TROPHOBLAST-INDUCED SPIRAL ARTERY REMODELLING AND UTEROPLACENTAL HAEMODYNAMICS IN PREGNANT RATS WITH INCREASED BLOOD PRESSURE INDUCED BY HEME OXYGENASE INHIBITION.

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Declarations of interest: none.

ABSTRACT

Introduction

The aim of the present study was to determine the contribution of the heme oxygenase (HO) system to the adaptation of the uteroplacental circulation to pregnancy in the rat, and its relationship with the maintenance of blood pressure during late gestation.

Methods

The HO inhibitor, stannous mesoporphyrin (SnMP), or vehicle were administered intraperitoneally to virgin and midpregnant rats. Mean arterial pressure (MAP) was measured before and after the treatment, in the conscious rats. Uterine and radial arteries blood flow velocities were obtained from pregnant rats at days 14 and 19 of gestation using high frequency ultrasonography. Trophoblast invasion and spiral arteries remodelling were analyzed in the mesometrial triangle of pregnant rats by inmunohistochemistry.

Results

HO activity inhibition during late gestation induced a significantly increase in the MAP of pregnant rats (114±1 mmHg vs 100±2 mmHg, $p<0.05$) but it did not affect this parameter in virgin rats (121±2 mmHg vs 124±3 mmHg). MAP elevation was associated with marked ($p<0.05$) decreases in the systolic and diastolic flow velocities in uterine and radial arteries, as compared with pregnant control rats. Furthermore, spiral arteries of pregnant rats treated with SnMP showed lower ($p<0.001$) proportion of lumen circumference covered by trophoblast (21±3%) and a higher ($p<0.05$) proportion of vascular smooth muscle (33±5%) than control pregnant rats (59±5% and 16±5%, respectively)

Discussion

These data indicate that HO system play an important role in the adaptation of the uteroplacental circulation to pregnancy and in the blood pressure regulation during late gestation.

Keywords

Trophoblast invasion, placenta, uteroplacental haemodynamics, vascular remodelling, hypertension, Doppler ultrasonography.

INTRODUCTION

Cardiovascular adaptation to gestation involves complex hemodynamic changes, including significant increases in cardiac output and blood volume, a marked reduction in the systemic resistance and a subsequent decline in blood pressure. Uteroplacental vasculature undergoes significant structural and functional modifications to ensure an adequate blood supply to the developing placenta and the fetus. In early stages of pregnancy, trophoblasts invade the uterine spiral arteries, replacing muscular and endothelial cells of the arterial wall and transforming them in low resistance vessels with an increased blood flow and reduced pressure (1). Furthermore, uterine, arcuate and radial arteries show a progressive dilation through gestation, thereby also contributing to the reduced resistance and the increased blood flow in the uteroplacental vascular bed (2, 3). Despite the intensive research linking uteroplacental blood flow alterations with several pregnancy diseases, such as hypertension, preeclampsia, intrauterine growth restriction and early pregnancy loss (4), the mechanisms leading to impaired adaptation of uteroplacental circulation in these disorders are still poorly understood.

Recent studies have reported that heme oxygenase (HO) system is an important regulator of uteroplacental function (5-8). HO, which catalyzes the conversion of heme to bilirubin and carbon monoxide (CO), is highly expressed in human, rat and mouse placenta (5, 9, 10) and its deficiency has been associated with growth restriction, placental dysfunction and fetal loss (5,6,11,12) . Recently, it has been also reported that the treatment of late pregnant rats with a HO inhibitor provokes hypertension associated with a decrease in placental vascular endothelial growth factor (VEGF), suggesting that the contribution of HO system to the regulation of arterial pressure during gestation may be mediated by the upregulation of proangiogenic factors (13). However, it is unknown whether hypertension induced by a decreased HO activity during pregnancy is related with an impaired trophoblast-induced spiral artery remodelling and/or with alterations in uteroplacental haemodynamics. Therefore, the aim of the current study was to investigate the contribution of the HO system to the adaptation of the uteroplacental

circulation to pregnancy in the rat, and its relationship with the maintenance of blood pressure during late gestation.

MATERIAL AND METHODS

Animals

All studies were performed in female Sprague Dawley (SD) rats purchased from the University of Murcia Animal Research Laboratory. The study was approved by the University Review Committee and experimental protocols were designed according to the National Institutes of Health Guide for the Care and Use of Laboratory Animals. Animals were housed in an environmentally controlled facility and were allowed free access to food and water. Rats (12 weeks of age) destined to become pregnant were mated overnight. The presence of sperm in vaginal smear on the following morning was considered as the first day of pregnancy.

HO-1 and HO-2 expression.

Implantation sites (placenta with associated mesometrial triangle) were obtained from pregnant rats at 8 (n=6), 14 (n=5) and 19 (n=4) days of gestation. Tissues samples were homogenized in ice-cold 50-mmol/L Tris-HCl buffer, pH 7.4, containing 1% NP-40, 0.25% sodium deoxycholate, 1-mmol/L EDTA, and 10% protease inhibitor cocktail (Sigma -Aldrich). Homogenates (50 μg) were electrophoresed on a 12% polyacrilamide gel and then transferred to a polyvinylidene difluoride membrane. Membranes were incubated for 1 h with a 1:1000 dilution of rabbit anti-rat HO-1 monoclonal or HO-2 polyclonal antibodies (Stressgen Biotechnologies). Afterwards, the membranes were incubated with horseradish peroxidase- conjugated anti-rabbit Ig G (Amersham) at a dilution of 1:2000. Chemiluminescence detection was performed with the Amersham ECL detection kit according to the manufacturer´s instructions. Protein expression changes were quantified by densitometry analysis and presented as the ratio of HO proteins to β-actin expression in samples run in the same gel.

