

Uterine Artery Remodeling in Pseudopregnancy Is Comparable to That in Early Pregnancy¹

Olivier W.H. van der Heijden,^{3,4,6,7} Yvonne P.G. Essers,^{3,6} Marc E.A. Spaanderman,^{3,6}
Jo G.R. De Mey,^{5,7} Guillaume J.J.M. van Eys,^{4,7} and Louis L.H. Peeters^{2,3,6}

Departments of Obstetrics and Gynecology,³ Molecular Genetics,⁴ and Pharmacology and Toxicology,⁵ and from the Research Institutes GROW⁶ and CARIM,⁷ University Maastricht, 6202 AZ Maastricht, The Netherlands

ABSTRACT

During pregnancy, the luminal diameter and wall mass of the uterine artery (UA) increase, most likely in response to the increased hemodynamic strain resulting from the chronically elevated uterine blood flow (UBF). In this remodeling process, the phenotype of vascular smooth-muscle cells (VSMC) is transiently altered to enable VSMC proliferation. These phenomena are already seen during early pregnancy, when the rise in UBF is still modest. This raises the question whether the newly instituted endocrine environment of pregnancy is involved in the onset of the pregnancy-related UA remodeling. We tested the hypothesis that the conceptus is not essential for the onset of UA remodeling of pregnancy. Six control and 18 pseudopregnant (Postcopulation Days 5, 11, and 17; $n = 6$ per subgroup) C57Bl/6 mice were killed and UAs were dissected and processed for either morphometric analysis or immunohistochemistry. The latter consisted of staining UA cross sections for the differentiation markers smooth muscle α -actin and smoothelin, and for the proliferation marker MKI67. We analyzed the UA changes in response to pseudopregnancy by ANOVA. Data are presented as mean \pm SD. By Day 11 of pseudopregnancy, the UA lumen was 25% wider and the media cross-sectional area 71% larger than in control mice. These differences were accompanied by reduced smoothelin expression and increased proliferation of UA medial VSMC. All UA morphological differences had returned or were in the process of returning to baseline values by Day 17 of pseudopregnancy. The structural and cellular aspects of UA remodeling as seen at midpregnancy are also seen in pseudopregnancy. These results support the concept that the conceptus does not contribute to the initiation of UA remodeling. We suggest that ovarian hormones trigger the onset of UA remodeling.

arterial remodeling, conceptus, mice, pregnancy, pseudopregnancy, uterine artery, uterus

INTRODUCTION

Mammalian pregnancy is characterized by important alterations in the maternal circulatory function [1]. Their importance is indicated by the clinical experience that complications of advanced pregnancy, such as fetal growth

restriction, are preceded by a defective hemodynamic adaptation in early pregnancy [2]. With advancing pregnancy, the rapidly increasing uterine blood flow (UBF) [3] is paralleled by structural changes in the uterine artery (UA) wall [4], which include medial hypertrophy and hyperplasia [5] along with dedifferentiation and proliferation of UA vascular smooth-muscle cells (VSMC) [6]. These structural and cellular adjustments serve to adapt the UA wall to the chronically elevated blood flow [7]. In mice, impaired UA remodeling in pregnancy, as previously observed with maternal aging [6] and in the absence of endothelial nitric oxide synthase (NOS3) [8], coincides with poor pregnancy outcome.

