Placental oxygen transfer reduces hypoxia/reoxygenation swings in fetal blood in a sheep model of gestational sleep apnea

Isaac Almendros[1,2,3], Paula Martínez-Ros[4], Nuria Farré[5,6,7], Mónica Rubio-Zaragoza[8,9], Marta Torres[2,10], Álvaro J. Gutiérrez-Bautista[11], José M. Carrillo-Poveda[8,9], Joaquín J. Sopena-Juncosa[8,9], David Gozal[12], Antonio Gonzalez-Bulnes[13], Ramon Farré[1,2,3,*]

[1]Unitat de Biofísica i Bioenginyeria, Facultat de Medicina i Ciències de la Salut, Universitat de Barcelona, Barcelona, Spain. [2]CIBER de Enfermedades Respiratorias, Madrid, Spain
[3]Institut d'Investigacions Biomediques August Pi Sunyer, Barcelona, Spain. [4]Animal Production and Health Department, Veterinary Faculty, Universidad Cardenal Herrera-CEU Universities, Valencia, Spain. [5]Department of Cardiology, Hospital del Mar, Barcelona, Spain. [6]Heart Diseases Biomedical Research Group (GREC), Hospital del Mar Medical Research Institute-IMIM, Barcelona, Spain. [7]Department of Medicine, Universitat Autònoma de Barcelona, Barcelona, Spain. [8]Bioregenerative Medicine and Applied Surgery Research Group, Animal Medicine and Surgery Department, Veterinary Faculty, Universidad Cardenal Herrera-CEU Universities, Valencia, Spain. [9]García Cugat Foundation for Biomedical Research, Barcelona, Spain. [10]Servei de Pneumologia, Hospital Clínic, Barcelona, Spain. [11]Anaesthesia Unit, Veterinary Teaching Hospital, Animal Medicine and Surgery Department, Veterinary Faculty, Universidad Cardenal Herrera-CEU Universities, Valencia, Spain. [12]Department of Child Health, The University of Missouri School of Medicine, Columbia, MO, USA. [13]Department of Animal Reproduction. SGIT-INIA, Madrid, Spain

Short title: Fetal hypoxia in maternal sleep apnea

Corresponding author:
Prof. Ramon Farré
Unitat Biofísica i Bioenginyeria
Facultat de Medicina i Ciències de la Salut.
Casanova 143
08036 Barcelona, Spain
Email: rfarre@ub.edu

ABSTRACT

AIM: Obstructive sleep apnea (OSA), characterized by events of hypoxia-reoxygenation, is highly prevalent in pregnancy, negatively affecting the gestation process and particularly the fetus. Whether the consequences of OSA on the fetus and offspring are mainly caused by systemic alterations in the mother or by direct effect of intermittent hypoxia in the fetus is unknown. In fact, how apnea-induced hypoxemic swings in OSA are transmitted across the placenta remains to be investigated. The aim of this study was to test the hypothesis, based on a theoretical background on the dampening effect of oxygen transfer in the placenta, that oxygen partial pressure (PO_2) swings resulting from obstructive apneas mimicking OSA are mitigated in the fetal circulation. METHODS: To this end, 4 anesthetized ewes close to term pregnancy were subjected to obstructive apneas consisting of 25-s airway obstructions. Real time PO_2 was measured in the maternal carotid artery and in the umbilical vein using fast-response fiberoptic oxygen sensors. RESULTS: The amplitude of PO_2 swings in the umbilical vein were considerably smaller (3.1±1.0 *vs.* 21.0±6.1 mmHg (m±SE); $p<0.05$). Corresponding estimated swings in fetal and maternal oxyhemoglobin saturation tracked PO_2 swings. CONCLUSION: This study provides novel insights into fetal oxygenation in a model of gestational OSA, and highlights the importance of further understanding the impact of sleep-disordered-breathing on fetal and offspring development.

NEWS & NOTEWORTHY:

This study in an airway-obstruction sheep model of gestational sleep apnea provides novel data on how swings in oxygen partial pressure (PO_2) translate from maternal to fetal blood. Real-time simultaneous measurement of PO_2 in maternal artery and in umbilical

vein shows that placenta transfer attenuates the magnitude of oxygenation swings. These data prompts to further investigate to what extent maternal apneas could induce similar direct oxidative stress in fetal and in maternal tissues.

Keywords: fetal oxygenation, intermittent hypoxia, pregnancy apnea.

INTRODUCTION

Obstructive sleep apnea (OSA) is a highly prevalent syndrome in the general population (54) and has been recently identified as particularly frequent among pregnant women, affecting 8% - 45% in mid/late pregnancy (46,52). OSA affects pregnant women either because the patient already suffered the syndrome prior to pregnancy or because gestational changes promote OSA incidence (24,52). Given that the major risk factor for OSA is obesity (65) ─ a current worldwide pandemic (50) particularly affecting young individuals approaching reproductive age (34) ─, it is expected that the prevalence of OSA in pregnancy will further increase in the upcoming years. Besides the well-known long-term consequences of OSA (e.g. increase in morbidity and mortality by cardiocirculatory, metabolic, neurocognitive and malignant diseases (25,28,33,38,61), OSA poses a specific challenge during pregnancy. Indeed, both clinical and animal studies have shown that OSA imposes significant risk accrual for the emergence diabetes and pre-eclampsia in pregnant women, while increased risk for metabolic syndrome is apparent in offspring both short-term and long-term, and even transmitted across generations (3,13,35,37,40,52,69).

