Histol Histopathol (2016) 31: 415-424

http://www.hh.um.es

Histology and Histopathology

From Cell Biology to Tissue Engineering

Effects of catecholaminergic nerve lesion on endometrial development during early pregnancy in mice

Yulan Dong*, Guanhui Liu*, Zixu Wang, Jing Li, Jing Cao and Yaoxing Chen

Laboratory of Veterinary Anatomy, College of Animal Medicine, China Agricultural University, Beijing, China

*Yulan Dong and Guanhui Liu contributed equally to the study

Summary. Maternal stress is common during pregnancy and the postnatal period. This stress typically activates the sympathetic nervous system which releases catecholamines. This study explored the influence of sympathectomy by using neurotoxin 6-hydroxydopamine (6-OHDA) on embryo implantation, and investigated the influence mechanism of sympathectomy on reconstruction of endometrial structure during early pregnancy. In the 6-OHDA-treated mice, uterine glands in the endometrium developed poorly, and the gland epithelia were arranged irregularly during early pregnancy. Furthermore, vacuoles, karyopykosis and plasmarrhexis appeared in some gland epithelia. The percentage of uterine glands and the density of proliferating cell nuclear antigen (PCNA) positivity were dramatically decreased, and Fas ligand (FasL) expression was decreased in cells from pregnancy days 5-9 (E5-9) in the treated group. Antioxidant enzyme activity levels in uteri were lower but the malondialdehyde (MDA) levels were higher in the 6-OHDA mice than those in the control mice at E5-9. Similarly, the number of inducible nitric oxide synthase (iNOS) positive cells was significantly increased during early pregnancy following treatment with 6-OHDA. Our results have indicated that peripheral catecholaminergic nerve lesions induced by 6-OHDA cause adverse pregnancy outcomes through disruption of endometrial gland development, which increases oxidative stress and iNOS expression in the endometrium. Thus, catecholaminergic nerves might favourably influence blastocyst implantation, foetal survival and development during early pregnancy by oxidative state regulation and endometrial gland reconstruction.

Key words: Catecholaminergic nerve, Endometrial gland, FasL, Oxidative stress, Pregnant mice

Introduction

Maternal stress is common during pregnancy and the postnatal period. From 2009-2010, approximately 75% of mothers in the USA suffered from at least one stressful event before delivery of their child (Centres for Disease Control and Prevention, Pregnancy Risk Assessment Monitoring System). Over 25% of mothers in South Asia have been reported to suffer from depressive disorder during the third trimester of pregnancy (Rahman et al., 2003). In addition, approximately 54% and 37.1% of mothers in China experienced antenatal anxiety and depression, respectively (Lee et al., 2007). Stress typically triggers the following two endocrine systems: (1) the hypothalamic-pituitary-adrenal (HPA) axis and glucocorticoid production; and (2) the sympathetic

Offprint requests to: Prof. Yaoxing Chen, Laboratory of Veterinary Anatomy, College of Animal Medicine, China Agricultural University, Haidian, Beijing, 100193, P.R. China. e-mail: yxchen@cau.edu.cn

DOI: 10.14670/HH-11-684

Abbreviations. 6-OHDA, 6-hydroxydopamine; DAB, 3′3-diaminobenzidine tetrahydrochloride; FasL, Fas ligand; GSH-PX, glutathione peroxidise; iNOS, inducible nitric oxide synthase; MDA, malondialdehyde; NET, NE transport body; NO, nitric oxide; PCNA, proliferating cell nuclear antigen; ROS, reactive oxygen species; SOD, superoxide dismutase; T-AOC, total antioxidant capacity; VEGF, vascular epithelial growth factor.

nervous system and catecholamine release (epinephrine and norepinephrine (NE)). The communication between pregnancy and sympathetic nerves which secrete catecholamine has long been studied (Fried et al., 1986; Dong et al., 2007, 2012; Brauer, 2008). For example, sympathetic denervation has been reported to cause the number of implanted embryos to decrease to 64.4% (on pregnancy day 7 (E7)), and to result in small-sized embryos in mice (Dong et al., 2007) and rats (Mac Donald and Airaksinen, 1981).

The mechanism of action of sympathetic nerve during pregnancy is unclear. Most previous reports have focused on cardiac pacing (Curione et al., 2005), blood pressure (Carter, 2012; Jarvis et al., 2012) and immunity (Minagawa et al., 1999; Dong et al., 2007) during pregnancy. A recent review by Latini et al. (2008) has emphasised that remodelling of sympathetic innervation in rodent and human uteri during the oestrous cycle and gestation and alterations in the biological mechanisms of uterine innervation might play significant roles in infertility and spontaneous abortion. However, some papers have suggested that sympathetic nerves have more dramatic effects on myometrial and endometrial development. Uterine innervation is abundant within the vascular zone, which is interposed between these two smooth muscle layers (Zoubina et al., 1998), and the fibres in the endometrium are mainly associated with blood vessels at the myometrial border (Haase et al., 1997; Zoubina et al., 1998). A change in the catecholamine concentration in the uterus has been previously reported to influence the rate of contractions and myometrial sensitivity to relaxin (Rudzik and Miller, 1962). Furthermore, sympathetic innervation has been shown to modulate electromyographic activity from the myometrium and mesometrium in nonpregnant ovariectomised sheep under oestradiol supplementation, and chemical sympathectomy has been reported to induce a decrease in spontaneous activity (Massmann et al., 1992). Furthermore, evidence suggests that catecholamines act as scavengers of free radicals and that they are potent antioxidants (Jodko and Litwinienko, 2010; Alani et al., 2014).

Despite these advances, the mechanisms underlying induction of the development of uterus endometrial plasticity by sympathetic nerves are not fully understood. In particular, whether sympathetic nerves influence the proliferation and apoptosis of uterine gland epithelial cells is unknown. 6-OHDA is a hydroxylated derivative of catecholamine that is similar in structure (Blum et al., 2001). Therefore, the current study aimed to explore the influence of sympathetic nerves on the reconstruction of endometrial structure during early pregnancy.

