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Contraceptive Vaccines Targeting Factors Involved in Establishment of Pregnancy

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Abstract

Problem—Current methods of contraception lack specificity and are accompanied with serious side effects. A more specific method of contraception is needed. Contraceptive vaccines can provide most, if not all, the desired characteristics of an ideal contraceptive.

Approach—This article reviews several factors involved in the establishment of pregnancy, focusing on those that are essential for successful implantation. Factors that are both essential and pregnancy-specific can provide potential targets for contraception.

Conclusion—Using database search, 76 factors (cytokines/chemokines/growth factors/others) were identified that are involved in various steps of the establishment of pregnancy. Among these factors, three, namely chorionic gonadotropin (CG), leukemia inhibitory factor (LIF), and preimplantation factor (PIF), are found to be unique and exciting molecules. Human CG is a well-known pregnancy-specific protein that has undergone phase I and phase II clinical trials, in women, as a contraceptive vaccine with encouraging results. LIF and PIF are pregnancy-specific and essential for successful implantation. These molecules are intriguing and may provide viable targets for immunocontraception. A multiepitope vaccine combining factors/antigens involved in various steps of the fertilization cascade and pregnancy establishment, may provide a highly immunogenic and efficacious modality for contraception in humans.

Keywords

Contraceptive vaccine; pregnancy-specific factors; implantation; immunocontraception

Introduction

With the continually increasing world population, there is an urgent need for an alternative form of contraception. Currently, available methods, including most used modalities, namely steroid contraceptives and intrauterine devices, haveseveral serious side effects. A more targeted, less invasive approach to contraception is desired. Contraceptive vaccines (CV) would provide an ideal alternative. CV would be easy to administer, less expensive, readily available, and more importantly, would be specific. By targeting factors that are essential for establishment of pregnancy, a CV would block the action of a factor(s) and prevent the onset of pregnancy. One of the essential factors that has been extensively studied is the chorionicgonadotropin (CG). Human chorionic gonadotropin (hCG) is a major systemic regulator of embryo development, implantation and is secreted by the implanting trophoblast,[1] making it an ideal pregnancy-specific target for CV development. Several

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forms of hCG vaccines have undergone clinical trialsin women, both phase I and phase II, displaying positive contraceptive effects.[2, 3] While the outlook of the hCG vaccine looks promising, research on additional potential targets continue with an ultimate goal of finding a vaccine that is more immunogenic and efficacious. This article will review the additional factors that are involved in the development and implantation of the embryo, with a focus on those that have been shown to be essential for normal embryonic development and/or implantation and pregnancy-specific. The long-term goal is to target these molecules for the development of highly specific, non-steroidal, and efficacious vaccine for birth control.

Discussion

1. Factors Involved in Various Stages of Establishment of Pregnancy

The PubMed database (www.pubmed.gov) was searched using the following keywords: secreted/pregnancy/fertilization/implantation/embryo development/pregnancy-specific/ molecules. Further focus was placed on those articles that were relevant to murine or human implantation and pregnancy. The search identified 76 cytokines, chemokines, growth factors, integrins, and miscellaneous factors involved in the establishment of pregnancy. Their molecular and functional parameters are summarized in Tables I–III. These 76 factors are grouped into five categories depending upon which stage of pregnancy establishment they are primarily involved in and described below(Fig 1).

a. FactorsInvolved inEarlyEmbryonicDevelopment—After fertilization, the resultant zygote undergoes a series of divisions and modifications before progressing to the blastocyst stage. Several factors promote growth and proliferation of these early embryos. Tumor necrosis factor α (TNFα) has been shown to bind early mouse embryos and may promote embryonic development,[4] however, it has deleterious effects at high levels.[5, 6] Insulin has been shown to stimulate DNA, RNA, protein synthesis,[7] and increase the rate at which these cells proliferate during the early diploid and tetraploid stages.[8] Higher levels of insulin-like growth factor (IGF) binding protein 3 (IGFBP-3) have been correlated to the increased embryonic development.[9] The embryo secretes platelet activating factor (PAF) that promotes embryo development.[10] Blocking the action of PAF with an antagonist prevents implantation.[11] Transforming growth factor (TGF)-β1 plays an important role in the development of the blastocyst.[12] TGF-β1 null mice produce embryos that are arrested at the morula stage, not developing to a blastocyst.[13] Granulocyte macrophage colony-stimulating factor (GM-CDF) enhances the viability and proliferation of blastomeres in early embryos.[14, 15] Insulin-like growth factor (IGF)-II also promotes the progression to the blastocyst stage. IGF-II antisense oligodeoxynucleotides (ODN) decreasethe rate that embryos enterintothe blastocyst stage.[16] Another factor affecting the growth and development of early embryos is growth hormone (GH). Patients with *in vitro* fertilization (IVF) failures have been shown to have GH deficiency. Supplementation with GH improves embryo quality and fertilization ratesin these patients.[17]

