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Steroid Hormones and Uterine Vascular Adaptation to Pregnancy

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Abstract

Pregnancy is a physiological state that involves a significant decrease in uterine vascular tone and an increase in uterine blood flow, which is mediated in part by steroid hormones, including estrogen, progesterone, and cortisol. Previous studies have demonstrated the involvement of these hormones in the regulation of uterine artery contractility through signaling pathways specific to the endothelium and the vascular smooth muscle. Alterations in endothelial nitric oxide synthase expression and activity, nitric oxide production, and expression of enzymes involved in PGI₂ production contribute to the uterine artery endothelium-specific responses. Steroid hormones also have an effect on calcium-activated potassium channel activity, PKC signaling pathway and myogenic tone, and alterations in pharmacomechanical coupling in the uterine artery smooth muscle. This review addresses current understanding of the molecular mechanisms by which steroid hormones including estrogen, progesterone, and cortisol modulate uterine artery contractility to alter uterine blood flow during pregnancy with an emphasis on the pregnant ewe model.

Keywords

Uterine artery; pregnancy; steroid hormone

Pregnancy is a physiological state encompassing a vast array of mechanisms to promote nutritional sufficiency for the developing fetus. It is also a state that involves significant hormone-mediated adjustments, mainly mediated by those of estrogen and progesterone, which are elevated during this period. These sex steroids play a significant role in causing molecular, physiological, and anatomical adjustments within the uterus to create an environment most advantageous for fetal growth. During the process of angiogenesis, structural changes encompass expansion of volume and the outgrowth of newly formed arterioles, which function to increase vascular surface area. Physiological changes may involve adjustments in vascular reactivity, which, in turn, affects the susceptibility of the artery to the vasoconstrictive or vasodilatory effects to various agonists circulating within the uterine artery. A discussion of such changes typical of normal pregnancy cannot be made without an in-depth analysis of molecular events, including alterations in gene and protein expression of key enzymes and mediators. In essence, pregnancy is a collective event of molecular, physiological, and anatomical adaptations that contribute to the final functional response of elevated uterine blood flow. The process of vasodilation, which is the focus of this review, is a critical event that involves signaling pathways at the level of both the endothelium and the vascular smooth muscle (VSM) within the uterine artery. This review will focus on the literature related to steroid hormone-mediated adaptations at the level of the endothelium and the VSM within the uterine artery of pregnant ewes.

STEROID HORMONES AND UTERINE BLOOD FLOW IN PREGNANCY

During the course of gestation, uterine blood flow (UBF) increases more than 40-fold and occurs in 3 distinct stages.¹ In sheep, the first trimester involves an increase in the microvascular volume as well as vasodilation of the uterine artery to create an environment most suitable for embryo implantation. Once implantation is complete, angiogenesis and further remodeling of the uterine vascular bed ensues. During the third trimester, there is an observable exponential rise in UBF, which is established by the increase in maternal uterine artery vasodilation. The significance of this elevation in UBF is the increased nutrient and substrate delivery through the uterine artery that acts as a direct conduit from the mother to the placenta. Numerous studies have focused on the sex steroid-related mechanisms by which the uterine artery vasodilation occurs. Estrogen and progesterone are significant in facilitating this adaptation, and although not as widely studied, there is also increasing evidence supporting the view that cortisol may play a role in association with the ovarian sex steroids to regulate UBF.

ESTROGEN

Estrogen-Mediated UBF

Effect of estrogen on systemic and uterine blood flow—UBF fluctuates along with changes in the steroid profile over the course of the estrous cycle and pregnancy. During the follicular phase of the estrous cycle, which is characterized by an increased estrogen-to-progesterone ratio, UBF is increased. During the subsequent luteal phase, which is characterized by high progesterone and low estrogen, UBF is decreased.^{2,3} Alternatively, during pregnancy, which is characterized by high progesterone and estrogen, UBF is consistently shown to rise.⁴ Because UBF patterns change along with the steroid hormone profile, various studies are focused on the pathways by which these vascular responses are induced. Estradiol-17 β (E2 β) is a potent vasodilator that has been used to assess ovarian steroid hormone effects on hemodynamic parameters. Previous studies in ovariectomized nonpregnant ewes have shown that systemic E2 β infusion alters cardiovascular parameters by increasing heart rate and cardiac output and decreasing systemic vascular resistance.⁵ Systemic infusion of E2 β also increases UBF; however, local infusion increases UBF without altering systemic parameters, which suggests that uterine response to estrogen is mediated locally and is independent of systemic effects.⁵ Most in vivo studies to date focused primarily on acute and nongenomic effects of estrogen on relaxation of the uterine artery, observed at the concentrations substantially higher than physiological concentrations.⁵⁻¹⁰ The genomic action of physiologically relevant concentrations of the steroid hormone on uterine artery contractility and myogenic tone and their adaptation to pregnancy remain poorly understood. Nevertheless, the differences between pharmacological and physiological responses of UBF to estrogen have been recognized.¹¹

Role of estrogen receptor (ER) signaling in UBF—Much of the current literature is supporting the involvement of steroid receptors in modulating vasculature to control blood flow. First, it has been established that ERs are present in both the endothelial and the VSM of the uterine artery.^{12,13} At the molecular level, the predominant biological effects of estrogen are exerted by ERs (ER α and ER β) that belong to the steroid hormone superfamily of nuclear receptors.¹ To date, ER subtypes described include ER α and ER β , which mediate estrogenic effects in target tissues.¹ Once bound to the hormone, these receptors act as transcription factors that bind to specific sites on DNA to regulate gene expression.¹ This results in an increase or decrease in mRNA transcripts depending on the tissue being examined. This phenomenon is described as the genomic response and may take hours to days for the initial effect to be seen, which is in contrast to the nongenomic response wherein the effect is immediate but often shorter in duration. It is known that these receptors are present in multiple tissues and species,

including human and sheep uterine arteries, but the functional response triggered by these receptors is more difficult to establish.¹³⁻¹⁵ There is a growing body of evidence to suggest that increased UBF is an ER-mediated process.^{16,17} First, different estrogens including E2 β , estrone, and estriol have been shown to elicit the same pattern and efficacy of estrogen-mediated elevation in UBF.^{6,7,16,18} Second, Lineweaver-Burk plots illustrated that catechol estrogens and estradiol had common y intercepts, which suggests that they bind to the same receptors.^{16,19} Third, sheep treated with the pure ER antagonist ICI 182780 demonstrated a decrease in the E2 β -induced elevation of UBF as a result of the induced blockade of ERs.¹⁶ These observations demonstrate that ERs are, at minimum, partially responsible for estrogen-mediated increase in UBF. The presence of ERs in both endothelial and VSM cells of the uterine artery suggests that both cell types are potential targets for elevated estrogen during pregnancy.¹³

Estrogen-Mediated Uterine Artery Endothelial Adaptations to Pregnancy

Trends in eNOS expression during estrogen exposure—Numerous studies in the past have implicated nitric oxide (NO) as one of the main mediators in the endothelium-dependent pathway for estrogen-induced uterine vasodilation.^{9,10,20} Observations showed that inhibition of NO synthase (NOS) attenuated the estrogen-induced increase in UBF by 60% to 70%.^{9,10} The endothelial nitric oxide synthase (eNOS) that is located in the uterine artery endothelium is partially responsible for the vasodilatory effects during late gestation.^{3,21} The trend in eNOS expression appears to be associated with the trend in estrogen levels. For instance, eNOS protein expression in the uterine artery endothelium is increased during periods of estrogen-dominated states, including normal pregnancy and the follicular phase of the ovarian cycle.²¹ Alternatively, eNOS protein and mRNA levels are lowest in ovariectomized animals and during the luteal phase, which is characterized by low estrogen levels.²¹ These findings do not prove that estrogen alone is directly responsible for eNOS levels and activity but is suggestive of the association between increased estrogen and eNOS expression.