The mechanisms that regulate UA remodeling during pregnancy are poorly understood. In (nonpregnant) conditions of chronically elevated blood flow, arterial remodeling appears to be triggered by the mechanical forces exerted on the vessel wall [9]. Previously, in a study in pregnant mice, we described that the UA structure in the first half of pregnancy had changed profoundly [6] in spite of an only modest rise in UBF, but in a condition of markedly increasing circulating levels of a wide range of vasoactive substances [10]. The contribution of nitric oxide to these changes seems modest, as UA remodeling was impaired but not absent in pregnant *Nos3* knock-out mice [8]. The stimulus for UA remodeling in early pregnancy may come from the conceptus, although a recent study has shown that, during ectopic pregnancies, spiral arteries undergo structural alterations similar to those observed during intrauterine pregnancies [11]. Moreover, during the first trimester of human pregnancy, spiral arteries remodel before an increase in local blood flow [12]. This raises the question whether UA remodeling in pregnancy differs from arterial remodeling in other blood vessels because of the involvement of the endocrine environment. The UA contains estrogen receptors [13], and cellular responses to estrogen are mediated by both genomic and nongenomic mechanisms [14]. Therefore, ovarian hormones in general, and estrogen in particular, may play a role in UA remodeling during pregnancy.

To address this issue, we tested the hypothesis that the rapidly growing conceptus, with its progressively growing metabolism and endocrine output, does not contribute appreciably to the initiation of UA remodeling in pregnancy. To this end, we assessed in pseudopregnant mice the cellular and structural changes of UA using age-matched control mice as a reference. Pseudopregnancy in mice lasts approximately 10–12 days. The endocrine profile is characterized by increasing circulating levels of sex steroids resembling those of normal pregnancy [15] without the concomitant appearance of trophoblast factors. Information about the effect of pseudopregnancy on the UA will improve our insight in the contribution of the conceptus to the initial UA remodeling in pregnancy.

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²Correspondence: Louis L.H. Peeters, Department of Obstetrics and Gynecology, University Hospital Maastricht, azM, P.O. Box 5800, 6202 AZ Maastricht, The Netherlands. FAX: 31 43 387 4765; e-mail: lpe@sgyn.azm.nl

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MATERIAL AND METHODS

Animal care and experimental procedures were performed according to the guidelines of the institutional committee for the welfare of laboratory animals of the University of Maastricht.

Animal Preparation

Control animals were aged-matched virgin female C57BL/6 mice ($n = 6$) purchased from Charles River (Maastricht, The Netherlands). All mice were 12–14 wk of age and virgin at the time of study. They had free access to food and water and were maintained on a 12L:12D cycle. Pseudopregnancy was induced by mating with an experienced vasectomized male of similar age. The recovery of a vaginal semen plug was considered to correspond with Day 1 of pseudopregnancy. We subdivided 18 pseudopregnant mice into three equal groups and killed them on postcopulation Days 5, 11, and 17. All animals were weighed before sacrifice.

Tissue Preparation

As previously described [6], mice were anesthetized using an intraperitoneal injection of pentobarbital (10 mg/kg). Briefly, a midline incision was made from the abdomen to the neck. We dissected one main uterine and one carotid artery under a stereomicroscope (Zeiss) and stored these tissue aliquots at -80°C for later immunohistochemistry. Meanwhile, we left the contralateral uterine and carotid arteries in situ for perfusion and tissue fixation. To this end, we perfused the remainder of the arterial tree at 80–100 mm Hg with phosphate-buffered saline and 4% phosphate-buffered formalin, pH 7.4, both containing 0.1 mg/ml sodium-nitroprusside (Sigma Chemical, St. Louis, MO), through a catheter inserted into the left ventricular apex. This procedure enabled vessel fixation at the maximal diameter for the prevailing in vivo arterial pressure.

Histology, Morphometry, and Immunohistochemistry

The remaining main uterine and carotid artery, together with the abdominal and thoracic aortas, from control and pseudopregnant mice were dissected, fixed overnight in 4% phosphate-buffered formalin, and stored in ethanol. A segment of the carotid artery (3 mm in length) and a segment (1–2 mm) of the uterine artery at midpoint of the uterine arcade were embedded in paraffin and transversely sectioned in 4- μm slices. Cross sections were stained with Lawson solution (Boom, Meppel, The Netherlands) to visualize the internal and external elastic laminae. Internal and external circumferences, demarcated by the internal and external elastic laminae, were measured. From these values, lumen radius, media cross-sectional area (CSA), and media thickness were calculated for each section [16]. Additional cross sections were stained with eosin and hematoxylin, and the number of VSMC nuclei in the medial layer of the vessel wall of each cross section was counted. In addition, cross sections were stained with Sirius red to determine the collagen content in the media of each vessel. Cross sections stained with Lawson solution were used to determine the medial elastin content in each vessel. A computerized morphometry system (Sigma Scan; Jandel Scientific, Corte Madera, CA) was applied for both procedures.

