Effect of Oxidative Stress on the Estrogen-NOS-NO-K\textsubscript{Ca} Channel Pathway in Uteroplacental Dysfunction: Its Implication in Pregnancy Complications

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During pregnancy, the adaptive changes in uterine circulation and the formation of the placenta are essential for the growth of the fetus and the well-being of the mother [1]. Throughout pregnancy, cardiac output rises by increasing heart rate and stroke volume, reaching ~50% above prepregnancy baseline in the third trimester. Systemic vascular resistance decreases by ~20% in the second trimester, leading to reduced mean arterial blood pressure. In addition, blood volume increases by 40-50%. Nevertheless, marked changes also occur at the maternal-fetal interface. The placenta formation and structural and physiological remodeling of uterine arteries lead to the establishment of the low-resistance uteroplacental circulation. In human and sheep, uterine blood flow increases from 20 to 50 ml/min in nonpregnant state to ≥1000 ml/min at near-term pregnancy. Elevated steroid hormones such as 17β-estradiol (E\textsubscript{2})\textsuperscript{β} and progesterone are believed to play an important role in the cardiovascular adaptation during pregnancy [2–4].

Aberrant uteroplacental adaptation leads to pregnancy complications such as preeclampsia and intrauterine (fetal) growth restriction (IUGR). These complications are associated with diminished uteroplacental blood flow [5, 6]. Both preeclampsia and IUGR are major causes of maternal and/or fetal morbidity and mortality. Accumulating evidence suggests that preeclampsia and IUGR also have detrimental effects on the health of both the mother beyond pregnancy and offspring. Women with a history of preeclampsia have increased risk of cardiovascular disease [7]. Moreover, offspring born from preeclamptic pregnancy also have high incidence of high blood pressure and stroke later in life [8, 9]. Similarly, IUGR is associated with increased prevalence of metabolic syndrome, diabetes, and cardiovascular disease in later life of offspring [10, 11].

Although the etiologies of preeclampsia and IUGR are not fully elucidated, placental insufficiency (or uteroplacental...
vascular insufficiency), the inability to deliver an adequate supply of oxygen and nutrients to the fetus due to reduced blood flow to the placenta, is generally considered as a major contributor to the development of these disorders. Soleymanlou et al. revealed a remarkable similarity of global gene expression in hypoxia-treated placenta explants, high-altitude placentas, and preeclamptic placentas [12], implying an important causative role of hypoxia in these complications. This notion is further substantiated by observations in animal models in which gestational hypoxia imitated placentals insensitivity, reduced fetal growth, and induced preeclampsia-like symptoms [13–15].

Oxidative stress is defined as an imbalance between oxidants and antioxidants in favor of the oxidants [16]. Prolonged hypoxia is shown to elicit oxidative stress [17]. Consistently, placental insufficiency also promotes oxidative stress in preeclampsia, IUGR, and high-altitude pregnancy [18, 19]. Accumulating evidence suggests a critical role of reactive oxygen species (ROS) in the pathogenesis of pregnancy complications [20, 21]. However, the mechanistic insights into ROS-induced maladaptation of uteroplacental circulation remain largely elusive. In this article, we provide a succinct review of effects of oxidative stress on $E_2$ signaling pathways in the uteroplacental circulation in pregnancy complications.

2. $E_2 \beta$ Signaling and Uteroplacental Circulation in Physiological and Pathophysiological Conditions

2.1. Estrogen and Estrogen Receptors (ERs) in Normal Pregnancy and Pregnancy Complications. Both $E_2 \beta$ and its metabolites are essential for the success of pregnancy. Starting from approximately week 9 of gestation, the placenta becomes the primary site of estrogen synthesis involving enzymes such as aromatase (CYP19) and hydroxysteroid 17β-dehydrogenases 1 (HSD17B1, 17β-HSD1) [22]. Circulating estrogen rises progressively throughout pregnancy, and plasma $17\beta$-estradiol ($E_2$) level at term is ~100-fold higher than that in nonpregnant subjects. Similarly, $E_2 \beta$ metabolites produced by cytochrome P450s and catechol-O-methyltransferase (COMT) such as catecholestradiols also elevated during pregnancy [23]. However, estrogen biosynthesis and metabolism are apparently impaired in pregnancy complications. Maternal plasma $E_2$ levels are significantly lower in preeclamptic [24–26] and IUGR [27] pregnancies. Low circulating $E_2$ was also observed in high-altitude human and sheep pregnancy [28–30], although one study showed an increase in plasma estrogen [31]. The metabolism of $E_2 \beta$ is also impaired in preeclampsia, leading to reduced 2-methoxyestrone and 2-methoxyestradiol [25, 32]. It appears that the reduced circulating levels of $E_2 \beta$ and its metabolites in pregnancy complications are the result of dysregulation of steroidogenic enzyme expression in the placenta. Preeclamptic placenta displayed deficiency of aromatase, HSD17B1, and COMT [24, 25, 32–34]. The impaired estrogen steroidogenesis and metabolism in these disorders are evidently caused by placental insufficiency. Aromatase in cultured human trophoblast cells and in trophoblast cell line JEG-3 was downregulated by hypoxia [24, 35], and the expression of placental aromatase was reduced in a rabbit model of placental ischemia [24]. Aberrant production of $E_2 \beta$ and its metabolites could contribute to the pathogenesis of pregnancy complications due to their key roles in regulating trophoblast invasion, angiogenesis, and uterine vascular tone, which will be discussed in later sections.

Estrogen produces its plethoric effects via interacting with its receptors involving both nongenomic and genomic mechanisms. To elicit genomic actions, estrogen binds to the nuclear estrogen receptor $\alpha$ (ERα) or estrogen receptor $\beta$ (ERβ). The receptors become dimerized and bind to the estrogen response element (ERE) located in the target gene promoter, triggering or suppressing gene expression [36]. Estrogen can also activate membrane G-protein-coupled estrogen receptor (GPER, or GPR30) and membrane-associated ERα and ERβ, which in turn stimulate adenylate cyclase to generate cAMP or activate kinases such as tyrosine kinase Src, phosphoinositide 3-kinase (PI3K), extracellular-signal-regulated kinase (ERK), and protein kinase B (PKB or AKT) [37]. Activation of membrane or membrane-associated estrogen receptors can lead both acute and long-term effects. The presence of ERα, ERβ, and GPER in uterine arteries and the placenta has been demonstrated by real-time polymerase chain reaction (PCR), Western blot, and immunohistochemistry [38–41]. The expression of all forms of estrogen receptors in uterine arteries and the placenta increases as pregnancy advances [38–40, 42]. The maintenance or upregulation of ERs in the uteroplacental tissues apparently requires continuous estrogen stimulation. Ovariectomy in sheep reduced ERβ expression in the endothelium of uterine arteries [42]. In addition, chronic treatment with $E_2 \beta$ in vivo and ex vivo significantly increased ERα expression in uterine arteries [40, 42]. The expression of GPER in HTR8/SVneo cells derived from first trimester extraplacental and placental extravillous explants was also upregulated by $E_2 \beta$ [43].

