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Regulation of embryonic implantation[☆]

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Abstract

The preimplantation embryo produces several factors during its development to signal its presence to the maternal organism. This paper will focus on the role of two distinctive cytokine and growth factor systems (interleukin-1 (IL-1) system and the vascular endothelial growth factor (VEGF) system) during early embryonic development and implantation.

IL-1 receptor is expressed in the endometrium of various species and antagonising the biological effects of IL-1 leads to implantation failure in mice. We could show that this is due to an endometrial, not an embryonic effect. Furthermore, we could detect the expression of all components of the IL-1 system in preimplantation embryos from mice and humans. We could show a possible influence of IL-1 on other systems involved in embryonic implantation, including invasion (MMPs/TIMPs) and angiogenesis (VEGF), therefore suggesting a role of this cytokine family during early embryonic development.

Immediately after contact to the endometrium, the embryo must induce angiogenesis to ensure its survival, VEGF is a potent angiogenetic growth factor. We have shown a cyclic regulation of the soluble VEGF-receptor, sflt, in human endometrium and have detected the expression of the transmembraneous VEGF-receptors, Flt-1 and kinase insert domain containing receptor (KDR) throughout the menstrual cycle. Furthermore, we have shown that the VEGF gene is one of the earliest genes activated during human preimplantation embryo development, giving rise to the assumption that VEGF is crucial for embryonic development.

Keywords: Embryonic implantation; Vascular endothelial growth factor; Infertility

1. Introduction

Infertility and pregnancy wastage affect one of every nine couples in Western Europe and in the United States. The molecular events of embryonic attachment to the endometrial epithelium and subsequent invasion and nidation into the stroma have long been of interest, scientifically to reproductive biologists and clinically to couples with infertility or habitual abortion and to the physicians caring for them. In order to achieve a successful pregnancy in the human, two major conditions have to be fulfilled: during the 4–5 days of transport through the fallopian tube, the embryo must undergo a series of complex maturation processes and, in the same time, a receptive endometrium must have developed. Human endometrium undergoes characteristic cyclic changes of proliferation and secretion and, without embryonic implantation, the endometrium is shed and the menstrual bleeding occurs. Uterine endometrium therefore is the anatomic prerequisite for the continuation of our species and its main purpose during the reproductive age is to communicate with, receive, nourish and protect the implanting blastocyst [1].

Understanding the factors involved in preimplantation embryo development and embryo–maternal interaction which result in the complex maturation of the embryo and eutopic implantation is crucial for reproductive medicine. Attempts to overcome the low success rates of human in vitro fertilization therapy by increasing the number of embryos per transfer often result in multiple-gestation pregnancies. These are not only associated with increased evidence of maternal and neonatal complications, but are also cause for concern on the part of medical economists. The total costs for delivery and neonatal care for triplet-pregnancies were calculated with US$ 109,765 and assisted reproduction techniques (ART) were responsible for 77% of higher order pregnancies [2]. On the other hand, even by increasing the number of embryos per transfer, the pregnancy rate will never be 100%.

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The preimplantation embryo produces several factors during its development to signal its presence to the maternal organism. The appropriate interaction between the preimplantation embryo and maternal endometrium is at least partly controlled by paracrine cytokines and this subject is extensively covered by several reviews [1,3–6]. Cytokine- and growth factors and their corresponding receptors have, on the mRNA-level, been detected in blastomeres and in preimplantation embryos from different species as well as in the human endometrium throughout the menstrual cycle. Although it is known that both, the endometrium and the preimplantation embryo express several of these cytokine/growth factor–receptor pairs during the time of implantation and although there is general agreement that both, endometrial and embryonic factors are involved in successful implantation, there is only limited knowledge about the actual role of these factors. A better understanding of these factors during early embryonic development and implantation could possibly lead to improved in vitro culture conditions and enhance the outcome of human IVF.

