CHAPTER 1

INTRODUCTION

Anatomy of the Uterus

General Features

The uterine wall in rodents consists of the outer mesometrium, inner endometrium and the intermediate myometrium (Figure 1.1; Papka et al., 1985). The myometrium consists of layers of longitudinal and circular smooth muscle cells (Figure 1.2); a layer of connective tissue containing blood vessels lies between the two smooth muscle layers. The inner smooth muscle layer borders the endometrial layer (Zoubina & Smith, 2000; Latini et al., 2008). The endometrial layer acts as a lining to the myometrium (Jones & Lopez, 2006). It is composed of 2 layers, the stratum functionalis and the stratum basalis. The stratum functionalis consists of epithelial cells and uterine glands; blood vessels are concentrated in the stratum basalis. The uterus connects to the ovary through the oviduct and to the cervix through the pelvic wall. In humans, supportive ligaments link the uterus to the ovary and cervix. (Jones & Lopez, 2006).

Histological changes in the uterus during the estrous cycle of the rat

The estrous cycle consists of 4 stages: proestrous, estrous, metestrous and diestrous. Intrafollicular oocytes develop into mature oocytes at proestrous; these oocytes degenerate if fertilization does not occur. Ovulation occurs as the rat proceeds from proestrous to estrous. Formation of corpora lutea occurs at late estrous and early diestrous. Development of the follicle starts one day before the start of proestrous (Maeda et al., 2000).

FIGURE 1.1. Diagram of a Cross Section through a Rodent Uterus showing the Main Layers.

Diagram based on mouse uterus. The diagram does not show linea uteri, thick bands of longitudinal muscle on the anti-mesometrial side of the uterus. Linea uteri have been found in rats but it is not known if they are present in mice.

Modified from Finn & Porter. (1975)

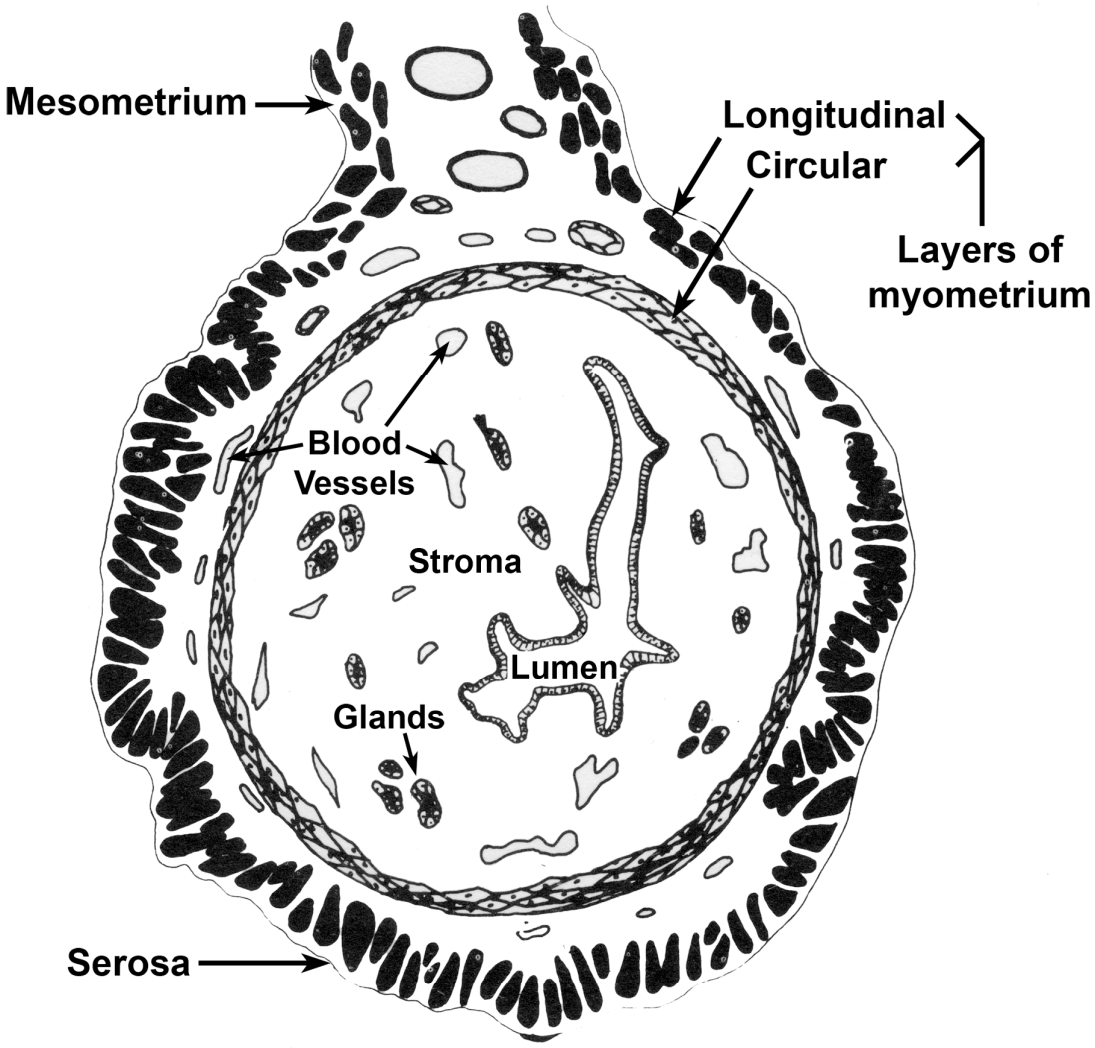
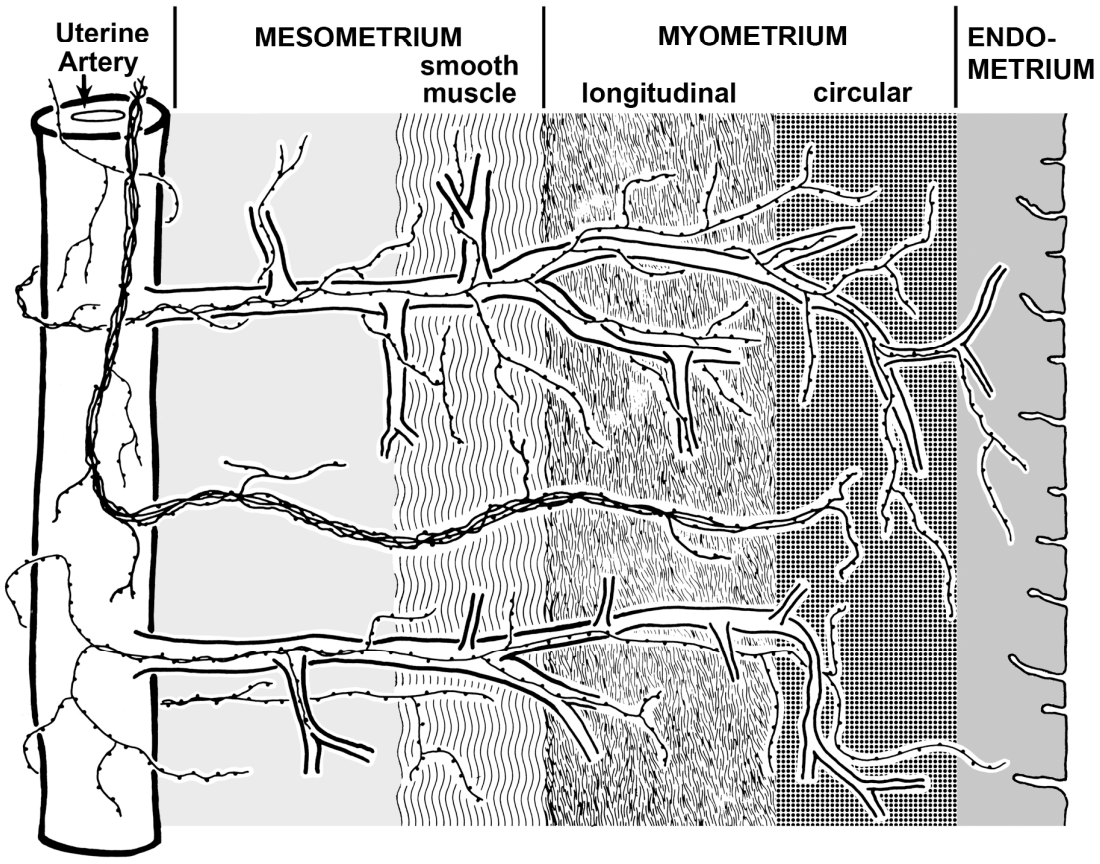

