



Contents lists available at ScienceDirect

## Taiwanese Journal of Obstetrics &amp; Gynecology

journal homepage: [www.tjog-online.com](http://www.tjog-online.com)

## Original Article

## Soluble epoxide hydrolase in the human placenta throughout gestation

Tai-Ho Hung<sup>a, b, \*</sup>, Szu-Fu Chen<sup>c</sup>, T'sang-T'ang Hsieh<sup>a</sup><sup>a</sup> Department of Obstetrics and Gynecology, Taipei Chang Gung Memorial Hospital, Taiwan<sup>b</sup> College of Medicine, Chang Gung University, Taoyuan, Taiwan<sup>c</sup> Department of Physical Medicine and Rehabilitation, Cheng Hsin Rehabilitation Medical Center, Taipei, Taiwan

## ARTICLE INFO

## Article history:

Accepted 19 August 2019

## Keywords:

Soluble epoxide hydrolase

Pregnancy

Placenta

Reoxygenation

## ABSTRACT

**Objective:** To investigate the spatial and temporal changes of soluble epoxide hydrolase (sEH) in the human placenta throughout gestation and to study the effects of hypoxia-reoxygenation (HR) on the expression of sEH in villous explants *in vitro*.

**Materials and methods:** Placental samples were obtained from women of different gestation and grouped as early (8–12 weeks, n = 10), mid- (16–28 weeks, n = 6), and late gestation (38–39 weeks, n = 10) according to gestational age. Immunohistochemistry, western blot, and real-time quantitative PCR were used to assess the cellular distribution and temporal changes of sEH. Villous explant cultures were used to study the effect of HR (8 h at 2% oxygen, followed by 16 h at 8% oxygen, two cycles) on the expression of sEH.

**Results:** Using a mouse monoclonal antibody against human sEH, immunoreactivity of sEH was observed mainly localized in the cytotrophoblasts and, to a lesser extent, the syncytiotrophoblast in the villous tissues throughout gestation. Compared to villous tissues of early gestation, the levels of sEH mRNA and protein were significantly increased in villous samples of mid- and late gestation. Furthermore, villous explants subjected to HR had significantly higher levels of sEH mRNA and protein compared to villous tissues kept at 8% oxygen throughout the experiment.

**Conclusion:** Our results indicate that sEH is likely to play an essential role in the development of human placenta and HR is a possible factor regulating the expression of sEH in the placenta.

© 2019 Taiwan Association of Obstetrics & Gynecology. Publishing services by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

## Introduction

Arachidonic acid (AA), a polyunsaturated fatty acid generated from the phospholipids of cell membrane by the phospholipase A<sub>2</sub>, is the precursor of several biologically and clinically important eicosanoids involved in the inflammatory process [1]. There are three major pathways involved in AA metabolism: the cyclooxygenase (COX) pathway, the lipoxygenase (LOX) pathway, and the cytochrome P450 (CYP450) enzymes pathway. The COX pathway results in the formation of prostaglandins (PG) such as PGE<sub>2</sub>, PGF<sub>2</sub>, and PGI<sub>2</sub>, and thromboxane A<sub>2</sub>. The LOX pathway forms hydroperoxyeicosatetraenoic acids and dihydroeicosatetraenoic acid,

which are subsequently converted to hydroxyeicosatetraenoic acids and leukotrienes (LTB). The CYP450 pathway forms epoxyeicosatrienoic acids (EETs) and dihydroxyacids. Unlike most metabolites of the COX and LOX pathways, EETs and metabolites of CYP450 enzymes have primarily vasodilatory, antihypertensive, anti-inflammatory, and natriuretic properties [2,3].

Soluble epoxide hydrolase (sEH), which adds water across the epoxide to give the corresponding dihydroxyeicosatrienoic acids (DHETs), is the leading enzyme to metabolize EET in many tissues [4]. Through its metabolism of the EETs, sEH contributes to the regulation of vascular tone, nociception, angiogenesis, and the inflammatory response in many disease states [5]. Indeed, increasing evidence suggests that pharmacological inhibition of sEH stabilizes endogenous EETs, thus preventing the development of hypertension, atherosclerosis, heart failure, fatty liver, and multiple organ fibrosis [3,5,6].