The purpose of this review is to discuss the data on some of the cytokine/growth factor–receptor pairs that are produced by the endometrium and embryo which potentially have functional roles in the process of preimplantation embryo development and implantation. Since the space of this article is limited, we can not discuss all these important cytokines and growth factors extensively. We will therefore focus on the role for two of the cytokine and growth factor systems we have been extensively studying over the last few years, the interleukin-1 (IL-1) system and the vascular endothelial growth factor (VEGF) system.

2. Interleukin-1 system

The Interleukin-1-system is composed of two agonists, Interleukin-1α (IL-1α) and -1β (IL-1β), one antagonist, the Interleukin-1 receptor antagonist (IL-1ra) and two membrane-bound receptors, Interleukin-1 receptor type I (IL-1R tI) and II (IL-1R tII) [7]. All components of the IL-1 family in humans are located on chromosome 2 and the protein-, DNA- and RNA-structures are all well characterized for many species. Both agonists are initially synthesized as precursor proteins of 31 kDa. The mature proteins have a molecular weight of 17 kDa and although the amino acid sequences have a similarity of only ~22%, they induce the same biological responses [8]. There is also a high similarity between the cDNA-sequences of IL-1α and -β in mice and humans [9]. Interleukin-1 receptors type I and II both possess a transmembrane domain and their extracellular portions are homologous with similar binding affinities for the agonists and antagonist; there is also a soluble form of the IL-1R tII. The IL-1 receptor type I is found in low numbers on almost all cell surfaces whereas IL-1R tII is found primarily on white blood cells. Only the binding of either IL-1α or -β to the IL-1 receptor type I results in signal transduction [10], with receptor type II and the soluble IL-1 receptor acting as competitors of the receptor type I [11]. The IL-1 receptor antagonist binds with a high affinity to both receptors and prevents signal transduction by IL-1α and -β [12].

The IL-1 system is intimately involved in implantation and preimplantation embryo development. In humans, the IL-1R tI has been detected in total human endometrium [13] and, more specifically, in endometrial epithelial cells with a maximal protein- and mRNA-expression during the luteal phase [14]—the time of embryonic attachment and implantation. IL-1β-mRNA was detected in secretory human endometrium beginning on day 23 of the menstrual cycle [15]. Recently, De los Santos et al. [16] detected all major components of the IL-1-system, namely IL-1β, IL-1ra and IL-1R tI immunohistochemically in single human preimplantation embryos and we could detect all major components of the IL-1 system in single mouse embryos on mRNA-level [17,18]. In vitro fertilized, cultured human embryos have been shown to produce both IL-1α and IL-1β, and high concentrations (>60 and >80 pg/ml) of these cytokines in culture media have been correlated with successful implantation after intrauterine transfer of these embryos [19], although other authors could not detect IL-1α or -β in culture fluids of human embryos [20]. We have also demonstrated the presence of IL-1β and IL-1R tI-mRNA and -proteins in the human fallopian tube in all phases of the menstrual cycle in epithelial and stromal cells of the human tubal mucosa [21], as well as the presence of IL-1R tI-mRNA in human preimplantation embryos grown from tripronuclear zygotes [22]. This allows the preimplantation embryo to communicate with maternal surfaces through its IL-1-production during the first 5 days of preimplantation development during tubal transport. In another study, single blastomeres from 12 human preimplantation embryos could be examined by RT/nested PCR for their expression of β-actin- IL-1β, IL-1ra and IL-1R tI-mRNA [23]. The mRNA for β-actin and IL-1R tI were detected in all blastomeres (100%) whereas IL-1β could be detected in only 9 of the blastomeres (75%). IL-1ra was expressed in only 2 (17%) of the blastomeres and those were simultaneously positive for IL-1β. Both IL-1ra positive embryos were arrested in development before reaching blastocyst stage. Five embryos (three of them IL-1β-mRNA positive and two IL-1β-mRNA negative) were transferred as blastocysts, none of the transfers resulting in a pregnancy. It appeared that preimplantation embryos expressing IL-1ra mRNA in a detectable amount appear more likely to be arrested in early developmental stages.