Although our understanding of the pathological mechanisms playing a role in the deleterious effects of OSA in pregnancy is scarce, current data available from systemic and end-organ research in children and adults suggest that gestational OSA may negatively impact on the fetus via two different ways: (i) the fetus is exposed to the systemic alterations induced by OSA in the mother. Indeed, as any other patient with OSA, a pregnant woman suffering from this sleep breathing disorder will be subjected to intermittent hypoxemia and hypercapnia, sleep fragmentation, and increased negative swings in intrathoracic pressures, resulting in a variety of systemic effects that could

potentially affect the fetus. For instance, increased sympathetic activation (48,51) and subsequent blood pressure alterations, or maternal circulating cytokines and exosomes (released as a result of oxidative stress and inflammation (2,36,41,42,59) could influence fetal homeostasis via their transfer through the placental barrier (22,29,66). (ii) OSA may induce fetal alterations via intermittent hypoxia developing in the fetus. Indeed, during the process of gas exchange in the placenta, fetal blood is directly exposed to the maternal intermittent hypoxemia induced by OSA, and hence all fetal tissues are potentially exposed to hypoxia/reoxygenation events. Noteworthy, there is existing evidence of hypoxia in the human placenta in OSA (57). However, how intermittent hypoxemia is translated from the mother to the fetus in OSA has not been explored to date.

From the fetal viewpoint, the placenta plays a similar role to the lungs after birth, i.e., the organ in which blood gases are interchanged with the environment, Figure 1 (17,67). Specifically, fetal blood extracts oxygen from maternal blood, similar blood extracting oxygen from alveolar gas. In both cases, oxygen transfer is passively driven by a gradient of oxygen concentration through a thin and high-surface barrier. There are, however, important differences between the alveolo-capillary barrier and the placental barrier. For instance, O_2 diffusing capacity and O_2 transfer rate are one order of magnitude higher in the lungs than in the placenta, O_2 tissue consumption and acid transfer are significant in the placenta and insignificant in the lungs, and Bohr and Haldane effects are double in the placenta and single in the lungs (45). Moreover, in the alveolo-capillary barrier, oxygen transfer takes place through the wall of a short and thin capillary with a blood transit time of slightly less than 1 s, facilitating fast equilibration between oxygen partial pressures (PO_2) in the alveolus and the blood. In contrast, oxygen transfer through the human placental barrier (Figure 1.A) is achieved as fetal blood circulates from the

111 umbilical arteries to the capillaries inside the placental villi, and returns to the umbilical
112 vein, whereas maternal blood percolates through a relatively high volume intervillous
113 compartment outside this arboreous structure (67). As a result, PO_2 in the fetal blood
114 leaving the placenta through the umbilical vein is considerably lower than PO_2 in the
115 maternal artery (6,8,32). Remarkably, maternal blood circulation through the human
116 intervillous space is a process with a washout time of ≈30 s (9). Such relatively high
117 circulation time does not pose a problem under normal stationary conditions of
118 oxygenation, but can hinder gas exchange during events such as those that characterize
119 OSA, in light of the fast-rate oxygen desaturation/re-oxygenation times, which can last as
120 short as 10 seconds or longer (44). Accordingly, it would be anticipated that placental
121 oxygen transfer would also reduce the amplitude of hypoxia/reoxygenation swings in fetal
122 blood.

123 Therefore, the aim of this work was to test the hypothesis that PO_2 swings resulting
124 from obstructive apneas mimicking OSA are reduced in fetal blood compared to maternal
125 blood because of the dampening effect of oxygen transfer in the placenta. To this end, we
126 carried out first ever real-time measurements of PO_2 in maternal arterial blood, and in
127 umbilical venous fetal blood during application of obstructive apneas realistically
128 mimicking OSA in a sheep model.
129

METHODS

The study was carried out on 4 near-term pregnant ewes (140 ± 0.8 days of gestation; ~95% of the total length of sheep pregnancy), aged 4-6 years-old and with mean weight of 61.5 ± 8.5 kg, from a commercial meat crossbreed. The experimental protocol was assessed and approved by the CEU Cardenal Herrera University Committee of Ethics in Animal Research and by the relevant regional authorities (report 2019/VSC/PEA/0007) according to the Spanish Policy for Animal Protection (RD53/2013), which meets the European Union Directive 2010/63/UE.

Animals were fasted from food but not from water 12 hours before surgery. Ewes were sedated with intravenous administration of midazolam (0.5 $mg \cdot kg^{-1}$; Midazolam Normon 15 $mg \cdot ml^{-1}$, Normon, Spain). Oxygen (3 $liters \cdot min^{-1}$) was administered using a facemask for 10 minutes. Afterwards, anesthesia was induced with intravenous propofol (Propofol Lipuro 10 $mg \cdot ml^{-1}$, B. Braun Melsungen AG, Germany) to proceed with endotracheal intubation. Anesthesia was maintained with Sevoflurane (room air). Ewes were then placed in sternal recumbency and epidural bupivacaine 0.5% (7.5 ml; Bupivacaina, B. Braun, Spain) was administered for intraoperative analgesia (5 ml). In case of intraoperative sign of nociception, rescue analgesia was administered with intravenous fentanyl (3 $\mu gr \cdot kg^{-1}$; Fentadon 50 $\mu gr \cdot ml^{-1}$, Dechra Veterinary Products, Spain). Neuromuscular blockage was induced with cisatracurium (0.5 $mg \cdot kg^{-1}$; Cisatracurio 2 $mg \cdot ml^{-1}$ EFG, Pfizer, Spain). All animals were mechanically ventilated with room air during the procedure with a tidal volume and respiratory rate suitable to maintain end-tidal CO_2 (35-45 mmHg).

