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Estrogen-Induced Uterine Vasodilation in Pregnancy and Preeclampsia

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Abstract

Normal pregnancy is associated with dramatically increased estrogen biosynthesis whose role is believed to raise uterine blood flow to facilitate the bi-directional maternal-fetal exchanges of gases (O_2 and CO_2), to deliver nutrients, and exhaust wastes to support fetal development and survival. Constrained uterine blood flow in pregnancy is a leading cause of preeclampsia with fetal growth restriction, rendering investigations of uterine hemodynamics to hold a high promise to inform pathways as targets for therapeutic interventions for preeclampsia. The mechanisms of estrogen-induced uterine vasodilation in pregnancy have long been attributed to enhanced endothelium production of nitric oxide, but clinical trials targeting this pathway that dominates uterine hemodynamics have achieved no to little success. Emerging evidence has recently shown a novel proangiogenic vasodilatory role of hydrogen sulfide in regulating uterine hemodynamics in pregnancy and preeclampsia, provoking a new field of perinatal research in searching for alternative pathways for pregnancy disorders especially preeclampsia and intrauterine growth restriction. This minireview is intended to summarize the nitric oxide pathway and to discuss the emerging hydrogen sulfide pathway in modulating estrogen-induced uterine vasodilation in pregnancy and preeclampsia.

Keywords: Estrogens; Uterine vasodilation; Nitric oxide; Hydrogen sulfide; Pregnancy; Preeclampsia

Introduction

Normal pregnancy is associated with profound uterine artery (UA) dilation, reflected as ~20–50-fold rises in uterine blood flow (UtBF) in late pregnant (P) *vs.* nonpregnant (NP) state, resulting in a large volume of maternal blood to be delivered to the maternal-fetal interface.[1–3] UtBF carries out the bidirectional maternal-fetal gas (ie, O_2 and CO_2) exchanges and provides the sole nutrient sources to support fetal and placental growth and survival.[2–4] Abnormal UA Doppler flow is linked to preeclampsia[5] and constrained UtBF results in intrauterine growth restriction (IUGR),[5,6] clearly demonstrating that UtBF is the rate-limiting factor for pregnancy health. Investigation of the mechanisms and pathways controlling UA vascular adaptations to pregnancy has been one of the major focuses of perinatal research because they can inform therapeutical and/or preventive targets for treating preeclampsia and IUGR.

Three periodic rises in UtBF occur in pregnancy; the first is associated with vasodilation and initiation of an increase in microvascular volume probably due to ovarian hormones, that is, estrogen and progesterone, which are necessary for optimizing the intrauterine environment for embryo implantation. Once the embryo is implanted and during placentation de novo formation of the placental vascular bed further remodels the maternal uteroplacental vascular bed, resulting in the second up to 2–4-fold rise in UtBF during this time in ovine pregnancy. The third exponential rise in UtBF occurs in the third trimester of gestation, which is essential for the nutrient delivery required for the exponential fetal growth that occurs at this time and maintenance of fetal well-being. This third peak rise in UtBF is due to vasodilation of the maternal UAs because at the time the uterine vascular bed exhibits minimal growth although the fetal placental vascular bed continues to grow.[7]

Decades of extensive studies have identified many endocrine regulatory pathways and UA local autocrine and paracrine factors that regulate uterine hemodynamics, including steroid hormones estrogen and progesterone, angiogenic growth factors, and endothelium-derived vasodilators such as nitric oxide (NO). Unfortunately, clinical trials targeting all known pathways controlling UtBF, including the NO pathway that dominates the field since the 1990s, have to date achieved no to little success. Inadequate understanding of how UA hemodynamics is regulated during normal pregnancy and how this is impaired in preeclampsia must be blamed for this major obstacle in developing effective treatments for preeclampsia with IUGR. Nonetheless, more studies to identify new

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pathways that contribute to the physiological UA vascular adaptations to normal pregnancy and their maladaptations to preeclampsia are warranted. This minireview is intended to discuss uterine hemodynamic regulation in normal pregnancy and preeclampsia with a focus on the physiological roles of estrogens and the underlying mechanisms.

