**Human Reproduction** vol.14 no.7 pp.1885–1889, 1999

# Pregnancy is not associated with altered morphology of the femoral artery

**Sofija Jovanović[1,3,4], Zdenka Blagojević[2], Verica Mrvić[2], Zora Nikolić[2] and Aleksandar Jovanović[1,3]**

[1]Division of Cardiovascular Diseases, Department of Medicine, Guggenheim-7, Mayo Clinic and Foundation, Rochester, MN 55905, USA and [2]Department of Anatomy, Veterinary Faculty, Belgrade, Yugoslavia

[3]Present address: Department of Child Health, Ninewells Hospital and Medical School, University of Dundee, Dundee DD1 4HN, Scotland, UK

[4]To whom correspondence should be addressed: Department of Child Health, Ninewells Hospital and Medical School, University of Dundee, Dundee DD1 4HN, Scotland, UK

**While pregnancy is associated with adjustments in cardiovascular function, the morphology of the vascular system during pregnancy has been generally viewed as being very stable. However, recently we have demonstrated that pregnancy remodels the aorta and the carotid artery. In the present study, we assessed the morphological characteristics of the guinea-pig femoral artery during different stages of pregnancy using light and electron microscopy. There were no significant differences between external and internal diameters, wall thickness, total cross-sectional area and cross-sectional areas of lumen, intima, media, and adventitia of femoral arteries from non-pregnant and early-, mid- and late-pregnant guinea-pigs ($n$ = 8–10). In previous studies, we have shown that the morphology of vascular smooth muscle and endothelial cells in the aorta and the carotid artery may be altered by pregnancy. Therefore, to test this possibility we measured diameters as well as cross-sectional areas of femoral arterial muscle and endothelial cells using electron microscopy. These parameters, at the electron microscopy level, were also not significantly changed by pregnancy ($n$ = 8–10). It is concluded that the morphology of the guinea-pig femoral artery is not altered during pregnancy. In this regard, this study demonstrated that pregnancy-induced vascular remodelling varies between blood vessels that undergo the same functional alterations. Therefore, this may suggest that pregnancy-induced changes in blood flow through different vascular beds are not the most important factor involved in vascular remodelling observed during pregnancy. Rather, it is possible that haemodynamic-independent factors regulate pregnancy-mediated structural changes of the vascular wall.**

*Key words:* endothelium/femoral artery/pregnancy/remodelling/stereology

## Introduction

It is known that cardiac output is increased during pregnancy, which is associated with increased rates of perfusion to both reproductive and non-reproductive organs (Rosenfeld, 1977). This vascular adaptation of mothers to pregnancy is considered to be due to blunted vascular response to vasoconstrictors on the one hand (Weiner *et al*., 1991; Grbović and Jovanović, 1996, 1997; Jovanović *et al*., 1998b), and increased response to vasodilators on the other (Weiner *et al*., 1989), although this concept has been repeatedly challenged (Jovanović *et al*., 1994a, 1995a,b,c, 1997).

While pregnancy is associated with adjustments in cardiovascular function, the morphology of the vascular system during pregnancy has been generally viewed as being very stable (Rosenfeld, 1977; Peeters *et al*., 1980). However, recently we have demonstrated that pregnancy remodels the aorta and the carotid artery (Jovanović and Jovanović, 1997, 1998). Specifically, it has been shown that pregnancy induces hypotrophy of vascular endothelium in guinea pig aorta and carotid artery as well as hypotrophy of vascular smooth muscle in the guinea-pig carotid artery (Jovanović and Jovanović, 1997, 1998). Therefore, in the present study, we addressed further the effect of pregnancy on the morphological characteristics of the cardiovascular system, i.e. we assessed the morphological characteristics of the guinea-pig femoral artery during different stages of pregnancy.

Here, we report that pregnancy does not alter the morphology of the femoral artery.

