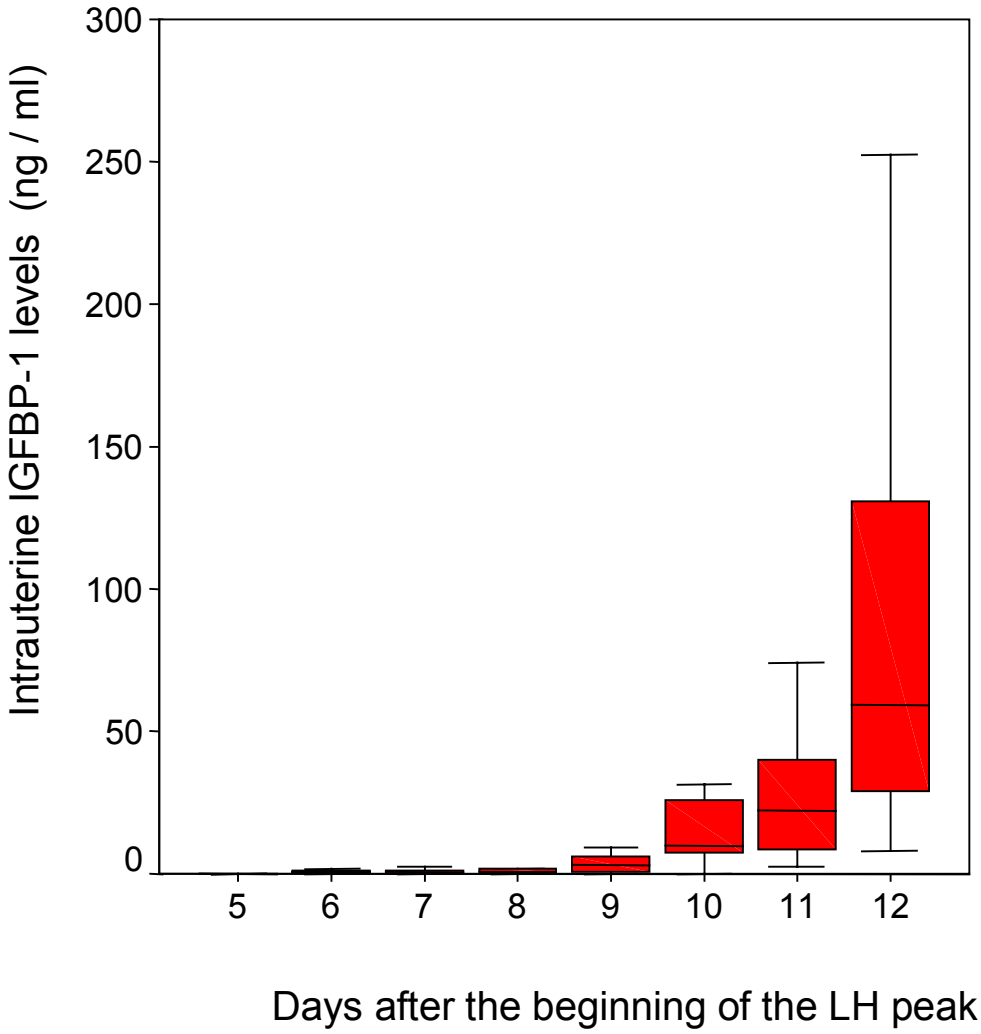

Figure 2

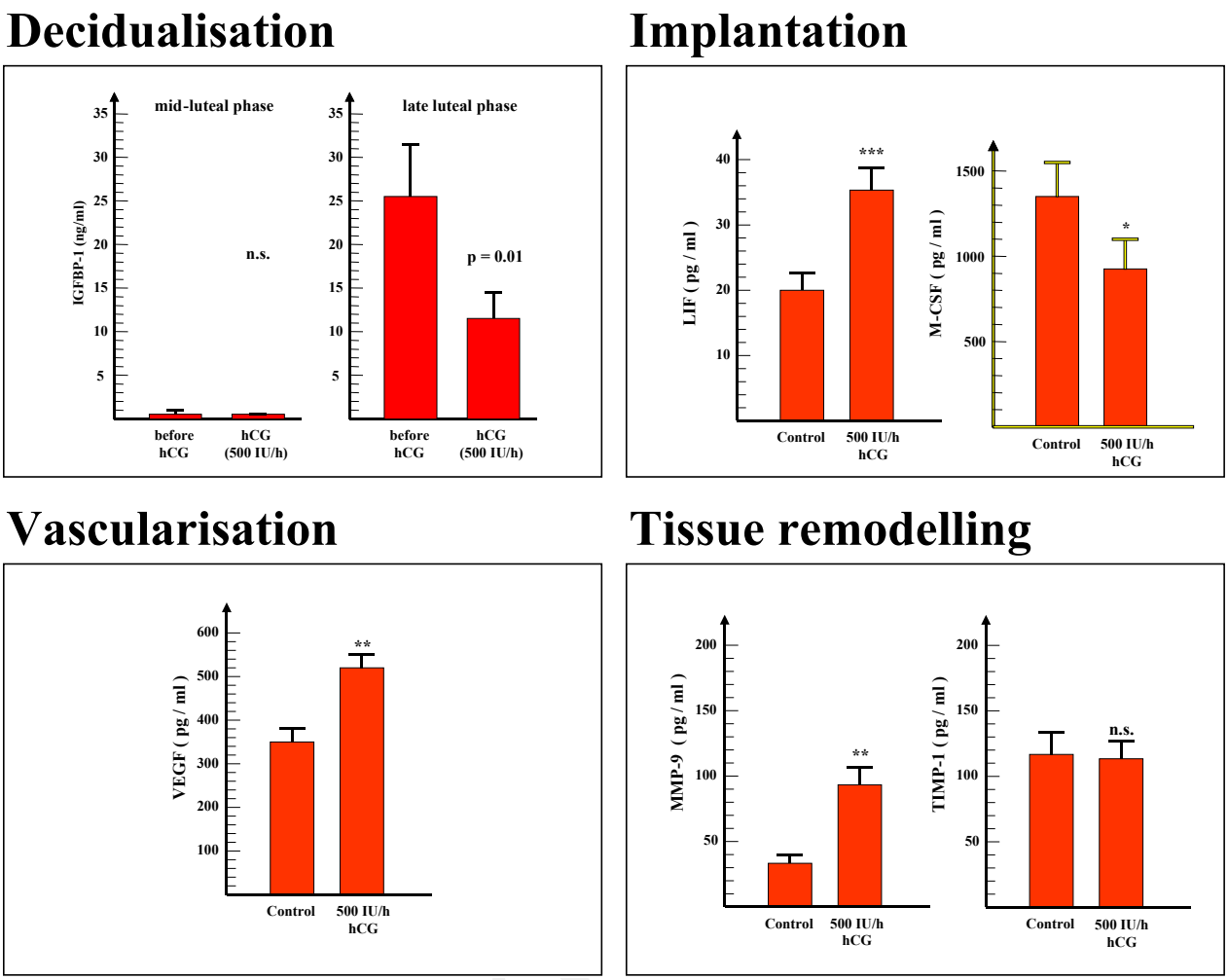

Figure 3

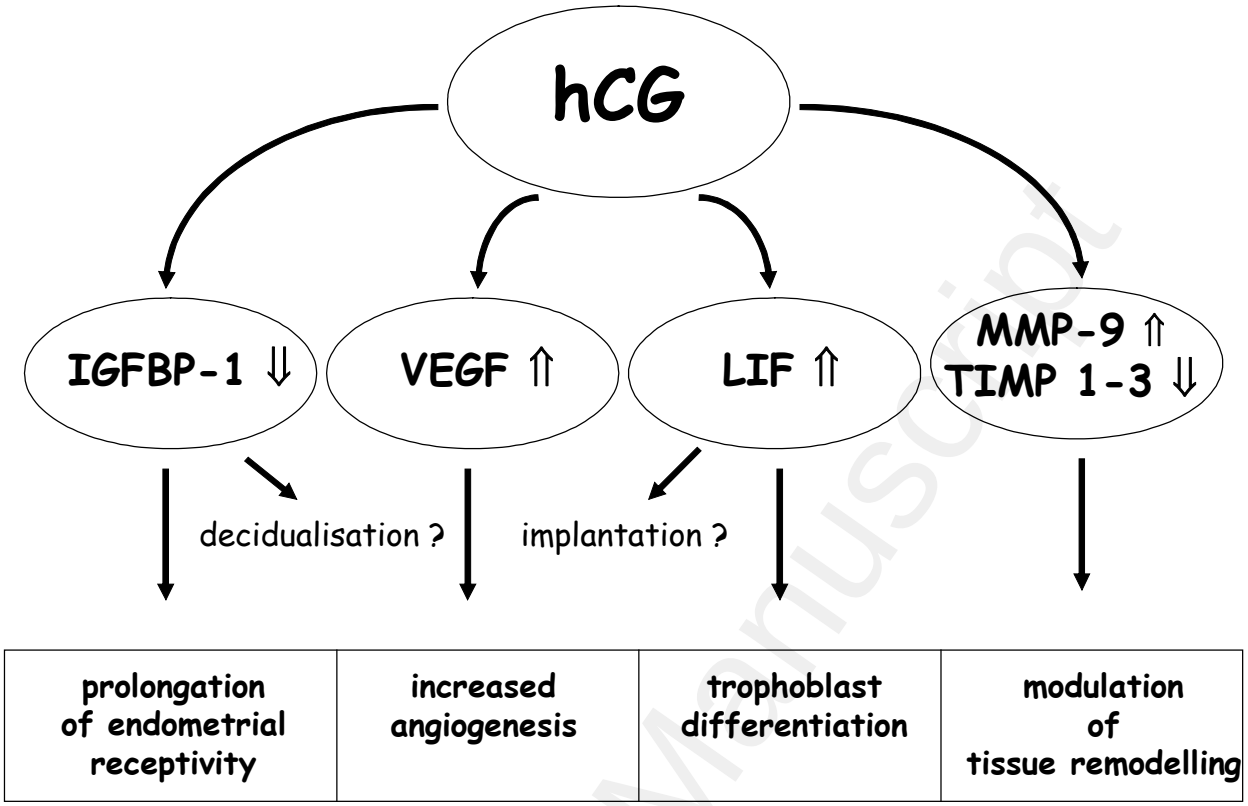

Figure 4

Figure Legends

Figure 1 : Intrauterine microdialysis device (IUMD).

The IUMD consists of two 5 gauche balloon catheters connected by microdialysis tubing (MW cut-off: 2000 kDa). The system is inserted into the uterine cavity, gently blocked and continuously perfused with sterile saline (30 μl/min). The paracrine milieu of a very early pregnancy was simulated by the application of low concentrations of urinary hCG (50 IU/h). The response of the tissue was dynamically assessed in the effluent.

Figure 2 : Intrauterine IGFBP-1 levels at various time-points during the secretory phase of the endometrial cycle.

A total of 92 women underwent an intrauterine microdialyisis procedure at between days 5 and 12 after the beginning of the LH-peak (x-axis). The intrauterine IGFBP-1 levels showed a highly significant increase at day 10 ($p<0.001$).

