Journal of Reproductive Immunology 172 (2025) 104736

Contents lists available at ScienceDirect

Journal of Reproductive Immunology

journal homepage: www.elsevier.com/locate/jri

Autoantibody-mediated disruption of the epidermal growth factor system during implantation and pregnancy

Aiko Aoki [a,b], Toshitaka Sugi [a,*], Kei Kawana [b]

[a] Laboratory for Recurrent Pregnancy Loss, Sugi Women's Clinic, Yokohama, Japan
[b] Department of Obstetrics and Gynecology, Nihon University School of Medicine, Tokyo, Japan

ARTICLE INFO

Keywords:
Epidermal growth factor
Implantation
Placental dysfunction
Preeclampsia
Pregnancy loss

ABSTRACT

Japan has a relatively high incidence of coagulation factor XII (FXII) and protein S deficiencies, which are undeniable risk factors for recurrent pregnancy loss (RPL). FXII and protein S share common epidermal growth factor (EGF)-like domains. Patients with RPL who have protein S and FXII deficiencies carry anti-protein S and anti-FXII antibodies (anti-FXII), respectively. Epitope mapping of these antibodies revealed that they have EGF-like domain epitopes, which cross-react with EGF. EGF plays a role in cell growth and differentiation, and its effects on the reproductive system, particularly in implantation and placental development, are well documented. We developed an anti-EGF antibody detection system to investigate the correlation between anti-EGF antibodies (anti-EGF) and other RPL-associated risk factors. Anti-EGF demonstrated a correlation not only with anti-FXII and anti-protein S but also with anti-phosphatidylserine/prothrombin antibodies (aPS/PT). Although the aPS/PT epitope lies within the thrombin region, which lacks an EGF-like domain, it nevertheless cross-reacts with EGF, indicating their pathogenicity to the EGF system as well as anti-FXII and anti-protein S. This review summarizes the history of antibody discovery and epitope mapping studies and discusses the pathogenic potential of these antibodies in pregnancy morbidity through their effects on reproductive biology.

1. Introduction

Japan has a relatively high incidence of coagulation factor XII (FXII) and protein S deficiencies, which are undeniable risk factors for recurrent pregnancy loss (RPL; Morita et al., 2019; Tsuda et al., 2020). Some studies have shown that anti-protein S autoantibodies (anti-protein S) can cause acquired protein S deficiency (D'Angelo et al., 1993; Sorice et al., 1994). For example, reduced free protein S levels have been reported in many patients with human immunodeficiency virus infection, with an overall anti-protein S positivity rate of 28.6 %, occurring more frequently in patients with protein S levels below 50 % (Sorice et al., 1994). Sorice et al. (1996) further demonstrated the possibility of protein S activity inhibition by anti-protein S.

Similarly, Gallimore et al. (1998) reported a high incidence (20.9 %) of apparent true FXII deficiency among patients who tested positive for lupus anticoagulant (LA). They hypothesized that some LA-positive patients develop anti-FXII antibodies (anti-FXII), and that immune complex formation contributes to reduced FXII levels. Supporting this, Jones et al. (1999) identified anti-FXII antibodies in plasma from LA-positive patients using enzyme-linked immunosorbent assay and surface plasmon resonance. Patients with anti-FXII were found to have significantly lower FXII levels compared with antibody-negative patients (Jones et al., 2000). This indicated that the immune complex formation and subsequent sequestration reduced the FXII levels. They also reported that anti-FXII exhibited a strong and statistically significant association with recurrent fetal loss (odds ratio [OR] 5.4, $P = 0.025$) (Jones et al., 2001). Thus, autoantibodies against FXII, rather than FXII deficiency itself, is a potential risk factor for thromboembolism and RPL.

The potential presence of these autoantibodies prompted us to develop autoantibody detection assays. It is also crucial to identify their epitopes to discuss their pathogenicity in reproduction. We started with the detection of the epitope of anti-FXII through direct binding studies and found that its binding sites consist of amino acids 1–30 (IPP30) in the FXII heavy chain (Inomo et al., 2008). Subsequently, another epitope was detected in the second epidermal growth factor (EGF)-like domain, with a prevalence of 23.0 % (23 out of 100) among patients with RPL (Sato et al., 2019). In addition, protein S autoantibodies were detected in 20.0 % (20 out of 100) of patients with RPL with the epitopes of anti-protein S also located within the 1–4 EGF-like domains (Sato et al.,

* Correspondence to: Laboratory for Recurrent Pregnancy Loss, Sugi Women's Clinic, 2-12-1–7 F, Shinyokohama, Kohoku, Yokohama, Kanagawa 222-0033, Japan.
E-mail address: sugi@sugi-wc.jp (T. Sugi).

https://doi.org/10.1016/j.jri.2025.104736
Received 17 July 2025; Received in revised form 3 October 2025; Accepted 6 October 2025
Available online 9 October 2025

Table 1
Current evidence on the growth factor properties of coagulation proteins and EGF as well as the potential effects of autoantibodies in patients with RPL.

Protein	Function as coagulant /anticoagulant	Current evidence of the growth-promoting effect of the protein	Epitopes of autoantibodies found in patients with RPL	Cross-reactivity of the epitopes of autoantibodies to EGF	Potential effects of EGF-related autoantibodies in patients with RPL on reproduction (hypothesis)
Protein S	Cofactor of protein C	Mitosis of hVSMC	1–4 EGF-like domains	Yes	Defects of endothelial cell proliferation /poor angiogenesis of SA ↓ RIF /early pregnancy loss?
FXII	Inhibition of platelet aggregation	Proliferation of human hepatoma cells Umbilical vein endothelial cells proliferation and angiogenesis	Amino-terminal of heavy chain (IPP30)	No	
			second EGF-like domain	Yes	
		Mitosis of aortic smooth muscle cell lines			
Prothrombin	Inactive precursor of thrombin	Potent mitogen for hVSMC* Adhesion and spreading of endothelial cells*	B4 domain (heparin-binding exosite)	Yes	Insufficient placental development ↓ FGR /preeclampsia?
EGF	None	Luminal epithelial proliferation in nonpregnant mice Acquisition of invasive phenotype in trophoblasts CT differentiation and syncytial formation	EGF	–	

*Reported function of thrombin. Antibodies whose epitopes cross-react with EGF are classified as "EGF-related autoantibodies." We hypothesized that "EGF-related autoantibodies" and anti-EGF itself negatively influence on cell proliferation, angiogenesis, and placental development, which may cause RIF, early pregnancy loss, FGR, and preeclampsia. RPL, recurrent pregnancy loss; FXII, factor XII; EGF, epidermal growth factor; hVSMC, human vascular smooth muscle cells; CT, cytotrophoblast; SA, spiral artery; RIF, recurrent implantation failure; FGR, fetal growth restriction

2018). The binding sites of the second EGF-like domain of FXII and the 1–4 EGF-like domains of protein S cross-react with EGF (Sato et al., 2018; Sato et al., 2019).