Hemodynamic adaptations of UA in normal pregnancy

Once conceived, the blood volume and flow rate in the main UAs increase gradually with the placental and fetal growth rates in the first and second trimesters; however, in the third trimester, the flow rate explodes proportionally so that a huge amount of blood is delivered to perfuse the placenta, essentially keeping pace with the exponential fetal growth rate. This is evidenced by the fact that compared to 1%–2% of total cardiac output is distributed to the uterine circulation in NP ewes, approximately 15%–20% of total cardiac output is distributed to the uterine circulation in late (day 130 in gestation, term ~145 days, equivalent to the third trimester in humans) pregnant ewes.[2] The blood delivered from the UA to the maternal-fetal unit provides the sole sources of nutrients and O_2 needed to support fetal and placental development and survival during pregnancy. Meanwhile, the uterine veins in parallel circulate the low oxygen blood back to the mother's circulation so that the metabolic wastes and respiratory gas (CO_2) of the fetus can be exhausted. Therefore, the uterine circulatory system is the lifeline for the fetus and UtBF is the rate-limiting factor for maternal and fetal well-being during pregnancy. To accommodate the dramatic pregnancy-dependent increase in UtBF, the UAs must undergo significant structural and functional changes adaptive to pregnancy.

In eutherian mammals including humans, UAs arise from the internal iliac arteries. Blood delivered by these arteries, joined by a blood supply from the ovarian arteries, are the principal transportation system for delivering the oxygen/nutrient-rich arterial blood to the arcuate branches within the myometrium and to the radial arteries continuing as the distal spiral arteries in the uterine endometrium.[8] Collectively these vessels with descending sizes form the uterine vascular system, composed of endothelial cells (ECs) covering the inner luminal surface, vascular smooth muscle cells (SMCs) forming the thick layer of vessel wall, and extracellular matrix that stabilizes the vessels. During pregnancy, UA is remodeled to be with increased lumen diameter and length and reduced myogenic tone leading to lower vascular resistance, thus resulting in increased UtBF. Pregnancy-dependent UA vascular remodeling has been recently detailed in an excellent review article by Osol and Moore,[9] in which they have also discussed the underlying mechanisms.

Estrogen-induced UA vasodilation in pregnancy

During the menstrual cycle, UtBF increases gradually from early or middle proliferative to secretory phase, from 22.4 ± 7.3 mL/min in the proliferative phase to 30.7 ± 13.7 mL/min in secretory phase, in association with maximum perfusion occurring in the mid-secretory phase in NP women.[10,11] Interestingly, blood flow in radial arteries does not parallel the changes in UtBF.[11] The regional changes in blood flow in the uterine vascular tree indicate that not only are vascular adaptations in the main UAs and thus UtBF under the control of specific mechanisms but also changes in steroid hormones (ie, estrogens and progesterone) are speculated to be critical physiological regulators of UtBF during the menstrual cycle. There are, however, conflicting findings regarding the correlation of UtBF with maternal circulating estradiol and progesterone in the menstrual cycle. For example, some studies reported no significant correlation of serum estradiol with uterine hemodynamics[11,12] and others even reported a reduction in UtBF during the follicular phase when serum estradiol levels are high.[13] Although the exact mechanisms regarding how UtBF varies during the menstrual cycle require further investigation, color Doppler ultrasound examination in women has proposed a role of local factors of the dominant follicle that reduce ovarian artery blood flow, indirectly causing a decrease in UtBF in the follicular phase.[12]

In primates and humans, pregnancy-associated rises in UtBF are associated with a dramatic up to a 1000-fold increase in maternal plasma total estrogens, which are predominantly produced by the placenta in the last two-thirds of pregnancy.[14] However, the predominant estrogens produced in pregnancy are relatively weak compared with estradiol-17β ($E_2\beta$) because estriol (E3) is the major estrogens in pregnant women and estrone (E1) plus sulfoconjugated estrogens are the major estrogens in ovine pregnancy.[15] The purpose for this enhanced estrogen synthesis remains unclear; but it is believed to play a key role in modulating maternal cardiovascular changes adaptative to pregnancy, including a fall in systemic vascular resistance, rise in cardiac output, development of attenuated pressor responses, and the exponential rise in UtBF in the last third of gestation.[16]

When administrated in intact or ovarietomized (OVX) NP sheep, $E_2\beta$ can cause blood flow to rise in several organs throughout the body with the greatest effects occurring in reproductive tissues, especially the uterus.[17] The selective uterine vasodilatory effect of estrogens is, however with no doubt, of major physiological significance during pregnancy-associated UA dilation because circulating estrogens are significantly elevated in pregnancy.[3,18] Plasma levels of total estrogens are ~300 pM in the secretory phase, rise to ~1000 pM in the proliferative phase, and aggressively increases with advancing gestation age and concentrations just before parturition can reach as much as 1000 times greater to that of early gestation.[19] UtBF rises sharply with elevated endogenous estrogens in the follicular phase and pregnancy,[18] providing the evidence of a physiological role of endogenous estrogens in uterine vasodilation. Furthermore, daily $E_2\beta$ (1 μg/kg body weight) treatment increases baseline UtBF by 30%–45% for up to 10 days in intact and OVX NP ewes[20]; acute $E_2\beta$ treatment provokes rapid (20–30 minutes) and even more robust up to 10-fold rise in UtBF within 90–120 minutes.[21] Thus, these physiological and pharmacological studies have provided convincing evidence for the uterine potent vasodilatory effects of estrogens.