Figure 3 : Effects of hCG on paracrine markers of several endometrial functions in the human female in vivo.

The effect of intrauterine application of hCG on several endometrial secretory parameters was investigated by intrauterine microdialysis. While there was a time-dependent inhibition of IGFBP-1 and M-CSF, LIF, VEGF and MMP-9 were significantly stimulated by the hormone. No effect on TIMP-1 secretion was observed in vivo.

Figure 4 : Hypothetical model of hCG directly regulating human implantation.

Coordinated effects of hCG on several endometrial functions may lead to prolongation of endometrial receptivity, increased angiogenesis, modulation of implantation parameters and tissue remodelling.

Reference List

Benveniste,R., Speeg,K.V., Jr., Carpenter,G., Cohen,S., Lindner,J., and Rabinowitz,D., 1978. Epidermal growth factor stimulates secretion of human chorionic gonadotropin by cultured human choriocarcinoma cells. J. Clin. Endocrinol. Metab 46, 169-172.

Bernardini,L., Moretti-Rojas,I., Brush,M., Rojas,F.J., and Balmaceda,J.P., 1995. Status of hCG/LH receptor and G proteins in human endometrium during artificial cycles of hormone replacement therapy. J. Soc. Gynecol. Investig. 2, 630-635.

Bhattacharya,S., Banerjee,J., Sen,S., and Manna,P.R., 1993. Human chorionic gonadotropin binding sites in the human endometrium. Acta Endocrinol. (Copenh) 129, 15-19.

Bonduelle,M.L., Dodd,R., Liebaers,I., Van Steirteghem,A., Williamson,R., and Akhurst,R., 1988. Chorionic gonadotrophin-beta mRNA, a trophoblast marker, is expressed in human 8-cell embryos derived from tripronucleate zygotes. Hum. Reprod. 3, 909-914.

Bonnamy,P.J., Benhaim,A., and Leymarie,P. 1993., Uterine luteinizing hormone/human chorionic gonadotropin-binding sites in the early pregnant rat uterus: evidence for total occupancy in the periimplantation period. Endocrinology 132, 1240-1246.