b. Factors Affecting Development ofBlastocyst—Once the blastocyst has formed, it must undergo changes that allow for implantation. A few key systemic factors regulate this process. LIF is an essential factor whose expression is under the control of progesterone. LIF controls the expression of several implantation-related genes, such as heparin-binding EGF-like growth factor (HB-EGF), amphiregulin, epiregulin, insulin-like growth factor binding protein 3 (IGFBP-3), immunoresponsive gene 1 homolog (IRG-1), and cochlin. [18–21] Gene knockout and LIF antagonist studies in mice have shown that deleting the LIF/LIF receptor gene or impeding the interaction of LIF with the receptor results in implantation failure.[22, 23] HB-EGF promotes the development of blastocysts through the hatching stage as well as the motility and attachment of the blastocyst.[24]

Several growth factors influence the growth and development of blastocyst. These include TGF-α, basic fibroblast growth factor (FGF-2),[25] hepatocyte growth factor(HGF),[26] platelet-derived growth factor (PDGFA),[27] and acrogranin. TGF-α has been demonstrated to stimulate DNA and protein synthesis in blastocysts as well asincrease the rate of blastocoel expansion. Administration of TGF-α antisense ODN significantly reduces the rate of blastocoel expansion.[28] Rate of blastocoel expansion is shown to increase in the presence of acrogranin. Not only does it affect expansion, it also promotes blastocyst hatching and outgrowth. Anti-acrogranin antibodies reduce these effects *in vitro* and also prevent the 8-cell embryos to develop to blastocysts.[29, 30] The inner cell mass (ICM) continually increases in cell number as the blastocyst develops. IGF-I, IGF-II, and leptin have all been reported to increase the number of ICM in cultured blastocysts.[16, 31, 32] In order for the blastocyst to adhere to the uterus, it must first become activated. The outgrowth and adhesion of blastocysts is inhibited by the addition of Dickkopf-1 (DKK-1)antisense ODN, suggesting an important role for DKK-1 in blastocyst activation.[33]

c. Factors Impacting Implantation—Migration of the blastocyst to the implantation site is controlled by many factors. Several chemokines, including CCL-4 and CX3CL-1, promote blastocyst migration.[34] Extravillous trophoblast (EVT) migration is also induced by a handful of growth factors. Epidermal growth factor (EGF) can stimulate trophoblast migration[35] using the PI3K/AKT and MAP kinase signaling pathways.[36] Along with EGF, IGF-I can also induce EVT migration. The α5β1 and αvβ3 integrins have been shown to play essential roles in this pathway.[37, 38] FGF-2 may also play a role in preparing the blastocyst for migration.[25] Several factors, such as macrophage inhibitory cytokine 1 (MIC-1),[39] can act to regulate the migration.

