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Review

Review

Ephrin and Eph receptor signaling in female reproductive physiology and pathology†

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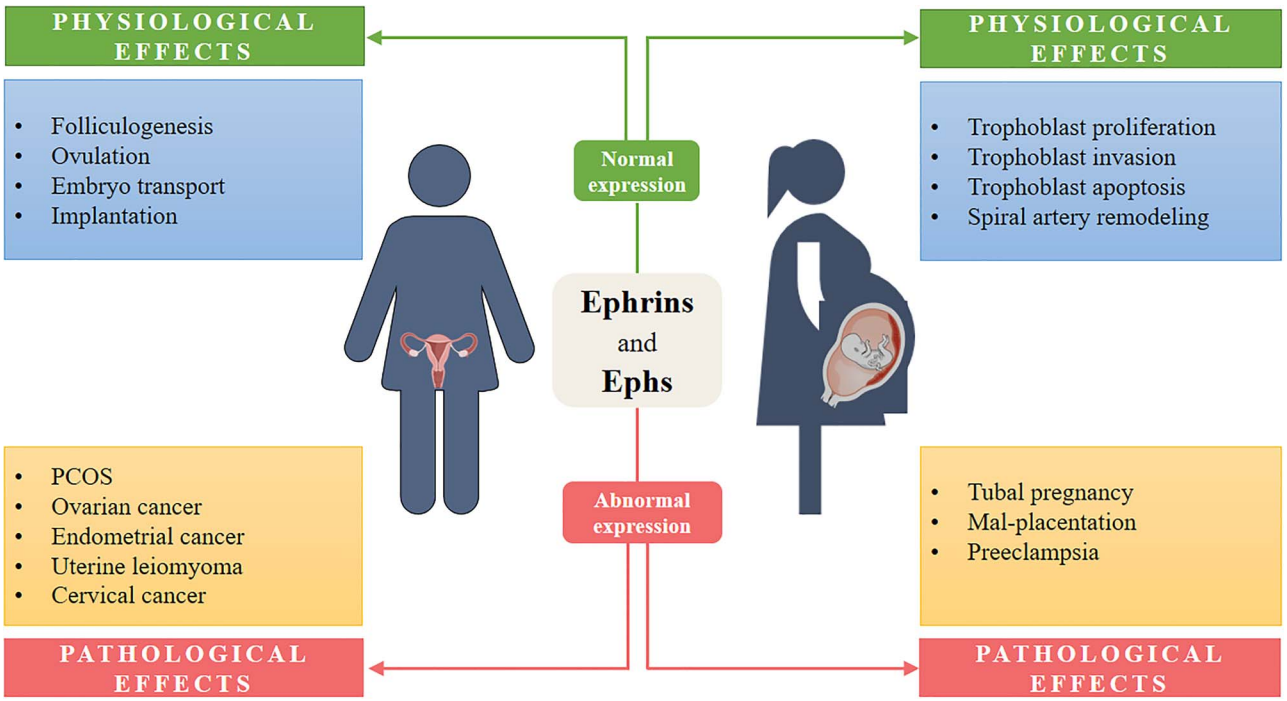
Abstract

Ephrins are ligands of Eph receptors (Ephs); both of which are sorted into two classes, A and B. There are five types of ephrin-As (ephrin-A1–5) and three types of ephrin-Bs (ephrin-B1–3). Also, there are 10 types of EphAs (EphA1–10) and six types of EphBs (EphB1–6). Binding of ephrins to the Eph receptors activates signaling cascades that regulate several biological processes such as cellular proliferation, differentiation, migration, angiogenesis, and vascular remodeling. Clarification of their roles in the female reproductive system is crucial to understanding the physiology and pathology of this system. Such knowledge will also create awareness regarding the importance of these molecules in diagnostic, prognostic, and therapeutic medicine. Hence, we have discussed the involvement of these molecules in the physiological and pathological events that occur within the female reproductive system. The evidence so far suggests that the ephrins and the Eph receptors modulate folliculogenesis, ovulation, embryo transport, implantation, and placentation. Abnormal expression of some of these molecules is associated with polycystic ovarian syndrome, ovarian cancer, tubal pregnancy, endometrial cancer, uterine leiomyoma (fibroids), cervical cancer, and preeclampsia, suggesting the need to utilize these molecules in the clinical setting. To enhance a quick development of this gradually emerging field in female reproductive medicine, we have highlighted some "gaps in knowledge" that need prospective investigation.

Summary sentence

The ephrin and Eph receptors are expressed throughout the female reproductive system, regulate the functioning of this system and associate with a number of obstetric and gynecologic disorders.

Graphical Abstract



Key words: ephrin, Eph receptor, ovary, endometrium, placenta, preeclampsia.

Introduction

Eph family receptor-interacting proteins (ephrins) are ligands of Eph (erythropoietin-producing human hepatocellular carcinoma cell) receptors. These receptors constitute the largest known subfamily of receptor protein-tyrosine kinases [1]. Based on their structure and linkage to the cell membrane, ephrins are categorized into ephrin-As and ephrin-Bs (Figure 1). There are five types of ephrin-As (ephrin-A1–5) and three types of ephrin-Bs (ephrin-B1–3). The Eph receptors (Ephs) are also categorized into EphAs and EphBs, based on their interactions with either ephrin-As or ephrin-Bs. EphAs are subdivided into 10 classes (EphA 1–10) and EphBs are subdivided into six classes (EphB1–6). EphA9 and EphB5 are not present in the human genome. Ephrin-As bind to EphAs and ephrin-Bs bind to EphBs (Table 1) [2, 3]. However, there are some exceptions. Ephrin-A5 can bind to EphB2 whereas ephrin-B2 and ephrin-B3 can bind to EphA4 (Table 1) [4]. Since both ephrins and Ephs are membrane-bound proteins (Figure 1), their binding and intracellular signaling activation occur via direct cell–cell interactions. Ephrins and Ephs undergo *cis*- and *trans*-interactions to mediate cellular processes such as proliferation, differentiation, migration, angiogenesis, and vascular remodeling (Figure 2) [3, 5, 6] via forward and reverse signaling which are jointly termed, bidirectional signaling. Forward signaling occurs in Eph receptor-expressing cells whereas reverse signaling occurs in ephrin-expressing cells (Figure. 2) [6].

The success of reproduction is partly dependent on the normal formation and functioning of the ovaries, fallopian tubes, endometrium, and the placenta. These processes are regulated by the interplay of several signaling molecules such as hormones, cytokines, and growth factors. Impairment in these signaling pathways often result in several female-specific disorders such as polycystic ovarian syndrome (PCOS), ovarian cancer, tubal pregnancy, endometrial cancer, uterine leiomyoma (fibroids), cervical cancer, and preeclampsia [7–12]. In this review, we have discussed the involvement of ephrin and Eph receptor signaling in the physiological and pathological events that are associated with the ovary, fallopian tube, endometrium, cervix, and the placenta.

Table 1. The ephrins and the corresponding Eph receptors they bind.

Ephrin	Eph receptors
Ephrin-A1	EphA1, EphA2, EphA3, EphA4, EphA5, EphA6, EphA7, EphA8, EphA10
Ephrin-A2	EphA1, EphA2, EphA3, EphA4, EphA5, EphA6, EphA7, EphA8, EphA10
Ephrin-A3	EphA1, EphA2, EphA3, EphA4, EphA5, EphA6, EphA7, EphA8, EphA10
Ephrin-A4	EphA1, EphA2, EphA3, EphA4, EphA5, EphA6, EphA7, EphA8, EphA10
Ephrin-A5	EphA1, EphA2, EphA3, EphA4, EphA5, EphA6, EphA7, EphA8, EphA10, EphB2
Ephrin-B1	EphB1, EphB2, EphB3, EphB4, EphB6
Ephrin-B2	EphA4, EphB1, EphB2, EphB3, EphB4, EphB6
Ephrin-B3	EphA4, EphB1, EphB2, EphB3, EphB4, EphB6

Ephrins and Eph receptors in ovarian biology

Oogenesis, folliculogenesis, and ovulation are the major morpho-physiological events in ovarian biology. The oogonia proliferate