Materials and methods

Animals and 6-OHDA treatments

Eight- to nine-week-old Kunming white female (25-35 g) and male (35-45 g) mice which obtained from Beijing Biological Products Company (Beijing, China) were used in this study. The mice were housed under conventional conditions and were fed a standard diet. This study was performed in accordance with the Guidelines for Animal Experimentation of China Agriculture University. After an adaptive period of one week, one hundred female mice were divided equally into two groups and were treated daily with a 0.01% L-ascorbic acid antioxidant vehicle in sterile saline (0.01 mL/g body weight) containing 6-OHDA (Sigma, St. Louis, MO, USA) at a doses of 0 (control group, n=50) or 100 mg/kg body weight (6-OHDA-treated group, n=50) by intraperitoneal injection for five days. Mice in the oestrous stage were immediately identified by performing vaginal smears with Wright's staining each evening. Oestrous mice were mated with male mice. On E1, E3, E5, E7 and E9, the animals (n=10 per time point) were euthanised by cervical dislocation under deep nembutal anaesthesia (50 mg/g body weight).

Histology and immunohistochemistry

Uterine tissue was immediately immersed in 4% paraformaldehyde in 0.1M PBS (pH 7.4), fixed overnight (4°C), dehydrated in a graded ethanol series, and embedded in paraffin. Sections (5 μm) were mounted on gelatinised glass slides and deparaffinised, rehydrated and rinsed with 0.01M PBS. For the histological experiments, sections were stained with haematoxylin and eosin. The ratios of the areas of all uterine glands and total uterine walls were measured from five cross-sections of the uterus of each animal using the Image Pro Plus software (IPP 6.0, Media Cybernetics, Silver Spring, MD, USA).

For immunohistochemistry, sections were treated with 3% hydrogen peroxide in methanol for 30 min and incubated with 5% normal goat serum (Sigma, Inc., USA) for 20 min at room temperature. After rinsing with PBS, the sections were incubated overnight at 4°C with a monoclonal mouse anti-human proliferating cell nuclear antigen (PCNA; 1:200 in PBS, p8825, Sigma, St. Louis, MO, USA), rabbit polyclonal Fas ligand (FasL; 1:50 in PBS, ab21233, Abcam Plc, Cambridge, UK), or inducible nitric oxide synthase (iNOS; 1200 in PBS, sc-651, Beijing Zhongshan, Inc., Beijing, China) primary antibody. Then, the sections were rinsed in PBS and incubated with a biotinylated donkey anti-mouse antibody for PCNA (SAB3701101, 1:200, Sigma, St. Louis, MO, USA) or a goat anti-rabbit (FasL, iNOS) secondary antibody (1:100, SAB3700848, Sigma) for 2 h at room temperature. After washing, the tissues were incubated with streptavidin-horseradish peroxidase (1:200, S2438, Sigma, St. Louis, MO, USA) for 2 h at room temperature. Immunoreactivity was visualised by incubation in 0.01M PBS containing 0.05% 3′3-diaminobenzidine tetrahydrochloride (DAB, Sigma, St. Louis, MO, USA) and 0.003% hydrogen peroxide for 10 min in the dark. The sections were mounted after a final rinse. The specificity of immunostaining was assessed by omitting the primary antibody incubation step.

Positive cells were observed in twenty visual fields from each slide, and five cross sections of the uterus of each animal were chosen. The number of positive cells was counted under a microscope (Bx51, Olympus, Japan), and the number of cells per mm^2 was calculated using Image-Pro Plus 6.0.

Oxidative stress-related enzymes and total antioxidant capacity and the oxidative product malondialdehyde

Uterine tissue without embryos was immediately frozen on dry ice and stored at -80°C. Experimentally, 10% of uterine homogenate in a 0.9% saline solution was prepared and centrifuged (x1000 g, 20 min) to detect oxidative production and antioxidative enzymes. Superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) are well-known scavenger enzymes that protect cells from oxidative stress, and malondialdehyde (MDA) is responsible for the induction of oxidative stress. T-AOC, SOD, GSH-Px and MDA assay kits were purchased from NanJing JianCheng Bioengineering Institute (Nanjing, China). The SOD, GSH-Px and T-AOC levels are expressed as U/mg·protein. The MDA level is expressed as nmol/mg·protein.

Data analysis

Data are expressed as the means ± SD. The independent-sample T test was used to analyse the results. Differences at a $P<0.05$ were considered statistically significant.

Results

6-OHDA caused poor development of the uterine glands in early pregnant mice

The structure of the uterine glands changed significantly in the 6-OHDA-treated mice. Fig. 1 shows that the uterine glands in the endometrium developed poorly during early pregnancy in the treated groups, and the gland epithelium was arranged irregularly. Furthermore, some pathologies, such as vacuoles, karyopykosis and plasmarrhexis, appeared in some epithelia. As showed in Table 1, the percentage of uterine glands in uterus walls decreased from (2.48±0.10%) at E1 to (0.30±0.02%) at E9 in the control groups and decreased from (1.55±0.06%) at E1 to (0.12±0.02%) at E9 in the treated groups. The area percentages of uterine glands in uterine walls decreased by 37.5%, 38.9%, 41.7%, 61.4% and 60.0%, respectively from E1-9 in the treated groups compared with the control groups. There were significant differences at E1-E9 between the control and 6-OHDA-treated groups ($P<0.01$).

Endometrial proliferation was delayed in early pregnant mice by 6-OHDA

PCNA is a protein expressed in cell nuclei and reflects the state of cell proliferation. PCNA-positive epithelia appeared round and oval with yellowish colour, and positive particles were located in nuclei (Fig. 1). PCNA was scattered in uterine cavity epithelia, gland epithelia and the muscular layer at E1 and E3. It was primarily localised to decidual cells of the endometrium and was found in few cells in the muscular layer, but it was not observed in the cavity epithelium or gland epithelium at E5 and E7. PCNA expression was strong in decidual cells, although it varied in different areas of the uterus. The density of PCNA-positive cells in the endometrium increased from 459.5±20.2/mm^2 at E1 to a peak at E7 3246.7±31.2/mm^2 in the control mice, and then decreased to 1698.6±157.4/mm^2 at E9. The densities of PCNA-positive decidual cells decreased by 27.2%, 30.2%, 40.7%, 27.2% and 27.7% from E1-E9 ($P<0.01$) in the 6-OHDA-treated groups compared with the control groups. Gland epithelial cell proliferation varied with the development of pregnancy. Normally, the proliferation index of the glandular epithelium was higher during pre-implantation (E3). The proliferation indices of the glandular epithelium decreased significantly by 35.2% and 11.3%, respectively, at E1 and E3 in the treated groups compared with the control groups, but there were few PCNA-positive gland epithelia cells after implantation in the control and treated mice (Table 2).