FXII and protein S are broadly recognized as coagulation factors; however, studies indicating that they are potential growth factors in the reproductive system are scarce. Protein S and FXII have also been reported to play a role in the proliferation of vascular smooth muscle cells (VSMCs) and vascular endothelial cells (Benzakour et al., 1995; Kanthou and Benzakour, 2000; Gordon et al., 1996; LaRusch et al., 2010). As spiral arteries are composed of vascular endothelial cells and VSMCs (Lyall, 2002), protein S and FXII may play a role in their angiogenesis (Staff et al., 2022). FXII is localized in predecidual cells and the glandular epithelium during the late secretory phase and in decidual cells in the first trimester, highlighting its role in implantation and placental development (Kawato et al., 2009).

Although research on epidermal growth factor receptor (EGFR) and its downstream signaling has advanced in oncology, studies in the field of the reproductive system remain scarce. EGF administration in nonpregnant female mice induced luminal epithelial cell proliferation, whereas anti-EGF antibodies (anti-EGF) administration inhibited estrogen-induced luminal epithelial cell proliferation (Nelson et al., 1991). EGF promotes an invasive phenotype of cytotrophoblast (CT) in the uterine myometrium (Bass et al., 1994), induces CT differentiation *in vitro* (Morrish et al., 1987), and triggers extensive syncytial formation (Morrish et al., 1997).

Studies on autoantibodies against FXII and protein S are scarce, but we conducted epitope mapping and found that the epitopes within their EGF-like domains often cross-react with EGF (Sato et al., 2018; Sato et al., 2019). However, in the absence of EGF-like domains, epitopes of anti-phosphatidylserine/prothrombin antibodies (aPS/PT) cross-reacts with EGF (Aoki et al., 2025). Thrombin itself has been reported to act as a potent mitogen for VSMCs (Kanthou et al., 1992). With accumulating evidence that autoantibodies against FXII, protein S, and prothrombin in patients with RPL frequently recognize EGF, we present this comprehensive review. Autoantibodies whose epitopes cross-react with EGF are classified as "EGF-related autoantibodies." This introduction describes the history of epitope mapping studies of autoantibodies detected in the patients with RPL. Table 1 and the figures summarize existing evidence on the growth factor properties of these proteins, along with hypotheses about the potential effects of EGF-related autoantibodies on reproductive system function in living organisms.

2. Epitope mapping studies

2.1. Anti-FXII autoantibodies recognize N-terminal amino acids 1–30 in the FXII heavy chain, enhancing γ-thrombin-induced platelet aggregation in vitro and inducing thrombosis in vivo

FXII knockout mice are viable and fertile, exhibiting normal pregnancy outcomes and litter sizes (Pauer et al., 2004; Iwaki et al., 2006). Although one study identified FXII deficiency as a risk factor for RPL (Schved et al., 1989), another study found no such association (Matsuura et al., 2001). Epitope mapping analysis showed that the antibodies in most patients with RPL recognized the FXII heavy chain but not the light chain (Inomo et al., 2008). Moreover, the antigen-binding site of anti-FXII consists of amino acids 1–30 (IPP30) of the FXII heavy chain, known as the FXII binding site for platelet glycoprotein Ib alpha (Inomo et al., 2008). Antibodies collected from 13 of 17 (76.5 %) patients with RPL who tested positive for plasma anti-FXII recognized the synthetic peptide IPP30. Bradford et al. (2000) reported that activated FXII inhibits thrombin–platelet interactions. However, its platelet binding was inhibited by monoclonal antibodies with epitopes mapped to amino acids 1–28 and 134–153 within the fibronectin type I domain (Pixley et al., 1987; Clarke et al., 1989). Therefore, anti-FXII species that recognize IPP30 in patients with RPL may also inhibit FXII–platelet interactions, thereby causing thrombosis and pregnancy loss. Our previous study (Sato et al., 2015) supported this hypothesis by showing that the exogenous addition of polyclonal anti-IPP30 antibodies significantly enhanced γ-thrombin-induced platelet aggregation *in vitro*. LDC27, located in domain 3 of kininogens and serving as the epitope for

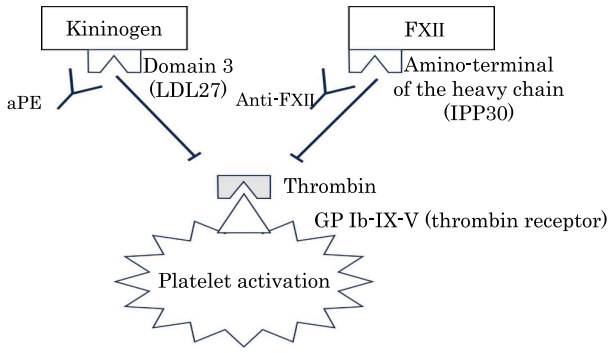

Fig. 1. aPE and anti-FXII enhance platelet aggregation, Kininogens and FXII bind to the platelet thrombin receptor GP Ib-IX-V via LDC27 in domain 3 of kininogens and IPP30 in the N-terminal of the FXII heavy chain, respectively. Furthermore, they prevent thrombin-induced platelet aggregation by inhibiting thrombin–platelet binding. However, when bound by aPE, which recognizes LDC27, and by anti-FXII, which recognizes IPP30, kininogens and FXII are prevented from binding to the thrombin receptor, causing thrombin-induced platelet aggregation. aPE, antiphosphatidylethanolamine antibodies; anti-FXII, anti-factor XII antibodies.

anti-phosphatidylethanolamine antibodies (aPE), and IPP30 in the N-terminal region of the FXII heavy chain bind to the platelet thrombin receptor and inhibit thrombin-induced platelet aggregation (Fig. 1). Kininogens and FXII are prevented from binding to the thrombin receptor when bound by aPE, which recognizes LDC27, and by anti-FXII, which recognizes IPP30, thereby triggering thrombin-induced platelet aggregation (Sato et al., 2015). Laser-light scattering aggregometry (PA-200; Kowa, Tokyo, Japan) showed that the prothrombotic potency of polyclonal anti-IPP30 and anti-LDC27 antibodies adheres to the following hierarchy: (anti-LDC27 and anti-IPP30) > (anti-IPP30) > (anti-LDC27). The pathogenicity of these antibodies, assessed *in vivo* in mice passively immunized with anti-LDC27 and anti-IPP30, resulted in a slight increase in fetal resorption, pronounced thrombosis, and enhanced placental hemorrhage and apoptosis, accompanied by reduced platelet counts. Moreover, the decidua exhibited reduced mitotic activity, impaired trophoblast giant cell invasion, and increased numbers of shrunken cells (Velayuthaprabhu et al., 2011).