An electronically driven 3-way valve connected at the entrance of the endotracheal tube was used for allowing application of a minimum of 12 obstructive apneas (25 s duration; 1 apnea each 2 min) by diverting mechanical ventilation to a ventilator test lung connected to the 3-way valve. The ewe carotid artery was exposed for measurement of maternal blood oxygenation and a caesarean section was carried out to expose the umbilical vein for measuring fetal blood oxygenation. After completing the whole experimental procedure, which lasted ≈90 min per ewe, the animals were immediately euthanized with an overdose of intravenous sodium pentobarbital (150 $mg \cdot kg^{-1}$, Dolethal; Vétoquinol, Madrid, Spain).

To measure blood PO_2 at the input (maternal artery) and output (umbilical vein) of the placental system, we used fiber-optic O_2 sensors similar to those previously employed to measure real-time in vivo blood PO_2 (26). Two identical 0.5 mm-diameter needles incorporating a retractable, miniaturized (~50 μm tip diameter; nominal accuracy: ±0.2%O_2 at 20%O_2) and fast (nominal response time in liquid < 2 s) sensor (OXR50, PyroScience, Aachen, Germany) were introduced into the blood vessels. PO_2 signals from both oxygen sensors were recorded simultaneously by a dual oxygen meter (FireStingO2, PyroScience, Aachen, Germany) and digitally stored for subsequent analysis. This meter also carried out automatic temperature compensation by using the reference signal form a shielded submersible temperature sensor (TSUB36, PyroScience, Aachen, Germany) placed into the ewe's esophagus. Immediately before and after use in each animal, the calibration and response time of both oxygen sensors was checked by subsequent fast submersion into water at room air equilibrium (21% O_2) and into an anoxic solution of 0.1 M sodium ascorbate and NaOH (0% O_2). In each experiment, the mean of maximum, minimum and

175 oxygen swings from at least 4 of the last apneic events in each ewe were computed. The
176 data are presented as mean±SE and the differences in these variables were compared by
177 paired t-tests. Differences were considered statistically significant when p values were
178 <0.05.

179 The oxygen dissociation curves for fetal and adult ovine blood were used to
180 determine the oxygen saturation (SaO_2) corresponding to each measured value of PO_2 in
181 the umbilical vein of the fetus and in the ewe carotid artery. To this end, we used the
182 relationship $\log(PO_2)=K_1-K_2\cdot pH+K_3\cdot\log(SaO_2/(100-SaO_2))$ with $K_1=4.522$, $K_2=0.404$,
183 $K_3=0.362$ for the adult sheep blood and $K_1=4.849$, $K_2=0.492$, $K_3=0.384$ for the fetal sheep
184 blood, corresponding to 38 ºC (47), for pH=7.4.
185

RESULTS

Obstructive apneic events imposed to the pregnant ewe for 25 s induced reductions in blood oxygenation in the mother and in the fetus as indicated by PO_2 levels measured in the maternal artery blood and in the umbilical vein, respectively (Figure 2). Figure 3A shows the mean (±SE) of baseline and nadir PO_2 values in maternal and fetal blood. In both instances, the changes observed in PO_2 values during the imposed obstructive apneic events were significant (paired t-test; $p<0.05$). As shown in Figure 3B, maternal PO_2 values swing amplitudes were considerably and significantly smaller in the fetus: from 21.0 mmHg to 3.1 mmHg, respectively ($p<0.05$).

Figure 4 shows how the swings in blood PO_2 during obstructive apneas translate into swings in SaO_2. The dissociation curves of oxygen in adult (black) and fetal (red) ovine blood illustrate the well-known higher fetal blood affinity for oxygen. Vertical dashed lines indicate the range of PO_2 values measured during imposed apnea events (from Figure 3A) in the maternal (black) and fetal (red) blood and horizontal dashed lines correspond to the ranges of SaO_2 in both sets. As expected from the monotonous increase in PO_2-SO_2 relationship and despite the known differences in slopes, swings in oxyhemoglobin saturation were clearly smaller in the fetus when compared to the mother (8.5% vs 14.0%).

DISCUSSION

This study confirms the hypothesis that the placenta behaves as an oxygen transfer system that dampens the amplitude of blood hypoxia-reoxygenation events caused by obstructive apneas mimicking gestational OSA. As a result, oxygen swings in the umbilical vein are considerably smaller than concomitant changes in maternal arterial blood.

The present study was carried out in a sheep model rather than the most commonly used rodent models which have previously explored the effects of maternal OSA on the fetus and offspring (3,13,35,37,40). A minor, but certainly not negligible advantage of the ovine model is that the size of both mother and fetus (usually single-gestation) are very close to those in humans, thereby facilitating local measurements and tissue biopsy at different fetal organs and most importantly, allowing for implantation of telemetric sensors to monitor physiological signals in the developing fetus subjected to maternal OSA from mid to late gestation. Notwithstanding, the most important advantage of the ovine model is the fact that the preponderance of data and conclusions derived from the pregnant sheep is immediately applicable to human placental physiology (4,12,49), in particular when studying how different interventions in the mother alter fetal oxygenation (27,30,43,53,60,68). Besides the widely accepted suitability of the sheep gestation model, the structures of the human and sheep placentas (Figure 1) present differences that could impact on the dynamics of oxygen transfer during the fast, intermittent hypoxic events caused by apneas mimicking OSA. One main difference is in the number of layers in the barrier separating fetal and maternal blood. Whereas the human placenta is hemomonochorial -only one layer of syncytiotrophoblasts separates the maternal blood space from the fetal capillaries-, the sheep placenta is epitheliochorial -one layer of uterine

epithelium cells and one layer of trophoblast cells separate maternal and fetal capillaries-(21). Thus, the thicker placenta barrier in the sheep could result in more buffering or blunting of the dynamics of oxygen transfer from mother to fetus. By contrast, another main structural difference, specifically concerning the villous structure could attenuate oxygen swings in the human discoid placenta as compared to sheep placenta. Such difference is not in the fetal villous tree since in both species there are stem, intermediate and terminal villi which consist of stem arteries and veins, intermediate arterioles and venules, and terminal capillaries (4). The main difference is that in the sheep placenta maternal villi interdigitate fetal villi, whereas in the human placenta there are no villi but an intervillous space (Figure 1). Therefore, potential differences in the values of oxygen swing attenuation through the placenta in sheep and humans cannot be ruled out.