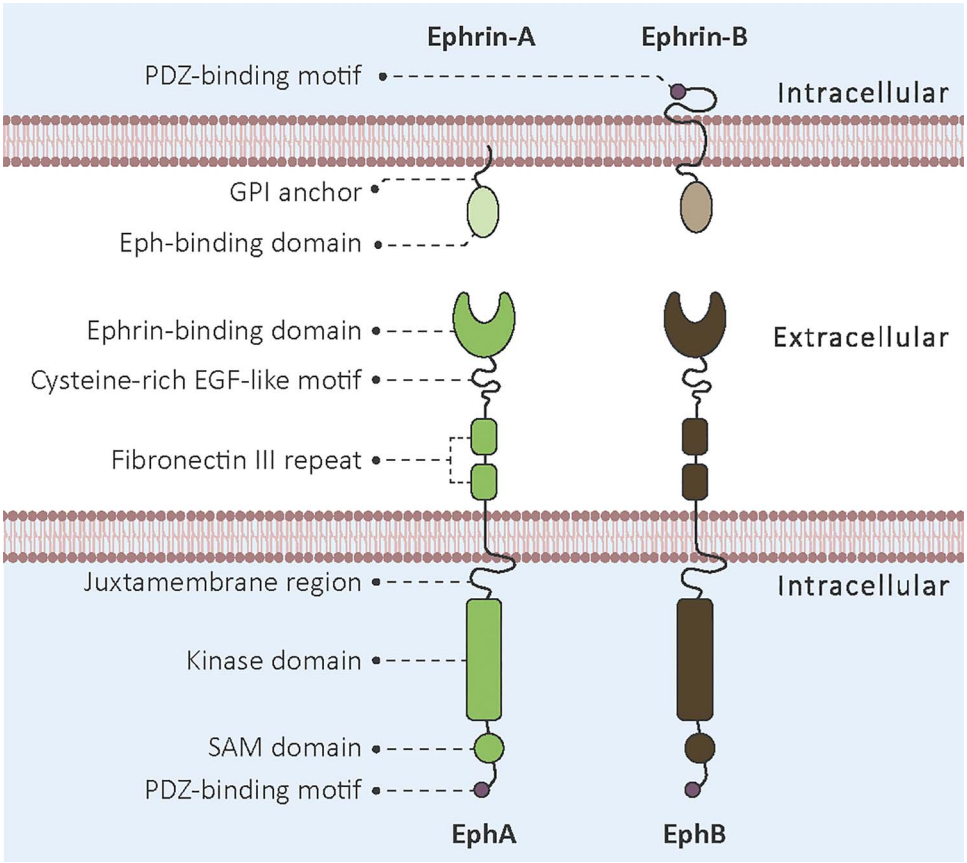


Figure 1. The general structure of the ephrins and the Eph receptors. Ephrin-As and ephrin-Bs have different structures. Ephrin-As are GPI-anchored to the plasma membrane whereas ephrin-Bs are transmembrane, and are connected to an intracellular PDZ-binding motif. On the other hand, EphAs and EphBs are similar in structure. They consist of an extracellular portion and an intracellular portion. The extracellular portion is composed of an ephrin-binding domain connected to two fibronectin type-III repeats by a cysteine-rich EGF-like motif. The intracellular portion consists of a juxtamembrane region that connects the extracellular portion of the receptor to a kinase domain which is linked to a SAM domain and a PDZ-binding motif in the cytoplasm. Ephs bind to ephrins through an extracellular Eph binding domain. Abbreviations: GP1, glycosylphosphatidyl inositol; PDZ, post synaptic density protein, Drosophila disc large tumor suppressor and zonula occludens-1 protein; SAM, sterile alpha motif.

and differentiate into primordial follicles. Activated primordial follicles - having a single layer of flattened granulosa cells (GCs) surrounding the primordial oocytes - sequentially differentiate into primary, secondary, and eventually antral (Graafian) follicles. A lot of the follicles undergo atresia whereas a few of them, under the stimulation of cyclic gonadotropins following puberty, reach the preovulatory stage. Under the influence of the preovulatory gonadotropin surge during each reproductive cycle, the dominant antral follicle releases the mature oocyte into the fimbriae of the fallopian tube whereas the remaining theca cells and GCs differentiate into the progesterone-secreting corpus luteum. These processes are precisely regulated in an orchestrated manner by many regulatory molecules (Figure 3) [13], including the ephrins and the Ephs (Figure 3).

Ephrin-A4, ephrin-A5, ephrin-B1, ephrin-B2, EphA3, EphA4, EphA45, EphA7, and EphA8 are expressed in the GCs [14–16]. EphA3, EphA5, and EphA8 expression in the GCs is regulated by follicle-stimulating hormone (FSH) via the protein kinase A (PKA) pathway [15]. This indicates that the GCs proliferation, differentiation, and steroidogenesis modulated by FSH may be mediated by ephrin and Eph receptor signaling. Investigation of this hypothesis is, thus, recommended. Human chorionic gonadotropin (hCG) also enhances the activities of the GCs by greatly influencing the expression of several genes in the GCs [17, 18]. Nonetheless, hCG is not a major determinant of ephrin/Eph receptor expression in the GCs [16]. Thus, the ephrin and Eph receptor system may regulate GCs morphophysiology, and this may largely occur via FSH signaling than hCG signaling. This hypothesis also merits investigation.

Decreased expression of ephrin-A5 leads to the downregulation of genes - such as progesterone receptor (PR), prostaglandin-endoperoxide synthase-2 (PTGS2), TNF-induced protein-6 (TNFAIP6), epiregulin (EREG), a disintegrin-like and metallopeptidase (reprolysin type) with thrombospondin type 1 motif-4 (ADAMTS4), and betacellulin (BTC) - involved in follicular growth and ovulation [19]. It also leads to subfertility associated with ovarian disorders [19]. Ephrin-A5 expression is elevated in large atretic follicles [14], indicating its involvement in follicular atresia. In a study, knockdown of ephrin-A5 promoted GCs proliferation via increased expression of proliferating cell nuclear antigen (PCNA) and the activation of the p-Akt and p-ERK1/2 pathways [20]. Also, it decreased GCs apoptosis by suppressing the expression of caspase-3, caspase-8, and BCL2-associated X protein (BAX). This resulted in increased release of estradiol without altering progesterone levels. The knockdown also inhibited the expression of tumor necrosis factor alpha (TNFα) [20], suggesting that ephrin-A5 induces GCs apoptosis by activating cell death receptors. Moreover,

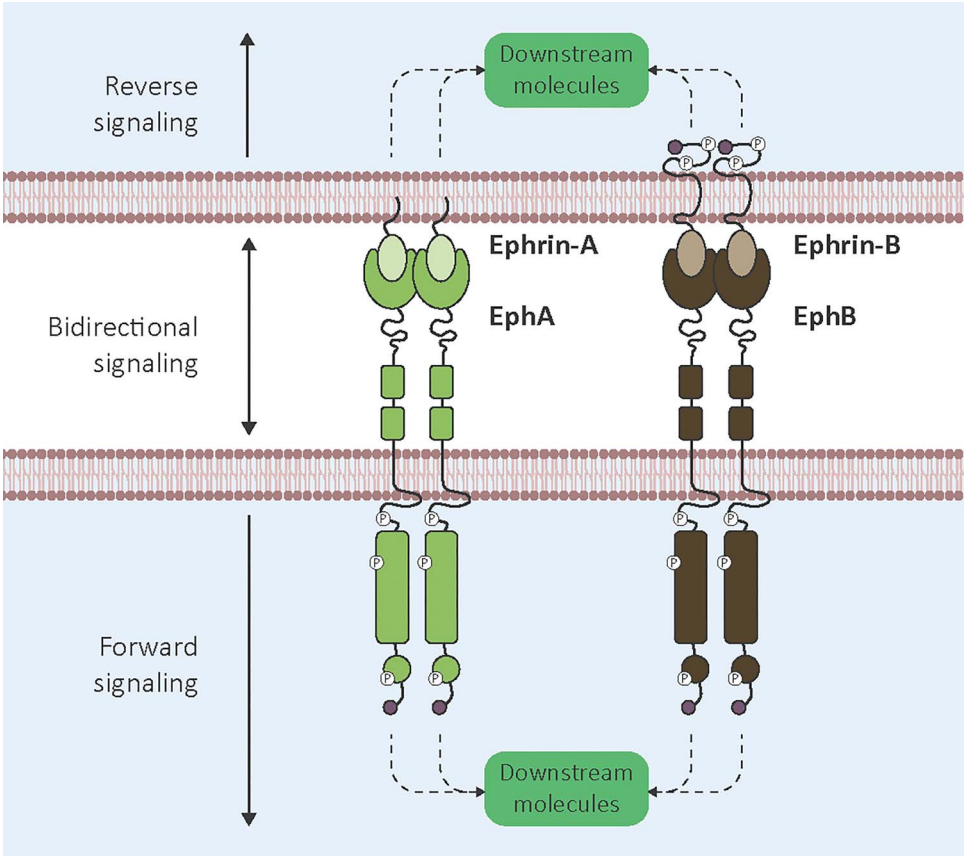


Figure 2. Mechanism of ephrin-Eph receptor bidirectional signaling. Binding of an ephrin to an Eph receptor causes a sequential clustering of several ephrins and Ephs, triggering autophosphorylation of the juxtamembrane region, the kinase domain and the SAM domain. This then elicits forward signaling in the Eph receptor-expressing cells and reverse signaling in the ephrin-expressing cells. Each signaling then activates several downstream molecules that mediate cellular functions. Abbreviations: SAM, sterile alpha motif; PDZ, post synaptic density protein, Drosophila disc large tumor suppressor and zonula occludens-1 protein.

it led to the respective upregulation and downregulation of bone morphogenetic protein-15 (BMP15) and growth differentiation factor-9 (GDF9) [20], two molecules which synergistically promote GCs proliferation via the ERK1/2 signaling pathway [21]. All these indicate that ephrin-A5 may regulate folliculogenesis (via GCs proliferation and steroidogenesis), follicular atresia and ovulation (Figure 3). Its ability to induce follicular atresia can help to maintain a healthy reproductive system, since a failed follicular atresia is associated with hormone-related cancers and chemoresistance. Nonetheless, confirmation of these roles of ephrin-A5 in vivo is highly recommended.

EphA7 promotes ovulation through the increased expression of CCAAT enhancer binding protein beta (C/EBPβ), Kruppel-like factor-4 (KLF4), and ADAMTS1 and increased secretion of luteinizing hormone and progesterone. Its expression is decreased in the GCs of PCOS patients [22]. This occurrence may account for the ovulatory impairment associated with this disease. Hence, its further investigation is recommended.