Mesometrium
Longitudinal
Circular
Layers of
myometrium
Blood
Vessels
Stroma
Lumen
Glands
Serosa

FIGURE 1.2. Diagram of the Neuroanatomy Rat Uterine Horn

Data derived from whole mount preparations in which the tubular wall has been cut open and stretched out flat. The diagram illustrates the general distribution of all classes of nerve fibers supplying the uterine horn and also shows location and orientation of the mesometrial smooth muscle.

Modified from Papka et al. (1985)

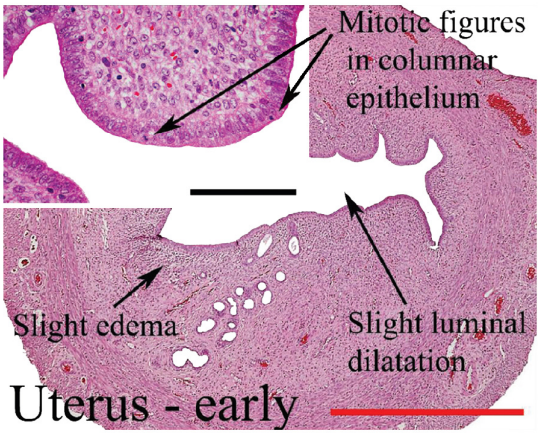

Uterine Artery
MESOMETRIUM
smooth muscle
MYOMETRIUM
longitudinal
circular
ENDO-METRIUM

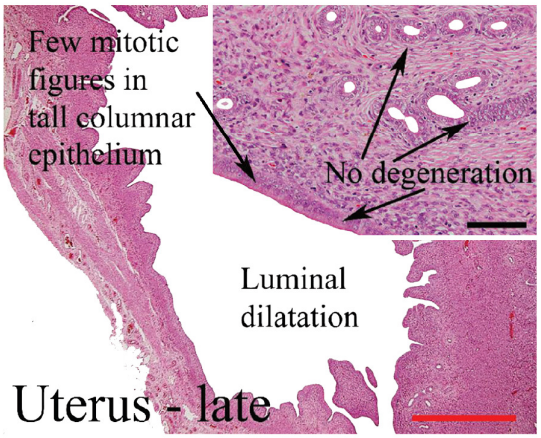
FIGURES 1.3A-1.3D. Histology of the rat uterus at different stages of the estrous cycle

Histological changes in the rat uterus during the estrous cycle. Paraffin section stained with haematoxylin and eosin. **A**, Proestrous. **B**, Estrous. **C**, Metestrous. **D**, Diestrous. Black bars = 100µm, red bars = 1 mm.

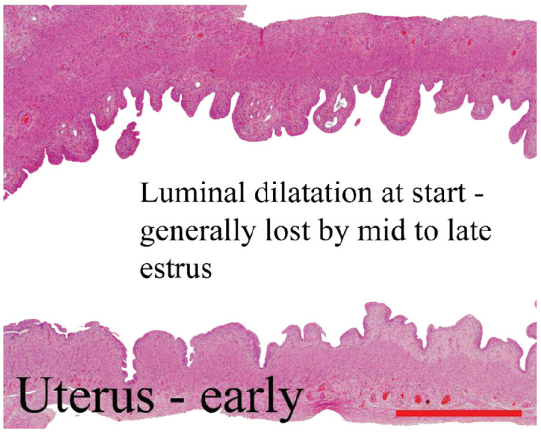
Modified from Westwood (2008)