Measurement of arterial pressure in conscious rats.

To determine the role of HO in regulating arterial pressure during gestation, the effects of a HO inhibitor, stannous mesoporphyrin (SnMP), on mean arterial pressure (MAP)

were compared in virgin and pregnant rats. SnMP (Frontier Scientific) was administered intraperitoneally at a dose of 50 μmol/kg on day 14 of gestation. A similar dose of SnMP was used in the study of George et al (13). These authors shown that SnMP reduced significantly HO activity in the liver and the placenta of pregnant rats, 5 days after its administration. Studies were conducted in 4 groups of rats: virgin rats treated with vehicle (50 mmol $Na_2 CO_3$) (n=10) or SnMP (n=10) and pregnant rats treated with vehicle (n=10) or SnMP (n=10).

On day 17 of pregnancy, rats were anesthetized and instrumented with indwelling carotid catheters (V/3 tubing, SCI), which were tunnelled under the skin and exteriorized at the back of the neck. On gestational day 19, conscious rats were placed in individual restraining cages and the carotid catheter was connected to a pressure transducer. After a 60 minute stabilization period, MAP was monitored for 40 minutes using a data acquisition system (PowerLab System). At the end of the experiment, animals were euthanized and the reproductive tract was removed. The number of healthy embryos and resorption sites was counted and placental tissues were obtained to HO activity measurement. Implantation sites, including placenta and mesometrial triangle, were also obtained from these animals to quantify trophoblast remodelling using immunohistochemistry.

Measurement of HO activity

To demonstrate the inhibitory effect of SnMP, HO activity was compared in the implantation sites (placenta with associated mesometrial triangle) from pregnant control rats and pregnant rats treated with the HO inhibitor. HO activity was measured by monitoring the conversion of bilirubin by tissue lysate exposure. To achieve that, homogenates obtained from the implantation sites at day 19 of gestation were combined with 2mM glucose-6phosphate, 0.2 U of glucose-6-phosphate dehydrogenase, 0.8 mM of nicotinamide-adenine dinucleotide phosphate and 20.0 μM hemin, in a total reaction volume of 1200 μl. After 1 hour of incubation at 37º C, bilirubin was extracted with chloroform and its concentration was determined spectrophotometrically using the difference in absorbance at wavelengths from λ460 to λ530 nm with an absorption coefficient of 40 mM/cm. Activity was expressed as nanograms of bilirubin per microgram of protein per hour.

Immunohistochemistry.

From 1 to 3 implantations sites of the pregnant rats from each group, including placenta and mesometrial triangle, were dissected out and submerged in fixative and left at room temperature for one day. After fixation, the tissues were dehydrated and embedded in Paraplast Plust according to standard procedures. Sets of 10 parallel sections were cut from each implantation site parallel to the mesometrial-fetal axis. Parallel sections from these sets were stained with the periodic acid Schiff (PAS) method, and immunostained for keratin (KRT) and alpha smooth muscle actin (ACTA). PAS method was used to detect fibrinoid deposition inside the arterial wall (14-16). KRT was used to identify trophoblast cells and was detected by a mouse anti-Pan KRT antibody (clone MNF116, DAKO) at a dilution of 1:50. ACTA was used to monitored smooth muscle cells and was detected by a mouse anti-ACTA antibody (clone 1A4, DAKO) at a dilution of 1:100. Both antibodies were detected with a peroxidase conjugated goat anti-mouse IgG (Sigma-Aldrich) followed by diaminobenzidine tetrahydrochloride (DAB; Sigma-Aldrich) according to standard procedures. Normal rat serum (10% Sigma-Aldrich) was added during the secondary antibody incubation to decrease non-specific reactions.

Quantitative analysis of spiral artery histology

All stained sections were scanned a 40X with a microscope slide scanner (SCN400F Leika) and visualized with a digital software (Image Hub, Leika) at 10X magnification. The number of total, invaded and non-invaded spiral artery sections, were counted in the whole mesometrial triangle. Additionally, all invaded spiral artery from each stained section and the same number of non-invaded were copied out in individual parallel windows and evaluated by trophoblast invasion and spiral artery remodelling with an image processing program (Image J). Arterioles with a lumen contour less than 150 μm were excluded from this study. Total length of the each spiral artery contour and the proportion of the lumen circumference covered by endovascular trophoblast, vascular smooth muscle and fibrinoid were measured. The presence of endovascular trophoblast, vascular smooth muscle and fibrinoid were expressed as % of the spiral artery contour length.

Ultrasonography studies.

Uterine and radial arteries blood flow velocities were obtained from pregnant rats at days 14 and 19 of gestation, before and after treatment with vehicle (n=7) or SnMP (n=7). Ultrasound scanning was performed using a high-resolution micro-ultrasound system (Vevo® 3100, VisualSonics, Toronto, Canadá). This has a range of single-element and high-frequency transducers with different frequencies and focal depths. MX250 (axial resolution: 50 μm; frequency: 25 MHz) and MX400 (axial resolution: 75 μm; frequency: 40 MHz) transducers were used to examine uterine hemodynamics. The selected transducer was mounted on the Visualsonics integrated rail system incorporating a micromanipulator for precision adjustment of the probe position and orientation. Later, B-mode was activated to locate and visualize the arteries. Imaging depth was set to at 3 to 10 mm and frame-rate was 20 to 30 frames/s. Blood flow evaluation was carried out by Doppler mode. The frequency of emission was 21 to 24 MHz in MX250 probe and 30 MHz in MX400 probe. Pulse-repetition frequency set at 3 to 30 kHz and wall filter was 100 to 250 Hz. Pulsed Doppler gate set between 0.2 to 0.5 mm and the angle between the Doppler beam and the vessel was < 60º.