Segments of frozen uterine arteries at midpoint of the uterine arcade and carotid arteries were cross-sectioned on a cryostat (5 μm) and mounted on gelatin-coated slides for immunohistochemistry. To determine the expression of vascular proteins during remodeling, vessels were stained for

smooth muscle α -actin (ACTA2) and smoothelin (SMTN), a vascular smooth-muscle cell differentiation marker [17]. Meanwhile, to quantify proliferation of VSMC during the process of remodeling, vessels were stained for the proliferation marker MKI67. Briefly, after blocking endogenous peroxidase activity, sections were treated with an avidin-biotin blocking kit (Vector Laboratories) followed by incubation with the biotinylated mouse monoclonal smoothelin antibody (R4A, 1:40). Smooth muscle α -actin and MKI67 reactivity were assessed by incubating the sections with a rabbit polyclonal antibody either against ACTA2 (1:3000, Sigma) or MKI67 (1:50, DAKO) followed by incubation with a swine anti-rabbit antibody (1:1000, Amersham Life Sciences). All sections were counterstained with hematoxylin. For negative controls, sections were incubated with the second antibody only and showed no immunoreactivity. All sections were evaluated blindly by three independent observers, and sections for ACTA2 and SMTN were scored semiquantitatively according to four levels of intensities: from 0, if no staining was detected, to 4, if the entire medial layer was stained. Proliferating VSMC, identified by MKI67 staining, displayed dark brown nuclei, while nondividing cells displayed blue nuclei because of counterstaining with hematoxylin. VSMC nuclei were counted in the media of each artery and the total number of proliferating and nonproliferating cells could be quantified. From these values, we calculated the number of proliferating VSMC as a proportion of total VSMC in each artery.

Statistical Analysis

All data are expressed as mean \pm SD, and as percentage change relative to the control reference values. Using ANOVA with Bonferoni correction, we tested the differences between pseudopregnant and control reference data. A probability of less than 0.05 was considered to indicate a statistically significant difference.

RESULTS

Mean body weight in the control (C) group was 20.0 ± 0.9 g. Maternal weight increased during pseudopregnancy to reach a maximum of 22.9 ± 1.4 g by Day 11 ($P < 0.05$, Table 1).

Uterine Artery Morphological Changes During Pseudopregnancy

Pseudopregnancy resulted in a 25% larger UA lumen radius ($P < 0.05$ as compared with control; Fig. 1A and Table 1) and a 71% larger UA media CSA on Day 11 of pseudopregnancy ($P < 0.05$ as compared with control; Fig. 1B and Table 1). These differences were accompanied by a larger UA media thickness ($P < 0.05$ as compared with control; Fig. 1C and Table 1) and wall-to-lumen ratio ($P < 0.05$ as compared with control; Table 1). All UA morphological differences had returned to baseline values by Day 17 of pseudopregnancy (Fig. 1, A–C, and Table 1).

Smooth-Muscle Cell and Extracellular Matrix Changes in the UA During Pseudopregnancy

Pseudopregnancy induced changes in the UA VSMC phenotype consistent with dedifferentiation and enhanced proliferation of the media UA VSMC. This was reflected by a significantly lower expression of SMTN (Fig. 2C) in concert with a significantly higher number of MKI67-positive nuclei in the UA medial layer (Fig. 2B) and significantly more VSMC

TABLE 1. Maternal weight and uterine artery structure during murine pseudopregnancy.