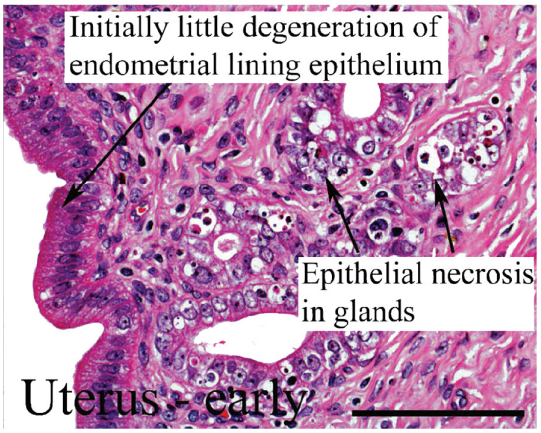
A. Proestrous

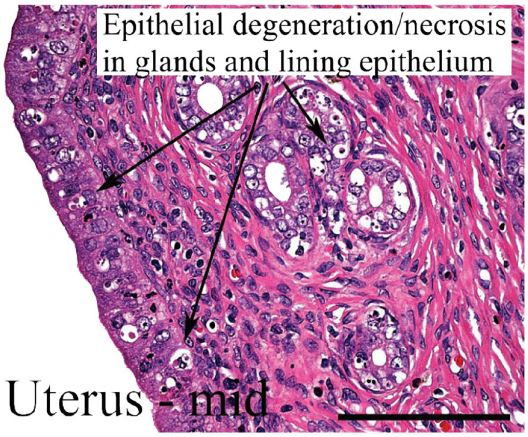




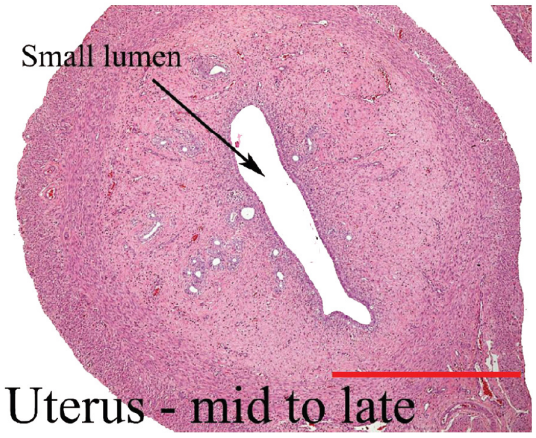
B. Estrous



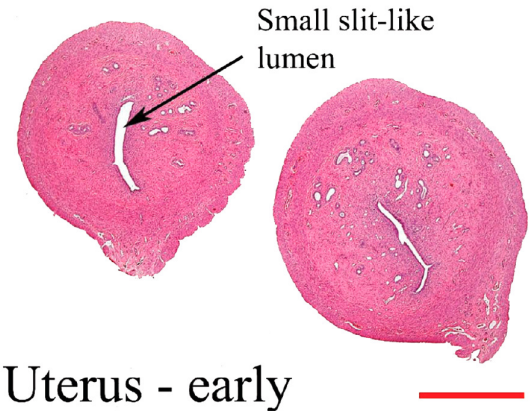


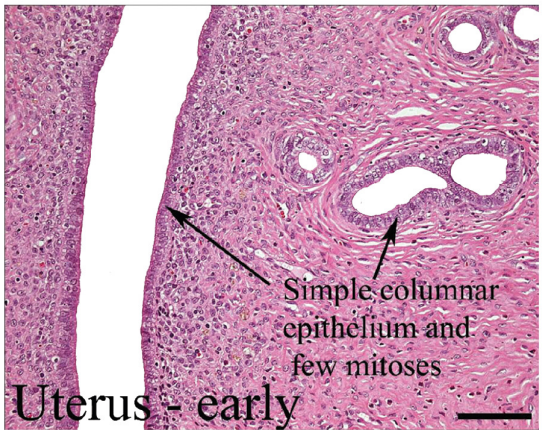


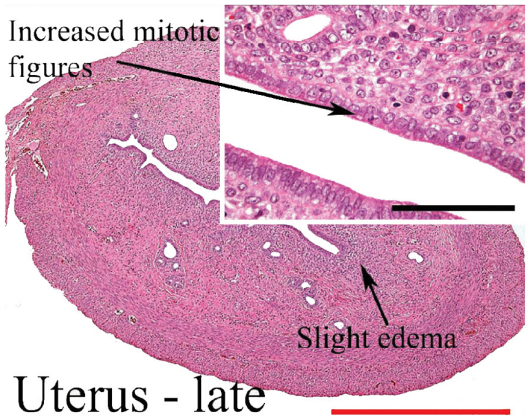
C. Metestrous



D. Diestrous







During early proestrous, the epithelium lining of the endometrium becomes cuboidal to columnar (Figure 1.3A); mitoses increase and the lumen of the uterus becomes larger towards the end of the stage (Figure 1.3B; Westwood, 2008). Estrous starts with disintegrating epithelial cells and glands (Figure 1.3B). The dilation of lumen is usually lost by the end of the stage (Figure 1.3B). Mitoses disappear (Westwood, 2008). During metestrous the epithelial cells continue to disintegrate along with mitotic activity (Figure 1.3C; Westwood, 2008). Diestrous is characterized by the presence of a small slit-like lumen (Figure 1.3D), absence of a notable vasculature and a columnar epithelial lining with a few degenerating cells (Figure 1.3D). Mitoses are uncommon at the start of the stage but gradually increase towards late diestrous. Late diestrous also shows a slight stromal oedema (Figure 1.3D; Westwood, 2008).

Innervation of the non-pregnant uterus

The uterus is innervated by sympathetic, parasympathetic and sensory nerves (Zoubina et al., 1998; Bae et al., 2001). The innervation of the uterus changes significantly during the estrous cycle in non-pregnant rats (Zoubina et al., 1998). These changes have been linked to the changes in the levels of oestrogen during the estrous cycle (Zoubina et al., 1998).

Sympathetic innervation

Sympathetic nerves are found in all regions of the uterus. Zoubina et al. (1998) used dopamine β hydroxylase (DBH) as a marker for sympathetic innervation and found that DBH-immunoreactive fibres were high in density in the vascular zone and in similar concentrations throughout the other layers of the uterus, including longitudinal smooth muscle, circular smooth muscle and the endometrium. The nerves ran parallel to the smooth muscle cells in the muscle layers (Bae et al., 2001). Zoubina et al (1998) also

noted that the density of sympathetic innervation was similar in the estrous and proestrus stages of the estrous cycle but that these two stages had lower densities compared to metestrus and diestrus. The innervation also varied in the different layers of the uterine wall and in different uterine regions. The ovarian end of the uterus had the highest density of DBH-immunoreactive fibres. The density of sympathetic nerves in the longitudinal smooth muscle was higher than in the circular muscle in the ovarian region while the endometrium had higher densities of DBH-immunoreactive fibres compared to the smooth muscle in the middle and cervical regions of the uterus. The smooth muscle of the cervical region showed very little variation during the estrous cycle. Bae et al, (2000) reported that the density of sympathetic nerves was greater in the myometrium compared to the endometrium using both DBH- and tyrosine hydroxylase (TH)-immunoreactivity as markers for sympathetic nerves (Bae et al., 2001). All studies have report that sympathetic nerves are often found close to blood vessels (Papka et al., 1985; Haase et al., 1997; Zoubina et al., 1998; Bae et al., 2001).

Nakanishi et al. (1969) treated human uteri with formaldehyde vapours to produce fluorescence in nerve containing catecholamines, including noradrenaline (NA). They found that in human non-pregnant uteri the density of NA nerves was highest around blood vessels and slowly decreased in density towards the anti-mesometrial side of the uterus. They also found that the cervical region had the richest supply of nerves.