Rats were anaesthetized with inhaled isoflurane, the hair was removed and a depilatory cream was applied to increase ultrasound penetration. Animals were placed in a supine position on a heated imaging platform whit integrated temperature sensor and ECG electrodes. Heart rate, respiratory rate and rectal temperature (36.5-37.5 ºC) were monitored throughout the experiment. A warmed gel was used as an ultrasound coupling medium. Doppler waveforms were obtained from the uterine artery near the uterocervical junction, close to the ileac artery and behind the urinary bladder. For locate uterine artery, the 40 MHz ultrasound probe was positioned in the diagonal plane, over one centimeter up vaginal vent using the rail system. B-mode was activated to locate and visualize the urinary bladder. Doppler waveforms from radial arteries were obtained using the 40 MHz probe during the second week of pregnancy and the 25 MHz probe during the third week. Ultrasound evaluation was performed in four embryos in each rat, two in the left horn and two in the right horn. The probe was placed in transversal position under thorax, at first on the right side and later, on the left side. With the rail system, probe was moving from top to down to localize each embryo and acquire the image.

Doppler flow velocity waveforms were obtained in the uterine and radial arteries from both experimental groups of pregnant rats on day 19 of gestation. The highest

point of the systolic waveform was considered as the peak systolic velocity (PSV) and the point of the diastolic waveform was considered as the end diastolic velocity (EDV). Both PSV and EDV were measured from at least five consecutive cardiac cycles. Mean velocity (Vm) and velocity time integral (VTI) were measured by outlining five consecutive heartbeat cycles. Ultrasound was performed by the same trained operator. Data were transferred to an ultrasound image workstation for subsequent analysis (Vevo LAB 3.0.0).

Statistical analysis.

GB-STAT version 6.5 was used for statistical analysis. All data are presented as means ± SE. The results were subjected to analysis of variance (ANOVA) for repeated measures and Fisher´s test. A level of $p < 0.05$ was considered significant.

RESULTS

HO-1 and HO-2 expression.

A representative Western blot of HO-1 and HO-2 expression at the implantation site during pregnancy is shown in figure 1A. Densitometry analysis (figure 1B) revealed that both isoforms are expressed at implantation sites throughout gestation. HO-1 and HO-2 levels peaked on day 14 of gestation, being significantly higher than the levels observed on day 8 of pregnancy. Additionally, both isoforms declined slightly at late gestation.

Arterial pressure in conscious rats.

As can be seen in figure 2A, pregnant control rats, on day 19 of gestation, showed levels of MAP significantly lower than virgin control rats (100±2 mmHg vs. 121±2 mmHg, respectively). The inhibition of HO activity with SnMP induced an increase ($p<0.05$) in MAP in pregnant rats (114 ±1 mmHg) but not in virgin rats (124± 3 mmHg). Furthermore, blood pressure elevation in pregnant rats was associated with a significative reduction in placental HO activity at 19 day of gestation (10.4±0.5 vs. 6.9±0.2 ng/bilirubin/µg protein/h) (figure 2B), demonstrating that 5 days after administration, the dose of SnMP used in our study had a significant inhibitory effect on

HO activity. Pregnant rats with an increased blood pressure at late gestation had also higher ($p<0.05$) fetal resorption rate (19%) than control rats (5%).

Endovascular trophoblast and associated remodelling in the whole mesometrial triangle.

A total of 13 and 12 implantation sites from 6 pregnant control rats and 7 pregnant rats treated with the HO inhibitor, respectively, were examined. Figure 3 shows trophoblast invasion in the mesometrial triangle from a pregnant control rat (3A) and a pregnant rat treated with the HO inhibitor (3B).This figure also shows parallel cross-sections of spiral arteries inmunostained for KRT, PAS and ACTA from a control rat (3C,3E,3G, respectively) and from a pregnant treated rat (3D,3F,3H, respectively). As previously described (14), endovascular trophoblast into the mesometrial triangle spiral arteries did not form a continuous covering of the whole circumference of the vessel (figure 3C, 3D) and fibrinoid material was observed underneath the luminal trophoblast layer (figure 3G, 3H). We also observed fragmentation of the vascular smooth muscle in invaded vessels (figure 3E, 3F). On day 19 of gestation, the percentage of spiral arteries invaded by trophoblast was similar in both groups (19% vs. 18%, respectively). However, the proportion of lumen circumference covered by endovascular trophoblast in the arterial cross sections was significantly ($p<0.001$) lower in pregnant rats treated with the HO inhibitor (21±3 %) than in control pregnant rats (59±5 %) (Table 2). The amount of fibrinoid in the spiral arteries, expressed as a percentage of the lumen contour (Table 2), showed a similar pattern, with lower ($p<0.05$) fibrinoid deposition (22±3%) in pregnant rats with diminished HO activity than in control rats (42±7 %). Contrarily, the length of the vascular smooth muscle layer versus the total vessel contour was significantly higher (33±5%) in pregnant rats with a reduction in HO activity than in pregnant control rats (16±5%) (Table 2).

Uterine and radial arteries blood velocity.