EETs, DHETs, and sEH have been detected in many human tissues including the placenta and fetal membranes [7–10].

\* Corresponding author. Department of Obstetrics and Gynecology, Taipei Chang Gung Memorial Hospital, 199 Dun-hua North Road, Taipei 105, Taiwan. Fax: +886 2 27197368.

E-mail address: [thh20@cgmh.org.tw](mailto:thh20@cgmh.org.tw) (T.-H. Hung).

Compared to nonpregnant women, significantly higher plasma EETs concentrations were noted in pregnant women [11]. Furthermore, concentrations of EETs are much higher in fetal than that in maternal plasma and erythrocytes, suggesting that feto-placental unit contribute to EETs biosynthesis and metabolism during pregnancy [11]. Nevertheless, the role of sEH in the human placental development remains unclear. Therefore, the objectives of this study were to investigate the spatial and temporal changes of sEH in the human placenta throughout gestation and to study the effects of hypoxia-reoxygenation (HR) on the expression of sEH in villous explants *in vitro*.

## Materials and methods

Conduction of this study was approved by the Institutional Review Board of Chang Gung Memorial Hospital (No. 201802304B0). All placental samples were obtained with written informed consent. The materials and chemicals used in this study were purchased from Sigma Chemical Co., St. Louis, MO, USA, except where other suppliers are stated individually.

### Collection of placental samples

Placental samples were obtained from the following sources or scenarios: elective termination of gestations ranging from 8 to 24 weeks with documented embryonic or fetal cardiac activity by ultrasound; pregnancies complicated by cervical incompetence; spontaneous preterm deliveries ranging from 24 to 28 weeks in the absence of signs, symptoms, and histological evidence of chorioamnionitis; and elective repeat cesarean section prior to the onset of labor. In the current study, samples were grouped according to gestational age as follows: early gestation (8–12 weeks,  $n = 10$ ), mid-gestation (16–28 weeks,  $n = 6$ ), and late gestation (38–39 weeks,  $n = 10$ ). Placental samples from early gestation were submitted for chromosomal study to confirm normal karyotypes. All fetuses delivered were examined by obstetricians for any gross malformations.

For mid- and late gestation, villous tissues were randomly sampled from five distinct sites from the maternal side after the placenta was delivered. Each site was mid-distance between the cord insertion and the periphery of the placenta and midway between the chorionic and basal plates. The villous samples were quickly washed in phosphate-buffered saline (PBS) to clear the maternal blood and fixed in 4% paraformaldehyde or frozen in liquid nitrogen and stored at  $-70^{\circ}\text{C}$  for further processing. All villous samples were collected and processed within 10 min after delivery.

### Villous explant culture and condition of HR

We obtained placentas from 8 women with normal term pregnancies who underwent elective cesarean deliveries prior to the onset of labor and performed villous explant cultures as described previously [12]. Briefly, villous samples were dissected into pieces weighing 5–10 mg and placed in culture medium; six to eight such pieces were suspended at the gas–liquid interface in individual Costar Netwell® inserts (24 mm diameter, 500  $\mu\text{m}$  mesh) suspended in 3 mL of culture medium. After an overnight equilibration in culture media at  $37^{\circ}\text{C}$  and 8% $\text{O}_2$ /5%  $\text{CO}_2$ /balanced  $\text{N}_2$  in a humidified incubator, the medium was refreshed the following day and the explants were subjected to HR condition (8 h at 2% oxygen, followed by 16 h at 8% oxygen, two cycles) or kept at 8% oxygen throughout the experiment as the normoxic control. Forty-eight hours later, the villous samples were briefly washed with PBS and snap-frozen in liquid nitrogen for further processing.