	Control ^a (n = 6)	Day 5 ^a (n = 6)	Day 11 ^a (n = 6)	Day 17 ^a (n = 6)
Body weight (g)	20.1 ± 0.9	21.0 ± 0.9	22.9 ± 1.4*	22.0 ± 1.2*
Radius (μm)	60 ± 4	71 ± 6	75 ± 4*	69 ± 4
Media CSA (μm ²)	1896 ± 373	2226 ± 302	3239 ± 358*	2344 ± 352
Media thickness (μm)	5 ± 0.3	5.1 ± 0.3	5.8 ± 0.4*	5.2 ± 0.4
Wall to lumen ratio	0.08 ± 0.01	0.08 ± 0.01	0.09 ± 0.02*	0.08 ± 0.01

^aData are expressed as mean ± SD; **P* < 0.05 relative to control mice.

per cross section (Fig. 2A) on Day 11 of pseudopregnancy. These differences were neither accompanied by a consistent change in the expression of ACTA2 (Fig. 2D) nor by a consistent change in UA collagen and elastin density (data not shown). In contrast with the increased lumen radius and media CSA and the reduced expression of SMTN (which were no longer statistically significant by Day 17), the number of media VSMC was still significantly elevated on Day 17 of pseudopregnancy (Fig. 2A). The smaller media CSA in combination with the stable number of VSMC indicates smooth-muscle cell atrophy.

Structural and Cellular Changes in Nonuterine Arteries During Pseudopregnancy

Pseudopregnancy did not induce structural or cellular changes in the carotid artery and abdominal and thoracic aortas (data not shown).

DISCUSSION

The present study was designed to test the hypothesis that the conceptus does not contribute appreciably to the initiation of UA remodeling during pregnancy. To this end, we studied morphological and cytological changes of the UA in a pseudopregnant mouse model. Our results indicate that the UA responds to pseudopregnancy by a transient 1.7- and 1.2-fold increase in media CSA and radius, respectively, demonstrating arterial wall growth and luminal expansion. In addition, with advancing pseudopregnancy, we observed smooth-muscle cell hyperplasia and a transient opposite change in the expression of SMTN and MKI67 in the UA media VSMC, indicating smooth-muscle cell dedifferentiation and proliferation. These structural and cellular adaptations appear to be specific to the UA because they do not occur in other parts of the arterial tree. Collectively, these results demonstrate that the conceptus is not involved in the initial remodeling response of the UA in an endocrine environment that mimics early pregnancy [15].