Figure 4A shows a representative Doppler flow velocity waveforms obtained in the uterine and radial arteries from both experimental groups of pregnant rats on day 19 of gestation. As shown in figure 4B and 4C, PSV and EDV increased significantly from day 14 to day 19 in uterine and radial arteries of pregnant control rats. However, in the pregnant rats treated with the HO activity inhibitor, these parameters were similar on

days 14 and 19 of gestation. In figure 4A it can be also observed that on day 19 of gestation, PSV and EDV were lower in uterine and radial arteries of pregnant rats with reduced HO activity than in control animals. Vm and VTI followed the same pattern (table 1), showing marked increase on day 19 of gestation in control rats and no changes in the pregnant rats with diminished HO activity.

DISCUSSION

The main purpose of the present study was to determine the importance of HO system in regulating the adaptation of uteroplacental circulation and blood pressure to late gestation in the rat. Our major findings were that reduced HO activity during late gestation led to deficient trophoblast invasion and defective spiral artery remodelling which were associated with marked alterations in uterine and radial arteries haemodynamic, and a significative increase in blood pressure.

The results of this study show that both isoforms of HO are expressed in the rat implantation sites during pregnancy. Specifically, we observed the highest levels of HO-1 and HO-2 at mid-pregnancy, coinciding with the onset of trophoblast invasion into the rat uterine decidua (17) and suggesting that HO may be involved in this process, and therefore in the maternal vascular remodelling induced by these cells. A similar gestational pattern of both HO isoforms expression was previously described in rat uterine and placental tissues (18). Although the days of gestation analyzed were different, these authors found that uterine and placental HO-1 and HO-2 protein, and ARNm levels reached a peak at about day 16 of pregnancy, coinciding also with early stages of uterine trophoblastic invasion (18). In the human placental bed, endovascular and interstitial cytotrophoblasts express HO-1 and HO-2 suggesting that the activity of both HO isoforms could contribute to the control of trophoblast invasion and placental function (5,19). In this regard, it has been reported that the inhibition of HO in isolated human placenta produced a significant constriction in the placental vasculature, implying that the HO-CO system may participate in the regulation of blood flow in this organ (5). However, the role of HO in the trophoblast-dependent remodelling of human spiral arteries are poorly understood. We evaluated endovascular trophoblast invasion and vascular transformation in the mesometrial triangle of pregnant rats treated with the HO inhibitor, SnMP. Similar to humans, pregnant rats have a haemochorial placenta, with a deep intrauterine trophoblast invasion and a trophoblast-dependent spiral artery

remodelling (17,20). In the rat, trophoblasts penetrate into the uterine decidua between day 14 and 15 of pregnancy and extend into the myometrium as gestation advances (17). Vascular invasion of the mesometrial triangle is maximal at 18 days of gestation and is associated with loss of smooth muscle layer in the invaded vessels (17). We administered the HO inhibitor at day 14 of gestation, coinciding with the onset of trophoblast invasion, and we evaluated the remodelling of spiral arteries induced by these cells in the mesometrial triangle at day 19 of gestation. Vascular transformation of uterine arteries was reduced in pregnant rats treated with the HO inhibitor compared with normal pregnant animals. At day 19 of gestation, uterine arteries of rats with reduced placental HO activity showed a lower proportion of arterial lumen contours covered by trophoblast and more vascular smooth muscle than contours of the same vessels in normal pregnant rats, suggesting that HO enzyme contributes to structural changes in the uterine mesometrial vasculature. A greater proportion of vascular smooth muscle in the spiral arteries of rats with reduced HO activity may result in higher resistance to blood flow in these vessels and subsequently a deficient blood supply to the placenta and the fetuses. This is the first study showing that HO-CO system is involved in trophoblast-induced spiral artery remodelling, during late gestation. Although the ratio of wall to lumen diameter of spiral arteries has been found increased in pregnant HO-1 deficient mice compared with control animals, these studies were done during early gestation and they do not include data quantifying trophoblast invasion (21). In the mouse, this process is also initiated at midgestation, but in contrast with the rat, the invasion is restricted to the uterine mesometrial decidua and has a lesser endovascular contribution (17,20). For that reason, despite deep trophoblast invasion in human pregnancy occurs earlier than in the rat, pregnant rat is considered a better experimental model than the mouse for studies of uterine remodelling during pregnancy.

Impaired remodelling of maternal spiral arteries in our study was associated with significant alterations in uterine haemodynamics, an elevated number ($P<0.05$) of reabsorbed pups and higher levels of blood pressure at late gestation, suggesting that the increase in this parameter, induced by the reduction of HO activity in pregnant rats, may be a consequence of an increased resistance in the uterine vasculature and insufficient blood supply to the uteroplacental unit. In this regard, it has been shown that mechanical reduction in uterine perfusion pressure provokes hypertension in pregnant rats (22) and mice (23) but not in nonpregnant animals. Although in our study the

increase in blood pressure observed at late gestation in response to HO inhibition is not excessive, the results suggest a considerable impairment of vascular adaptation to gestation. This is supported by the data obtained from non treated pregnant rats showing values of MAP around 14 mm Hg lower than treated pregnant rats. Finally these results are also consistent with studies showing that alterations of uterine artery blood flow are present in women with gestational hypertension and preeclampsia (24).