Estrogen receptors (ERs) and estrogen-induced UA dilation

Blockade of estrogen actions using the ER antagonist ICI 182,780 inhibits exogenous estrogen-induced rise in UtBF in OVX sheep by ~70% and endogenous estrogen-induced UA dilation in the follicular phase and pregnant ewes by 35%–40%.[3] These studies established a primary proximal physiologic cause and effect relationship between endogenous estrogen-mediated ER activation and estrogen-induced UA dilation.

The biological functions of estrogens are known to be elicited by signaling via multiple ERs including ERα and ERβ and G protein-coupled ER (GPR30/GPER).[22] The vascular effects of estrogens are mediated by both "genomic" and "nongenomic" pathways, such as ERα, ERβ, and GPER, which are found in ECs and SMCs in various vascular beds,[23–25] including sheep UA.[26,27] The genomic pathway is mediated by ERα/ERβ in the nucleus of target cells, where the ligated receptors function as ligand-activated transcription factors to regulate gene expression via interactions with estrogen-responsive elements (EREs) in the gene promoter[26] or crosstalk between ligated ERs with other ERE-interacting transcription factors. The nongenomic estrogen signaling is mediated by plasma membrane ERs (ERα, ERβ, and GPER1).[28,29] In the "extranuclear" mode, estrogen signaling is initiated in seconds to minutes via binding to membrane ER, activating multiple protein kinases[30,31] that activate downstream proteins to elicit biological functions or nuclear transcription factors to regulate latent gene expression.[32] Moreover, estrogen signaling can be even more complicated by the different and even opposite roles that ERα and ERβ may play in regulating cellular responses to estrogens, depending on cell types and cellular microenvironment.[33]

GPER has been regarded as a specific membrane ER.[34] Although GPER interacts with $E_2\beta$ with estimated binding affinities of 3–6 nM,[35] this is much lower as compared with its binding affinities for classical ERs that are in the range of 0.1–1.0 nM.[36] It also interacts with anti-estrogens such as tamoxifen and the nonspecific ER antagonist ICI 182,780,[35] as well as many other ER modulators,[29] which makes it difficult to elucidate the specific role of GPER in target cells. Thus, the development of specific pharmacological tools, including highly selective GPER agonist G1 and antagonist G15[37] has greatly facilitated the characterization of GPER function. G1 binds GPER with high affinity ($K_d = 10$ nM) without binding to ERα/β at concentrations as high as 10 μM, whereas G15, with a similar structure as G1, but lacks the ethanone moiety that forms hydrogen bonds involved in receptor activation, displays a $K_d > 10$ μM for binding ERα/β.[38]

Both ERα and ERβ are expressed in ovine and human UAs and their expression has been shown to be differentially regulated in pregnancy.[26,27,39] ERβ is preferentially upregulated in pregnant UA.[33] Pregnancy augmented UA ER expression may be due to elevated endogenous estrogens because UA ER in OVX NP sheep and rats can be stimulated by exogenous $E_2\beta$ in vivo and by $E_2\beta$ treatment of UA rings from NP ewes in vitro.[27,40]. In an ex vivo study using wire myography to examine the specific roles of ERα and ERβ in estrogen-induced vasodilation in human UA and placental arteries, $E_2\beta$ treatment and activation of ERα by its specific agonist PPT (1,3,5-tris (4-hydroxyphenyl)-4-propyl-1H-pyrazole) or ERβ by its specific agonist DPN (2,3-bis(4-hydroxyphenyl) propionitrile) provoke significant and similar relaxation effects in myometrial arteries. In contrast, $E_2\beta$ and DPN are less effective in relaxing placental arteries than that in myometrial arteries; whilst PPT was ineffective.[39] The small arteries isolated from myometrial biopsies used in the study do not answer a question regarding the specific roles of ERα and ERβ in the response of the main UA to estrogens. However, these findings demonstrate that ERα and ERβ display vascular bed-specific roles in mediating the acute vasodilatory responses of estrogens in the human maternal-fetal interface vasculatures.