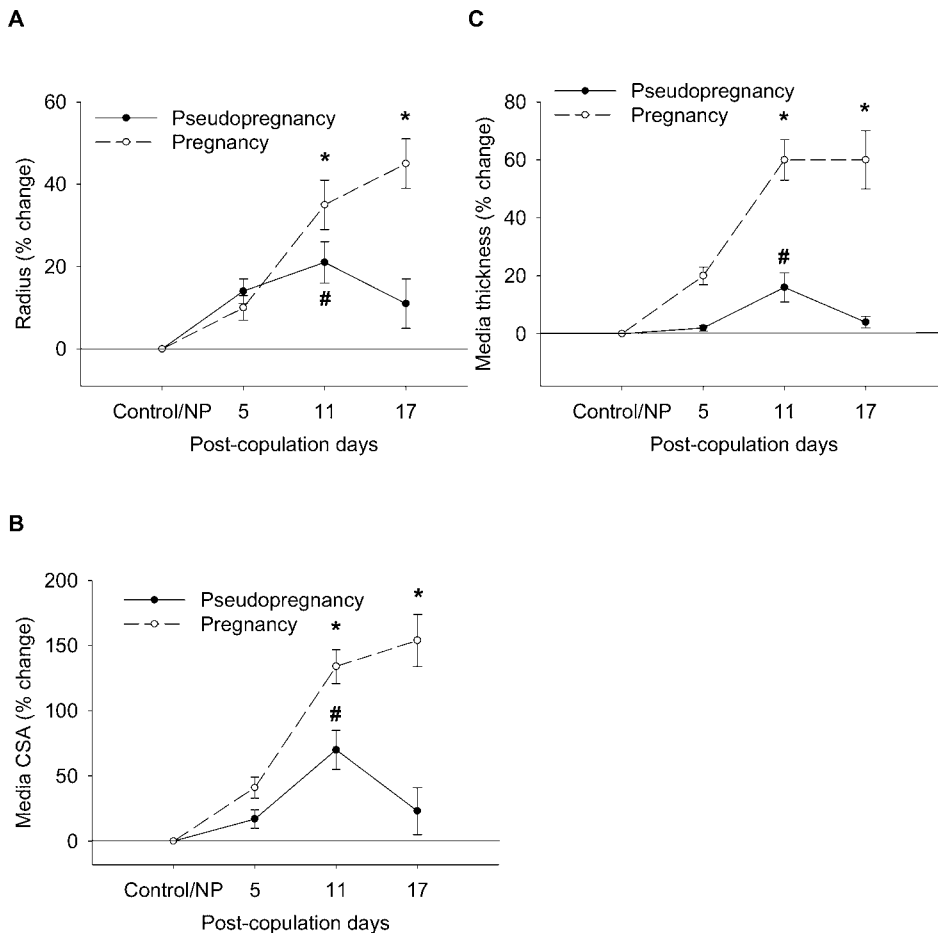
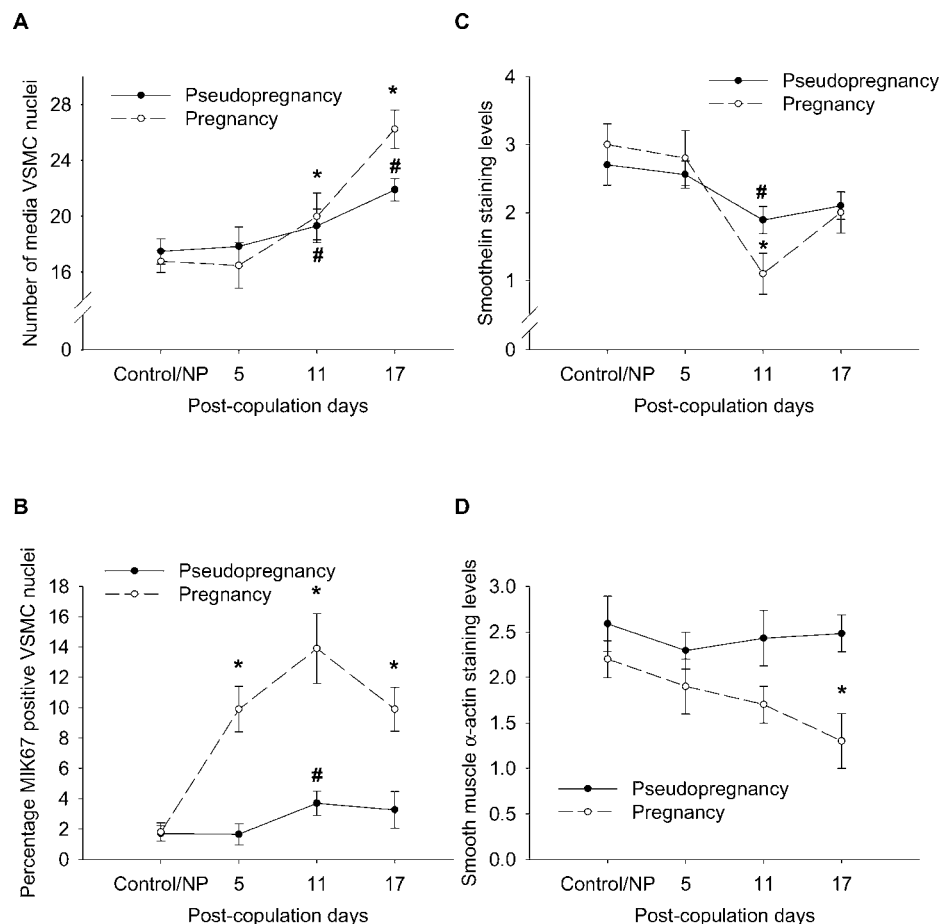