Mechanisms of eNOS activation—Mechanisms accounting for the elevation in eNOS activity during pregnancy have been proposed.⁴ Initially, it was thought that the regulation of eNOS involves changes in protein expression or increased cytosolic calcium concentrations ([Ca²⁺]_i), which, in turn, promotes calcium-activated calmodulin binding.²² However, eNOS can also form NO through [Ca²⁺]_i-insensitive pathways as presented by previous studies that demonstrated the activation eNOS in the presence of basal [Ca²⁺]_i but absence of elevated [Ca²⁺]_i.^{4,23-27} It has also been shown that there are kinases that function independently of Ca²⁺ to target several phosphorylation sites on eNOS.^{4,28-30} This, in turn, either mediates the activation of eNOS or causes an increase in the eNOS sensitivity to fluctuations in cytosolic [Ca²⁺]_i.^{4,28-30} Although investigators have managed to identify the phosphorylation sites of several key eNOS amino acid residues that are potential sites of regulation, it has been shown that changes in phosphorylation of these residues is not a sufficient predictor of activity in pregnant uterine artery endothelial cells.²² It is suggested that the reasoning behind this is that the activity of eNOS involves a multiplicity of effects that interact with one another including phosphorylation, influence of Ca²⁺, cofactors, and subcellular location.²²

Role of ER-dependent signaling in NO production—There is evidence to show that ER stimulation is an event that is associated with eNOS expression during pregnancy in the uterine artery.^{31,32} Previous studies with E2 β and the membrane-impermeable E2 β -bovine serum albumin conjugate showed that the activation of either the plasma membrane or cytosolic ER was sufficient to induce eNOS expression.³² This suggests that the immediate, acute vasodilatory effects of estrogen mediated by plasma membrane ER stimulation and the long-term, genomic-mediated mechanisms mediated by cytosolic ER, which has transcriptional factor activity, are both involved. Additional mechanistic studies have also shown that eNOS

activation is blocked by treatment with specific ER antagonists, blocked by inhibition of tyrosine kinases or mitogen-activated protein kinase, and unaffected by actinomycin D.³¹ It is evident that the estrogen-induced rise in NO production in the uterine artery endothelium is at least, in part, mediated by mitogen-activated protein signaling and ER-dependent mechanisms.³² It is established that eNOS possesses multiple phosphorylation sites for kinases including ERK2/1, which can either stimulate or inhibit eNOS activity depending on the endothelial cell type.³¹⁻³⁵ In the case of cultured uterine artery endothelial cells, the effect of ERK2/1 has been shown to be stimulatory.^{28,32} E2 β caused ERK2/1 activation in a time-dependent and dose-dependent manner, which, in turn, increased NO production in cultured uterine artery endothelial cells.³² On the inhibition of the ERK pathway by PD98059, however, the E2 β -mediated eNOS activation and NO production were prevented.³² Although eNOS is also shown to possess phosphorylation sites for the Akt kinase, the inhibition of Akt activation by pretreatment with LY294004 does not inhibit estrogen-mediated NO production.^{32,36,37} It appears that ERK2/1 signaling, but not Akt, is involved in estrogen-induced activation of eNOS and NO production in uterine artery endothelial cells.³²

Role of tissue-specific factors in the responsiveness to estrogen—eNOS is not exclusive to the uterine artery but can also be found in nonreproductive tissues. However, not all of these tissues have eNOS whose expression is altered by estrogen and progesterone. The eNOS abundance changes during sex steroid treatment in the uterine artery; however, no alterations are reported for the omental, renal, or mammary artery eNOS.³ Consistent with these findings, estrogen is shown to increase blood flow in the uterine artery but has no effect on blood flow of the omental artery.¹² This suggests that the uterine artery possesses unique characteristics that set it apart from other arteries. One possible explanation may be related to the differences in receptor density and distribution in the target tissues. The levels of ER α and ER β are documented to vary among different vascular beds and the relative ratio of these is critical for the overall biological response to estrogen.¹³ Although ERs are present in the omental artery endothelium, their levels are not altered by the ovarian cycle, ovariectomy, or hormone replacement therapy.¹² The uterine artery, however, possesses ER α and ER β , which are differentially regulated in response to sex steroids.¹² For instance, investigations in sheep studies have shown that exogenous estrogen, progesterone, or combination of these 2 steroids increases endothelial ER β levels; however, only estrogen treatment, but not progesterone or their combination, increases ER α expression.¹² An in vitro study with cultured uterine artery endothelial cells has shown that estrogen treatment has no effect on ER α or ER β mRNA but caused the downregulation of their associated proteins.³⁸ The suggested hypothesis is that estrogen activates the proteasomal pathway to mediate the degradation of ER.³⁸ This is evidenced by studies showing that the use of the irreversible proteasome inhibitor, lactacystin, is sufficient to block ER α and ER β degradation.³⁸ Insulin, which is a known reversible inhibitor of protein degradation, inhibits degradation for the ER α subtype but not for the ER β subtype.³⁹ The selective action of insulin may be attributed to a pathway that blocks ubiquitin ligase activity, which is specific for ER α but not for ER β .³⁸ Further studies delineating the differences in regulation of the ER isoforms is required.

Role of caveolin-1 in the regulation of ER during estrogen exposure—A mechanism of interest that accounts for the estrogen-mediated regulation of ER involves the sequestering effect of caveolin-1 at the plasma membrane. Caveolin-1 functions as a plasma membrane protein that sequesters eNOS to render it less active or inactive.^{10,40} It acts as an endogenous inhibitor of eNOS by binding to it, which results in the inactivation of this enzyme. Once caveolin-1 is dissociated from the complex, eNOS activity is restored and thereby NO production ensues.⁴¹ Caveolin-1 has been shown to colocalize with ER α and eNOS to form a functional regulatory complex at the plasma membrane of uterine artery endothelial cells.^{13,42} Although ER β is also present on the plasma membrane, its association with caveolin-1

remains unknown because previous attempts of detection by immunocytochemical analysis have been unsuccessful.^{13,42} An understanding of the significance of the individual roles of the ER isoforms is of interest for future studies.

Effect of pregnancy or steroid treatment in the regulation of ER isoforms—In cultured uterine artery endothelial cells isolated from pregnant ewes, ER α mRNA levels are shown to be significantly greater than that of ER β mRNA levels, which strengthens the argument that the α variant plays a more important role in carrying out the effects of estrogen, at least for this particular type of cell.¹³ In another study, however, Western analysis has shown that in intact sheep pregnancy is associated with the increase of only the β variant when compared with the luteal phase.¹² In ovariectomized vehicle sheep, only the ER β variant, but not the ER α variant, is significantly reduced. Exogenous estrogen, progesterone, or combination of the 2 was sufficient to increase endothelial ER β levels; however, only estrogen treatment, but not progesterone or their combination, increased ER α expression.¹² It is unclear which of the 2 isoforms is more significant with regard to estrogen-mediated vasodilation of the uterine artery; however, these findings support the notion that ER α and ER β are differentially regulated in response to ovarian sex steroids.

Estrogen-Mediated Uterine Artery Smooth Muscle Adaptation to Pregnancy

Role of VSM-dependent pathways in estrogen-mediated uterine artery vasodilation—Many studies have focused on the endothelium/NO-dependent pathway for the investigation of estrogen-induced uterine vasodilation. However, this only accounts for approximately 65% to 70% of uterine vasodilation, which implicates the involvement of alternative pathways that may be endothelium independent.^{9,13} Recently, estrogen has been shown to target the uterine artery VSM through various strategies that include the cyclic guanosine monophosphate (cGMP) pathway and activation of VSM potassium channels.¹⁷ Rosenfeld et al⁹ have reported several interesting findings regarding the cGMP pathway. E2 β infusion is shown to increase cGMP secretion and UBF; however, inhibition of NOS with L-NAME decreased cGMP secretion by approximately 66%.⁹ Although L-NAME was able to lower cGMP secretion in both pregnant and nonpregnant animals, UBF fell in the nonpregnant group but was unaffected in the pregnant group.⁹ It is evident that the cGMP mechanism, although interesting and particularly applicable to nonpregnant UBF, is not the predominating strategy during pregnancy. With regard to the effects of NOS, NO production was previously thought to be an endothelium-specific mechanism but there is increasing support that NOS in the VSM also may be involved. Salhab et al⁴³ presented for the first time that nNOS is present in uterine vascular myocytes and that its expression is increased by long-term but not acute estrogen exposure. However, the individual contribution of this particular isoenzyme during pregnancy is still not known. It may be interesting to address the association between VSM ER-mediated signaling to the previously mentioned endothelium-independent pathways. The smooth muscle is a potential target for estrogen because both ER subtypes, α and β , have been found in uterine artery smooth muscle cells.¹³ Treatment with the ER antagonist ICI 182780 has been shown to affect UBF but these studies are not informative of the individual contribution of VSM as opposed to the entire uterine artery as a whole.¹⁶ It may be worthwhile to address ex vivo studies on the uterine artery to determine the functional effect of receptor antagonism with regard to the smooth muscle layer.