Edwards,R.G., Talbert,L., Israelstam,D., Nino,H.V., and Johnson,M.H., 1968. Diffusion chamber for exposing spermatozoa to human uterine secretions. Am. J. Obstet. Gynecol. 102, 388-396.

Fazleabas,A.T., Donnelly,K.M., Srinivasan,S., Fortman,J.D., and Miller,J.B., 1999. Modulation of the baboon (Papio anubis) uterine endometrium by chorionic gonadotrophin during the period of uterine receptivity. Proc. Natl. Acad. Sci. U. S. A 96, 2543-2548.

Freidman,S., Gurevich,M., and Shemesh,M., 1995. Bovine cyclic endometrium contains high-affinity luteinizing hormone/human chorionic gonadotropin binding sites. Biol. Reprod. 52, 1020-1026.

Garcia-Lloret,M.I., Morrish,D.W., Wegmann,T.G., Honore,L., Turner,A.R., and Guilbert,L.J., 1994. Demonstration of functional cytokine-placental interactions: CSF-1 and GM-CSF stimulate human cytotrophoblast differentiation and peptide hormone secretion. Exp. Cell Res. 214, 46-54.

Hamani,C., Luer,M.S., and Dujovny,M., 1997. Microdialysis in the human brain: review of its applications. Neurol. Res. 19, 281-288.

Herr,F., Liang,O.D., Herrero,J., Lang,U., Preissner,K.T., Han,V.K., and Zygmunt,M., 2003. Possible angiogenic roles of insulin-like growth factor II and its receptors in uterine vascular adaptation to pregnancy. J. Clin. Endocrinol. Metab 88, 4811-4817.

Hoshina,M., Boothby,M., Hussa,R., Pattillo,R., Camel,H.M., and Boime,I., 1985. Linkage of human chorionic gonadotrophin and placental lactogen biosynthesis to trophoblast differentiation and tumorigenesis. Placenta 6, 163-172.

Irwin,J.C. and Giudice,L.C., 1998. Insulin-like growth factor binding protein-1 binds to placental cytotrophoblast alpha5beta1 integrin and inhibits cytotrophoblast invasion into decidualized endometrial stromal cultures. Growth Horm. IGF. Res. 8, 21-31.

Jensen,J.D. and Odell,W.D., 1988. Identification of LH/hCG receptors in rabbit uterus. Proc. Soc. Exp. Biol. Med. 189, 28-30.

Licht,P., Cao,H., Lei,Z.M., Rao,C.V., and Merz,W.E., 1993. Novel self-regulation of human chorionic gonadotropin biosynthesis in term pregnancy human placenta. Endocrinology 133, 3014-3025.

Licht,P., Cao,H., Zuo,J., Lei,Z.M., Rao,V., Merz,W.E., and Day,T.G., Jr., 1994. Lack of self-regulation of human chorionic gonadotropin biosynthesis in human choriocarcinoma cells. J. Clin. Endocrinol. Metab 78, 1188-1194.

Licht,P., Losch,A., Dittrich,R., Neuwinger,J., Siebzehnrubl,E., and Wildt,L., 1998. Novel insights into human endometrial paracrinology and embryo-maternal communication by intrauterine microdialysis. Hum. Reprod. Update. 4, 532-538.

Licht,P., Russu,V., Lehmeyer,S., Moll,J., Siebzehnrubl,E., and Wildt,L., 2002. Intrauterine microdialysis reveals cycle-dependent regulation of endometrial insulin-like growth factor binding protein-1 secretion by human chorionic gonadotropin. Fertil. Steril. 78, 252-258.

Licht,P., Russu,V., Lehmeyer,S., and Wildt,L., 2001. Molecular aspects of direct LH/hCG effects on human endometrium--lessons from intrauterine microdialysis in the human female in vivo. Reprod. Biol. 1, 10-19.

Licht,P., Russu,V., Lehmeyer,S., Wissentheit,T., Siebzehnrubl,E., and Wildt,L., 2003a. Cycle dependency of intrauterine vascular endothelial growth factor levels is correlated with decidualization and corpus luteum function. Fertil. Steril. 80, 1228-1233.

Licht,P., von Wolff,M., Berkholz,A., and Wildt,L., 2003b. Evidence for cycle-dependent expression of full-length human chorionic gonadotropin/luteinizing hormone receptor mRNA in human endometrium and decidua. Fertil. Steril. 79 Suppl 1, 718-723.

Lin,J., Lei,Z.M., Lojun,S., Rao,C.V., Satyaswaroop,P.G., and Day,T.G., 1994. Increased expression of luteinizing hormone/human chorionic gonadotropin receptor gene in human endometrial carcinomas. J. Clin. Endocrinol. Metab 79, 1483-1491.