Uterine haemodynamics in our study was analyzed by Doppler ultrasonography. Doppler velocities, PSV, EDV, Vm and VTI increased in the uterine and radial arteries toward the end of pregnancy in normal pregnant rats. However these parameters did not change in the uterine vessels of rats with decreased HO activity. A progressive increase in systolic and diastolic velocities through gestation has been found in uterine arteries of rats and mice (25) indicating a direct relationship between these parameters and the gradual enhancement of blood flow to the utero-placental unit that occurs during pregnancy. These findings are consistent with a recent study showing that ligation of uterine vessels in mice causes a significant decline in uterine artery systolic blood velocity measured by Doppler ultrasonography (25). In human pregnancy, it has been reported that PSV and EDV show a marked elevation since early pregnancy, which is strongly correlated with the duration of gestation, therefore indicating change of compliance and resistance in the uterine circulation from the first weeks of gestation (26). On the other hand, Vm in the uterine artery has been also correlated with the gestational age in pregnant woman, demonstrating that this parameter can be also a good indicator of uterine vascular perfusion (27). Taken together, these data would indicate that a disminished HO activity may lead to an inadequate uteroplacental blood flow and a suboptimal blood supply to the fetuses. Although the contribution of the HO system to the uteroplacental circulation adaptation to pregnancy is clear in our study, the mechanism underlying this effect is unknown. Similar to our results, several studies have shown that a defective trophoblast invasion is associated with uterine artery Doppler alterations (15,28-30). However, uterine and radial arteries do not show trophoblast invasion. Thus, there are evidences indicating that flow through spiral arteries is not the only determinant of the Doppler uterine arterial waveform in pregnancy (3). In this regard, uterine artery waveforms from abdominal pregnancies show similar changes during gestation as seen in intrauterine pregnancies, despite the absence of spiral arteries trophoblast invasion (31,32).Therefore, we hypothesized that HO may be involved in the structural and functional changes observed in uterine and

radial arteries during pregnancy. During gestation, these vessels undergo enlargement in calibre and axial growth, which are trophoblast independent and probably mediated by hypertrophy and hyperplasia of smooth muscle and endothelial cells (2). HO system could contribute to these structural changes through its actions regulating VEGF levels, given that this factor induces vasodilation, stimulates endothelial mitosis and it has been associated with hypervascularization and vascular enlargement (2). This hypothesis is supported by the study of George et al. (13), which have demonstrated that the increase in blood pressure induced by the administration of SnMP during late gestation is associated with a significant reduction in VEGF, therefore suggesting that lower levels of this proangiogenic factor may be mediating altered uterine and radial arteries remodelling, and uterine vasoconstriction induced by HO activity inhibition. Consistent with these results, it has also reported that HO-1 induction attenuates the hypertension and the decreased levels of VEGF induced by placental ischemia, suggesting that vascular endothelial dysfunction secondary to decreased uterine perfusion pressure might be improved in response to an inductor of this HO isoform (33). Finally, disminished levels of CO in response to reduced HO activity may also contribute directly to the reduction in uterine blood flow observed in our study.

On the other hand, the decrease in uterine blood flow induced by the inhibition of the HO activity could be partially mediating the alterations in trophoblast dependent transformation of spiral arteries. In this regard, it has been suggested that preconditioning of the spiral arteries precedes trophoblast invasion (11), and it has been reported that some maternal vessels undergo morphological changes without interaction with these cells (34). Lyall et al. (5) demonstrated that HO-2 expression was reduced in placental endothelial cells of pregnancy disorders as preeclampsia or fetal growth restriction, suggesting that a reduction in CO levels in these cells could alter spiral artery dilatation prior to trophoblast invasion. One possible explanation would be that a reduced uterine blood flow and the shear stress could influence negatively the interactions between endothelial and vascular smooth muscle cells in the spiral arteries remodelling process (34). Consistent with this hypothesis it has been reported that human pregnancies with a low resistance uterine artery flow pattern obtained by Doppler ultrasound are associated with a more extensive trophoblastic invasion of the decidual vessels than pregnancies with a high resistance flow pattern (28).

In summary, the data obtained in this study indicate that HO-CO system play an important role in the adaptation of the uteroplacental circulation and the blood pressure

regulation during late gestation. Although the mechanisms mediating these effects have not been fully elucidated in the present study, our results appear to indicate that both trophoblast dependent and independent processes are involved. We speculate that the inhibition of the HO activity during late gestation could induce a reduction in uterine and radial arteries blood flow which may be contributing partly to the altered spiral arteries remodelling dependent of the trophoblast cell invasion. We also hypothesized that an increased resistance in the uterine circulation, during the last stages of gestation, can be a possible cause of the hypertension secondary to the HO activity inhibition. Further studies are necessary to determine the contribution of HO-CO system to the structural changes occurring in uterine and radial arteries during late pregnancy, and the impact that an inadequate transformation of these vessels in response to gestation could have in pregnancy disorders including hypertension, preeclampsia, and growth restriction.

Acknowledgements

This work was supported by the Subdirección General de Proyectos de Investigación of Ministerio de Economía y Competitividad, Spain (BFU2013-49098-R and PI16/01556) and Fundación Séneca-Agencia de Ciencia y Tecnología de la Región de Murcia en el marco del PCTIRM 2011-2014 (19422/PI/14).
We wish to thank Carlos Manuel Martínez for his hepful advice about images.