Previous studies suggest that although FXII deficiency appears to be a cause of RPL, the underlying factor may actually be anti-FXII autoantibodies. The presence of anti-FXII can explain the conflicting findings. In fact, one group has shown a significant correlation between anti-FXII and RPL (Jones et al., 2001).

2.2. Anti-FXII recognize the second EGF-like domain in the FXII heavy chain, and the binding site cross-reacts with EGF, which promotes cell proliferation

Of the 100 plasma samples from patients with RPL, 23 (23.0 %) recognized the synthetic peptide ASQ41, which covers the second EGF-like domain in the FXII heavy chain. Among the 23 anti-ASQ41-positive plasma samples, 13 (56.5 %) recognized the 22-residue segment C-terminal half of ASQ41 and 17 (73.9 %) recognized recombinant human EGF (hEGF). Affinity-purified anti-FXII, which recognizes ASQ41, also recognizes recombinant EGF family proteins, such as EGF and heparin-binding EGF-like growth factor (HB-EGF; Sato et al., 2019).

FXII is not only a coagulation factor but also a growth factor that promotes proliferation and angiogenesis (Schmeidler-Sapiro et al., 1991; Gordon et al., 1996; LaRusch et al., 2010). FXII has been shown to enhance the human hepatoma cell proliferation (Schmeidler-Sapiro et al., 1991) and to exert mitogenic effects on several EGF-sensitive cell types (Gordon et al., 1996). Moreover, it stimulates Akt phosphorylation and extracellular signal-related kinase 1/2 (ERK1/2) via the EGFR,

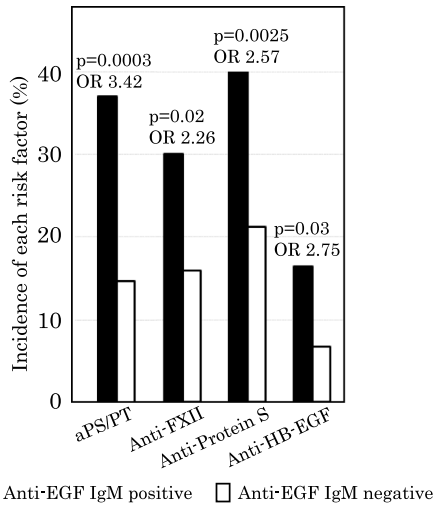

Fig. 2. Incidence of each risk factor in anti-EGF–positive and anti-EGF–-negative patients with RPL and/or repeated implantation failure (n = 223), Plasma samples from 223 patients with RPL and/or repeated implantation failure were analyzed. Anti-EGF antibodies were significantly associated with anti-FXII, anti-protein S, and anti-HB-EGF antibodies. Interestingly, anti-EGF antibodies were also associated with aPS/PT (unpublished data). Among all the autoantibodies, the *P*-value between anti-EGF and aPS/PT was the lowest. RPL, recurrent pregnancy loss; OR, odds ratio; anti-EGF, anti-epidermal growth factor antibodies; aPS/PT, anti-phosphatidylserine/prothrombin antibodies; anti-FXII, anti-factor XII antibodies; anti-HB-EGF, anti-heparin binding EGF-like growth factor antibodies; IgM, immunoglobulin M.

specific integrins, and the urokinase plasminogen activator receptor, thereby promoting endothelial cell proliferation, growth, and angiogenesis (LaRusch et al., 2010). The *FXII* mRNA levels in the endometrium rose during the secretory phase and were markedly elevated in decidual tissues in early pregnancy (Kawato et al., 2009). In human decidualized cells *in vitro*, FXII is expressed in response to estrogen and progesterone stimulation, highlighting its role in implantation (Kawato et al., 2009). Anti-FXII may cause pregnancy loss by blocking EGF signals on multiple biogeneses.

2.3. Anti-protein S binding sites are located in EGF-like domains, and the binding site cross-reacts with EGF, which may disrupt the EGF system

Of the 100 patients with RPL, 20 (20.0 %) were seropositive for anti-protein S. On immunoblot analysis, anti-protein S recognized Gla-domain-free protein S, particularly the three fragments of EGF-like domains: EGF1–2, EGF3–4, and EGF1–4. Notably, anti-protein S also recognized EGF proteins. The regions crucial for Ca^{2+} binding in protein S potentially involve EGF 2–4. Anti-protein S and polyclonal recombinant hEGF antibodies recognize protein S in the absence of Ca^{2+} but not in its presence, likely due to Ca^{2+}-induced conformational changes. These results indicate that anti-protein S in patients with RPL recognize EGF-like domains in protein S (Sato et al., 2018). Beyond coagulation, protein S potentially plays a role in other biological processes, including apoptosis, angiogenesis/vasculogenesis, and cancer progression (Suleiman et al., 2013). During early and late pregnancy, protein S accumulates around damaged placental trophoblastic cells, suggesting that it potentially protects or restores damaged villi and exerts physiological effects on the placenta (Matsumoto et al., 2008). Autoantibodies that recognize EGF-like domains in protein S may contribute to angiogenesis inhibition and apoptotic cell clearance during normal pregnancy. Anti-protein S in patients with RPL may be associated with not only thrombophilia but also EGF system disruption.