Calcium-activated K⁺ channels modulate uterine artery VSM activity during estrogen exposure—In addition to the cGMP pathway, K⁺ channels are also significant because of their ability to regulate basal arterial tone.⁴⁴ This is mediated by hyperpolarization of the smooth muscle membrane, which blocks Ca²⁺ entry through voltage-gated channels to promote vasorelaxation of the artery.⁴⁴ In the VSM, there are several types of potassium channels expressed, which include voltage-dependent K⁺ (K_V) channels, calcium-activated

K⁺ channels (K_{Ca}), ATP-sensitive K⁺ channels (K_{ATP}), and inward rectifier K⁺ (K_{ir}) channels.⁴⁵ Of these, the calcium-activated potassium channel, also known as the BK_{Ca} channel, is most commonly studied in pregnancy.⁴⁴ In nonpregnant and pregnant ewes, selective blockade of BK_{Ca} in the uterine artery is shown to attenuate E2 β -induced rise in UBF.^{44,46} Infusion of tetraethylammonium (TEA) to induce a blockade of BK_{Ca} channels caused a dose-dependent attenuation of E2 β -mediated vasodilation, which is similar to the effect of L-NAME infusion alone.^{9,46} When TEA and L-NAME were infused together, there was complete inhibition of E2 β -induced rise in UBF although the basal UBF is left intact.⁴⁶ This suggests that the 2 pathways may interact, but there still may be another mechanism involved.¹⁷ The mechanism of action of E2 β may involve direct binding to the β -subunit of the BK_{Ca} channel or by an indirect pathway by which E2 β binds to a receptor to trigger an enzymatic cascade that results in the activation of the channel downstream.^{46,47} Acute, nongenomic mechanisms may be involved because UBF is elevated immediately after TEA infusion is stopped.⁴⁶ Interestingly, although acute or daily E2 β treatment has no effect on BK_{Ca} density, daily treatment increases the β_1 -subunit in the proximal and distal uterine arterial VSM, which may alter the channel's responsiveness/sensitivity to estrogen.⁴⁸ Previous studies have shown that changes in the α : β_1 stoichiometry alone without alterations in channel density affects the BK_{Ca} channel's sensitivity to E2 β .⁴⁷⁻⁴⁹ Consistent with these findings, β_1 -subunit knockout models have demonstrated greater systemic pressor responses, including elevated vascular tone and hypertension.^{43,50,51} This suggests that the elevation in β_1 -subunit mRNA and protein to increase channel responsiveness to E2 β is a potential strategy for uterine artery vasorelaxation at the level of the VSM during pregnancy.

Role of alternative K⁺ channels in the regulation of uterine artery contractility—

With regard to other types of K⁺ channels, the current literature suggests that inhibition of the low-conductance K_{ATP} channel had no effect on baseline or E2 β -mediated UBF.¹⁷ It was also shown that inhibition of the K_v channel did not affect local parameters, including the basal UBF or uterine vascular resistance at doses <1.0 mM, although the mean arterial pressure was elevated.¹⁷ Of all the mentioned K⁺ channels, BK_{Ca} is the most well studied with respect to the vasorelaxatory mechanisms at the level of the smooth muscle in sheep. In gilts, the potential-sensitive channel (PSC) is an important target for estrogen. The endometrial tissue in gilts has been shown to convert E2 β and estrone to 4-hydroxylated forms, which are then transported to the uterine arterial vasculature.⁵² Once transported to the uterine artery, these estrogens bind and block perivascular α -adrenergic receptor activity, which reduces vasoconstriction.² In another study, it was also shown that the catechol estrogen 4-hydroxylated estradiol blocked Ca²⁺ uptake through PSCs, thereby reducing uterine artery contractility.⁵² The decreased Ca²⁺ uptake through the PSCs reduces cellular PKC activity, thereby reducing uterine arterial tone.^{53,54} These studies suggest that the catechol estrogens are also important in regulating uterine artery contractility through smooth muscle-dependent mechanisms.

Role of estrogen in the regulation of uterine artery myogenic tone—Myogenic activity is an intrinsic property of the VSM, in response to pressure or stretch. It is modulated by paracrine or endocrine substances but does not require the nerves or the endothelium to occur. The physiological importance of the myogenic response in the regulation of UBF in human pregnancy has been demonstrated in myometrial arteries in term pregnant women.^{55,56} Myogenic tone has been found either increased or decreased in pregnant uterine arteries in rats, mice, and rabbits.⁵⁷⁻⁵⁹ Recent studies in sheep have demonstrated clearly that pressure-induced myogenic response is significantly decreased, and the distensibility of the uterine artery is increased in pregnant animals.⁶⁰ Similar findings have shown decreased pressure-induced myogenic tone of uterine, mesenteric, and renal arteries in pregnant mice and rats.^{59,61-63} Among other mechanisms, numerous studies have demonstrated an important role of PKC in regulating arterial myogenic response.⁶⁴⁻⁶⁸ It has been demonstrated that PKC

plays a key role in the regulation of myogenic tone of resistance-sized uterine arteries, and the reduced myogenic tone in the pregnant uterine artery is primarily mediated by a decrease in PKC signaling pathway.^{60,69,70} Consistent with these findings, it has been shown that pregnancy is associated with attenuated arterial PKC activity.^{53,54,71-73} In ovine uterine arteries, PKC-mediated contractions were significantly depressed in pregnant animals.^{70,74} Recently, studies in an ex vivo tissue culture model system showed that chronic treatment (48 hours) of nonpregnant ovine uterine arteries with physiologically relevant concentrations of E2 β (0.3-10 nM) significantly decreased the PKC-mediated contractions.⁷⁵ In contrast, acute treatment with the steroid hormone had no effect on the PKC-mediated contractions. The similar temporal response of the rise in UBF was demonstrated in ovary-intact ewes, showing the maximal increase of UBF at approximately 45 to 55 hours in animals treated physiologically with the steroid hormones.¹¹ In accordance with the genomic effects of the steroid hormone observed in the nonpregnant uterine arteries, treatment of the pregnant uterine arteries for 48 hours with the ER antagonist ICI 182780 significantly increased PKC-mediated contractions as well as pressure-induced myogenic tone and eliminated the difference of PKC-mediated myogenic contractions between nonpregnant and pregnant uterine arteries.^{75,76} Because myogenic tone plays an important role in the regulation of vascular resistance and blood flow to various organs, the decreased myogenic tone of the uterine artery is likely to contribute significantly to the adaptation of uterine vascular hemodynamics in pregnancy.

PROGESTERONE

Progesterone-Mediated UBF

Alterations in UBF during progesterone treatment—Previous studies have reported that estradiol and/or progesterone may be responsible for the regulation of UBF.⁷³ This is based on findings that an elevation in the estrogen-to-progesterone ratio, which is characteristic of late pregnancy and the follicular phase of the estrous cycle, is an event that parallels elevations in UBF.⁷³ Alternatively, during the luteal phase, which is characterized by high plasma progesterone concentrations and low estrogen, UBF is restored to basal levels.² In nonpregnant sheep models, it appears that exogenous progesterone when administered alone has no vasodilatory effect on the uterine vascular bed but has an inhibitory effect when infused in combination with estrogen.⁷⁷⁻⁸⁰ With regard to the spatial distribution of UBF, it is shown that progesterone favors caruncular flow whereas estradiol favors flow to the uterine cervix and myometrium.⁷⁷ Early in pregnancy, the caruncles receive about 27% of the total UBF but by late pregnancy this rises to 82%, which may be because of progesterone.⁸¹ In sheep, it is shown that estrogen triggers estrogen and progesterone receptor transcription and synthesis whereas progesterone downregulates both but the physiological consequence of this is unknown.⁸² Many studies have examined the effect of estrogen with regard to the pregnant uterine artery but information available on progesterone is limited and often controversial,^{2, 83-93} possibly because of the relative difficulty of in vivo studies with prolonged treatment of a progesterone receptor antagonist in pregnant animals. The following will discuss the literature available on the signaling transduction pathways by which progesterone alters vascular reactivity of the uterine artery.