GPER is also expressed in various arteries including pregnant UA in rats.[25] In humans, G1 relaxes human internal mammary artery ex vivo,[41] but is unable to relax pressurized human myometrial and placental arteries ex vivo in myography studies.[39] However, activation of GPER by $E_2\beta$ or G1 can result in a pregnancy-dependent decrease in uterine vascular tone in uterine radial artery via activating NO/cyclic guanosine monophosphate (cGMP) pathway in rats.[25] These studies suggest that GPER displays vascular-bed and potentially species-dependent vascular effects of estrogens in the uterine and systemic vascular beds. Nonetheless, the specific roles of the classical ERα and ERβ and membrane GPER in estrogen-induced and pregnancy-associated uterine vasodilation remain to be incompletely understood, although the process has been shown to be clearly mediated by specific ER-dependent mechanisms in sheep models in vivo.[3]

Estrogens and uterine vascular maladaptation to preeclampsia

Preeclampsia is a human pregnancy disorder clinically defined as new onset hypertension and proteinuria, and often with edema after the 20th week of gestation.[42] Approximately 10 million pregnant women develop preeclampsia annually in the world,[43] raising perinatal mortality ~5-fold, causing death of 45,000 babies in the United States alone and killing approximately 76,000 pregnant women worldwide.[43,44] Preeclampsia predisposes the mother and her child to a significantly higher risk in metabolic diseases such as cardiovascular and diabetes later in life, representing a major public health threat.

The pathogenesis of preeclampsia remains partially understood but this is believed to be a two-stage disease composed of: (1) perturbation in placentation in the first trimester due to shallow trophoblast invasion and impaired spiral artery (the distal branch of UA vascular tree) remodeling and (2) constrained UtBF lead to placenta ischemia/hypoxia that further stimulates placental production of harmful factors, which in turn result in maternal inflammation and uterine and systemic EC damage/vascular dysfunction.[45] Nonetheless, in patients with preeclampsia, the UA flow velocity decreases by ~26%,[46] and uteroplacental perfusion is reduced by ~50%,[47] as a result of increased vascular resistance compared with normal pregnancy.[48] This clearly suggests that maternal and fetal well-being during pregnancy and after birth is largely controlled at the level of UtBF, making studies of uterine

hemodynamics to hold high promise to inform pathways and targets for developing treatments for preeclampsia and IUGR.

In primates and human pregnancy, estrogens are mainly produced by the placenta to promote angiogenesis and vasodilation, which are two key mechanisms to cause UtBF to rise.[14,16,49] Estrogen synthesis is greatly decreased in pregnant women with preeclampsia.[50] Estrogen metabolism is also dysregulated in preeclamptic women.[50] Mice lacking catechol-O-methyltransferase (the enzyme that converts $E_2\beta$ to catechol-estrogens) develop preeclampsia-like symptoms; supplementation of estrogens can lessen the preeclampsia phenotype in pregnant catechol-O-methyltransferase$^{-/-}$ mice.[51] Thus, aberrant production and metabolism of estrogens play a key role in the pathogenesis of preeclampsia.

Current treatments for preeclampsia aim to normalize blood pressure rather than targeting the placenta pathology, but none is satisfactory; hypertension is reduced transiently, allowing Caesarean delivery to be set up. Delivery before term remains the only current effective treatment for severe preeclampsia, clearly testifying urgent unmet medical needs for patients with preeclampsia. In theory, it must be very difficult to develop therapeutics for preeclampsia by targeting placenta defects since a fully functional human placenta forms around week 15 in gestation before clinical manifestations of preeclampsia are diagnosed at the 20th week of gestation.[52] Since resistant myometrial UA (radial and arcuate arteries) plays a key role in regulating maternal blood pressure during pregnancy,[53] improving UtBF provides the most attractive target for managing clinical preeclampsia (ie, hypertension) so that gestation can be extended to avoid premature delivery. Unfortunately, clinical trials targeting NO signaling or other known pathways to improve UtBF and uterine perfusion have hitherto achieved no or little success in preeclampsia,[54] urging more studies to identify new pathways.