Several studies have demonstrated a correlation between RPL or fetal

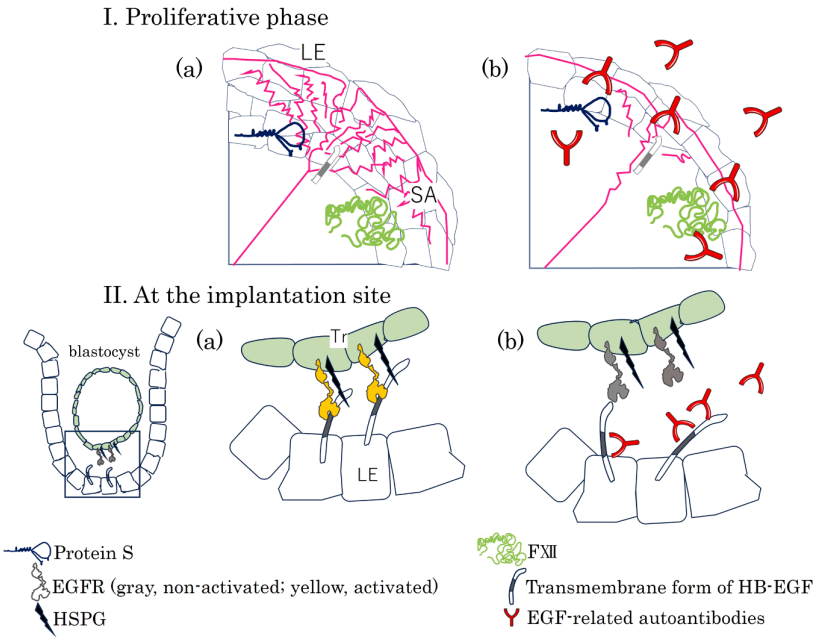

Fig. 3. Hypothesis of impaired implantation caused by epidermal growth factor (EGF) system disruption. (I) EGF plays a role in endometrial thickening and mature SA formation during the proliferative phase (a). Under EGF-related autoantibodies, endometrial proliferation is insufficient, and SA are sparse and possibly have poor quality (b). (II) HB-EGF–expressing cells cause the trophectoderm to express EGFR through juxtacrine secretion signaling. The transmembrane form of HB-EGF mediates blastocyst adhesion by interacting with HSPG (a). The antigen–antibody reaction is potentially inhibited by EGF-related autoantibodies (b). EGF, epidermal growth factor; LE, luminal epithelium; SA, spiral arteries; Tr, trophectoderm; FXII, coagulation factor XII; EGFR, epidermal growth factor receptor; HB, heparin binding; HSPG, heparan sulfate proteoglycan.

loss after 22 weeks of gestation and protein S deficiency (Rey et al., 2003; Robertson et al., 2006). Japan has a high prevalence (1.6 %–1.8 %) of congenital protein S deficiency, particularly protein S Tokushima (p.Lys196Glu), a genetic mutation and molecular defect detected in the EGF2 domain of protein S (Hayashi et al., 1994) and associated with mid-to-late-pregnancy loss (Sato et al., 2022). Protein S antibodies are similarly pathogenic to protein S Tokushima, as they commonly affect the EGF1–2 domains of protein S, which is directly involved in activated protein C cofactor function. Notably, the incidence of anti-protein S-positive patients is higher than that of protein S Tokushima-positive patients in mid-to-late-pregnancy loss affected patients (Sato et al., 2022).

2.4. Anti-EGF is associated with anti-protein S, anti-FXII, and anti-phosphatidylserine/prothrombin antibodies (aPS/PT)

As anti-FXII and anti-protein S recognize the EGF-like domains of their respective proteins and cross-react with EGF, an experimental detection system for anti-EGF autoantibodies was previously developed (Sato and Sugi, 2020). Plasma samples from 223 patients who experienced RPL and/or repeated implantation failure were analyzed, and anti-EGF showed significant correlations with anti-FXII, anti-protein S, and anti-HB-EGF antibodies (Fig. 2). Notably, anti-EGF were also associated with aPS/PT (unpublished data). Among all autoantibody pairs, the *P*-value between anti-EGF and aPS/PT was the lowest. The unpublished data was preliminary at that time, but we later confirmed the association and published the epitope mapping of prothrombin as an original article, which is described below (Aoki et al., 2025).

2.5. Antigenic aPS/PT binding sites in patients with RPL and EGF exhibit cross-reactivity

Out of 26 aPS/PT-positive patients who experienced pregnancy loss, 18 (69.2 %) also tested positive for anti-EGF. Among those 18 patients, 14 (77.7 %) recognized α-thrombin, particularly targeting the B4 domain—its primary heparin-binding exosite epitope (Aoki et al., 2025). Competitive binding analysis showed the cross-reactivity of the antigenic binding sites of anti-prothrombin antibodies with EGF, which is significant as prothrombin lacks an EGF-like domain, such as those in FXII and protein S. This finding indicates the pathogenicity of anti-prothrombin contributing to the disruption of the EGF system. Thrombin is a potent mitogen for human VSMCs (Kanthou et al., 1992) that induces the adhesion and spreading of endothelial cells, thereby promoting repair mechanisms in the injured luminal surface of vessels (Bar-Shavit et al., 1991). As the heparin-binding exosite acts as the binding site for these autoantibodies, we suggest that their pathogenicity is also potentially associated with blood coagulation. In patients with antiphospholipid syndrome, anti-prothrombin antibodies are strongly linked to anti-protein C and S antibodies (Pengo et al., 1996). Our study supports this association, as the antigenic binding sites of anti-prothrombin antibodies and anti-protein S demonstrate cross-reactivity.

3. Further hypothesis toward the future

Acquired autoantibodies (including anti-protein S, anti-FXII, and aPS/PT) are relatively common in patients with RPL and share a competitive binding site with anti-EGF. Protein S, FXII, and prothrombin are known coagulation factors, and autoantibodies against them can cause coagulation abnormalities. However, as all these autoantibodies named as EGF-related autoantibodies also recognize EGF family proteins, there may be a novel pathogenic mechanism beyond coagulation abnormalities. Based on previous reports on the effects of the EGF system on the reproductive system, the pathogenicity of EGF-related autoantibodies in fertility is subsequently discussed.