FIG. 1. Effect of pseudopregnancy on uterine artery (UA) structure. **A**) Radius, media CSA (**B**), and media thickness (**C**) of UA in pseudopregnant mice (whole line) and pregnant mice (dashed line, adapted from van der Heijden et al. [6]). Data are presented as percentage change relative to control mice (for the pseudopregnant mice group; whole line) and nonpregnant (NP) mice (for a pregnant mice group; dashed line, adapted from van der Heijden et al. [6]), and shown as mean ± SD. # *P* < 0.05 as compared with control; * *P* < 0.05 as compared with NP mice.

FIG. 2. Effects of pseudopregnancy on cytological properties of uterine artery (UA) smooth-muscle cells. **A**) Number of media vascular smooth-muscle cell (VSMC) nuclei, percentage MKI67-positive media VSMC nuclei (**B**), smoothelin (**C**), and smooth muscle α -actin immunoreactivity (**D**) of UA in pseudopregnant mice (whole line) and pregnant mice (dashed line, adapted from van der Heijden et al. [6]); # $P < 0.05$ as compared with control for pseudopregnant mice; * $P < 0.05$ as compared with nonpregnant (NP) mice for a pregnant mice group (adapted from van der Heijden et al. [6]). Data are shown as mean \pm SD.



Previously, we have shown that the UA responds to pregnancy by an increase in wall mass and lumen diameter [6, 8, 16], a response described as outward hypertrophic remodeling [18]. Arterial wall growth was characterized by a transient dedifferentiation of the media smooth-muscle cell in concert with VSMC proliferation. This is illustrated in Figures 1 and 2 with dashed lines. Whether this remodeling response is triggered by the increased metabolic and nutritional demands of the growing fetuses remains obscure. Because spiral arteries have been shown to undergo structural alterations in the absence of invading trophoblast [11], factors other than those from the conceptus may be involved in this remodeling response.

In the present study, pseudopregnancy resulted in transient outward UA remodeling. Maximal increases in lumen diameter (1.2-fold) and wall mass (1.7-fold) were observed by 11 days postcopulation, reaching levels approaching those previously observed in 11-days pregnant mice (1.33- and 2.3-fold, respectively) [6, 8, 16]. Moreover, these increases are considerably larger than those at midpregnancy in *Nos3* knock-out mice (1.07- and 1.23-fold, respectively) [8]. Collectively, these findings indicate that, while the growing fetuses may be required for the maintenance of UA remodeling throughout pregnancy, maternal factors are responsible for the initiation of UA structural changes through mechanisms that involve endothelial nitric oxide synthase.

Ovarian steroids influence cardiovascular function by direct and indirect effects on the vascular wall [14]. Estrogen is a potent vasodilator of the uterine vasculature [19, 20] and is involved in vascular growth responses [21]. Administration of estrogen induces a >10-fold increase in uterine blood flow [20], a process mediated in part by the increased expression

[19, 22] and stimulation of NOS3 [23, 24]. Estrogens can modulate arterial remodeling either by enhancing or inhibiting VSMC proliferation [21, 25]. While exogenous administration of estrogen has been shown to inhibit proliferation and migration of VSMC in vitro [25], pregnancy has been shown to stimulate growth [26] and DNA synthesis [27] of uterine smooth-muscle cells. This is in line with our findings that the increase in UA wall mass seems to result from VSMC hyperplasia, as indicated by the concomitant transient increase and decrease in MKI67 and smoothelin expression, respectively (Fig. 2, B and C). This change in VSMC gene expression is consistent with transient UA VSMC dedifferentiation, which is necessary to enable smooth-muscle cell proliferation. The outward UA remodeling in both pregnancy and pseudopregnancy may differ from arterial remodeling elsewhere by the presence of an extra stimulus for smooth-muscle cell proliferation in the UA. The actions of estrogens on the UA wall are mediated through estrogen receptors. However, the estrogen effect may be in part indirect. As a matter of fact, the angiogenic peptide vascular endothelial growth factor (VEGF) has been found to act as potent vasodilator of uterine vessels [28], and its expression may contribute to arterial remodeling [29]. The presence of estrogen enhances VEGF-induced UA dilation in pregnant rats [30], suggesting that VEGF is involved in pregnancy-related arterial remodeling [28].