Once at the site of implantation, the blastocyst attaches to the uterine epithelium. Prokineticin 1 (PROK-1) promotes the gene expression of many implantation related genes, such as cyclooxygenase 2 (COX-2), LIF, interleukin (IL)-6, IL-8, and IL-11, that allow for attachment to the uterus.[40, 41] LIF, along with progesterone, lead to the upregulation of IRG-1.[19] Antisense ODN leads to suppression of IRG-1 expression, resulting in impairment of embryo implantation.[42] Members of IL-1 family of cytokines are important in adhesion of blastocyst. IL-1β stimulates IL-8 production that is necessary for implantation.[43] IL-1α and IL-1β secreted by the embryo mediate pathways involving integrins. Both of these growth factors appear to target endometrial epithelial β3 integrin, preparing the blastocyst for adhesion.[44] IL-1α upregulates integrin expression and induces changes that result in a more invasive phenotype.[45] Both IL-1α and IL-1β have been detected in the sera of women undergoing in vitro fertilization (IVF) having higher implantation rates, suggesting that they may have an important role.[46] IL-1 receptor antagonist (IL-1Ra) inhibits the actions of IL-1α and IL-1β by down-regulating integrins.[47] CX3CL-1 regulates the expression of adhesion molecules, such as secreted phosphoprotein 1 (SPP1) and matrix metalloproteinases (MMPs), that mediate attachment of the implanting blastocyst.[48] SPP1 co-localizes with leukocytes and macrophages and may allow for attachment to the luminal epithelium through SPP1-positive macrophages.[49] In the ovine model, SPP1 was demonstrated to bind integrins (αvβ3 and α5β1) on the conceptus and luminal epithelium.[50] Along with integrins, trophinin is involved in blastocyst binding to the uterine epithelium.[51] Acrogranin and DKK-1 are both essential adhesion factors. The inhibition or removal of these factors reduces adhesion.[30, 33] Other factors involved in attachment are mucin-1 (MUC-1),[52, 53] heparan sulfate proteoglycans (HSPGs), [54, 55] and PIF. PIF is an embryo-derived peptide playing an essential role in adhesion.[56, 57]

As the blastocyst attaches, various molecules participate in the timing and spacing of the embryo, at least in the murine model. Lysophosphatidic acid 3 (LPA3) and cytosolic phospholipase A2α (cPLA2α) regulate embryo spacing. Mice deficient in either of these molecules have delayed implantation and abnormal spacing of embryos, resulting in smaller litter size, and, in some cases, pregnancy failure.[58–60] HB-EGF-deficient micealso display delayed implantation.[61]

Invasion of the blastocyst upon adhesion to the uterus involves various factors. Adrenomedullin enhances invasion of trophoblasts *in vitro*.[62] Mice with reduced expression of adrenomedullin also demonstrate reduced fertility and defectin invasion.[63, 64] Other factors mediating invasiveness are HGF, leptin and IGFBP-1. Both HGF and leptin induce cytotrophoblast modifications that regulate invasiveness.[26, 45, 65] IGFBP-1 acts to inhibit IGF-I activity, preventing invasion.[66]

d. Factors Involving in Uterine Receptivity and Decidualization—Maintenance of corpus luteum (CL) is important for establishing and maintaining pregnancy. Factors such as vascular endothelial growth factor (VEGF)[67] and hCG[1] both participate in CL maintenance. CL secretes several hormones that allow for the establishment of pregnancy. Most importantly, it secretes progesterone that allows for the decidualization of the endometirum. Activin A is also secreted by the CL, promoting decidualization by preventing T cell activation,[68] upregulating MMPs,[69] and secreting IL-11.[70] IL-11 signaling through binding to its receptor is required for the development of decidua.[71, 72] IL-11 receptor null mice have defective decidualization and, as a result, are infertile.[73] IL-6 also promotes implantation and decidualization by stimulating leptin secretion and MMP activity.[74] IL-6-deficient mice show a decreasein viable implantation sites resulting in reduced fertility.[75] Another important regulator of decidualization is prolactin (PRL). Mice lacking the PRL receptor exhibit implantation failures.[76] hCG is responsible for the expression or upregulation of many factors that participate in the implantation process. Not only does hCG induce expression of two important implantation factors, LIF and IL-6,[77] it also induces expression of COX-2.[78] The COX-2 biosynthesizes prostaglandins, like prostaglandin E2 (PGE_2), which affect uterine receptivity. Inhibition of COX-2 results in inhibition of stromal cell expression, leading to decidualization failure.[79, 80] Homebox (HOX)A proteins, HOXA10 and HOXA11, are involved in stromal cell differentiation required for decidualization.[81–83] Mice expressing HOXA10 mutants show stromal cell and decidualization defects that result in implantation failure.[82, 84] A few integrins, α4β1 and αvβ3, have also been implicated in having a role in decidualization.[55] Other factors that present possible roles in regulation of decidualization include MIC-1[85] and connective tissue growth factor(CTGF).[86–88]

