Human Reproduction Update 2000, Vol. 6, No. 4 pp. 351–363 

# Vascular effects of environmental oestrogens: implications for reproductive and vascular health

**Raghvendra K.Dubey[1,2,*], Marinella Rosselli[2], Bruno Imthurn[2], Paul J.Keller[2] and Edwin K.Jackson[1]**

[1]Center for Clinical Pharmacology, Department of Medicine, University of Pittsburgh Medical Center, Pittsburgh, USA and [2]University Hospital Zurich, Department of Obstetrics and Gynecology, Clinic for Endocrinology, Zurich, Switzerland

**Environmental oestrogens are defined as xenobiotics structurally resembling oestrogen, and are divided into two broad categories, xeno-oestrogens and phyto-oestrogens. Environmental oestrogens may contribute importantly to the increased incidence of reproductive disorders in the modern environment. Although the mechanisms by which environmental oestrogens induce their deleterious effects on the reproductive system remain poorly defined, it is likely that the vascular effects of these compounds play a critical role. In this regard, oestradiol strongly regulates both angiogenesis and vascular remodelling by influencing the growth and function of vascular endothelial cells (EC) and smooth muscle cells (SMC). Since blood vessels, by undergoing angiogenesis, vascular regression and vascular remodelling, actively participate in the normal functioning of reproductive organs, environmental oestrogens—by mimicking or antagonizing the vascular effects of oestradiol—may induce abnormalities in vascular function and structure leading to reproductive disorders such as pre-eclampsia, endometriosis, impaired follicular development, inefficient implantation, impotence and infertility. The purpose of the present review is to summarize the evidence regarding the vascular effects of xeno-oestrogens and phyto-oestrogens and to discuss the implications for these effects on the reproductive system.**

*Keywords:* angiogenesis/endothelial cells/environmental oestrogens/smooth muscle cells/vascular remodelling

## TABLE OF CONTENTS



## Introduction

### *Role of the vasculature in reproductive physiology and pathophysiology*

Blood vessels form an integral part of every reproductive organ, and play an active role in reproductive physiology and pathophysiology. The vasculature serves as a conduit to supply reproductive organs with nutrients, oxygen, paracrine factors, endocrine hormones and cells for immunological defence, restricts penetration of deleterious agents into reproductive tissues, and regulates the temperature of reproductive organs. In males, vasodilatation and dynamic changes within the vasculature are key regulators of penile erection. Thus, the formation (angiogenesis), structural adaptation (vascular remodelling) and functional regulation (vasodilation/vasoconstriction) of blood vessels in reproductive tissues are critical elements for a healthy reproductive system.

Angiogenesis and its antithesis, i.e. blood vessel regression, are involved in the physiology and pathophysiology of multiple reproductive organs including the ovaries, uterus, oviduct and placenta. For instance, ovarian angiogenesis is a requirement for the early stages of folliculogenesis and luteal growth, and also plays an important role in the process of follicular atresia and luteal regression (Suzuki *et al*., 1998). Changes in the microvasculature prelude the formation of the interstitial gland of the ovary, and thecal capillary angiogenic processes support a gradual increase of ovarian blood flow during follicle growth (Macchiarelli *et al.*, 1993). On the other hand, reduced thecal blood flow is associated with advanced atresia of antral follicles (Carson *et al*., 1986).

Angiogenic processes in the ovary and uterus occur in a cyclic fashion. In the ovary, the massive sprouting of blood vessels in the growing follicle occurs during the first third of the ovarian

*To whom correspondence should be addressed at: Center for Clinical Pharmacology, Department of Medicine, 623 Scaife Hall, 200 Lothrop Street, University of Pittsburgh Medical Center, Pittsburgh, PA 15213-2582, USA. Tel: 412-648-1875; Fax: 412-648-7107; e-mail: dubey@med1.dept-med.pitt.edu

cycle. However, during the luteolysis and for several weeks thereafter, all newly formed vessels regress, suggesting that both angiogenesis and regression of blood vessels are essential for regulating ovarian function and folliculogenesis. Moreover, recent data provide convincing evidence that the regulated angiogenesis within the ovary not only participates in the development of the follicles, but also is a prerequisite for embryo implantation. In this regard, human studies show that high-grade follicular vascularity is associated with increased pregnancy rates, suggesting a possible link between follicular vascularity and implantation potential (Chui *et al.*, 1997). Direct evidence for the effects of vascularity on embryo implantation comes from in-vitro fertilization studies, where the pregnancy rates are significantly higher for transferred embryos derived from follicles with high-grade vascularity as compared with follicles with medium- and low-grade vascularity (Bhal *et al*., 1999). In contrast, no correlation exists between follicular morphology and vascularity grade, suggesting that follicular vascularity plays an important role in the health of follicles and is a better predictor of successful implantation.

Differences in the degree of perifollicular vascularity correlate with differences in the dissolved oxygen content of the corresponding follicular fluid. Oocytes with cytoplasmic and chromosomal disorders and embryos with multinucleated blastomeres and limited developmental ability are derived predominantly from underoxygenated follicles (Van Blerkom *et al*., 1997; Van Blerkom, 1998), suggesting that oxygen content increases with vascularity and facilitates healthy oocyte development. Indeed, a relationship between follicular blood velocity, oocyte recovery and the production of high-grade preimplantation embryos is documented by several studies (Nargund *et al*., 1996; Van Blerkom, 1998). These findings provide strong evidence that the vasculature plays a key role in regulating folliculogenesis and is involved in determining successful embryo implantation and pregnancy. However, the possibility that the vascular insufficiency identified in infertility patients is due to disturbance of the hypothalamus–pituitary–ovarian axis cannot be ruled out, and this needs to be further investigated.

Vascularization in the uterus involves angiogenesis and plays a pivotal role during the normal menstrual cycle and during pregnancy. Hyperplastic and hypertrophic growth of uterine arterial endothelial cells (EC) and smooth muscle cells (SMC) is increased in pregnancy (Cipolla and Osol, 1994), and this remodelling plays an important role in viable pregnancy. Indeed, lack of modulation of vascularity in humans exposed to diethylstilboestrol may contribute to miscarriages and obstetric complications such as intrauterine growth retardation and pre-eclampsia (Salle *et al*., 1996). Statistically significant differences in flow velocity within the ovary and uterine arteries are reported between fertile and infertile women (Kurjak *et al*., 1991), and high vascular resistance in uterine arteries and ovarian arteries in the luteal phase reduce the take-baby-home rate (Tinkanen *et al*., 1994). Therefore, abnormalities in angiogenesis may lead to reproductive dysfunction.

Abnormalities in vascular tone and structure within some reproductive organs participates in the pathophysiology of diseases of the reproductive system. For example, abnormalities in ovarian and uterine diastolic blood flow may cause infertility (Kurjak *et al*., 1991). Discordant uterine artery pulsatility indexes in the first trimester are significantly associated with pregnancy loss (Leible *et al*., 1998), and structural changes (endothelial basophilia, vacuolation, arterial dilations, hypertrophied SMC layers) within spiral arteries of the endometrium can occur both during intrauterine and ectopic pregnancies (Craven *et al*., 1998). Increased blood flow within the tubal arteries and neovascularization of blood vessels around the oviduct may occur in ectopic pregnancy (Kirchler *et al*., 1993; Abramov *et al*., 1997b). Moreover, during ectopic pregnancy blood vessels originating from the omentum often surround tubal masses (Adachi *et al.*, 1984). However, it is unclear whether the vascular changes are the consequence of the implanted embryo within the oviduct or a causative factor in the abnormal implantation. Marked increases in stromal vascularization in the endometrium occurs in subjects with adenomyosis (Ota *et al*., 1998). The active participation of abnormalities within the vasculature is associated with endometriosis (Nisolle *et al*., 1993), and vascular abnormalities (occlusive disorders, endothelial dysfunction and endothelial barrier/permeability disorders) within the placenta also contribute to the pathophysiology of pre-eclampsia (Haller *et al*., 1998; Roberts, 1998; Rosselli *et al*., 1998). Vascular disorders (atherosclerotic occlusion) within the hypogastric–vaginal/clitoral arterial bed are associated with diffuse vaginal and clitoral fibrosis, vaginal engorgement insufficiency and clitoral erectile dysfunction (Park *et al*., 1997). Similarly, vascular abnormalities are a major contributor to erectile disorders in males (De Luca *et al*., 1996; Adams *et al*., 1997). Taken together, the above observations indicate that the vasculature plays a dynamic role in the physiology of most reproductive functions, and that vascular dysfunction participates importantly in reproductive disorders.

## Processes regulating the vascular system of reproductive organs

### *Cellular processes in vascular remodelling*

Angiogenesis of microvessels in the ovary and uterus during follicular development, menstrual cycle and pregnancy is mediated largely by EC (Augustin *et al*., 1995; Goodger and Rogers 1995; Suzuki *et al*., 1998; Obermair *et al*., 1999). The cyclic nature of these processes suggests the involvement of both hormones and growth factors (Shifren *et al*., 1996). The angiogenic process is triggered by increased local expression of growth factors such as vascular endothelial growth factor (VEGF), which is a potent mitogen for EC. Increased expression of VEGF increases vascular permeability and triggers the disruption of a normal vessel, and the migration of EC along the gradient expression of VEGF towards the tissue. Subsequently, these migratory EC form a well-perfused microcapillary (Van Blerkom *et al*., 1997; Goede *et al*., 1998; Reynolds and Redmer, 1998). In the ovary, initiation of the angiogenic process is associated with increases in oestradiol generation, and regression of the newly formed microvessels is accompanied by a fall in oestradiol concentrations, suggesting that the angiogenic process is oestradiol-regulated (Shweiki *et al.*, 1993). Interestingly, oestradiol has been shown to induce VEGF synthesis and induce EC migration and proliferation (Schnaper *et al*., 1996). Under physiological conditions, the angiogenesis process is cyclic. However, abnormalities in angiogenesis has

pathological consequences in diseases such as ovarian cancer (Santin *et al*., 1999), tumours and ascites (Zebrowski *et al*., 1999), endometriosis (Willemse *et al*., 1997) and ovarian hyperstimulation syndrome and infertility (Abramov *et al.*, 1997a).

In contrast to angiogenesis, the vascular remodelling process associated with occlusive disorders in the reproductive system (e.g. pre-eclampsia, impotence) involves damage and dysfunction of EC, as well as SMC proliferation. Vaso-occlusive disorders are associated with a reduction in the lumen cross-sectional area of blood vessels as a result of plaque formation or thickening of the vascular wall, and both processes involve vascular SMC. In normal blood vessels, vascular SMC and EC quiescence is maintained by the balanced generation of SMC/EC activators versus SMC/EC inhibitors (Figure 1). In contrast, in a diseased blood vessel, loss of this delicate balance triggers a cascade of events resulting in an increase in SMC activity including migration, proliferation, hypertrophy and extracellular matrix synthesis (Figure 1). Similarly, imbalance in the generation of growth activators and inhibitors is responsible for regulating EC growth (Figure 1) and plays an important role in the pathophysiology of angiogenic and neovascularization processes.

### *Autocrine/paracrine factors and vascular remodelling*

Homeostasis within a normal blood vessel is maintained by the appropriate production of a battery of vascular SMC activators [angiotensin II (Ang II), endothelin, noradrenaline, platelet-derived growth factor (PDGF), epidermal growth factor (EGF), vasopressin, interleukin-β (IL-β), VEGF, etc.) and inhibitors (nitric oxide, prostacyclin, adenosine, atrial natriuretic peptide, etc.; Figure 2). Imbalance in the generation of vasoconstrictors versus vasodilators, i.e. increased generation of vasoconstrictors or decreased generation of vasodilators, results in increased contractility of the vasculature and reduction in the vascular lumen size. Sustained changes in pressure or flow induces EC damage and dysfunction, which subsequently results in altered generation of endothelium-derived factors involved in the regulation of vascular homeostasis, vascular cell growth, as well as matrix production (Bobik and Campbell, 1993; Ross, 1993) and contributes to the vascular remodelling process and vaso-occlusive disorders.