Ephrin-B1 is moderately expressed in theca cells and at a low level in GCs of the antral follicles. After ovulation, there is a rapid increase in ephrin-B1 expression in luteinizing GCs, but a decrease in its expression in luteinizing theca cells. Also, ephrin-B1, ephrin-B2, EphB1, EphB2, and EphB4 are expressed in the corpus luteum during the early luteal phase [23]. Since intense angiogenesis takes place in the corpus luteum to enable the hormone-producing cells to access the nutrients, oxygen, and the hormone precursors that are vital to the synthesis and the release of high quantities of progesterone to help establish and maintain early pregnancy [24, 25]; and the ephrins and Ephs have been found to enhance angiogenesis in other parts of the body [26, 27], it is possible that ephrin-B1, ephrin-B2, EphB1, EphB2, and EphB4 enhance angiogenesis in the corpus luteum. We recommend prospective studies to investigate this hypothesis as well as any other physiological significance of the expression patterns of these molecules in the corpus luteum.

Ephrins and Eph receptors in ovarian cancer

Ovarian cancer is the second most common malignancy after breast cancer in women above 40 years of age, and the fifth leading cause of cancer-related deaths in women. Despite the awareness that has been created about ovarian cancer, its curative and survival trends have not changed significantly as the early diagnosis of this disease remains a challenge [28]. Perhaps, the ephrin and EPh receptor system can be helpful in understanding the pathogenesis of this disease, and contribute to its early diagnosis.

Ephrin-A1, ephrin-A5, EphA1, and EphA2 are upregulated in ovarian cancer cells. Increased expression of ephrin-A1 and ephrin-A5 correlates with a shorter patient survival, but increased expression of EphA1 does not correlate significantly with patient survival [29]. Upregulation of EphA2 is also significantly associated with

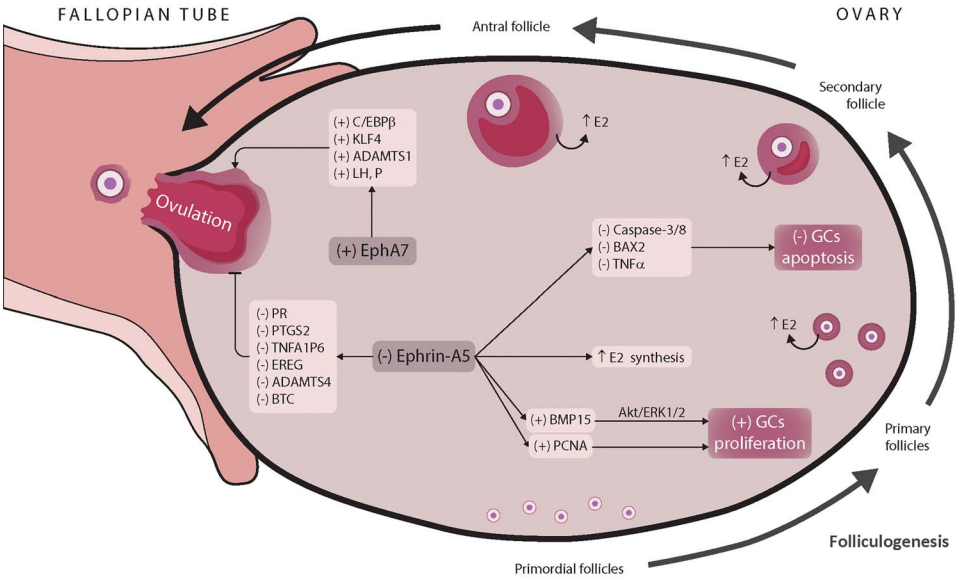


Figure 3. Mechanistic involvement of ephrins and Eph receptors in folliculogenesis and ovulation. Downregulation of ephrin-A5 promotes GCs proliferation via the upregulation of BMP15 and PCNA. BMP15 enhances GCs proliferation by activating the Akt/ERK1/2 signaling pathway. Also, downregulation of ephrin-A5 reduces the rate of GCs apoptosis by decreasing the expression of caspase-3, caspase-8, BAX, and TNFα. Moreover, it promotes estradiol production through an unknown mechanism. Interestingly, the downregulation of the molecule also inhibits ovulation via the downregulation of PR, PTGS2, TNFA1P6, EREG, ADAMTS4, and BTC. Hence, lower levels of ephrin-A5 may facilitate folliculogenesis but inhibit ovulation and vice versa. It is very possible that primary and secondary follicles express low levels of ephrin-A5 to enhance their development whereas antral follicles express higher levels of ephrin-A5 to enhance ovulation. This reflects a major involvement of ephrin-A5 in female fertility. On the other hand, upregulation of EphA7 promotes ovulation via the increased expression of C/EBPβ, KLF4, and ADAMTS1 and increased secretion of LH and progesterone. Abbreviations: GCs, granulosa cells; PCNA, proliferating cell nuclear antigen; BMP15, bone morphogenetic protein-15; BAX, BCL2-associated X protein; TNFα, tumor necrosis factor alpha; PR, progesterone receptor; PTGS2, prostaglandin-endoperoxide synthase-2; TNFAIP6, TNF-induced protein-6; EREG, epiregulin, ADAMTS4, a disintegrin-like and metallopeptidase (reprolysin type) with thrombospondin type 1 motif-1; BTC, betacellulin; C/EBPβ, CCAAT enhancer binding protein beta; KLF4, Kruppel-like factor-4 (KLF4); LH, luteinizing hormone.

a shorter patient survival, and it is a good prognostic marker of ovarian carcinomas. The expression of ephrin-A1 or EphA2 has no association with the age of the patient, histological type, and stage of the cancer [30].

The expression of EphA5 is gradually reduced from normal tissues to the more malignant forms of tumor. That is; EphA5 expression is sequentially and significantly decreased from normal fallopian tubes, benign ovarian serous tumors, ovarian serous borderline tumors to ovarian serous carcinomas [31]. This decreased expression is more often found in high-grade and advanced stage ovarian serous carcinomas. Regardless of this, there is no significant relationship between the expression of EphA5 and age at diagnosis, diameter, and metastasis of the disease [31]. It is very imperative to find out whether or not EphA5 expression is associated with patient survival.

EphA8 mRNA level is significantly higher in ovarian cancer tissues than in normal ovarian tissues or normal fallopian tube tissues [32]. Similarly, EphA8 protein level is significantly higher in ovarian cancer tissues than in normal ovarian tissues, benign ovarian tumors, and borderline tumors [32]. High EphA8 protein level is associated with higher age at diagnosis, higher stages, presence of metastasis, positive ascetic fluid, and higher serum levels of cancer antigen-125 (CA125); and can be an independent prognostic marker of poor overall survival in ovarian cancer patients [32].

Low expression of EphB1 is observed in ovarian serous carcinomas, and it is associated with high-tumor grade, metastasis, and a high proliferation rate, but not with the stage, age at diagnosis, and diameter of the carcinoma. The downregulation of EphB1 is associated with metastasis and poorer survival of the patients [33]. Ephrin-B1, ephrin-B2, EphB2, EphB3, and EphB4 are upregulated in ovarian cancers [29, 34]. The expressions of Ephrin-B2 and EphB4 are directly proportional to the stage of the cancer [34]. EphB6 expression is decreased in benign epithelial ovarian tumors, is decreased in ovarian serous borderline tumors and highly decreased in ovarian serous carcinomas. This decreased expression associates significantly with grade and stage of the cancer but inversely related to the proliferation rate of the cancerous cells. Patients with negative or weak expression of EphB6 have a poorer outcome than those with positive expression [35].

These reports indicate that the ephrins, except those not yet studied in ovarian cancers, are upregulated in the disease. Concerning the Ephs, while some are upregulated others are downregulated in ovarian cancers. In as much as this expression pattern helps to understand the pathogenesis of the disease, investigations of the mechanisms underlying this differential expression pattern will also enhance a detailed understanding of ovarian carcinogenesis.

Ephrins and Eph receptors in fallopian tube biology

The fallopian tube is the site for fertilization and preimplantation development of the embryo. It serves as a conduit for the transportation of sperms, secondary oocytes, and embryos. It consists of four main parts: fimbriae, infundibulum, ampulla, and isthmus.

The mucosa of the fallopian tube is lined with ciliated columnar epithelial cells. The swaying movement of the cilia and the rhythmic muscular contractions, termed peristaltic waves, facilitate the transport functions of the fallopian tube. These processes are controlled by molecules such as cytokines and hormones [36]. Impairment in these functions can cause tubal pregnancy and infertility [37, 38].

Ephrin and Eph receptor signaling is an important regulator of cell-to-cell interactions, and plays crucial roles in cell migration and adhesion during embryonic development [39]. EphA2 is expressed in both the ciliated and secretory cells of the fallopian tube epithelia in temporospatial-independent manner. The morphophysiology of the fallopian tube epithelium, including that of the ciliated and secretory cells, is modulated by the fluctuating sex hormones during the menstrual cycle [40]. Nevertheless, the levels of EphA2 in the fallopian tube epithelia remain constant throughout the menstrual cycle, regardless of the menstrual phase or the fallopian tube region [41]. This shows that EphA2 expression in the fallopian tube is not affected by the sex hormones. EphA2 can be activated by ephrin-A1 in human fallopian tubal epithelial cells, leading to its phosphorylation (Pho-EphA2) which can attenuate the adhesiveness of the fallopian tube [42]. Since tubal pregnancy is a consequence of molecular derangements in cell adhesion mediators and altered cilia activity within the tubal microenvironment [43, 44], disruption in ephrin and Eph receptor signaling in the fallopian tube can trigger certain pathological events such as tubal pregnancy. Intriguingly, elevated EphA2 expression and a decreased Pho-EphA2 are observed in the epithelia of the fallopian tube during tubal pregnancy [41].