Lopata,A. and 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.

Miyamoto,A. and Schams,D., 1991. Oxytocin stimulates progesterone release from microdialyzed bovine corpus luteum in vitro. Biol. Reprod. 44, 1163-1170.

Nordenvall,M., Ulmsten,U., and Ungerstedt,U., 1989. Influence of progesterone on the sodium and potassium concentrations of rat uterine fluid investigated by microdialysis. Gynecol. Obstet. Invest 28, 73-77.

Perrier,d.S., Charlet-Renard,C., Berndt,S., Dubois,M., Munaut,C., Goffin,F., Hagelstein,M.T., Noel,A., Hazout,A., Foidart,J.M., and 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.

Pollard,J.W., Hunt,J.S., Wiktor-Jedrzejczak,W., and Stanley,E.R., 1991. A pregnancy defect in the osteopetrotic (op/op) mouse demonstrates the requirement for CSF-1 in female fertility. Dev. Biol. 148, 273-283.

Reshef,E., Lei,Z.M., Rao,C.V., Pridham,D.D., Chegini,N., and 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.

Robinson,J.E., 1995. Microdialysis: a novel tool for research in the reproductive system. Biol. Reprod. 52, 237-245.

Sawai,K., Matsuzaki,N., Kameda,T., Hashimoto,K., Okada,T., Shimoya,K., Nobunaga,T., Taga,T., Kishimoto,T., and Saji,F., 1995. Leukemia inhibitory factor produced at the fetomaternal interface stimulates chorionic gonadotropin production: its possible implication during pregnancy, including implantation period. J. Clin. Endocrinol. Metab 80, 1449-1456.

Segaloff,D.L. and Ascoli,M., 1993. The lutropin/choriogonadotropin receptor ... 4 years later. Endocr. Rev. 14, 324-347.

Shi,Q.J., Lei,Z.M., Rao,C.V., and Lin,J., 1993. Novel role of human chorionic gonadotropin in differentiation of human cytotrophoblasts. Endocrinology 132, 1387-1395.

Simon,C., Gimeno,M.J., Mercader,A., Frances,A., Garcia,V.J., Remohi,J., Polan,M.L., and Pellicer,A., 1996. Cytokines-adhesion molecules-invasive proteinases. The missing paracrine/autocrine link in embryonic implantation? Mol. Hum. Reprod. 2, 405-424.

Stewart,C.L., Kaspar,P., Brunet,L.J., Bhatt,H., Gadi,I., Kontgen,F., and Abbondanzo,S.J., 1992. Blastocyst implantation depends on maternal expression of leukaemia inhibitory factor. Nature 359, 76-79.

Stewart,E.A., Sahakian,M., Rhoades,A., Van Voorhis,B.J., and Nowak,R.A., 1999. Messenger ribonucleic acid for the gonadal luteinizing hormone/human chorionic gonadotropin receptor is not present in human endometrium. Fertil. Steril. 71, 368-372.

Tabibzadeh,S. and Babaknia,A., 1995. The signals and molecular pathways involved in implantation, a symbiotic interaction between blastocyst and endometrium involving adhesion and tissue invasion. Hum. Reprod. 10, 1579-1602.

Tabibzadeh,S., Kaffka,K.L., Kilian,P.L., and Satyaswaroop,P.G., 1990. Human endometrial epithelial cell lines for studying steroid and cytokine actions. In Vitro Cell Dev. Biol. 26, 1173-1179.

Wilcox,A.J., Baird,D.D., and Weinberg,C.R., 1999. Time of implantation of the conceptus and loss of pregnancy. N. Engl. J. Med. 340, 1796-1799.

Ziecik,A.J., Stanchev,P.D., and Tilton,J.E., 1986. Evidence for the presence of luteinizing hormone/human chorionic gonadotropin-binding sites in the porcine uterus. Endocrinology 119, 1159-1163.

Zygmunt,M., Herr,F., Keller-Schoenwetter,S., Kunzi-Rapp,K., Munstedt,K., Rao,C.V., Lang,U., and Preissner,K.T., 2002. Characterization of human chorionic gonadotropin as a novel angiogenic factor. J. Clin. Endocrinol. Metab 87, 5290-5296.

Zygmunt,M., McKinnon,T., Herr,F., Lala,P.K., and Han,V.K. 2005. HCG increases trophoblast migration in vitro via the insulin-like growth factor-II/mannose-6 phosphate receptor. Mol. Hum. Reprod. 11: 261-267.