Leukocytes are recruited to help prepare the uterus for implantation. Many cytokines and chemokines are involved in the initiation of this essential inflammatory response. Colony stimulating factor (CSF)-1, CSF-2, and CSF-3 all serve as chemoattractants in the recruitment of macrophages to the uterus.[89, 90] Homozygous crosses of mice lacking CSF-1 result in infertility.[91] Upregulation of IL-8, CCL-2, and RANTES by progesterone has been demonstrated *in vitro*.[92] CCL2 recruits macrophages, monocytes, natural killer (NK) cells, and T-cells in the endometrium.[89, 93–95] CCL3, CCL4, CCL5 (RANTES), and CCL7 are also involved in the recruitment of macrophages and natural killer (NK) cells.[85, 89, 94, 96] IL-8 upregulates several inflammatory response genes.[97, 98] Stimulation of stromal cells *in vitro* with IL-23 shows an increase in IL-8 expression.[99] Another chemokine responsible for upregulating the inflammatory response is CXCL1. [94, 100] Recent research suggests that this inflammatory environment is mediated by the trophoblast through toll-like receptors (TLRs).[101] Other factors involved in the inflammatory response are PGE_2[1] and L-selectin. [102, 103]

e. Immunomodulatory Regulators—Possibly the most critical aspect of successful pregnancy is maternal tolerance of the implanting embryo. Several cytokines act to suppress an immune response to the blastocyst. The IL-12/IL-18 system is important in managing immune responses. Alterations to the IL-12 or IL-18 levels have been associated with recurrent implantation failure.[104] IL-18 has the ability to increase perforin expression and cytolytic potentials of uterine NK (uNK) cells[105] and its absence or overexpression can lead to implantation failure. [106] IL-15, on the other hand, is thought to regulate uNK cells.[85] Essential interleukins mediating maternal tolerance are IL-10 and IL-27. Mice lacking IL-10 exhibit fetal resorption due to an increased activation of cytotoxic uNK cells.[107] Neutralization of IL-27 in mice also results in increased fetal resorption.[108] Glycodelin is a pregnancy-specific protein shown to increase IL-10 production and reduce the expression of costimulatory molecules in monocyte-derived dendritic cells, suggesting a role in preventing an immune response.[109, 110] TNFα is known to cause spontaneous abortion in mice and women.[5, 6, 111, 112] Tumor necrosis factor-like weak inducer of apoptosis (TWEAK) is thought to protect against the deleterious effect of TNFα, by controlling uNK cell cytotoxicity and regulating of IL-15 and IL-18.[108, 113] TNFα and interferon γ(IFNγ) cause spontaneous abortion by binding to their receptors, which are expressed in the presence of lipopolysaccharide (LPS). An *in vivo* model of spontaneous abortion has been created in mice by injecting mated mice with LPS. Addition of TGF-β3 to this model increased the success of pregnancy by promoting a regulatory T-cell response.[111] Studies have implicated corticotrophin-releasing hormone (CRH) in the regulation of the immune response through killing of activated T-cells. Administration of CRH antibodies on day 3–8 of pregnancy results in implantation failure in 60% of cases.[114] A CRH receptor antagonist, antalarmin, also decreases implantation and live embryos as well as FasL expression, suggesting its role in T-cell regulation.[115] Other notable factors involved in immune tolerance are hCG,[116] PIF,[57] interferon-stimulated 17 kDa protein (ISG-15),[117] and α-fetoprotein.[118]

2. Immunocontraceptive Targets

The purpose of this article is to review the factors that are involved in the establishment of pregnancy and delineate which of these factors are essential and pregnancy-specific. By selecting the proteins that are essential and pregnancy-specific, it is ensured that targeting these molecules will reduce fertility without affecting any other molecule and process. Research in this area has been rapidly progressing over the past decade. The pregnancy-specific protein, hCG, was initially used for detection of pregnancy in women. Now, it is being investigated as a contraceptive target for development of a birth control vaccine. Several vaccines based on the βsubunit of hCG incorporating various carriers and adjuvants have undergone phase I and phase II clinical trialsin women. A study completed in 1994 by Talwar et al recorded that women administered an hCG vaccine developed antibody titers that prevented pregnancy. Only 1 pregnancy occurred in over 1224 cycles observed in these vaccinated women.[2] Another trial demonstrated that an HSD-hCG vaccine was reversible and that titers below the protective threshold showed no teratogenic effect on pregnancy outcome.[3]