The above reports indicate that EphA2 regulates the tubal motility of the embryo but this effect is independent on the sex hormones. Increased phosphorylation of EphA2 decreases the adhesiveness of the fallopian tube to facilitate embryonic transport (Figure 4). Hence, decreased Pho-EphA2 increases tubal adhesiveness to cause tubal pregnancy. The mechanisms responsible for the decreased rate of EphA2 phosphorylation during tubal pregnancy merits investigation. Also, the expression and roles of the ephrins and the other Ephs in the fallopian tube should be studied.

Ephrins and Eph receptors in uterine biology

The uterus is anatomically divided into four regions: the fundus (which is the uppermost rounded portion of the uterus), the body (corpus), the cervix, and the cervical canal. The uterus has three layers: endometrium, myometrium, and perimetrium. The endometrium is made up of the stratum basalis and the stratum functionalis. The stratum functionalis, in response to estrogen and progesterone, undergoes numerous morphological changes during the menstrual cycle; and it is discharged when no implantation occurs. Some of these changes are the appearance of pinopodes in the epithelium, decidualization of the stromal cells, and angiogenesis. These processes are regulated by growth factors, chemokines, cytokines, hormones, and cell adhesion molecules [45, 46] including the ephrins and the Ephs.

Ephrins and Eph receptors in endometrial changes

EphA3 is expressed in the mesenchymal stromal cells and perivascular spiral arterioles of the stratum basalis and the stratum functionalis, and enhances endometrial angiogenesis. This expression varies across the menstrual cycle, as EphA3 is less expressed in the proliferative phase but markedly expressed in the secretory phase [47]. In spite of this, the role of the ephrins and the Ephs in endometrial changes during the menstrual cycle has not been explored. It is already established that hypoxia promotes endometrial repair after menstruation [48, 49]. Interestingly, the expression of EphA3 in the stromal cells is facilitated by hypoxia via hypoxia inducible factor-1 alpha signaling [47]. Hypoxia similarly enhances the expression of ephrin-A1 and ephrin-A3 in the stromal cells. However, it attenuates the expression of ephrin-A4, ephrin-A5, ephrin-B2, EphA2, EphB2, EphB3, and EphB4 [47]. Thus, the expressions of the ephrins and the Ephs mediate the hypoxia-induced endometrial repair that follows menstruation. Investigation of the exact roles of the ephrins and the Ephs in endometrial repair is highly recommended. The strong and positive relationship between ephrin-A1/ephrin-A3 and EphA3, during hypoxia of the endometrial cells [47], somehow suggests that ephrin-A1 and ephrin-A3 preferentially signal through EphA3 in endometrial cells, and so should be investigated.

Ephrins and Eph receptors in embryo implantation

Embryo implantation in the uterus is a crucial step to a successful pregnancy. This process is partly dependent on sufficient uterine receptivity, during when adhesion-promoting molecules increase whereas adhesion-inhibitory molecules decrease. The embryo may utilize repulsive forces generated by the ephrin and Eph receptor system for its timely attachment to and subsequent invasion through the decidua basalis [50].

Ephrin-A1, ephrin-A2, ephrin-A3, and ephrin-A4 are expressed on blastocysts; and EphA1, EphA2, and EphA4 are expressed in the decidua [51]. The expression of ephrin-A1, ephrin-A2, ephrin-A3, and ephrin-A4 on the blastocyst transiently decreases around the implantation period. Also, the expression of EphA1 in the decidua and the expression of ephrin-A1 and ephrin-A3 on the blastocyst decrease at the implantation sites [51]. In assessing the role of this decreased expression in implantation, recombinant Eph-A1 was made to bind to the surface of ephrin-A-bearing trophectoderm cells. Interestingly, the binding resulted in the inhibition of blastocyst attachment. This points to a crucial role of the ephrin-A and EphA receptor system in regulating the initial blastocyst-maternal contact during the cross-talk period that precedes implantation [51].

In a follow-up study, these researchers investigated the physiological effects of blastocysts on EphA-bearing endometrial epithelial cells by using the permeability assay to examine the effects of ephrin A1 on cell-to-cell dissociation of Ishikawa cells. They observed that recombinant ephrin A1-conjugated beads were able to bind to the Ishikawa cells, indicating that EphA receptors expressed on the Ishikawa cells had the ability to interact with ephrin A1. In that study, ephrin A1 stimulation induced EphA2 phosphorylation and reduced the cell-to-cell barriers in a monolayer culture of the Ishikawa cells [52].

The expression of the ephrin-As on the blastocyst and the expression of EphA1 in the decidua prior to implantation mean that they play certain roles which are not yet known; and their downregulation, a requirement for a successful embryo implantation, suggests that these molecules facilitate the adhesion of the blastocyst to the decidua in a concentration-dependent manner (Figure 4). Failure of these molecules to be downregulated to favorable levels can, therefore, trigger tubal pregnancy or miscarriage.

Ephrins and Eph receptors in endometrial cancers

Endometrial cancers are the most common cancers of the uterus. There are type I endometrial cancers and type II endometrial cancers. Type I endometrial cancers account for 70–85% of the cases, and

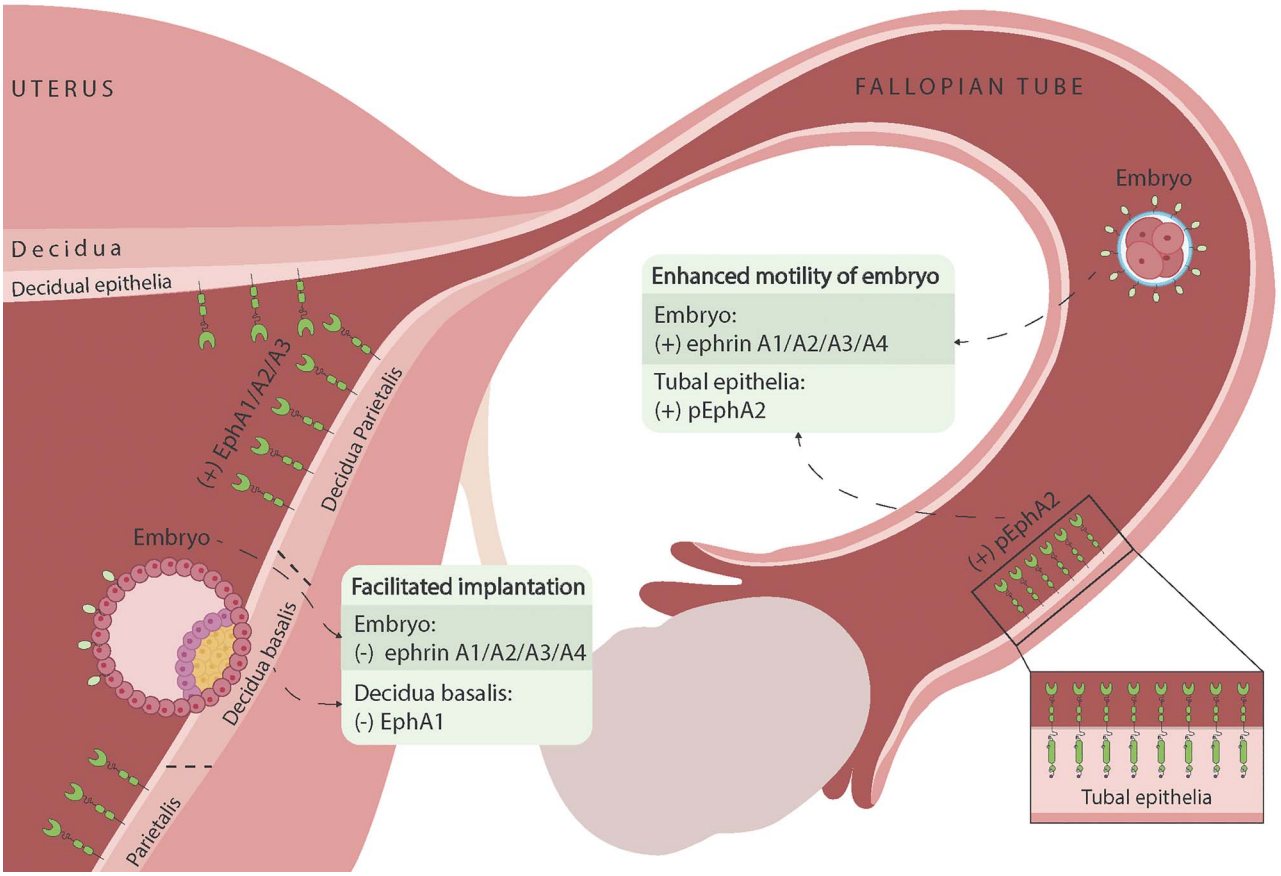


Figure 4. Mechanistic involvement of ephrins and Eph receptors in embryo motility and implantation. The ciliated and secretory cells of the fallopian tube epithelia express EphA2 which becomes phosphorylated (Pho-EphA2) to decrease the adhesiveness of the embryo to the fallopian tube so as to enhance its motility. Within the fallopian tube, the embryo expresses high levels of ephrin A1, ephrin-A2, ephrin-A3, and ephrin-A4. However, when the embryo reaches the uterus, the expression of these ephrins decreases to make embryo implantation possible. In the decidua parietalis, there is a high expression of EphA1, EphA2 and EphA3. The expression of EphA1 decreases at the implantation sites (decidual basalis) to enhance embryo implantation.

comprise low-grade endometroid carcinomas which express estrogen receptor (ER) and/or PR. In contrast, type II endometrial cancers represent about 10–20% of the cases, and comprise nonendometroid carcinomas which are associated with higher risk of recurrence and poor survival, due to their high-grade and their ability to deeply invade the myometrium and spread to neighboring tissues [53, 54].