## Materials and methods

Non-pregnant female guinea-pigs and early-pregnant (day 22 of pregnancy), mid-pregnant (day 44 of pregnancy) and late-pregnant (days 64–66 of pregnancy) guinea-pigs were used in this study (8–10 in each group), as previously described (Jovanović and Jovanović, 1997, 1998). Briefly, guinea-pigs were anaesthetized with sodium pentobarbital (40 mg/100 g, i.p.), the femoral artery was removed, cut, fixed in 10% buffered neutral formaldehyde (48 h) (Fluka, Buchs, Switzerland), dehydrated through a graded series of ethanol solutions (70–100%) and embedded in paraffin wax. Resultant blocks were cut at 3 μm (Sorval JB-4 microtome, Newtown, CT, USA). Sections were stained with haematoxylin and eosin and each section was examined under ×20 magnification (Olympus Vanox microscope, Tokyo, Japan). For electron microscopy femoral arteries were cut into rings (1 mm long), fixed in 3% glutaraldehyde (Fluka) for 24 h and after rinsing in 0.1 mol/l cacodylate buffer (pH 7.4) (Fluka) postfixed in 2% osmium tetroxide in cacodylate solution (Fluka) (1 h). The specimens were dehydrated in increasing concentrations (70–100%) of ethanol, and then passed through propylene oxide and embedded in Araldite (Jovanović and Jovanović, 1997, 1998;

Jovanović *et al*., 1998a). Ultrathin sections (50–70 nm) were cut with a diamond knife. The sections were stained with uranyl acetate (Fluka) and Reynold's lead citrate (Fluka) and then examined and photographed using a Philips EM 400-HMG electron microscope (Eindhoven, The Netherlands). The investigation conformed with the 'Guide for the Care and Use of Laboratory Animals' (NIH publication 85–23, revised 1985).

### *Morphometric and stereological analysis*

*Light microscopy*

Sections that included the entire circumference of each ring were cut from five different blocks of each animal and viewed with light microscopy. For each block five sections were examined. Maximal and minimal internal and external diameters were determined at magnification ×20, and these values were used to determine the mean values of internal and external diameters. Wall thickness was calculated by subtraction of the two diameters and dividing by 2 (Jovanović and Jovanović, 1997, 1998; Jovanović *et al*., 1998a). Cross-sectional areas were determined using the Weibel M42 standard point-counting system (Weibel, 1979; Jovanović and Jovanović, 1997, 1998). The cross-sectional areas were calculated using the following equation (Weibel, 1979; Lee *et al*., 1983): $A_m = (P_i/P_t) \times A_g$; where $A_m$ = cross-sectional area, $P_i$ = number of points falling on specific layer, $P_t$ = total points of test grid and $A_g$ = area of the whole test grid for the appropriate magnification (×20). Correction for eccentricity due to sectioning angle with reference to the long axis of the vessel was used for all calculations (Lee *et al*., 1983; Jovanović and Jovanović, 1997, 1998; Jovanović *et al*., 1998a): correction = $d_1/d_2$ where $d_1$ = minimal radius of vessel, $d_2$ = maximal radius of vessel. The definite form of the equation used was $A_m = (P_i/P_t) \times Ag \times (d_1/d_2)$.

*Electron microscopy*

Morphometric analyses were made from 20 random electron micrographs (five micrographs per block, four blocks per aorta) obtained at ×2800–7900 magnification. The size of endothelial and smooth muscle cells, defined as cross-sectional area (Lee *et al*., 1983), were determined using the B 100 standard point-counting system (Weibel, 1979). To avoid errors in making average estimates of cell objects without having information about individual objects, the point counting was performed by eye, and the cross-sectional area of each individual cell profile was determined.

Cross-sectional area was calculated using the following equation $A = P_i \times d'$ (Weibel, 1979); where A is the cell cross-sectional area; $P_i$ is the number of points falling on cell cross-sectional area; d is the distance between the nearest points of the grid; d′ is the distance between the nearest points of the grid corrected for magnification: d′ = d/magnification (Weibel, 1979; Lee *et al*., 1983).