Table 1. Ratio of uterine glands between 6-OHDA-treated groups and control mice during early pregnancy (unit:%).

groups	E1	E3	E5	E7	E9
Control	2.48±0.10	2.16±0.39	1.32±0.11	1.27±0.07	0.30±0.02
6-OHDA	1.55±0.06**	1.32±0.32**	0.77±0.08**	0.49±0.26**	0.12±0.02**

** indicates highly significant difference ($P<0.01$).

Table 2. PCNA expression indices between the 6-OHDA-treated groups and the control mice during early pregnancy.

Site	Groups	E1	E3	E5	E7	E9
Endometrium (unit: number/mm2)	Control	459.5±20.2	1961.4±15.3	2413.5±245.3	3246.7±31.2	1698.6±157.4
	6-OHDA	334.3±35.2	1368.6±5.8	1430.1±332.7	2364.8±30.9	1228.3±97.0
Glandular epithelium (unit: %)	Control	32.7±6.2	90.0±2.4	—	—	—
	6-OHDA	21.2±4.8**	79.8±4.5**	—	—	—

"—" indicates glandular epithelia were negative; ** indicates highly significant difference ($P<0.01$).

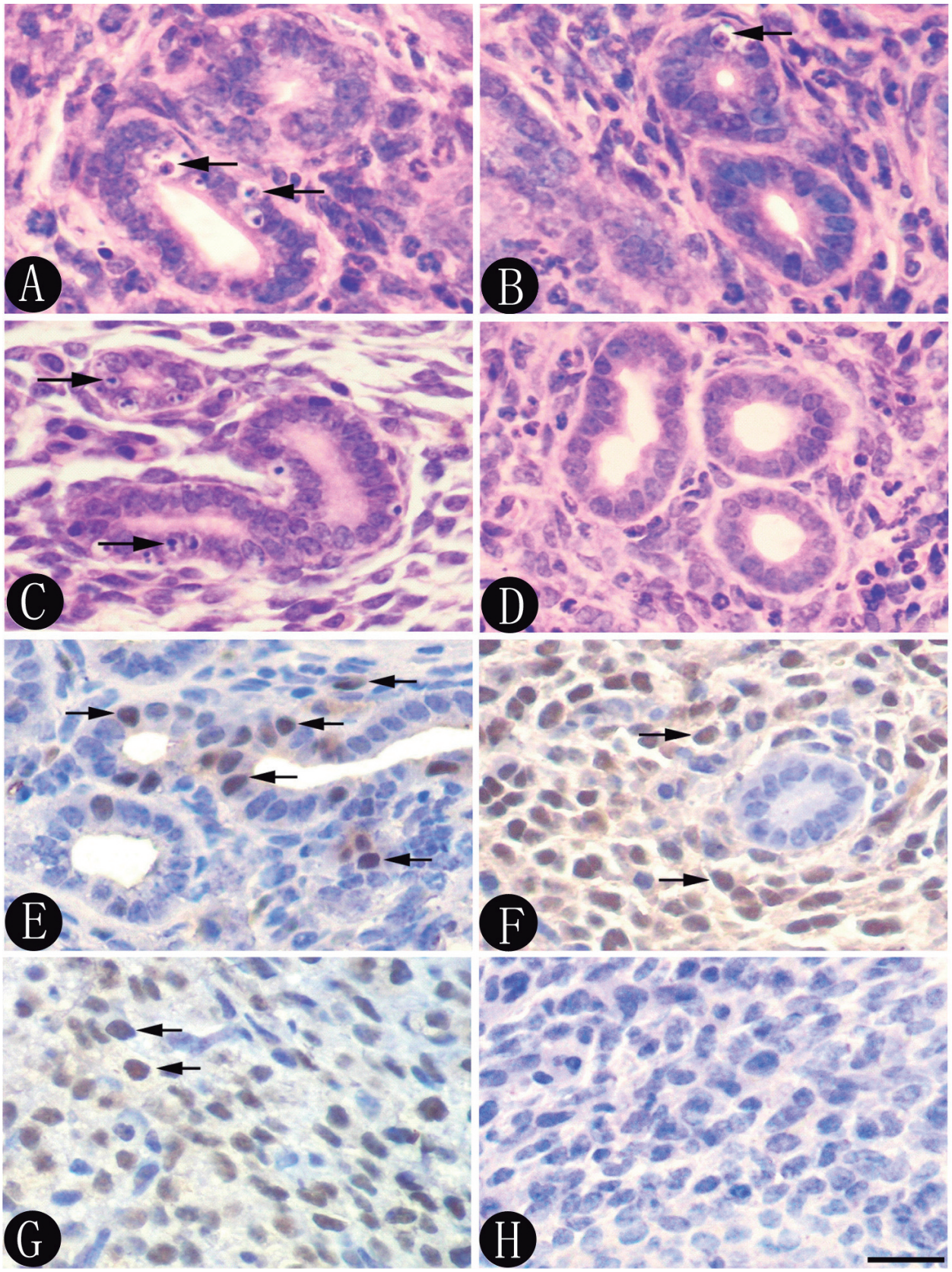

Fig. 1. Morphology of and PCNA expression in the uterine endometria of 6-OHDA-treated and control mice during early pregnancy. **A and B.** 6-OHDA-treated group - the glandular epithelium was arranged irregularly and exhibited vacuole variation, as indicated by the arrows. **A** (E1) and **B** (E3). **C.** 6-OHDA-treated group - the glandular epithelium exhibited pyknosis at E5, as indicated by the arrows. **D.** Control groups - the glandular epithelium exhibited a regular arrangement and was well developed at E3. **E.** PCNA was expressed in the glandular epithelium and interstitial cells, as indicated by the arrows (E1). **F.** PCNA positivity was highly prevalent in decidual cells, but not in the glandular epithelium, as indicated by the arrows (E5). **G.** PCNA positivity was highly prevalent in decidual cells, as indicated by the arrows (E5). **H.** Negative control. Scale bar: 50 μm.