## Immunohistochemistry

Immunohistochemistry for sEH immunoreactivity was performed as previously described [13]. Briefly, formaldehyde-fixed, paraffin embedded sections were dewaxed in xylene twice, 5 min for each, and then rehydrated in serial descending concentrations of ethanol (100%, 90%, 70%, 50% and distilled water, 5 min for each) before being subjected to immunohistochemistry. After pretreatment with heat-induced epitope retrieval method using sodium citrate buffer (10 mM sodium citrate, 0.05% Tween 20, pH 6.0), endogenous peroxidase activity was quenched with 3%  $\text{H}_2\text{O}_2$  in methanol for 10 min, and non-specific binding was blocked with 10% normal goat serum in PBS with 0.1% Tween-20 (PBST) for 30 min. Slides were then reacted with a mouse anti-human sEH (A-5) monoclonal antibody (1:10 dilution; catalog no. SC-166961; Santa Cruz Biotechnology, Inc. Dallas, TX, USA), diluted with 5% normal goat serum in PBS-T at  $4^{\circ}\text{C}$  overnight. The sections were incubated with biotinylated goat anti-mouse IgG at 1:200 dilution for 30 min at room temperature, washed in PBS-T, and then incubated in avidin-biotin-peroxidase solution according to the instructions for the Vectastain Elite ABC kit (Vector Laboratories, Burlingame, CA, USA) for another 30 min. Diaminobenzidine tetrahydrochloride was used as the peroxidase substrate. The specificity of staining reaction was assessed in several control procedures, including omission of the primary antibody and substitution of the primary antibody with non-immune mouse isotypic IgG. Sections were viewed and photographed under a differential interference contrast microscope (Nikon Eclipse 80i, Nikon Corporation, Tokyo, Japan).

### Western blot

Western blotting was performed as previously detailed [14]. Fifty to 100  $\mu\text{g}$  of cytosolic proteins were separated by 8–12% SDS-PAGE, transferred to nitrocellulose membranes, and probed with the mouse anti-human sEH (A-5) monoclonal antibody (1:200 dilution; catalog no. SC-166961; Santa Cruz Biotechnology) at  $4^{\circ}\text{C}$  overnight. Horseradish peroxidase-linked secondary antibodies were used in combination with enhanced chemiluminescence to visualize the target protein bands on autoradiography films. The relative intensity of protein signals was normalized to the corresponding  $\beta$ -actin (clone AC-15, Sigma) density and quantified by densitometric analysis using the Image J analysis software (National Institutes of Health, Bethesda, MD, USA; <http://rsb.info.nih.gov/ij/>).

### Real-time quantitative PCR

Total RNA was extracted from villous tissues using RNeasy Mini Kits (Qiagen) and then subjected to reverse transcription using SuperScript II RNase H reverse transcriptase according to the manufacturers' instructions (Invitrogen). Real-time quantitative PCR analysis was performed with an ABI PRISM 7900 sequence detector (Applied Biosystems). Assay-on-Demand TaqMan primers and probes for human *EPHX2* (Hs00157403\_m1) from Applied Biosystems were used. 18S ribosomal RNA (Hs99999901\_s1) was used as an endogenous control. Thermal cycling was initiated with a 2-min incubation at  $50^{\circ}\text{C}$ , followed by a first denaturation step of 10 min at  $95^{\circ}\text{C}$ , and then 40 cycles of  $95^{\circ}\text{C}$  for 15 s and  $60^{\circ}\text{C}$  for 1 min. All samples were analyzed on the same run, and each sample was run in triplicate. Relative quantities of sEH and 18S ribosomal RNA were calculated by the comparative threshold cycle method as previously described [15].

### Statistical analysis

Data are presented as the mean  $\pm$  SEM, analyzed and plotted using Prism 7 for Mac OS X, version 5.0d (GraphPad Software, Inc., La Jolla, CA, USA). Differences between two groups were computed with the Student's *t*-test. A *P* value of  $<0.05$  is considered to be statistically significant.

## Results

### *Localization of immunoreactivity of sEH in the villous tissues of different stages of gestation*

We first studied the localization of sEH immunoreactivity in the villous tissues of different stages of gestation and its spatial change throughout gestation. Using a mouse monoclonal antibody against human sEH, immunoreactivity of sEH was mainly localized at the cytotrophoblasts and, to a lesser extent, the syncytiotrophoblast in the villous tissues from early gestation (Fig. 1a). Compared to villous tissues of early gestation, there was no significant spatial change in the immunostaining of sEH in villous samples of mid- and late gestation (Fig. 1b, c). There was essentially no staining at all in the negative controls with omission or replacement of the primary antibody with the same mouse isotypic IgG (Fig. 1d).