Using glyoxylic acid-induced catecholamine fluorescence, Papka et al. (1985) reported noradrenergic fibers around the branches of the main uterine artery and travelling alone in the mesometrium. NA axons were particularly dense around arteries in the mesometrium. NA axons also ran parallel to mesometrial muscle cells, also shown by Bae et al (2001). The smooth muscle and arteries of the myometrium were also supplied with NA fibers. Axons immunoreactive for neuropeptide Y (NPY) were found around arteries

in the mesometrium. In the myometrium, the fibers occurred around blood vessels and in both the longitudinal and circular smooth muscle layers.

Alm & Lundberg. (1988) used TH, DBH and NPY as markers to study sympathetic nerves in non-pregnant guinea-pig uteri. All three neurochemicals showed identical distributions but the numbers of axons immunoreactive for each marker varied. Although NPY co-localises with NA in the fibers innervating blood vessels of the female reproductive system (Markiewicz et al., 2003), there were more TH and DBH axons than NPY axons. All three types of axons occurred in large nerve trunks and as fine varicose nerves in the myometrium.

As mentioned previously, the sympathetic innervation of the uterus changes during the estrous cycle. Varying hormone levels are probably the reason for these changes (Zoubina et al., 1998; Zoubina & Smith, 2000). This hypothesis is supported by the reduction in the density of DBH-immunoreactive nerves that occurs after administration of oestrogen in ovariectomized rats (Zoubina et al., 2001; Brauer, 2008). Sympathetic hyper innervation occurs in the estrogen receptor alpha knock-out mouse, suggesting that oestrogen acts through this receptor to cause changes in the innervation of the uterus (Zoubina & Smith, 2001). Oestrogen may influence uterine innervation by acting on sympathetic neurons or their targets or both (Zoubina & Smith, 2000). This hormonal influence on the innervation of the uterus during the estrous cycle is selective for sympathetic innervation (Zoubina & Smith, 2000). Although sensory innervation revealed by CGRP-immunofluorescence was found to decrease slightly during the estrous stage, there was no significant difference between the different phases of the estrous cycle. (Zoubina et al., 1998; Zoubina & Smith, 2001). Parasympathetic nerves marked by immunofluorescence for vasoactive intestinal polypeptide (VIP) or vesicular acetylcholine

transporter (VAChT) did not show significant variation during the estrous cycle (Morales et al., 1995; Zoubina & Smith, 2001).

NA can cause contraction or relaxation of the myometrial smooth muscle (Traurig & Papka, 1993; Papka et al., 1996). β-adrenergic receptor mechanisms promote relaxation and α-adrenergic mechanisms promote contraction (Traurig & Papka, 1993). NPY has been reported to be vasoconstrictive in different vascular beds, including in the female reproductive system (Papka et al., 1985; Markiewicz et al., 2003). In uterine blood vessels, both NA and NPY are responsible for constriction. NA is responsible for quick, short-lasting contractions whereas NPY is responsible for slow, long-lasting contractions (Traurig & Papka, 1993). During pregnancy, the arteries directly supplying the uterus are unresponsive to both NA and NPY (Traurig & Papka, 1993).

Parasympathetic innervation

VIP has been used as a marker for parasympathetic nerves in the uterus (Zoubina et al., 1998; Bae et al., 2001). Parasympathetic nerves occurred in the smooth muscle layers, endometrium and the vascular zone. In the endometrium, VIP immunoreactive fibers were found in the region of the endometrial glands. The circular muscle layer and the blood vessels had the highest densities of VIP-immunoreactive nerves and the cervical region of the uterus had a higher density than the ovarian region. The density of parasympathetic nerves did not change markedly during the estrous cycle (Morales et al., 1995).

Nitric oxide synthase (NOS), the enzyme that produces nitric oxide, is also present in parasympathetic nerves in the uterus. NOS-immunoreactive axons are found mainly in the vascular smooth muscle but also around blood vessels (Majewski et al., 1995). Nerves synthesizing nitric oxide (NO) in the uterine cervix can be parasympathetic or sensory (Papka et al., 1995).

NO relaxes both vascular and non-vascular smooth muscle (Papka et al., 1995) and is vasodilatory (Majewski et al., 1995).

Sensory innervation

Substance P (SP) and calcitonin gene related peptide (CGRP) are released by sensory neurons (Kingsley, 2000) and are considered to be markers for these nerves. The cell bodies of sensory neurons lie in the dorsal root ganglia (DRG) and these neurons provide sensory innervation to the female reproductive system as well as all other regions of the body (Papka & Traurig, 1993). There are two types of neurons identified by morphology in DRG. They are the large light (L- or A-type) neurons and the small dark (SD- or B-type) neurons (Lawson, 1995). Large neurons have myelinated axons (A fibers) and fast conduction velocities. Small neurons have unmyelinated axons (C fibers) (Kingsley, 2000) and slow conduction velocities (Kingsley, 2000). Several neuropeptides occur in DRG neurons, including SP, CGRP, neurokinin A (NKA), somatostatin (SOM), vasoactive intestinal peptide (VIP), galanin, opioid peptides and atrial natriuretic peptide (reviewed by (Lawson, 1995). SP is present in 18 to 20% of neurons in rat lumbar DRG, mostly in small neurons and sometimes in medium sized neurons. CGRP is present in 30 to 60% of neurons in rat and chick lumbar DRG mostly in small neurons and sometimes occurring in medium sized and large neurons. SP-, CGRP- and NKA-containing axons in the uterus originate from dorsal root ganglia at the thoracic, lumbar and sacral levels (Papka & Traurig, 1993).

Sensory nerves identified by immunoreactivity for CGRP have been found in all regions of the uterus (Morales et al., 1995) and occur in similar densities to nerves containing VIP (Bae et al., 2001). The myometrial vascular plexus had the highest density of CGRP-immunoreactive axons while the longitudinal and smooth muscle layers showed

a medium level of innervation. In the endometrium, CGRP-positive axons were present around blood vessels and glands. Alm & Lundberg. (1988) reported a high density of SP and CGRP fibers in the endometrium; CGRP fibers are also found in the smooth muscle of the myometrium and ran along with blood vessels. Co-existence of SP and CGRP fibers was seen by double-immunostaining. Sensory innervation did not vary with the estrous cycle, similar to parasympathetic innervation. However, unlike the parasympathetic nerves, sensory nerves were found in higher densities in the ovarian region than in the cervical region (Bae et al., 2001). Papka et al. (1985) showed that SP-immunoreactive nerves are distributed similar to NPY-immunoreactive nerves but with a lower density and that SP axons are present as free nerve endings in all layers of the uterus (Papka et al., 1985).