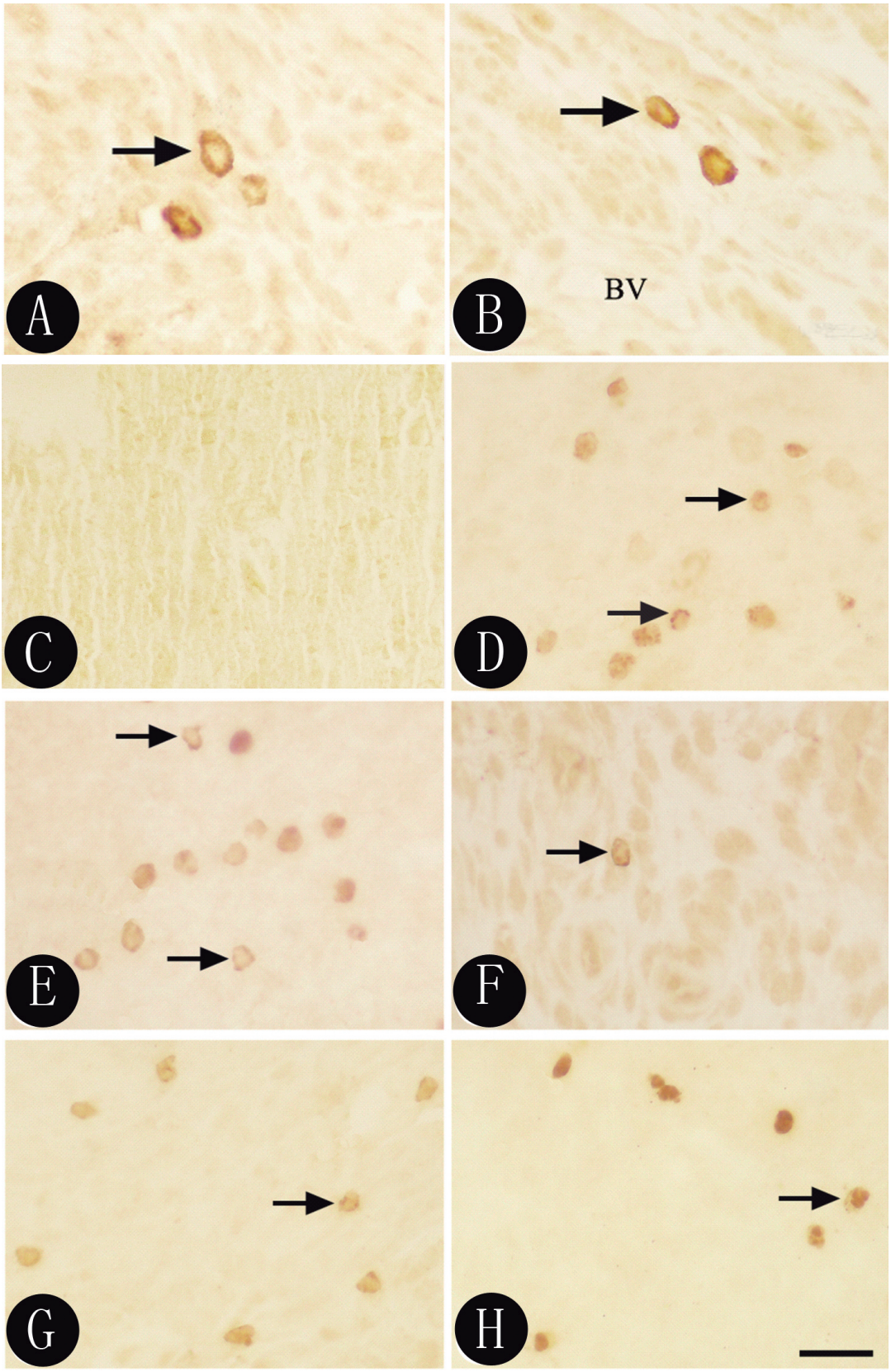

Fig. 2.
Immunohistochemistry staining for FasL and iNOS in endometrial cells of mice during early pregnancy. FasL expression is shown in cells in **A** (E5, control group) and **B** (E7, control group). FasL-positive cells are indicated by the arrows, which point to the vascular layer of the endometrium. iNOS-positive cells are indicated by brown staining in the endometrial stroma in **D** (E1), **E** (E3), **F** (E5), **G** (E7) and **H** (E9). iNOS-positive cells are indicated by the arrows. The negative control is shown in **C**. BV: blood vessel. Scale bar: 20 μm.

FasL expression induced by 6-OHDA in the uterine endometria of early pregnant mice

FasL-positive cells were round or oval with a claybank colour, and positive granules were located in the cytoplasm but not in the nucleus (Fig. 2). No FasL expression was detected in the endometria in either of the groups; instead, it was mostly expressed near blood vessels and in the mesometrium before E3. The number of FasL-positive cells was the highest (44.3±3.8/mm^2) in the control group, and it was decreased by 28.9% (P<0.01) in the treated group at E5 (Table 3). Further, FasL expression was decreased by 14.6% (P<0.05) at E7 and by 13.5% (P<0.05) at E9 in the treated mice compared with the control mice.

Table 3. Numbers of FasL-positive cells in endometria between 6-OHDA-treated groups and control mice during early pregnancy (unit: number/mm^2).

Groups	E1	E3	E5	E7	E9
Control	—	—	44.3±3.8	30.2±2.5	31.8±2.8
6-OHDA	—	—	31.5±3.1**	25.8±2.5*	27.5±2.1*

"—" indicates FasL expression was negative; * indicates significant difference (P<0.05), ** indicates highly significant difference (P<0.01)