### *Temporal changes of the levels of sEH protein and mRNA in the villous tissues of different stages of gestation*

Compared to villous tissues from early gestation, the levels of sEH were significantly increased in villous samples from mid- and late gestation (Fig. 1e). In parallel with the changes of sEH protein at different stages of gestation, we found there was an increase in the levels of sEH mRNA in villous tissues of mid- and late gestation than that of early gestation (Fig. 1f).

### *HR leads to increased levels of sEH mRNA and protein in villous explants*

Compared to villous tissues kept at 8% oxygen throughout the experiment (normoxic controls), villous tissues subjected to HR had significantly higher levels of sEH mRNA and protein (Fig. 2).

## Discussion

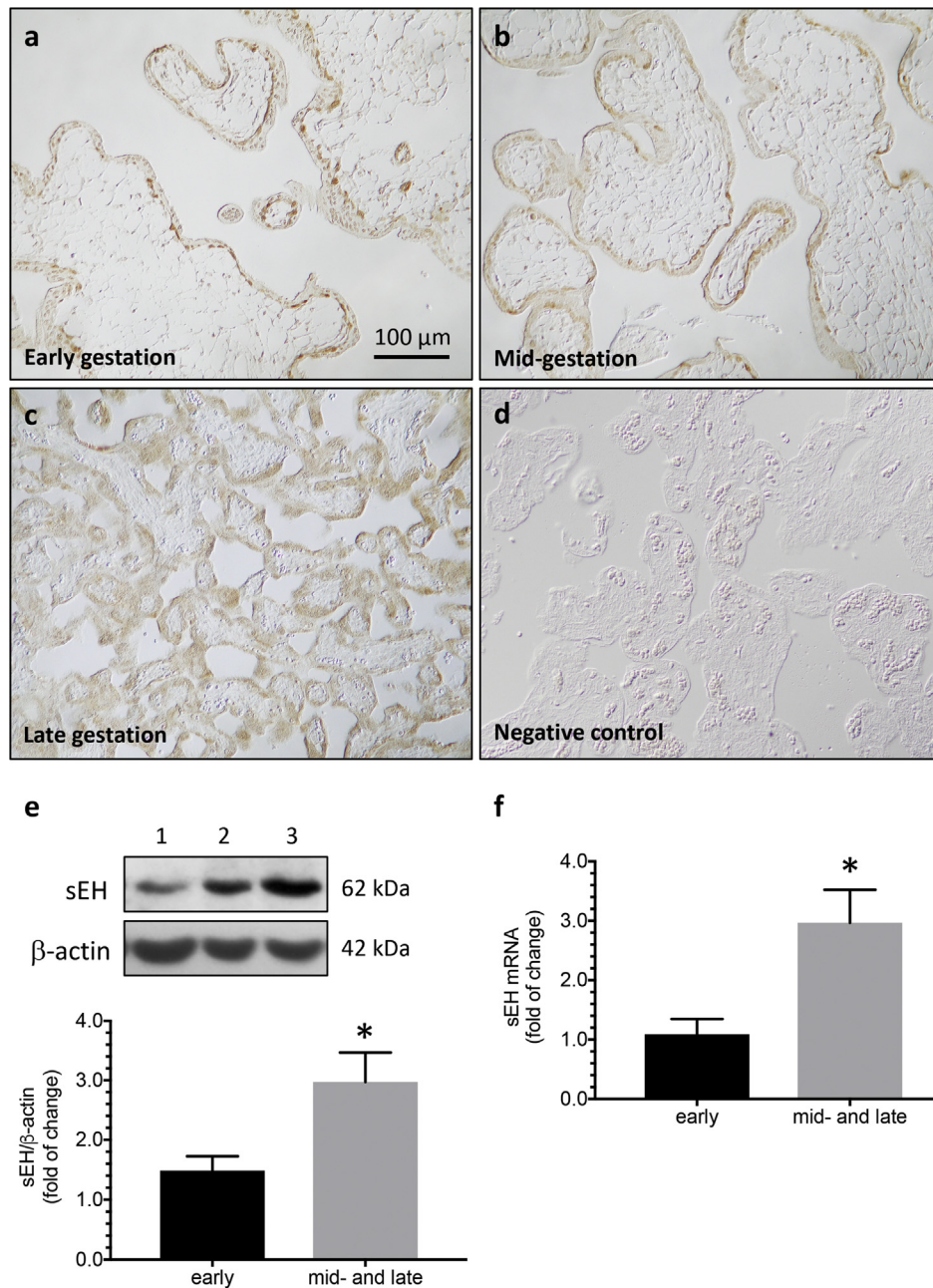
sEH has been demonstrated in the human myometrium and gestational tissues including the placenta and fetal membranes. However, results regarding the cellular distribution of sEH in the placenta are inconsistent. Dalle Vedove et al. found that sEH had a nuclear localization within the mesenchymal cells in the term placental villi and in the vascular wall of the umbilical cord [8]. In contrast, Cizkova and Tauber found that immunoreactivity of sEH is strictly limited to cytotrophoblast in embryonic and fetal stages of the development [16]. In the term placenta, sEH is expressed in cytotrophoblast but expression in syncytiotrophoblast is also detectable. However, these results were based on a limited sample size. By analyzing villous tissues from different gestational stages, we confirmed that immunoreactivity of sEH was mainly localized at the cytotrophoblasts and, to a lesser extent, the syncytiotrophoblast in the villous tissues of early gestation (before 12 weeks of gestation). Cellular distribution of sEH does not change significantly throughout gestation, though the staining intensity in the syncytiotrophoblast seemed stronger in villous tissues of mid- and late gestation than that in the villous tissues of early gestation.