A valve placed at the entrance of the ewe airway was used to realistically mimic the events of upper airway collapse that characterize OSA. A 25-s valve occlusion elicited hypoxia and reoxygenation with timing characteristics similar to those recorded in OSA patients (44). However, it could be possible that different amplitudes in the hypoxemic swings were induced in case that 25-s occlusions were applied chronically (as obstructions are experienced by OSA patients) instead of acutely as in this study. Mechanical ventilation was applied with room air and not with oxygen-enriched air to induce hypoxic events under the atmospheric normal conditions as occurs in OSA patients. In fact, the baseline values of PO_2 in maternal artery and umbilical vein (71.5 mmHg and 23.2 mmHg, respectively; Figure 3A) were virtually the same as those previously reported in anesthetized pregnant sheep (71). It should be mentioned, however, that our sheep were not obese, in contrast with most pregnant OSA patients in whom it has been reported that obesity modulates

umbilical cord oxygen values (58). Moreover, potential effects of anesthesia on sympathetic activity, hormones, uteroplacental blood flow and umbilical blood could not be ruled out. Although the baseline maternal PO_2 was lower than in non-anesthetized animals, we should remark that arterial PO_2 in pregnant women in the supine position is considerably lower during pregnancy (90.1 mmHg) when compared to paired postpartum control (99.2 mmHg) (70). Moreover, although a conventional baseline arterial PO_2 of ~100 mmHg is applicable to most humans, ≈7.5% of the world population (≈580 million people) inhabits areas at an altitude higher than 1,400 m (16) presenting a baseline arterial PO_2 below 80 mmHg (20). Interestingly, the prevalence and severity of sleep disordered breathing is substantially higher in highlanders compared with lowlanders (55). However, the most relevant variable for the present study, which focused on the short-time dynamics of placental oxygen transmission, was realistic, since the maternal arterial swings produced in our model (21.0 mmHg, Figure 3) were similar to the ones observed in pregnant women with severe OSA (24).

The main blood oxygenation variable measured in this study was PO_2 since, according to Fick's law, this is the primary variable determining oxygen transfer by diffusion through the placental barrier. Moreover, PO_2 can currently be directly measured in real time by fast-response fiber-optical sensors which do not require that blood is pulsating for properly functioning. Hence, PO_2 measurements were preferred despite the fact that SaO_2 is the variable used to non-invasively monitor hypoxemia in OSA patients in a clinical setting. Indeed, whereas arterial SaO_2 in the ewe could be measured by pulse oximetry, this technique could not be used for real time measuring oxygenation in the umbilical vein since this vessel is not pulsatile, a basic and essential requirement of pulse

oximetry. However, given that blood oxygen content is mainly determined by SaO_2 since the amount of dissolved oxygen transported by blood is very low as compared with the oxygen carried by hemoglobin, we computed the values of SaO_2 corresponding to the measured PO_2 values. To this end, we used available data describing the dissociation curves of oxygen in the maternal and fetal blood of the sheep (47) to describe the changes in SaO_2 induced by the obstructive apneas (Figure 4). This figure, derived for T=38 ºC and pH=7, illustrates that the higher O_2 reserve in fetal blood contributes to attenuate the transient hypoxic events effects induced by obstructive apneas. However, it should be mentioned that *in vivo* the maternal and fetal dissociation curves tend to be closer since maternal arterial blood is slightly alkalotic and hypocarbic, while fetal blood is slightly acidotic and hypercarbic, while fetal temperature is slightly higher than mother temperature (45).

The hypothesis that the placenta attenuates the amplitude of hypoxia-reoxygenation swings when oxygen is transferred from maternal to fetal blood is based on the anatomical structure of this temporary organ (Figure 1). Although oxygen transport through the placenta has been theoretically investigated with complex placental models, all studies to date have focused on the physiological conditions of steady state oxygen transport (10,14,15,62,64), and fast transient conditions in placental oxygen transfer, such as those occurring in OSA have not been addressed. Figure 5A depicts a simplified scheme of the maternal section of the human placenta (Figure 1.A) from a circulatory viewpoint. Blood flow (V') enters the system from the maternal artery, is mixed with the blood volume (V_0) of the intervillous space and leaves the placenta through the maternal vein. As PO_2 of blood in the intervillous space is higher than in the fetal blood, which circulates along the villi, and given that both blood compartments are separated by a membrane permeable to oxygen, there is a passive diffusion process of oxygen proportional to the PO_2 gradient

298 across the membrane (Figure 1). This process finally determines the PO_2 of blood leaving
299 the fetal portion of the placenta through the umbilical vein. However, given than oxygen
300 transfer in the placenta does not follow a model of concurrent vessels, but rather reflects
301 flow in a mixed-pool model (Figure 5A) (6), there is no full equilibration of the PO_2
302 between maternal and fetal blood. In fact, in both human and ovine pregnancy there is an
303 almost constant baseline shift, with PO_2 in the umbilical vein registering 10-20 mmHg
304 lower than in the uterine vein (6,32) despite their different models (pool vs. concurrent
305 vessels). Whereas PO_2 closely equilibrates at the "end capillary" of the placenta, measured
306 differences in venous PO_2 reflect vascular shunts, perfusion-perfusion inequalities, and
307 placental O_2 consumption, which are factors present in both the pool and concurrent models
308 (32,45). Therefore, although oxygen transfer across the placenta is mainly determined by
309 maternal arterial PO_2, any reduction in maternal arterial PO_2 is always translated into a
310 smaller decrease in umbilical vein PO_2 (8).