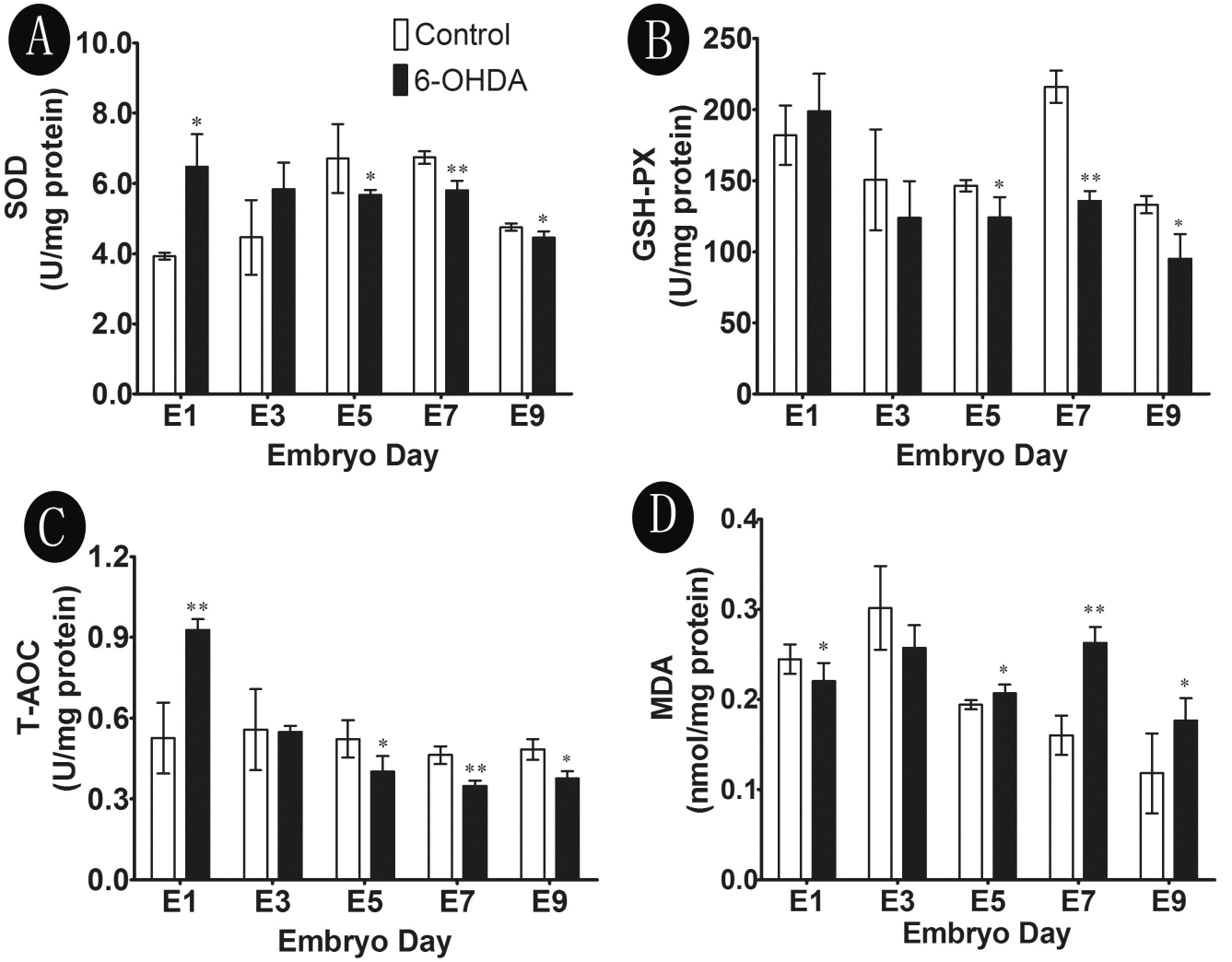

Fig. 3. Changes in oxidative stress in the uterus of mice during early pregnancy. The bar charts show the relative mean normalised expression levels of SOD **(A)**, GSH-Px **(B)**, T-AOC **(C)** and MDA **(D)** in the uteri of the control and 6-OHDA mice from E1-9. **P<0.01 and *P<0.05 vs. the control counterparts.

Table 4. Numbers of iNOS-positive cells in endometria between 6-OHDA-treated groups and control mice during early pregnancy (unit: number/mm^2).

Groups	E1	E3	E5	E7	E9
Control	585.7±28.0	169.4±3.1	3.9±0.3	20.8±1.3	10.8±0.7
6-OHDA	1032.2±38.5**	246.9±5.2**	7.0±0.5**	39.4±2.2**	17.2±1.3**

** indicates highly significant difference (P<0.01)

iNOS positivity induced by 6-OHDA in uterine cells from early pregnant mice

The immunohistochemistry results (Fig. 2) revealed that iNOS-positive granules were located in the cytoplasm, and cells appeared round or oval with a claybank colour. Most of the positive cells were scattered in the mesenchyma (or decidua) and vessels. The density and distribution of positive cells changed with pregnancy development. There were a few positive cells at E1, but no expression was detected at E3 in the muscle layer. In the endometrium, the number of iNOS-positive cells was the highest (585.7±28.0/mm^2) at E1, and it then decreased to the lowest number (3.9±0.3/mm^2) at E5 during early pregnancy in the control mice (Table 4). The numbers of iNOS-positive cells were significantly increased by 76.2%, 45.7%, 79.5%, 89.4% and 59.3% from E1-E9 (P<0.01) in the treated mice compared with the controls. Thus, 6-OHDA increased iNOS expression during early pregnancy.

Antioxidant capacity and oxidative stress induced by 6-OHDA in the uteri of early pregnant mice

The SOD activity levels in the uteri of the treated mice decreased from E1 to E9, and they decreased dramatically by 15.48% (P<0.05), 13.84% (P<0.01), 6.24% (P<0.05) from E5-9 compared with those in the uteri of the control groups (Fig. 3). The GSH-PX activity levels were 15.22% (P<0.05), 37.10% (P<0.01) and 28.51% (P<0.05) lower in the treated mice from E5 to E9 compared with those in the control mice. The T-AOC levels decreased from (0.56±0.15) U/mg·protein in the control mice to (0.55±0.02) U/mg·prot in 6-OHDA mice at E3 and they significantly decreased by 23.19% (P<0.05), 24.84% (P<0.01) and 22.14% (P<0.05) at E5, E7 and E9 in the 6-OHDA mice versus the control mice. However, the MDA levels in the 6-OHDA mice were 6.50%, 63.67% and 49.60% higher compared with those in the control mice at E5 (P<0.05), E7 (P<0.01) and E9, respectively (P<0.05). Therefore, 6-OHDA significantly increased the oxidative stress level after implantation (E5-E9).