Progesterone-Mediated Uterine Endothelial Adaptations to Pregnancy

Effect of progesterone on NO production in uterine artery endothelium—

Progesterone administration alone is sufficient to induce eNOS protein expression in the uterine artery endothelium; however, treatment in combination with estrogen or estrogen alone induces greater expression.^{3,41} Estrogen may have a greater effect with regard to eNOS expression than progesterone and there are several strategies that may account for this. Caveolin-1, which functions as an endogenous inhibitor of eNOS by mediating the sequestration of this enzyme at the plasma protein, has been shown to be differentially regulated in response to steroid

treatments.⁴¹ The data confirmed that estrogen and its combination with progesterone down-regulated caveolin-1; however, progesterone alone was shown not to have an effect.⁴¹ These findings suggest that the effect of estrogen on the regulation of eNOS expression is greater than that of progesterone; however, when systemic NO was measured, it was shown that the levels for these is higher during progesterone or combined progesterone and estrogen treatment as opposed to treatment with estrogen alone.³ Systemic NO is not necessarily indicative of the local, uterine levels because these values are based on the total NO production from different vascular beds. It has been suggested that progesterone mediates stimulation of other isoforms of NOS including the iNOS in other organs/tissues.³

Effect of progesterone on PGI₂ production in uterine artery endothelium—In addition to NO, the eicosanoid prostacyclin (PGI₂) is another important endothelium-derived vasodilator that is elevated during pregnancy.⁹⁴⁻⁹⁶ There are several key enzymes responsible for PGI₂ production, which includes phospholipase A2 (PLA₂), cyclooxygenase 1 (COX-1), and prostacyclin synthetase (PGIS).⁹⁶ Alteration in the expression of these enzymes is a potential strategy by which ovarian sex steroids regulate vascular reactivity. It was demonstrated that E2 β and progesterone treatment caused the rise in levels of cPLA₂ and COX-1 in the uterine artery endothelium.⁹⁶ COX-1, which is a rate-limiting enzyme for PGI₂ synthesis, has been shown to be elevated during pregnancy and to a lesser degree during the follicular phase in the uterine artery endothelium.⁹⁶⁻⁹⁸ COX-1 expression is blocked by the use of antiprogestins or the ER antagonist ICI 182780, which illustrates that sex steroids have an effect on COX-1 expression.^{96,99,100} In the case of the PGI₂ synthetase in the uterine artery endothelium, expression is elevated by E2 β treatment but was attenuated by the combination treatment.⁹⁶ These findings suggest that sex steroids have an effect on the expression of enzymes involved in PGI₂ production but the mechanisms remain to be determined.⁹⁶

Progesterone-Mediated Smooth Muscle Adaptations to Pregnancy

Role of progesterone in α 1-adrenergic and PKC-mediated contractions in uterine artery—Ford⁵⁴ has previously shown several findings on the effects of progesterone at the level of the uterine arterial smooth muscle during pregnancy. UBF during pregnancy is a physiological state that involves 2 important calcium-mediated events: phasic contractility and tone. Phasic contractility, which is typically <10 minutes in duration, mediate short-term contractions through the activation of α 1-adrenergic receptors triggering a series of downstream events that result in the increase of inositol 1,4,5-trisphosphate (Ins(1,4,5)P₃) followed by the release of intracellular calcium stores. Alternatively, tonic or long-term contractility involves activation of PKC-DAG complexes.⁵⁴ In the uterine artery, progesterone is reported to be the only sex steroid that directly regulates the α 1-adrenergic/calmodulin system.^{54,101} Progesterone is thought to modulate phasic contractility by the increase of α 1-adrenergic receptor numbers on the uterine arterial smooth muscle membranes.⁵⁴ In doing so, this sensitizes the uterine vasculature to the circulating adrenal catecholamines, resulting in an increased vasoconstrictive response. During the estrous cycle in gilts, it has been shown that there is a strong correlation between systemic progesterone concentrations, number of α 1 receptors in the uterine artery, and in vitro contractile response to nerve stimulation.^{52,101} These findings are consistent with the observation that higher vasoconstrictive responses to phenylephrine (α 1-adrenergic receptor agonist) are measured in the luteal phase uterine arteries (high progesterone, low estrogen) than the estrous uterine arteries (high estrogen, low progesterone) as well as in pregnant uterine arteries when progesterone levels are high.^{32,52,74,102-105}

From the physiological perspective, during pregnancy, the uterine vasculature acts as a low-resistance shunt to accommodate the large increase in uteroplacental blood flow required for

normal fetal development. In addition to increased endothelial NO synthesis/release, pregnancy is associated with a transient and reversible sympathetic denervation of the uterus and uterine artery, which is associated with a profound decrease in contractions of smooth muscle to electric field stimulation.^{32,106,107} Although the decreased sympathetic innervation and the increased NO release maintain low uterine vascular tone in pregnancy, sympathetic denervation, as well as the action of progesterone, may sensitize the postsynaptic α 1-adrenergic pathway and increase the ability of nonsynaptic α 1-adrenergic-mediated contractions in the uterine artery. This may be important for the mother to protect herself under stresses and allow a redistribution of blood by contracting the uterine artery to circulating catecholamines. Indeed, UBF was found significantly reduced under the stress of short-term exercise in pregnant women and sheep.¹⁰⁸ This may be achieved in part by enhanced intracellular Ca^{2+} mobilization through activation of α 1-adrenoceptors, resulting in activation of myosin light chain kinase and phosphorylation of MLC_{20} . On the other hand, the significant decrease in PKC-mediated sustained contractions contributes to maintaining low basal vascular tone of the uterine artery during pregnancy and helps maintain low vascular tone in response to increased blood flow through the uterine artery during pregnancy. It has been shown that progesterone plays a role in decreasing PKC-mediated contractions and myogenic tone of the uterine artery.^{75,76}

CORTISOL

Cortisol-Mediated UBF

During pregnancy in humans and sheep, maternal plasma cortisol levels are shown to approximately double, which has important implications for fetal development and growth.^{109,110} When maternal cortisol levels exceed or fall below the physiological limits, such as during moments of hypercorticism or hypocorticism, it is shown that incidences of fetal growth restriction, prematurity, and death are elevated.¹¹¹ Although it is shown that either extremes have the ability to exert similar biological responses, such as restricted growth rates, the mechanisms involved may be dissimilar. It is hypothesized that during periods of low maternal cortisol, the resulting impaired placental perfusion mediates the restrictions on fetal growth.¹¹² Alternatively, during periods of elevated cortisol, growth restrictions may be caused by factors such as alterations in fetal hormone levels, placental structure, or impaired growth factor function.¹¹²

Effect of elevated maternal cortisol and increased UBF—There is increasing evidence to support an association between maternal plasma cortisol concentrations and UBF.¹¹² One such study in pregnant ewes at late gestation found that UBF was not elevated in the adrenalectomized low-cortisol group, whereas an elevation in UBF was observed in the control and high-cortisol groups.¹¹² In this study, from 120 to 130 days gestation, all the ewes in the intact control and high cortisol groups experienced increments in UBF whereas this increase was absent in the low-cortisol group: mean increase 470 ± 148 mL/min in the control group; 262 ± 95 mL/min in the high-cortisol group; and -26 ± 41 mL/min in the low-cortisol group.¹¹² Although this study does not indicate the mechanism for the change in UBF, it is supportive of the hypothesis that cortisol has a direct or indirect effect on uteroplacental perfusion.¹¹²

Mechanism of action of cortisol on uteroplacental perfusion—Cortisol affects both systemic and local uterine parameters to regulate UBF. The systemic mechanism by which maternal cortisol regulates uteroplacental perfusion during late gestation involves adjustments in maternal plasma volume.^{112,113} It has been suggested that maternal adrenal secretion indirectly sustains fetal physiological parameters through adjustments in maternal plasma volume.¹¹³ These adrenal secretions include cortisol and aldosterone, which are required to sustain maternal plasma volume, which, in turn, maintains fetal arterial pressure and fetal

arterial oxygen tension within normal values.¹¹² One hypothesis suggests that lowered maternal blood volume causes the reduction in maternal cardiac output, which, in turn, causes a reduction in uterine perfusion.¹¹² Cortisol also exerts local effects at the uterine arterial vascular bed through mechanisms at the level of the endothelium and the VSM, as will be discussed in the later part of this review.^{111,112}

Cortisol-Mediated Uterine Endothelial Adaptations to Pregnancy

Effect of cortisol on eNOS expression—It is apparent that cortisol is necessary to sustain normal vasodilation in the uterine artery of pregnant sheep, but the mechanisms involved are still not well understood. It is reported that cortisol decreases eNOS protein expression in both pregnant and nonpregnant uterine artery endothelial scrapings although a greater decrease is seen in the nonpregnant group.¹¹¹ Consistent with the trend in eNOS protein expression, cortisol decreases NO release by about 74% in the nonpregnant uterine arteries and by 44% in pregnant uterine arteries.¹¹¹ These findings suggest pregnancy attenuates cortisol-mediated inhibition of eNOS expression. In another study, adrenalectomized ewes (low-cortisol group) demonstrated a reduction in eNOS expression, which was contrary to what the authors expected.¹¹⁴ The authors reported that this may be because of poor perfusion rather than a direct effect of the corticosteroids on the endothelial cells.¹¹⁴ Shear stress is a known physiological activator of eNOS expression and activity and is reduced during moments of lowered blood flow, which may account for the reduction in eNOS expression in the adrenalectomized animals, demonstrating a fluctuating pattern of blood pressure that involves periods of low or no flow.^{114,115} There is no evident consensus as to whether cortisol is stimulatory or inhibitory on eNOS expression; however, it is apparent that alterations in eNOS expression do occur and pregnancy will influence the effect of cortisol on endothelium-dependent pathways.