A more recent protein of interest is LIF. Studies done in the mouse model have shown that hindering the interaction of LIF with its receptor will block implantation. Stewart et al mutated the LIF gene to express a truncated, non-functional LIF mutant. The mutated DNA was injected into blastocysts, and crossed the resulting F1 offsprings to create homozygous LIF-mutant mice. These mice demonstrated complete implantation failure.[22] Administration of a LIF antagonist conjugated to polyethylene glycol (PEG-LA), increased blocking implantation in mice.[23] More importantly, LIF is required for implantation not only in mice, but also in humans. LIF mRNA concentration peaks in human endometrium at the time of implantation.[119] Studies on endometrial explants from fertile and infertile women reveal that LIF production in cultures from infertile women and fertile women, using intrauterine devices (IUD), was significantly less than that of cultures from normally cycling fertile women.[120] A similar study showed immunostaining of LIF in biopsies from fertile women, was higher than that of infertile women.[121] Recently, it was discovered that LIF gene mutations in infertile women may account for poor IVF outcome, since maternal LIF expression is critical for implantation and successful pregnancy.[122] Our laboratory recently conducted a study using a vaccine targeting LIF and its receptors in the mouse model. Preliminary results are very exciting. The administration of the vaccine to female mice developed specific antibodies resulting in a reduction of fertility in the vaccinated female mice (Lemons and Naz, unpublished data).

Other interesting molecules include glycodelin,[102] oviduct-specific glycoprotein 1 (OVGP-1),[103, 123] trophinin and PIF. Glycodelin A has been shown to have immunosuppressive effects against the maternal response to spermatozoa.[109, 110, 124] Trophinin promotes activation of blastocyst for adhesion to uterine epithelium.[51] Trophinin is expressed by both trophoblast and endometrial epithelial cells and its expression seems to be regulated by hCG secretion.[125] PIF is an embryo-derived peptide detected in the serum just before implantation.[1] It has recently been shown to be essential for implantation by promoting adhesion, regulating immunity, and apoptosis.[56, 57]

3. Conclusions

The database review identified 76 various factors that are involved in several steps of establishment of pregnancy. At least three of these factors (hCG/LIF/PIF) were found to be essential and pregnancy-specific. These molecules, besides others, may provide viable target for immunocontraception. The contraceptive vaccines targeting factors involved in pregnancy establishment have two potential concerns: 1) Although these factors are involved in the early events of embryonic development and preimplantation, the vaccines against them are not contraceptives in true sense because they target the post-fertilization stages, and 2) They are “self” molecules and it may be a challenging proposition to induce enough antibodies to neutralize these factors. However, the findings of phase I and phase II clinical trials of hCG vaccine in women indicate that by using appropriate carriers and adjuvants, one can modulate the “self” molecule to break its tolerance and raise an immune response against these molecules in humans. Also, the hCG vaccine trials indicate that there is no teratogenic effect of the low titer residual antibodies left after the protective levels decline. The hCG vaccine trials in women have established the basis for developing a birth control vaccine, targeting various factors involved in establishment of pregnancy. A multiepitope vaccine combining factors/antigens involved in various steps of fertilization cascade and pregnancy establishment, may provide a highly immunogenic and efficacious modality for contraception in humans.

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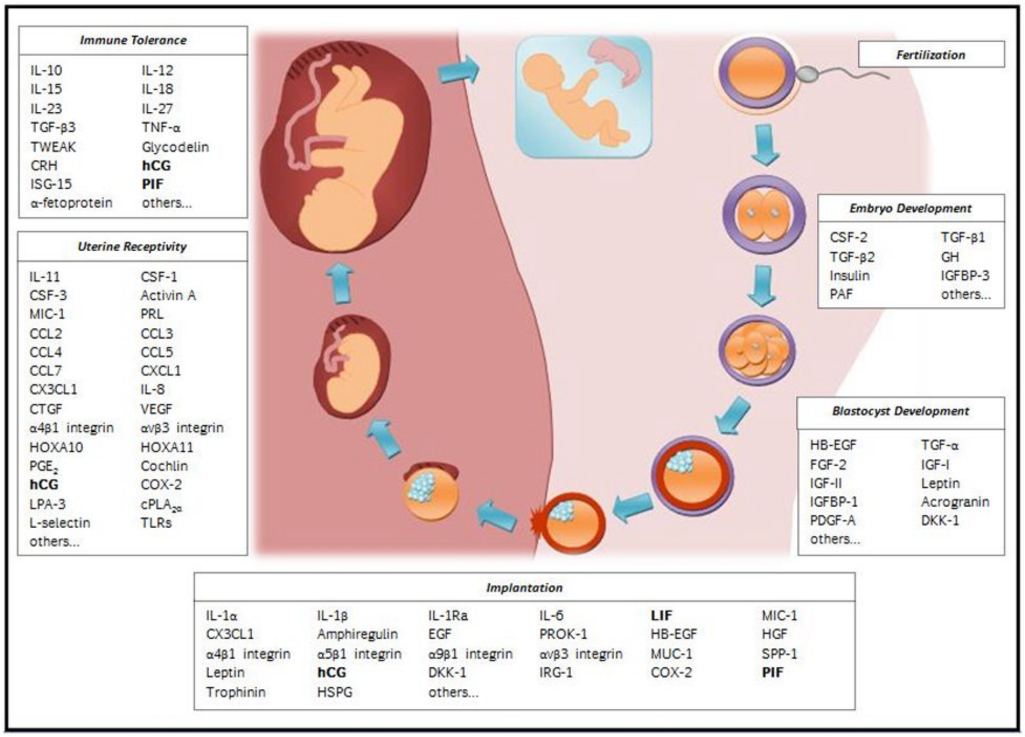