Discussion

A statistical report stated among the 6.5 million pregnancies in the United States in 2008, approximately 10%-25% of all pregnancies end in miscarriage (Ventura et al., 2012). Other surveys in China have revealed that rates of abortion vary 3.6-14.6% in humans (Gao et al., 1993; Liu and Gao, 2002; Hu et al., 2008). Among pregnancy failures in all stages, preimplantation occurs approximately 30% (Macklon et al., 2002; Stephenson et al., 2002; Philipp et al., 2003), and another 30% of pregnancies lost are in the immediate few weeks following implantation (Macklon et al., 2002). In addition, there are only a very limited percentage (0.5-5%) of the somatic cloning embryos to full-term development in sheep, cattle, goats, pigs, and mice, and the reason is mainly due to a high frequency of peri-implantation and postimplantation developmental arrest (Kato et al., 2000; Zhou et al., 2000), such as fetal losses associated with placental abnormalities during the first trimester in cattle (Hill et al., 2000; Ogura et al., 2013). Those showed that the implantation is an important stage and closely associates with embryos loss rates. Besides, there are various causes for early embryo miscarriage: uterine anatomical anomalies, endocrine infections, decreased maternal immune tolerance (Pandey et al., 2005), exposure to environmental stress factors (Kutteh, 1999; Sugiura-Ogasawara et al., 2002) and parental chromosomal disorders. Environmental stress factors could inactivate or activate catecholaminergic nerves, which innervate most organs and play dominant roles in organ blood flow regulation and cardiac performance. 6-OHDA, the specific neurotoxin for catecholaminergic neurons, was transported to induce semi-permanent damage of terminal of sympathetic nerve by noradrenergic receptor (Kostrzewa and Jacobovitz, 1974; Rotman, 1977; Penttilä Heikki et al., 2001). Usually 6-OHDA was used to establish parkinson's disease animal model with intracerebral injection (Hu et al., 2010), but with injection intraperitoneally was used to explore the role of sympathetic nerve on organs outside cranial cavity, like small intestine (Ke et al., 2011). Furthermore, local injection of 6-OHDA into the fatpads of popliteal and inguinal lymph nodes of rats resulted in a significant reduction of NE content in the lymph nodes, spleen and heart on 1, 5, and 14 days following treatment (Lorton et al., 1996), and re-innervation in spleen of young adult male rats proceeds initially along the splenic artery as it enters the hilus (1-5 days), extends into the hilar region (5-10 days), and later proceeds into the regions distal to the hilus (21-56 days) following 6-OHDA treatment (Lorton et al., 1990, 2009). Previously, we reported that the sympathetic nerve terminals of uterus in pregnant mice had significantly disappeared and the number of implanted embryos in treated mice decreased to 64.4% (at E7) after catecholaminergic denervation by 6-OHDA (Dong et al., 2007). Therefore, we hypothesised that catecholaminergic nerves might regulate implantation environment through endometrial development and blood flow during early pregnancy.

The endometrial glands synthesise or transport and

secrete substances that are essential for the survival and development of the embryo or foetus (Carson et al., 2000). Stewart has found that endometrial glands play a crucial role during the pre-implantation period of early pregnancy (Stewart and Cullinan, 1997). Certain secretory components from the endometrial glands, such as the mucin MUC-1 and glycodelin A, are taken up by the secondary yolk sac lining the exocoelomic cavity during pregnancy and might thereby assist in providing foetal nutrition (Burton et al., 2002). Moreover, vascular epithelial growth factor (VEGF) is highly expressed on endometrial gland epithelia for uterine angiogenesis and vascularisation and promotes the exchange of nutrients at the maternal-foetal interface (Haouzi et al., 2009). Thus, endometrial gland development in the uterus is crucial to blastocyst implantation and embryo survival. Some tube-like endometrial glands in the uterus are lined by columnar epithelia. Generally, in the non-pregnant uterus, the gland cavity is small in size and displays a round or oval shape. After conception, the glands become enlarged and elongated, presenting a contorted or waved appearance. Histological microscopy showed that the endometrial uterine glands developed poorly during early pregnancy in the 6-OHDA-treated mice, and that the gland epithelia were arranged irregularly. Furthermore, some pathologies, such as vacuoles, karyopykosis and plasmarrhexis, appeared in some epithelia. Further, the percentage of uterine glands greatly decreased during early pregnancy. Therefore, these poorly developed endometrial glands were harmful to the embryo. Bottalico has reported that NE transport body (NET) mRNA is expressed on the surfaces of uterine glandular epithelial cells and exhibits cyclical physical changes, demonstrating the relationship between the sympathetic nerves and uterine glands during pregnancy (Bottalico et al., 2003). Therefore, in this study, the destruction of catecholaminergic nerves by 6-OHDA was considered to inhibit the development of endometrial glands development during early pregnancy.

Uterine glandular epithelial cells are the functional cells of the endometrial glands. PCNA is a protein that is expressed in the cell nuclei and reflects the state of cell proliferation. Uterine gland epithelia displayed cell dysplasia, vacuolation and karyopyknosis in histological experiments. Our data indicated that the proliferation indices of the glandular epithelium were significantly decreased at E1 and E3 in the treated groups compared with the control groups. These results further clarified that the sympathetic nerves regulated the development of the endometrial glands during early pregnancy. Another study has reported that FasL is associated with the maintenance of immune privilege and with ensuring the protection and growth of placental tissues (Guvendag et al., 2008). It was noted that FasL expression appeared after implantation (E5, E7 and E9) in the control and treated mice, and it was lower in the treated mice compared with the control group on these days. Therefore, 6-OHDA inhibited both PCNA and FasL expression within uterine glandular epithelial cells. However, Pan and his collaborators reported that the expression of FasL, but not that of Fas, within the striatum and substantia nigra pars compacta increased after 6-OHDA lesions were created in a rat model of Parkinson's disease (Pan et al., 2007). The reasons for these differences may be complex. On one hand, the mechanisms of action of 6-OHDA might be different between peripheral organs and the brain. Neurons exposed to 6-OHDA in the brain exhibit dopaminergic loss, but an intraperitoneal injection of 6-OHDA results in increased susceptibility of noradrenergic nerve terminals to lesion formation compared with dopaminergic and adrenergic nerves. However, FasL is mainly present in haematopoietic cells and immune privileged sites, such as the eyes and testes (Brint et al., 2013). Furthermore, some reviews have found that Fas activation also results in non-apoptotic responses, such as cell proliferation or NF-kappaB activation (Wajant et al., 2003; Brint et al., 2013).

In addition, the endometrial injury caused by 6-OHDA might be related to free radicals and oxidative cellular damage. The results of this study showed that the level of antioxidant function was decreased and that MDA was up-regulated by 6-OHDA. In MN9D cells treated with 6-OHDA, apoptosis has been shown to be closely correlated with increases in reactive oxygen species (ROS) levels and down-regulation of SOD expression (Zhou et al., 2003). Nitric oxide synthases (NOSs) comprise a family of enzymes that catalyse the production of nitric oxide (NO) from L-arginine and induce oxidative stress (Knowles and Moncada, 1994). NO controls programmed cell death (apoptosis) through up-regulation of the tumour suppressor p53 and subsequent changes in the expression of pro- and anti-apoptotic Bcl-2 family members, cytochrome C relocation, caspase activation, chromatin condensation, and DNA fragmentation (Brune et al., 2000). Ota et al. have found that both iNOS and endothelial nitric oxide synthase (eNOS) expression increase during endometriosis and adenomyosis and have suggested that their expression is correlated with early embryo loss (Ota et al., 1998). In our study, the numbers of iNOS-positive cells increased obviously increased from E1-E9 in the treated groups compared with those in the control groups. These results further confirmed that 6-OHDA had an adverse impact on embryonic development by promoting oxidative cellular damage.