Cortisol-Mediated Uterine Smooth Muscle Adaptations to Pregnancy

It has been well documented that glucocorticoids are associated with increased arterial contraction and vascular response. By binding to its corresponding glucocorticoid receptors, these hormones have been shown to potentiate vasoactive responses to agents including angiotensin II, vasopressin, and norepinephrine.^{111,116} Based on these observations, it may seem counterintuitive that cortisol is increased during pregnancy. There are numerous pregnancy-specific mechanisms involved in the desensitization of the uterine artery to the constriction-promoting effects of cortisol.

Mechanisms involved in the attenuation of cortisol-mediated potentiation of uterine artery contractility during pregnancy—In sheep models, cortisol potentiates norepinephrine-induced contractions in the uterine artery of nonpregnant animals but this response is attenuated in pregnant animals.¹¹⁷ The difference in response between the pregnant and nonpregnant state is achieved by several key strategies that include variations in (a) ligand-receptor affinity, (b) 11-bHSD activity, and (c) α 1-adrenoceptor-mediated pharmacomechanical coupling.^{111,117} Although pregnancy does not alter glucocorticoid receptor density in the uterine artery, it can decrease the agonist-binding affinity of norepinephrine to α 1-adrenoceptors in cortisol-treated uterine arteries.¹¹¹ The apparent dissociation constant (K_A) values for norepinephrine were lower in the pregnant (5.2 ± 2.0 μ M) than nonpregnant (24.6 ± 6.5 μ M).¹¹¹ With regard to 11-bHSD activity in the uterine artery, inhibition of the enzyme had no effect on norepinephrine-induced contractions in the absence or presence of cortisol in nonpregnant uterine arteries.¹¹¹ In pregnant animals, however, the use of the 11-bHSD inhibitor, carbenoxolone, potentiated norepinephrine-mediated contractions of uterine arteries in the absence of cortisol.¹¹¹ These findings support the hypothesis that increased 11-bHSD activity in uterine artery smooth muscle cells may be one of the mechanisms by which pregnancy attenuates norepinephrine-induced contractions

in the uterine artery. Differences in pharmacomechanical coupling also account for the decreased sensitivity of the uterine artery to norepinephrine-induced contractions in the presence of cortisol during pregnancy. In the nonpregnant state, cortisol increases $\text{Ins}(1,4,5)\text{P}_3$ production for any given number of $\alpha 1$ -adrenoceptors occupied.¹¹⁷ In other words, cortisol improves the coupling of $\alpha 1$ -adrenoceptors to $\text{Ins}(1,4,5)\text{P}_3$ synthesis. This may occur on many different levels including enhanced receptor coupling to $\text{Ins}(1,4,5)\text{P}_3$ synthesis, improvement in $\text{Ins}(1,4,5)\text{P}_3$ efficiency to Ca^{2+} mobilization, and increased Ca^{2+} sensitivity of contractile myofilaments.¹¹⁷ During pregnancy, however, this increased coupling efficiency is not observed, which suggests that there is an unknown pregnancy-associated factor involved in preventing this. One possibility is that progesterone, which is elevated during pregnancy and is also reported to have antiglucocorticoid effects, may have bound to glucocorticoid receptors to block agonist-induced Ca^{2+} sensitization of smooth muscle.¹¹⁷

CONCLUDING REMARKS

It is evident that estrogen, progesterone, and cortisol have an effect on local uterine blood flow through their activity at the level of the endothelium and the VSM in the uterine artery; however, the finer details of the pathways discussed remain unknown and are the subject of further study. Many animal studies, although very informative, are limited because of the difficulty in controlling for external factors that may affect the final response of the tissue being studied. Many of these studies also use sex steroid doses that may not be within physiological limits. On the other hand, many in vitro studies are also limited in terms of physiological relevance because treatment and laboratory conditions will have an effect on the tissue response. In this review, there was much discussion on the effect of sex steroids on nitric oxide production, nitric oxide synthases, potassium channels, sex steroid receptors, adrenoceptors, and alterations in pharmacomechanical coupling. However, this is not to say that these are the only strategies by which sex steroids promote increased uteroplacental perfusion during pregnancy because there are, in fact, many others that have not been discussed. Many previous studies have examined the nongenomic effects of the steroid hormones, which provide much insight on the immediate responses of the uterine artery. However, pregnancy is a gradual, long-term physiological condition that involves genomic pathways and alterations in gene expression, and studies on these are in progress. A thorough understanding of the differences, similarities, and how the genomic and nongenomic pathways interact may provide a better understanding of how sex steroids regulate uterine artery contractility during pregnancy.

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REFERENCES

1. Hall JM, Couse JF, Korach KS. The multifaceted mechanisms of estradiol and estrogen receptor signaling. *J Biol Chem* 2001;276:36869–36872. [PubMed: 11459850]
2. Ford SP. Control of uterine and ovarian blood flow throughout the estrous cycle and pregnancy of ewes, sows and cows. *J Anim Sci* 55(suppl 2):32–42. [PubMed: 6765316]
3. Rupnow HL, Phernetton TM, Shaw CE, Modrick ML, Bird IM, Magness RR. Endothelial vasodilator production by uterine and systemic arteries. VII. Estrogen and progesterone effects on eNOS. *Am J Physiol Heart Circ Physiol* 2001;280:H1699–H1705. [PubMed: 11247782]
4. Yi FX, Magness RR, Bird IM. Simultaneous imaging of $[\text{Ca}^{2+}]_i$ and intracellular NO production in freshly isolated uterine artery endothelial cells: effects of ovarian cycle and pregnancy. *Am J Physiol Regul Integr Comp Physiol* 2005;288:R140–R148. [PubMed: 15297265]
5. Magness RR, Rosenfeld CR. Local and systemic estradiol-17 beta: effects on uterine and systemic vasodilation. *Am J Physiol* 1989;256:E536–E542. [PubMed: 2650565]