Multiple circulating, nerve-derived and autocrine–paracrine factors influence growth of SMC directly or in concert with each other (Figure 3) (Dubey *et al.*, 1997). Cells respond to changes in external stimuli by activation of a variety of signal transduction pathways (Figure 4) which culminate in stereotypical responses such as proliferation, growth arrest, hypertrophy, differentiation or apoptosis (Figure 4) (Davies *et al*., 1993; Ross, 1993; Dubey *et al*., 1997; Waltenberger, 1997). The major pathways via which external stimuli induce proliferative or hypertrophic growth involve mitogen-activated protein (MAP) kinase cascades (Waltenberger, 1997), [the extracellular signal-regulated kinase (ERK), the p38 MAP kinase and the stress-activated protein kinase or c-jun N-terminal kinase], phosphatidylinositol turnover, diacylglycerol formation, intracellular $Ca^{2+}$ flux (Bornfeld *et al*., 1994), protein kinase C (PKC) and tyrosine kinase activity (Dubey *et al*., 1997; Waltenberger, 1997), oxidation/peroxidation of membrane phospholipids (Dubey *et al*., 1999b) and polymerization of microtubule-associated proteins into microtubules (Inoue, 1981).

### *Vascular effects of oestrogen: cellular and biochemical mechanisms*

The role of oestradiol in vascular biology is evidenced by the findings that ovarian dysfunction and oestrogen deficiency are linked to vaso-occlusive disorders in postmenopausal women. As

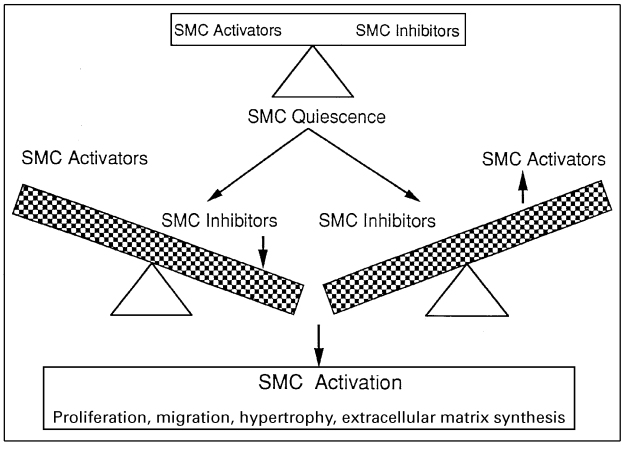


**Figure 1.** Regulation of vascular smooth muscle cell (SMC) and endothelial cell (EC) activity. In normal blood vessels, SMC and EC quiescence is maintained by the balanced generation (either endocrine, neural, paracrine or autocrine) of SMC/EC activators versus SMC/EC inhibitors. Imbalance in the generation of these factors under pathophysiological conditions results in SMC/EC activation, i.e. proliferation, migration, hypertrophy and extracellular matrix synthesis.

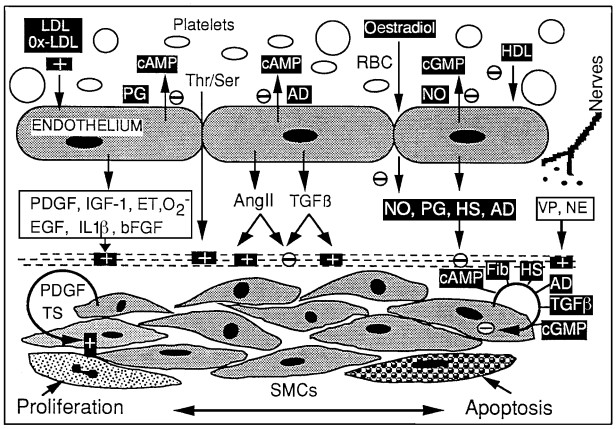


**Figure 2.** Role of endogenous factors in regulating vascular smooth muscle cell (SMC) number. Normally, there is an appropriate generation of growth inducers (+) and growth inhibitors (–) such that the rate of cellular proliferation is matched by the rate of programmed cell death (apoptosis). Abbreviations: cAMP = cyclic AMP; PG = prostaglandins; Thr = thrombin; Ser = Serotonin; Ad = adenosine; ADE = adenosine deaminase; cGMP = cyclic GMP; RBC = red blood cells; PDGF = platelet-derived growth factor; NO = nitric oxide; AngII = angiotensin II; HS = heparin/heparin sulphate; bFGF = basic fibroblast growth factor; IGF-1 = insulin-like growth factor-1; ET = endothelin; VEGF = vascular endothelial growth factor; ($O_2^-$) = oxygen free radical; TGFβ = transforming growth factor-β; EGF = epidermal growth factor; Fib = fibronectin; TS = thrombospondin; IL-1β = interleukin-1β; iNOS = inducible nitric oxide synthase; cNOS = constitutive nitric oxide synthase; VP = vasopressin; NE = norepinephrine (noradrenaline); MCP-1 = monocyte chemoattractant protein 1; HDL = high-density lipoprotein; LDL = low-density lipoprotein; Ox-LDL = oxidized-LDL.

compared with men, women within the reproductive age group are protected against vaso-occlusive diseases, and these differences decrease with the onset of menopause (for review, see Oparil *et al*., 1999). Oestrogen replacement therapy may reduce vaso-occlusive disorders in postmenopausal women (Oparil *et al*., 1999), although in a recent randomized trial, treatment with oestrogen plus progesterone did not reduce the overall incidence of coronary heart disease in postmenopausal women in whom the condition was established (Hulley *et al*., 1998). Moreover, inclusion of progestins maintains the decrease in risk for cardiovascular disease (Grodstein *et al*., 1996). Finally, premenopausal women who undergo premature surgical menopause (i.e. bilateral oophorectomy) and who do not use postmenopausal oestrogens have twice the risk of vaso-occlusive disorders than do age-matched premenopausal controls, whereas, if they use oestrogens, their incidence of vaso-occlusive disorders is the same as age-matched premenopausal women (Colditz and Stampfer, 1992).

The above findings provide evidence that oestradiol helps in maintaining homeostasis within the vasculature, and protects against vaso-occlusion. Possible mechanisms include alterations in plasma concentrations of lipoproteins [decrease in low-density lipoprotein (LDL), decrease in oxidized LDL formation, increase in high-density lipoprotein (HDL)], glucose, insulin and haemostatic factors, neutralization of free radicals, changes in endothelium-derived factors (decrease in endothelin, increase in nitric oxide and prostaglandins) and inhibition of neointima formation [inhibition of SMC migration and proliferation induced by various mitogens, i.e. PDGF, basic fibroblast growth factor (bFGF), insulin-like growth factor-1 (IGF-1) and Ang II] (Rosselli *et al*., 1994, 1995, 1998; Imthurn *et al*., 1997; Krasinski *et al*., 1997; Mendelsohn and Karas, 1999; Oparil *et al*., 1999). Although the precise biochemical pathways involved remain obscure, oestradiol may alter vascular SMC biology by inhibiting the MAP kinase (Dubey *et al*., 2000) and phosphatidylinositol/diacylglycerol pathways (Bornfeld *et al*., 1994).

In contrast to its effects on SMC growth, oestradiol induces growth of EC and is involved in the angiogenic process within the ovary and the uterus. The mitogenic effects of oestradiol on EC are associated with MAP kinase activation and release of growth factors such as bFGF and VEGF (Shweiki *et al*., 1993; Kim-Schulze *et al*., 1998; Reynolds and Redmer, 1998). Oestradiol prevents EC damage, induces the recovery of damaged endothelium (Krasinski *et al*., 1997; Spyridopoulos *et al*., 1997) and inhibits neointima formation and adhesion of platelets/leukocytes to endothelial cell surfaces. Oestradiol protects the vasculature by stimulating nitric oxide and prostacyclin synthesis (Malika *et al*., 1982; Gustafsson, 1997) from EC and by inducing vasorelaxation (Dubey *et al.*, 1997), inhibiting platelet aggregation (Dubey *et al.*, 1997), abrogating neutrophil-induced endothelium damage (Dubey *et al.*, 1997) and acting as an anti-mitogenic agent for vascular SMC (Dubey, 1994; Dubey *et al*., 1995, 1997).

One key molecule of importance in mediating the mitogenic effects of oestradiol on EC is VEGF. Both SMC and EC are known to synthesize VEGF, and oestradiol induces the synthesis of VEGF in EC (Suzuma *et al.*, 1999). In contrast to EC, VEGF is a weak mitogen for SMC proliferation, although it has been shown to induce SMC migration (Grosskreutz *et al*., 1999), suggesting that local generation of VEGF may selectively regulate EC growth in an autocrine–paracrine fashion. Indeed, in-vivo studies have provided evidence that infusion of VEGF protects against endothelial injury-induced vaso-occlusive disorders, and promotes rapid regeneration of endothelial cell layer as well as angiogenesis (Asahara *et al*., 1995; Weatherford *et al*., 1996). Because VEGF has been shown to mediate its effects on cell migration (a key step for angiogenesis) via nitric oxide generation, it is feasible that the effects of VEGF on EC growth

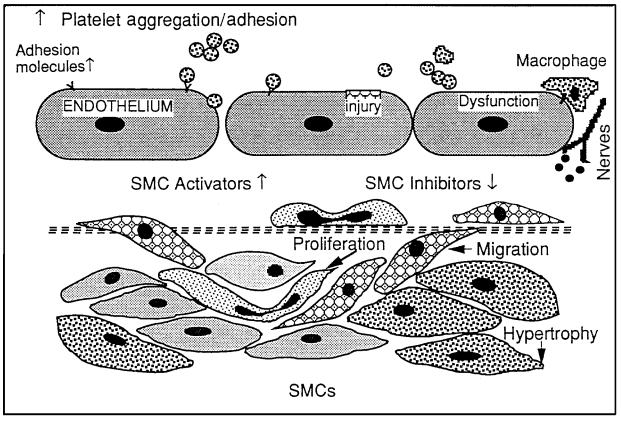


**Figure 3.** Cellular mechanisms involved in the vascular remodelling process leading to vaso-occlusive disorders. Damage and dysfunction of endothelial cells leads to platelet and leukocyte adhesion to endothelial cells, which triggers the production of vascular smooth muscle cell (SMC) activators and decreases the production of SMC inhibitors. This is followed by migration of SMC into the intima and proliferation, hypertrophy and reduced apoptosis of SMC in the intima and media. These changes are largely responsible for the vascular remodelling associated with vaso-occlusive disorders.