### *Statistical analysis*

For all vessel parameters, separate data measurements of diameters, wall thickness and cross-sectional profiles of vascular components from each animal were respectively pooled and an average value recorded, so that in the analysis each animal contributed only one value for each parameter. All data were tested by the Kolmogorov Goodness of Fit test and were found to be normally distributed. The results are expressed as means ± SEM with range of values in parenthesis; *n* refers to the number of animals. One-way analysis of variance (ANOVA) was used when more than two groups were analysed and the individual pregnant groups were compared with the non-pregnant one, which served as a control. A value of $P < 0.05$ was considered to be statistically significant.

**Table I.** Values of external and internal diameters and wall thickness of guinea-pig femoral artery in different stages of pregnancy. Values are expressed as mean ± SEM ($n$ = 8–10)

| Stage of pregnancy | External ($10^2$ μm) | Internal ($10^2$ μm) | Wall thickness ($10^2$ μm) |
|---|---|---|---|
| Non-pregnant | 13.9 ± 1.9 | 6.6 ± 0.5 | 3.66 ± 0.32 |
| Early-pregnant | 13.9 ± 17 | 6.4 ± 0.6 | 3.72 ± 0.39 |
| Mid-pregnant | 13.4 ± 1.9 | 5.7 ± 0.6 | 3.83 ± 0.43 |
| Late-pregnant | 13.2 ± 1.1 | 5.8 ± 0.4 | 3.87 ± 0.49 |

**Table II.** Values of minimal and maximal diameters of individual endothelial cells in guinea-pig femoral artery in different stages of pregnancy. Values are expressed as mean ± SEM ($n$ = 8–10)

| Stage of pregnancy | Minimal (μm) | Maximal (μm) |
|---|---|---|
| Non-pregnant | 3.1 ± 0.4 | 6.8 ± 0.6 |
| Early-pregnant | 3.2 ± 0.3 | 6.2 ± 0.9 |
| Mid-pregnant | 3.6 ± 0.5 | 6.4 ± 0.9 |
| Late-pregnant | 3.3 ± 0.4 | 6.3 ± 0.8 |

**Table III.** Values of minimal and maximal diameters of individual smooth muscle cells in guinea-pig femoral artery in different stages of pregnancy. Values are expressed as mean ± SEM ($n$ = 8–10)

| Stage of pregnancy | Minimal (μm) | Maximal (μm) |
|---|---|---|
| Non-pregnant | 3.9 ± 0.4 | 12.9 ± 2.1 |
| Early-pregnant | 4.2 ± 0.6 | 13.4 ± 1.7 |
| Mid-pregnant | 4.4 ± 0.7 | 14.1 ± 1.9 |
| Late-pregnant | 3.7 ± 0.6 | 13.3 ± 1.4 |

## Results

### *Light microscopy*

The values of external and internal diameters and wall thickness of femoral artery from guinea-pigs in different stages of pregnancy are shown in Table I. There were no statistically significant differences between those values ($n$ = 8–10). To examine more precisely the influence of pregnancy stage on the morphology of the femoral artery, we calculated the cross-sectional areas of different arterial layers in non-pregnant, early-, mid- and late-pregnant animals (Figure 1). The values of cross-sectional areas were not significantly different between animals in different stages of pregnancy (Figure 1; $n$ = 8–10).

### *Electron microscopy*

Obtained values of maximal and minimal diameters of endothelial and smooth muscle cells are presented in Tables II and III respectively. Photomicrographs of endothelial and muscle cells from non-pregnant and late-pregnant guinea-pig femoral arteries are presented in Figure 2, while values for cross-sectional areas of endothelial and muscle cells during all stages of pregnancy are presented in Figure 3. Statistical analysis revealed that diameters and cross-sectional areas of individual endothelial and smooth muscle cells were not significantly changed by pregnancy in both cases ($n$ = 8–10).