In mice, IL-1α and IL-1β have been detected and localized at mRNA- and protein-level in endometrial endothelial cells [24] in increasing levels from day 3 of pregnancy peaking between days 4 and 5 [25] with blastocyst implantation known to occur late on day 4. Systemically administered recombinant human IL-1ra given intraperitoneally from day 3 to 6 of pregnancy inhibited embryonic implantation in mice [26], therefore suggesting a role of the agonist (IL-1α and/or β) for attachment. Furthermore, it was demonstrated, that this prevention of embryonic implantation by IL-1ra in the mouse seems to be mediated by an effect on the endometrial epithelium, not on the preimplantation embryo. The mechanism by which IL-1ra interferes with embryonic attachment seems to be a direct effect on the endometrial epithelium by inhibition of transformation of the epithelial plasma membrane at the time of implantation, presumably related to the alteration of α4, αv, and β3 adhesion-molecules [27].

The expression of the IL-1-system mRNAs described in these studies may have possible implications for preimplantation embryo physiology and implantation. IL-1β and IL-1ra in human preimplantation embryos may influence the uterine endometrium in a paracrine manner and there are several mechanisms by which this may affect the process of implantation. IL-1 is known to induce the adhesion of some white blood cells like eosinophil and neutrophil granulocytes to endothelial cells [28] and this effect can be specifically antagonized by IL-1ra [29]. One possible mechanism for the attachment of the embryo to the endometrium may be the appropriate regulation of adhesion-molecules such as integrins at the implantation site. There is evidence that integrins, especially $\alpha_v\beta_3$-integrins that are expressed in human endometrium at days 20–24 of the menstrual cycle, may be necessary for embryonic attachment. They have therefore been considered a marker of uterine receptivity [30]. A recent study demonstrated that the β_3-integrin-subunit on the surface of human endometrial epithelial cells (EEC) could be upregulated by the coculture with a human preimplantation embryo. Furthermore, this effect was also achieved when IL-1α and/or IL-1β were added to the EEC-culture and blocked by administration of IL-1β plus anti-IL-1-antibody [5]. It was concluded that the appropriate stimulation of the IL-1R tI by binding of IL-1α and/or IL-1β might be responsible for initiating appropriate endometrial epithelial integrin expression and therefore might trigger the attachment and implantation. After attaching to the uterine surface, the embryo must traverse the epithelial layer and basement membrane to implant in the decidua for further development. The invasion process is associated with tissue remodeling of extracellular matrix and is regulated in part by matrix metalloproteinases (MMPs) [31,32]. The 92 kDa collagenase type IV [33–35] and their naturally occurring specific inhibitors, the tissue inhibitors of metalloproteinases (TIMPs) [36] seem to play an important role in these processes. Recently the influence of the interleukin-1 system on this invasion process was demonstrated [37]: the mRNA-expression as well as the protein activity as determined by zymogram analysis of the 92 kDa collagenase type IV in cultured human luteal phase endometrial stroma cells was upregulated by coculture with IL-1β in a dose dependent manner. This effect could be reversed by simultaneous coculture with anti-IL-1β-antibody and/or IL-1ra. The mRNA-expression of TIMP I and III in the same cell cultures were downregulated by coculture with IL-1β in a dose dependent manner and this effect could be reversed by simultaneous coculture with anti-IL-1β-antibody and/or IL-1ra. Co-culture of luteal phase endometrial stroma cells with transforming growth factor β_1 (TGF-β_1) had a reverse effect on the mRNA-expression patterns of the 92 kDa collagenase type IV, TIMP I and III, therefore acting as a counterbalance to IL-1β and limiting the potential for endometrial invasion. It was concluded from these data that the IL-1β synthesized by the trophoblast cells might modulate the embryonic invasiveness into the maternal endometrium, allowing the embryo to digest the extracellular matrix and the basement membrane.