Information on estrogen receptor expression in pregnancy complications is scant, and conflicting observations have been reported. ERα expression was described as increased, decreased, or unchanged in the preeclamptic placenta [44–46]. No conclusion could be drawn currently, and more rigorous studies are needed to clarify the discrepancy. The expression of ERα in uteroplacental tissues was suppressed in high-altitude pregnancy [40], and hypoxia appeared to be the causative factor responsible for ERα downregulation [45, 47]. Defective expression of ERα could have profound effects on uteroplacental function including gene expression. Intriguingly, the placental expression of ERβ appears to be differently affected in preeclampsia and IUGR. Whereas ERβ expression was reduced in the IUGR placenta [44], an upregulation of ERβ was observed in preeclamptic placentas [44, 45]. These observations suggest that the etiologies of preeclampsia and IUGR may differ. It remains to be determined whether/how the distinct regulations of ERβ contribute to the pathogenesis of these two complications. The placental expression of GPER was reduced in preeclamptic pregnancy [43, 48], which may lead to dysfunction of uteroplacental vessels.
2.2. Estrogen and the Regulation of Uteroplacental Circulation. Several lines of evidence have implicated a critical role of estrogen in the adaptation of the uteroplacental circulation. First, the high ratio of E2β to progesterone in the follicular phase was associated with increased blood to the uterus [49, 50]. Second, reduced uterine vascular resistance and increased uterine blood flow concurred with progressively rising plasma E2β levels during pregnancy [51–53]. Third, acute treatment with exogenous E2β markedly increased uterine blood flow and/or reduced uterine vascular resistance in nonpregnant animals [54–56]. Fourth, chronic administration of E2β into nonpregnant sheep also significantly increased uterine blood flow and/or reduced uterine vascular resistance [57, 58]. Ex vivo treatment of uterine arteries from nonpregnant sheep with E2β reduced uterine arterial myogenic tone [59]. The chronic effects of E2β simulated pregnancy-induced hemodynamic changes in the uterine circulation. Fifth, the nonselective ERα/ERβ antagonist ICI 182,780 reduced the increase in uterine blood flow induced by exogenous E2β in nonpregnant sheep and by endogenous E2β in the follicular phase of nonpregnant sheep by ~60% [53]. Intriguingly, the same antagonist also lowered basal uterine blood flow in pregnant sheep by 37% [53]. Importantly, E2β and its metabolites also play an important role in uteroplacental adaptation. E2β, 2-hydroxyestradiol, 4-hydroxyestradiol, and 4-methoxyestradiol were implicated in angiogenesis by promoting endothelial cell proliferation [60], whereas 2-methoxyestradiol promoted the differentiation of the cytotrophoblast to an invasive phenotype [61].

2.3. NO and Ca2+-Activated K+ (KCa) Channels in Regulating Uteroplacental Function. Nitric oxide (NO) is a gaseous messenger-generated nitric oxide synthase (NOS). NO contributes to the maintenance of cardiovascular homeostasis by regulating vasocontractility [62]. The large-conductance Ca2+-activated K+ (BKCa) channel is primarily expressed in vascular smooth muscle cells (VSMCs) and plays a pivotal role in regulating myogenic tone [63]. In VSMCs, the BKCa channel is a heteromeric assembly of the pore-forming α subunit and accessory β1 subunits [64]. The β1 subunit encoded by KCNMB1 increases the channel’s Ca2+ voltage sensitivity. Importantly, the BKCa channel is one of many targets of NO in the cardiovascular system [64]. Not surprisingly, the NO-cGMP-PKG-BKCa channel axis is implicated in the adaptation of uteroplacental circulation during pregnancy [65] (Figure 1).

Activation of either of ERα, ERβ, or GPER induced acute vasorelaxation of uterine arteries [66]. The acute estrogen effects in regulating uterine hemodynamics involved stimulation of endothelial NOS (eNOS) activity and increased NO release in endothelial cells (ECs) [38, 67] and activation of BKCa channels in VSMCs [67]. Stimulation of eNOS activity by estrogen in uterine arterial ECs required phosphorylation of the enzyme at serine 635 and serine 1177 mediated by PKG [64]. Important, E2β also directly activate BKCa channels in uterine arterial VSMCs [67], possibly via interacting with the accessory β1 subunit [69].

E2β could also exert its genomic effect to regulate the expression of both NOS and BKCa channels in uteroplacental tissues. Expression and function of eNOS [70–72] and the
BKCa channel β1 subunit [65, 73, 74] in uterine arteries were increased in the follicular phase and during pregnancy. The upregulation of eNOS and the BKCa channel β1 subunit in uteroplacental circulation during these two physiological states was apparently stimulated by estrogen as chronic treatment with exogenous E2β in intact nonpregnant animals [58, 75, 76] and in ex vivo cultured uterine arteries [73] elevated their abundance and activity.

In vivo studies revealed distinct contributions of eNOS and the BKCa channel to basal uterine blood flow in non-pregnant and pregnant sheep. Intrauterine arterial infusion of the NO synthase inhibitor L-nitro-arginine methyl ester (L-NAME) demonstrated minimal contribution of NO to basal uterine blood flow in both nonpregnant and pregnant sheep [77]. However, infusion of the BKCa channel blocker tetraethylammonium into uterine arteries revealed that at least half of the basal uterine blood flow is maintained by the BKCa channel in pregnant sheep, whereas the channel did not contribute to basal uterine blood flow in nonpregnant animals [67, 78]. These findings are reinforced by the observations that uterine arterial myogenic tone (i.e., the major constituent of vascular tone) of pregnant subjects was regulated by the BKCa channel [73], but not by the endothelium [79, 80]. Thus, estrogen-induced eNOS expression and activity during pregnancy are probably responsible for enhanced endothelium-dependent vasorelaxation in uterine arteries in response to given vasodilators [81, 82] and uterine artery remodeling [83], but not for regulating basal uterine vascular tone. In contrast, the upregulation of the BKCa channel is essential for the reduced uterine vascular tone during pregnancy. In addition, the upregulated BKCa channel also contributed to blunted vasoconstrictor responses in uterine arteries during pregnancy [65, 84]. Thus, the BKCa channel in uteroplacental circulation functions as a negative feedback control mechanism to prevent excessive vasoconstriction. Together, these findings reinforced the notion that E2β, through its acute and chronic actions on eNOS and BKCa channels, plays a pivotal role in uteroplacental adaptation.