Outward arterial remodeling serves to strengthen the vessel wall surrounding a wider lumen [31] and is needed to enable chronically elevated blood flow without a concomitant rise in wall shear stress [32]. The roles of maternal hormones and NOS3 in the initiation of this process may help prepare the

uterine vascular bed to supply nutrients and oxygen to the growing and developing fetuses at later stages of pregnancy.

In summary, the results of this study provide convincing evidence that UA remodeling during murine pseudopregnancy is similar to the one in early murine pregnancy. This supports our hypothesis that the process of UA remodeling in pregnancy is initiated without an appreciable role of the conceptus. A limitation of the present study is that these observations are made in mice and cannot be extrapolated to man or sheep.

REFERENCES

- Thornburg KL, Jacobson SL, Giraud GD, Morton MJ. Hemodynamic changes in pregnancy. *Semin Perinatol* 2000; 24:11–14.
- Duvekot JJ, Cheriex EC, Pieters FA, Peeters LL. Severely impaired fetal growth is preceded by maternal hemodynamic maladaptation in very early pregnancy. *Acta Obstet Gynecol Scand* 1995; 74:693–697.
- Dickey RP. Doppler ultrasound investigation of uterine and ovarian blood flow in infertility and early pregnancy. *Hum Reprod Update* 1997; 3:467–503.
- Osol G, Cipolla M. Pregnancy-induced changes in the three-dimensional mechanical properties of pressurized rat uteroplacental (radial) arteries. *Am J Obstet Gynecol* 1993; 168:268–274.
- Cipolla M, Osol G. Hypertrophic and hyperplastic effects of pregnancy on the rat uterine arterial wall. *Am J Obstet Gynecol* 1994; 171:805–811.
- van der Heijden OW, Essers YP, Simkens LH, Teunissen QG, Peeters LL, De Mey JG, van Eys GJ. Aging blunts remodeling of the uterine artery during murine pregnancy. *J Soc Gynecol Invest* 2004; 11:304–310.
- Cowan DB, Langille BL. Cellular and molecular biology of vascular remodeling. *Curr Opin Lipidol* 1996; 7:94–100.
- van der Heijden OW, Essers YP, Fazzi G, Peeters LL, De Mey JG, van Eys GJ. Uterine artery remodeling and reproductive performance are impaired in endothelial nitric oxide synthase-deficient mice. *Biol Reprod* 2005; 72:1161–1168.
- Langille BL. Arterial remodeling: relation to hemodynamics. *Can J Physiol Pharmacol* 1996; 74:834–841.
- Bird IM, Zhang L, Magness RR. Possible mechanisms underlying pregnancy-induced changes in uterine artery endothelial function. *Am J Physiol Regul Integr Comp Physiol* 2003; 284:R245–R258.
- Craven CM, Morgan T, Ward K. Decidual spiral artery remodelling begins before cellular interaction with cytotrophoblasts. *Placenta* 1998; 19:241–252.
- Jauniaux E, Watson AL, Hempstock J, Bao YP, Skepper JN, Burton GJ. Onset of maternal arterial blood flow and placental oxidative stress. A possible factor in human early pregnancy failure. *Am J Pathol* 2000; 157:2111–2122.
- Liao WX, Magness RR, Chen DB. Expression of estrogen receptors- α and - β in the pregnant ovine uterine artery endothelial cells in vivo and in vitro. *Biol Reprod* 2005; 72:530–537.
- Mendelsohn ME, Karas RH. The protective effects of estrogen on the cardiovascular system. *N Engl J Med* 1999; 340:1801–1811.