311 Another phenomenon that could reduce the amplitude of oxygenation swings across
312 the human placenta is illustrated in Figure 5.B. In the event of a transient decrease in
313 maternal arterial PO_2 (for instance caused by an obstructive apnea), PO_2 in the blood within
314 the intervillous space will not change immediately, since there is a mixing process between
315 the entering blood (V') and the blood (V_0) in the intervillous space. In fact, simply as a
316 result of mixing, if maternal PO_2 experiences a decreasing step, PO_2 in the intervillous
317 space would decrease exponentially with a time constant $\tau = V_0/V'$ (Figure 5B). For the
318 sake of simplicity this mixing model neglects the amount of oxygen flowing to fetal blood.
319 Another limitation of this simplified model is that the washout time may not exactly reflect
320 the effective intervillous transit time for gas exchange at the villous interface, since blood

flow within the intervillous space is heterogeneous and includes both fast and slow components. Despite the model limitations, it can be assumed that the intervillous space behaves as a first order dampening system effectively slowing the changes in PO_2 in maternal arterial blood. For the human placenta, τ can be estimated from published data for V' and V_0. De-Paula *et al*. (23) reported that near term of gestation the volume of maternal blood in the placenta is 428-644 ml (50%-90% percentile). Taking into account that the intervillous space is ≈56% of the placental volume (7), and that the fraction of uterine artery that flows to the intervillous space is ≈450 $ml \cdot min^{-1}$ (5,63), it can be estimated that τ ≈ 30-50 s. Interestingly, these τ values from data near gestation term should not be different at mid-gestation since, according to de-Paula *et al*. (23) and Browne *et al*. (8), V' and V_0 change in a similar proportion (by 2-2.5 fold) from gestation week 20 to term. Given that this time constant τ is similar or even longer than the rate of change of PO_2 in maternal arterial blood during obstructive apneas, its dampening effect in PO_2 swings must be considerable. Indeed, Figure 5.C shows one of the recordings of arterial PO_2 in an ewe during obstructive apneas mimicking OSA -which could be an example of the ones experienced by pregnant OSA patients (24)- and the result of applying a first order filter with τ of 30 s and 50 s to estimate the time course of PO_2 at the intervillous space. To this end, and following a conventional discrete-time realization of a first order filter, the output signal at time-point i (y_i) was computed from a combination of the input signal value at time-point i (x_i) and the previous output value (y_{i-1}) according to $y_i = \alpha \cdot x_i + (1- \alpha) \cdot y_{i-1}$, where $\alpha = \Delta/(\tau+\Delta)$, being Δ the data sampling time interval and τ the filter time constant (V_0/V'). The original swing amplitude of 30 mmHg is considerably reduced to 13 mmHg and 9 mmHg, respectively (Figure 5.C). Given that intervillous PO_2 is the main driver of

oxygen transfer across the human placenta, the swing attenuation induced by τ must contribute to reduce the swings in fetal blood oxygenation within the umbilical vein.

It should be mentioned, that this study was focused on measuring and comparing the real time measurements of umbilical venous and maternal arterial PO_2 to document the attenuations occurring in the fetus during apneas simulating OSA, and that studying the complex different mechanisms determining oxygen dynamics in the placenta and the fetus —explained in detail by Longo (45) and more recently reviewed by Carter (12) — was beyond the scope of the present study. This experimental work in a sheep model has several limitations, some of which have already been discussed, and these limitations must be considered when trying to translate the results to pregnant women with sleep apnea. Besides the obvious differences between human and sheep physiology in pregnancy, we should point out that apneas were applied acutely and not chronically, and the potential effects of apneas of different duration was not explored. Fetal variables such as temperature, heart rate and arterial pressure were not measured. In addition, the transit times used in the simplified simulation were estimated rather than measured. Moreover, blood oxygen content was not measured but estimated from measured PO2 values. Notwithstanding its limitations, this study highlights a relevant question on the potential impact of direct intermittent hypoxia on the fetus in gestational OSA. On the one hand, since the severity of hypoxemic swings is attenuated in the fetus, it could be anticipated that hypoxia-reoxygenation, and thus the oxidative stress induced in the various fetal organs and tissues would be lower than those experienced by adult tissues (1). Whereas this hypothesis seems plausible, we do not know how sensitive the fetus is to transient hypoxemic insults. It is well known that beyond maternal-fetal adaptation mechanisms for chronic hypoxia

such as living at high altitude (12), the fetus has adaptive mechanisms to tolerate acute hypoxia, such as that originating from umbilical cord compression (12,30). For instance, during acute hypoxia the fetus can counter a 50% reduction in oxygen delivery by increasing fractional extraction (12). However, these mechanisms have been observed during events lasting from several minutes to hours, a period much longer than those associated with transient OSA events. Whether such fetal protective mechanisms are activated and effective during much shorter hypoxemic events is unknown. It is also possible that the sensitivity and tolerance of fetal tissues to very short intermittent hypoxia, which has so far not been studied, is different than in adults (56). Moreover, the impact of intermittent hypoxemia, even of low amplitude, could be different in the fetus and in adults, since fetal mechanisms to regulate the distribution of blood flow among organs is markedly different from adults (11,12,30,31). These potential differences could modulate the epigenetic changes observed in the offspring following gestational intermittent hypoxia (18,19,39,40).