Expression/activity of placental eNOS in preeclamptic and IUGR pregnancies was reported as either unaltered [85, 86], decreased [44, 87], or increased [88, 89]. Whereas eNOS in placental choriionic plate arteries was downregulated in preeclampsia [90], this enzyme in uterine arteries was upregulated in high-altitude pregnancy [91]. Regardless of uteroplacental eNOS expression status, NO bioavailability in pregnancy complications appeared to be reduced due to substrate deficiency and enzyme inhibition. Both plasma and placental L-arginine levels were reduced in preeclampsia [86, 92]. In addition, the expression of arginase-2, which consumes eNOS’s substrate L-arginine, was increased in the placenta and in omental vessels of women with preeclampsia [86, 93]. The increased arginase-2 expression could be imitated by treating human umbilical vein endothelial cells (HUVECs) with preeclamptic plasma [93]. Moreover, HUVECs from IUGR pregnancy also displayed increased arginase-2 expression and activity and placental vessels exhibited impaired eNOS-dependent relaxation [89]. A deficiency of L-arginine would not only reduced eNOS-derived NO but also increase eNOS-mediated superoxide production leading to peroxynitrite (ONOO−) formation, evidenced by increased nitrotyrosine staining in villi and maternal vasculature of preeclamptic women [86, 94]. Similarly, nitrotyrosine staining was increased in the syncytiotrophoblast and extravillous trophoblast of high-altitude placenta [95]. Intriguingly, the circulating level of asymmetric dimethylarginine (ADMA), an endogenous NOS inhibitor, also increased in preeclamptic and IUGR pregnancies [96, 97]. Not surprisingly, NOS-dependent relaxation of placental choriionic arteries from IUGR pregnancy was impaired [98]. Moreover, both chronic blockade of NOS with L-NAME or knockout of eNOS in rodents increased maternal blood pressure and reduced fetal growth [99, 100], partly due to impaired uteroplacental vessel remodeling [101].

The expression and function of the BKCa channel in uteroplacental vessels are also impaired in pregnancy complications. It appears that the β1 subunit of the channel is selectively targeted, whereas the α subunit remains unaffected in these disorders. The BKCa channel β1 subunit was downregulated in placental choriionic plate arteries in preeclampsia [102] and uterine arteries in high-altitude pregnancy [103]. High-altitude pregnancy also suppressed the ability of estrogen to upregulate the expression of the BKCa channel β1 subunit in uterine arteries [103], leading to increased uterine arterial myogenic tone. BKCa channel-mediated vasorelaxation was also reduced in both pathological conditions. The impact of high-altitude pregnancy on the BKCa channel was simulated by ex vivo hypoxia [104], implicating a causative role of hypoxia in the downregulation of the BKCa channel β1 subunit. In a preeclampsia-like murine model induced by autoantibodies against angiotensin II type 1 receptor (AT1-AA), the expression of the BKCa channel β1 subunit and channel activity in mesenteric arteries was also reduced [105].

The intermediate-conductance (IKs) and small-conductance (SKs) Ca2+-activated K+ channels are predominately expressed in ECs and also mediate endothelium-dependent vasodilation [106]. The endothelium-derived hyperpolarizing factor (EDHF) causes hyperpolarization of VSMCs by activating IKs and SKs. Both IKs and SKs are expressed in uteroplacental tissues [90, 107, 108]. IKs and SKs are also expressed in VSMCs of uterine and placental choriionic plate arteries in addition to their expression in ECs [90, 107]. In the uteroplacental system, IKs and SKs participated in the regulation of contractility of uterine and placental vessels [90, 107, 109]. Moreover, SK3 was also involved in regulating uterine vascular remodeling and placental vasculatization [110, 111]. Like BKCa channels, E2β is required to maintain and to upregulate the expression and function of SKs in vasculature. Pregnancy via estrogen’s action upregulated the expression of SK2 and SK3 in uterine arteries [107]. Ovariectomy reduced SK3 activity in ECs and ablated the channel’s role in EDHF-mediated vasorelaxation in non-uterine arteries [112].

The expression and function of IK1, SK2, or SK3 in uteroplacental vessels and umbilical vessels were downregulated in high-altitude pregnancy and preeclampsia [90, 107, 113] as well as in a rat model of preeclampsia induced by testosterone [108]. Given the important role of estrogen in the
regulation of IKs and SKs in uteroplacental circulation, it is anticipated that impaired E2β-ER signaling could contribute to the downregulation of these ion channels in high-altitude and preeclamptic pregnancies.

Together, evidence presented in this section demonstrated critical roles of both estrogen synthesis and metabolism in the adaptation of uteroplacental circulation. Preeminent, E2β and its metabolites contribute to this adaptive process by promoting angiogenesis, trophoblast invasion, and remodeling and by lowering uterine vascular tone through upregulating activity and/or expression of both eNOS and KCa channels. However, the E2β-NOS-NO-KCa channel pathway is disrupted in pregnancy complications, which could contribute to the pathogenesis of these disorders.

3. Oxidative Stress and Pregnancy Complications

3.1. Cellular Sources of ROS and Antioxidant Defense. ROS are oxidants formed during oxygen metabolism, primarily produced during oxidative phosphorylation in the mitochondria and by oxidases such as NADPH oxidases (NOXs) and xanthine oxidase (XO) as well as uncoupled NOS [114, 115]. ROS include free radicals such as superoxide (O2·−) and hydroxyl radical (‘OH) and nonradical hydrogen peroxide (H2O2). In order to maintain redox hemostasis, mammalian cells have developed enzymatic and nonenzymatic defense mechanisms to balance the oxidative state. The major antioxidant enzymes involved in detoxifying ROS include superoxide dismutase (SOD), catalase, glutathione peroxidase (GPx), and peroxiredoxin (Prx) [116]. Nonenzymatic antioxidants include metabolic products such as glutathione (GSH), uric acid, and melatonin [117, 118].