It should be mentioned that, although there appears to be certain evidence for the important role of the IL-1 system in murine and human reproduction, the IL-1R $tI^{-/-}$ knockout mice, even while having smaller litter sizes when compared to the wildtype IL-1R $tI^{+/+}$ mice, were able to reproduce [38]. Transgenic models are excellent tools to examine functions driven by single genes. This, however, is not the case for most reproductive functions, which are based in redundancy from the processes of implantation to parturition. Implantation is one example wherein redundant mechanisms are critical. Transgenic models therefore cannot be considered the ultimate validation in physiologic processes of reproduction that depend on redundancy for the survival of the species [27].

3. Vascular endothelial growth factor system

After invading the maternal endometrium, embryonic development is characterized by a dramatic growth of blood vessels coincident with decidualization, development of vascular membranes, and placenta formation [39]. These active processes involve both angiogenesis, the growth of blood vessels by sprouting from a pre-existing endothelium [40], and vasculogenesis, the in situ formation of primordial vessels from hemangioblasts [41,42]. The vascular endothelial growth factor (VEGF) system is composed out of one agonist, VEGF, two transmembraneous receptors, the kinase insert domain containing receptor (KDR or Flk-1) and the fms-like tyrosine kinase (Flt-1), as well as one soluble receptor (sflt) that acts as an antagonist to VEGF [43]. The agonist, VEGF, is a dimeric heparin-binding glycoprotein that has been purified as a vascular permeability factor from various tumor cell lines [44,45] and that has been shown to increase the proliferative ability of vascular endothelial cells in vitro by acting as a highly specific mitogen for these cell type [46]. It induces angiogenesis and increases the permeabilization of blood vessels [45]. The vascular endothelial growth factor-A (VEGF-A) ligand system is composed out of five isoforms created by alternative splicing of the VEGF mRNA. The human VEGF proteins have been characterized to consist of 121, 145, 165, 189 and 206 amino acids ($VEGF_{121}$, $VEGF_{145}$, $VEGF_{165}$, $VEGF_{189}$, and $VEGF_{206}$) (Figs. 1 and 2). All isoforms contain the exons 1–5 and 8. They differ only by various combinations

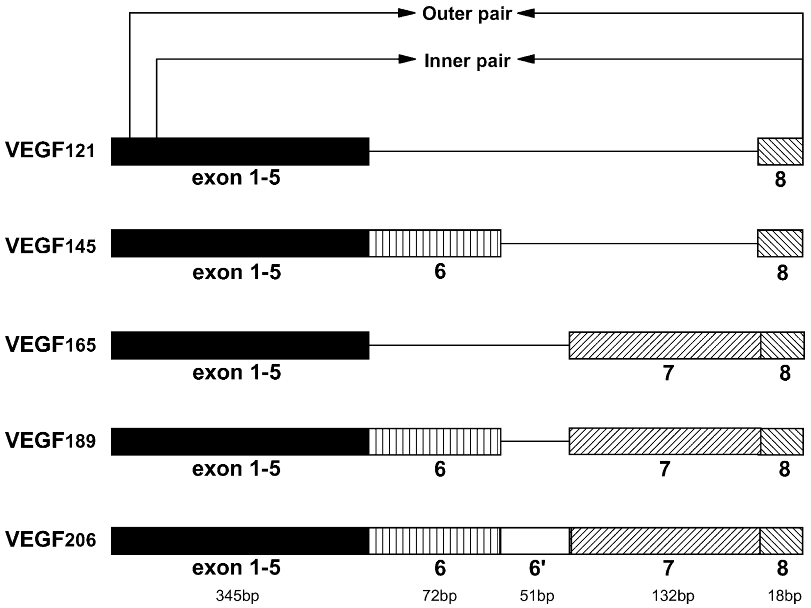

Fig. 1. Schematic illustration of the different VEGF-A isoforms. Five different isoforms are generated by mechanisms of alternative splicing. Different combinations of exons 6, 6′ and 7 are possible, whereas all isoforms contain the exons 1–5 and 8 [72].

of either no additional exon (VEGF$_{121}$), or addition of exon 6 (VEGF$_{145}$), exon 7 (VEGF$_{165}$), exons 6 and 7 (VEGF$_{189}$) or exons 6, 6′ and 7 (VEGF$_{206}$) [47–50]. Biologically, VEGF$_{121}$, VEGF$_{145}$ and VEGF$_{165}$ are secreted forms, whereas VEGF$_{189}$ and VEGF$_{206}$ appear to be bound to the cell surface [51].