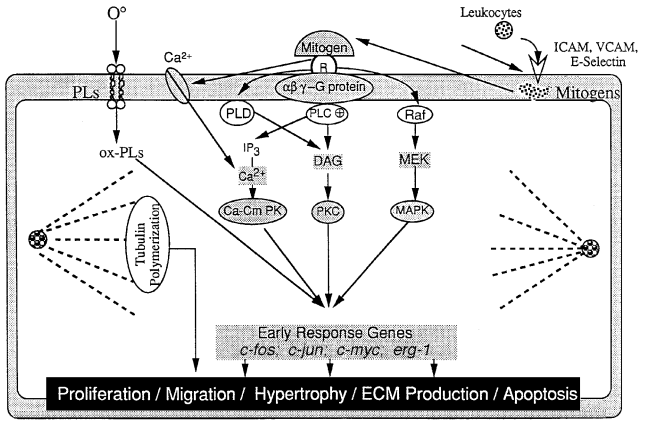


**Figure 4.** Signal transduction mechanisms by which endogenous mitogens can regulate vascular smooth muscle cell proliferation, migration, hypertrophy and extracellular matrix production. Abbreviations: PLD = phospholipase D; DAG = diacylglycerol; $IP_3$ = inositol-1,4,5-triphosphate; PKC = protein kinase C; $PLA_2$ = phospholipase $A_2$; Ca-Cm PK = calcium-calmodulin protein kinase; MAPK = mitogen-activated protein kinase; PAI-1 = plasminogen activator inhibitor-1; MEK = mitogen-activated protein kinase kinase; STAT = signal transducers and activators of transcription; JAK = Janus kinase; Oo = peroxyl free radical; PL = phospholipids; ox-PLs = oxidized phospholipids; PLC = phospholipase C.

are nitric oxide-mediated. Oestradiol not only induces VEGF synthesis but also induces the synthesis and expression of nitric oxide and VEGF receptor-2 in EC (Suzuma *et al*., 1999). This suggests that oestradiol may induce VEGF-mediated mitogenic effects via VEGF 2 receptors and nitric oxide synthesis. The mitogenic effects of VEGF on EC are increased in a synergistic fashion by bFGF. Because oestradiol induces bFGF as well as VEGF release from EC, it is feasible that the combined effects of these two molecules play a prominent role in regulating angiogenesis/neovascularization. The fact that VEGF synthesis is regulated by oestradiol, and that both VEGF and bFGF are synthesized within the ovary (Reynolds and Redmer, 1998), suggests that this molecule may play a critical role in mediating the cyclic angiogenic as well as neovascularization processes within the reproductive organs which are under oestrogen control, for example the ovary.

#### *Role of oestrogen receptors in vascular remodelling*

The vasculature expresses both α and β oestrogen receptors (ER); however, whether the vascular effects of oestrogen are receptor- or non-receptor-mediated is unresolved. Recent studies have shown that ICI 182780, a pure ER antagonist, blocks the growth effects of oestradiol on both EC and SMC (Schnaper *et al.*, 1996; Dubey *et al*., 2000). Others (Iafrati *et al*., 1997) have shown that oestradiol induces vasoprotective effects in mice lacking ERα. Because these rats express ERβ, it is feasible that the effects were β receptor-mediated. However, recent studies from our laboratory provide evidence that endogenous oestradiol metabolites that have low affinity for ER are potent inhibitors of SMC growth (Dubey *et al*., 1998a, 1999b). Thus, the anti-mitogenic effects of oestradiol may be in part ER-independent. This notion is supported by the very recent finding that oestradiol inhibits neointima formation in mice lacking ERβ (Karas *et al*., 1999). Finally, oestradiol metabolites have been shown to induce multiple biological effects on several reproductive and non-reproductive systems (Zhu and Conney, 1998).

With regard to the role of ER in regulating EC growth and regulating angiogenesis, several studies have provided evidence that the anti-apoptotic and mitogenic effects of oestradiol on EC are receptor-mediated. Preliminary findings also provide evidence that the effects of oestradiol on VEGF are receptor-dependent. Moreover, in mice expressing ERβ, but not ERα, oestradiol was unable to induce angiogenesis, suggesting that there was no angiogenic effect of ERβ. Moreover, in EC overexpressing ERα there is decreased cell proliferation, as well as synthesis of VEGF, suggesting that ERα may have a negative effect on oestradiol-induced angiogenesis and VEGF synthesis. The above findings, together with the fact that oestradiol induces the release of bFGF via an ER-dependent mechanism involving MAP kinase, suggests that the growth effects of oestradiol on EC depend on the interaction between oestradiol and functional ER.

The above findings, together with the fact that increases in oestradiol concentrations during follicular development, menstruation and pregnancy are associated with extensive vascular remodelling, provides convincing evidence that oestradiol plays a key role in regulating cyclic vascular changes within the reproductive system. Because oestradiol mediates its effects directly via ER as well as indirectly via generation of metabolites, it is feasible that environmental oestrogens cause reproductive dysfunction by inducing vascular abnormalities.

## Effects of environmental oestrogens on the vasculature

Environmental oestrogens may be divided into two major groups: (i) phyto-oestrogens, which are plant-derived; and (ii) xeno-oestrogens, which are human-made molecules released into the environment. Phyto-oestrogens are grouped into three main classes: isoflavones, lignans and coumestans. All phyto-oestrogens are metabolically converted to non-steroidal heterocyclic phenols with a structure similar to 17β-oestradiol. Xeno-oestrogens are a structurally diverse group of compounds including pesticides, components of plastics, hand creams, contraceptives, etc. Xeno-oestrogens likely to be present in the environment include diethylstilboestrol, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), polychlorinated hydroxybiphenyls (OH-PCB) and dichlorodiphenyltrichloro-ethane (DDT).

#### *Vascular effects of xeno-oestrogens*

##### *Diethylstilboestrol (DES)*

DES causes infertility in women, ectopic pregnancies, in-utero fetal deaths, pre-eclampsia, spontaneous miscarriages, late abortions and ovulatory disorders (Salle *et al*., 1996), and the effects of DES on the vasculature may importantly contribute to DES-induced reproductive dysfunction. Ultrasound studies indicate that DES increases the pulsatile index in the uterine artery and interferes with the pregnancy-induced vascular remodelling process. Abnormal vascularity of the DES-exposed uterus may explain miscarriages and obstetric complications such as intrauterine growth retardation or pre-eclampsia (Salle *et al.*, 1996). Indeed, administration of DES inhibits trophoblast maturation and development of fetal blood vessels (Scott and Adejokun, 1980). Cervical vascular malformation is the main cause of antepartum and intrapartum bleeding in DES-exposed subjects (Follen *et al*., 1985). DES treatment causes subfertility and ovarian dysfunction (Newbold *et al*., 1983), and histochemical studies have demonstrated that DES causes endothelial damage (Wordinger *et al*., 1984). Together, these findings suggest that DES adversely influences the angiogenesis process in the ovary as well as the uterus, and also influences follicular development and pregnancy-related vascularization (implantation and placenta). DES is no longer used in a clinical setting, and hence is less likely to be a prominent xeno-oestrogen influencing the reproductive system.

##### *2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD)*

TCDD induces multiple reproductive disorders, and studies both *in vivo* and *in vitro* have shown that TCDD influences vascular structure and function. In-vitro studies provide evidence that TCDD can regulate SMC growth in a biphasic manner (Weber *et al.*, 1996) by regulating intracellular mechanisms linked to protein kinase C and activator protein-1 (AP-1) transcription factor whose members, i.e. *fos* and *jun*, are known to regulate cell growth. Because growth of vascular cells contributes to the vascular remodelling process associated with cyclic angiogenesis as well as with vaso-occlusive disorders, it is feasible that the deleterious

effects of TCDD on the vasculature are associated with its deleterious effects on the reproductive system/function.

Indeed, in-vivo studies in rabbits (Brewster *et al*., 1988) demonstrated that intraperitoneal injections of TCDD cause ruffling, denudation and sloughing-off of EC within the aortic arch. Since angiogenesis within the ovary and uterus is essential for follicular development and implantation, TCDD-induced damage to EC may contribute to the abnormal oestrous cyclicity (ovulation), implantation and embryo development. Indeed, TCDD induces embryo toxicity by inducing apoptotic cell death in the embryonic vasculature (Cantrell *et al*., 1998). Moreover, reduced litter size fecundity (associated with abnormal follicle development) is observed in hamsters treated with TCDD (Gray and Ostby, 1995).

TCDD is also known to induce endometriosis, a condition characterized by intense vascularization. It is conceivable that TCDD-induced damage to the endothelium disrupts the endothelial barrier, triggers the local release of injury-induced growth factors which stimulate migration and proliferation of EC to form capillaries. Alternatively, TCDD-induced endothelial damage may lead to abnormal growth of SMC (Dubey *et al*., 1997), which in the presence of increased LDL (induced by TCDD) would facilitate plaque formation and generation of growth factors leading to neovascularization (Alpern-Elran *et al*., 1989). Indeed, treatment with TCDD induces macrophage-like cells in the intima and media of the vasculature, suggesting that TCDD may cause vascular damage similar to that observed in lipid-induced vaso-occlusive disorders. Interestingly, TCDD also causes a decrease in adipose tissue lipoprotein lipase activity and a concomitant increase in serum triglyceride concentration, thereby inducing hyperlipidaemia—a pro-atherosclerotic state (Brewster *et al*., 1988). These findings support the earlier observation that TCDD treatment increases plasma as well as aortic triglyceride concentrations in rabbits (Lovati *et al*., 1984), and similar effects are observed in humans exposed to TCDD (Zack and Suskind, 1980). Hence, it is feasible that TCDD induces vascular remodelling by influencing the growth of vascular cells.

In postmenopausal women, atherosclerosis in the coronary arteries correlates with atherosclerosis within the uterine artery (Crawford *et al*., 1997). Because TCDD causes hyperlipidaemia and EC damage, these effects may collectively result in vaso-occlusive disorders similar to those observed in atherosclerosis. Therefore, these vaso-occlusive effects of TCDD may play a critical role in inducing the deleterious effects on the reproductive system. In this regard, recent studies have provided evidence that atherosclerosis is associated with erectile dysfunction, impotence, female infertility and fetal growth retardation (Azadzoi and Goldstein, 1992; Berg *et al.*, 1994; Bujo *et al*., 1995). Moreover, vascular abnormalities are a key factor in the pathophysiology of polycystic ovarian syndrome, ovarian hyperstimulation syndrome, ovarian tumours and ascites, endometriosis and pre-eclampsia.

The mechanism by which TCDD may induce two different processes of vascular remodelling, i.e. neovascularization (angiogenesis) as well as vaso-occlusive disorders (atherosclerosis), remains unclear. However, based on its effects on the vascular cells it is feasible that TCDD induces EC injury/dysfunction (Brewster *et al*., 1988), and this leads to the generation of growth-promoting factors such as bFGF and PDGF. In turn, this would induce the expression of adhesion molecules and monocyte attachment (Dubey *et al*., 1997), which may then trigger SMC growth and induce vaso-occlusive disorder similar to atherosclerosis. The resulting occlusion would cause ischaemic conditions at the site of injury, leading to release of VEGF from vascular SMC and EC (Okuda *et al.*, 1998). Because VEGF is a potent mitogenic factor for EC it would trigger the endothelial capillary formation/angiogenesis and neovascularization. However, further studies are required to determine the net effects of TCDD on vascular remodelling, and more specifically on endothelial cell and SMC growth.

Finally, many of the effects of TCDD are diametrically opposed to the effects of oestradiol, i.e. oestradiol reduces hyperlipidaemia, protects endothelial cells against dysfunction and damage, and inhibits SMC growth and PKC activity (see above). This suggests that the mechanisms by which TCDD induces its effects on the vasculature are not the same as those activated by oestradiol. In this regard, TCDD binds to cytosolic arylhydrocarbon (Ah) receptors and induces cytochrome P4501A1 (CYP1A1) gene expression (Ou and Ramos, 1995; Stegeman *et al*., 1995). Inasmuch as CYP1A1 is expressed in SMC, benzo(a)pyrene (BaP)—which is found in cigarette smoke—induces vascular SMC growth (Sadhu *et al*., 1993) and CYP1A1 is a key enzyme that metabolizes procarcinogens to epoxide carcinogenic and pro-atherosclerotic intermediates (Thirman *et al*., 1994), it is feasible that TCDD indirectly enhances the vaso-occlusive effects of other aromatic hydrocarbons such as BaP by inducing CYP1A1. Moreover, because oestradiol does not bind to the Ah receptor, the binding of TCDD to the Ah receptor provides a mechanism that would explain the contrasting effects of TCDD and oestradiol on the vasculature.

### *Polychlorinated biphenyls (PCB)*

PCB are a mixture of biphenyl molecules that are chlorinated in different positions to produce a variety of chlorinated biphenyl congeners. Biologically, PCB congeners that are ortho-substituted with chlorine are more active than those which lack chlorines in the ortho position (non-ortho), because ortho-substituted PCB are able to attain a planar configuration. Also, the oestrogenic activity of chlorinated PCB can be further enhanced through metabolic hydroxylation at vacant para positions. Several lines of evidence suggest that PCB induce deleterious effects on the reproductive system via vascular actions.