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Figure 1

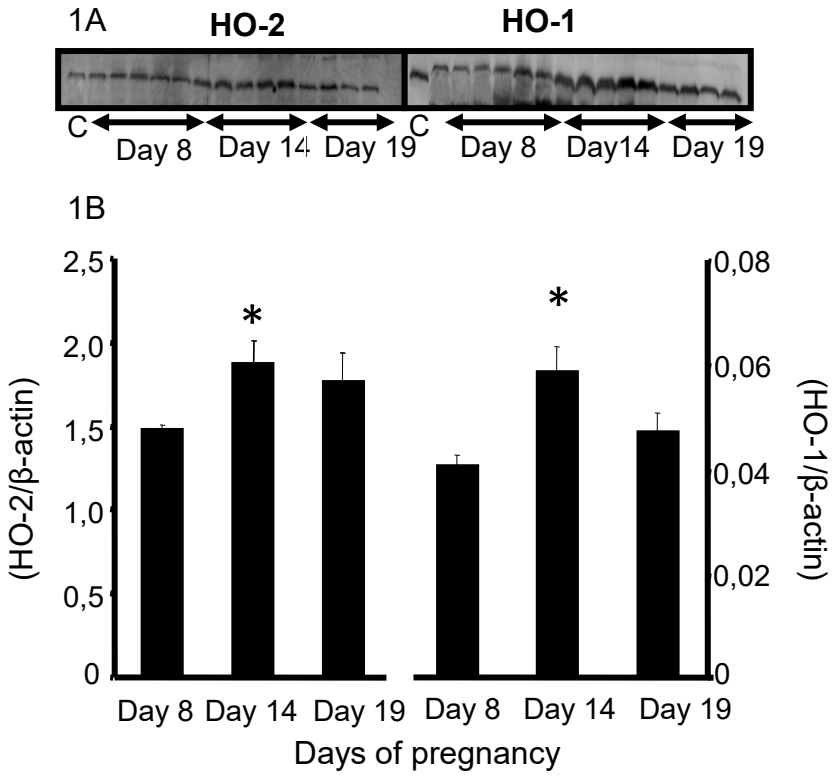


Fig. 1. -Expression of HO-2 and HO-1 at the implantation site through gestation. (A) Representative Western blot. C means control protein. (B) Quantification of protein expression using densitometry. Densitometric values are expressed relative to β-actin expression. * ¼ p < *0.05 vs. day 8.*

Figure 2

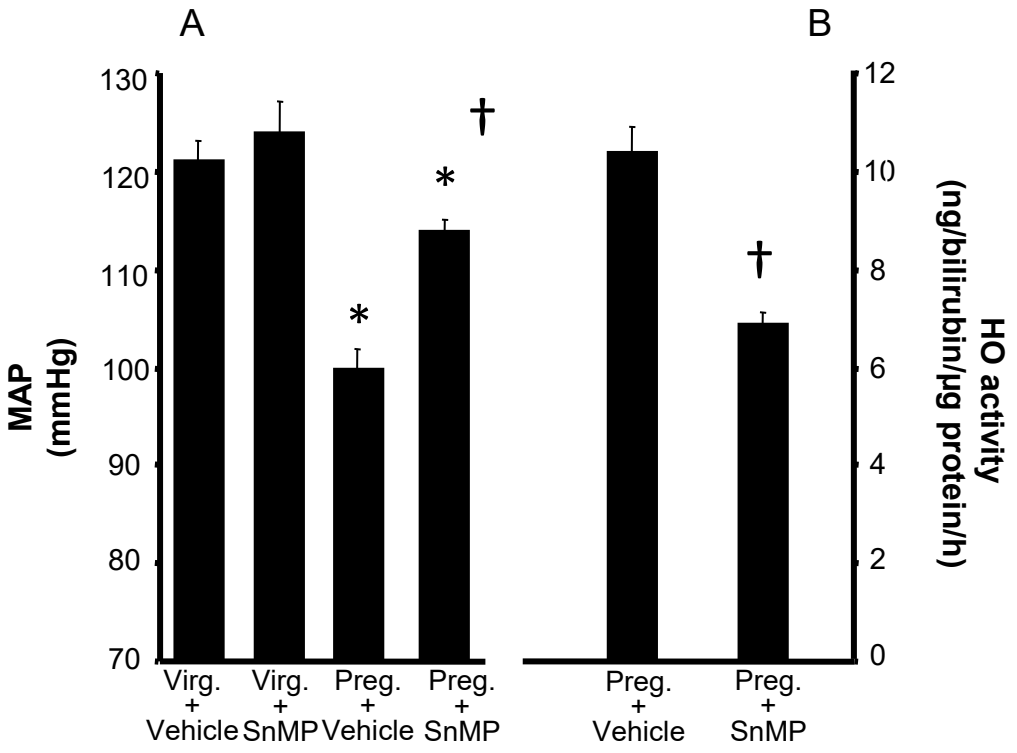


Fig. 2. -(A) Mean Arterial Pressure (MAP) in virgin (Vir.) and pregnant rats (Preg.) treated intraperitoneally with vehicle or stannous mesoporphyrin (SnMP). (B) HO activity at the implantation site in pregnant rats treated intraperitoneally with vehicle or SnMP. * ¼ p < *0.05 vs. virgin control rats. y ¼ p < 0.05 vs. pregnant control rats.*