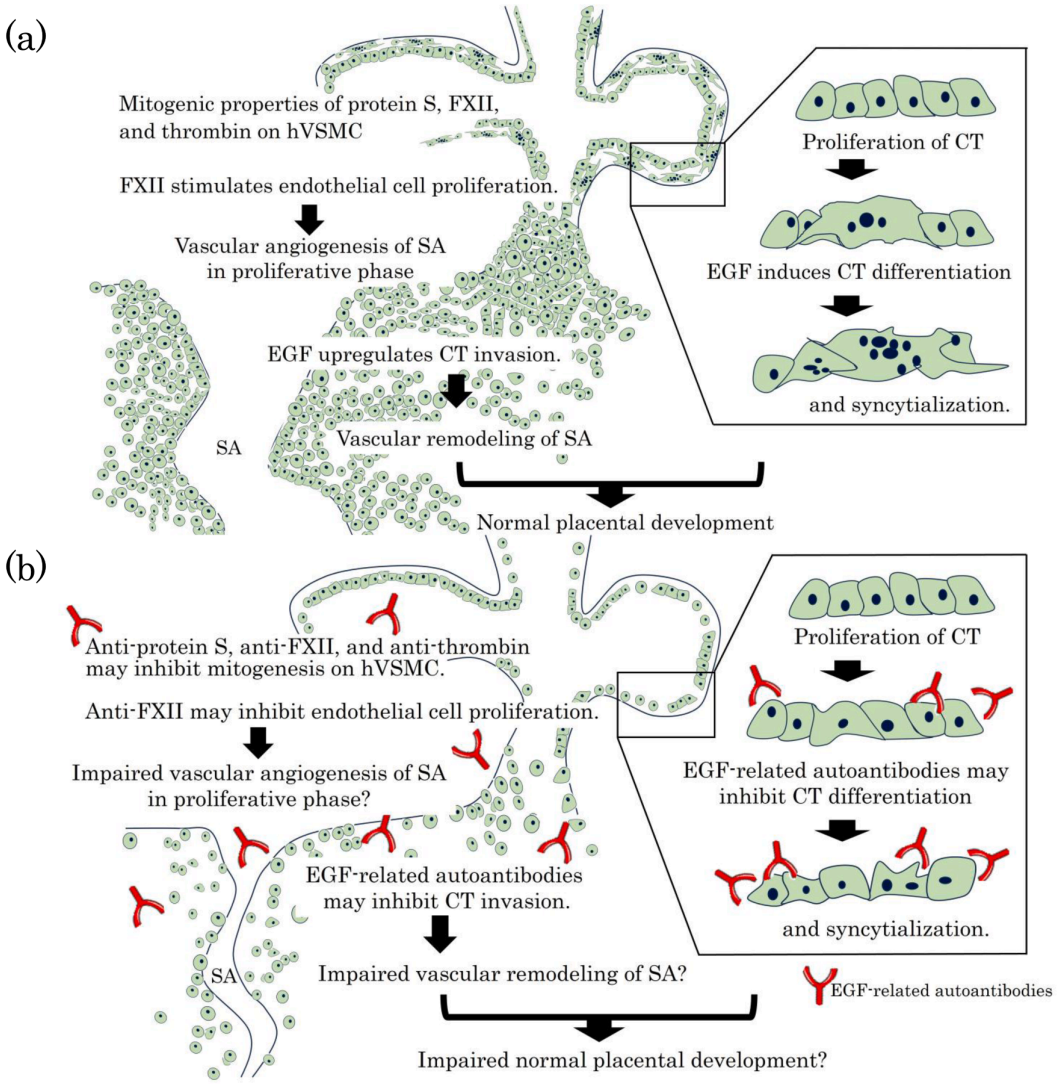

Fig. 4. Current evidence on the growth factor properties of coagulation proteins and epidermal growth factor (EGF) and hypothesis on the potential effects of EGF-related autoantibodies on normal placental development. (a) Current evidence on the growth factor properties of coagulation proteins and EGF and their effects on normal placental development. Since protein S, FXII, and thrombin exhibit growth factor effects, they may promote the vascular angiogenesis of SA during the proliferative phase. EGF promotes an invasive CT phenotype in the uterine myometrium, induces CT differentiation *in vitro*, and triggers extensive syncytial formation. These processes are essential in normal placental development. (b) Potential effects of EGF-related autoantibodies on normal placental development. EGF-related autoantibodies may inhibit the growth factor properties of protein S, FXII, thrombin, and EGF. These antibodies may inhibit the vascular angiogenesis of SA during the proliferative phase, as well as CT invasion, differentiation, and syncytialization, impairing placental development. EGF, epidermal growth factor; FXII, factor XII; CT, cytotrophoblast; hVSMC, human vascular smooth muscle cells; SA, spiral artery.

3.1. Autoantibody-mediated EGF system disruption may cause implantation failure

Several studies have indicated that protein S, FXII, and thrombin play a role in the proliferation of VSMCs. The addition of protein S to growth-arrested human VSMCs induces mitosis in a dose-dependent manner, which can also be inhibited by anti-human protein S pre-incubation (Benzakour et al., 1995; Kanthou and Benzakour, 2000). Gordon et al. (1996) performed an assay of aortic smooth muscle cell lines and demonstrated that FXII has an EGF-like domain and, similar to EGF, induces mitosis via the mitogen-activated protein kinase (MAPK) system. Thus, the proliferative effects of FXII are mediated through EGFR. Kanthou et al. (1992) found that thrombin expresses platelet-derived growth factor-A gene to promote mitosis in human VSMCs, indicating that thrombin exhibits growth factor-like activity.

FXII also plays a role in the proliferation of vascular endothelial cells. Gordon et al. (1996) demonstrated mitogenic effects of FXII on endothelial cells. LaRusch et al. (2010) found that FXII induces phosphorylation of ERK1/2 and Akt (Ser473) in human umbilical vein endothelial cell line and that MAP/ERK1 and phosphoinositol-3 kinase inhibitors inhibit these pathways, respectively. Furthermore, EGFR inhibitors inhibit ERK1/2 and Akt phosphorylation, indicating that FXII induces downstream signals involved in endothelial cell proliferation via the EGFR (LaRusch et al., 2010). These findings suggest that protein S, FXII, and thrombin may play a role in angiogenesis of spiral arteries (Staff et al., 2022). During the proliferative phase, spiral arteries sprout into the functional layer of the endometrium. Similar to other arteries, spiral arteries consist of vascular endothelial cells and VSMCs (Lyall, 2002). Therefore, the presence of EGF-related autoantibodies may increase their vulnerability.

Estrogen and progesterone regulate EGF expression in luminal epithelial and stromal cells (Wang et al., 1994). EGF administration in nonpregnant female mice promoted the proliferation of luminal epithelial cells, whereas anti-EGF administration inhibited estrogen-induced luminal epithelial cell proliferation (Nelson et al., 1991). Proliferation of the luminal epithelium and normal spiral arteries is crucial for implantation. The presence of potentially disruptive EGF-related autoantibodies may represent a novel cause of implantation failure. Supporting this, the rate of positive anti-protein S in patients who experienced recurrent implantation failure (19.2 %) is comparable to that in patients affected by recurrent early pregnancy loss (20.1 %) or mid-to-late-pregnancy loss (23.0 %; Sato et al., 2022).

Furthermore, HB-EGF plays a pivotal role in implantation, particularly at the site of molecular networks between the endometrium and blastocyst. Implantation involves three steps, namely, apposition, attachment, and penetration, in which HB-EGF may play a contributory role. *HB-EGF* mRNA is synthesized in the mouse uterine luminal epithelium, which is limited to the blastocyst apposition site (Das et al., 1994). Moreover, murine cell lines synthesizing transmembrane HB-EGF extensively adhered to day 4 mouse blastocysts, mediated by EGFR and heparan sulfate proteoglycan (Raab et al., 1996).

HB-EGF receptors include EGFR (Higashiyama et al., 1991) and HER4 (Elenius et al., 1997). Chobotova et al. (2002) found that ErbB4 is expressed at the human blastocyst frontline and exclusively adheres to HB-EGF-expressing cells. *In vitro* analysis showed that anti-FXII recognizes EGF and HB-EGF (Sato et al., 2019). According to these findings, we hypothesized that EGF-related autoantibodies inhibit EGF expression at the implantation site. Fig. 3 shows a schematic of the hypothesis of impaired implantation caused by EGF system disruption.