Fig. 1.

Schematic of the factors involved in the establishment of pregnancy. Factors that are essential and pregnancy-specific are represented in **bold.** These factors may provide interesting targets for contraception.

Table I

Cytokines Involved in the Establishment of Pregnancy

	Protein	Size	Human Gene	Role	Species
Interleukins	IL-1α	18 kDa	*IL1A*	induces changes for adhesion and invasion [44, 45]	human/mouse
	IL-1β	17.5 kDa	*IL1B*	induces changes for adhesion and stimulates IL-8 production [43, 44, 46]	human/mouse
	IL-1 receptor antagonist (IL-1Ra)	17 kDa	*lL1RN*	prevents adhesion [47]	mouse
	IL-6	26 kDa	*IL6*	stimulates leptin secretion and metalloproteinase activity [74]	human/mouse
	IL-10	18 kDa	*IL10*	decreases cytotoxic activation of uNK cells [107]	human/mouse
	IL-11	23 kDa	*IL11*	receptor signaling required for decidua development [71–73]	human/mouse
	IL-12	75 kDa	*IL12A/IL12B*	Immunomodulatory [104]	human
	IL-15	18 kDa	*IL15*	regulates IL-8 expression and uNK cells [85]	human
	IL-18	18 kDa	*IL18*	increases perforin expression and cytolytic potentials of uNK cells [105]	human
	IL-23	21 kDa	*IL23A/IL12B*	immunomodulatory, regulates IL-8 expression [99, 108]	human/mouse
	IL-27	27 kDa	*EBI3/IL30*	Immunomodulatory [108]	mouse
	leukemia inhibitory factor (LIF)	26 kDa	*LIF*	regulates expression of genes important in implantation [19, 20]	human/mouse
CSFs	Granulocyte colony-stimulating factor (G-CSF)	~19 kDa	*CSF3*	recruits macrophages to the uterus to prepare it for implantation [90]	mouse
	Granulocyte macrophage colony-stimulating factor (GM-CSF)	14.4 kDa	*CSF2*	enhances proliferation and viability of blastomeres [14, 15]	mouse
	Macrophage colony-stimulating factor (M-CSF)	~36 kDa	*CSF1*	recruits macrophages to the uterus to prepare it for implantation [89, 90]	mouse
TGFβ Superfamily	Activin A	24–28 kDa	*INHBA*	promotes decidualization; prevents activitation of T cells [68–70]	mouse
	Macrophage inhibitory cytokine (MIC-1)	25 kDa	*GDF15*	regulates trophoblast migration/invasion and decidualization [39, 85]	human
	Transforming growth factor β1 (TGFβ1)	25 kDa	*TGFB1*	regulate embryo development [12, 13]	human/mouse
	Transforming growth factor β2 (TGFβ2)	25 kDa	*TGFB2*	regulate embryo development [12]	human
	Transforming growth factor β3 (TGFβ3)	25 kDa	*TGFB3*	promotes a regulatory T cell response [111]	mouse
TNF Family	Tumor necrosis factor α (TNFα)	25 kDa	*TNF*	immunomodulatory, has deleterious effects at high levels [4–6]	human/mouse
	tumor necrosis factor-like weak inducer of apoptosis(TWEAK)	17 kDa	*TWEAK*	controls cytotoxicity, possibly through regulation of IL-15 and IL-18 [108, 113]	human/mouse