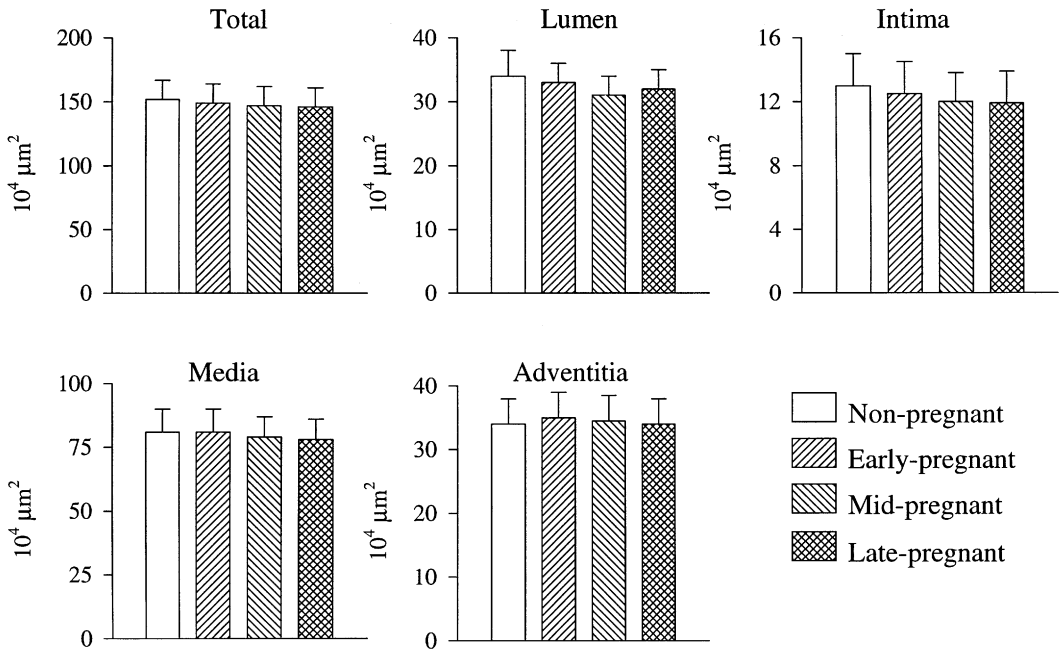


**Figure 1.** Total cross-sectional area and cross-sectional areas of the lumen, intima, media and adventitia of guinea-pig femoral artery at different stages of pregnancy. Bars represent mean ± SEM ($n$ = 8–10).