In conclusion, this study reveals that the oxygenated blood perfusing fetal tissues experiences attenuated hypoxic swings than those registered in maternal blood during obstructive apneas similar to the ones occurring in pregnant OSA patients, and highlights the importance of furthering our understanding on how obstructive apneas modulate the response of the fetal cardiovascular system and how relevant are the resulting hypoxia-reoxygenation events in fetal tissues. This information will be useful to better characterize the impact of gestational intermittent hypoxia in fetus development, and to clarify the mechanisms underlying the effects of intermittent hypoxia during gestation on long-term and transgenerational alterations in the offspring.

390

391

392 **ACKNOLEDGEMENTS**

393 The authors wish to thank Mr. Miguel A. Rodríguez (University of Barcelona) for
394 his excellent technical assistance and the CEU-Cardenal Herrera staff for their assistance
395 with animal care and handling. This work was supported in part by the Spanish Ministry of
396 Science, Innovation and Universities (SAF2017-85574-R).

REFERENCES

1. Almendros I, Farré R, Planas AM, Torres M, Bonsignore MR, Navajas D, Montserrat JM. Tissue oxygenation in brain, muscle, and fat in a rat model of sleep apnea: differential effect of obstructive apneas and intermittent hypoxia. Sleep, 34:1127-33, 2011.

2. Almendros I, Wang Y, Gozal D. The polymorphic and contradictory aspects of intermittent hypoxia. Am J Physiol Lung Cell Mol Physiol, 307: L129-40, 2014.

3. Badran M, Abuyassin B, Ayas N, Laher I. Intermittent hypoxia impairs uterine artery function in pregnant mice. J Physiol. 2019 Apr 19. doi: 10.1113/JP277775.

4. Barry JS, Anthony RV. The pregnant sheep as a model for human pregnancy. Theriogenology, 69:55-67, 2008.

5. Bartels, H., Moll, W., Metcalfe, J. Physiology of gas exchange in the human placenta. Am. J. Obstet. Gynecol, 84: 1714–1730, 1962.

6. Battaglia FC, Meschia G. Review of studies in human pregnancy of uterine and umbilical blood flows. Dev. Period Med, 17, 287–292, 2013.

7. Berglund L, Lilja A, Andersson J, Lindberg B, Ulin J, Långström B, Lundqvist H. Maternal blood volume in placenta of the rhesus monkey measured in vivo by positron emission tomography. Gynecol Obstet Invest, 31:1-7, 1991.

8. Browne VA, Julian CG, Toledo-Jaldin L, Cioffi-Ragan D, Vargas E, Moore LG. Uterine artery blood flow, fetal hypoxia and fetal growth. Philos Trans R Soc Lond B Biol Sci, 370(1663):20140068, 2015.

9. Burchell RC. Arterial blood flow into the human intervillous space. Am J Obstet Gynecol, 98: 303-11, 1967.

10. Burton GJ, Woods AW, Jauniaux E, Kingdom JC. Rheological and physiological consequences of conversion of the maternal spiral arteries for uteroplacental blood flow during human pregnancy. Placenta, 30:473-82, 2009.

11. Cahill LS, Zhou YQ, Seed M, Macgowan CK, Sled JG. Brain sparing in fetal mice: BOLD MRI and Doppler ultrasound show blood redistribution during hypoxia. J Cereb Blood Flow Metab, 34:1082-8, 2014.

12. Carter AM. Placental Gas Exchange and the Oxygen Supply to the Fetus. Compr Physiol, 5:1381-403, 2015.

13. Chen L, Zadi ZH, Zhang J, Scharf SM, Pae EK. Intermittent hypoxia in utero damages postnatal growth and cardiovascular function in rats. J Appl Physiol (1985), 124: 821-830, 2018.

14. Chernyavsky IL, Jensen OE, Leach L. A mathematical model of intervillous blood flow in the human placentone. Placenta, 31:44-52, 2010.

15. Clark AR, Lin M, Tawhai M, Saghian R, James JL. Multiscale modelling of the feto-placental vasculature. Interface Focus, 5:20140078, 2015.

16. Cohen JE, Small C. Hypsographic demography: the distribution of human population by altitude. Proc Natl Acad Sci U S A, 95:14009-14, 1998.

17. Cornelis G, Heidmann O, Degrelle SA, Vernochet C, Lavialle C, Letzelter C, Bernard-Stoecklin S, Hassanin A, Mulot B, Guillomot M, Hue I, Heidmann T, Dupressoir A. Captured retroviral envelope syncytin gene associated with the unique placental structure of higher ruminants. Proc Natl Acad Sci U S A. 2013; 110(9): E828-37.

18. Cortese R, Gileles-Hillel A, Khalyfa A, Almendros I, Akbarpour M, Khalyfa AA, Qiao Z, Garcia T, Andrade J, Gozal D. Aorta macrophage inflammatory and epigenetic changes in a murine model of obstructive sleep apnea: Potential role of CD36. Sci Rep, 7:43648, 2017.

19. Cortese R, Khalyfa A, Bao R, Andrade J, Gozal D. Epigenomic profiling in visceral white adipose tissue of offspring of mice exposed to late gestational sleep fragmentation. Int J Obes (Lond), 39:1135-42, 2015.

20. Crapo RO, Jensen RL, Hegewald M, Tashkin DP. Arterial blood gas reference values for sea level and an altitude of 1,400 meters. Am J Respir Crit Care Med, 160:1525-31, 1999.