Compared to villous tissues of early gestation, increased levels of sEH mRNA and protein were noted in the villous tissues of mid- and

late gestation. Although the mechanisms underlying the regulation of these changes remain unclear, we surmised that change of oxygen concentration is a possible factor for the following reasons. First, the oxygen level in the uterus at the time of implantation is low, with the averages of 15 mmHg and 18.9 mmHg reported [17,18]. By keeping metabolism running at a low level, such conditions protect the embryo from the teratogenic effects of reactive oxygen species during the critical phase of organogenesis [19,20]. Early placental development occurs under much the same conditions as aggregates of invading endovascular trophoblast plug the tips of the spiral arteries [21]. Intraplacental oxygen concentrations rise approximately three-fold at that time when the maternal circulation to the placenta is established at the end of the first trimester [22,23]. The increased oxygen concentration is essential to support the high levels of placental active transport and protein synthesis for the rapid fetal growth that characterizes the second and third trimesters. Second, such a profound change of oxygen concentration during the establishment of maternal circulation to the placenta can lead to oxidative stress [24]. The sEH gene promoter region contains recognition sites for a number of transcription factors including NF- $\kappa$ B [25], which can be activated by oxidative stress induced by hypoxia-reoxygenation [26]. Third, in other organ systems such as heart and brain, it has been demonstrated that the expression and activity of sEH increase after ischemia-reperfusion in mouse and rat models [27,28]. In this study, we found that villous explants subjected to HR had higher levels of sEH mRNA and protein than explants kept at normoxia throughout the experiment. These results support our hypothesis and reveal a possible mechanism underlying the regulation of sEH in human placenta during the transition from early to mid-gestation.