In-vitro studies provide evidence that PCB induce several pro-mitogenic effects on vascular cells. For example, PCB induce oxidative stress in cultured porcine EC (Toborek *et al*., 1995), and increase levels of intracellular calcium and disrupt the endothelial barrier function (Toborek *et al*., 1995). Treatment of cultured porcine pulmonary EC with PCB with various binding affinities to the Ah receptor markedly increase transendothelial transport of albumin, indicating that PCB disrupt endothelial barrier function (Hennig *et al*., 1999). Because EC dysfunction/damage, generation of free radicals and increases in intracellular calcium levels are associated with vaso-occlusive disorders, it is feasible that PCB induce vascular remodelling similar to that observed in atherosclerosis. This notion is further supported by the findings that PCB not only increase CYP1A1 activity in EC (implicated in atherosclerosis), but also enhance lipid (linoleic acid)-induced activation of CYP1A1 (Stegeman *et al*., 1995; Hennig *et al*.,

1999). Moreover, PCB increase expression of oxidative stress-sensitive nuclear transcription factor-kappa, which is known to increase at sites of vascular injury and atherosclerosis (Dubey *et al*., 1997). Finally, treatment of EC with PCB decreases production of tocopherol, which is known to induce anti-vaso-occlusive effects on the vasculature. Taken together, the above findings suggest that PCB may induce abnormal growth within the vasculature by either down-regulating endogenous growth inhibitory mechanisms or by inducing pro-mitogenic factors or pathways.

In-vivo animal studies have provided convincing evidence that PCB are damaging to the vasculature (Bäcklin *et al*., 1998). In mink exposed to Clophen A50 at a dose of 1.3 mg/day for 54 days starting before mating, a variety of lesions were found in the placental labyrinthine zone. These lesions comprised degeneration and detachment of maternal EC, thrombi, leakage of erythrocytes and fluid, focal trophoblastic disintegration and loss of fetal capillary integrity. Vascular lesions also appeared in stem arteries and in sinusoids on the fetal side of the placental labyrinthine zone. Electron microscopic studies have demonstrated that treatment with PCB causes surface irregularities in EC, displacement of nuclei to the apical cell surface, and dilation of cisternae of the rough endoplasmic reticulum. Similar effects of PCB have been reported in the murine cutaneous microvasculature (Bell, 1983). These structural changes suggest that the adverse effects of PCB may be due to abnormalities in glycan expression (Jones *et al*., 1997). Since PCB disrupt the assembly of phospholipid monolayers (Nelson *et al*., 1990), it is also feasible that they cause EC damage by interacting with membrane phospholipids (Toborek *et al*., 1995). Alternatively, free radicals generated in response to PCB (Toborek *et al*., 1995) would induce oxidation of membrane phospholipids and consequently damage the cell.

PCB are known to damage the EC and disrupt the cell barrier (Toborek *et al*., 1995), as well as the blood–brain barrier (Ness *et al*., 1994). Because brain capillaries forming the blood–brain barrier are similar to the placental vascular endothelial barrier, the disruptive effects of PCB on the placental vascular barrier might eventually result in exposure of the fetus to PCB and lead to fetal abnormalities. Indeed, PCB treatment decreases placental blood flow in guinea pigs (Hedman *et al*., 1985) and causes disseminated thrombosis followed by multiple haemorrhages in organs such as the lungs and brain, thus suggesting involvement of the vascular system (Jensen *et al*., 1977). In the same manner, increases in endothelial permeability are also associated with endometriosis (McLaren *et al*., 1996; Donnez *et al*., 1998), pre-eclampsia (Baker *et al*., 1995), polycystic ovary syndrome (PCOS; Agrawal *et al*., 1998), ovarian hyperstimulation syndrome (McClure *et al*., 1994; Krasnow *et al*., 1996), and ascites (Nagy *et al*., 1995), and PCB increase endothelial permeability by influencing the endothelial barrier. Thus, it is feasible that PCB may increase the incidence of reproductive disorders such as endometriosis by increasing endothelial permeability.

Interestingly, it has been reported (Kreiss *et al*., 1981) that in humans there is an association between blood pressure and PCB concentrations. PCB concentrations also correlate positively with gamma-glutamyl transpeptidase and serum cholesterol concentrations, and there is a positive association between severe arteriosclerosis and concentrations of organochlorine compounds in humans (Pines *et al*., 1986). Because PCB decrease the production of endogenous antioxidant and growth inhibitory molecules such as vitamin E, it is feasible that they may also induce abnormal growth within the vasculature, which eventually would lead to occlusion of vessels. This would result in reduced perfusion of the reproductive organs, and create a negative impact on the reproductive system. Finally, PCB accumulate in the follicular fluid (Baukloh *et al*., 1985; Schlebusch *et al*., 1989), and exposure to PCB induces detrimental effects on oocytes, fertilization, early cleavage (Kholkute *et al*., 1994) and implantation (Lindenau and Fischer, 1996). Alternatively, local accumulation of PCB may influence the cyclic angiogenesis process which is essential for the follicular development process. Apart from direct effects on the angiogenesis process, PCB may indirectly induce their effects by interfering with the actions of oestradiol. In this regard, it is well established that PCB bind to the ER as well as inducing the cytochrome P450 enzymes that metabolize oestradiol and reduce their concentrations (for details, see the review by Rosselli *et al.*, 1999).

#### *Dichlorodiphenyltrichloroethane (DDT)*

Compared with PCB and TCDD, much less is known regarding the effects of DDT on the vasculature. The relationship between DDT and vascular disease is supported by an observational study showing that concentrations of organochlorine pesticides (including DDT) are increased in patients with atherosclerosis (Pines *et al*., 1986). With regard to the effects of DDT on vascular SMC, it has been shown (Ruehlmann *et al*., 1998) that 4-octylphenol, *p*-nonylphenol and *o*,*p*'-DDT inhibit L-type $Ca^{2+}$ channels in vascular SMC and invoke a rapid and endothelium-independent relaxation of the coronary vasculature similar to that induced by 17β-oestradiol. Moreover, these authors provided evidence that these vasodilatory effects are mediated via a non-genomic pathway. In light of these findings, and the fact that oestradiol induces beneficial effects on the vasculature, it is difficult to explain how an agent which mimics the effects of oestradiol induces deleterious effects on the vasculature. Further studies are required to study the growth effects of these agents on SMC and EC.

### *Vascular effects of phyto-oestrogens*

Phyto-oestrogens are known to influence the biology of both vascular SMC and EC which play a key role in the vascular remodelling process. In this regard, phyto-oestrogens bind with varying affinity to both functional ER (α and β). Genistein, a phyto-oestrogen which binds exclusively to ERβ, as well as other phyto-oestrogens, have been shown to inhibit SMC growth, i.e. proliferation (DNA synthesis, change in cell number), extracellular matrix synthesis and cell migration, which participate in vascular remodelling as well as the development of new vessels (Figures 5–7) (Dubey *et al*., 1999a). Additionally, genistein inhibits endothelial microcapillary formation and neovascularization, and is a potent anti-angiogenic factor (Fotsis *et al*., 1993; Kruse *et al*., 1997). Moreover, a very recent finding provides evidence that genistein has a preventive action against injury-induced vaso-occlusive disorders, without inducing uterotrophic effects (Mäkela *et al*., 1999), suggesting a growth inhibitory role for genistein via the ERβ.

The regulatory effects of phyto-oestrogens on the biology of both vascular SMC and EC may be mediated via multiple

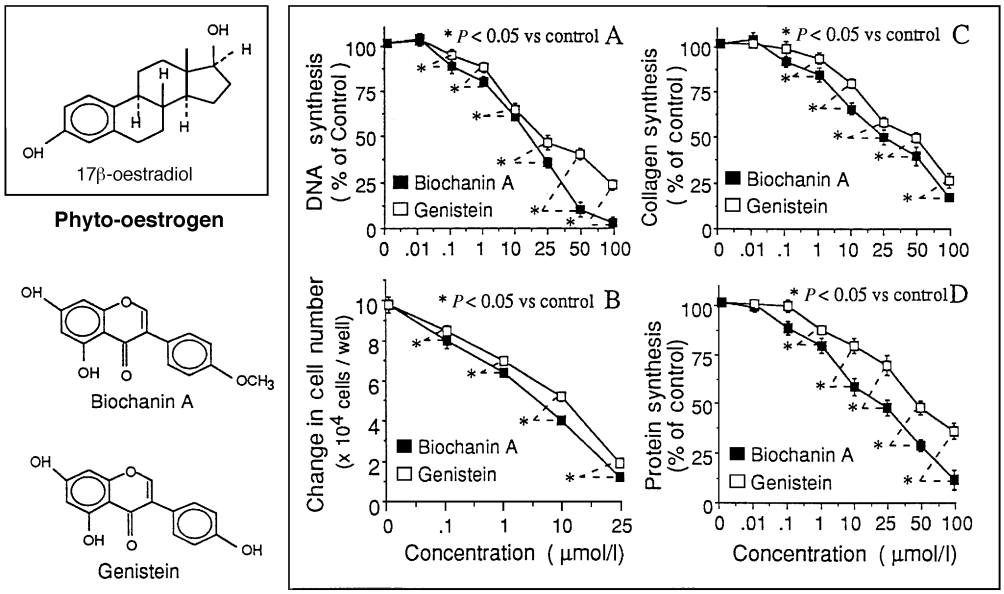


**Figure 5.** Effects of the phyto-oestrogens, biochanin A and genistein, which structurally resemble 17β-oestradiol, on fetal calf serum (2.5%)-induced growth of human aortic smooth muscle cells. (**A**) Effects on DNA synthesis as measured by [$^3$H]thymidine incorporation. (**B**) Effects on cell proliferation as assayed by counting cells after 4 days of treatment. (**C**, **D**) Effects on collagen and protein synthesis, respectively, as measured by assaying [$^3$H]proline and [$^3$H]leucine incorporation, respectively. Both biochanin A and genistein inhibited serum-induced SMC proliferation and synthesis of extracellular matrix proteins. [Reprinted and modified with permission of Dubey *et al.* (1999a) and *Hypertension*.]