3.2. Autoantibody-mediated EGF system disruption may cause preeclampsia

Extravillous trophoblast invasion into the uterine decidua and CT syncytialization are essential processes for normal placentation (Steegers et al., 2010; Gerbaud and Pidoux, 2015). EGF promotes an invasive phenotype of CTs in the uterine myometrium (Bass et al., 1994), induces CT differentiation *in vitro* (Morrish et al., 1987), and triggers extensive syncytial formation (Morrish et al., 1997). However, exogenous EGF–induced syncytial formation is inhibited by EGFR antibodies (Morrish et al., 1997).

Some studies support an association between EGF and its receptor, EGFR, and the development of preeclampsia. Expressions of EGF family proteins, including EGF, HB-EGF, and transforming growth factor α, are reduced in the basal plate of human preeclamptic placentas at a median gestational age of 32.6 weeks (Armant et al., 2015). Moreover, the expressions of EGFR and *ATF3*, one of the genes induced by oxidative stress and hypoxia, are upregulated in preeclamptic placentas, whereas EGFR signaling is downregulated (Moslehi et al., 2013).

Although these studies describe events observed in preeclamptic placentas, the underlying cause remains unclear. If EGF-related autoantibodies prevent EGF from binding to EGFR, the EGF family protein expression decreases, EGFR expression increases, and downstream EGFR signaling in the preeclamptic placenta is reduced. The presence of EGF-related autoantibodies could therefore explain the multiple findings reported to date. Velayuthaprabhu et al. (2011) demonstrated in a murine model that anti-FXII infusion increased placental apoptosis and the number of shrunken cells in the decidua, while reducing the number of mitotic cells and alleviating the invasiveness of trophoblast giant cells. Therefore, EGF-related autoantibodies in patients with RPL may inhibit the growth factor functions of FXII and induce placental dysfunction. A prospective cohort study involving pregnant women demonstrated that protein S activity below the 5th percentile in early pregnancy was a risk factor for preeclampsia. Moreover, the frequency of preeclampsia increased with declining FXII activity (Ebina et al., 2015). This study further supported the presence of EGF-related autoantibodies in patients with preeclampsia. Fig. 4a and b summarize current evidence on the growth factor properties of coagulation proteins and EGF, and illustrate the potential effects of EGF-related autoantibodies on placental development.

4. Conclusion

Anti-protein S, FXII, and prothrombin antibodies share competitive binding sites with anti-EGF. Considering the physiological effects of EGF proteins on implantation and placental development, EGF-related autoantibodies may play a role in pregnancy complications. Future studies are warranted to establish animal models to elucidate the pathogenicity of EGF-related autoantibodies *in vivo*.

CRediT authorship contribution statement

Aiko Aoki: Writing – original draft, Visualization, Data curation. **Toshitaka Sugi:** Writing – review & editing, Validation, Supervision, Project administration, Data curation, Conceptualization. **Kei Kawana:** Writing – review & editing, Validation.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

Aoki, A., Sugi, T., Kawana, K., Sugi, T., Sakai, R., 2025. Cross-reactivity of antigenic binding sites of antiphosphatidylserine/prothrombin antibodies in patients with pregnancy loss and epidermal growth factor. J. Reprod. Immunol. 167, 104399. https://doi.org/10.1016/j.jri.2024.104399.

Armant, D.R., Fritz, R., Kilburn, B.A., Kim, Y.M., Nien, J.K., Maihle, N.J., et al., 2015. Reduced expression of the epidermal growth factor signaling system in preeclampsia. Placenta 36, 270–278. https://doi.org/10.1016/j.placenta.2014.12.006.

Bar-Shavit, R., Sabbah, V., Lampugnani, M.G., Marchisio, P.C., Fenton 2nd, J.W., Vlodavsky, I., et al., 1991. An Arg-Gly-Asp sequence within thrombin promotes endothelial cell adhesion. J. Cell Biol. 112, 335–344. https://doi.org/10.1083/jcb.112.2.335.

Bass, K.E., Morrish, D., Roth, I., Bhardwaj, D., Taylor, R., Zhou, Y., et al., 1994. Human cytotrophoblast invasion is up-regulated by epidermal growth factor: evidence that paracrine factors modify this process. Dev. Biol. 164, 550–561. https://doi.org/10.1006/dbio.1994.1223.

Benzakour, O., Formstone, C., Rahman, S., Kanthou, C., Dennehy, U., Scully, M.F., et al., 1995. Evidence for a protein s receptor(s) on human vascular smooth muscle cells. Analysis of the binding characteristics and mitogenic properties of protein s on human vascular smooth muscle cells. Biochem J. 30, 481–485. https://doi.org/10.1042/bj3080481.

Bradford, H.N., Pixley, R.A., Colman, R.W., 2000. Human factor XII binding to the glycoprotein Ib-IX-V complex inhibits thrombin-induced platelet aggregation. J. Biol. Chem. 275, 22756–22763. https://doi.org/10.1074/jbc.m002591200.

Chobotova, K., Spyropoulou, I., Carver, J., Manek, S., Heath, J.K., Gullick, W.J., et al., 2002. Heparin-binding epidermal growth factor and its receptor ErbB4 mediate implantation of the human blastocyst. Mech. Dev. 119, 137–144. https://doi.org/10.1016/s0925-4773(02)00342-8.

Clarke, B.J., Côté, H.C., Cool, D.E., Clark-Lewis, I., Saito, H., Pixley, R.A., et al., 1989. Mapping of a putative surface-binding site of human coagulation factor XII. J. Biol. Chem. 264, 11497–11502. https://doi.org/10.1016/s0021-9258(18)60491-0.

D'Angelo, A., Valle, P.D., Crippa, L., Pattarini, E., Grimaldi, L., D'Angelo, S.V., 1993. Autoimmune protein s deficiency in a boy with severe thromboembolic disease. N. Engl. J. Med. 328, 1753–1757. https://doi.org/10.1056/NEJM199306173282405.

Das, S.K., Wang, X.-N., Paria, B.C., Damm, D., Abraham, J.A., Klagsbrun, M., et al., 1994. Heparin-binding EGF-like growth factor gene is induced in the mouse uterus temporally by the blastocyst solely at the site of its apposition: a possible ligand for interaction with blastocyst EGF-receptor in implantation. Development 120, 1071–1083. https://doi.org/10.1242/dev.120.5.1071.

Ebina, Y., Ieko, M., Naito, S., Kobashi, G., Deguchi, M., Minakami, H., et al., 2015. Low levels of plasma protein S, protein c and coagulation factor XII during early pregnancy and adverse pregnancy outcome. Thromb. Haemost. 114, 65–69. https://doi.org/10.1160/th14-11-0928.