EphA2 is upregulated in endometrial cancers, and associates positively with proliferation rate, myometrial invasion, disease stage, and tumor grade but inversely with ER and PR expression [55]. Patients whose endometrial cancers express higher levels of PR and ER have a good survival outcome [56], indicating that the expression of these receptors can be a mechanism that counteracts the disease. Ephrin-B2 and EphB4 are expressed in endometrial cancers but they are only upregulated in those cancers that express ER and PR [57]. Hence, they are upregulated in only type I endometrial cancers. This means that the expression of ephrin-B2 and EphB4 in endometrial cancers is partly dependent on ER and PR expression. Thus, while EphA2 expression may promote endometrial carcinogenesis, ephrin-B2 and EphB4 expression may inhibit endometrial carcinogenesis. Investigation of the molecular relationship between ephrin-B2/EphB4 and ER/PR is crucial to clarifying whether ephrin-B2 and EphB4 are potential therapeutic targets against endometrial cancers.

Ephrins and Eph receptors in uterine leiomyoma (fibroid or myoma)

Uterine leiomyomas (myomas or fibroids) are common benign tumors of the uterus. Their growth is determined by the rate of cell proliferation, cell differentiation, cell apoptosis, angiogenesis, and extracellular matrix deposition [58–60]. Ephrin-A4, ephrin-B2, ephrin-B3, EphA1–6, EphA8, EphB2, and EphB4 are upregulated in uterine leiomyomas [61]. Studies about the expression of EphA7 in this disease have yielded conflicting reports. Although in a study it was observed that EphA7 was downregulated in fibroids [61], in another study it was observed that EphA7 was upregulated in fibroids [62]. Apart from these conflicting reports, the roles of the ephrin and Eph receptor system in fibroids have not been reported. Nonetheless, the dysregulated expression of these molecules indicates their possible involvement in the pathogenesis of uterine leiomyomas, and so should be investigated.

Ephrins and Eph receptors in cervical cancer

The fourth most common female malignancy is cervical cancer [63]. EphB2 is upregulated in cervical cancers and promotes the invasiveness of the cancerous cells via the R-Ras signaling pathway [64]. This means that the pathogenesis of cervical cancer involves the upregulation of EphB2. Since human papillomavirus (HPV) is a

causative agent of cervical cancer [65], the upregulation of EphB2 may be associated with HPV infection or any other viral infection that causes cervical cancer. This hypothesis merits investigation.

Ephrins and Eph receptors in placentation

Placentation involves the formation of the cytotrophoblasts (CTBs) from the trophectoderm of the blastocyst, and its subsequent proliferation and differentiation into the syncytiotrophoblast (STB) and extravillous trophoblasts (EVTs). CTBs that differentiate into the STB undergo syncytialization whereas those that form the EVT lineages undergo epithelial to mesenchymal transition (EMT). During the process of EMT, the CTBs first differentiate to form noninvasive proximal column trophoblasts (PCTs), which further proliferate and differentiate into the nonproliferative but invasive distal column trophoblasts (DCTs). These trophoblasts then break through the overlying STB and anchor the embryo to the decidua. Interstitial EVTs (iEVTs) eventually develop from the anchoring villi and diffusively invade the decidua and the inner third of the myometrium to enhance the establishment of the histiotrophic nutrition and the uteroplacental circulation. Excessive invasion of the myometrium is prevented by the aggregation and fusion of the iEVTs to form giant cells. Derangement in each of these processes triggers abnormal placentation and its associated pregnancy complications such as preeclampsia and miscarriage (Figure 5) [7, 9–11, 66].

Ephrin-A1, ephrin-A4, and ephrin-A5, as well as EphA1, EphA2, and EphA4 are expressed in the human choriocarcinoma trophoblastic cells, called JEG3 cells. Both recombinant ephrin-A4 and recombinant EphA1 promote the invasiveness of JEG3 cells, via the upregulation of integrin $\alpha 5$, but do not affect the rate of proliferation of these cells [67]. EphA2 is also expressed in decidual endothelial cells, and enhances the proliferation and invasiveness of TEV1 cells by regulating ephrin-A1[68].

In the first two trimesters, EphA2 is expressed in the CTBs, PCTs, DCTs, Hofbauer cells, iEVTs and in decidual glands [69] but its role has not been reported. Also, ephrin-A1 is localized in the anchoring villi. However, during the third trimester, it is expressed in only the trophoblasts that have invaded the decidua [69]. This shows that ephrin-A1 is expressed in the placenta at each trimester of pregnancy in an extravillous cell lineage-specific manner, and may enhance the invasiveness of the EVTs. In placenta accreta, the expression of ephrin-A1 is normal and so may not have a role in the pathogenesis of this disease [69]. In preeclampsia, the integrity of ephrin-A1 expression is not known, but it is likely to be abnormal. The reason for this hypothesis is that matrix metalloproteinase-9 (MMP9), which coexpresses with and regulates ephrin-A1, is defective in the placentae of preeclamptic pregnancies [70, 71].

In early and midgestation, ephrin-B2 is expressed by the CTBs, STB, and EVTs and in the endothelial cells of the villous capillaries. At term, ephrin-B2 expression is generally diminished, as it is confined to the vascular endothelium, with irregular staining in the CTBs, STB, and EVTs [72]. Inhibition of ephrin-B2 suppresses the invasiveness of HTR8/SVneo cells through the downregulation of MMP2 and MMP9; and impairs the remodeling of spiral arteries through a decreased expression of placental growth factor [73]. In the placenta of pregnancies complicated by preeclampsia, ephrin-B2 is downregulated [74]. This suggests that ephrin-B2 facilitates placentation, and that its decreased placental expression is involved in the pathogenesis of preeclampsia.

In early gestation, EphB4 is expressed in the CTBs, STB, EVTs, giant cells, and mesenchymal cells of the villi, as well as in the subset of nucleated erythroid precursors, but not in the endothelial cells of the decidua [72]. At the 12th week of gestation, EphB4 is expressed at the apical side of the villi, as well as in the villous capillaries and the EVTs but these expressions are less as compared to those in early gestation [72]. At term, EphB4 expression is significantly reduced, with some still present at the apical side of the STB and in a subset of capillaries inside the villi, but neither in the CTBs, the EVTs, nor the giant cells [72]. Its expression in the EVTs is less as compared to that in the villous trophoblasts. It reduces the rate of proliferation but increases the rate of apoptosis of HTR8/SVneo cells by increasing the activity of caspase-3. It also inhibits the migration and invasiveness of these cells by decreasing MMP2 and MMP9 expression and suppressing the phosphatidyl inositol 3-kinase (PI3K) signaling pathway. Additionally, EphB4 is expressed in the decidual endothelial cells, and impairs spiral artery remodeling and endothelial integrity via a decreased expression of vascular endothelial growth factor-A (VEGF-A) [75]. Inhibition of EphB4 by microRNA-454 promotes the proliferation, migration, and invasion of trophoblast cells [76]. These observations suggest that EphB4 expression decreases when the CTBs proliferate and differentiate into the EVTs to quicken the rate at which the EVTs invade the decidua and remodel the spiral arteries, and failure of EphB4 to be downregulated can lead to abnormal placentation and its associated pregnancy complications (Figure 5B). This is confirmed by the observed upregulation of EphB4 in the placentae of preeclamptic women [75].

The ephrins and Ephs participate in the chemokine-induced migration of EVT cells. There is a transient switch in the expression of Ephs in the PCT and DCT cells. These cells upregulate ephrin-B1 and ephrin-B2 but downregulate EphB4 [77]. In the decidua, the invading iEVTs are guided toward the cells that express the arterial marker, ephrin-B2, and away from the cells that express the venous marker, EphB4/ephrin-B1. This results in the preferential remodeling of the arterioles, but not the veins [77]; and this is a hallmark of human placentation.

Both ephrin-B2 and EphB4 are expressed in the intravillous mesenchyme, either in the mesenchymal cells or in the newly formed capillaries. The expression of EphB4 is restricted to a subset of capillaries, and this probably indicates the venous fate of these vessels [72]. On the other hand, the expression of ephrin-B2 is evident in all capillaries during the first trimester. However, at term, the expression is restricted to a subset of the capillaries, probably the arterial capillaries. The period when positive EphB4 and negative ephrin-B2 capillaries are observed is nearly after the 10th week of gestation [72], indicating that arterial–venous endothelial differentiation may be promoted by an increased rate of blood flow at this period of gestation. This is further supported by the observed higher expression of ephrin-B2 in larger decidual arteries than in the smaller arteries [78].