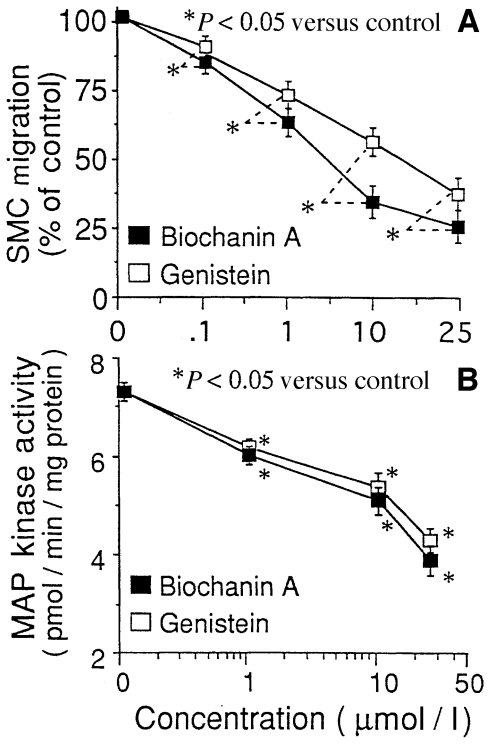


**Figure 6.** Effects of the phyto-oestrogens, biochanin A and genistein on platelet-derived growth factor BB (PDGF-BB)-induced growth of human aortic smooth muscle cells. (**A**) Inhibitory effects on smooth muscle cell (SMC) migration as assayed using the modified Boydens chamber. (**B**) Inhibitory effects of biochanin A and genistein on PDGF-BB-induced mitogen-activated protein kinase (MAP Kinase) activity. [Reprinted and modified with permission of Dubey *et al.* (1999a) and *Hypertension.*]

mechanisms. In this regard, phyto-oestrogens such as genistein are capable of inhibiting the key signal transduction pathway involved in vaso-occlusive disorders, as well as in carcinogenesis/angiogenesis. For example, genistein inhibits MAP kinase activity (Dubey *et al.*, 1999a); prevents neointima formation by inhibiting transforming growth factor-β (TGF-β1, TGF-β3) mRNA expression by 85%; inhibits the expression of integrins $\alpha_V\beta_3$ (Ward *et al.*, 1997), inhibits mitogen-induced tyrosine kinase activity and suppresses focal adhesion of SMC to fibronectin, cell spreading, and cytoskeletal reorganization (Hedin *et al.*, 1997); slows the phenotypic modulation of SMC and inhibits phospholipase C (PLC) -mediated $Ca^{2+}$ transients in SMC (Touyz and Schiffrin *et al.*, 1996); inhibits phospholipase D activity (Morton *et al.*, 1995); suppresses tumour necrosis factor-α (TNF-α) -stimulated expression of intracellular adhesion molecule 1 (ICAM-1); and prevents adhesion of monocytes to endothelial cells (Weber *et al.*, 1995). Finally, being phenolic in nature, the phyto-oestrogens express anti-oxidant effects and favourably influence the profile of cholesterol/lipids and inhibit platelet aggregation (Wilcox and Blumenthal, 1995) and protect against free radical-induced oxidative injury. The above findings, together with the observation that phyto-oestrogens inhibit growth of MCF-7 breast cancer cells (Wang *et al.*, 1996) and prevent angiogenesis by inhibiting EC growth (Fotsis *et al.*, 1993), suggest that phyto-oestrogens may protect against reproductive cancers/tumours either directly or by inhibiting angiogenesis. More importantly, the inhibitory effects of phyto-oestrogen on the vasculature may play a critical role in protecting the female reproductive system against cancer. Future studies are needed to investigate these possibilities.

Although as compared with xeno-oestrogens general consumption of phyto-oestrogens is not associated with reproductive disorders, ample evidence suggests that phyto-oestrogens can

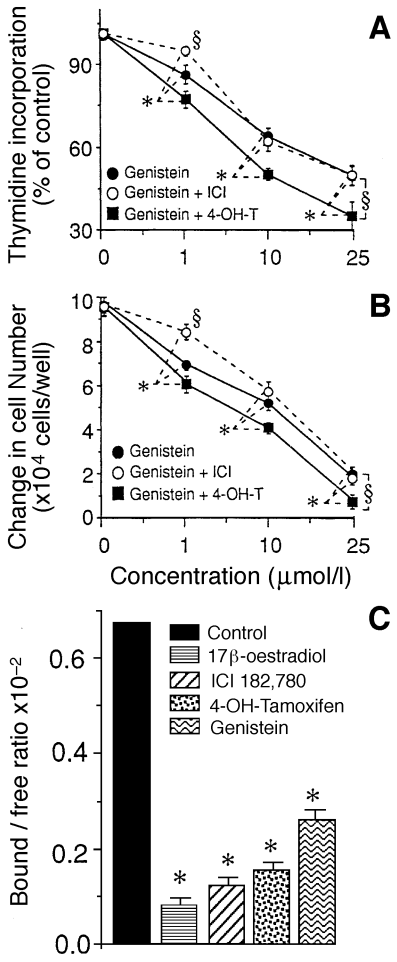


**Figure 7.** The inhibitory effects of genistein on fetal calf serum (2.5%)-induced thymidine incorporation (**A**) and cell proliferation (**B**) are not blocked by oestrogen receptor ligands ICI 182780 (ICI; 10 μmol/l) or 4-hydroxytamoxifen (4-OH-T; 1 μmol/l). ICI 182780, 4-OH-T, unlabeled 17β-oestradiol and genistein effectively block the binding of [$^3$H]17β-oestradiol (1 nmol/l) to cytosolic oestrogen receptors in human aortic smooth muscle cells (**C**). [Reprinted and modified with permission of Dubey *et al.* (1999a) and *Hypertension*.]

influence the reproductive system. In this regard, the consumption of large amounts of clover, which contains phyto-oestrogens (e.g. equol), was shown to cause reproductive disorders (clover disease) in sheep (Bennetts *et al*., 1946), as well as vascular abnormalities (Yeong *et al*., 1990). Moreover, in women taking soya extracts containing phyto-oestrogens, the time of follicular development and the menstruation cycle has been shown to be significantly prolonged (Phipps *et al*., 1993; Murkies *et al*., 1998). Because angiogenesis of the microvessels in the ovary and uterus during follicular development, menstrual cycle and pregnancy is largely mediated by EC and SMC, it is feasible that prolongation of the follicular development and menstruation cycle in women taking phyto-oestrogens is due to the anti-angiogenic effects of genistein in the ovary. Moreover, it suggests that the effects of phyto-oestrogens on the vasculature can influence reproductive function.

As genistein inhibits VEGF-induced angiogenesis (Xia *et al*., 1996), and since up-regulation of VEGF synthesis in the ovary is associated with angiogenesis while down-regulation is associated with regression of vessels (Lebovic *et al*., 1999), it is feasible that genistein may interfere with the angiogenic process in the ovary. Hence, genistein may be responsible for the prolongation of the follicular development seen in subjects consuming a soy-based diet. VEGF is also known as a permeability factor, and increased generation of VEGF is associated with permeability disorders in PCOS, ovarian hyperstimulation syndrome, endometriosis, ovarian and other reproductive tumours and ascites. Because, in contrast to oestradiol, genistein inhibits the effects of VEGF, it is feasible that phyto-oestrogens such as genistein may protect the vasculature against permeability disorders observed in the above pathological conditions.

Epidemiological studies also provide evidence that consumption of a phyto-oestrogen-rich diet, as seen in traditional Asiatic societies, is associated with a lower risk of breast and prostate cancer and vascular disease (Adlercreutz, 1990; Murkies *et al*., 1998). Because angiogenesis within the tumours plays a critical role in tumour progression, the inhibitory effects of phyto-oestrogens on vascular EC and SMC growth may be a critical mechanism by which phyto-oestrogens protect against cancer. Since endometriosis is also associated with increased neovascularization, it is feasible that phyto-oestrogens such as genistein may protect against endometriosis, and this possibility requires further investigation. In this regard, it is important to note that phyto-oestrogens such as genistein are capable of inhibiting the key signal transduction pathways involved in vaso-occlusive disorders as well as carcinogenesis**.**

Apart from angiogenesis, occlusion of blood vessels is also associated with infertility. In this regard, the association between infertility and obesity as well as hypercholesterolaemia is well established. Moreover, the severity of uterine artery atherosclerosis is significantly correlated with known risk factors for cardiovascular disease (Crawford *et al*., 1997). Additionally, women with PCOS are infertile and suffer from vaso-occlusive disorders, i.e. atherosclerosis (Guzick *et al*., 1996). Finally, occlusion of the internal iliac arteries associated with atherosclerosis results in erectile dysfunction and infertility (Azadozi and Goldstein, 1992). The above findings, together with the findings that a phyto-oestrogen-rich diet (soy protein) prevents fat diet-induced vaso-occlusive disorders in non-human primates (Anthony *et al*., 1997), and phyto-oestrogens inhibit key processes associated with vascular remodelling leading to vaso-occlusive disorders, suggest that phyto-oestrogens may protect the vasculature against occlusive disorders and prevent infertility.

Finally, it should be noted that the effects of phyto-oestrogens may largely be dose-related, i.e. at higher doses they may induce anti-angiogenic effects, whereas at lower concentrations they may prevent against vaso-occlusive disorders. Further studies are needed to study the interaction of phyto-oestrogens with growth factors such as VEGF and their effects on pathophysiology of angiogenesis within the reproductive system.

## How do environmental oestrogens exert their biological effects?

Since angiogenesis, as well as vascular remodelling, plays a critical role in the biology and pathophysiology of the reproductive system, it is important to dissect the mechanisms by which environmental oestrogens can influence the vasculature.

The mechanisms by which these compounds mediate their biological effects has been reviewed in detail (Rosselli *et al*, 1999), and is therefore discussed only briefly here. Ligand binding studies from our laboratory provide evidence for the presence of ER on human aortic SMC, and demonstrate that phyto-oestrogens bind to these receptors. However, the inhibitory effects of phyto-oestrogens on cell growth are actually enhanced in the presence of 4-OH-tamoxifen, an ER ligand with partial agonistic properties. Moreover, ICI 182780, a specific ER antagonist, only partially blocks the inhibitory effects of phyto-oestrogens. This suggests that the inhibitory effects of phyto-oestrogens are only in part ER-mediated. In this regard, recent studies demonstrate that phyto-oestrogens inhibit growth of ER-positive and ER-negative cell lines (Sathyamoorthy *et al*., 1994; Wang *et al*., 1996). In addition, other oestrogen-like compounds, for example the ER antagonists tamoxifen and 4-OH-tamoxifen, inhibit SMC growth and induce vasoprotective effects. Also, several lines of evidence suggest that the inhibitory effects of 17β-oestradiol on SMC growth are both receptor- and non-receptor-mediated. In this regard it is important to note that even though genistein binds to ER with high affinity, whereas biochanin A does not (Kuiper *et al*., 1998), both compounds inhibit SMC growth and MAP kinase activity to an equal extent (Dubey *et al*., 1999a). Taken together, these findings suggest phyto-oestrogens induce their effects on the vasculature in part via ER-independent pathways, the nature of which should be the subject of further studies.

In contrast to phyto-oestrogens that bind as effectively as oestradiol to ERα and ERβ, the affinity of xeno-oestrogens for ERα and ERβ is low (Kuiper *et al*., 1998). Hence, it is likely that the effects of xeno-oestrogens on the vasculature engage a different pathway than phyto-oestrogens and natural oestrogens. In this regard, the binding of xeno-oestrogens to the Ah receptors may be the key mechanism via which they induce their vascular effects. Further studies are needed to clarify the role of Ah receptors and the ABC-cassette transporters in mediating the effects of xeno-oestrogens on the vasculature.

Almost all data on the effects of xeno-oestrogens address how these compounds cause deleterious effects on the vasculature, whereas most studies focus on the beneficial effects of phyto-oestrogens. Is this disparate approach of investigating the two classes of environmental oestrogens the result of good and bad publicity, or the result of valid scientific reasoning? From the physiological and biochemical points of view, phyto-oestrogens not only resemble 17β-oestradiol, but also bind strongly to their receptors and activate oestrogen response elements. In contrast to phyto-oestrogens, xeno-oestrogens are weak ligands for ER, but have a high affinity for the Ah receptor. Because the Ah receptor is linked to mono-oxygenases (CYP450) and its stimulation by ligands such as TCDD might activate CYP450, xeno-oestrogens may stimulate the cascade of events leading to conversion of pro-carcinogens to carcinogens. For example, metabolism of benzo(a)-pyrene and other carcinogens induce vaso-occlusive disorders (Thirman *et al*., 1994). Moreover, abnormal angiogenesis as well as reproductive disorders occurs in mice lacking the protein arylhydrocarbon-receptor nuclear translocator (ARNT) (Maltepe *et al*., 1997). In contrast to xeno-oestrogens, phyto-oestrogens may reduce the risk of cancer, inhibit vascular remodelling associated with atherosclerosis, and decrease angiogenesis. In this regard, it is interesting to note that substituted flavones also act as Ah receptor agonists as well as antagonists (Lu *et al*., 1996) and inhibit CYP450 activity induced by TCDD (Lu *et al*., 1996), which is associated with the activation of pro-carcinogens to carcinogens (Sadhu *et al*., 1993; Thirman *et al*., 1994). If the effects of phyto-oestrogens and xeno-oestrogens are indeed mediated via different mechanisms, and the similarities lie only in the likeness of their structures, then these two classes of environmental oestrogens may induce differential effects on the vasculature, i.e. phyto-oestrogens are protective, whereas xeno-oestrogens are damaging. Indeed, in contrast to phyto-oestrogens, xeno-oestrogens such as TCDD bind to Ah receptors and stimulate the CYP450 *in vivo*, and this induces anti-oestrogenic effects by inducing oestradiol metabolism. Because abnormalities in oestradiol production as observed in PCOS are accompanied with vaso-occlusive disorders and infertility, TCDD may induce deleterious effects on the vasculature by reducing oestradiol synthesis. Similar to TCDD, many other xeno-oestrogens induce oestradiol metabolism and may induce their anti-oestrogenic effects by increasing oestradiol catabolism. One thing is clear; most of the information regarding the vascular effects of environmental oestrogens is with regard to phyto-oestrogens, and very little is known about the effects of xeno-oestrogens on the vasculature. Hence, before we can conclude that, as compared with oestradiol or phyto-oestrogens, environmental oestrogens induce deleterious effects or differential effects on the vasculature, more detailed information and/or research is needed regarding the effects of various xeno-oestrogens on the cellular, biochemical and molecular mechanisms involved in vascular biology.