In summary, our results have shown that peripheral catecholaminergic nerve lesions induced by 6-OHDA interfered with endometrial gland development, including inhibition of PCNA and FasL expression. The suppressive effects of 6-OHDA on the endometrium indicate that iNOS expression was promoted, which might have increased the oxidative stress level, consequently promoting cell apoptosis. Therefore, catecholaminergic nerves might have favourable effects on blastocyst implantation and foetal survival and development during early pregnancy by regulating the oxidative state and influencing endometrial reconstruction.

Acknowledgements. This work was supported by the National High-tech Research and Development Projects (863) (No. 2013AA102306), and the National Natural Science Foundation of China (Nos. 31272483, 30671514, and 31372332).
Conflicts of interest statement. The authors disclose that there are no conflicts of financial interest that could inappropriately influence the work.

References

Alani B., Salehi R., Sadeghi P., Zare M., Khodagholi F., Arefian E., Hakemi M. and Digaleh H. (2014). Silencing of Hsp90 chaperone expression protects against 6-hydroxydopamine toxicity in PC12 cells. J. Mol. Neurosci. 52, 392-402.

Blum D., Torch S., Lambeng N., Nissou M., Benabid A., Sadoul R. and Verna J. (2001). Molecular pathways involved in the neurotoxicity of 6-OHDA, dopamine and MPTP: contribution to the apoptotic theory in Parkinson's disease. Prog. Neurobiol. 65, 135-172.

Bottalico B., Pilka R., Larsson I., Casslen B., Marsal K. and Hansson S. (2003). Plasma membrane and vesicular monoamine transporters in normal endometrium and early pregnancy decidua. Mol. Hum. Reprod. 9, 389-394.

Brauer M. (2008). Cellular and molecular mechanisms underlying plasticity in uterine sympathetic nerves. Auton. Neurosci. 140, 1-16.

Brint E., O'Callaghan G. and Houston A. (2013). Life in the Fas lane: differential outcomes of Fas signaling. Cell. Mol. Life Sci. 70, 4085-4099.

Brune B., von Knethen A. and Sandau K. (2000). Nitric oxide (NO): an effector of apoptosis. Cell Dev. Differ. 6, 969-975.

Burton G., Watson A., Hempstock J., Skepper J. and Jauniaux E. (2002). Uterine glands provide histiotrophic nutrition for the human fetus during the first trimester of pregnancy. J. Clin. Endocrinol. Metab. 87, 2954-2959.

Carson D., Bagchi I., Dey S., Enders A., Fazleabas A., Lessey B. and Yoshinaga K. (2000). Embryo implantation. Dev. Biol. 223, 217-237.

Carter J. (2012). Sympathetic neural tone: large and in charge throughout pregnancy. J. Physiol. 590, 3411-3412.

Curione M., Cugini P., Napoli A., Colatrella A., Di Bona S., Cammarota C., Amato S., Castro C. and Fallucca F. (2005). A lower level of entropy in circadian rhythm of the sinus R-R intervals suggests a prevalence of the cardiac sympathetic regulation in early physiological pregnancy. Chronobiol. Int. 22, 711-722.

Dong Y., Chen Y., Wang Z., Naito J. and Chen J. (2007). Role of sympathetic nerves in early embryonic development and immune modulation of uterus in pregnant mice. Auton. Neurosci. 131, 87-93.

Dong Y., Chen Y., Wang Z. and Zhao J. (2012). Effects of chemical sympathectomy on the level of reproductive hormones and mast cells during mouse early pregnancy. Asian J. Anim. Vet. Adv. 7, 960-969.

Fried G., Hokfelt T., Lundberg J., Terenius L. and Hamberger L. (1986). Neuropeptide Y and noradrenaline in human uterus and myometrium during normal and pre-eclamptic pregnancy. Hum. Reprod. 1, 359-364.

Gao E.R., Deng X., He G., Fang K., Tang W. and Lou C. (1993). The analysis of abortion rate in Chinese married women. Reprod. Contraception (Chin) 13, 358-366.

Guvendag Guven E., Okur H. and Beksac M. (2008). Placental fas/fas ligand expression in early pregnancy losses. Am. J. Reprod. Immunol. 60, 1-7.

Haase E., Buchman J., Tietz A. and Schramm L. (1997). Pregnancy-induced uterine neuronal degeneration in the rat. Cell Tissue Res. 288, 293-306.

Haouzi D., Mahmoud K., Fourar M., Bendhaou K., Dechaud H., De Vos J., Rème T., Dewailly D. and Hamamah S. (2009). Identification of new biomarkers of human endometrial receptivity in the natural cycle. Hum. Reprod. 24, 198-205.

Hill J., Burghard R., Jones K., Long C., Looney C., Shin T., Spencer T., Thompson J., Winger Q. and Westhusin M. (2000). Evidence for placental abnormality as a major cause of mortality in first-trimester somatic cell cloned bovine fetuses. Biol. Reprod. 63, 1787-1794.

Hu L., Lu M., Tiong C., Dawe G., Hu G. and Bian J. (2010). Neuroprotective effects of hydrogen sulfide on Parkinson's disease rat models. Aging Cell 9,135-146.

Hu X., Bai Y., Zhao C. and An H. (2008). Study on natural abortion of hospitalized pregnant woman in Lanzhou city. Chin. J. Public Health (Chin) 24, 146-147.

Jarvis S., Shibata S., Bivens T., Okada Y., Casey B., Levine B. and Fu Q. (2012). Sympathetic activation during early pregnancy in humans. J. Physiol. 590, 3535-3543.

Jodko K. and Litwinienko G. (2010). Oxidative stress in the neurodegenerative diseases--potential antioxidant activity of catecholamines. Postepy Biochem. (Polish) 56, 248-259.

Kato Y., Tani T. and Tsunoda Y. (2000). Cloning of calves from various somatic cell types of male and female adult, newborn and fetal cows. J. Reprod. Fertil. 120, 231-237.

Ke Y., Liu W., Wang Z., Dong Y. and Chen Y. (2011). The Role of sympathectomy on the distribution of intraepithelial lymphocyte, mast cell, IgA+, CD4+ and CD8+ cell in intestine of mice. Asian J. Anim. Vet. Adv. 6, 935-943.