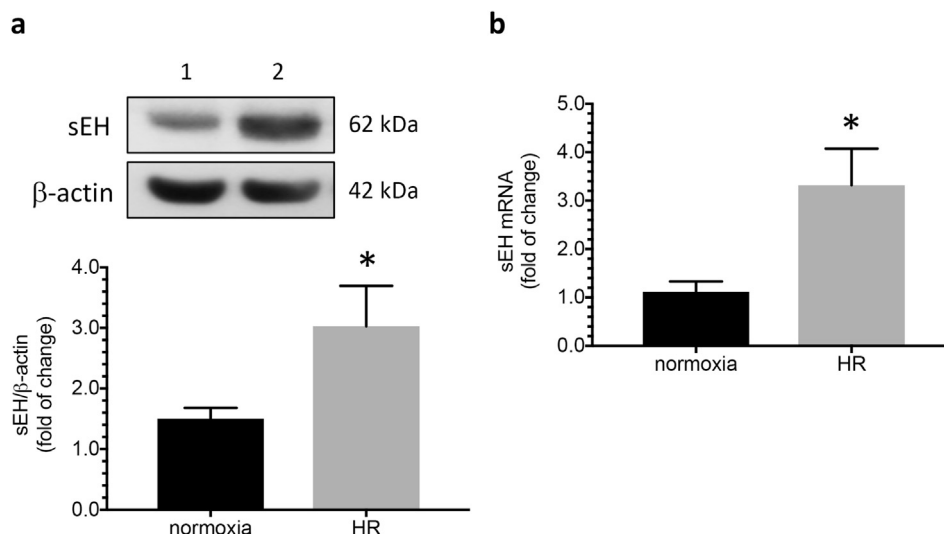
The biological significance of EETs, DHETs, and sEH in human pregnancy remains not clear. It was found that plasma levels of EETs are higher in pregnant women than that in nonpregnant women [11] and concentrations of EETs are much higher in fetal than that in maternal plasma and erythrocytes, indicating that fetoplacental unit contribute to EETs biosynthesis and metabolism during normal pregnancy [11]. The increased levels of EETs may facilitate uterine blood flow to provide the development of placenta and growth of the fetus. On the other hand, abnormal changes of EETs, DHETs, and sEH in the maternal circulation and placenta have been noted in pregnancy complications such as preeclampsia, though the results are still inconsistent. Herse et al. observed an elevation of plasma levels of 5,6- EET and 14,15-EET, as well as the corresponding DHETs in preeclamptic women compared with normotensive women in the latter two thirds of pregnancy [29]. Furthermore, Dalle Vedove et al. found increased levels of total EETs and reduced expression of sEH in the placenta from women with preeclampsia compared with normal pregnant women [8]. On the other hand, Santos and coworkers found that levels of total 14,15-DHET, which is a measurement of EET-dependent sEH activity, were higher in urine samples obtained from preeclamptic women compared to healthy pregnant women [30]. Accordingly, they hypothesized that sEH expression or activity is augmented in the preeclamptic women, reducing EET, and increasing blood pressure. This hypothesis is supported by a recent study showing that women with hypomethylation of the promoter region of *EPHX2* and *K55R* polymorphism were associated with significant increased risk of preeclampsia [31]. Further studies are needed to clarify the roles of placental EETs, DHETs, and sEH in normal pregnancies and pregnancies complicated by preeclampsia.

In conclusion, we found that sEH was continuously expressed in the placenta throughout gestation and that the levels of sEH increased with gestational age. Moreover, HR caused an increase in the levels of sEH protein and mRNA in villous explants *in vitro*.



**Fig. 1. Cellular distribution and temporal changes of sEH in the villous tissues of different stages of gestation.** (a–c) Immunoreactivity of sEH was mainly localized at the cytotrophoblasts and, to a lesser extent, the syncytiotrophoblast in the villous tissues throughout gestation. (d) There was essentially no staining at all in the negative controls with omission or replacement of the primary antibody with the same mouse isotypic IgG. (e) Compared to villous tissues from early gestation, the levels of sEH were significantly higher in villous samples from mid- and late gestation. Lane 1, villous tissues of 8 weeks' gestation; lane 2, villous tissues of 23 weeks' gestation; and lane 3, villous tissues of 38 weeks' gestation.  $\beta$ -actin was used to normalize loading variability. (f) There was an increase in the levels of sEH mRNA in villous tissues of mid- and late gestation than that of early gestation. A total of 10 villous samples from early gestation (8–12 weeks), 6 from mid-gestation (16–28 weeks), and 10 from late gestation (38–39 weeks) were analyzed. Data presented as mean  $\pm$  SEM. \*,  $P < 0.05$ , compared to villous tissues of early gestation.





**Fig. 2. HR leads to increased levels of sEH mRNA and protein in villous explants.** Compared to villous tissues kept at 8% oxygen throughout the experiment (normoxic controls), villous tissues subjected to HR had significantly higher levels of sEH protein (a) and mRNA (b). Lane 1, normoxic control; lane 2, HR.  $\beta$ -actin was used to normalize loading variability. Data presented as mean  $\pm$  SEM based on 8 individual experiments. \*,  $P < 0.05$ , compared to the normoxic controls.