SP contracts the smooth muscle of the myometrium (Traurig & Papka, 1993; Papka & Traurig, 1993) and also dilates uterine blood vessels (Papka & Traurig, 1993). CGRP relaxes the uterine vasculature and also uterine smooth muscle (Traurig & Papka, 1993; Yallampalli et al., 2002; Gangula et al., 2003). Myometrial contractions in the uterus of rats and humans are inhibited by CGRP (Gangula et al., 2003).

In addition to their effects on smooth muscle, SP and CGRP may also play other roles in the uterus. SP dilates blood vessels to facilitate transport of blood-borne sensitizing agents, like histamine, to sites of tissue damage (Kingsley, 2000). SP also supports the growth of smooth muscle (Kingsley, 2000).

Changes in uterine innervation during pregnancy

During pregnancy, there are significant changes in the sympathetic innervation of all layers of the uterus (Haase et al., 1997). The size of the uterine horn gradually increases as pregnancy progresses and this is accompanied by a decrease in the number of sympathetic

nerves innervating the uterus. At day 15 of a 20-21 day pregnancy in rats, the sympathetic nerves were fragmented and had swollen varicosities while these nerves had almost completely disappeared at days 18 and 19 of pregnancy (Klukovits et al., 2002; Chavez-Genaro et al., 2006). However, three to five days postpartum, the density of the sympathetic nerves was similar to that seen during early pregnancy (Chavez-Genaro et al., 2006). Uterine-projecting sympathetic neurons have also been reported to decrease in size during pregnancy in the rat (Latini et al., 2008). Similar results were obtained for sympathetic nerves in guinea pigs after immunohistochemical staining using TH and NPY as markers for sympathetic nerves (Fried et al., 1985).

Studies on TH activity in the guinea pig uterus have supported the loss of sympathetic innervation during pregnancy (Alm et al., 2007). TH activity was measured by the formation of H_3-3, 4-dihydroxyphenylalanine (DOPA) from H_3-tyrosine in tissue homogenates. TH activity of the non-pregnant uterine horn was 1.132 ± 0.147 pM $\times$ $mg^{-1}\times$ h^{-1}. In a foetus-containing uterine horn, this decreased to 0.204 ± 0.048 pM $\times$ mg^{-1} $\times$ h^{-1} at early gestation (20 to 25 days of a 65-70 day pregnancy in guinea pigs). At mid-gestation (30 to 40 days pregnancy), the value was 0.048 ± 0.012 pM $\times$ mg^{-1} $\times$ h^{-1}. By 60 to 65 days, TH activity decreased to unmeasurable levels. An empty uterine horn showed similar reductions in TH activity during pregnancy but had a 90% reduction at 60 – 65 days of pregnancy (Alm et al., 2007). Along with reductions in TH activity, a decrease in NA levels in uterine horns devoid of foetuses also occurred in unilateral pregnancies (Owman, 1981; Brauer, 2008)

Fried et al. (1986) reported a significant decrease in the sympathetic innervation of the human uterus during pregnancy. A decrease in the density of NPY-immunoreactive axons was found in the second trimester (13 to 28 weeks of a 36 week pregnancy) while at term they were practically absent (Owman, 1981). Seventeen non-pregnant women had

NA concentrations of 410 ± 82 pmol of NA per gram of myometrial strip (w/w) and NPY concentrations of 2.14 ± 0.91 pmol/g of myometrial strip (w/w). In pregnant women, there were 23.7 ± 6.3 pmol of NA per gram of myometrial strip (w/w; n=14) and 0.17 ± 0.05 pmol of NPY per gram of myometrial strip (w/w; n=11; Fried et al., 1986). These findings provide conclusive evidence that pregnancy also reduces sympathetic innervation of the uterus in humans.

Although there is good data showing that pregnancy causes sympathetic denervation of the uterus in laboratory mammals and humans, sensory innervation of the human uterus during pregnancy has not extensively been examined. In the guinea pig uterus, (Alm & Lundberg, 1988) found a complete disappearance of sensory nerves identified by SP-, CGRP- and neurokinin A (NKA)-immunoreactivity during pregnancy. However, this result was based on only a very small sample of tissue (8 to 20 sections of 15µm thickness from each of 6 to 10 guinea pigs.).Traurig et al. (1984) have shown a decrease in the concentration of SP in the rat uterus at day 19 of pregnancy using radioimmunoassay (Traurig et al., 1984).

There is also evidence to indicate that parasympathetic innervation and expression of NOS change during pregnancy. Three different isoforms of NOS are expressed in the uterus, inducible NOS (iNOS), vascular endothelial constitutive NOS (eNOS) and the neuronal constitutive NOS (nNOS; Bansal et al., 1997; Thomson et al., 1997; Riemer et al., 1997; Norman et al., 1999; Massmann et al., 1999). Western blotting has shown that nNOS was expressed in the non-pregnant but not in the pregnant rat uterus. Immunohistochemistry revealed nNOS fibers in the longitudinal and circular smooth muscle of the non-pregnant rat uterus but not in the pregnant rat uterus (Riemer et al., 1997). Only 1 × 1 cm samples of full thickness uterine tissue was studied in both instances (Riemer et al., 1997). A reduction in the number of NO-synthesizing axons in the pregnant

rat uterus detected with NADPH-diaphorase was observed by Natuzzi et al. (1993). However, in the pregnant sheep uterus, no decrease in nNOS activity or protein was observed on the last day of pregnancy; NO-synthesizing nerve fibers were not studied (Massmann et al., 1999). In humans, western blotting and immunohistochemistry have shown that nNOS expression increases early in the third trimester but decreases late in the third trimester and during pregnancy (Norman et al., 1999). However, this finding is disputed by Thomson et al. (1997), who showed that nNOS activity and localization do not change during pregnancy in the human uterus, using arginine to citrulline conversion assay and immunocytochemistry for nNOS.

In all of the studies described above, most of the data concerning the innervation of the uterus have been collected from sections. Since sections only show a small sample of the nerves present in the uterus, whole mounts, which enable all nerves to be seen and quantified, would give a much more reliable picture of the innervation of pregnant and non-pregnant uterus.