Hypoxia regulates the expression of ephrin-B2 and EphB4. During hypoxia, there is a significant, constant, and time-dependent increase in the expression of ephrin-B2 whereas there is a moderate but transient increase in the expression of EphB4 [72]. Nonetheless, downregulation of ephrin-B2 and upregulation of EphB4 are evident in the placenta during preeclampsia [74]. Ephrin-B2 and EphB4 are direct targets of the upregulated micro-RNA-20b in these placentae, and their differential expression suggests that they are regulated by other molecules, such as micro-RNA-17 and microRNA-20a [74]. This indicates that hypoxia can trigger preeclampsia via the upregulation of EphB4 (Figure 5B). Nevertheless, the upregulatory effects

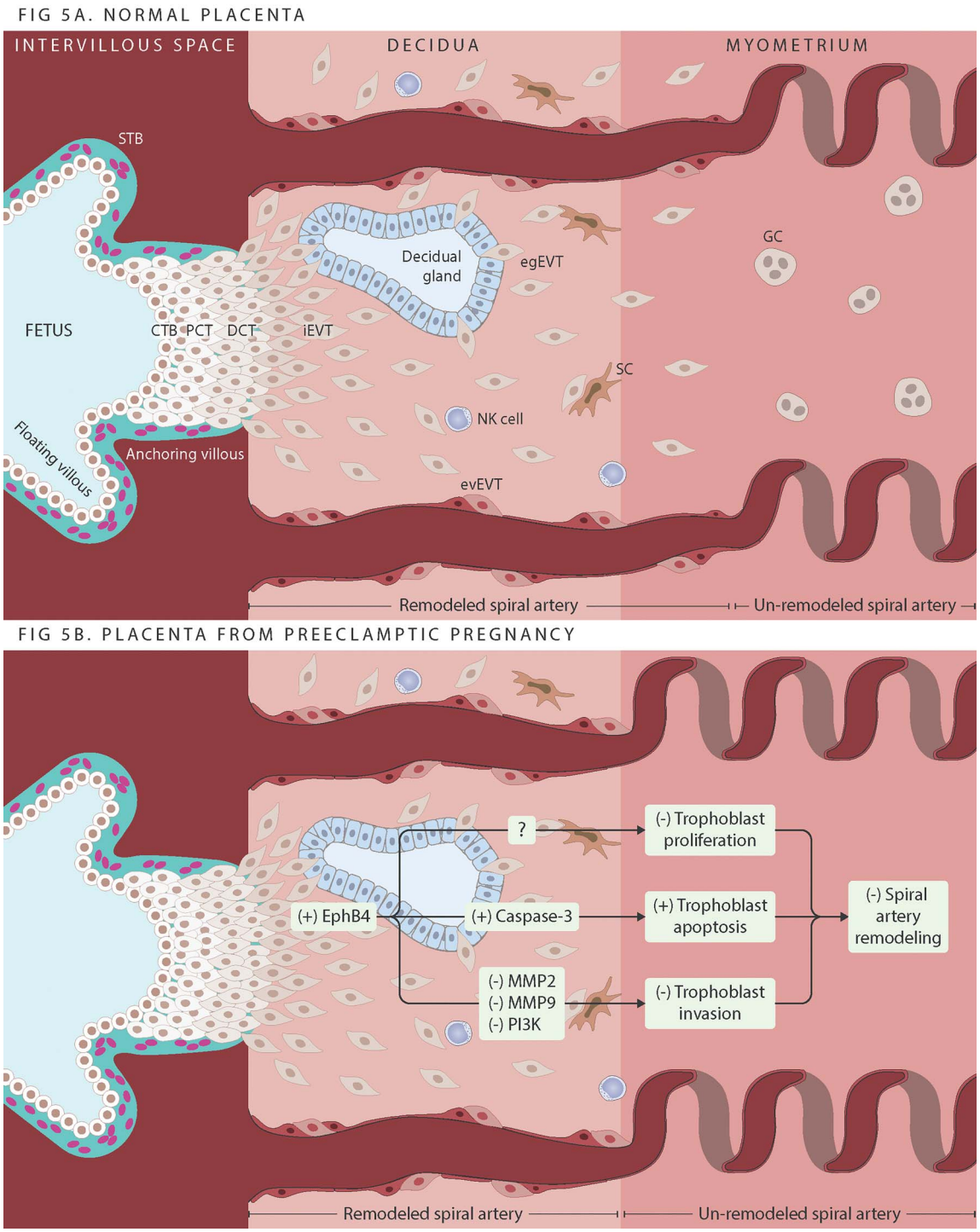


Figure 5. (B) Mechanistic involvement of EphB4 in abnormal placentation and preeclampsia. Upregulation of EphB4 inhibits trophoblast proliferation via unknown mediators and signaling pathways. It increases the rate of trophoblast apoptosis via the upregulation of caspase-3. Also, it inhibits trophoblast invasion through a decreased expression of MMP2, MMP9, and PI3K. All these lead to shallow spiral artery remodeling, such that uteroplacental circulation becomes defective to trigger preeclampsia. Abbreviations: EphB4, erythropoietin-producing human hepatocellular carcinoma cell B receptor-4; MMP2, matrix metalloproteinase-2; MMP9, matrix metalloproteinase-9; PI3K, phosphatidylinositol-3-kinase; NK, natural killer; SC, stromal cell.

of hypoxia on ephrin-B2 and the downregulation of ephrin-B2 in the placenta during preeclampsia challenge the proposition that hypoxia triggers preeclampsia. It is very needful to determine the relationship between these two molecules and hypoxia inducible factors.

The expression and role of ephrin-A3 in placentation have not been reported. However, microRNA-210, which is upregulated in the placenta during preeclampsia, inhibits trophoblast migration and invasion by repressing ephrin-A3 to cause preeclampsia [71].

This indicates that ephrin-A3 may be involved in placental formation and malplacentation-mediated pathologies, and so needs to be investigated.

Conclusion

The ephrin and Eph receptor system is an emerging field in female reproductive biology. The few studies available have shown that coexpression of the ephrins and the Ephs is a significant occurrence that regulates the physiology and pathology of the female reproductive system.

Decreased expression of ephrin-A5 in the GCs can enhance folliculogenesis but inhibit ovulation whereas increased expression of EphA7 promotes ovulation (Figure 3). Downregulation of EphA7 in the GCs is associated with PCOS and hence a potential therapeutic target in treating the disease. During ovarian carcinogenesis, ephrin-A1, ephrin-A5, ephrin-B1, ephrin-B2, EphA2, EphA8, EphB2, EphB3, and EphB4 are upregulated whereas EphA5, EphB1, and EphB6 are downregulated. Ephrin-B2 and EphB4 are upregulated in only type I endometrial cancers whereas EphA2 is upregulated in both type I and type II endometrial cancers. In fibroids, Ephrin-A4, ephrin-B2, ephrin-B3, EphA1–6, EphA8, EphB2, and EphB4 are upregulated; and in cervical cancers, EphB2 is upregulated. Thus, these molecules can serve as high-grade and low-grade diagnostic biomarkers, distinguishing biomarkers and prognostic biomarkers of these diseases as well as therapeutic targets in treating each of them. Nevertheless, the transcription factors and the signaling pathways activated by these molecules to mechanistically influence the proliferation, migration, invasion, and angiogenesis of these pathologies need further investigation.

Phosphorylation of EphA2 promotes the tubal transport of the embryo by decreasing the adhesiveness of the fallopian tube to the embryo. Decreased rate of EphA2 phosphorylation is involved in the pathogenesis of tubal pregnancy. When the blastocyst arrives in the uterus, ephrin-A1, ephrin-A2, ephrin-A3, and ephrin-A4 expression on the blastocyst and EphA1 expression in the decidua basalis transiently decrease to make implantation possible (Figure 4). The expression of these molecules in the uterine epithelia and in the decidua indicates their possible involvement in decidualization. Hence, it is very necessary to investigate this hypothesis.

As the placenta forms, ephrin-A1 and EphA2 promote trophoblast proliferation, whereas ephrin-A4, EphA1, and EphA2 promote trophoblast invasion yet their expression status in abnormal placentation is not known. Downregulation of ephrin-B2 and upregulation EphB4 in the placenta lead to abnormal placentation and preeclampsia (Figure 5B). There are other female-specific reproductive disorders - such as endometriosis, miscarriage, and placenta accreta - in which the involvement of the ephrin and Eph receptor system is not known. It is very imperative to investigate the involvement of this system in the pathogenesis of each of these diseases. Since DNA methylation is a major event in the dysregulation of genes, prospective studies should investigate the role of DNA methylation in the expression pattern of the ephrin and Eph receptor system in the physiological and pathological processes that occur in the female reproductive system. These studies should also identify the specific ephrin-Eph receptor binding that occurs in each process. Such findings will greatly enhance our understanding about female reproductive physiology and pathology, and assist in the designing of novel and proactive therapeutic interventions against the various gynecologic and obstetric disorders.

Conflict of interest

The authors have declared that no conflict of interest exists.

Ethical guidelines

Ethical issues are not applicable to this study since the study neither involved animal nor human subjects.