These results suggest that oxygen regulates the expression of sEH and that sEH may be important for placental development during human pregnancy.

#### Funding statement

This work was supported by grants from the Ministry of Science and Technology, Taiwan (106-2314-B-182A-146, 107-2314-B-182A-099) and Chang Gung Memorial Hospital (CMRP3G0281, CMRPG1J0071).

#### Declaration of Competing Interest

All the authors declare that they have no conflicts of interest.

#### Acknowledgments

The authors are grateful to the staff of the Delivery Unit of Taipei Chang Gung Memorial Hospital for their assistance in obtaining the placental material and to the Tissue Bank and the Taipei Common Laboratory of Chang Gung Memorial Hospital for providing technical support.

#### References

- [1] Samuelsson B. Arachidonic acid metabolism: role in inflammation. *Z Rheumatol* 1991;50(Suppl. 1):3–6.
- [2] Deng Y, Theken KN, Lee CR. Cytochrome P450 epoxygenases, soluble epoxide hydrolase, and the regulation of cardiovascular inflammation. *J Mol Cell Cardiol* 2010;48:331–41.
- [3] Imig JD. Epoxides and soluble epoxide hydrolase in cardiovascular physiology. *Physiol Rev* 2012;92:101–30.
- [4] Spector AA. Arachidonic acid cytochrome P450 epoxygenase pathway. *J Lipid Res* 2009;50(Suppl):S52–6.
- [5] Harris TR, Hammock BD. Soluble epoxide hydrolase: gene structure, expression and deletion. *Gene* 2013;526:61–74.
- [6] Morisseau C, Hammock BD. Impact of soluble epoxide hydrolase and epoxyeicosanoids on human health. *Annu Rev Pharmacol Toxicol* 2013;53:37–58.
- [7] Corriveau S, Berthiaume M, Rousseau E, Pasquier JC. Why eicosanoids could represent a new class of tocolytics on uterine activity in pregnant women. *Am J Obstet Gynecol* 2009;201:420 e1–7.
- [8] Dalle Vedove F, Fava C, Jiang H, Zanconato G, Quilley J, Brunelli M, et al. Increased epoxyeicosatrienoic acids and reduced soluble epoxide hydrolase expression in the preeclamptic placenta. *J Hypertens* 2016;34:1364–70.
- [9] Schafer WR, Zahradnik HP, Arbogast E, Wetzka B, Werner K, Breckwoldt M. Arachidonate metabolism in human placenta, fetal membranes, decidua and myometrium: lipoxygenase and cytochrome P450 metabolites as main products in HPLC profiles. *Placenta* 1996;17:231–8.
- [10] Zhang JH, Pearson T, Matharoo-Ball B, Ortori CA, Warren AY, Khan R, et al. Quantitative profiling of epoxyeicosatrienoic, hydroxyeicosatetraenoic, and dihydroxyeicosatetraenoic acids in human intrauterine tissues using liquid chromatography/electrospray ionization tandem mass spectrometry. *Anal Biochem* 2007;365:40–51.
- [11] Jiang H, McGiff JC, Fava C, Amen G, Nesta E, Zanconato G, et al. Maternal and fetal epoxyeicosatrienoic acids in normotensive and preeclamptic pregnancies. *Am J Hypertens* 2013;26:271–8.
- [12] Hung TH, Chen SF, Li MJ, Yeh YL, Hsieh TT. Differential effects of concomitant use of vitamins C and E on trophoblast apoptosis and autophagy between normoxia and hypoxia-reoxygenation. *PLoS One* 2010;5:e12202.
- [13] Hung TH, Chen SF, Hsu JJ, Hsieh CC, Hsueh S, Hsieh TT. Tumour necrosis factor- $\alpha$  converting enzyme in human gestational tissues from pregnancies complicated by chorioamnionitis. *Placenta* 2006;27:996–1006.
- [14] Hung TH, Hsieh TT, Wu CP, Li MJ, Yeh YL, Chen SF. Mammalian target of rapamycin signaling is a mechanistic link between increased endoplasmic reticulum stress and autophagy in the placentas of pregnancies complicated by growth restriction. *Placenta* 2017;60:9–20.
- [15] Hung TH, Chen SF, Wu CP, Li MJ, Yeh YL, Hsieh TT. Micronized progesterone pretreatment affects the inflammatory response of human gestational tissues and the cervix to lipopolysaccharide stimulation. *Placenta* 2017;57:1–8.
- [16] Cizkova K, Tauber Z. Time-dependent expression pattern of cytochrome P450 epoxygenases and soluble epoxide hydrolase in normal human placenta. *Acta Histochem* 2018;120:513–9.
- [17] Yedwab GA, Paz G, Homonnai TZ, David MP, Kraicer PF. The temperature, pH, and partial pressure of oxygen in the cervix and uterus of women and uterus of rats during the cycle. *Fertil Steril* 1976;27:304–9.
- [18] Ottosen LD, Hindkaer J, Huth M, Petersen DE, Kirk J, Ingerslev HJ. Observations on intrauterine oxygen tension measured by fibre-optic microensors. *Reprod Biomed Online* 2006;13:380–5.
- [19] Leese HJ. Quiet please, do not disturb: a hypothesis of embryo metabolism and viability. *Bioessays* 2002;24:845–9.
- [20] Jauniaux E, Gulbis B, Burton GJ. The human first trimester gestational sac limits rather than facilitates oxygen transfer to the foetus—a review. *Placenta* 2003;24(Suppl. A):S86–93.
- [21] Burton GJ, Jauniaux E, Watson AL. Maternal arterial connections to the placental intervillous space during the first trimester of human pregnancy: the Boyd collection revisited. *Am J Obstet Gynecol* 1999;181:718–24.
- [22] Jauniaux E, Watson AL, Hempstock J, Bao YP, Skepper JN, Burton GJ. Onset of maternal arterial blood flow and placental oxidative stress. A possible factor in human early pregnancy failure. *Am J Pathol* 2000;157:2111–22.
- [23] Rodesch F, Simon P, Donner C, Jauniaux E. Oxygen measurements in endometrial and trophoblastic tissues during early pregnancy. *Obstet Gynecol* 1992;80:283–5.
- [24] Hung TH, Burton GJ. Hypoxia and reoxygenation: a possible mechanism for placental oxidative stress in preeclampsia. *Taiwan J Obstet Gynecol* 2006;45:189–200.
- [25] Tanaka H, Kamita SG, Wolf NM, Harris TR, Wu Z, Morisseau C, et al. Transcriptional regulation of the human soluble epoxide hydrolase gene EPHX2. *Biochim Biophys Acta* 2008;1779:17–27.
- [26] Cindrova-Davies T, Spasic-Boskovic O, Jauniaux E, Charnock-Jones DS, Burton GJ. Nuclear factor-kappa B, p38, and stress-activated protein kinase