- Mann RJ, Keri RA, Nilson JH. Transgenic mice with chronically elevated luteinizing hormone are infertile due to anovulation, defects in uterine receptivity, and midgestation pregnancy failure. *Endocrinology* 1999; 140:2592–2601.
- Hilgers RH, Bergaya S, Schiffers PM, Meneton P, Boulanger CM, Henrion D, Levy BI, De Mey JG. Uterine artery structural and functional changes during pregnancy in tissue Kallikrein-deficient mice. *Arterioscler Thromb Vasc Biol* 2003; 23:1826–1832.
- van der Loop FT, Schaart G, Timmer ED, Ramaekers FC, van Eys GJ. Smoothelin, a novel cytoskeletal protein specific for smooth muscle cells. *J Cell Biol* 1996; 134:401–411.
- Gibbons GH, Dzau VJ. The emerging concept of vascular remodeling. *N Engl J Med* 1994; 330:1431–1438.
- 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.
- Magness RR, Phernetton TM, Zheng J. Systemic and uterine blood flow distribution during prolonged infusion of 17 β -estradiol. *Am J Physiol* 1998; 275:H731–743.
- Lamping KG, Christensen LP, Tomanek RJ. Estrogen therapy induces collateral and microvascular remodeling. *Am J Physiol Heart Circ Physiol* 2003; 285:H2039–2044.
- 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:H2134–2142.
- Weiner CP, Knowles RG, Moncada S. Induction of nitric oxide synthases early in pregnancy. *Am J Obstet Gynecol* 1994; 171:838–843.
- Weiner CP, Lizasoain I, Baylis SA, Knowles RG, Charles IG, Moncada S. Induction of calcium-dependent nitric oxide synthases by sex hormones. *Proc Natl Acad Sci U S A* 1994; 91:5212–5216.
- Ling S, Dai A, Dilley RJ, Jones M, Simpson E, Komesaroff PA, Sudhir K. Endogenous estrogen deficiency reduces proliferation and enhances apoptosis-related death in vascular smooth muscle cells: insights from the aromatase-knockout mouse. *Circulation* 2004; 109:537–543.
- Keyes LE, Moore LG, Walchak SJ, Dempsey EC. Pregnancy-stimulated growth of vascular smooth muscle cells: importance of protein kinase C-dependent synergy between estrogen and platelet-derived growth factor. *J Cell Physiol* 1996; 166:22–32.
- Keyes LE, Majack R, Dempsey EC, Moore LG. Pregnancy stimulation of DNA synthesis and uterine blood flow in the guinea pig. *Pediatr Res* 1997; 41:708–715.
- Ni Y, May V, Braas K, Osol G. Pregnancy augments uteroplacental vascular endothelial growth factor gene expression and vasodilator effects. *Am J Physiol* 1997; 273:H938–944.
- Sho E, Komatsu M, Sho M, Nanjo H, Singh TM, Xu C, Masuda H, Zarins CK. High flow drives vascular endothelial cell proliferation during flow-induced arterial remodeling associated with the expression of vascular endothelial growth factor. *Exp Mol Pathol* 2003; 75:1–11.
- Stormont JM, Meyer M, Osol G. Estrogen augments the vasodilatory effects of vascular endothelial growth factor in the uterine circulation of the rat. *Am J Obstet Gynecol* 2000; 183:449–453.
- De Mey JG, Schiffers PM, Hilgers RH, Sanders MM. Toward functional genomics of flow-induced outward remodeling of resistance arteries. *Am J Physiol Heart Circ Physiol* 2005; 288:H1022–1027.
- Girerd X, London G, Boutouyrie P, Mourad JJ, Safar M, Laurent S. Remodeling of the radial artery in response to a chronic increase in shear stress. *Hypertension* 1996; 27:799–803.