Knowles R. and Moncada S. (1994). Nitric oxide synthases in mammals. Biochem. J. 298, 249-258.

Kostrzewa R. and Jacobovitz D. (1974). Pharmacological actions of 6-hydroxydopamine. Pharmacol Rev. 26, 199-288.

Kutteh W. (1999). Recurrent pregnancy loss: an update. Curr. Opin. Obstet. Gynecol. 11, 435-439.

Latini C., Frontini A., Morroni M., Marzioni D., Castellucci M. and Smith P. (2008). Remodeling of uterine innervation. Cell Tissue Res. 334, 1-6.

Lee A., Lam S., Sze Mun Lau S.M., Chong C., Chui H. and Fong D. (2007). Prevalence, course, and risk factors for antenatal anxiety and depression. Obstet. Gynecol. 110, 1102-1112.

Liu B. and Gao E.R. (2002). Risk factors for spontaneous abortion of Chinese married women at reproductive age. Chin. J. Public Health (Chin) 18, 890-892.

Lorton D., Bellinger D., Hewitt D., Felten S. and Felten D. (1990). Noradrenergic reinnervation of the rat spleen following chemical sympathectomy with 6-hydroxydopamine: pattern and time course of reinnervation. Brain Behav. Immun. 4, 198-222.

Lorton D., Bellinger D., Duclos M., Felten S. and Felten D. (1996). Application of 6-hydroxydopamine into the fatpads surrounding the draining lymph nodes exacerbates adjuvant-induced arthritis. J. Immunol. 67, 103-113.

Lorton D., Lubahn C., Sweeney S., Major A., Lindquist C.A., Schaller J., Washington C. and Bellinger D.L. (2009) Differences in the injury/sprouting response of splenic noradrenergic nerves in Lewis rats with adjuvant-induced arthritis compared with rats treated with

6-hydroxydopamine. Brain Behav. Immun. 23, 276-285.

Mac Donald E. and Airaksinen M. (1981). Studies on the mechanism of action of the abortive effect of 6-hydroxydopamine in rats. Med. Biol. 59, 111-115.

Macklon N., Geraedts J. and Fauser B. (2002). Conception to ongoing pregnancy: the 'black box' of early pregnancy loss. Hum. Reprod. Update 8, 333-343.

Massmann G., Figueroa J., Hoffman G. and Nathanielsz P. (1992). Role of the sympathetic innervation on uterine electromyographic activity in nonpregnant ovariectomized sheep under estrogen supplementation. Biol. Reprod. 46, 483-491.

Minagawa M., Narita J., Tada T., Maruyama S., Shimizu T., Bannai M., Oya H., Hatakeyama K. and Abo T. (1999). Mechanisms underlying immunologic states during pregnancy: possible association of the sympathetic nervous system. Cell. Immunol. 196, 1-13.

Ogura A., Inoue K. and Wakayama T. (2013). Recent advancements in cloning by somatic cell nuclear transfer. Philos. Trans. R. Soc. Lond. B 368, 1-12.

Ota H., Igarashi S., Hatazawa J. and Tanaka T. (1998). Endothelial nitric oxide synthase in the endometrium during the menstrual cycle in patients with endometriosis and adenomyosis. Fertil. Steril. 69, 303-308.

Pan J., Zhao Y., Wang Z., Jin L., Sun Z. and Chen S. (2007). Expression of FasL and its interaction with Fas are mediated by c-Jun N-terminal kinase (JNK) pathway in 6-OHDA-induced rat model of Parkinson disease. Neurosci. Lett. 428, 82-87.

Pandey M., Rani R. and Agrawal S. (2005). An update in recurrent spontaneous abortion. Arch. Gynecol. Obstet. 272, 95-108.

Penttilä Heikki K., von Smitten Karl A. and Waris T. (2001). Disappearance of catecholamine fluorescence from the adrenergic nerves in arterial grafts in rats: an experimental fluorescence histochemical study. Scand. J. Plast. Reconstr. Surg. Hand Surg. 35, 123-128.

Philipp T., Philipp K., Reiner A., Beer F. and Kalousek D. (2003). Embryoscopic and cytogenetic analysis of 233 missed abortions: factors involved in the pathogenesis of developmental defects of early failed pregnancies. Hum. Reprod. 18, 1724-1732.

Rahman A., Iqbal Z. and Harrington R. (2003). Life events, social support and depression in childbirth: perspectives from a rural community in the developing world. Psychol. Med. 33, 1161-1167.

Rotman A. (1977). The mechanism of action of neurocytotoxic compounds. Life Sci. 21, 891-900.

Rudzik A. and Miller J. (1962). The effect of altering the catecholamine content of the uterus on the rate of contractions and the sensitivity of the myometrium to relaxin. J. Pharmacol. Exp. Ther. 138, 88-95.

Stephenson M., Awartani K. and Robinson W. (2002). Cytogenetic analysis of miscarriages from couples with recurrent miscarriage: a case-control study. Hum. Reprod. 17, 446-451.

Stewart C. and Cullinan E. (1997). Pre-implantation development of the mammalian embryo and its regulation by growth factors. Dev. Genet. 21, 91-101.

Sugiura-Ogasawara M., Furukawa T., Nakano Y., Hori S., Aoki K. and Kitamura T. (2002). Depression as a potential causal factor in subsequent miscarriage in recurrent spontaneous aborters. Hum. Reprod. 17, 2580-2584.

Ventura S., Curtin S., Abma J. and Henshaw S. (2012) Estimated pregnancy rates and rates of pregnancy outcomes for the United States, 1990-2008. Natl. Vital Stat. Rep. 60, 1-21.

Wajant H., Pfizenmaier K. and Scheurich P. (2003). Non-apoptotic Fas signaling. Cytokine Growth Factor Rev. 14, 53-66.

Zhou Q., Boulanger L. and Renard J. (2000). A simplified method for the reconstruction of fully competent mouse zygotes from adult somatic donor nuclei. Cloning 2, 35-44.

Zhou Y., Chen X., Zhu Y., Fang F., Chen L. and Chen Y. (2003). JNK signaling pathway mediate MPTP- induced apoptosis in subs tantia nigra neurons. J. Fujian. Med. Univ. (Chin) 37, 125-127.

Zoubina V., Fan Q. and Smith P. (1998). Variations in uterine innervation during the estrous cycle in rat. J. Comp. Neurol. 397, 561-571.

Accepted November 10, 2015