Pregnancy induced changes in uterine blood vessels

In humans, uterine blood flow increases 10-fold during pregnancy in order to deliver adequate nutrients and oxygen to the growing foetus. In rats, uterine blood flow increases 70-fold (Page et al., 2002) whereas in pigs there is a 15-fold increase (Guenther et al., 1988). In order to meet the requirement for increased blood flow, the vessels that bring blood to the uterus undergo significant remodelling during pregnancy (Page et al., 2002). Most of the work on vascular remodelling during pregnancy has been done in the guinea-pig, where uterine arteries increase in length and diameter (Mione et al., 1988; Guenther et al., 1988). The length of the uterine artery in pregnant guinea pigs was 11.1 ± 2.0 cm compared to 3.5 ± 0.5 mm in the non-pregnant state while the diameter increased from 1.5

± 0.5 mm to 3.2 ± 0.7 mm (Mione et al., 1988). The diameter of the arterial lumen also increased by 157% during pregnancy (Mione et al., 1990). In addition, there is an increase in the number and volume of arterial smooth muscle cells (Mione et al., 1988) but a decrease in the collagen to elastin ratio (Guenther et al., 1988). In the non pregnant uterine artery of the guinea pig, SP-immunoreactive axons formed a sparse plexus around the uterine artery; NPY and VIP formed a dense plexus of immunoreactive fibres whereas CGRP immunoreactive fibres provided a moderate innervation (Mione et al., 1988). The CGRP, VIP and SP-immunoreactive fibres run along the main axis of the uterine artery (Mione et al., 1988). Significant denervation of the uterine artery occurs during pregnancy (Mione et al., 1988; Guenther et al., 1988). In the pregnant guinea pig, the density of NPY immunoreactive fibres decreased by 42%; VIP immunoreactive fibres, by 26%, CGRP immunoreactive fibres, by 45%; and SP immunoreactive fibres, by 25% (Mione et al., 1988). However, subsequent work showed an increase in NPY-immunoreactive innervation during pregnancy in guinea pig uterine artery (Mione et al., 1990).

The uterine vein of the rat remodels structurally during pregnancy with significant alterations in size, mechanical properties, matrix composition, cellular composition and density of nerves showing glyoxylic acid-induced catecholamine fluorescence. There is also an increase in lumen diameter, distensibility, number of dividing cells, mitotic indices in vascular smooth muscle and endothelial cells and also the venous diameter along with a decrease in elastin content in the uterine venous wall. Significant denervation of the uterine veins is observed (Page et al., 2002).

In pregnant guinea pigs, Fried & Thoresen. (1990) tested the effects of NPY and NA on uterine arterial pressure and velocity of blood flow. NPY (10-1000 pmol) caused a 15.4 - 46.4% increase in uterine arterial pressure compared to control while NA (10-1000 pmol) caused a 5 - 57.5% increase. NA (10-1000 pmol) also caused a 5.9 - 30.2% decrease in the

velocity of uterine blood flow whereas NPY had no effect on velocity (Fried & Thoresen, 1990). These date reflect the known vasoconstrictor effects of NPY and NA and the necessity for sympathetic denervation of blood vessels during pregnancy in order to accommodate the increase in blood flow.

Hypertensive disorders of pregnancy

Hypertensive disorders of pregnancy are differentiated on the basis of epidemiological features, pathophysiology and effects on mother and baby. These disorders include gestational hypertension, chronic hypertension, pre-eclampsia and eclampsia. Understanding the changes in uterine innervation during a normal pregnancy will aid in discovering the cause for pre-eclampsia, a hypertensive disorder of pregnancy where the expected denervation of the uterus does not occur as in a normal pregnancy.

Gestational hypertension is defined as the presence of elevated blood pressure after mid-gestation without proteinuria, i.e., presence of protein in the urine (Roberts et al., 2003; Leeman & Fontaine, 2008). This is a broad provisional diagnosis and 50% of women diagnosed with gestational hypertension develop diagnostic features of pre-eclampsia between 24 and 35 weeks of pregnancy (Leeman & Fontaine, 2008). It has been reported that women who develop severe gestational hypertension need to be treated like those who develop severe pre-eclampsia (Leeman & Fontaine, 2008). Severe gestational hypertension leads to highly unfavourable perinatal outcomes, such as a higher rate of preterm delivery and small for gestational age infants in comparison to mild pre-eclampsia (Leeman & Fontaine, 2008).

High blood pressure (140/90 mm Hg or more) which occurs before mid-pregnancy or persisting high blood pressure 12 weeks after delivery is defined as **chronic hypertension** (Roberts et al., 2003; Leeman & Fontaine, 2008). Development of pre-eclampsia along

with existing hypertension results in outcomes worse than *de novo* pre-eclampsia for both mother and child (Roberts et al., 2003), such as higher rates of caesarean delivery, intrauterine growth restriction, foetal death and postpartum haemorrhage.

Pre-eclampsia is diagnosed by a systolic blood pressure of ≥140 mm Hg or diastolic blood pressure of ≥90 mm Hg and proteinuria of ≥300 mg per 24 hours (Roberts et al., 2003). Pre-eclampsia can also be identified by laboratory tests for the HELLP (Hemolysis, Elevated Liver enzymes and Low Platelet count) syndrome. As the name implies, this syndrome is diagnosed by the presence of broken red blood cells (hemolysis), elevated liver enzymes and a low platelet count and is only seen in severe pre-eclampsia (Leeman & Fontaine, 2008). Other diagnostic features of pre-eclampsia include renal insufficiency, liver disease, neurological problems and haematological disturbances. Pre-eclampsia affects babies as well as mothers, leading to intrauterine growth restriction, pre term birth, low birth weight and perinatal death (Roberts, 2003). Delivery is the only cure for pre-eclampsia.

Eclampsia occurs when pre-eclampsia proceeds to the stage of life-threatening seizures (Roberts et al., 2003; Leeman & Fontaine, 2008). An eclamptic seizure can occur in mid-gestation, during delivery and up to 48 hours after delivery (Roberts et al., 2003; Leeman & Fontaine, 2008). Eclamptic seizures may also occur in women with slightly elevated blood pressure and no proteinuria (Leeman & Fontaine, 2008)

Pre-eclampsia

Pre-eclampsia affects between 5 to 8% of all pregnancies (Quinn, 2005). Between 50,000 and 370,000 women are affected by pre-eclampsia every year in industrialised countries. In developing countries between 1.5 and 8 million women develop pre-eclampsia annually. In addition, pre-eclampsia is responsible for 40,000 maternal deaths

each year in developing countries and 20% of babies born to pre-eclamptic women are either still born or die perinatally (Villar et al., 2003). Premature (induced) delivery prevents maternal deaths in industrialised nations (Roberts et al., 2003). Risk factors for pre-eclampsia include (adapted from Nelson-Piercy, 2003)

1. General
    - Age < 20 years, > 35 years
    - Obesity
2. Genetic
    - Mother (20-25% risk of developing pre-eclampsia)
    - Sister (35-40% risk of developing pre-eclampsia)
3. Obstetric
    - Previous pre-eclampsia
    - Primiparity
    - Long Birth interval
    - Multiple pregnancy
    - Hydatidiform mole (an unusual growth inside the uterus that forms at the start of pregnancy).
    - Hydrops (abnormal accumulation of fluid in body tissues or cavities)
    - Triploidy (presence of 3 chromosomes in the cell)
    - In vitro fertilization, using surgically obtained sperm or donor eggs
4. Medical
    - Hypertension
    - Diabetes
    - Renal disease
    - Systemic lupus erythematosus/anti phospholipid syndrome

- Inherited thrombophilia: abnormalities in coagulation.