References

1. Tuzi NL, Gullick WJ. Eph, the largest known family of putative growth factor receptors. *Br J Cancer* 1994; **69**:417–421.
2. Pitulescu ME, Adams RH. Eph/ephrin molecules—A hub for signaling and endocytosis. *Genes Dev* 2010; **24**:2480–2492.
3. Kullander K, Klein R. Mechanisms and functions of Eph and ephrin signalling. *Nat Rev Mol Cell Biol* 2002; **3**:475–486.
4. Himanen J-P, Chumley MJ, Lackmann M, Li C, Barton WA, Jeffrey PD, Vearing C, Geleick D, Feldheim DA, Boyd AW, Henkemeyer M, Nikolov DB. Repelling class discrimination: Ephrin-A5 binds to and activates EphB2 receptor signaling. *Nat Neurosci* 2004; **7**:501–509.
5. Committee EN. Unified nomenclature for Eph family receptors and their ligands, the ephrins. *Cell* 1997; **90**:403–404.
6. Dudanova I, Klein R. The Axon's balancing act: Cis- and trans-interactions between Ephs and Ephrins. *Neuron* 2011; **71**:1–3.
7. Adu-Gyamfi EA, Fondjo LA, Owiredu WKBA, Czika A, Nelson W, Lamptey J, Wang Y, Ding Y. The role of adiponectin in placentation and preeclampsia. *Cell Biochem Funct* 2020; **38**:106–117.
8. Fondjo LA, Gyamfi EAA, Owiredu WKBA, Turpin CA, Mante DA, Anto EO. Maternal serum adiponectin, leptin and adiponectin-leptin ratio as possible biomarkers of preeclampsia. *Edorium J Gynecol Obs* 2016; **2**:41–47.
9. Adu-Gyamfi EA, Lamptey J, Duan F, Wang Y-X, Ding Y-B. The transforming growth factor β superfamily as possible biomarkers of preeclampsia: A comprehensive review. *Biomark Med* 2019; **13**:1321–1330.
10. Adu-Gyamfi EA, Ding Y-B, Wang Y-X. Regulation of placentation by the transforming growth factor beta superfamily. *Biol Reprod* 2020; **102**:18–26.
11. Adu-Gyamfi EA, Wang Y-X, Ding Y-B. The interplay between thyroid hormones and the placenta: A comprehensive review. *Biol Reprod* 2020; **102**:8–17.
12. Appiah Adu-Gyamfi E, Tanam Djankpa F, Nelson W, Czika A, Kumar Sah S, Lamptey J, Ding Y-B, Wang Y-X. Activin and inhibin signaling: From regulation of physiology to involvement in the pathology of the female reproductive system. *Cytokine* 2020; **133**:155105.
13. Jones ASK, Shikanov A. Follicle development as an orchestrated signaling network in a 3D organoid. *J Biol Eng* 2019; **13**:2.
14. Hatzirodos N, Irving-Rodgers HF, Hummitzsch K, Harland ML, Morris SE, Rodgers RJ. Transcriptome profiling of granulosa cells of bovine ovarian follicles during growth from small to large antral sizes. *BMC Genomics* 2014; **15**:24.
15. Buensuceso AV, Deroo BJ. The ephrin signaling pathway regulates morphology and adhesion of mouse granulosa cells in vitro. *Biol Reprod* 2013; **88**:21–25.
16. Xu Y, Zagoura D, Keck C, Pietrowski D. Expression of Eph receptor tyrosine kinases and their ligands in human granulosa lutein cells and human umbilical vein endothelial cells. *Exp Clin Endocrinol Diabetes* 2006; **114**:590–595.
17. Ophir L, Yung Y, Yerushalmi GM, Baum M, Machtinger R, Maman E, Hourvitz A. An optimized model for hCG stimulation of human mural granulosa cell culture. *Reprod Biol* 2019; **19**:67–74.
18. Bildik G, Akin N, Esmaeilian Y, Hela F, Yakin K, Onder T, Urman B, Oktem O. hCG improves luteal function and promotes progesterone output through the activation of JNK pathway in the luteal granulosa cells of the stimulated IVF cycles. *Biol Reprod* 2020; **102**:1270–1280.

19. Buensuceso AV, Son AI, Zhou R, Paquet M, Withers BM, Deroo BJ. Ephrin-A5 is required for optimal fertility and a complete ovulatory response to gonadotropins in the female mouse. *Endocrinology* 2016; **157**:942–955.
20. Worku T, Wang K, Ayers D, Wu D, Ur Rehman Z, Zhou H, Yang L. Regulatory roles of ephrinA5 and its novel signaling pathway in mouse primary granulosa cell apoptosis and proliferation. *Cell Cycle* 2018; **17**:892–902.
21. Sanfins A, Rodrigues P, Albertini DF. GDF-9 and BMP-15 direct the follicle symphony. *J Assist Reprod Genet* 2018; **35**:1741–1750.
22. Li S, Zhai J, Liu J, Di F, Sun Y, Li W, Chen Z-J, Du Y. Erythropoietin-producing hepatocellular A7 triggering ovulation indicates a potential beneficial role for polycystic ovary syndrome. *EBioMedicine* 2018; **36**:539–552.
23. Egawa M, Yoshioka S, Higuchi T, Sato Y, Tatsumi K, Fujiwara H, Fujii S. Ephrin B1 is expressed on human luteinizing granulosa cells in corpora lutea of the early luteal phase: The possible involvement of the B class Eph-ephrin system during corpus luteum formation. *J Clin Endocrinol Metab* 2003; **88**:4384–4392.
24. Gaytan F, Morales C, Garcia-Pardo L, Reymundo C, Bellido C, Sanchez-Criado JE. A quantitative study of changes in the human corpus luteum microvasculature during the menstrual cycle. *Biol Reprod* 1999; **60**:914–919.
25. Fraser HM, Wulff C. Angiogenesis in the corpus luteum. *Reprod Biol Endocrinol* 2003; **1**:1–8.
26. Brantley-Sieders DM, Chen J. Eph Receptors and Ephrins: Role in Vascular Development and Tumor Angiogenesis. In: *Tumor Angiogenesis*. New York, USA: Springer; 2008: 121–134.
27. Kuijper S, Turner CJ, Adams RH. Regulation of angiogenesis by Eph–ephrin interactions. *Trends Cardiovasc Med* 2007; **17**:145–151.
28. Stewart C, Ralyea C, Lockwood S. Ovarian cancer: an integrated review. In: *Seminars in oncology nursing*. Amsterdam, Netherlands: Elsevier; 2019.
29. Herath NI, Spanevello MD, Sabesan S, Newton T, Cummings M, Duffy S, Lincoln D, Boyle G, Parsons PG, Boyd AW. Over-expression of Eph and ephrin genes in advanced ovarian cancer: Ephrin gene expression correlates with shortened survival. *BMC Cancer* 2006; **6**: 144.
30. Han L, Dong Z, Qiao Y, Kristensen GB, Holm R, Nesland JM, Suo Z. The clinical significance of EphA2 and Ephrin A-1 in epithelial ovarian carcinomas. *Gynecol Oncol* 2005; **99**:278–286.
31. Chen X, Wang X, Wei X, Wang J. EphA5 protein, a potential marker for distinguishing histological grade and prognosis in ovarian serous carcinoma. *J Ovarian Res* 2016; **9**:83.
32. Liu X, Xu Y, Jin Q, Wang W, Zhang S, Wang X, Zhang Y, Xu X, Huang J. EphA8 is a prognostic marker for epithelial ovarian cancer. *Oncotarget* 2016; **7**:20801.
33. Wang H, Wen J, Wang H, Guo Q, Shi S, Shi Q, Zhou X, Liu Q, Lu G, Wang J. Loss of expression of EphB1 protein in serous carcinoma of ovary associated with metastasis and poor survival. *Int J Clin Exp Pathol* 2014; **7**:313.
34. Alam SM, Fujimoto J, Jahan I, Sato E, Tamaya T. Coexpression of EphB4 and ephrinB2 in tumour advancement of ovarian cancers. *Br J Cancer* 2008; **98**:845–851.
35. Gu Y, Li F, Qian N, Chen X, Wang H, Wang J. Expression of EphB6 in ovarian serous carcinoma is associated with grade, TNM stage and survival. *J Clin Pathol* 2016; **69**:448–453.
36. Li S, Winuthayanon W. Oviduct: Roles in fertilization and early embryo development. *J Endocrinol* 2017; **232**:R1–R26.
37. Tempfer CB, Dogan A, Tischoff I, Hilal Z, Rezniczek GA. Chronic ectopic pregnancy: Case report and systematic review of the literature. *Arch Gynecol Obstet* 2019; **300**:651–660.
38. Guan J, Watrelot A. Fallopian tube subtle pathology. *Best Pract Res Clin Obstet Gynaecol* 2019; **59**:25–40.
39. Fujiwara H, Yoshioka S, Tatsumi K, Kosaka K, Satoh Y, Nishioka Y, Egawa M, Higuchi T, Fujii S. Human endometrial epithelial cells express ephrin A1: Possible interaction between human blastocysts and endometrium via Eph-ephrin system. *J Clin Endocrinol Metab* 2002; **87**:5801–5807.
40. Zhu J, Xu Y, Rashedi AS, Pavone ME, Kim JJ, Woodruff TK, Burdette JE. Human fallopian tube epithelium co-culture with murine ovarian follicles reveals crosstalk in the reproductive cycle. *Mhr Basic Sci Reprod Med* 2016; **22**:756–767.
41. Jiang H, Yang X-Y, Zhu W-J. Dysregulated erythropoietin-producing hepatocellular receptor A2 (EphA2) is involved in tubal pregnancy via regulating cell adhesion of the fallopian tube epithelial cells. *Reprod Biol Endocrinol* 2018; **16**:84.
42. Yang X-Y, Zhu W-J, Jiang H. Activation of erythropoietin-producing hepatocellular receptor A2 attenuates cell adhesion of human fallopian tube epithelial cells via focal adhesion kinase dephosphorylation. *Mol Cell Biochem* 2012; **361**:259–265.
43. Kemp B, Kertschanska S, Kadyrov M, Rath W, Kaufmann P, Huppertz B. Invasive depth of extravillous trophoblast correlates with cellular phenotype: A comparison of intra-and extrauterine implantation sites. *Histochem Cell Biol* 2002; **117**:401–414.
44. Lyons RA, Saridogan E, Djahanbakhch O. The reproductive significance of human fallopian tube cilia. *Hum Reprod Update* 2006; **12**:363–372.
45. Zhang S, Lin H, Kong S, Wang S, Wang H, Wang H, Armant DR. Physiological and molecular determinants of embryo implantation. *Mol Aspects Med* 2013; **34**:939–980.
46. Yoshinaga K. A historical review of blastocyst implantation research. *Biol Reprod* 2018; **99**:175–195.
47. To C, Farnsworth RH, Vail ME, Chheang C, Gargett CE, Murone C, Llerena C, Major AT, Scott AM, Janes PW. Hypoxia-controlled EphA3 marks a human endometrium-derived multipotent mesenchymal stromal cell that supports vascular growth. *PLoS One* 2014; **9**:e112106.
48. Maybin JA, Murray AA, Saunders PTK, Hirani N, Carmeliet P, Critchley HOD. Hypoxia and hypoxia inducible factor-1α are required for normal endometrial repair during menstruation. *Nat Commun* 2018; **9**:1–13.
49. Okada H, Kido T. The influence of hypoxia on angiogenesis and metabolism in human endometrial stromal cells. *Fertil Steril* 2019; **112**:e132–e133.
50. Fujiwara H, Ono M, Sato Y, Imakawa K, Iizuka T, Kagami K, Fujiwara T, Horie A, Tani H, Hattori A. Promoting roles of embryonic signals in embryo implantation and placentation in cooperation with endocrine and immune systems. *Int J Mol Sci* 2020; **21**:1885.
51. Fujii H, Tatsumi K, Kosaka K, Yoshioka S, Fujiwara H, Fujii S. Eph–ephrin a system regulates murine blastocyst attachment and spreading. *Dev Dyn an Off Publ Am Assoc Anat* 2006; **235**:3250–3258.
52. Fujii H, Fujiwara H, Horie A, Sato Y, Konishi I. Ephrin A1 induces intercellular dissociation in Ishikawa cells: Possible implication of the Eph–ephrin a system in human embryo implantation. *Hum Reprod* 2011; **26**:299–306.
53. Suarez AA, Felix AS, Cohn DE. Bokhman redux: Endometrial cancer "types" in the 21st century. *Gynecol Oncol* 2017; **144**:243–249.
54. Murali R, Soslow RA, Weigelt B. Classification of endometrial carcinoma: More than two types. *Lancet Oncol* 2014; **15**:e268–e278.
55. Kamat AA, Coffey D, Merritt WM, Nugent E, Urbauer D, Lin YG, Edwards C, Broaddus R, Coleman RL, Sood AK. EphA2 overexpression is associated with lack of hormone receptor expression and poor outcome in endometrial cancer. *Cancer* 2009; **115**:2684–2692.
56. Zhang Y, Zhao D, Gong C, Zhang F, He J, Zhang W, Zhao Y, Sun J. Prognostic role of hormone receptors in endometrial cancer: A systematic review and meta-analysis. *World J Surg Oncol* 2015; **13**: 208.
57. Dong L, Cheng X, Zhou L, Huang Q, Li J, Yi C. Overexpression of erythropoietin-producing hepatocyte receptor B4 and ephrin-B2 is associated with estrogen receptor expression in endometrial adenocarcinoma. *Oncol Lett* 2017; **13**:2109–2114.
58. Islam MS, Ciavattini A, Petraglia F, Castellucci M, Ciarmela P. Extracellular matrix in uterine leiomyoma pathogenesis: A potential target for future therapeutics. *Hum Reprod Update* 2017; **24**:59–85.
59. Chegini N. Proinflammatory and profibrotic mediators: Principal effectors of leiomyoma development as a fibrotic disorder. In: *Seminars in*