ROS at low levels can act as intracellular second messengers to modulate cellular responses. The very short lifetime and diffusion distance of O2·− and ‘OH make them unsuitable to function as signaling molecules. In contrast, H2O2 mediates reversible oxidation of cysteine residues in proteins, which can alter protein activities and functions [119]. These proteins include enzymes (i.e., mitogen-activated protein kinases (MAPKs), tyrosine kinases, and protein tyrosine phosphatases) and transcription factors (i.e., activator protein-1 (AP-1), nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB), and hypoxia-inducible factor 1 (HIF-1)). When ROS production overwhelms the intrinsic antioxidant defense, due to either increased ROS formation or reduced ability to neutralize ROS or both, oxidative stress arises. As a consequence, ROS attack cellular components, leading to potential cell/tissue damage.

3.2. Normal Pregnancy Is a Mild State of Oxidative Stress. The metabolic activity of the placenta is high in order to meet the growth of both the placenta and the fetus, leading to increased ROS production during normal pregnancy. It has long been proposed that pregnancy is a state of oxidative stress [120, 121]. This notion is supported by the following observations: (1) increased levels of superoxide O2·−, 8-isoprostaglandin F2α (8-iso-PGF2α), and malondialdehyde (MDA) in the circulation and the placenta [122–124]; (2) reduced circulating expression and activity of enzymatic antioxidants such as SOD, GPx, and catalase [123, 125]; and (3) decreased levels of nonenzymatic antioxidants including uric acid, vitamin C, vitamin E, and GSH [123, 126]. Notably, the increased ROS production in early pregnancy plays an important role in trophoblast proliferation, differentiation, invasion, and angiogenesis [127, 128]. As gestation advances, placental SODs and catalase as well as total antioxidant capacity also increase [129, 130], which counters the increased ROS generation. Thus, a relatively physiological balance between oxidants and antioxidants is maintained in normal pregnancy.

3.3. Pregnancy Complications Are Associated with Heightened Oxidative Stress. Both acute and chronic hypoxia has been shown to elevate ROS [17, 131]. Mitochondria and NOXs are the major sources of ROS in response to oxygen deprivation [132–134]. Placental insufficiency is believed to be a critical element in the pathogenesis of preeclampsia and IUGR. Not surprisingly, these disorders display heightened oxidative stress compared to normal pregnancy. Apparently, both overproduction of ROS and reduction of antioxidant defense contribute to the heightened oxidative stress in pregnancy complications. The activity and expression of oxidant enzymes such as NOXs and XO increased in the preeclamptic placenta and/or circulation [135–137]. In contrast, levels and activity of circulating and placental antioxidant enzymes such as SOD, catalase, and GPx as well as thioredoxin (Trx) were decreased in preeclamptic and IUGR pregnancies [135, 138, 139]. Similarly, activities of SOD, GPx, and Trx reductase (TrxR) were reduced in placentas from high-altitude pregnancy [95]. Moreover, mitochondria in the placenta became dysfunctional in pregnancy complication. Mitochondria appear to be damaged as evidenced by swelling and broken cristae in the preeclamptic placenta [140]. Respiratory chain enzyme expression and activity of mitochondrial complexes were suppressed in preeclamptic and IUGR placentas as well as in placentas from high-altitude pregnancy [140–142], uncoupling respiration from oxidative phosphorylation. Furthermore, circulating and placental nonenzymatic antioxidants including GSH, vitamin C, and melanin were lower in preeclampsia and IUGR [139, 143, 144]. Concomitantly, both complications exhibited higher ROS [124, 145, 146] and oxidative stress markers [147–149] in the circulation and the placenta, leading to lipid peroxidation and oxidative DNA damage [144, 149]. Increased nitrotyrosine immunostaining was observed in villous vessels of the placenta [94, 135] and systemic vessels [150] in preeclamptic pregnancy, suggesting that preeclampsia promotes NO uncoupling and OONO− generation.

3.4. Animal Models Replicate Oxidative Stress in Pregnancy Complications. The elevated oxidative stress in preeclampsia and IUGR has been imitated in animal models. Increased placental O2·− was observed in an eNOS−/− mouse model of fetal growth restriction [151]. The reduced uterine perfusion pressure model reduced levels of SODs and GPx, increased levels of MDA, and decreased mitochondrial complexes I and II expression [152–154] in rat placentas. Rodent models
of preeclampsia and/or IUGR also promoted eNOS uncoupling in the aorta and placenta [14, 155] and decreased placental GSH content [156]. Therefore, similar to human pregnancy complications, an imbalance between oxidant and antioxidant systems apparently accounts for the heightened oxidative stress in these animal models and hypoxia appeared to be a major cause of the heightened oxidative stress in these models. Increased uterine arterial ROS generation was detected in a sheep model of high-altitude pregnancy due to increased NOX2 expression, which could be replicated by ex vivo hypoxic treatment of uterine arteries [157]. Naïve high-altitude pregnant sheep exhibited higher circulating MDA than low-altitude pregnant sheep and native high-altitude pregnant sheep [30]. In addition, gestational hypoxia increased levels of 4-hydroxynonenal (4-HNE), a lipid peroxidation product, in rat placentas [158].

In conclusion, oxidative stress is an inherent feature of normal pregnancy and plays an important role in the development of the placenta. The uteroplacental system is particularly vulnerable to oxidative stress. When unchecked, oxidative stress becomes augmented and could give rise to pathological conditions such as preeclampsia and IUGA, harming both the mother and the fetus. Therefore, oxidative can play both physiological and pathological roles in the progression and outcome of pregnancy.

4. Regulation of Eβ Production and Eββ Signaling Pathway by ROS in Pregnancy Complications

As aforementioned, Eβ is an essential element in the adaptation of the uteroplacental circulation during pregnancy. Given heightened oxidative stress in preeclampsia, IUGR and high-altitude pregnancy, and diverse effects of ROS on macromolecules, it is not surprising that excessive ROS plays a critical role in the pathogenesis of these complications by disrupting the Eββ signaling pathway. ROS could directly or indirectly exert their detrimental effects on the targets, and their actions could be acute or chronic. Unfortunately, there is limited information regarding the impacts of ROS on the Eββ signaling pathway in uteroplacental circulation under pathophysiological conditions. In this section, findings from both uteroplacental and nonuteroplacental tissues/cells will be discussed.