21. Dahl Andersen, M, Alstrup, AKO, Duvald, CS, Mikkelsen, E, Vendelbo, M, Ovesen, PG & Pedersen, M. Animal models in fetal medicine and obstetrics. In *Animal Models for Human Diseases*. InTechOpen. 2018. http://dx.doi.org/10.5772/intechopen.74038

22. Dahlgren J, Samuelsson AM, Jansson T, Holmäng A. Interleukin-6 in the maternal circulation reaches the rat fetus in mid-gestation. Pediatr Res, 60: 147-51, 2006.

23. de-Paula CF, Ruano R, Campos JA, Zugaib M. Placental volumes measured by 3-dimensional ultrasonography in normal pregnancies from 12 to 40 weeks' gestation. J Ultrasound Med, 27:1583-90, 2008.

24. Edwards N, Blyton DM, Hennessy A, Sullivan CE. Severity of sleep-disordered breathing improves following parturition. Sleep, 28: 737-41, 2005.

461 25. Farré N, Farré R, Gozal D. Sleep Apnea Morbidity: A Consequence of Microbial-
462 Immune Cross-Talk? Chest, 154 :754-759, 2018.

463 26. Formenti F, Chen R, McPeak H, Murison PJ, Matejovic M, Hahn CE, Farmery AD,
464 et al. Intra-breath arterial oxygen oscillations detected by a fast oxygen sensor in an animal
465 model of acute respiratory distress syndrome. Br J Anaesth, 114: 683-8, 2015.

466 27. Gardner DS, Giussani DA, Fowden AL. Hindlimb glucose and lactate metabolism
467 during umbilical cord compression and acute hypoxemia in the late-gestation ovine fetus.
468 Am J Physiol Regul Integr Comp Physiol, 284:R954-64, 2003.

469 28. Gileles-Hillel A, Kheirandish-Gozal L, Gozal D. Biological plausibility linking
470 sleep apnea and metabolic dysfunction. Nat Rev Endocrinol, 12: 290-8, 2016.

471 29. Girard S, Sebire G. Transplacental Transfer of Interleukin-1 Receptor Agonist and
472 Antagonist Following Maternal Immune Activation. Am J Reprod Immunol, 75: 8-12,
473 2016.

474 30. Giussani DA, Unno N, Jenkins SL, Wentworth RA, Derks JB, Collins JH,
475 Nathanielsz PW. Dynamics of cardiovascular responses to repeated partial umbilical cord
476 compression in late-gestation sheep fetus. Am J Physiol, 273: H2351-60, 1997.

477 31. Giussani DA. The fetal brain sparing response to hypoxia: physiological
478 mechanisms. J Physiol, 594:1215-30, 2016.

479 32. Goplerud JM, Delivoria-Papadopoulos M. Physiology of the placenta—gas
480 exchange. Ann Clin Lab Sci, 15: 270-8, 1985.

481 33. Gozal D, Farré R, Nieto FJ. Obstructive sleep apnea and cancer: Epidemiologic
482 links and theoretical biological constructs. Sleep Med Rev, 27: 43-55, 2016.

483 34. Gozal D, Kheirandish-Gozal L. The obesity epidemic and disordered sleep during
484 childhood and adolescence. Adolesc Med State Art Rev, 21: 480-90, 2010.

485 35. Gozal D, Reeves SR, Row BW, Neville JJ, Guo SZ, Lipton AJ. Respiratory effects
486 of gestational intermittent hypoxia in the developing rat. Am J Respir Crit Care Med, 167:
487 1540-7, 2003.

488 36. Izci-Balserak B, Pien GW. Sleep-disordered breathing and pregnancy: potential
489 mechanisms and evidence for maternal and fetal morbidity. Curr Opin Pulm Med, 16: 574-
490 82, 2010.

491 37. Johnson SM, Randhawa KS, Epstein JJ, Gustafson E, Hocker AD, Huxtable AG,
492 Baker TL, Watters JJ. Gestational intermittent hypoxia increases susceptibility to

493 neuroinflammation and alters respiratory motor control in neonatal rats. Respir Physiol
494 Neurobiol, 256: 128-142, 2018.

495 38. Kerner NA, Roose SP. Obstructive Sleep Apnea is Linked to Depression and
496 Cognitive Impairment: Evidence and Potential Mechanisms. Am J Geriatr Psychiatry, 24:
497 496-508, 2016.

498 39. Khalyfa A, Cortese R, Qiao Z, Ye H, Bao R, Andrade J, Gozal D. Late gestational
499 intermittent hypoxia induces metabolic and epigenetic changes in male adult offspring
500 mice. J Physiol, 595: 2551-2568, 2017.

501 40. Iqbal W, Ciriello J. Effect of maternal chronic intermittent hypoxia during gestation
502 on offspring growth in the rat. Am J Obstet Gynecol, 209: 564.e1-9, 2013.

503 41. Khalyfa A, Kheirandish-Gozal L, Gozal D. Circulating exosomes in obstructive
504 sleep apnea as phenotypic biomarkers and mechanistic messengers of end-organ morbidity.
505 Respir Physiol Neurobiol, 256: 143-156, 2018.

506 42. Lavie L. Oxidative stress in obstructive sleep apnea and intermittent hypoxia--
507 revisited--the bad ugly and good: implications to the heart and brain. Sleep Med Rev, 20:
508 27-45, 2015.

509 43. Lee SJ, Hatran DP, Tomimatsu T, Peña JP, McAuley G, Longo LD. Fetal cerebral
510 blood flow, electrocorticographic activity, and oxygenation: responses to acute hypoxia. J
511 Physiol, 587: 2033-47, 2009.

512 44. Lim DC, Brady DC, Po P, Chuang LP, Marcondes L, Kim EY, Keenan BT, Guo X,
513 Maislin G, Galante RJ, Pack AI. Simulating obstructive sleep apnea patients' oxygenation
514 characteristics into a mouse model of cyclical intermittent hypoxia. J Appl Physiol (1985),
515 118: 544-57, 2015.