Estrogen-induced uterine vasodilation in pregnancy by the NO pathway

Many investigators have attempted to address the mechanisms underlying the vasodilatory effects of estrogens in the uterine vasculature since the 1970s. It was, however, until the discovery of NO as the endothelium-derived vasodilator in the 1980s that a major pathway was identified for estrogen-induced uterine vasodilation. Endogenous NO is synthesized from L-arginine by a family of NO synthases (NOS), including endothelial NOS (eNOS/NOS3), neuronal NOS (nNOS/NOS2), and inducible NOS (iNOS). Endothelium-derived NO diffuses into the surrounding SMC where it activates the soluble guanylyl cyclase (sGC) to generate the second messenger cGMP; increased cGMP further activates protein kinase G (PKG) to relax blood vessels, thus resulting in vasodilation and increasing blood flow.[55] Van Buren *et al.*[56] first reported that when infused intraarterially into the UA, a nonspecific NOS inhibitor N^G-nitro-L-arginine-methyl ester (L-NAME) dose-dependently decreases the maximum $E_2\beta$-induced increase in UtBF in sheep. Many follow-up studies not only confirmed this major finding but also reported that estrogen-induced rise in UtBF requires increased production of cGMP.[3,57] The role of the NO/cGMP pathway in uterine vasodilation has promoted significant interest in perinatal research, and numerous studies have concluded that the NOS enzyme responsible for pregnancy- and agonist (estrogens)-stimulated UA NO production is primarily eNOS, which is exclusively expressed in the EC in all species studied, including sheep,[31,57] mice,[58] rats,[59] nonhuman primates,[60] and women.[61] UA endothelial eNOS expression is upregulated by exogenous estrogens in OVX animals receiving estrogen replacement therapy and elevated endogenous estrogens during pregnancy, which is mediated by genomic estrogen signaling to upregulate *eNOS* transcription via nuclear ERα interactions with the EREs in eNOS promoter.[62] In addition, ERα is present on the plasma membrane caveola of UA ECs,[26,31,63] mediating estrogen-stimulated rapid (within minutes) NO production via eNOS phosphorylation via extracellular signal-activated kinases ½[31] and possibly protein kinase B (PKB/Akt).[30]

Numerous studies have also demonstrated that the eNOS-derived NO is a focal mediator of uterine vasodilation since NO acts downstream of numerous UA dilators including estrogens, vascular endothelial growth factor, and angiotensin II (reviewed by Bai *et al.*[62]). The role of UA endothelium eNOS-derived NO in estrogen-induced and pregnancy-dependent rise in uterine vasodilation leads to increased cGMP production which in turn results in activation of various potassium channels such as ATP-dependent (K_{ATP}), voltage-dependent (Kv), and large conductance calcium-activated and voltage dependent (BK_{Ca}) potassium channels.[62] However, infusion of BK_{Ca} blockers, but not other K^+ channel blockers, intra-arterially in UA significantly blocks estrogen-induced and baseline pregnancy-associated rises in UtBF in sheep,[64] suggesting a primary role of SMC BK_{Ca} downstream of eNOS-NO/cGMP in regulating uterine hemodynamics.

BK_{Ca} channels are tetramers of the α subunit (BKα), which can be complemented with the regulatory subunits, including the β (1–4) and γ (1–4) isoforms.[65] BKβ1 subunit is essential for increasing voltage sensitivity when intracellular free Ca^{2+} is beyond 1 μM.[66] The γ1–γ4 are auxiliary subunits that greatly modify channel activity in mammalian cells.[67] The expression and their physiological and pathological functions of SMC BK_{Ca} channels have been well-studied in other tissues in mammalians,[68] but their distribution and function remain to be explored in UA SMC. Previous studies have shown UA SMC expression of α and β1[69] and γ1[70] of BK_{Ca}. BKα is constitutively expressed, whereas pregnancy and estrogen significantly upregulate BKβ1 expression in UA.[71] BKγ1 is upregulated 7-fold in mouse UA in pregnancy and female mice lacking *BKγ1* gene is with blunted UA dilation and develops preeclampsia-like conditions during pregnancy.[70] Other BK subunits, including β3, β4, and γ2–3 mRNAs are also detected in pregnant human UA and cultured primary human UA SMC.[72] However, the role of these subunits in UA SMC BK_{Ca} activation pertaining to estrogen-induced UA dilation in pregnancy is unknown.

UtBF is reduced and placental vascularization is deranged in *eNOS*-deficient mice in association with impaired spiral artery remodeling and IUGR.[58,73] Animals treated with L-NAME to inhibit endogenous NO production develop preeclampsia-like symptoms.[74] These studies show

a causal role of dysregulated NO signaling in uterine and placental hemodynamics, forming the foundational knowledge base that has provoked many clinical trials to explore the NO pathway as a potential therapy for preeclampsia; and many preclinical and clinical trials have been performed through increasing endogenous NO production via L-arginine supplementation or decreasing NO metabolism by suppressing cyclic nucleotide phosphodiesterases that breaks down cGMP.[75] A beneficial effect of L-arginine was confirmed in seven controlled randomized trials in which L-arginine supplementation significantly reduces the risk of pregnant women with established or suspected preeclampsia.[76] Bolstering endogenous NO signaling using a phosphodiesterase 5 inhibitor Sildenafil (Viagra) and its derivatives have been extensively studied for treating preeclampsia. Sildenafil promotes a significant increase in UtBF in rats[77] and has shown promising results in many preclinical studies in animal models of preeclampsia. In human studies, gestation is extended for 4 days in severe preeclamptic patients.[78] However, a most recent clinical trial shows beneficial effects of sildenafil in treating pregnant women with a high risk of IUGR, but unfortunately, the trial has been halted because infants born from women receiving the drug died from pulmonary hypertension.[79]