	Protein	Size	Human Gene	Role	Species
Hormones	Growth hormone (GH)	22 kDa	*GH1/GH2*	effects quality of embryo and fertilization rate [17]	human
	Prolactin (PRL)	24 kDa	*PRL*	promotes decidualization [76]	human/mouse

Table II

Chemokines and Growth Factors Involved in the Establishment of Pregnancy

	Protein	Size	Human Gene	Role	Species
Chemokines	CCL-2 (MCP-1)	~11 kDa	*CCL2*	recruits monocytes, macrophages and T cells in the endometrium [89, 92, 93, 95]	human/mouse
	CCL-3 (MIP1α)	7.9 kDa	*CCL3*	recruits macrophages[94]	human/mouse
	CCL-4 (MIP-1β)	7.62 kDa	*CCL4*	recruits macrophages and NK cells; promotes trophoblast migration [34, 85]	human
	CCL5 (RANTES)	8 kDa	*CCL5*	recruits macrophages; high levels negatively affect fertilization [89, 92, 94]	human/mouse
	CCL-7 (MCP-3)	8.5 kDa	*CCL7*	recruits macrophages and NK cells; implantation requires a downregulation [85, 96]	mouse
	CXCL1 (GRO1; KC)	~11 kDa	*CXCL1*	upregulates the inflammatory response [94, 100]	human/mouse
	IL-8 (CXCL8)	8.5 kDa	*IL8*	regulates expression of inflammatory response genes [100, 112]	human
	CX3CL1 (fractalkine)	90 kDa	*CX3CL1*	recruits macrophages and NK cells; promotes trophoblast migration; regulates gene expression for adhesion [34, 48, 98]	human
EGF Family	Amphiregulin (AREG)	9.5–16.5 kDa	*AREG*	regulated by LIF, important in implantation [18, 19]	mouse
	Epidermal growth factor (EGF)	~6 kDa	*EGF*	stimulates trophoblast migration/invasion [35, 36]	human/mouse
	Heparin binding EGF-like growth factor (HB-EGF)	22 kDa	*HB-EGF*	regulated by LIF; promotes development of blastocyst, motility, attachment and invasion [18, 24]	human/mouse
	Transforming growth factor α (TGFα)	17 kDa	*TGFA*	increases the rate of blastocoel expansion [28]	mouse
Growth Factors	Acrogranin/progranulin	68 kDa	*GRN*	promotes blastocyst hatching, adhesion and outgrowth [29, 30]	mouse
	Basic fibroblast growth factor (FGF2, bFGF)	18–22 kDa	*FGF2*	prepares blastocyst for migration [25]	mouse
	Connective tissue growth factor (CTGF)	~38 kDa	*CTGF*	regulates uterine function [87, 88]	human/mouse
	Hepatocyte growth factor (HGF)	78 kDa	*HGF*	regulates cytotrophoblast differentiation and depth of invasion [26]	human
	Platelet-derived growth factor (PDGF-A)	16 kDa	*PDGFA*	promotes trophoblast outgrowth [27]	mouse
	Prokineticin 1 (PROK1)	9.7 kDa	*EGVEGF*	promotes expression of implantation-related genes (i.e. LIF) [40,41]	human
	Vascular endothelial growth factor (VEGF-A)	45 kDa	*VEGFA*	maintains corpus lutuem[67]	human