## Conclusions and future directions

Based on the available literature, it is clear that both phyto-oestrogens and xeno-oestrogens influence the vasculature. In this regard, phyto-oestrogens are clearly vasoprotective, whereas—due to lack of data—the effects of xeno-oestrogens on the vasculature remain poorly defined. Because the vasculature is an integral and active component of reproductive organs, anti-vaso-occlusive agents such as phyto-oestrogens may exert beneficial effects on the reproductive system. In contrast, agents such as DES, TCDD, PCB and DDT, which induce vascular damage, may cause vaso-occlusive disorders that might lead to reduced perfusion of the reproductive organs and consequently result in abnormal function. However, in order to better understand the effects of environmental oestrogens on reproductive function, future studies should evaluate the role of blood vessels in reproductive organ physiology and assess whether the effects of environmental oestrogens on the vasculature are associated with reproductive dysfunction. Specifically, the following questions should be addressed: (i) What is the influence of environmental oestrogens on the growth (angiogenesis) and function of endothelial cells and on the release of endothelium-derived vasoactive factors?; (ii) What are the effects of environmental oestrogens, particularly xeno-oestrogens, on cellular processes contributing to vaso-occlusive disorders such SMC proliferation, migration, hypertrophy and extracellular matrix synthesis?; (iii) How do environmental oestrogens affect intracellular mechanisms (biochemical and molecular) involved in regulating SMC growth and are these effects oestrogen receptor-mediated or non-receptor-mediated?; and (iv) Do environmental oestrogens

interfere with the protective actions of 17β-oestradiol on the vasculature? Hopefully, future studies will better define the effects of environmental oestrogens on the cellular, biochemical and molecular processes associated with vascular remodelling and vaso-occlusive disorders within the reproductive system.

## Acknowledgements

These studies were supported by the Swiss National Science Foundation (32-54172.98, 32-45986.95 and 32-45986.99); the National Institutes of Health (HL-55314, HL-35909); Schering (Schweiz) AG, Switzerland; and IBSA, Switzerland.

## References

Abramov, Y., Barak, V., Nisman, B. and Schenker, J.G. (1997a) Vascular endothelial growth factor plasma levels correlate to the clinical picture in severe ovarian hyperstimulation syndrome. *Fertil. Steril.*, **67**, 261–265.

Abramov, Y., Nadjari, M., Shushan, A. *et al*. (1997b) Doppler findings in chronic ectopic pregnancy: case report. *Ultrasound Obstet. Gynecol.*, **9**, 344–346.

Adachi, A., Segen, J. and Li, J.K. (1984) Ovarian vein thrombosis mimicking ectopic pregnancy. *N. Y. State J. Med.*, **84**, 567–568.

Adams, M.A., Banting, J.D., Maurice, D.H. *et al*. (1997) Vascular control mechanisms in penile erection: phylogeny and the inevitability of multiple and overlapping systems. *Int. J. Impot. Res.*, **9**, 85–91.

Adlercreutz, H. (1990) Western diet and western diseases: some hormonal and biochemical mechanisms and associations. *Scand. J. Clin. Lab. Invest.*, **50** (Suppl. 210), 3–23.

Agrawal, R., Conway, G., Sladkevicius, P. *et al*. (1998) Serum vascular endothelial growth factor and Doppler blood flow velocities in *in vitro* fertilization: relevance to ovarian hyperstimulation syndrome and polycystic ovaries. *Fertil. Steril*., **70**, 651–658.

Alpern-Elran, H., Morog, N., Robert, F. *et al*. (1989) Angiogenic activity of the atherosclerotic carotid artery plaque. *J. Neurosurg.*, **70**, 942–945.

Anthony, M.S., Clarkson, T.B., Bullock, B.C. and Wagner, J.D. (1997) Soy protein versus soy phyto-oestrogens in the prevention of diet-induced coronary artery atherosclerosis of male cynomolgus monkeys. *Arterioscler. Thromb. Vasc. Biol.*, **17**, 2524–2531.

Asahara, T., Bauters, C., Zheng, L.P. *et al*. (1995) Synergistic effect of vascular endothelial growth factor and basic fibroblast growth factor on angiogenesis *in vivo*. *Circulation*, **92** (9 suppl.); 11365–11371.

Augustin, H.G., Braun, K., Telemenakis, I. *et al*. (1995) Ovarian angiogenesis. Phenotypic characterization of endothelial cells in a physiological model of blood vessel growth and regression. *Am. J. Pathol*., **147**, 339–351.

Azadzoi, K.M. and Goldstein, I. (1992) Erectile dysfunction due to atherosclerotic vascular disease: the development of an animal model. *J. Urol.*, **47**, 1675–1681.

Bäcklin, B.-M., Persson, E., Jones, C.J.P. and Dantzer, V. (1998) Polychlorinated biphenyl (PCB) exposure produces placental vascular and trophoblastic lesions in mink (*Mustela vison*): a light and electron microscopic study. *APMIS*, **106**, 785–799.

Baker, P.N., Krasnow, J., Roberts, J.M. and Yeo, K.T. (1995) Elevated serum levels of vascular endothelial growth factor in patients with preeclampsia. *Obstet. Gynecol.*, **86**, 815–821.

Baukloh,V., Bohnet, H.G., Trapp, M. *et al*. (1985) Biocides in human follicular fluid. *Ann. N. Y. Acad. Sci*., **442**, 240–250.

Bell, M. (1983) Ultrastructural features of the murine cutaneous microvasculature after exposure to polychlorinated biphenyl compounds (PCBs) and benzo-(a)-pyrene. *Virchows Arch. B. Cell Pathol. Incl. Mol. Pathol.*, **42**, 131–142.

Bennetts, H.W., Underwood, E.J. and Shier, F.L. (1946) A specific breeding problem of sheep on subterranean clover pastures in western Australia. *Aust. Vet. J*., **22**, 2–12.

Berg, K., Roald, B. and Sande, H. (1994) High Lp(a) lipoprotein level in maternal serum may interfere with placental circulation and cause fetal growth retardation. *Clin. Genet.*, **46**, 52–56.

Bhal, P.S., Pugh, N.D., Chui, D.K. *et al*. (1999) The use of transvaginal power Doppler ultrasonography to evaluate the relationship between perifollicular vascularity and outcome in in-vitro fertilization treatment cycles. *Hum. Reprod.*, **14**, 939–945.

Bobik, A. and Campbell, J.H. (1993) Vascular derived growth factors: cell biology, pathophysiology, and pharmacology. *Pharmacol. Rev*., **45**, 1–42.

Bornfeld, K.E., Raines, E.W., Nakano, T. *et al*. (1994) Insulin like growth factor-1 and platelet derived growth factor BB induce directed migration of human arterial smooth muscle cells via signalling pathways that are distinct from those of proliferation. *J. Clin. Invest.*, **93**, 1266–1274.

Brewster, D.W., Bombick, D.W. and Matsumura, F. (1988) Rabbit serum hypertriglyceridemia after administration of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD). *J. Toxicol. Environ. Health*, **25**, 495–507.

Bujo, H., Yamamoto, T., Hayashi, K. *et al*. (1995) Mutant oocyte low density lipoprotein receptor gene family member causes atherosclerosis and female infertility. *Proc. Natl Acad. Sci. USA*, **92**, 9905–9909.

Cantrell, S.M., Joy-Schlezinger, J., Stegeman, J.J. *et al*. (1998) Correlation of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin-induced apoptotic cell death in the embryonic vasculature with embryotoxicity. *Toxicol. Appl. Pharmacol.*, **148**, 24–34.

Carson, R., Findlay, J., Mattner, P. and Brown, B. (1986) Relative levels of thecal blood flow in atretic and non-atretic ovarian follicles of the conscious sheep. *Aust. J. Exp. Biol. Med. Sci.*, **64**, 381–387.

Chui, D.K., Pugh, N.D., Walker, S.M. *et al*. (1997) Follicular vascularity – the predictive value of transvaginal power Doppler ultrasonography in an in-vitro fertilization programme: a preliminary study. *Hum. Reprod*., **12**, 191–196.

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.

Colditz, G.A. and Stampfer, M.J. (1992) Cardiovascular effects of menopause and oestrogen replacement. The epidemiological evidence. In Swartz, D.P. (ed.), *Hormone Replacement Therapy*. Williams & Wilkins, Baltimore, 1992, pp. 101–137.

Craven, C.M., Morgan, T. and Ward, K. (1998) Decidual spiral artery remodelling begins before cellular interaction with cytotrophoblasts. *Placenta*, **19**, 241–252.

Crawford, B.S., Davis, J. and Harrigill, K. (1997) Uterine artery atherosclerotic disease: histologic features and clinical correlation. *Obstet. Gynecol.*, **90**, 210–215.

Davies, M.J., Gordon, J.L., Gearing, A.J.H. *et al*. (1993) The expression of the adhesion molecules ICAM-1, VCAM-1, PECAM and E-selectin in human atherosclerosis. *J. Pathol.*, **171**, 223–229.

De Luca, V., Pescatori, E.S., Taher, B. *et al*. (1996) Damage to the erectile function following radical pelvic surgery: prevalence of veno-occlusive dysfunction. *Eur. Urol.*, **29**, 36–40.

Donnez, J., Smoes, P., Gillerot, S. *et al*. (1998) Vascular endothelial growth factor (VEGF) in endometriosis. *Hum. Reprod.*, **13**, 1686–1690.

Dubey, R.K. (1994) Vasodilator-derived nitric oxide inhibits fetal calf serum- and angiotensin-II-induced growth of renal arteriolar smooth muscle cells. *J. Pharmacol. Exp. Ther.*, **268**, 402–408.

Dubey, R.K., Jackson, E.K. and Luscher, T.F. (1995) Nitric oxide inhibits angiotensin II-induced migration of rat aortic smooth muscle cell. Role of cyclic-nucleotide and angiotensin1 receptors. *J. Clin. Invest.*, **96**, 141–149.

Dubey, R.K., Jackson, E.K., Rupperecht, H. and Sterzel, R.B. (1997) Factors controlling growth and matrix production in vascular smooth muscle and glomerular mesangial cells. *Curr. Opin. Nephrol. Hypertension*, **6**, 88–105.

Dubey, R.K., Gillespie, D.G., Imthurn, B. *et al*. (1998a) Differential effects of clinically used oestrogen(s) on mitogen-induced growth of human aortic smooth muscle cells. *Hypertension*, **32**, 625 (abstract).

Dubey, R.K., Gillespie, D.G., Jackson, E.K. and Keller, P.J. (1998b) 17β-oestradiol, its metabolites and progesterone inhibit cardiac fibroblast growth. *Hypertension*, **31**, 522–528.

Dubey, R.K., Gillespie, D.G., Imthurn, B. *et al*. (1999a) Phyto-oestrogens inhibit growth and MAP Kinase activity in human aortic smooth muscle cells. *Hypertension*, **33** (II), 177–182.