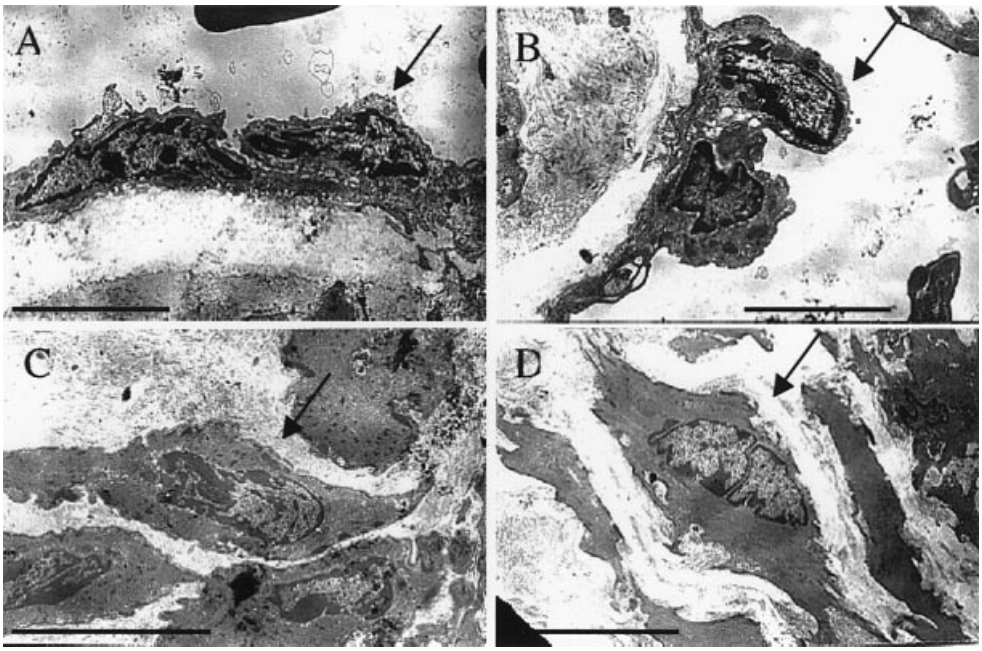


**Figure 2.** Original photomicrographs of endothelial (**A** and **B**) and muscle cells (**C** and **D**) from non-pregnant (**A** and **C**) and late-pregnant (**B** and **D**) guinea-pig femoral artery. Arrows delineate endothelial (**A** and **B**) and muscle cells (**C** and **D**). Scale bars represent 5 µm.

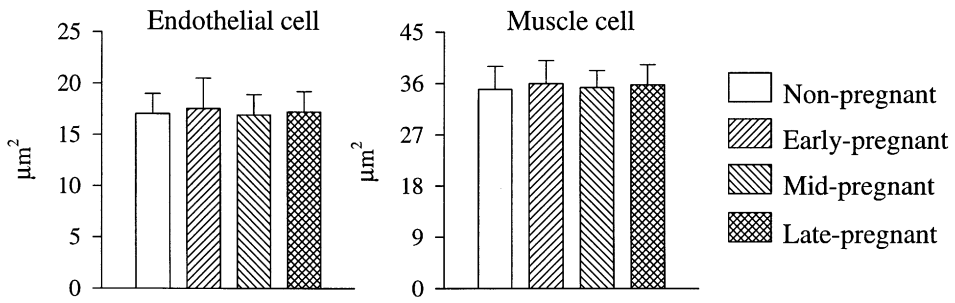


**Figure 3.** Cross-section areas of endothelial and smooth muscle cells from guinea-pig femoral artery in different stages of pregnancy. Bars represent mean ± SEM ($n$ = 8–10).

## Discussion

In different animal species, including humans, it has been reported that pregnancy is associated with changes of blood flow throughout the body (Peeters *et al*., 1980; Easterling *et al*., 1991; Magness *et al*., 1991). It has been postulated that this cardiovascular adaptation to pregnancy may be due to altered vascular reactivity toward neurotransmitters and humoral factors, leading to general maternal vasodilatation (Conrad *et al*., 1991; Grbović and Jovanović, 1996; Jovanović *et al*., 1998b).

As opposed to the aorta and the mesenteric and uterine arteries, the effect of pregnancy on the function and morphology of the femoral artery has been less investigated. In fact, there are no reports that addressed the outcome of haemodynamic changes on the femoral artery morphology. So far, it has been demonstrated that the reactivity of the femoral artery to vasodilator substances is increased during pregnancy (Ahokas *et al.*, 1991). Previously, Awal *et al*. (1995) have shown that, as observed in the uterine artery (Osol and Cipolla, 1993; Cipolla and Osol, 1994), pregnancy increases the diameter of the femoral artery. In contrast, here we demonstrate that the diameter of the guinea-pig femoral artery is not altered by pregnancy. This disagreement may be due to the differences in species examined in these two studies. Specifically, Awal *et al*. (1995) performed their study on rat blood vessels, while the current study used guinea-pigs. Guinea-pigs were specifically used in the present study, having in mind that functional changes in the cardiovascular system during guinea-pig pregnancy are apparently similar to that in humans (Jovanović *et al*., 1994b,c, 1995b,c,d, 1998c).