- mitogen-activated protein kinase signaling pathways regulate proinflammatory cytokines and apoptosis in human placental explants in response to oxidative stress: effects of antioxidant vitamins. *Am J Pathol* 2007;170:1511–20.
- [27] Ding Y, Li Y, Zhang X, He J, Lu D, Fang X, et al. Soluble epoxide hydrolase activation by S-nitrosation contributes to cardiac ischemia-reperfusion injury. *J Mol Cell Cardiol* 2017;110:70–9.
- [28] Tu R, Armstrong J, Lee KSS, Hammock BD, Sapirstein A, Koehler RC. Soluble epoxide hydrolase inhibition decreases reperfusion injury after focal cerebral ischemia. *Sci Rep* 2018;8:5279.
- [29] Herse F, Lamarca B, Hubel CA, Kaartokallio T, Lokki AI, Ekholm E, et al. Cytochrome P450 subfamily 2J polypeptide 2 expression and circulating epoxyeicosatrienoic metabolites in preeclampsia. *Circulation* 2012;126:2990–9.
- [30] Santos JM, Park JA, Joiakim A, Putt DA, Taylor RN, Kim H. The role of soluble epoxide hydrolase in preeclampsia. *Med Hypotheses* 2017;108:81–5.
- [31] Sari I, Pinarbasi H, Pinarbasi E, Yildiz C. Association between the soluble epoxide hydrolase gene and preeclampsia. *Hypertens Pregnancy* 2017;36:315–25.