Dubey, R.K., Tyurina, Y.Y., Tyurin, V.A. *et al*. (1999b) Oestrogen and tamoxifen metabolites protect smooth muscle cell membrane phospholipids against peroxidation and inhibit cell growth. *Circ. Res.*, **84**, 229–239.

Dubey, R.K., Jackson, E.K., Gillespie, D.G. *et al*. (2000) Clinically used oestrogens differentially inhibit human aortic smooth muscle cell growth and MAP kinase activity. *Arterioscler. Thromb. Vasc. Biol.*, **20**, 964–972.

Follen, M.M., Fox, H.E. and Levine, R.E. (1985) Cervical vascular malformation as a cause of antepartum and intrapartum bleeding in three diethylstilbestrol-exposed progeny. *Am. J. Obstet. Gynecol.*, **153**, 890–891.

Fotsis, T., Pepper, M., Adlercreutz, H. *et al*. (1993) Genistein, a dietary

derived inhibitor of *in vitro* angiogenesis. *Proc. Natl Acad. Sci. USA*, **90**, 2690–2694.

Goede, V., Schmidt, T., Kimmina, S. *et al*. (1998) Analysis of blood vessel maturation processes during cyclic ovarian angiogenesis. *Lab. Invest*., **78**, 1385–1394.

Goodger, A.M. and Rogers, P.A. (1995) Blood vessel growth in the endometrium. *Microcirculation*, **2**, 329–343.

Gray, L.E., Jr and Ostby, J.S. (1995) *In utero* 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) alters reproductive morphology and function in female rat offspring. *Toxicol. Appl. Pharmacol*., **133**, 285–294.

Grodstein, F., Stampfer, M.J., Manson, J.E. *et al*. (1996) Postmenopausal oestrogen and progestin use and the risk of cardiovascular disease. *N. Engl. J. Med.*, **335**, 453–461.

Grosskreutz, C.L., Anand-Apte, B., Duplaa, C. *et al*. (1999) Vascular endothelial growth factor-induced migration of vascular smooth muscle cells *in vitro*. *Microvasc. Res.*, **58**, 128–136.

Gustafsson, J.-A. (1997) Oestrogen receptor β – getting in on the action ? *Nature Med.*, **3**, 493–494.

Guzick, D.S., Talbott, E.O., Sutton-Tyrrell, K. *et al*. (1996) Carotid atherosclerosis in women with polycystic ovary syndrome: initial results from a case-control study. *Am. J. Obstet. Gynecol*., **174**, 1224–1229.

Haller, H., Hempel, A., Homuth, V. *et al*. (1998) Endothelial-cell permeability and protein kinase C in pre-eclampsia. *Lancet*, **351**, 945–949.

Hedin, U., Thyberg, J., Roy, J. *et al*. (1997) Role of tyrosine kinases in extracellular matrix-mediated modulation of arterial smooth muscle cell phenotype. *Arterioscler. Thromb. Vasc. Biol.*, **17**, 1977–1984.

Hedman, C., Bjellin, L. and Martensson, L. (1985) The influence of 2,2',4,4',5,5'-hexachlorobiphenyl on the placental blood flow in guinea pigs at a late stage of gestation. *Environ. Res.*, **38**, 293–300.

Hennig, B., Slim, R., Toborek, M. and Robertson, L.W. (1999) Linoleic acid amplifies polychlorinated biphenyl-mediated dysfunction of endothelial cells. *J. Biochem. Mol. Toxicol.*, **13**, 83–91.

Hulley, S., Grady, D., Bush, T. *et al*. (1998) Randomized trial of oestrogen plus progestin for secondary prevention of coronary heart disease in postmenopausal women. *JAMA*, **280**, 605–613.

Iafrati, M.D., Karas, R.H., Aronovitz, M. *et al*. (1997) Oestrogen inhibits vascular injury response in oestrogen receptor alpha-deficient mice. *Nature Med.*, **3**, 545–548.

Imthurn, B., Rosselli, M., Jaeger, A.W. *et al*. (1997) Differential effects of hormone-replacement therapy on endogenous nitric oxide (nitrate/nitrite) levels in postmenopausal women substituted with 17β-oestradiol valerate and cyproterone acetate or medroxyprogesterone acetate. *J. Clin. Endocrinol. Metab.*, **82**, 388–394.

Inoue, S. (1981) Cell division and the mitotic spindle. *J. Cell Biol.*, **91**, 131s–147s.

Jensen, S., Kihlström, J.E., Olsson, M. *et al*. (1977) Effects of PCB and DDT on mink (*Mustela vison*) during the reproductive season. *Ambio*, **6**, 239–239.

Jones, C.J., Backlin, B.M., Stoddart, R.W. and Dantzer, V. (1997) Environmental pollutants as aetiological agents in female reproductive pathology: placental glycan expression in normal and polychlorinated biphenyl (PCB)-exposed mink (*Mustela vison*). *Placenta*, **18**, 689–699.

Karas, R.H., Hodgin, J.B., Kwoun, M. *et al*. (1999) Oestrogen inhibits the vascular injury response in oestrogen receptor beta-deficient female mice. *Proc. Natl Acad. Sci. USA*, **96**, 15133–15136.

Kholkute, S.D., Rodriguez, J. and Dukelow, W.R. (1994) The effects of polybrominated biphenyls and perchlorinated terphenyls on *in vitro* fertilization in the mouse. *Arch. Environ. Contam. Toxicol.*, **26**, 208–211.

Kim-Schulze, S., Lowe, W.L., Jr and Schnaper, H.W. (1998) Oestrogen stimulates delayed mitogen-activated protein kinase activity in human endothelial cells via an autocrine loop that involves basic fibroblast growth factor. *Circulation*, **98**, 413–421.

Kirchler, H.C., Seebacher, S., Alge, A.A. *et al*. (1993) Early diagnosis of tubal pregnancy: changes in tubal blood flow evaluated by endovaginal color Doppler sonography. *Obstet. Gynecol.*, **82**, 561–565.

Kransow, J.S., Berga, S.L., Guzick, D.S. *et al*. (1996) Vascular permeability factor and vascular endothelial growth factor in ovarian hyperstimulation syndrome: a preliminary report. *Lancet*, **65**, 552–555.

Krasinski, K., Spyridopoulos, I., Asahara, T. *et al*. (1997) Oestradiol accelerates functional endothelial recovery after arterial injury. *Circulation*, **95**, 1768–1772.

Kreiss, K., Zack, M.M., Kimbrough, R.D. *et al*. (1981) Association of blood pressure and polychlorinated biphenyl levels. *JAMA*, **245**, 2505–2509.

Kruse, F.E., Joussen, A.M., Fotsis, T. *et al*. (1997) Inhibition of neovascularization of the eye by dietary factors exemplified by isoflavonoids. *Opthalmologie*, **94**, 152–156.

Kuiper, G.G.J.M., Lemmen, J.G., Carlsson, B. *et al*. (1998) Interaction of oestrogenic chemicals and phyto-oestrogens with oestrogen receptor β. *Endocrinology*, **139**, 4252–4263.

Kurjak, A., Kupesic-Urek, S., Schulman, H. and Zalud, I. (1991) Transvaginal color flow Doppler in the assessment of ovarian and uterine blood flow in infertile women. *Fertil. Steril.*, **56**, 870–873.

Lebovic, D.I., Mueller, M.D. and Taylor, R.N. (1999) Vascular endothelial growth factor in reproductive biology. *Curr. Opin. Obstet. Gynecol.*, **11**, 255–260.

Leible, S., Cumsille, F., Walton, R. *et al*. (1998) Discordant uterine artery velocity waveforms as a predictor of subsequent miscarriage in early viable pregnancies. *Am. J. Obstet. Gynecol.*, **179**, 1587–1593.

Lindenau, A. and Fischer, B. (1996) Embryotoxicity of polychlorinated biphenyls (PCBs) for preimplantation embryos. *Reprod. Toxicol.*, **10**, 227–230.

Lovati, M., Galbrussera, M., Franeschini, G. *et al*. (1984) Increased plasma and aortic triglycerides in rabbits after administering 2,3,7,8-tetrachloro-di-benzo-*p*-dioxin. *Toxicol. Appl. Pharmacol.*, **75**, 91–97.

Lu, Y.-F., Santostefano, M., Cunningham, B.D.M. *et al*. (1996) Substituted flavones as aryl hydrocarbon (Ah) receptor agonists and antagonists. *Biochem. Pharmacol.*, **51**, 1077–1087.

Macchiarelli, G., Nottola, S.A., Vizza, E. *et al*. (1993) Microvasculature of growing and atretic follicles in the rabbit ovary: a SEM study of corrosion casts. *Arch. Histol. Cytol.*, **56**, 1–12.

Mäkela, S., Savolainen, H., Aavik, E. *et al*. (1999) Differentiation between vasculoprotective and uterotrophic effects of ligands with different binding affinities to oestrogen receptors alpha and beta. *Proc. Natl Acad. Sci. USA*, **96**, 7077–7082.

Malika, U.M., Wahlberg, L., Viinikka, L. and Ylikorkala, O. (1982) Regulation of prostacyclin and thromboxane production by human umbilical vessels. The effect of oestradiol and progesterone in a superfusion model. *Prostaglandins Leukotr. Med.*, **8**, 115–124.

Maltepe, E., Schmidt, J.V., Baunoch, D. *et al*. (1997) Abnormal angiogenesis and responses to glucose and oxygen deprivation in mice lacking the protein ARNT. *Nature*, **386**, 403–407.

McClure, N., Healy, D.L., Rogers, P.A. *et al*. (1994) Vascular endothelial growth factor as capillary permeability agent in ovarian hyperstimulation syndrome. *Lancet*, **344**, 235–236.

McLaren, J., Prentice, A., Charnock-Jones, D.S. and Smith, S.K. (1996) Vascular endothelial growth factor (VEGF) concentrations are elevated in peritoneal fluid of women with endometriosis. *Hum. Reprod.*, **11**, 220–223.

Mendelsohn, M.E. and Karas, R.H. (1999) The protective effects of estrogen on the cardiovascular system. *N. Engl. J. Med.*, **340**, 1801–1811.

Morton, C., Wilkie, N. and Boarder, M.R. (1995) Tyrosine phosphorylation, MAPK and PLD in AII stimulated mitogenesis. *Biochem. Soc. Trans.*, **23**, 426S.

Murkies, A.L., Wilcox, G. and Davis, S.R. (1998) Clinical Review 92: Phyto-oestrogens. *J. Clin. Endocrinol. Metab.*, **83**, 297–303.

Nagy, J.A., Masse, E.M., Herzberg, K.T. *et al*. (1995) Pathogenesis of ascites tumor growth: vascular permeability factor, vascular hyperpermeability, and ascites fluid accumulation. *Cancer Res.*, **55**, 360–368.

Nargund, G., Bourne, T.H., Doyle, P.E. *et al*. (1996) Associations between ultrasound indices of follicular blood flow, oocyte recovery and perimplantation embryo quality. *Hum. Reprod.*, **11**, 109–113.

Nelson, A., Auffret, N. and Borlakoglu, J. (1990) Interaction of hydrophobic organic compounds with mercury adsorbed dioleoylphosphatidylcholine monolayers. *Biochim. Biophys. Acta*, **1021**, 205–216.

Ness, D.K., Schantz, S.L. and Hansen, L.G. (1994) PCB congeners in the rat brain: selective accumulation and lack of regionalization. *J. Toxicol. Environ. Health*, **43**, 453–468.

Newbold, R.R., Bullock, B.C. and McLachlan, J.A. (1983) Exposure to diethylstilbestrol during pregnancy permanently alters the ovary and oviduct. *Biol. Reprod.*, **28**, 735–744.