Elenius, K., 1997. Activation of HER4 by heparin-binding EGF-like growth factor stimulates chemotaxis but not proliferation. EMBO J. 16, 1268–1278. https://doi.org/10.1093/emboj/16.6.1268.

Gallimore, M.J., Jones, D.W., Winter, M., 1998. Factor XII determinations in the presence and absence of phospholipid antibodies. Thromb. Haemost. 79, 87–90. https://doi.org/10.1055/s-0037-1614225.
Gerbaud, P., Pidoux, G., 2015. Review: an overview of molecular events occurring in human trophoblast fusion. Placenta 36, S35–S42. https://doi.org/10.1016/j.placenta.2014.12.015.
Gordon, E.M., Venkatesan, N., Salazar, R., Tang, H., Schmeidler-Sapiro, K., Buckley, S., et al., 1996. Factor XII-induced mitogenesis is mediated via a distinct signal transduction pathway that activates a mitogen-activated protein kinase. Proc. Natl. Acad. Sci. 93, 2174–2179. https://doi.org/10.1073/pnas.93.5.2174.
Hayashi, T., Nishioka, J., Shigekiyo, T., Saito, S., Suzuki, K., 1994. Protein s tokushima: abnormal molecule with a substitution of glu for Lys-155 in the second epidermal growth factor-like domain of protein s. Blood 83, 683–690. https://doi.org/10.1182/blood.v83.3.683.683.
Higashiyama, S., Abraham, J.A., Miller, J., Fiddes, J.C., Klagsbrun, M., 1991. A heparin-binding growth factor secreted by macrophage-like cells that is related to EGF. Science 251, 936–939. https://doi.org/10.1126/science.1840698.
Inomo, A., Sugi, T., Fujita, Y., Matsubayashi, H., Izumi, S., Mikami, M., 2008. The antigenic binding sites of autoantibodies to factor XII in patients with recurrent pregnancy losses. Thromb. Haemost. 99 (02), 316–323. https://doi.org/10.1160/th07-07-0447.
Iwaki, T., Castellino, F., 2006. Plasma levels of bradykinin are suppressed in factor XII-deficient mice. Thromb. Haemost. 95, 1003–1010. https://doi.org/10.1160/th06-03-0128.
Jones, D.W., Gallimore, M.J., Harris, S., Winter, M., 1999. Antibodies to FXII associated with lupus anticoagulant. Thromb. Haemost. 81, 387–390. https://doi.org/10.1055/s-0037-1614483.
Jones, D.W., Gallimore, M.J., Mackie, I.J., Harris, S.L., Winter, M., 2000. Reduced factor XII levels in patients with the antiphospholipid syndrome are associated with antibodies to factor XII. Br. J. Haematol. 110, 721–726. https://doi.org/10.1046/j.1365-2141.2000.02251.x.
Jones, D.W., Mackie, I.J., Gallimore, M.J., Winter, M., 2001. Antibodies to factor XII and recurrent fetal loss in patients with the anti-phospholipid syndrome. Br. J. Haematol. 113, 550–552. https://doi.org/10.1046/j.1365-2141.2001.02776.x.
Kanthou, C., Benzakour, O., 2000. Cellular effects and signalling pathways activated by the anti-coagulant factor, protein s, in vascular cells. Adv. Exp. Med. Biol. 476, 155–166. https://doi.org/10.1007/978-1-4615-4221-6_13.
Kanthou, C., Parry, G., Wijelath, E., Kakkar, V.V., Demoliou-Mason, C., 1992. Thrombin-induced proliferation and expression of platelet-derived growth factor-A chain gene in human vascular smooth muscle cells. FEBS Lett. 314, 143–148. https://doi.org/10.1016/0014-5793(92)80961-f.
Kawato, H., Tabata, T., Minoura, H., Murabayashi, N., Ma, N., Wang, D.F., et al., 2009. Factor XII gene expression in endometrial stromal cells during decidualisation. Reprod. Fertil. Dev. 21, 840. https://doi.org/10.1071/rd08301.
LaRusch, G.A., Mahdi, F., Shariat-Madar, Z., Adams, G., Sitrin, R.G., Zhang, W.M., et al., 2010. Factor XII stimulates ERK1/2 and akt through uPAR, integrins, and the EGFR to initiate angiogenesis. Blood 115, 5111–5120. https://doi.org/10.1182/blood-2009-08-236430.
Lyall, F., 2002. The human placental bed revisited. Placenta 23, 555–562. https://doi.org/10.1053/plac.2002.0850.
Matsumoto, M., Tachibana, D., Nobeyama, H., Nakano, A., Nakai, Y., Nakayama, M., et al., 2008. Protein s deposition at placenta: a possible role of protein s other than anticoagulation. Blood Coagul. Fibrinolysis 19, 653–656. https://doi.org/10.1097/mbc.0b013e3283001d1f.
Matsuura, T., Kobayashi, T., Asahina, T., Kanayama, N., Terao, T., 2001. Is factor XII deficiency related to recurrent miscarriage? Semin Thromb. Hemost. 27, 115–120. https://doi.org/10.1055/s-2001-14069.
Morita, K., Ono, Y., Takeshita, T., Sugi, T., Fujii, T., Yamada, H., et al., 2019. Risk factors and outcomes of recurrent pregnancy loss in Japan. J. Obstet. Gynaecol. 45, 1997–2006. https://doi.org/10.1111/jog.14083.
Morrish, D.W., Bhardwaj, D., Dabbagh, L.K., Marusyk, H., Siy, O., 1987. Epidermal growth factor induces differentiation and secretion of human chorionic gonadotropin and placental lactogen in normal human placenta. J. Clin. Endocrinol. Metab. 65, 1282–1290.
Morrish, D.W., Dakour, J., Li, H., Xiao, J., Miller, R., Sherburne, R., et al., 1997. In vitro cultured human term cytotrophoblast: a model for normal primary epithelial cells demonstrating a spontaneous differentiation programme that requires EGF for extensive development of syncytium. Placenta 18, 577–585. https://doi.org/10.1016/0143-4004(77)90013-3.
Moslehi, R., Mills, J.L., Signore, C., Kumar, A., Ambroggio, X., Dzutsev, A., 2013. Integrative transcriptome analysis reveals dysregulation of canonical cancer molecular pathways in placenta leading to preeclampsia. Sci. Rep. 3, 2407. https://doi.org/10.1038/srep02407.