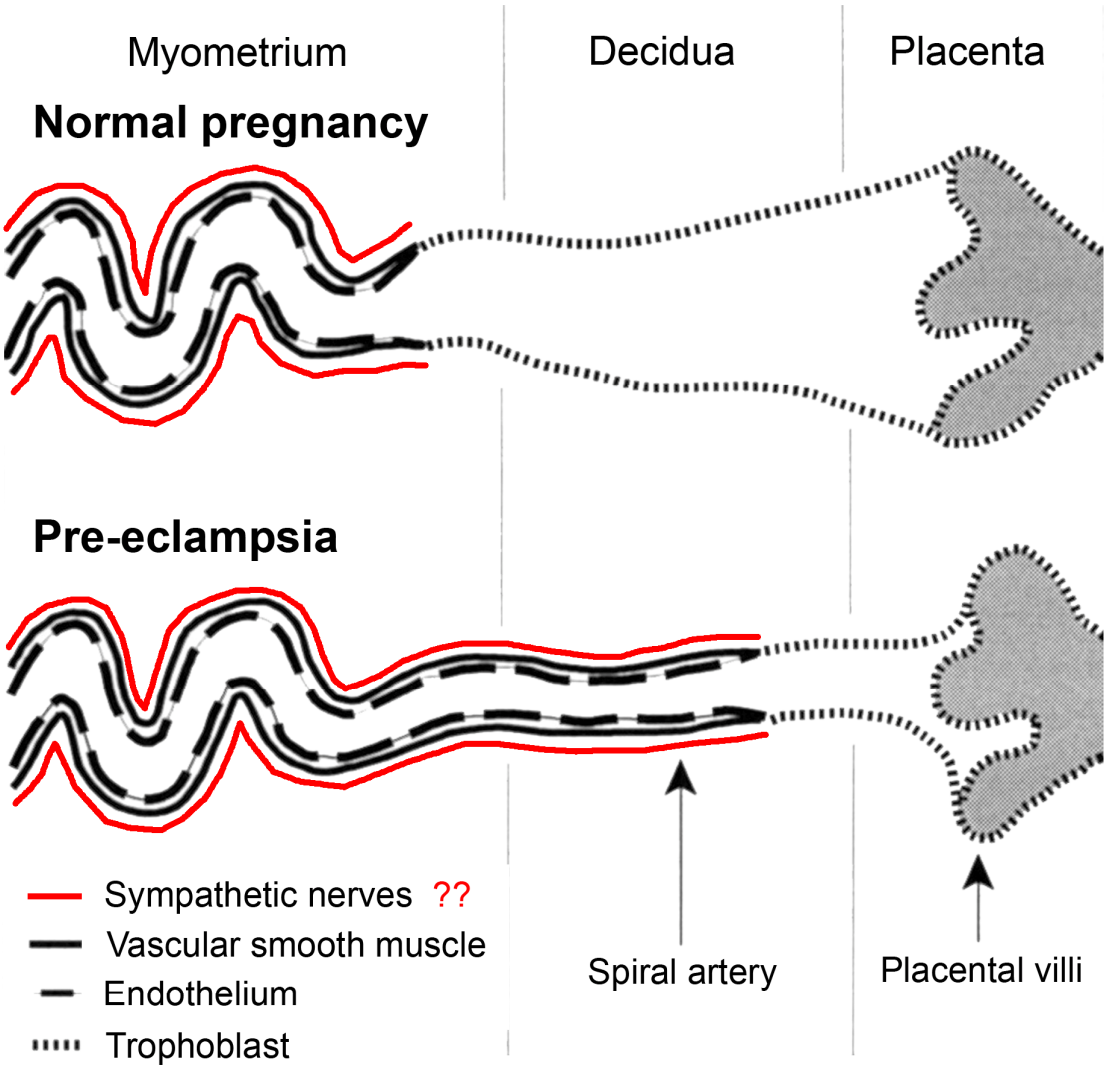
The pathophysiology of pre-eclampsia remains unclear but there are 2 features critical for its development (Roberts, 2003)

Stage one – reduced placental perfusion in pre-eclampsia

Stage one of pre-eclampsia is characterized by reduced perfusion of the placenta, which is due to defects in implantation and the failure of the blood vessels to remodel (Roberts et al., 2003; Roberts, 2003; Roberts, 2007). This important relation to placental perfusion was reported in 1939 (Page, 1939). Creating reduced perfusion by blocking arteries in rats and baboons has also resulted in signs and symptoms similar to pre-eclampsia in humans (Granger et al., 2006; Makris et al., 2007) and clinical results have confirmed the impaired remodelling of the blood vessels in human pre-eclampsia (VanWijk et al., 2000; Quinn, 2005). The smooth muscle and elastic tissues of the spiral arteries that supply the placenta are lost during a healthy pregnancy (Roberts, 2007); this results in arteries that are large, distended and unresponsive to constrictive vascular stimuli (VanWijk et al., 2000; Quinn, 2005). In pre-eclampsia, these changes do not occur in one-third of the arteries (VanWijk et al., 2000) and hence these vessels are narrower than in a normal pregnancy. The changes in the spiral arteries are initiated by trophoblast invasion of the placental bed (Figure 1.4; VanWijk et al., 2000). Defective trophoblast invasion results in the arteries retaining their endothelial and muscular lining so that vessels are narrow and reactive (VanWijk et al., 2000). The intervillus blood vessels also fail to remodel in pre-eclampsia. They do not increase in diameter and retain their vascular smooth muscle. All of these failures in remodelling result in a massive reduction in intervillus blood flow (Roberts, 2007).

FIGURE 1.4. Trophoblast invasion into the spiral arteries in the placental bed in normal pregnancy and in pre-eclampsia.

From VanWijk, M. J et al. (2000)


Myometrium
Decidua
Placenta
Normal pregnancy
Pre-eclampsia
Sympathetic nerves ??
Vascular smooth muscle
Endothelium
Trophoblast
Spiral artery
Placental villi

Stage two of pre-eclampsia

After placental perfusion decreases, blood flow to other maternal organs can also be compromised. In the liver, necrosis and haemorrhage occur along with reduced perfusion. The heart and brain suffer from subendocardial necrosis and petechial haemorrhage, respectively, along with reduced perfusion. The changes seen in the kidney include glomerular endotheliosis, which is characterised by the swelling of glomerular capillaries. Such changes have not been seen in other forms of hypertension and are therefore exclusive to pre-eclampsia. Three factors have been identified as the pathophysiological changes that result in reduced perfusion. These are vasoconstriction, the coagulation cascade and reduced plasma volume (Roberts et al., 2003).