Hydrogen sulfide (H_2S) and estrogen-induced and pregnancy-associated uterine vasodilation

As mentioned above, numerous clinical trials targeting all known pathways controlling uterine vasodilation have hitherto only achieved no to little success in preeclampsia and IUGR, urging more studies to search for new pathways underlying uterine hemodynamic regulation. Of note is that the dominating NO/cGMP pathway only partially accounts for estrogen-induced and pregnancy-associated uterine vasodilation since L-NAME inhibits at most ~70% estrogen-induced rise in UtBF[3,56,57] and ~25% baseline pregnancy-associated rise in UtBF[80] in sheep in vivo. These studies clearly suggest that mechanisms in addition to NO are involved in mediating estrogen-induced uterine vasodilation in pregnancy. To this end, our recent studies have shown that enhanced H_2S serves as a novel UA dilator that may be accountable for the mechanisms behind NO to comprehend uterine hemodynamic regulation.

Endogenous H_2S is synthesized from L-cysteine by two pyridoxal-5′-phosphate-dependent enzyme cystathionine β-synthase (CBS) and cystathionine γ-lyase (CSE) of the *trans*-sulfuration pathway. H_2S is now accepted as the third "gasotransmitter" after NO and carbon monoxide. Both enzymes are expressed in the human body but with highly tissue/cell-specific expression patterns and in some tissues one enzyme is sufficient for H_2S biosynthesis but in others, both are needed.[81] CSE knock-out mice develop pronounced hypertension with reduced serum H_2S levels and blunted vasodilation,[82] showing H_2S as a physiological relaxant. Endogenous H_2S is also a potent proangiogenic factor.[83] Enhanced H_2S production by trophoblast and endometrial stroma is a paracrine factor that regulates placental and endometrial angiogenesis.[84,85] Trophoblast-derived H_2S also maintains early pregnancy by regulating maternal-fetal interface immune hemostasis thus protecting the semi-allograft placenta and fetus from rejection in pregnancy.[86]

The CBS/CSE-H_2S system has been recently identified to be present in the UA in ewes[87] and women.[88] Systemic vasculature produces H_2S mainly via upregulating endothelial CSE expression or activity, which is a potent physiological vasorelaxant[82] and proangiogenic factor.[83] However, in OVX NP sheep, estrogen replacement therapy stimulates UA H_2S production in association with SMC and EC CBS upregulation without altering CSE expression in vivo.[89] Moreover, UA H_2S production is significantly augmented in association with elevated endogenous estrogens in the follicular/proliferative phase and pregnancy in ewes[87] and women,[88] with EC/SMC CBS upregulation without altering CSE[87,88] and other H_2S synthesizing enzymes, that is, 3-mercaptopyruvate sulfurtransferase and cysteine aminotransferase.[88] A slow-releasing H_2S donor GYY4237 dose-dependently relaxes phenylephrine-preconstricted UA rings from both pregnant and NP rats, but with significantly greater potency in the pregnant state.[88] Moreover, the vasodilatory effect of H_2S in pregnancy is potentially vascular bed-specific because GYY4237 does not dilate mesentery artery in pregnant rats.[88] The H_2S donor also dose-dependently dilates pregnant UA in women ex vivo.[88] Thus, these findings show that exogenous H_2S can stimulate pregnancy-dependent UA vasodilation, directly supporting H_2S as the new UA vasodilator.

The stimulatory effects of estrogens on UA EC and SMC CBS expression have been confirmed in cultured ovine UA EC and SMC cells.[90,91] Unlike in vivo studies, $E_2β$ also stimulates CSE expression ovine UA EC and SMC in vitro,[90,91] showing that in vitro cell models cannot always be used to completely mirror in vivo conditions although these ovine cell models have been widely accepted for mechanistic studies of UA vascular adaptations to normal pregnancy. Nonetheless, these studies demonstrate that CBS is the key enzyme responsible for augmented UA H_2S production in response to exogenous and endogenous estrogens. In addition, augmented UA EC and SMC CBS mRNA and protein expression in NP OVX sheep by estrogen replacement therapy[89] and during pregnancy[87,88] suggests that activation of genomic estrogen signaling to upregulate *CBS* transcription is involved. Indeed, this notion is supported by the fact that $E_2β$ stimulates CBS mRNA and protein expression in UA EC and SMC in vitro by upregulating *CBS* transcription via mechanisms involving both ERα and ERβ.[90,91]