To test definitively the possibility that changes in the morphology of the femoral artery during pregnancy do occur, we applied morphometry and stereology on electron micrographs, as previously described (Jovanović and Jovanović, 1997, 1998; Jovanović *et al*., 1998a, 1999). This analysis confirmed our findings obtained with light microscopy, and provided more evidence for the notion that the morphology of the intima and media of the guinea-pig femoral artery is not altered by pregnancy. Therefore, the use of precise morphometric and stereological methods, at the level of both light and electron microscopy, allows us to conclude that pregnancy is without effects on the morphology of the guinea-pig femoral artery. Previously, we have reported that pregnancy remodels guinea-pig aorta and carotid artery (Jovanović and Jovanović, 1997, 1998) in a similar way. However, during pregnancy blood flow is increased through the aorta, but decreased through the carotid artery (Rosenfeld, 1977). Here, we demonstrated that the femoral artery, which is characterized by pregnancy-associated functional alterations, is not morphologically changed by pregnancy. Taken together, these studies, as well as the present findings, may suggest that pregnancy-induced changes in blood flow through different vascular beds are not the most important factor involved in the vascular remodelling observed during pregnancy. Rather, it is possible that haemodynamic-independent factors regulate pregnancy-mediated structural changes of the vascular wall.

## Acknowledgements

This work was supported by the Merck Company Foundation and the American Heart Association.

## References

Ahokas, R.A., Mercer, B.M. and Sibai, B.M. (1991) Enhanced endothelium-derived relaxing factor activity in pregnant, spontaneously hypertensive rats. *Am. J. Obstet. Gynecol.*, **165,** 801–807.

Awal, M.A., Matsumoto, M. and Nishinakagawa, H. (1995) Morphometrical changes of the arterial walls of main arteries from heart to the abdomino–inguinal mammary glands of rat from virgin through pregnancy, lactation and post weaning. *J. Vet. Med. Sci.*, **57,** 251–256.

Cipolla, M. and Osol, G. (1994) Hypertrophic and hyperplastic effects of pregnancy on the rat uterine arterial wall. *Am. J. Obstet. Gynecol.*, **171,** 805–811.

Conrad, K.P., Barrera, S.A., Friedman, P.A. and Schmidt, V.M. (1991) Evidence for attenuation of myo-inositol uptake, phosphoinositide turnover and inositolphosphate production in aortic vasculature of rats during pregnancy. *J. Clin. Invest.*, **87,** 1700–1709.

Easterling, T.R., Benedetti, T.J., Schmucker, B.C. *et al.* (1991) Maternal hemodynamics and aortic diameter in normal and hypertensive pregnancies. *Obstet. Gynecol.*, **78,** 1073–1077.

Grbović, L. and Jovanović, A. (1996) Effect of the vascular endothelium on contractions induced by prostaglandin $F_{2\alpha}$ in isolated pregnant guinea-pig uterine artery. *Hum. Reprod.*, **11,** 2041–2047.

Grbović, L. and Jovanović, A. (1997) Indomethacin reduces prostaglandin $F_{2\alpha}$-induced contraction of guinea-pig uterine artery with both intact and denuded endothelium. *Prostaglandins*, **53,** 371–379.

Jovanović, S. and Jovanović, A. (1997) Remodeling of guinea-pig aorta during pregnancy: a selective alterations of endothelial cells. *Hum. Reprod.*, **12,** 2297–2302.

Jovanović, S. and Jovanović, A. (1998) Pregnancy is associated with hypotrophy of carotid artery endothelial and smooth muscle cells. *Hum. Reprod.*, **13,** 1074–1078.

Jovanović, A., Grbović, L., Drekić, D. and Novaković, S. (1994a) Muscarinic receptor function in the guinea-pig uterine artery is not altered during pregnancy. *Eur. J. Pharmacol.*, **258,** 185–194.

Jovanović, A., Grbović, L. and Tulić, I. (1994b) Predominant role for nitric oxide in the relaxation induced by acetylcholine in human uterine artery. *Hum. Reprod.*, **9,** 387–393.

Jovanović, A., Grbović, L. and Tulić, I. (1994c) Endothelium-dependent relaxation in human uterine artery in response to acetylcholine. *Eur. J. Pharmacol.*, **256,** 131–139.