4.1. ROS and Estrogen Synthesis. Aromatase and HSD17B1, two key enzymes in estrogen biosynthesis catalyze the interconversion between testosterone and E2β and between estrone and E2β, respectively, using cofactors NADPH [159, 160]. In fact, NADPH is a key component against cellular oxidation. Maintaining an adequate NADPH/NADP+ ratio is essential to activities of these enzymes and E2β generation. In HUVECs, high glucose elevated ROS [161] but reduced the NADPH level [162]. Lowering the NADPH/NADP+ ratio markedly reduced the conversion of estrone to E2β in HEK293 cells [163]. Interestingly, the reduced E2β level in preeclamptic placental explants was mimicked by the treatment of placental explants from normal pregnancy with H2O2 [164]. Moreover, H2O2 treatment of the homogenate of the human ovary suppressed aromatase activity, which could be prevented by GPx [165]. These observations suggest that oxidative stress could impair estrogen synthesis by suppressing key enzyme activities.

4.2. ROS and Estrogen Receptor Expression. ERα expression is also subject to ROS modulation. In general, the expression of ERα is negatively regulated by ROS. The following observations were made in cancer cell lines. In MCF-7 cells, a brief treatment with glucose oxidase, which catalyzes the oxidation of glucose to H2O2 and D-glucono-δ-lactone, resulted in marked ERα level reduction 24 hours after the treatment [166]. Chronic (16 hours) H2O2 treatment of ZR-75-1 cells also decreased ERα protein level [167]. The detrimental effect of H2O2 on ERα expression could be normalized by increasing antioxidant capacity. Overexpression of Prx-1, a H2O2 scavenger, ablated H2O2-induced downregulation of ERα, whereas inhibition of Prx-1/2 activity with adenanthin promoted ERα downregulation [167].

4.3. ROS and NO Production. NOS are also regulated by ROS. ROS affect NO production apparently through altering eNOS expression/activity and eNOS cofactors. In HUVECs, H2O2 treatment for 2 hours increased eNOS phosphorylation of serine 1177 and enzyme activity, whereas catalase did the opposite [168]. However, H2O2 was found to decrease NO bioavailability in porcine aortic ECs by inactivation of eNOS cofactors without altering enzyme activity [169]. Long-term treatment with H2O2 or superoxide treatment resulted in downregulation of eNOS in HUVECs [170, 171]. NOXs appeared to be major sources of ROS responsible for eNOS downregulation. HUVECs from women with preeclampsia exhibited NOX2 upregulation and eNOS downregulation [113]. In addition, the upregulation of NOX4 by angiotensin II and high glucose promoted eNOS uncoupling, leading to increased generation of O2·− and OONO− in glomerular mesangial cells [172, 173]. Thus, it is expected that inhibiting oxidant generation or enhancing antioxidant defense could potentially normalize the adverse effect of ROS on eNOS. As expected, eNOS expression was partially rescued or restored by NOX inhibitor apocynin or overexpression of SOD2 [113, 174]. Administration of the GSH synthase inhibitor buthionine sulfoximine into rats decreased total GSH level in the liver, reduced urinary excretion of NOx, and increased nitrotyrosine staining in the kidney without altering renal eNOS level [175].

4.4. ROS and KCa Channels. ROS display complex actions toward the BKCa channel. H2O2 could be stimulatory or inhibitory on BKCa channel activity depending on experimental conditions. H2O2 increased BKCa channel activity in human and porcine artery VSMCs and HUVECs [176–178], whereas it decreased BKCa channel-mediated currents in porcine renal artery ECs and vascular smooth muscle-type BKCa channel reconstituted in HEK2929 cells [179]. A study by Tang et al. revealed that both cysteine and methionine residues of the BKCa channel were subject to redox modulation [180]. Interestingly, oxidation of cysteine and methionine produced opposite regulations of BKCa channel activity.
Whereas cysteine oxidation decreased BK\textsubscript{Ca} channel currents, methionine oxidation increased channel activity. Moreover, oxidation of a cysteine residue near the Ca\textsuperscript{2+} bowl of the BK\textsubscript{Ca} channel \(\alpha\) subunit by H\textsubscript{2}O\textsubscript{2} almost abolished physiological activation of the channel [181]. It is likely that distinct actions of H\textsubscript{2}O\textsubscript{2} on the BK\textsubscript{Ca} channel resulted from selectively targeting cysteine and methionine residues. Whereas O\textsubscript{2}•− did not alter currents mediated by the BK\textsubscript{Ca} channel, ONOO− exhibited an inhibitory effect on BK\textsubscript{Ca} channel activity [182, 183]. It appears that the BK\textsubscript{Ca} channel in uterine artery VSMCs of high-altitude pregnant sheep is under tonic inhibition by ROS. An acute application of antioxidants such as N-acetylcysteine (NAC), the NOX inhibitor apocynin, and the synthetic SOD/catalase mimic EUK-134 partially reversed gestational hypoxia-induced suppression of BK\textsubscript{Ca} channel-mediated currents and vasorelaxation [104, 184]. As NOX2 was upregulated in gestational hypoxia, the superoxide generated by this enzyme and its dismutation product H\textsubscript{2}O\textsubscript{2} probably contributed to the gestational hypoxia-induced suppression of BK\textsubscript{Ca} channel activity/function in uterine arteries [157]. IK channel-mediated currents in HUVECs were also inhibited by the superoxide donors, xanthine/xanthine oxidase (X/XO) mixture [185].

In addition to direct modulation of K\textsubscript{Ca} channel activity, ROS also exert a significant impact on the expression of K\textsubscript{Ca} channels. High-altitude pregnancy increased uterine vascular tone owing to NOX2 overexpression and KCNMB1 down-regulation as well as decreased BK\textsubscript{Ca} channel activity [103, 157]. These detrimental effects could be simulated by ex vivo hypoxic treatment of uterine arteries of low-altitude pregnancy [104]. A cause-and-effect relationship was established by the observation that antioxidants apocynin and NAC largely eliminated gestational hypoxia-induced reduction of KCNMB1 expression and channel activity [104, 157]. In addition, estrogen–induced upregulation of the BK\textsubscript{Ca} channel \(\beta\) subunit and channel activity in uterine arteries was eradicated by gestational hypoxia, which was restored by NAC in ex vivo experiments [104, 184]. Similarly, preeclampsia reduced the expression of KCNMB1 along with upregulation of NOX2 and superoxide in HUVECs [113]. Importantly, the KCNMB1 downregulation was partially rescued by treating cultured HUVECs with apocynin [113]. The KCNMB1 downregulation appeared to be directly induced by ROS. Exposure of the cultured human coronary artery VSMCs to H\textsubscript{2}O\textsubscript{2} for 12 hours led to reduced KCNMB1 expression [186]. These observations signal a contributing role of ROS in the dysfunction of the BK\textsubscript{Ca} channel in utero-placental circulation. Targeting KCNMB1 expression by ROS is also observed in diabetes. The BK\textsubscript{Ca} channel \(\beta\) subunit protein level was downregulated in diabetic mouse aorta, which was accompanied by increased expression of NOX1 and NOX4, decreased expression of SOD and catalase, and elevated O\textsubscript{2}•− generation [186].