Nisolle, M., Casanas-Roux, F., Anaf, V. *et al*. (1993) Morphometric study of the stromal vascularization in peritoneal endometriosis. *Fertil. Steril.*, **59**, 681–684.

Obermair, A., Preyer, O. and Leodolter, S. (1999) Angiogenesis in gynecology and obstetrics. *Wien. Klein. Wochenschr.*, **111**, 262–277.

Okuda, K., Tsurumaru, K., Suzuki, S. *et al*. (1998) Hypoxia and endothelin-1 induce VEGF production in human vascular smooth muscle cells. *Life Sci.*, **63**, 477–484.

Oparil, S., Levine, R.L. and Chen, Y.F. (1999) Hormones and vasoprotection. *Hypertension*, **33** (II), 170–176.

Ota, H., Igarashi, S. and Tanaka, T. (1998) Morphometric evaluation of

stromal vascularization in the endometrium in adenomyosis. *Hum. Reprod.*, **13**, 715–719.

Ou, X. and Ramos, K.S. (1995) Regulation of cytochrome P4501A1 gene expression in vascular smooth muscle cells through aryl hydrocarbon receptor-mediated signal transduction requires a protein synthesis inhibitor. *Arch. Biochem. Biophys*., **316**, 116–122.

Park, K., Goldstein, I., Andry, C. *et al*. (1997) Vasculogenic female sexual dysfunction: the hemodynamic basis for vaginal engorgement insufficiency and clitoral erectile insufficiency. *Int. J. Impot. Res.*, **9**, 27–37.

Phipps, W.R., Martini, M.C. and Lampe, J.W. (1993) Effect of flaxseed ingestion on the menstrual cycle. *J. Clin. Endocrinol. Metab.*, **77**, 1215–1219.

Pines, A., Cucos, S., Ever-Hadani, P. *et al*. (1986) Levels of some organochlorine residues in blood of patients with arteriosclerotic disease. *Sci. Total Environ.*, **54**, 135–155.

Reynolds, L.P. and Redmer, D.A. (1998) Expression of the angiogenic factors, basic fibroblast growth factor and vascular endothelial growth factor, in the ovary. *J. Anim. Sci.*, **76**, 1671–1681.

Roberts, J.M. (1998) Endothelial dysfunction in preeclampsia. *Semin. Reprod. Endocrinol.*, **16**, 5–15.

Ross, R. (1993) The pathogenesis of atherosclerosis: a perspective for the 1990s. *Nature*, **362**, 801–809.

Rosselli, M., Keller, P.J., Kern, F. *et al*. (1994) Oestradiol inhibits mitogen induced proliferation and migration of human aortic smooth muscle cells: implications for cardiovascular disease in women. *Circulation*, **90**, I-87 (abstract).

Rosselli, M., Imthurn, B., Keller, P.J. *et al*. (1995) Circulating nitric oxide (nitrite/nitrate) levels in postmenopausal women substituted with 17β-oestradiol and norethisterone acetate: a two year follow-up study. *Hypertension*, **25**, 848–853.

Rosselli, M., Keller, P.J. and Dubey, R.K. (1998) Role of nitric oxide in the biology, physiology and pathophysiology of reproduction. *Hum. Reprod. Update*, **4**, 3–24.

Rosselli, M., Rheinhardt, K., Imthurn, B. *et al*. (1999) Cellular and biochemical mechanisms by which environmental oestrogens may influence the reproduction function. *Hum. Reprod. Update*, **6**, 332–350.

Ruehlmann, D.O., Steinert, J.R., Valverde, M.A. *et al*. (1998) Environmental oestrogenic pollutants induce acute vascular relaxation by inhibiting L-type $Ca^{2+}$ channels in smooth muscle cells. *FASEB J.*, **12**, 613–619.

Sadhu, D.N., Merchant, M., Safe, S.H. and Ramos, K.S. (1993) Modulation of protooncogene expression in rat aortic smooth muscle cells by benzo(a)pyrene. *Arch. Biochem. Biophys.*, **300**, 124–131.

Salle, B., Sergeant, P., Awada, A. *et al*. (1996) Transvaginal ultrasound studies of vascular and morphological changes in uteri exposed to diethylstilbestrol *in utero*. *Hum. Reprod.*, **11**, 2531–2536.

Santin, A.D., Hermonat, P.L., Ravaggi, A. *et al*. (1999) Secretion of vascular endothelial growth factor in ovarian cancer. *Eur. J. Gynaecol. Oncol.*, **20**, 177–181.

Sathyamoorthy, N., Wang, T.T. and Phang, J.M. (1994) Stimulation of pS2 expression by diet-derived compounds. *Cancer Res.*, **54**, 957–961.

Schlebusch, H., Wagner, U., van der Ven, H. *et al*. (1989) Polychlorinated biphenyls: the occurrence of the main congeners in follicular and sperm fluids. *J. Clin. Chem. Clin. Biochem.*, **27**, 663–667.

Schnaper, H.W., McGowan, K.A., Kim-Schulze, S. and Cid, M.C. (1996) Oestrogen and endothelial cell angiogenic activity. *Clin. Exp. Pharmacol. Physiol.*, **23**, 247–250.

Scott, J.N. and Adejokun, F. (1980) Placental changes due to administration of diethylstilbestrol (DES). *Virchows Arch. B. Cell Pathol. Incl. Mol. Pathol.*, **34**, 261–267.

Shifren, J.L., Tseng, J.F., Zaloudek, C.J. *et al*. (1996) Ovarian steroid regulation of vascular endothelial growth factor in the human endometrium: implications for angiogenesis during the menstrual cycle and in the pathogenesis of endometriosis. *J. Clin. Endocrinol. Metab.*, **81**, 3112–3118.

Shweiki, D., Itin, A., Neufeld, G. *et al*. (1993) Patterns of expression of vascular endothelial growth factor (VEGF) and VEGF receptors in mice suggest a role in hormonally regulated angiogenesis. *J. Clin. Invest.*, **91**, 2235–2243.

Spyridopoulos, I., Sullivan, A.B., Kearney, M. *et al*. (1997) Oestrogen-receptor-mediated inhibition of human endothelial cell apoptosis. Oestradiol as a survival factor. *Circulation*, **95**, 1505–1514.

Stegeman, J.J., Hahn, M.E., Weisbrod, R. *et al*. (1995) Induction of cytochrome P4501A1 by aryl hydrocarbon receptor agonists in porcine aorta endothelial cells in culture and cytochrome P4501A1 activity in intact cells. *Mol. Pharmacol.*, **47**, 296–306.

Suzuki, H.M., Sasano, H., Takaya, R. *et al*. (1998) Cyclic changes of vasculature and vascular phenotypes in normal ovaries. *Hum. Reprod.*, **13**, 953–959.

Suzuma, I., Mandai, M., Takagi, H. *et al*. (1999) 17β-oestradiol increases VEGF receptor-2 and promotes DNA synthesis in retinal microvascular endothelial cells. *Invest. Ophthalmol. Vis. Sci.*, **40**, 2122–2129.

Thirman, M.J., Albrecht, J.H., Krueger, M.A. *et al*. (1994) Induction of cytochrome CYP1A1 and formation of toxic metabolites of benzo(a)pyrene by rat aorta: a possible role in atherogenesis. *Proc. Natl Acad. Sci. USA*, **91**, 5397–5401.

Tinkanen, H., Kujansuu, E. and Laippala, P. (1994) Vascular resistance in uterine and ovarian arteries: its association with infertility and the prognosis of infertility. *Eur. J. Obstet. Gynecol. Reprod. Biol.*, **57**, 111–115.

Toborek, M., Barger, S.W., Mattson, M.P. *et al*. (1995) Exposure to polychlorinated biphenyls causes endothelial cell dysfunction. *J. Biochem. Toxicol.*, **10**, 219–226.

Touyz, R.M. and Schiffrin, E.L. (1996) Tyrosine kinase signaling pathways modulate angiotensin II-induced calcium ($[Ca^{2+}]i$) transients in vascular smooth muscle cells. *Hypertension*, **27**, 1097–1103.

Van Blerkom, J. (1998) Epigenetic influences on oocyte development competence: perifollicular vascularity and intrafollicular oxygen. *J. Assist. Reprod. Genet.*, **15**, 226–234.

Van Blerkom, J., Antczak, M. and Schrader, R. (1997) The developmental potential of the human oocyte is related to the dissolved oxygen content of follicular fluid: association with vascular endothelial growth factor levels and perifollicular blood flow characteristics. *Hum. Reprod.*, **12**, 1047–1055.

Waltenberger, J. (1997) Modulation of growth factor action. Implications for the treatment of cardiovascular diseases. *Circulation*, **96**, 4083–4094.

Wang, T.T.Y., Sathyamoorthy, N. and Phang, J.M. (1996) Molecular effects of genistein on oestrogen receptor mediated pathways. *Carcinogenesis*, **17**, 271–275.

Ward, M.R., Agrotis A., Kanellakis, P. *et al*. (1997) Inhibition of protein tyrosine kinases attenuates increases in expression of transforming growth factor-beta isoforms and their receptors following arterial injury. *Arterioscler. Thromb. Vasc. Biol.*, **17**, 2461–2470.

Weatherford, D.A., Sackman, J.E., Reddick, T.T. *et al*. (1996) Vascular endothelial growth factor and heparin in a biologic glue promotes human aortic endothelial cell proliferation with aortic smooth muscle cell inhibition. *Surgery*, **120**, 433–439.

Weber, C., Negrescu, E., Erl, W. *et al*. (1995) Inhibitors of protein tyrosine kinase suppress TNF-stimulated induction of endothelial cell adhesion molecules. *J. Immunol.*, **155**, 445–451.

Weber, T.J., Chapkin, R.S., Davidson, L.A. and Ramos, K.S. (1996) Modulation of protein kinase C-related signal transduction by 2,3,7,8-tetrachlorodibenzo-*p*-dioxin exhibits cell cycle dependence. *Arch. Biochem. Biophys.*, **328**, 227–232.

Wilcox, J.N. and Blumenthal, B.F. (1995) Thrombotic mechanisms in atherosclerosis: potential impact of soy proteins. *J. Nutr.*, **125** (3 Suppl.), 631S–638S.

Willemse, F., Nap, M., de Bruijn, H.W. and Hollema, H. (1997) Quantification of vascular density and of lumen and vessel morphology in endometrial carcinoma. evaluation of their relation to serum levels of tissue polypeptide-specific antigen and CA-125. *Anal. Quant. Cytol. Histol.*, **19**, 1–7.

Wordinger, R.J., Highman, B., Townsend, J.W. and Greenman, D.L. (1984) Histochemical and ultrastructural characteristics of the endometrial connective tissue stroma from mice continuously fed diethylstilbestrol. *Virchows Arch. B. Cell Pathol. Incl. Mol. Pathol.*, **47**, 247–261.

Xia, P., Aiello, L.P., Ishii, H. *et al*. (1996) Characterization of vascular endothelial growth factor's effect on the activation of protein kinase C, its isoforms, and endothelial cell growth. *J. Clin. Invest.*, **98**, 2018–2026.

Yeong, M.L., Swinburn, B., Kennedy, M. and Nicholson, G. (1990) Hepatic veno-occlusive disease associated with comfrey ingestion. *J. Gastroenterol. Hepatol.*, **5**, 211–214.

Zack, J.A. and Suskind, R.R. (1980) The mortality experience of workers exposed to tetrachloro-dibenzo-*p*-dioxin in a trichlorophenol process accident. *J. Occup. Med.*, **22**, 11–44.

Zebrowski, B.K., Liu, W., Ramirez, K. *et al*. (1999) Markedly elevated levels of vascular endothelial growth factor in malignant ascites. *Ann. Surg. Oncol.*, **6**, 373–378.

Zhu, B.T. and Conney, A.H. (1998) Functional role of oestrogen metabolism in target cells. Review and perspectives. *Carcinogenesis*, **19**, 1–27.

*Received on January 31, 2000; accepted on June 2, 2000*