reproductive medicine, vol. **28**. New York, USA: © Thieme Medical Publishers; 2010: 180–203.
60. Rafique S, Segars JH, Leppert PC. *Mechanical signaling and extracellular matrix in uterine fibroids. Seminars in reproductive medicine*, vol. **35**. New York, USA: Thieme Medical Publishers; 2017: 487–493.
61. Jiang Y, Suo G, Sadarangani A, Cowan B, Wang JYJ. Expression profiling of protein tyrosine kinases and their ligand activators in leiomyoma uteri. *Syst Biol Reprod Med* 2010; **56**:318–326.
62. Yu L, Moore AB, Dixon D. Receptor tyrosine kinases and their hormonal regulation in uterine leiomyoma. *Semin Reprod Med* 2010; **28**:250–259.
63. Bermudez A, Bhatla N, Leung E. Cancer of the cervix uteri. *Int J Gynecol Obstet* 2015; **131**:S88–S95.
64. Gao Q, Liu W, Cai J, Li M, Gao Y, Lin W, Li Z. EphB2 promotes cervical cancer progression by inducing epithelial-mesenchymal transition. *Hum Pathol* 2014; **45**:372–381.
65. Wentzensen N, Arbyn M. HPV-based cervical cancer screening-facts, fiction, and misperceptions. *Prev Med (Baltim)* 2017; **98**:33–35.
66. Gyamfi EAA. *Maternal serum levels of adiponectin and leptin in non-pregnant, normal pregnant and preeclamptic women*. Kumasi, Ghana: Kwame Nkrumah University of Science and Technology, 2015.
67. Fujiwara H, Nishioka Y, Matsumoto H, Suginami K, Horie A, Tani H, Matsumura N, Baba T, Sato Y, Araki Y. Eph-Ephrin a system regulates human choriocarcinoma–derived JEG-3 cell invasion. *Int J Gynecol Cancer* 2013; **23**:576–582.
68. Yang Y, Min J. Effect of ephrin-A1/EphA2 on invasion of trophoblastic cells. *J Huazhong Univ Sci Technol Medical Sci* 2011; **31**:824–827.
69. Goldman-Wohl D, Greenfield C, Haimov-Kochman R, Ariel I, Anteby EY, Hochner-Celnikier D, Farhat M, Yagel S. Eph and ephrin expression in normal placental development and preeclampsia. *Placenta* 2004; **25**: 623–630.
70. Ilan N, Madri JA. New paradigms of signaling in the vasculature: Ephrins and metalloproteases. *Curr Opin Biotechnol* 1999; **10**:536–540.
71. Zhang Y, Fei M, Xue G, Zhou Q, Jia Y, Li L, Xin H, Sun S. Elevated levels of hypoxia-inducible microRNA-210 in pre-eclampsia: New insights into molecular mechanisms for the disease. *J Cell Mol Med* 2012; **16**: 249–259.
72. Chennakesava CS, Di Santo S, Ziemiecki A, Schneider H, Andres A-C. Differential expression of the receptor tyrosine kinase EphB4 and its ligand Ephrin-B2 during human placental development. *Placenta* 2006; **27**:959–967.
73. Luo Q, Liu X, Zheng Y, Zhao Y, Zhu J, Zou L. Ephrin-B2 mediates trophoblast-dependent maternal spiral artery remodeling in first trimester. *Placenta* 2015; **36**:567–574.
74. Wang W, Feng L, Zhang H, Hachy S, Satohisa S, Laurent LC, Parast M, Zheng J, Chen D. Preeclampsia up-regulates angiogenesis-associated microRNA (ie., miR-17,-20a, and-20b) that target ephrin-B2 and EPHB4 in human placenta. *J Clin Endocrinol Metab* 2012; **97**: E1051–E1059.
75. Liu X, Hu Y, Zheng Y, Liu X, Luo M, Liu W, Zhao Y, Zou L. EPHB4 regulates human trophoblast cell line HTR-8/SVneo function: Implications for the role of EPHB4 in preeclampsia. *Biol Reprod* 2016; **95**: 61–65.
76. Wang F, Yan J. MicroRNA-454 is involved in regulating trophoblast cell proliferation, apoptosis, and invasion in preeclampsia by modulating the expression of ephrin receptor B4. *Biomed Pharmacother* 2018; **107**:746–753.
77. Red-Horse K, Kapidzic M, Zhou Y, Feng K-T, Singh H, Fisher SJ. EPHB4 regulates chemokine-evoked trophoblast responses: A mechanism for incorporating the human placenta into the maternal circulation. *Development* 2005; **132**:4097–4106.
78. Korff T, Braun J, Pfaff D, Augustin HG, Hecker M. Role of ephrinB2 expression in endothelial cells during arteriogenesis: Impact on smooth muscle cell migration and monocyte recruitment. *Blood, J Am Soc Hematol* 2008; **112**:73–81.