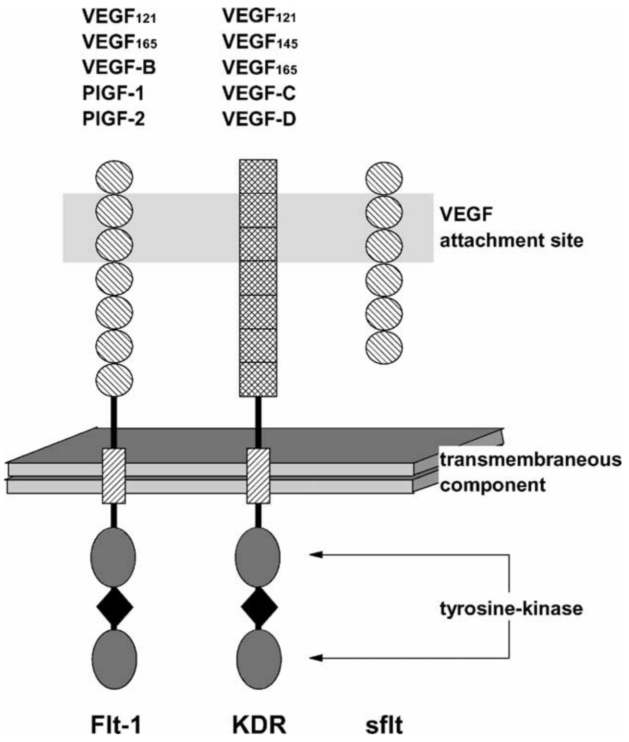

Fig. 2. Schematic illustration of VEGF-receptors Flt-1, KDR and sflt, and the interaction of these receptors with different growth factors.

There are three transmembraneous tyrosine kinase receptors VEGFR-1 (flt-1), VEGFR-2 (KDR or flk-1) and VEGFR-3 (flt-4) that bind to all secreted VEGF-isoforms [51]. Recently the transmembraneous neuropilin-1 receptor, a receptor from the collapsin/semaphorin family that mediates neuronal cell guidance was reportedly expressed on endothelial cells and specifically bound to the VEGF-isoform 165 [52]. Both, the kinase insert domain containing receptor (KDR or FLK-1) [53,54] and the fms-like tyrosine kinase (Flt-1) [55] contain a single membrane spanning domain, seven extracellular immunglobulin-like domains and an intracellular kinase-insert domain, they share 33% identity in the amino acid sequence [56]. Binding of VEGF to either of the receptors induces autophosphorylation and signal transduction but initiation of a biological response appears to be facilitated through the binding of VEGF to KDR rather than to Flt-1 [57].

There are data from knockout mice lacking specific components of the VEGF-system: VEGF$^{+/-}$ [58,59], Flt-1$^{-/-}$ [60], as well as KDR$^{-/-}$ [61] knock-out mice do not produce viable offspring. Embryos with functional inactivation of one VEGF allele (VEGF$^{+/-}$) show several malformations in the vascular system resulting in lethality on days 11 and 12 of pregnancy, thus suggesting a dose dependent regulation of fetal vascular development by VEGF. KDR is expressed on the surface of hemangioblasts and KDR$^{-/-}$ embryos fail to produce mature haematopoietic cells as well as endothelial cells. KDR therefore seems to be involved in the differentiation of both cell lines derived from hemangioblasts. Flt-1$^{-/-}$ embryos do produce endothelial cells, but these cells fail to combine to normal blood vessels. The specific roles of KDR and Flt-1 in vascular development and function are, however, not totally clear.