Figure 3

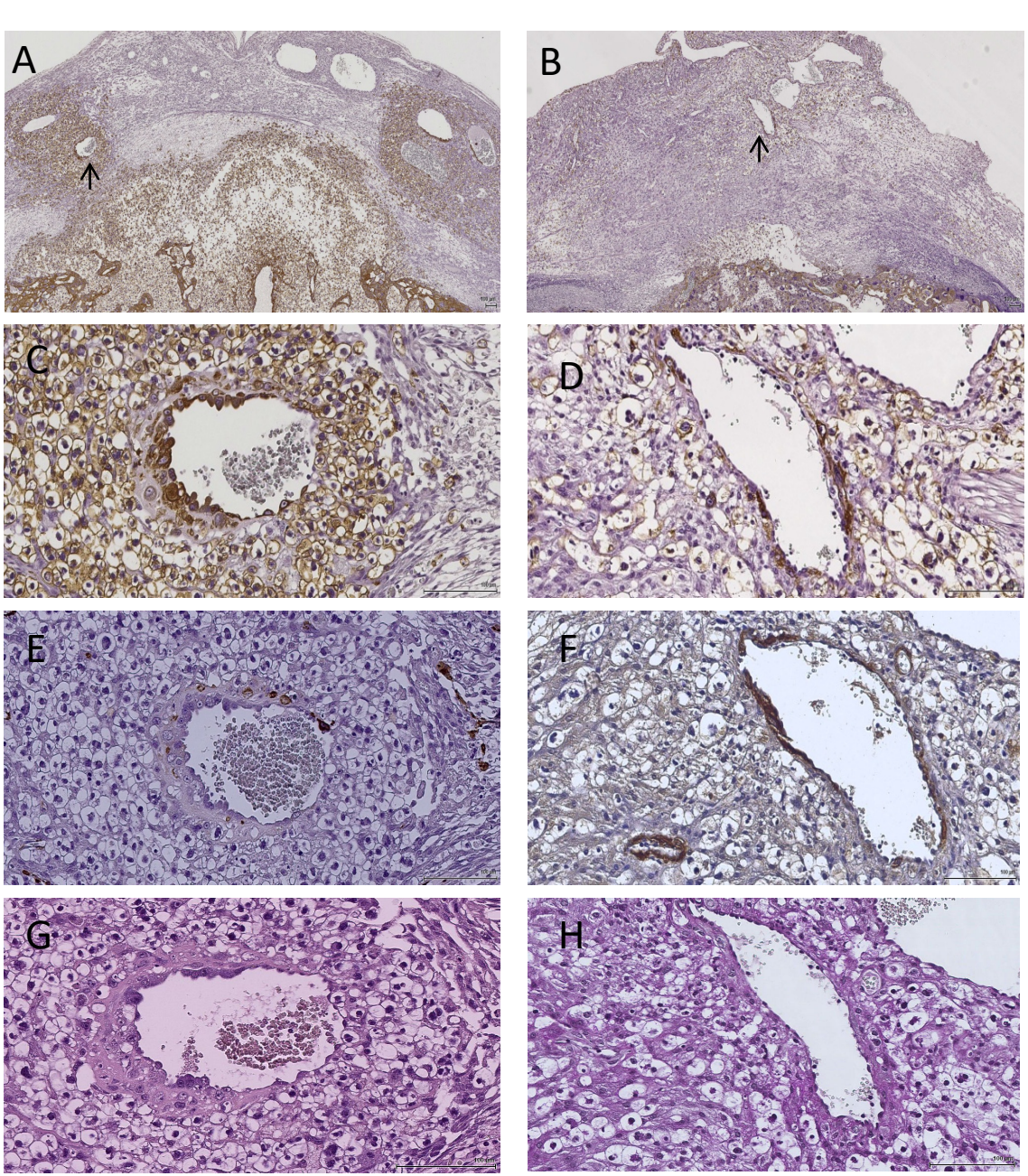
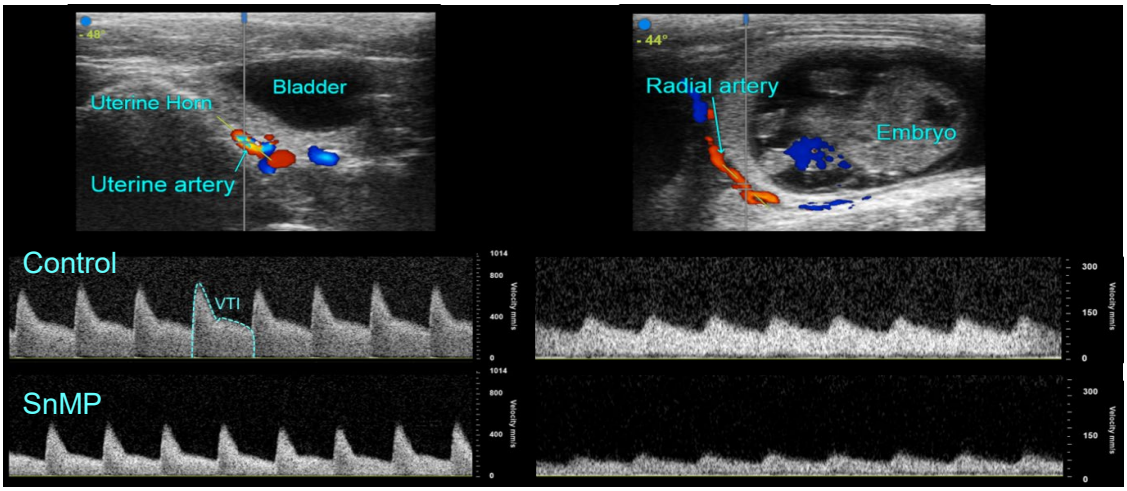


Fig. 3. Mesometrial triangle at day 19 of pregnancy of a control pregnant rat (A) and a pregnant rat treated with SnMP (B) stained for KRT. Arrows on A and B indicate the localization of the vessel magnified below. Parallel cross-sections of a spiral artery inmunostained for KRT, ACTA and PAS from a pregnant control rat (C, E,G) and from a pregnant rat treated with SnMP (D,F,H).