Interestingly, $E_2β$ can stimulate rapid (within minutes) H_2S production in cultured human umbilical cord EC, which is achieved by ERα interactions with Gαi-2/3 on the plasma membrane, resulting in activation of GC/cGMP which in turn can phosphorylate CSE to increase H_2S production. Importantly, blocking the pathway can blunt estrogen-induced aorta vasodilation in mice, suggesting that activation of plasma membrane ERα-mediated nongenomic activation of endothelial CSE/H_2S production plays a role in estrogen-induced systemic vasodilation.[92] In addition, CBS phosphorylation on serine[227] by PKG also results in enzyme activation and increased H_2S production. Whether GPER or ERβ also play a role in the nongenomic activation of CSE/CBS remains to be explored. Although it is not reported whether estrogen stimulates rapid H_2S biosynthesis in UA EC and SMC, it is

warranted to determine if nongenomic estrogen signaling leading to CBS and/or CSE phosphorylation and activation thereby increasing H_2S production is a mechanism to contribute to the initiation of estrogen-induced rise in UtBF within 15–30 minutes in animal models.[21,93,94]

Activation of SMC K_{ATP} channels was the first mechanism reported to mediate the vasodilatory effect of H_2S in systemic arteries.[95] However, we have recently reported that H_2S stimulates relaxation of human UA and rat vas deferens via activating SMC BK_{Ca} channels without activation of K_{ATP} channels,[72,96,97] although how H_2S activates BK_{Ca} channels needs to be determined.

Human IUGR is associated with reduced uterine spiral artery CSE expression,[98] suggesting that H_2S signaling is impaired in pregnancy complications associated with reduced UtBF. Pregnancy also upregulates placental trophoblast H_2S production, which is a placental vasodilator[98] and angiogenesis promoter[84] and maintains early pregnancy via regulating maternal-fetal interface immune hemostasis.[86] It is also well-documented that pregnancy-upregulated placental CBS/CSE-H_2S signaling is reduced in preeclampsia.[99] Importantly, in animal models of preeclampsia induced by overexpression of soluble vascular endothelial growth factor (VEGF) receptor 1 (soluble fms-like tyrosine kinase-1, sFlt1) to disrupt vascular endothelial growth factor signaling, H_2S donors can rescue the animals from developing new onset hypertension and proteinuria and partially restored IUGR.[100] These promising findings show a great potential of targeting H_2S signaling to treat preeclampsia. However, research in H_2S in uterine hemodynamics is still in its very early stage. More investigations are needed to determine a definite physiological role of enhanced H_2S signaling in uterine vascular adaptation in normal pregnancy and a pathophysiological role of dysregulated H_2S signaling in preeclampsia and other pregnancy complications.

Summary

Estrogen-induced uterine vasodilation in pregnancy is mediated by ER-dependent augmentation of local UA production of orchestrated vasodilators, among which NO has been in general considered to play a leading role. More recently, evidence has emerged to show that enhanced local UA H_2S production is a novel UA dilator for mediating estrogen-induced uterine vasodilation in pregnancy and preeclampsia (Fig. 1). It is possible that H_2S may play an even more important role than NO in mediating estrogen-induced uterine vasodilation because NO is produced by eNOS that is mainly expressed in EC while H_2S is produced by CBS that is upregulated by both EC and SMC. H_2S production is thus expected to be with a