Vasoconstriction in Pre-eclampsia

Vasoconstriction and reduced plasma volume are particularly important in the reduced blood flow observed in pre-eclampsia (Roberts et al., 2003). Vasoconstriction is the narrowing of blood vessels, which results in reduced blood flow. Vasoconstriction and vasodilation are controlled by sympathetic nerves and parasympathetic nerves, respectively. Therefore, changes in the structure and function of sympathetic nerves could underlie some of the symptoms of pre-eclampsia. As mentioned above, pregnancy results in significant denervation of the uterus. Studies in humans have shown that in a pre-eclamptic pregnancy, innervation is higher than in a normal pregnancy. Normal pregnant women had a uterine NA content of 23.7 ± 6.3 pmol/g (w/w) and NPY content of 0.17 ± 0.05 pmol/g (w/w) whereas pre-eclamptic patients had a NA content of 98.8 ± 9.9 pmol/g (w/w) and NPY content of 0.88 ± 0.16 pmol/g w/w (Fried et al., 1986). These results show that NA and NPY are elevated in a pre-eclamptic pregnancy compared to a normal pregnancy. NA and NPY are sympathetic vasoconstrictors that cause an increase in blood

pressure; NA also decreases the velocity of blood flow (Fried & Thoresen, 1990).. O'Shaughnessy et al. (1983) have shown an increase in norepiephrine, dopamine and epinephrine content in pre-eclampsia compared to a normal human pregnancy using a radioenzymatic assay. A comparison of sympathetic nerve activity in pregnant, non pregnant, non-pregnant hypertensive and pre-eclamptic women showed an increase in sympathetic vasoconstrictor activity in women with pre-eclampsia that returned to normal after delivery (Schobel et al., 1996). Although sympathetic vasoconstrictor discharge to skeletal muscle alone was studied, sympathetic activity that is elevated during pre-eclampsia and returns to normal after delivery suggests that the impaired response of sympathetic neurons responsible for vasoconstriction may be at least partly responsible for the reduced blood blow characteristic of pre-eclampsia. Parasympathetic nerves that control vasodilation have not been studied in any detail during pregnancy or pre-eclampsia in either animals or humans and there is very limited data on NOS synthesizing nerves during pregnancy.

Existing animal models of pre-eclampsia

As mentioned previously, there is clear evidence of defects in the remodelling of uterine innervation during pre-eclampsia. In order to further investigate the involvement of defective remodelling of the innervation of the uterus and uterine blood vessel in the pathophysiology of pre-eclampsia, an effective animal model of pre-eclampsia is necessary. This would aid in quantifying the changes in the innervation of the pre-eclamptic uterus and in determining the causes for these innervation changes. This knowledge will be useful in suggesting treatment and preventive strategies for pre-eclampsia. Neurotrophic factors such as nerve growth factor (NGF) have been suggested as possible causes for the remodelling of uterine innervation (Latini et al., 2008). One of

the future directions of this project is to develop a physiologically relevant animal model of pre-eclampsia to investigate what factors might lead to defective remodelling of innervation. Existing animal models of pre-eclampsia are described below. None of these models address the question of whether excessive vasoconstriction plays a role in the development of pre-eclampsia. Furthermore, changes in the innervation of uterine blood vessels have not been examined in any of the models.

Reduced Uterine Perfusion Pressure (RUPP) Model

Granger and colleagues published the RUPP model of pre-eclampsia in 2006 (Granger et al., 2006). In this model, a silver clip is placed around the aorta below the renal arteries and on the main uterine artery at its ovarian end. This surgery reduces uterine perfusion pressure by 40%. Clipping the blood vessels resulted in elevated arterial pressure (20 to 30 mmHg), proteinuria, increased oxidative stress (increased production of reactive oxygen species which was measured by an increase in 8-isoprostane which is a marker of oxidative stress), reduced renal plasma flow and glomerular filtration rate and intrauterine growth restriction, all of which are features of pre-eclampsia. However, the model is not truly physiological because the arteries are blocked whereas in pre-eclampsia reduction in blood flow is attributed to increased vasoconstriction and not blockage.

Uteroplacental ischemia (UPI)

Makris et al. (2007) developed the UPI model, in which one uterine artery was ligated, to cause a 30 to 50% reduction in placental perfusion in the corresponding uterine horn. This model shows symptoms of pre-eclampsia, including hypertension, proteinuria, renal histological changes and plasma biochemical changes. Here again, similar to the RUPP model, the artery is blocked and hence this is also not an ideal physiological model to study reduced blood flow in pre-eclampsia.

Soluble fms-like tyrosine kinase (s FLT-1) and soluble endoglin (s Eng)

s Flt-1 and s Eng are circulating factors that are derived from the placenta and are found in elevated levels in a pre-eclamptic pregnancy (Venkatesha et al., 2006). Injection of these factors into animals causes symptoms of pre-eclampsia, including an increase in mean arterial pressure, foetal growth restriction, renal and liver changes, hemolysis and vascular damage (Venkatesha et al., 2006). However, it is not clear how these factors relate to the vasoconstriction seen in preeclampsia. It is possible that their release is triggered by hypoperfusion of the placenta so elevated levels are a consequence not a cause of vasoconstriction.

Angiotensin receptor agonistic autoantibodies

Angiotensin II is a hypertensive peptide, the effects of which result from activation of the angiotensin II receptor type 1a ($AT1_A$ receptor). Injecting immunoglobulin from pre-eclamptic women into mice activates the AT1 receptor and causes pre-eclamptic symptoms (Zhou et al., 2008). Hypertension, proteinuria, impaired renal function, placental abnormalities, intrauterine growth restriction and increased sFLT1 concentrations all occur in this pre-eclamptic animal model. However, the innervation of uterine blood vessels has not been studied.

Aims and Hypothesis

General Hypothesis

Pregnancy causes plastic changes in the sympathetic, parasympathetic and sensory innervation of the uterus.

Aims:

- To develop a method to assess sympathetic, parasympathetic and sensory innervation of the entire non-pregnant and pregnant rat uterus using whole mount preparations.
- To describe in detail the sympathetic, parasympathetic and sensory innervation of the non-pregnant and pregnant rat uterus.

Biotechnology Significance

Understanding the changes in the innervation of the uterus during pregnancy and the changes in the neurons providing the innervation will enable future studies on determining the exact causes of pre-eclampsia and may suggest new drug therapies to treat this condition.