Nelson, K.G., Takahashi, T., Bossert, N.L., Walmer, D.K., McLachlan, J.A., 1991. Epidermal growth factor replaces estrogen in the stimulation of female genital-tract growth and differentiation. Proc. Natl. Acad. Sci. USA 88, 21–25. https://doi.org/10.1073/pnas.88.1.21.
Pauer, H.-U., Renné, T., Hemmerlein, B., Legler, T., Fritzlar, S., Adham, I., et al., 2004. Targeted deletion of murine coagulation factor XII gene-a model for contact phase activation in vivo. Thromb. Haemost. 92, 503–508. https://doi.org/10.1160/th04-04-0250.
Pengo, V., Biasiolo, A., Brocco, T., Tonetto, S., Ruffatti, A., 1996. Autoantibodies to phospholipid-binding plasma proteins in patients with thrombosis and phospholipid-reactive antibodies. Thromb. Haemost. 75, 721–724. https://doi.org/10.1055/s-0038-1650355.
Pixley, R.A., Stumpo, L.G., Birkmeyer, K., Silver, L., Colman, R.W., 1987. A monoclonal antibody recognizing an icosapeptide sequence in the heavy chain of human factor XII inhibits surface-catalyzed activation. J. Biol. Chem. 262, 10140–10145. https://doi.org/10.1016/s0021-9258(18)61089-0.
Raab, G., Kover, K., Paria, B.C., Dey, S.K., Ezzell, R.M., Klagsbrun, M., 1996. Mouse preimplantation blastocysts adhere to cells expressing the transmembrane form of heparin-binding EGF-like growth factor. Development 122, 637–645. https://doi.org/10.1242/dev.122.2.637.
Rey, E., Kahn, S.R., David, M., Shrier, I., 2003. Thrombophilic disorders and fetal loss: a meta-analysis. Lancet 361, 901–908. https://doi.org/10.1016/s0140-6736(03)12771-7.
Robertson, L., Wu, O., Langhorne, P., Twaddle, S., Clark, P., Lowe, G.D.O., et al., 2006. Thrombophilia in pregnancy: a systematic review. Br. J. Haematol. 132, 171–196. https://doi.org/10.1111/j.1365-2141.2005.05847.x.
Sato, Y., Sugi, T., Sakai, R., 2015. Autoantibodies to factor XII and kininogen-dependent antiphosphatidylethanolamine antibodies in patients with recurrent pregnancy loss augment platelet aggregation. Am. J. Rep. Immunol. 74, 279–289. https://doi.org/10.1111/aji.12402.
Sato, Y., Sugi, T., Sakai, R., 2018. Antigenic binding sites of anti-protein s autoantibodies in patients with recurrent pregnancy loss. Res Pr. Thromb. Haemost. 2, 357–365. https://doi.org/10.1002/rth2.12081.
Sato, Y., Sugi, T., Sakai, R., 2019. Anti-factor XII autoantibodies in patients with recurrent pregnancy loss recognize the second epidermal growth factor–like domain. TH Open 03, e263–e272. https://doi.org/10.1055/s-0039-1695709.
Sato, Y., Sugi, T., 2020. Autoantibodies to epidermal growth factor (EGF) in patients with recurrent pregnancy loss and repeated implantation failure. J. Reprod. Immunol. 142, 103231. https://doi.org/10.1016/j.jri.2020.103231.
Sato, Y., Sugi, T., Sakai, R., Kuma, H., 2022. The role of epidermal growth factor-like domain-related abnormalities, protein s tokushima, and anti-protein s autoantibodies in pregnancy loss. J. Reprod. Immunol. 150, 103476. https://doi.org/10.1016/j.jri.2022.103476.
Schmeidler-Sapiro, K.T., Ratnoff, O.D., Gordon, E.M., 1991. Mitogenic effects of coagulation factor XII and factor XIIa on HepG2 cells. Proc. Natl. Acad. Sci. 88, 4382–4385. https://doi.org/10.1073/pnas.88.10.4382.
Schved, J.F., Gris, J.C., Neveu, S., Dupaigne, D., Mares, P., 1989. Factor XII congenital deficiency and early spontaneous abortion. Fertil. Steril. 52, 335–336. https://doi.org/10.1016/s0015-0282(16)60866-x.
Sorice, M., Arcieri, P., Griggi, T., Circella, A., Misasi, R., Lenti, L., et al., 1996. Inhibition of protein s by auto antibodies in patients with acquired protein s deficiency. Thromb. Haemost. 75, 555–559. https://doi.org/10.1055/s-0038-1650320.
Sorice, M., Griggi, T., Arcieri, P., Circella, A., d'Agostino, F., Ranieri, M., et al., 1994. Protein s and HIV infection: the role of anticardiolipin and anti- protein s antibodies. Thromb. Res. 73, 165–175. https://doi.org/10.1016/0049-3848(94)90095-7.
Staff, A.C., Fjeldstad, H.E., Fosheim, I.K., Moe, K., Turowski, G., Johnsen, G.M., et al., 2022. Failure of physiological transformation and spiral artery atherosis: their roles in preeclampsia. Am. J. Obstet. Gynecol. 226, S895–S906. https://doi.org/10.1016/j.ajog.2020.09.026.
Steegers, E.A., Von Dadelszen, P., Duvekot, J.J., Pijnenborg, R., 2010. Pre-eclampsia. Lancet 376, 631–644.
Suleiman, L., Négrier, C., Boukerche, H., 2013. Protein S: a multifunctional anticoagulant vitamin K-dependent protein at the crossroads of coagulation, inflammation, angiogenesis, and cancer. Crit. Rev. Oncol. Hematol. 88, 637–654. https://doi.org/10.1016/j.critrevonc.2013.07.004.
Tsuda, H., Noguchi, K., Oh, D., Bereczky, Z., Lee, L.H., Kang, D., et al., 2020. Racial differences in protein s tokushima and two protein c variants as genetic risk factors for venous thromboembolism. Res. Pr. Thromb. Haemost. 4, 1295–1300. https://doi.org/10.1002/rth2.12440.
Velayuthaprabhu, S., Matsubayashi, H., Sugi, T., Nakamura, M., Ohnishi, Y., Ogura, T., et al., 2011. A unique preliminary study on placental apoptosis in mice with passive immunization of anti-phosphatidylethanolamine antibodies and anti-factor XII antibodies. Am. J. Reprod. Immunol. 66, 373–384. https://doi.org/10.1111/j.1600-0897.2011.01008.x.
Wang, X.N., Das, S.K., Damm, D., Klagsbrun, M., Abraham, J.A., Dey, S.K., 1994. Differential regulation of heparin-binding epidermal growth factor-like growth factor in the adult ovariectomized mouse uterus by progesterone and estrogen. Endocrinology 135, 1264–1271. https://doi.org/10.1210/endo.135.3.8070372.