The expression of SK and IK channels is also regulated by ROS in pregnancy complications. Pregnancy/estrogen–induced upregulation of SK2 (K\textsubscript{Ca}2.2) and SK3 (K\textsubscript{Ca}2.3) channel expression/activity in ovine uterine arteries was diminished at high altitude [107], and a causative role of ROS was evidenced by the reversal of gestational hypoxia-induced detrimental effects with NAC [184]. Treatment of human uterine microvascular ECs with serum from preeclamptic women also reduced SK3 and IK1 expression, which was reversed by silencing NOX4 with siRNA or treatment with a membrane-permeable SOD [187]. The reduced expression of SK3 and IK1 (K\textsubscript{Ca}3.1) in the placenta, umbilical vessels, and HUVECs was also associated with the upregulation of NOX2 or NOX4 and heightened oxidative stress in preeclamptic pregnancy [113, 187, 188]. The contributing role of ROS to the downregulation of SK\textsubscript{Ca} and IK\textsubscript{Ca} channels was substantiated based on the following findings: (1) restoration of channel expression by antioxidants such as apocynin, tempol, and tiron and (2) simulation of the downregulation by oxidants such as superoxide generated by exogenous X/XO mixture and H\textsubscript{2}O\textsubscript{2} [113, 188, 189].

Overwhelming evidence suggests that the E\textsubscript{2}β-NOS--NO-K\textsubscript{Ca} channel pathway in utero-placental tissue is a target of oxidative stress in pregnancy complications. Overall, excessive ROS inhibited E\textsubscript{2}β synthesis and estrogen receptor expression. In addition, NOS and K\textsubscript{Ca} channel expression/activity could also be suppressed by oxidative stress, leading to reduced NO bioavailability and impaired K\textsubscript{Ca} functions.

5. The Interplay among Hypoxia, ROS, and Epigenetic Modifications in Pregnancy Complications

Although it is now well-recognized that placental insufficiency and oxidative stress are important contributors to the pathogenesis of preeclampsia and IUGR, the mechanisms underlying their actions in these complications are not fully resolved. Recent studies have identified epigenetic modifications as important mechanisms underlying various human diseases [190]. In this section, we will try to establish a link among hypoxia, ROS, and epigenome in preeclampsia and IUGR.

5.1. ROS in O\textsubscript{2} Sensing. HIFs are transcription factors and function as master regulators of cellular responses to hypoxia. HIFs are heterodimers composed of a HIF-\(\alpha\) subunit (HIF-1\(\alpha\) and HIF-2\(\alpha\)) and a constitutively expressed HIF-1\(\beta\) subunit. Under normoxia, HIF-\(\alpha\) subunits are hydroxylated on proline residues by the O\textsubscript{2}–-dependent prolyl hydroxylases (PHDs), resulting in ubiquitination and successive proteasomal degradation by the von Hippel–Lindau protein (pVHL) E3-ubiquitin ligase. In hypoxia, PHD activity is suppressed. Subsequently, HIF-\(\alpha\) is accumulated, translocated into the nucleus, and dimerized with HIF-1\(\beta\), leading to gene expression by binding to hypoxia-responsive element (HRE) in the promoter of the target gene. Interestingly, ROS appear to participate in cellular oxygen sensing and hypoxic activation of HIFs. ROS generated by mitochondrial complex III in response to hypoxia were found to stabilize HIF-1\(\alpha\) [132, 191]. The stabilization of HIF-1\(\alpha\) was mimicked by exogenous H\textsubscript{2}O\textsubscript{2} and by genetic suppression of SOD2 under normoxia [191, 192]. However, HIF-1\(\alpha\) stabilization was attenuated by silencing Rieske iron-sulfur protein of complex III and by enzymatic and nonenzymatic antioxidants

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ROS produced by NOXs could also lead to accumulation of HIF-1α [196, 197] and HIF-2α [198, 199]. ROS stabilized HIF-α apparently through suppressing the ability of the PHDs to hydroxylate HIF-α protein [200]. ROS-mediated stabilization of HIFs thus constitutes an important mechanism for hypoxia to stimulate gene expression.

5.2. Crosstalk between ROS and Epigenome. Whereas genome confers genetic information for making and maintaining an organism, the epigenome describes all the chemical modifications to DNA and histone proteins. Epigenetic modifications of the genome determine how the information in genes is expressed by switching genes on and off without altering the DNA sequence. The major mechanisms of the epigenetic modification include DNA methylation, histone modifications, and noncoding-RNA-based silencing [201]. Several lines of evidence suggest existence of a crosstalk between ROS and epigenetic modifications. ROS are found to promote DNA hypermethylation by altering DNA methylation/demethylation machineries and enzyme recruitment. In vitro studies demonstrated that H2O2 treatment increased expression/activity of DNA methyltransferases (DNMTs) [202–204], although many of these studies were conducted in cancer cell lines. In addition, H2O2 could facilitate DNA methylation by recruiting DNMT1 to the CpG sites in gene promoters [203, 205]. The linking of ROS induced by hypoxia and other stimuli to DNA hypermethylation was further confirmed by findings that antioxidants such as NAC and apocynin were able to prevent both ROS-induced global methylation or specific gene methylation [202, 206, 207] and upregulation of DNMTs [202]. ROS could also impair DNA demethylation. In a cell-free system, H2O2 suppressed enzymatic activity of ten-eleven translocation (TET) dioxygenase [208]. The catalytic activity of TETs requires vitamin C and Fe2+ as cofactors [209, 210]. To maintain an active dioxygenase enzyme, vitamin C is required to reduce Fe3+ to Fe2+. Thus, vitamin C depletion in pregnancy complications [144, 211] would reduce TET activity. Histone modifications are also subject to ROS regulation. It is found that increasing oxidative stress by H2O2 upregulated histone deacetylase 1 (HDAC1) in cancer cell lines [204]. Prolonged treatment with H2O2 or increased global histone methylation marks H3K4me3 and H3K27me3 in human bronchial epithelial cells [208]. It appears that ROS produced from both mitochondria and NOX promotes microRNA-210 (miR-210) generation. Whereas Nox4 siRNA partially decreased hypoxia-induced miR-210 expression, mitochondrial complexes I and III inhibitors rotenone and antimycin increased miR-210 biogenesis in adipose-derived stem cells [212].