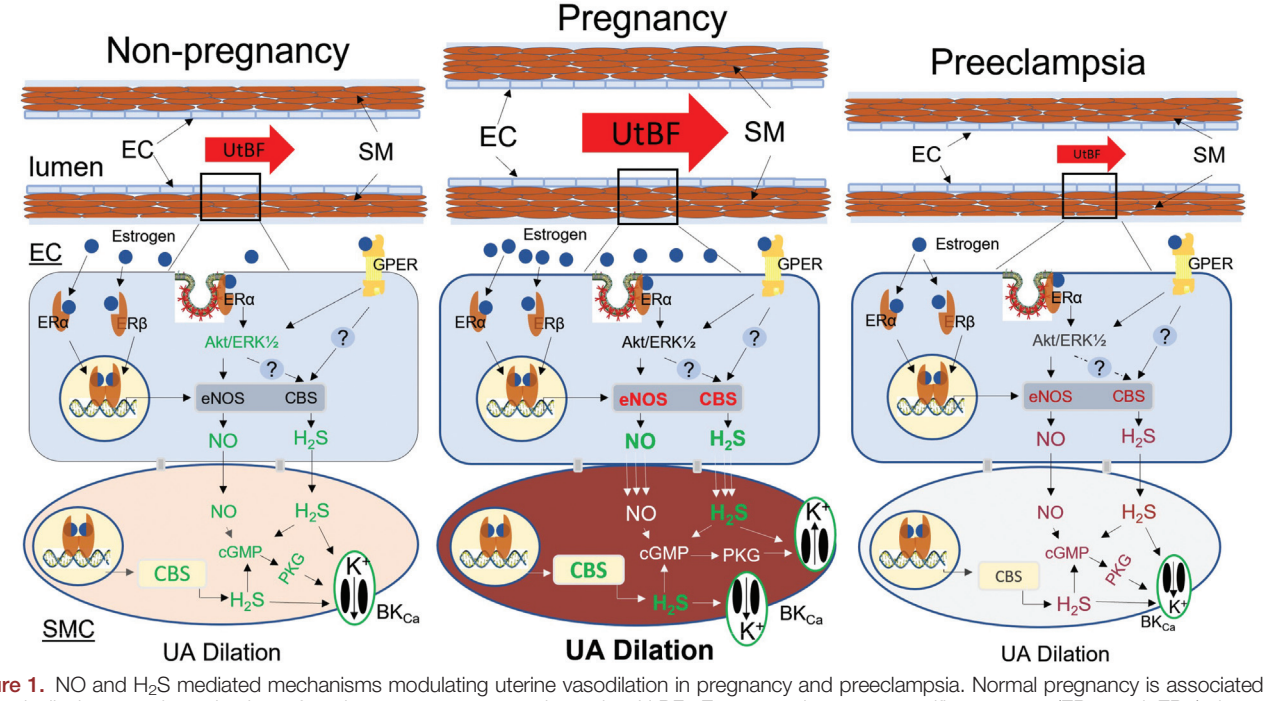

Figure 1. NO and H_2S mediated mechanisms modulating uterine vasodilation in pregnancy and preeclampsia. Normal pregnancy is associated with dramatically increased production of endogenous estrogens that raise UtBF. Estrogens increase specific receptor (ERα and ERβ)-dependent endothelium (EC) expression of eNOS to produce NO that has been recognized as a leading player to mediate estrogen-induced uterine vasodilation in pregnancy. Most recent emerging evidence also shows that estrogens stimulate ERα and ERβ dependent EC and SM expression of CBS to produce H_2S. In addition, by binding with plasma membrane ER including caveolar ERα and the GPER, estrogen can stimulate rapid production of NO and possibly H_2S by activating eNOS and CBS via posttranslational mechanisms such as phosphorylation by protein kinases including PKB (Akt), extracellular signal-activated kinases (ERK½), and PKG. Enhanced NO and H_2S collectively open the large conductance calcium-activated and voltage-dependent potassium (BK_{Ca}) channels to hyperpolarize SM resulting in UA relaxation. In preeclampsia, estrogen production decreases and its metabolism is also impaired to further result in the deactivation of NO- and H_2S-mediated mechanisms modulating uterine vasodilation that contributes to the clinical manifestations of preeclampsia. Words in bright color indicate stimulation and words in dimmed color indicate deactivation. CBS: Cystathionine β-synthase; cGMP: Cyclic guanosine monophosphate; EC: Endothelial cells; ER: Estrogen receptor; ERK1/2: Extracellular signal-activated kinases 1/2; eNOS: Endothelial NOS; GPER: G protein-coupled ER; H_2S: Hydrogen sulfide; NO: Nitric oxide; NOS: NO synthases; PKB: Protein kinase B; PKG: Protein kinase G; SM: Smooth muscle; UA: Uterine artery; UtBF: Uterine blood flow.

much great quantity than NO production in UA upon estrogen stimulation in pregnancy, considering that the tube-shaped main UA is composed of a single layer of EC and multi-layer of SMCs. Clinical trials targeting the best-studied NO pathway have been essentially failed in treating preeclampsia, urging more studies necessitated to explore the therapeutic potential of H_2S in preeclampsia. Nonetheless, research on H_2S maternal physiology and placental biology is in its very early stage. Although a few studies have shown a beneficial effect of H_2S in animal models of preeclampsia,[100] a clinical trial of H_2S in preeclampsia with IUGR will mostly become a reality when a physiological and pathophysiological role of H_2S can be established in future studies to provide a thorough understanding of the biosynthesis, metabolism, and mechanisms of action of H_2S during uterine and possibly systemic vascular adaptations to pregnancy and preeclampsia.

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Conflicts of Interest

None.

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