There is also a soluble form of the Flt-1 receptor called sflt [62] generated by alternative splicing of the Flt-1-mRNA, which encodes a protein similar to the Flt-1 protein but without the transmembraneous region and the intracellular kinase-insert domain. This soluble receptor acts as a specific high-affinity antagonist of VEGF function by competitively binding VEGF and therefore preventing the agonist–receptor interaction and the induction of a biological response.

Transmembraneous VEGF-receptors are expressed mostly on endothelial cells [54,60,61] and hemangioblasts [63], but Flt-1 could as well be detected in human trophoblast- and choriocarcinoma-cells [64,65]. The soluble receptor has initially been detected in endothelial cells [62]. Recently, sflt-mRNA and protein could be detected in villous trophoblast cells of the human placenta and the protein could also be detected within the circulation of pregnant women, suggesting a role in blocking angiogenesis locally in the placenta and probably in the pathogenesis of preeclampsia [66]. VEGF and its receptors have also been identified in several reproductive tissues, including corpus luteum, ovarian follicles, endometrial vessels and embryonic implantation sites in mice [67] as well as in giant trophoblast cells and early yolk sac [63] and in the human placenta [47], fallopian tube and ovary [68].

In the human endometrium, VEGF was detected on both, the mRNA and protein-level throughout the menstrual cycle with a maximal expression in secretory endometrium at the luteal phase; the protein was localized in glandular epithelial cells [69]. Furthermore, in a study to determine the quantitative mRNA-expression of the transmembraneous receptors Flt-1 and KDR as well as the soluble receptor sflt in human endometrium throughout the menstrual cycle, it was shown that, while mRNA for transmembraneous receptors Flt-1 and KDR was expressed at almost constant levels throughout the menstrual cycle, the soluble receptor, sflt, showed a 3× higher level of expression during the proliferative phase when compared to the secretory phase. The expression of Flt-1-, KDR- and sflt-mRNA was detected in both, isolated endometrial epithelial and stromal cell fractions [70]. This downregulation of sflt, which functions as a soluble antagonist, during the luteal phase may act to sensitize the maternal endothelial receptors to angiogenetic stimuli secreted by the implanting embryo.

Knowing that functional transmembraneous VEGF-receptors are present at low levels throughout the entire

Table 1
Cytokines, growth factors and their receptors in endometrium and in preimplantation embryos

	Endometrium	Preimplantation embryo
Interleukin-1		
IL-1α	Human: 108	Human: 89, 93
	Mouse: 29	Mouse: 59
IL-1β	Human: 52, 99	Human: 30, 64, 89
	Mouse: 29	Mouse: 48, 59, 62
IL-1 receptor antagonist (IL-1ra)	Human: 101	Human: 30, 64
		Mouse: 48, 59, 62
IL-1 receptor type I (IL-1R tI)	Human: 98, 99	Human: 30, 63, 64
		Mouse: 48, 62
Colony stimulating factor (CSF)		
CSF-1	Human: 4, 27, 79	Human: 53, 120
	Mouse: 9	Mouse: 5, 78
CSF-receptor (c-fms)	Human: 80	Human: 92
	Mouse: 9	Mouse: 78
Leukemia inhibitory factor and LIF-receptor	Human: 6, 16, 17, 60	Human: 16, 18, 92
	Mouse: 10	Mouse: 26, 75
Transforming growth factor		
TGF-α	Human: 19	Human: 46, 108
	Mouse: 86	Mouse: 112
TGF-β	Human: 8	Human: 7, 20, 43, 90
	Mouse: 82, 83, 86	Mouse: 111
Epidermal growth factor		
EGF	Human: 19	Human: 113
	Mouse: 81, 118	Mouse: 41, 50, 117
EGF-receptor	Human: 19	Human: 113
	Mouse: 2, 81, 83	Mouse: 28
Vascular endothelial growth factor		
VEGF	Human: 67, 68	Human: 96
VEGF antagonist (sflt)		Human: 65
VEGF-receptors (Flt-1, KDR)		Human: 65