Conversely, ROS production could be altered by epigenetic modifications of genes for enzymatic oxidants and antioxidants. It appears that hypermethylation promotes oxidative stress, whereas demethylation boosts antioxidation. In human pulmonary arterial hypertension, a CpG island in an enhancer region of intron 2 and another in the promoter of SOD2 were hypermethylated in pulmonary arterial smooth muscle cells (PASMCs) owing to upregulation of DNMT1 and DNMT3b, leading to downregulation of the antioxidant enzyme [213]. Similarly, hypoxia also reduced SOD2 expression in the rat carotid body via hypermethylation of a single CpG dinucleotide close to the transcription start site [214]. H2O2 promoted methylation of a CpG island in the catalase promoter and downregulated catalase [215]. TET1 deficiency produced by TET1 siRNA enhanced H2O2-induced increase apoptosis of cerebellar granule cells [216], suggesting that TET1-mediated demethylation may upregulate antioxidant mechanisms to counter oxidative stress. Histone modification also contributes to the hemostasis of the oxidant-antioxidant system. The expression/activity of SOD3 in the lung from human idiopathic pulmonary arterial hypertension was reduced, and this downregulation could be reversed by the treatment of PASMCs with class I HDAC inhibitors or HDAC3 siRNA [217], suggesting that histone deacetylation negatively regulates SOD3 expression. In contrast, histone deacetylation mediated by HDAC3 upregulated NOX4 in HUVECs as HDAC3 siRNA and pan-HDAC inhibitor scriptaid reduced NOX4 expression [218]. Furthermore, miRs also participate in the regulation of mitochondrial metabolism and function. The downregulation of iron-sulfur cluster assembly enzyme (ISCU) in mitochondria by miR-210 in hypoxia would block electron exit from complex I, promoting its leakage to generation of ROS [219]. Overall, it appears that there exists a positive feed-forward loop between ROS generation and epigenetic modifications.

5.3. Epigenetic Mechanisms in Regulating Uteroplacental Circulation during Normal Pregnancy. In sheep, the upregulation of ERα in uterine arteries was conferred by epigenetic mechanism [220]. The specificity protein 1- (Sp1-) binding site (Sp1-520) at the promoter of the ERα encoding gene ESR1, to which Sp1 or Sp1-ERα binds, was essential for E2β-stimulated promoter activity. The CpG dinucleotide of this site was hypermethylated in nonpregnant animals, and the gene is thus kept quiescent. However, the Sp1 site became less methylated in pregnant animals and enabled the expression of the gene, leading to increased ERα mRNA and protein abundance in uterine arteries and subsequent attenuation of uterine vascular tone.

E2β also epigenetically upregulates KCNMB1 expression in uterine arteries [221, 222]. Similar to ERα, the CpG dinucleotide in the Sp1-binding site (-380) at the promoter of KCNMB1 was highly methylated in uterine arteries of nonpregnant sheep, resulting in gene silence. During pregnancy, E2β through ERα stimulated TET1 (TET1 encoding gene) promoter activity and gene expression. The upregulation of TET1 in turn promoted Sp1-380 demethylation of the KCNMB1 promoter. Consequently, the expression of KCNMB1 and the activity of the BKCa channel increased in uterine arteries, leading to reduced myogenic tone.

5.4. Aberrant Epigenetic Modifications in Pregnancy Complications. Epigenetic mechanisms play an important role in the pathophysiological processes of pregnancy complications. Global hypermethylation was observed in pre-eclamptic placenta [223, 224]. In addition, various genes including ESR1 and KCNMB1 in the uterine arteries of
high-altitude pregnant sheep [52, 220, 221, 225] and IGF1, HSD11B2, H19, and HLA-G in the placenta from preeclamptic and IUGR pregnancies [224, 226, 227] were hypermethylated. The increased methylation in the uteroplacental tissues was accompanied by upregulation of DNMT1 and DNMT3b expression/activity [224, 225, 227, 228] and downregulation of TET1, TET2, and TET3 expression [52, 227, 229, 230]. Pregnancy complications also alter histone modification in the placenta. JMJD6 histone demethylase activity was suppressed in preeclamptic placenta [231]. Moreover, miR-210 was also upregulated in both uterine arteries and placenta of high-altitude pregnancy [52, 142]. Increased miR-210 level was also observed in preeclamptic and IUGR placenta [140, 230, 232]. These changes undoubtedly would contribute to the aberrant expression of key elements in the E2β-NOS-NO-KCa pathway in uteroplacental circulation.

The aforementioned changes in epigenetic modifications of the uteroplacental system in pregnancy complications are apparently caused by hypoxia/ischemia. HIF-1α overexpression in uteroplacental tissues is a characterized feature in pregnancy complications and high-altitude pregnancy [157, 233, 234]. Both ex vivo hypoxia treatment of tissues or pharmacologically induced hypoxia in intact animal models induced the expression of DNMTs and miR-210 [142, 225, 235] and repressed both histone demethylase activity [231] and TETs expression/activity [235, 236]. Although not investigated in the uteroplacental tissues, studies conducted in other tissues/cells suggest that hypoxia-induced alterations in epigenetic machineries is HIF-1α-dependent. DNMT1, DNMT3b, and miR210 all contain hypoxia-responsive element (HRE) in their promoters, and the binding of HIF-1α to HRE stimulates the expression of these genes [237]. Hypoxia via HIF-1α also induced the expression of histone demethylases JHDM1B/KDM2B and JARID1B/KDM5B, which demethylate the activating mark H3K4me2/3, leading to gene repression [238]. The E2β metabolite 2-methoxyestradiol is an endogenous HIF inhibitor [239]. The reduced 2-methoxyestradiol level in preeclampsia probably contributes to aberrant epigenetic modifications in uteroplacental tissues due to the relief of HIF inhibition.