Figure 4



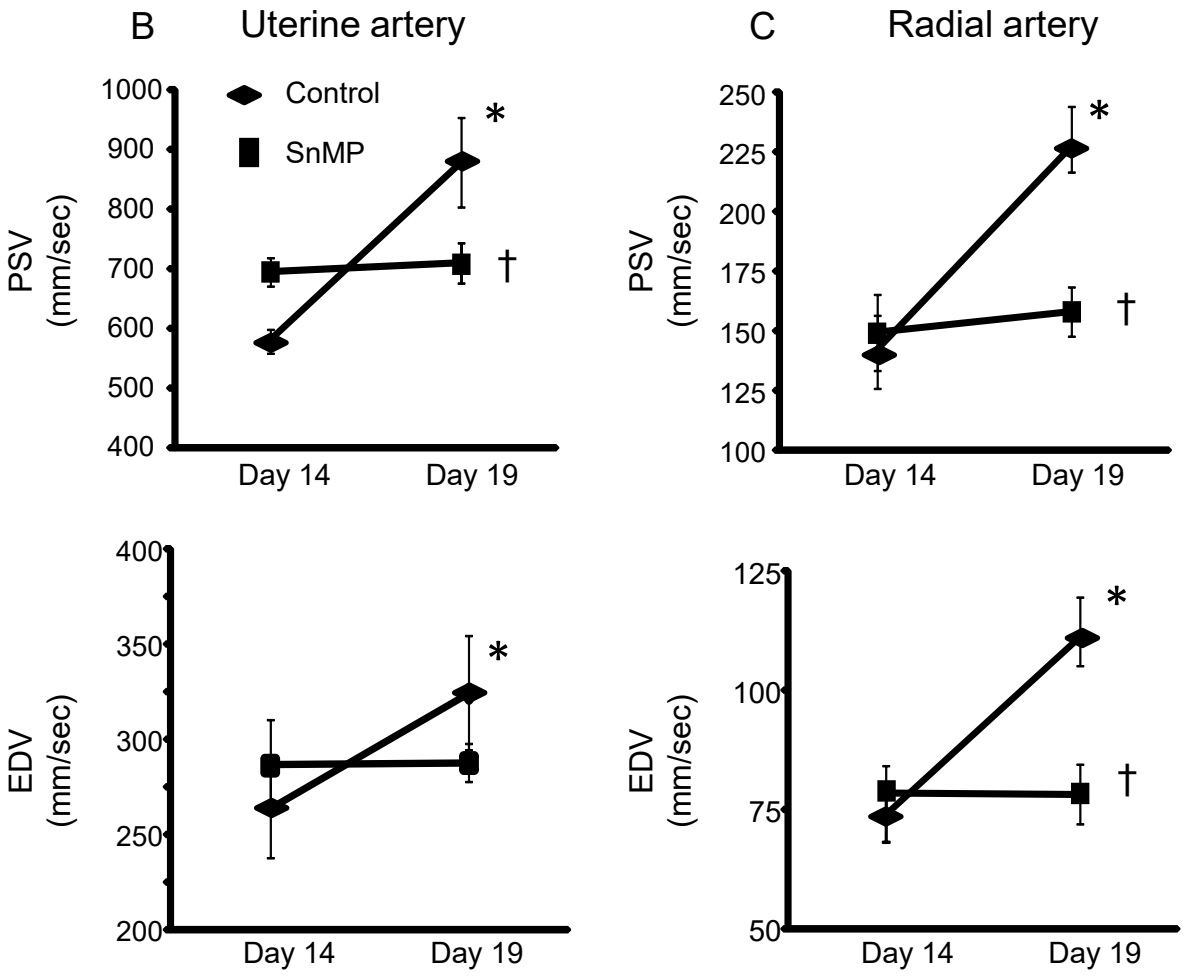


Fig. 4. -(A) Doppler flow velocity waveforms obtained at day 19 of gestation in the uterine and radial arteries of a pregnant control rat (Control) and of a pregnant rat treated with stannous mesoporphyrin (SnMP). (B) Changes in the uterine artery peak systolic (PSV) and end diastolic velocity (EDV) from day 14 to day 19 of gestation in pregnant rats treated with vehicle or the HO inhibitor, SnMP. (C) Changes in the radial artery PSV and EDV from day 14 to day 19 of gestation in pregnant rats treated with vehicle or the HO inhibitor, SnMP. * ¼ p < *0.05 vs. day 14 of gestation.*

Table 1.-Mean velocity (Vm) and velocity time integral (VTI) obtained in uterine and radial arteries from control pregnant rats and pregnant rats treated with SnMP, at days 14 and 19 of gestation.*=p<0.05 vs. day 14.†=p<0.05 vs. control pregnant rats.

	Uterine Artery				Radial Artery			
	Vm		VTI		Vm		VTI	
	Day 14	Day 19	Day 14	Day 19	Day 14	Day 19	Day 14	Day 19
Control	414±19	573±49*	64±5	86±8*	100±9	164±12*	17±2	26±2*
SnMP	479±21	487±20	73±4	70±1	108±10	112±8 †	17±1	17±1 †

Table 2.- Endovascular trophoblast (EF), fibrinoid (F) and vascular smooth muscle (VSM) in spiral arteries of the whole mesometrial triangle of control pregnant rats and pregnant rats treated with SnMP, expressed as % of the total spiral artery contour length.

	Control	SnMP
% ET	59 ± 5	21 ± 3*
% F	42 ± 7	22 ± 3*
% VSM	16 ± 5	33 ± 5*

*=$p<0.05$ vs. control pregnant rats.