6. Killam AP, Rosenfeld CR, Battaglia FC, Makowski EL, Meschia G. Effect of estrogens on the uterine blood flow of oophorectomized ewes. *Am J Obstet Gynecol* 1973;115:1045–1052. [PubMed: 4734886]
7. Magness RR, Parker CR Jr, Rosenfeld CR. Systemic and uterine responses to chronic infusion of estradiol-17 beta. *Am J Physiol* 1993;265:E690–E698. [PubMed: 8238494]
8. Magness RR, Phernetton TM, Zheng J. Systemic and uterine blood flow distribution during prolonged infusion of 17beta-estradiol. *Am J Physiol* 1998;275:H731–H743. [PubMed: 9724274]
9. Rosenfeld CR, Cox BE, Roy T, Magness RR. Nitric oxide contributes to estrogen-induced vasodilation of the ovine uterine circulation. *J Clin Invest* 1996;98:2158–2166. [PubMed: 8903336]
10. Van Buren GA, Yang DS, Clark KE. Estrogen-induced uterine vasodilatation is antagonized by L-nitroarginine methyl ester, an inhibitor of nitric oxide synthesis. *Am J Obstet Gynecol* 1992;167:828–833. [PubMed: 1530046]
11. Gibson TC, Phernetton TM, Wiltbank MC, Magness RR. Development and use of an ovarian synchronization model to study the effects of endogenous estrogen and nitric oxide on uterine blood flow during ovarian cycles in sheep. *Biol Reprod* 2004;70:1886–1894. [PubMed: 14985241]
12. Byers MJ, Zangl A, Phernetton TM, Lopez G, Chen DB, Magness RR. Endothelial vasodilator production by ovine uterine and systemic arteries: ovarian steroid and pregnancy control of ER-alpha and ER-beta levels. *J Physiol* 2005;565:85–99. [PubMed: 15774511]
13. Liao WX, Magness RR, Chen DB. Expression of estrogen receptors-alpha and -beta in the pregnant ovine uterine artery endothelial cells in vivo and in vitro. *Biol Reprod* 2005;72:530–537. [PubMed: 15564597]
14. Batra S, Iosif S. Nuclear estrogen receptors in human uterine arteries. *Gynecol Obstet Invest* 1987;24:250–255. [PubMed: 3428713]
15. Lantta M, Karkkainen J, Lehtovirta P. Progesterone and estradiol receptors in the cytosol of the human uterine artery. *Am J Obstet Gynecol* 1983;147:627–633. [PubMed: 6638107]
16. Magness RR, Phernetton TM, Gibson TC, Chen DB. Uterine blood flow responses to ICI 192 780 in ovariectomized oestradiol-17 beta-treated, intact follicular and pregnant sheep. *J Physiol* 2005;565:71–83. [PubMed: 15774510]
17. Rosenfeld CR, Roy T, Cox BE. Mechanisms modulating estrogen-induced uterine vasodilation. *Vascul Pharmacol* 2002;38:115–125. [PubMed: 12379958]
18. Resnik R, Killam AP, Battaglia FC, Makowski EL, Meschia G. The stimulation of uterine blood flow by various estrogens. *Endocrinology* 1974;94:1192–1196. [PubMed: 4462611]
19. Rosenfeld CR, Jackson GM. Induction and inhibition of uterine vasodilation by catechol estrogen in oophorectomized, nonpregnant ewes. *Endocrinology* 1982;110:1333–1339. [PubMed: 7060529]
20. Bird IM, Zhang L, Magness RR. Possible mechanisms underlying pregnancy induced increases in uterine artery endothelial function. *Am J Physiol* 2003;284:R245–R258.
21. Magness RR, Sullivan JA, Li Y, Phernetton TM, Bird IM. Endothelial vasodilator production by uterine and systemic arteries. VI. Ovarian and pregnancy effects on eNOS and NOx. *Am J Physiol Heart Circ Physiol* 2001;280:H1692–H1698. [PubMed: 11247781]
22. Cale JM, Bird IM. Dissociation of endothelial nitric oxide synthase phosphorylation and activity in uterine artery endothelial cells. *Am J Physiol Heart Circ Physiol* 2006;290:1433–1445.
23. Alderton WK, Cooper CE, Knowles RG. Nitric oxide synthases: structure, function and inhibition. *Biochem J* 2001;357:593–615. [PubMed: 11463332]
24. Boo YC, Sorescu GP, Bauer PM, et al. Endothelial NO synthase phosphorylated at SER635 produces NO without requiring intracellular calcium increase. *Free Radic Biol Med* 2003;35:729–741. [PubMed: 14583337]
25. Fleming I, Busse R. Molecular mechanisms involved in the regulation of the endothelial nitric oxide synthase. *Am J Physiol Regul Integr Comp Physiol* 2003;284:R1–R12. [PubMed: 12482742]
26. Takahashi S, Mendelsohn ME. Synergistic activation of endothelial nitric-oxide synthase (eNOS) by HSP90 and Akt: calcium-independent eNOS activation involves formation of an HSP90-Akt-CaM-bound eNOS complex. *J Biol Chem* 2003;278:30821–30827. [PubMed: 12799359]
27. Yi FX, Zhang AY, Campbell W, Zou AP, VanBremen C, Li PL. Simultaneous in situ monitoring of intracellular Ca^{2+} and NO in endothelium of coronary arteries. *Am J Physiol Heart Circ Physiol* 2002;283:H2725–H2732. [PubMed: 12388315]

28. Bird IM, Sullivan JA, Di T, et al. Pregnancy-dependent changes in cell signaling underlie changes in differential control of vasodilator production in uterine artery endothelial cells. *Endocrinology* 2000;141:1107–1117. [PubMed: 10698187]
29. Di T, Sullivan JA, Magness RR, Zhang L, Bird IM. Pregnancy-specific enhancement of agonist-stimulated ERK1/2 signaling in uterine artery endothelial cells increases Ca^{2+} sensitivity of endothelial nitric oxide synthase as well as cytosolic phospholipase A2. *Endocrinology* 2001;142:3014–3026. [PubMed: 11416023]
30. Gifford SM, Cale JM, Tsoi S, Magness RR, Bird IM. Pregnancy-specific changes in uterine artery endothelial cell signaling in vivo are both programmed and retained in primary culture. *Endocrinology* 2003;144:3639–3650. [PubMed: 12865347]
31. Chen Z, Yuhanna IS, Galcheva-Gargova Z, Karas RH, Mendelsohn ME, Shaul PW. Estrogen receptor- α mediates the nongenomic activation of endothelial nitric oxide synthase by estrogen. *J Clin Invest* 1999;103:401–406. [PubMed: 9927501]
32. Chen DB, Bird IM, Zheng J, Magness RR. Membrane estrogen receptor-dependent extracellular signal-regulated kinase pathway mediates acute activation of endothelial nitric oxide synthase by estrogen in uterine artery endothelial cells. *Endocrinology* 2004;145:113–125. [PubMed: 14512434]
33. Bernier SG, Haldar S, Michel T. Bradykinin-regulated interactions of the mitogen-activated protein kinase pathway with the endothelial nitric-oxide synthase. *J Biol Chem* 2000;275:30707–30715. [PubMed: 10899167]
34. Hisamoto K, Ohmichi M, Kanda Y, et al. Induction of endothelial nitric-oxide synthase phosphorylation by the raloxifene analog LY117018 is differentially mediated by Akt and extracellular signal-regulated protein kinase in vascular endothelial cells. *J Biol Chem* 2001;276:47642–47649. [PubMed: 11595733]
35. Wyatt AW, Steinert JR, Wheeler-Jones CP, et al. Early activation of the p42/p44MAPK pathway mediates adenosine-induced nitric oxide production in human endothelial cells: a novel calcium-insensitive mechanism. *FASEB J* 2002;16:1584–1594. [PubMed: 12374781]
36. Dimmeler S, Fleming I, Fisslthaler B, Hermann C, Busse R, Zeiher AM. Activation of nitric oxide synthase in endothelial cells by Akt-dependent phosphorylation. *Nature* 1999;399:601–605. [PubMed: 10376603]
37. Michell BJ, Griffiths JE, Mitchelhill KI, et al. The Akt kinase signals directly to endothelial nitric oxide synthase. *Curr Biol* 1999;9:845–848. [PubMed: 10469573]
38. Tschugguel W, Dietrich W, Zhegu Z, Stonek F, Kolbus A, Huber JC. Differential regulation of proteasome-dependent estrogen receptor α and β turnover in cultured human uterine artery endothelial cells. *J Clin Endocrinol Metab* 2003;88:2281–2287. [PubMed: 12727987]
39. Russell-Jones DL, Umpleby M. Protein anabolic action of insulin, growth hormone and insulin-like growth factor-1. *Eur J Endocrinol* 1996;135:631–642. [PubMed: 9025701]
40. Michel T, Feron O. Nitric oxide synthases: which, where, how, and why? *J Clin Invest* 1997;100:2146–2152. [PubMed: 9410890]
41. Chen DB, Jia S, King AG, et al. Global protein expression profiling underlines reciprocal regulation of caveolin 1 and endothelial nitric oxide synthase expression in ovariectomized sheep uterine. *Biol Reprod* 2006;74:832–838. [PubMed: 16436525]
42. Chambliss KL, Shaul PW. Estrogen modulation of endothelial nitric oxide synthase. *Endocr Rev* 2002;23:665–686. [PubMed: 12372846]
43. Salhab WA, Shaul PW, Cox BE, Rosenfeld CR. Regulation of types I and III NOS in ovine uterine arteries by daily and acute estrogen exposure. *Am J Physiol Heart Circ Physiol* 2000;278:H2134–H2142. [PubMed: 10843913]
44. Rosenfeld CR, Cornfield DN, Roy T. Ca^{2+} -activated K^{+} channels modulate basal and estradiol-induced rises in uterine blood flow in ovine pregnancy. *Am J Physiol Heart Circ Physiol* 2001;281:H422–H431. [PubMed: 11406511]
45. Brayden JE. Potassium channels in vascular smooth muscle. *Clin Exp Pharmacol Physiol* 1996;23:1069–1076. [PubMed: 8977162]
46. Rosenfeld CR, White RE, Roy T, Cox BE. Calcium-activated potassium channels and nitric oxide coregulate estrogen-induced vasodilation. *Am J Physiol Heart Circ Physiol* 2000;279:319–328.