Table III

Integrins and Other Factors Involved in the Establishment of Pregnancy

	Protein	Size	Human Gene	Role	Species
Integrins	α4β1 integrin	~280 kDa	*ITGA4/ITGB1*	important in implantation and decidualization [55]	human
	α5β1 integrin	~265 kDa	*ITGA5/ITGB1*	essential for the migration of extravillous trophoblasts (IFG-I-induced) [37]	human
	α9β1 integrin	~230 kDa	*ITGA9/ITGB1*	important in implantation [26]	human
	αvβ3 integrin	~230 kDa	*ITGAV/ITGB3*	involved in EVT migration (IGF-I-induced), important in implantation and decidualization [18,38,55]	human
Other Factors	Adrenomedullin	6 kDa	*ADM*	involved in invasion and pinopode formation [62–64]	human/mouse
	α-fetoprotein	70 kDa	*AFP*	inhibits the immune response [118]	mouse
	Cochlin (COCH)	~60 kDa	*COCH*	regulated by LIF; marker for uterine receptivity? [21]	mouse
	Corticotropin-releasing hormone (CRH)	~5 kDa	*CRH*	promotes implantation by regulating FasL expression [114, 115]	human/mouse
	Cyclooxygenase-2 (COX-2)	72 kDa	*PTGS2*	synthesizes prostaglandins; required for fertilization, implantation and decidualization [79]	mouse
	Cytosolic phospholipase A2α (cPLA$_{2\alpha}$)	85 kDa	*cPLA2α*	provides arachidonic acid for synthesis of PGs by COX2; deficiency results in abnormal spacing and delayed implantation [60]	mouse
	Dickkopf-1 (DKK-1)	~25 kDa	*DKK1*	required for blastocyst outgrowth and adhesion [33]	mouse
	Glycodelin	28 kDa	*PAEP*	involved in sperm-oocyte binding and prevention of the inflammatory response [109, 110, 124]	human
	Heparan sulfate proteoglycans (HSPG)	>500kDa	n/a	Expressed on the trophectoderm of blastocyst during the attachment phase of implantation [54]	mouse
	Human chorionic gonadotropin (hCG)	37.6 kDa	*CGB*	responsible for progesterone production and LIF expression; maintains the corpus luteum; also involved in angiogenesis, attachment and immune tolerance [1, 77, 116]	human
	Homebox A10 (HOXA-10)	~40 kDa	*HOXA10*	required for decidualization and successful implantation [82–84]	human/mouse
	Homebox A11 (HOXA-11)	~35 kDa	*HOXA11*	required foruterine stromal and glandular cell differentiation [81]	mouse
	Immunoresponsive gene 1 homolog (IRG1)	~52 kDa	*IRG1*	regulated by progesterone and LIF; important for implantation [19, 42]	mouse
	Insulin	5.8 kDa	*INS*	increases cell proliferation of early stage embryos [7, 8]	mouse
	Insulin-like growth factor I (IGF-I)	7.65 kDa	*IGF1*	increases number of cells in inner cell mass [31]	mouse

Protein	Size	Human Gene	Role	Species
Insulin-like growth factor II (IGF-II)	7.5 kDa	*IGF2*	involved in oocyte maturation and development of the embryo to blastocyst stage [9, 16]	human/mouse
Insulin-like growth factor binding protein 1 (IGFBP-1)	~25 kDa	*IGFBP1*	limits trophoblast growth and inhibits IGF-I activity [26, 66]	human
Insulin-like growth factor binding protein 2(IGFBP-3)	~40 kDa	*IGFBP3*	upregulated by LIF; involved in oocyte maturation and embryo development 9, 19	human/mouse
Interferon-induced 17 kDa protein (ISG15)	17 kDa	*ISG15*	induced in the endometrium in response to the implanting conceptus; immunomodulatory? [117]	human/mouse
Leptin	16 kDa	*LEP*	involved in blastocyst development; mediates the invasiveness of the cytotrophoblast [32, 45, 65]	human/mouse
Lysophosphatidic acid receptor 3 (LPA3)	40 kDa	*LPAR3*	regulates uterine receptivity [58, 59]	mouse
L-selectin	43 kDa	*SELL*	plays an early role in the homing of leukocytes the uterus, regulating uterine receptivity [102, 103]	human/mouse
Mucin 1 (MUC-1)	>300 kDa	*MUC1*	involved in embyro attachment [1, 53]	mouse/human
Oviduct-specific glycoprotein (OVGP1; MUC-9)	120 kDa	*OVGP1*	enhances binding of sperm to the zona pellucida [123]	human
Platelet activating factor (PAF)	~524 kDa	n/a	stimulates early embryo development [10, 11]	human/mouse
Preimplantation factor (PIF)	0.6–1.8 kDa	n/a	regulates immunity, promotes adhesion and invasion, and regulates apoptotic processes[56, 57]	human
Prostaglandin E_2 (PGE_2)	352 kDa	n/a	involved in the inflammatory response in the endometrium required for implantation [1]	human
Secreted phosphoprotein 1 (SPP1)	44 kDa	*SPP1*	allows for attachment to the luminal epithelium; induces focal adhesions [49, 50]	mouse
Trophinin	69 kDa	*TRO*	involved in activation of the trophectoderm for adhesion [51]	human/mouse