Intriguingly, hypoxia-induced TET1 repression in uterine arteries was mediated by miR-210 and the binding of miR-210 to the 3′-untranslated region (3′UTR) of TET1 mRNA resulted in degradation of the transcript [52]. The overall effects of upregulation of DNMT3b and downregulation of TET1 in uterine arteries promoted ESR1 and KCNMB1 hypermethylation and gene repression [52, 220, 221, 225, 235]. ERα and the BKCa channel are two key elements contributing to reduced uterine vascular tone in pregnancy [59, 73]. Consequently, the downregulation of both ERα and the BKCa channel impaired pregnancy-induced attenuation of uterine vascular tone, leading to maladaptation of uteroplacental circulation [40, 47, 225] (Figure 2). Increased DNA methylation may also contribute to impaired spiral artery remodeling. The downregulation of TET2 reduced in vitro trophoblast migration and invasion [230]. The overexpression of miR-210 in the preeclamptic placenta suppressed ISCU and impaired mitochondrial respiration [140, 142, 232]. It is probably that both the miR-210-mediated mitochondrial dysfunction and DNA hypermethylation (indirectly via downregulating TETs) disrupt trophoblast invasion and impair spiral artery remodeling in high-altitude pregnancy and pregnancy complications. In addition, miR-210 also targeted potassium channel modulatory factor 1 (KCMF1) and thrombospondin type I domain-containing 7A (THSD7A), which could also contribute to the impaired trophoblast invasion [240, 241]. The expression of CYP19A1 and HSD17B1 is also regulated by DNA methylation. Methylation of CpG islands in the promoters of both genes suppressed their expression [242, 243]. Although not examined in the placenta, it is probably DNA methylation-mediated downregulation of aromatase and HSD17B1 also occurs in preeclampsia, IUGR, and high-altitude pregnancy. Furthermore, the expression of HSD17B1 was downregulated by miR-210 in preeclamptic placenta [33]. The epigenetic modifications of key enzymes in estrogen biosynthesis could then reduce circulating E2β level in pregnancy complications.

6. Concluding Remarks

Preeclampsia and IUGR are leading causes of maternal and perinatal mortality and morbidity and have great impacts
on maternal and offspring health. Unfortunately, there is currently no cure for them. Preeclampsia, IUGR, and high-altitude pregnancy all exhibit uteroplacental hypoxia/ischemia and oxidative stress concurrently. Moreover, these pregnancy complications are associated with altered epigenome. There exist interplays among ROS, HIFs, and epigenome. The ROS-HIF pathway appears to be a potential cause in the changes of epigenetic modifications in these complications. In uterine arteries, HIF-1α apparently functions as an important link between ROS and aberrant epigenetic modifications, leading to disrupted E2β-BKCa axis and increased uterine vascular tone. In the placenta, the ROS-HIF-epigenome interplay impairs estrogen synthesis, trophoblast invasion, and spiral artery transformation. Both preeclampsia and IUGR are multifactorial disorders. What we know about these complications is only the tip of the iceberg. Further studies are needed to advance our understanding on the pathogenesis of them in order to develop effective therapeutics.

**Abbreviations**

3’UTR: 3’-Untranslated region  
4-HNE: 4-Hydroxynonenal  
8-iso-PGF2α: 8-Isoprostaglandin F2α  
ADMA: Asymmetric dimethylarginine  
AP-1: Activator protein-1  
AT1-AA: Autoantibodies against angiotensin II type 1 receptor  
BKCa: Large-conductance Ca2+-activated K+ channel  
cAMP: Cyclic adenosine monophosphate  
cGMP: Cyclic guanosine monophosphate  
COMT: Catechol-O-methyltransferase  
CpG: Cytosine-guanine dinucleotide  
CYP19: Aromatase  
CYP19A1: The gene encoding aromatase  
DNMT: DNA methyltransferase  
E2β: 17β-Estradiol  
ECs: Endothelial cells  
EDHF: Endothelium-derived hyperpolarizing factor  
eNOS: Endothelial nitric oxide synthase  
ERα: Estrogen receptor α  
ERβ: Estrogen receptor β  
ERK: Extracellular signal-regulated kinase  
ERE: Estrogen response element  
ESR1: The gene encoding ERα  
GPER (GPR30): G-protein-coupled estrogen receptor  
GPx: Glutathione peroxidase  
GSH: Glutathione  
H19: The gene encoding imprinted maternally expressed transcript  
HRE: Hypoxia-responsive element  
HDAC: Histone deacetylase  
HIF: Hypoxia-inducible factor  
HLA-G: The gene encoding major histocompatibility complex, class I, G  
H2O2: Hydrogen peroxide  
HRE: Hypoxia-responsive element  
HSD11B2: The gene encoding hydroxysteroid 11β-dehydrogenase 2  
HSD17B1 (17β-HSD1): Hydroxysteroid 17β-dehydrogenases 1  
HUVEC: Human umbilical vein endothelial cell  
IGF1: The gene encoding insulin-like growth factor 1  
IK: Intermediate-conductance Ca2+-activated K+ channel  
IUGR: Intrauterine growth restriction  
ISCU: Iron-sulfur cluster scaffold  
KCa: Ca2+-activated K+ channel  
KCMF1: Potassium channel modulatory factor 1  
KCNB1: The gene encoding BKCa channel β subunit 1  
L-NAME: L-Nitro-arginine methyl ester or Nω-nitro-L-arginine methyl ester  
MDA: Malondialdehyde  
MAPKs: Mitogen-activated protein kinases  
miR: MicroRNA  
NAC: N-Acetylcysteine  
NADPH: Reduced form of NADP+  
NF-κB: Nuclear factor kappa-light-chain-enhancer of activated B cells  
NO: Nitric oxide  
NOS: Nitric oxide synthases  
NOX: NADPH oxidase  
O2·−: Superoxide  
·OH: Hydroxyl radical  
ONOO−: Peroxynitrite  
PASMC: Pulmonary arterial smooth muscle cell  
PDH: Prolyl hydroxylases  
PCR: Polymerase chain reaction  
PI3K: Phosphoinositide 3-kinase  
PKB (AKT): Protein kinase B  
PKG: Protein kinase G  
Prx: Peroxiredoxin  
PVHL: von Hippel–Lindau protein  
ROS: Reactive oxygen species  
siRNA: Small interfering RNA  
SK: Small-conductance Ca2+-activated K+ channel  
SOD: Superoxide dismutase  
Sp1: Specificity protein 1  
TET: Ten-eleven translocation dioxigenase  
THSD7A: Thrombospondin type I domain containing 7A  
Trx: Thioredoxin  
TrxR: Thioredoxin reductase  
VSMCs: Vascular smooth muscle cells
Conflicts of Interest

None of the authors has any conflict of interests to disclose.

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References


R. R. Magness, T. M. Phernetton, T. C. Gibson, and D. B. Chen, “Uterine blood flow responses to ICI 182 780 in ovariectomized oestradiol-17β-treated, intact follicular and


[138] Y. Wang and S. W. Walsh, "Antioxidant activities and mRNA expression of superoxide dismutase, catalase, and glutathione