47. Valverde MA, Rojas P, Amigo J, et al. Acute activation of Maxi-K channels (hSlo) by estradiol binding to the beta subunit. *Science* 1999;285:1929–1931. [PubMed: 10489376]
48. Nagar D, Liu X, Rosenfeld CR. Estrogen regulates beta 1-subunit expression in Ca^{2+} -activated K^{+} channels in arteries from reproductive tissues. *Am J Physiol Heart Circ Physiol* 2005;289:H1417–H1427. [PubMed: 15923308]
49. Dick GM, Sanders KM. (Xeno)estrogen sensitivity of smooth muscle BK channels conferred by the regulatory beta-1 subunit: a study of beta-1 knockout mice. *J Biol Chem* 2001;276:34594–34599. [PubMed: 11454866]
50. Bell DR, Rensberger HJ, Koritnik DR, Koshy A. Estrogen pretreatment directly potentiates endothelium-dependent vasorelaxation of porcine coronary arteries. *Am J Physiol Heart Circ Physiol* 1990;258:H377–H383.
51. Forstermann U, Schmidt HHHW, Pollack JS, et al. Isoforms of nitric oxide synthase. Characterization and purification from different cell types. *Biochem Pharmacol* 1991;42:1849–1857. [PubMed: 1720618]
52. Stice SL, Ford SP, Rosazza JP, Van Orden DE. Interaction of 4-hydroxylated estradiol and potential-sensitive Ca^{2+} channels in altering uterine blood flow during the estrous cycle and early pregnancy in gilts. *Biol Reprod* 1987;36:369–375. [PubMed: 2437970]
53. Farley DB, Ford SP. Evidence for declining extracellular calcium uptake and protein kinase C activity in uterine arterial smooth muscle during gestation in gilts. *Biol Reprod* 1992;46:315–321. [PubMed: 1319752]
54. Ford SP. Control of blood flow to the gravid uterus of domestic livestock species 1,2. *J Anim Sci* 1995;73:1852–1860. [PubMed: 7673079]
55. Kublickiene KR, Cockell AP, Nisell H, Poston L. Role of nitric oxide in the regulation of vascular tone in pressurized and perfused resistance myometrial arteries from term pregnant women. *Am J Obstet Gynecol* 1997;177:1263–1269. [PubMed: 9396927]
56. Kublickiene KR, Kublickas M, Lindblom B, Lunell NO, Nisell H. A comparison of myogenic and endothelial properties of myometrial and omental resistance vessels in late pregnancy. *Am J Obstet Gynecol* 1997;176:560–566. [PubMed: 9077607]
57. Cipolla MJ, Binder ND, Osol G. Myoendometrial versus placental uterine arteries: structural, mechanical, and functional differences in late-pregnant rabbits. *Am J Obstet Gynecol* 1997;177:215–221. [PubMed: 9240609]
58. Osol G, Cipolla M. Interaction of myogenic and adrenergic mechanisms in isolated, pressurized uterine radial arteries from late-pregnant and nonpregnant rats. *Am J Obstet Gynecol* 1993;168:697–705. [PubMed: 8438952]
59. Veerareddy S, Cooke CL, Baker PN, Davidge ST. Vascular adaptations to pregnancy in mice: effects on myogenic tone. *Am J Physiol Heart Circ Physiol* 2002;283:H2226–H2233. [PubMed: 12388224]
60. Xiao D, Buchholz JN, Zhang L. Pregnancy attenuates uterine artery pressure-dependent vascular tone: role of PKC/ERK pathway. *Am J Physiol Heart Circ Physiol* 2006;290:H2337–H2343. [PubMed: 16399857]
61. Grandley RE, Conrad KP, McLaughlin MK. Endothelin and nitric oxide mediate reduced myogenic reactivity of small renal arteries from pregnant rats. *Am J Physiol Regul Integr Comp Physiol* 2001;280:R1–R7. [PubMed: 11124127]
62. Meyer MC, Brayden JE, McLaughlin MK. Characteristics of vascular smooth muscle in the maternal resistance circulation during pregnancy in the rat. *Am J Obstet Gynecol* 1993;169:1510–1516. [PubMed: 8267055]
63. Meyer MC, Osol G, McLaughlin M. Flow decreases myogenic reactivity of mesenteric arteries from pregnant rats. *J Soc Gynecol Investig* 1997;4:293–297.
64. Davis MJ, Hill MA. Signaling mechanisms underlying the vascular myogenic response. *Physiol Rev* 1999;79:387–423. [PubMed: 10221985]
65. Hill MA, Falcone JC, Meininger GA. Evidence for protein kinase C involvement in arteriolar myogenic reactivity. *Am J Physiol* 1990;259:H1586–H1594. [PubMed: 2240255]
66. Lagaud G, Gaudreault N, Moore ED, Van Breemen C, Laher I. Pressure-dependent myogenic constriction of cerebral arteries occurs independently of voltage-dependent activation. *Am J Physiol Heart Circ Physiol* 2002;283:H2187–H2195. [PubMed: 12388215]

67. Nakayama K, Obara K, Tanabe Y, Saito M, Ishikawa T, Nishizawa S. Interactive role of tyrosine kinase, protein kinase C, and Rho/Rho kinase systems in the mechanotransduction of vascular smooth muscles. *Biorheology* 2003;40:307–314. [PubMed: 12454420]
68. Osol G, Laher I, Cipolla M. Protein kinase C modulates basal myogenic tone in resistance arteries from the cerebral circulation. *Circ Res* 1991;68:359–367. [PubMed: 1991343]
69. Xiao D, Huang X, Longo LD, Pearce WJ, Zhang L. Regulation of baseline Ca^{2+} sensitivity in permeabilized uterine arteries: effect of pregnancy. *Am J Physiol Heart Circ Physiol* 2006;291:H413–H420. [PubMed: 16501025]
70. Xiao D, Zhang L. ERK MAP kinases regulate smooth muscle contraction in ovine uterine artery: effect of pregnancy. *Am J Physiol Heart Circ Physiol* 2002;282:H292–H300. [PubMed: 11748074]
71. Kanashiro CA, Alexander BT, Granger JP, Khalil RA. Ca^{2+} -insensitive vascular protein kinase C during pregnancy and NOS inhibition. *Hypertension* 1999;34:924–930. [PubMed: 10523386]
72. Kanashiro CA, Cockrell KL, Alexander BT, Granger JP, Khalil RA. Pregnancy-associated reduction in vascular protein kinase C activity rebounds during inhibition of NO synthesis. *Am J Physiol* 2000;278:R295–R303.
73. Magness RR, Rosenfeld CR, Carr BR. Protein kinase C in uterine and systemic arteries during ovarian cycle and pregnancy. *Am J Physiol* 1991;260:E464–E470. [PubMed: 2003600]
74. Xiao D, Zhang L. Adaptation of uterine artery thick- and thin-filament regulatory pathway to pregnancy. *Am J Physiol Heart Circ Physiol* 2005;288:H142–H148. [PubMed: 15358611]
75. Xiao, D.; Zhang, L. Direct effects of estrogen and progesterone on PKC-mediated contractions of the uterine artery; Paper presented at: the 15th World Congress of Pharmacology (IUPHAR 2006); Beijing, China. July 2–7, 2006;
76. Yang, S.; Xiao, D.; Zhang, L. Role of sex steroid hormones and ERK1/2 in pregnancy-suppressed uterine artery myogenic tone; Paper presented at: SSR 2008; Kona, Hawaii. May 27–31, 2008;
77. Anderson SG, Hackshaw BT, Still JG, Greiss FC Jr. Uterine blood flow and its distribution after chronic estrogen and progesterone administration. *Am J Obstet Gynecol* 1977;127:138–142. [PubMed: 831495]
78. Caton D, Abrams RM, Clapp JF, Barron DH. The effect of exogenous progesterone on the rate of blood flow of the uterus of ovariectomized sheep. *Q Exp Physiol* 1974;59:225–231.
79. Greiss FC, Anderson SG. Effect of ovarian hormones on the uterine vascular bed. *Am J Obstet Gynecol* 1970;107:829–836. [PubMed: 5429013]
80. Resnik R, Brink GW, Plumer MH. The effect of progesterone on estrogen-induced uterine blood flow. *Am J Obstet Gynecol* 1977;128:251–254. [PubMed: 871140]
81. Rosenfeld CR, Morriss FH Jr, Makowski EL, Meschia G, Battaglia FC. Circulatory changes in the reproductive tissues of ewes during pregnancy. *Gynecol Invest* 1974;5:252–268. [PubMed: 4461407]
82. Meikle A, Tasende C, Sosa C, Garofalo EG. The role of sex steroid receptors in sheep female reproductive physiology. *Reprod Fertil Dev* 2004;16:385–394. [PubMed: 15315737]
83. Buhimschi I, Yallampalli C, Chwalisz K, Garfield RE. Preeclampsia-like conditions produced by nitric oxide inhibition: effects of L-arginine, D-arginine and steroid hormones. *Hum Reprod* 1995;10:2723–2730. [PubMed: 8567800]
84. Deichert U, Albrand-Thielmann C, van de Sandt M. Doppler-sonographic pelvic blood flow measurements and their prognostic value in terms of luteal phase and implantation. *Hum Reprod* 1996;11:1591–1593. [PubMed: 8921096]
85. Habara T, Nakatsuka M, Konishi H, Asagiri K, Noguchi S, Kudo T. Elevated blood flow resistance in uterine arteries of women with unexplained recurrent pregnancy loss. *Hum Reprod* 2002;17:190–194. [PubMed: 11756386]
86. Magness, RR.; Rosenfeld, CR. The role of steroid hormones in the control of uterine blood flow. In: Rosenfeld, CR., editor. *Reproductive and Perinatal Medicine. X. Perinatology Press; Ithaca, NY: 1989. p. 234–271.*
87. Magness, RR.; Rosenfeld, CR.; Meeting NACDA-NIH. Endometrial Function and Dysfunctional Uterine Bleeding. American Association for the Advancement of Science Press; Washington, DC: 1992. Steroid control of blood vessel function; p. 107–120.
88. Pecins-Thompson M, Keller-Wood M. Effects of progesterone on blood pressure, plasma volume, and responses to hypotension. *Am J Physiol* 1997;272:R377–R385. [PubMed: 9039032]