menstrual cycle, it was recently demonstrated that VEGF-mRNA-expression is initiated already during the first cleavages of human preimplantation embryo development [71]. To further examine whether the mRNA detected in this study encoded for alternatively spliced mRNAs resulting in freely secreted proteins or proteins bound to cell-surface heparansulfate proteoglycans, the authors initiated another study [72]. Human blastocysts unsuitable for transfer ($n = 19$) were examined by reverse transcription/hemi-nested polymerase chain reaction for their expression of VEGF-mRNA splicevariants. $VEGF_{121}$ mRNA was detected in 88%, $VEGF_{145}$ mRNA in 100%, $VEGF_{165}$ in 71%, and $VEGF_{189}$ in 24% of blastocysts. There was co-expression of mRNA for $VEGF_{121}$ and $VEGF_{145}$ only in 29% blastocysts, of mRNA for $VEGF_{165}$ and $VEGF_{145}$ only in 12%, and of $VEGF_{121}$, $VEGF_{145}$ and $VEGF_{165}$ in 59% blastocysts. $VEGF_{206}$ mRNA could not be detected (Table 1). It was demonstrated that blastocysts express the mRNAs encoding for the free VEGF proteins, enabling the implanting embryo to immediately induce angiogenesis at the implantation site by binding to the endometrial receptors. Furthermore, $VEGF_{145}$ mRNA was expressed in 100% of blastocysts, suggesting a role of this splice variant for implantation [72].

There are clearly other cytokines and growth factors involved in endometrial functioning and in the process of implantation that should be discussed in greater detail, for example leukaemia inhibitory factor (LIF) and insulin-like growth factors (IGFs). LIF is the only growth factor that has been proven to be absolutely mandatory for the process of implantation, since transgenic mice lacking the gene for LIF can conceive, but the embryos do not implant [73]. However, when LIF is exogenously administered to LIF-deficient mice, the implantation rates revert to normal. Furthermore, when $LIF^{-/-}$-blastocysts are transferred into the uteri of wildtype ($LIF^{+/+}$) pseudopregnant mice, these embryos do implant normally [73]. There are several indications for a possible interaction of other cytokines and growth factors with LIF: transforming growth factor β (TGF-β), tumor necrosis factor α (TNF-α), epidermal growth factor (EGF) and IL-1β increase the in vitro expression of LIF in human endometrium [74]. LIF itself seems to stimulate the expression of the proinvasive enzymes matrix metalloproteinases 9 (MMP9) and urokinase-type plasminogen activator (uPA) in mice [75], and therefore possibly increases the invasive potential of the implanting embryo. LIF seems to enhance in vitro blastocyst development of mouse embryos [76,77] and ovine embryos [78] and also in human IVF [79].

4. Conclusion

As explained previously, this article can unfortunately not cover all the important cytokines and growth factors that are present in the human endometrium in detail. We have focused this article on the IL-1 system and the VEGF system and have tried to explain the possible interactions of these two systems with other cytokine and growth factor families. The interested reader is asked to study the list of references to intensify the information provided here. It is clear that cytokines, growth factors and their corresponding receptors that are produced in the endometrium and the preimplantation embryo have important roles in the maternal–fetal interaction during the peri-implantation period. Although the absolute necessity for embryonic implantation has only been demonstrated for LIF in the mouse; other cytokines and growth factors have important functional roles in this process. Although many of these cytokines and growth factors can be knocked out in transgenic mice without leading to complete reproductive failure, some of these knockouts show reproductive inefficiency and pregnancy wastage. Further studies on cytokine and growth factor expression in the endometrium and in preimplantation embryos will provide a better understanding of their role in human infertility and pregnancy lass, and open the way to find new treatment options.

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