Jovanović, A., Grbović, L. and Jovanović, S. (1995a) Effect of the vascular endothelium on noradrenaline-induced contractions in non-pregnant and pregnant guinea-pig uterine arteries. *Br. J. Pharmacol.*, **114,** 805–815.

Jovanović, A., Grbović, L. and Jovanović, S. (1995b) $K^+$ channel blockers do not modify relaxation of guinea-pig uterine artery evoked by acetylcholine. *Eur. J. Pharmacol.*, **280,** 95–100.

Jovanović, A., Grbović, L., Jovanović, S. and Zikić, I. (1995c) Effect of pregnancy on vasopressin-mediated responses in guinea-pig uterine arteries with intact and denuded endothelium. *Eur. J. Pharmacol.*, **280,** 101–111.

Jovanović, A., Grbović, L., Zikić, I. and Tulić, I. (1995d) Characterization of arginine vasopressin actions in human uterine artery: lack of role of the vascular endothelium. *Br. J. Pharmacol.*, **115,** 1295–1301.

Jovanović, A., Jovanović, S. and Grbović, L. (1997) Endothelium-dependent relaxation in pregnant guinea-pig uterine artery in response to acetylcholine. *Hum. Reprod.*, **12,** 1805–1809.

Jovanović, S., Blagojević, Z. and Mrvić, V. (1998a) Morphometrical and stereological analysis of nonpregnant female guinea-pig aorta. *Acta Veterinaria (Belgrade)*, **48,** 191–195.

Jovanović, A., Jovanović, S. and Grbović, L. (1998b) Characterization of oxytocin actions in guinea-pig isolated uterine artery: the effect of pregnancy. *Eur. J. Pharmacol*, **343,** 35–42.

Jovanović, A., Jovanović, S., Tulić, I. and Grbović, L. (1998c) Predominant role for nitric oxide in the relaxation induced by vasoactive intestinal polypeptide in human uterine artery. *Mol. Hum. Reprod.*, **4,** 71–76.

Jovanović, S., Grantham, A.J., Tarara, J.E. *et al.* (1999) Increased number of cardiomyocytes in cross-sections from tachycardia-induced cardiomyopathic hearts. *Int. J. Mol. Med.*, **3,** 153–155.

Lee, R.M.K.W., Forrest, B.J., Garfield, E.R. and Daniel, E.E. (1983)

Ultrastructural changes in mesenteric arteries from spontaneously hypertensive rats. A morphometric study. *Blood Vessels*, **20,** 72–91.

Magness, R.R., Rosenfeld, C.R. and Carr, B.R. (1991) Protein kinase C in uterine and systemic arteries during ovarian cycle and pregnancy. *Am. J. Physiol.*, **260,** E464–E470.

Osol, G. and Cipolla, M. (1993) Pregnancy-induced changes in the three-dimensional mechanical properties of pressurized rat uteroplacental (radial) arteries. *Am. J. Obstet. Gynecol.*, **168,** 268–274.

Peeters, L.L.H., Grutters, G. and Martin, C.R. (1980) Distribution of cardiac output in the unstressed pregnant guinea-pig. *Am. J. Obstet. Gynecol.*, **138,** 1177–1184.

Rosenfeld, C.R. (1977) Distribution of cardiac output in ovine pregnancy. *Am. J. Physiol.*, **232,** H231–H235.

Weibel, E.R. (1979) *Stereological Methods. Vol. 1. Practical Methods for Biological Morphometry.* Academic Press, San Francisco.

Weiner, C., Martinez, E., Zhu, L.K. *et al.* (1989) *In vitro* release of endothelium-derived relaxing factor by acetylcholine is increased during the guinea pig pregnancy. *Am. J. Obstet. Gynecol.*, **161,** 1599–1605.

Weiner, C., Zhu, L.K., Thompson, L. *et al.* (1991) Effect of pregnancy on endothelium and smooth muscle: their role in reduced adrenergic sensitivity. *Am. J. Physiol.*, **261,** H1275–H1283.

*Received on December 29, 1998; accepted on March 22, 1999*