89. Reynolds LP. Utero-ovarian interactions during early pregnancy: role of conceptus-induced vasodilation. *J Anim Sci* 1986;62(suppl 2):47–61. [PubMed: 3533875]
90. Reynolds LP, Magness RR, Ford SP. Uterine blood flow during early pregnancy in ewes: interaction between the conceptus and the ovary bearing the corpus luteum. *J Anim Sci* 1984;58:423–429. [PubMed: 6706875]
91. Roesch DM, Keller-Wood M. Progesterone rapidly reduces arterial pressure in ewes. *Am J Physiol* 1997;272:H386–H391. [PubMed: 9038960]
92. Roman-Ponce H, Caton D, Thatcher WW, Lehrer R. Uterine blood flow in relation to endogenous hormones during estrous cycle and early pregnancy. *Am J Physiol* 1983;245:R843–R849. [PubMed: 6660330]
93. Wallace JM, Bourke DA, Da Silva P, Aitken RP. Influence of progesterone supplementation during the first third of pregnancy on fetal and placental growth in overnourished adolescent ewes. *Reproduction* 2003;126:481–487. [PubMed: 14525530]
94. Magness RR, Mitchell MD, Rosenfeld CR. Uteroplacental production of eicosanoids in ovine pregnancy. *Prostaglandins* 1990;39:75–88. [PubMed: 2106715]
95. Magness RR, Shideman CR, Habermehl DA, Sullivan JA, Bird IM. Endothelial vasodilator production by uterine and systemic arteries. V. Effects of ovariectomy, the ovarian cycle, and pregnancy on prostacyclin synthase expression. *Prostaglandins Other Lipid Mediat* 2000;60:103–118. [PubMed: 10751641]
96. Rupnow HL, Phernetton TM, Modrick ML, Wiltbank MC, Bird IM, Magness RR. Endothelial vasodilator production by uterine and systemic arteries. VIII. Estrogen and progesterone effects on cPLA2, COX-1, and PGIS protein expression. *Biol Reprod* 2002;66:468–474. [PubMed: 11804964]
97. Habermehl DA, Janowiak MA, Vagnoni KE, Bird IM, Magness RR. Endothelial vasodilator production by uterine and systemic arteries. IV. Cyclooxygenase isoform expression during the ovarian cycle and pregnancy in sheep. *Biol Reprod* 2000;62:781–788. [PubMed: 10684824]
98. Janowiak MA, Magness RR, Habermehl DA, Bird IM. Pregnancy increases ovine uterine artery endothelial cyclooxygenase-1 expression. *Endocrinology* 1998;139:765–771. [PubMed: 9449651]
99. Jun SS, Chen Z, Pace MC, Shaul PW. Estrogen upregulates cyclooxygenase-1 gene expression in ovine fetal pulmonary artery endothelium. *J Clin Invest* 1998;102:176–183. [PubMed: 9649571]
100. Kim JJ, Wang J, Bamba C, Das SK, Dey SK, Fazleabas AT. Expression of cyclooxygenase-1 and -2 in the baboon endometrium during the menstrual cycle and pregnancy. *Endocrinology* 1999;140:2672–2678. [PubMed: 10342857]
101. Ford SP, Reynolds LP, Farley DB, Bhatnagar RK, VanOrden DE. Interaction of ovarian steroids and periarterial alpha-1 adrenergic receptors in altering uterine blood flow during the estrous cycle of gilts. *Am J Obstet Gynecol* 1984;150:480–484. [PubMed: 6093534]
102. Annibale DJ, Rosenfeld CR, Kamm KE. Alterations in vascular smooth muscle contractility during ovine pregnancy. *Am J Physiol* 1989;256:H1282–H1288. [PubMed: 2719128]
103. Annibale DJ, Rosenfeld CR, Stull JT, Kamm KE. Protein content and myosin light chain phosphorylation in uterine arteries during pregnancy. *Am J Physiol* 1990;259:C484–C489. [PubMed: 2399969]
104. D'Angelo G, Osol G. Regional variation in resistance artery diameter responses to alpha-adrenergic stimulation during pregnancy. *Am J Physiol* 1993;264:H78–H85. [PubMed: 8430865]
105. D'Angelo G, Osol G. Modulation of uterine resistance artery lumen diameter by calcium and G protein activation during pregnancy. *Am J Physiol* 1994;267:H952–H961. [PubMed: 8092300]
106. Klukovits A, Gaspar R, Santha P, Jancso G, Falkay G. Functional and histochemical characterization of a uterine adrenergic denervation process in pregnant rats. *Biol Reprod* 2002;67:1013–1017. [PubMed: 12193415]
107. Nelson SH, Steinsland OS, Johnson RL, Suresh MS, Gifford A, Ehardt JS. Pregnancy-induced alterations of neurogenic constriction and dilation of human uterine artery. *Am J Physiol* 1995;268:H1694–H1701. [PubMed: 7733373]
108. Lotgering FK, Gilbert RD, Longo LD. Maternal and fetal responses to exercise during pregnancy. *Physiol Rev* 1985;65:1–36. [PubMed: 3880895]
109. Keller-Wood M. Inhibition of stimulated and basal ACTH by cortisol during ovine pregnancy. *Am J Physiol Regul Integr Comp Physiol* 1996;271:R130–R136.

110. Noltén WE, Rueckert PA. Elevated free cortisol index in pregnancy: possible regulatory mechanisms. *Am J Obstet Gynecol* 1981;139:492–498. [PubMed: 6258437]
111. Xiao D, Huang X, Bae S, Ducsay CA, Zhang L. Cortisol-mediated potentiation of uterine artery contractility: effect of pregnancy. *Am J Physiol Heart Circ Physiol* 2002;283:H238–H246. [PubMed: 12063296]
112. Jensen E, Wood CE, Keller-Wood M. Chronic alterations in ovine maternal corticosteroid levels influence uterine blood flow and placental and fetal growth. *Am J Physiol Regul Integr Comp Physiol* 2005;288:R54–R61. [PubMed: 15231491]
113. Jensen E, Wood C, Keller-Wood M. The normal increase in adrenal secretion during pregnancy contributes to maternal volume expansion and fetal homeostasis. *J Soc Gynecol Investig* 2002;9:362–371.
114. Li F, Wood CE, Keller-Wood M. Adrenalectomy alters regulation of blood pressure and endothelial nitric oxide synthase in sheep: modulation by estradiol. *Am J Physiol Regul Integr Comp Physiol* 2007;293:R257–R266. [PubMed: 17459913]
115. Malek AM, Izumo S, Alper SL. Modulation by pathophysiological stimuli of the shear stress-induced up-regulation of endothelial nitric oxide synthase expression in endothelial cells. *Neurosurgery* 1999;45:334–344. [PubMed: 10449079]
116. Yagil Y, Krakoff LR. The differential effect of aldosterone and dexamethasone on pressor responses in adrenalectomized rats. *Hypertension* 1988;11:174–178. [PubMed: 3343046]
117. Xiao D, Huang X, Pearce WJ, Longo LD, Zhang L. Effect of cortisol on norepinephrine-mediated contractions in ovine uterine arteries. *Am J Physiol Heart Circ Physiol* 2003;284:H1142–H1151. [PubMed: 12531736]