516 45. Longo LD. Respiratory gas exchange in the placenta. In: Handbook of Physiology.
517 Section 3: The respiratory system. Volume IV. Gas Exchange: 351-401. American
518 Physiological Society, Bethesda, MD, 1987.

519 46. Louis JM, Koch MA, Reddy UM, Silver RM, Parker CB, Facco FL, Redline S,
520 Nhan-Chang CL, Chung JH, Pien GW, Basner RC, Grobman WA, Wing DA, Simhan HN,
521 Haas DM, Mercer BM, Parry S, Mobley D, Carper B, Saade GR, Schubert FP, Zee PC.
522 Predictors of sleep-disordered breathing in pregnancy. Am J Obstet Gynecol, 218: 521.e1-
523 521.e12, 2018.

524 47. Meschia G, Hellegers A, Blechner JN, Wolkoff S, Barron DH. A comparison of the
525 oxygen dissociation curves of the bloods of maternal, fetal and newborn sheep at various
526 pHs. Q J Exp Physiol Cogn Med Sci, 46: 95-100, 1961.

48. Mifflin S, Cunningham JT, Toney GM. Neurogenic mechanisms underlying the rapid onset of sympathetic responses to intermittent hypoxia. J Appl Physiol (1985), 119: 1441-8, 2015.

49. Morrison JL, Berry MJ, Botting KJ, Darby JRT, Frasch MG, Gatford KL, Giussani DA, Gray CL, Harding R, Herrera EA, Kemp MW, Lock MC, McMillen IC, Moss TJ, Musk GC, Oliver MH, Regnault TRH, Roberts CT, Soo JY, Tellam RL. Improving pregnancy outcomes in humans through studies in sheep. Am J Physiol Regul Integr Comp Physiol, 315: R1123-R1153, 2018.

50. NCD Risk Factor Collaboration (NCD-RisC). Trends in adult body-mass index in 200 countries from 1975 to 2014: a pooled analysis of 1698 population-based measurement studies with 19·2 million participants. Lancet, 387(10026):1377-1396, 2016.

51. Oyarce MP, Iturriaga R. Contribution of Oxidative Stress and Inflammation to the Neurogenic Hypertension Induced by Intermittent Hypoxia. Front Physiol, 9: 893, 2018.

52. Pamidi S, Kimoff RJ. Maternal Sleep-Disordered Breathing. Chest, 153: 1052-1066, 2018.

53. Parraguez VH, Mamani S, Cofré E, Castellaro G, Urquieta B, De Los Reyes M, Astiz S, Gonzalez-Bulnes A. Disturbances in Maternal Steroidogenesis and Appearance of Intrauterine Growth Retardation at High-Altitude Environments Are Established from Early Pregnancy. Effects of Treatment with Antioxidant Vitamins. PLoS One, 10:e0140902, 2015.

54. Peppard, P. E., Young, T., Barnet, J. H., Palta, M., Hagen, E. W., Hla, K. M. Increased prevalence of Sleep-Disordered Breathing in Adults. Am. J. Epidemiol, 177: 1006–1014, 2016.

55. Pham LV, Meinzen C, Arias RS, Schwartz NG, Rattner A, Miele CH, Smith PL, Schneider H, Miranda JJ, Gilman RH, Polotsky VY, Checkley W, Schwartz AR. Cross-Sectional Comparison of Sleep-Disordered Breathing in Native Peruvian Highlanders and Lowlanders. High Alt Med Biol, 18:11-19, 2017.

56. Prabhakar NR, Semenza GL. Adaptive and maladaptive cardiorespiratory responses to continuous and intermittent hypoxia mediated by hypoxia-inducible factors 1 and 2. Physiol Rev, 92:967-1003, 2012.

57. Ravishankar S, Bourjeily G, Lambert-Messerlian G, He M, De Paepe ME, Gündoğan F. Evidence of Placental Hypoxia in Maternal Sleep Disordered Breathing. Pediatr Dev Pathol, 18: 380-6, 2015.

58. Richardson BS, Ruttinger S, Brown HK, Regnault TRH, de Vrijer B. Maternal body mass index impacts fetal-placental size at birth and umbilical cord oxygen vàlues with implications for regulatory mechanisms. Early Hum Dev, 112: 42-47, 2017.

59. Ryan S, Taylor CT, McNicholas WT. Systemic inflammation: a key factor in the pathogenesis of cardiovascular complications in obstructive sleep apnea syndrome? Thorax, 64: 631-6, 2009.

60. Sales F, Peralta OA, Narbona E, McCoard S, De Los Reyes M, González-Bulnes A, Parraguez VH. Hypoxia and Oxidative Stress Are Associated with Reduced Fetal Growth in Twin and Undernourished Sheep Pregnancies. Animals (Basel), 8(11), 2018.

61. Sánchez-de-la-Torre M, Campos-Rodriguez F, Barbé F. Obstructive sleep apnea and cardiovascular disease. Lancet Respir Med, 1: 61-72, 2013.

62. Serov AS, Salafia C, Grebenkov DS, Filoche M. The role of morphology in mathematical models of placental gas exchange. J Appl Physiol (1985), 120:17-28, 2016.

63. Serov AS, Salafia CM, Brownbill P, Grebenkov DS, Filoche M. Optimal villi density for maximal oxygen uptake in the human placenta. J Theor Biol, 364:383-96, 2015.

64. Serov AS, Salafia CM, Filoche M, Grebenkov DS. Analytical theory of oxygen transport in the human placenta. J Theor Biol, 368:133-44, 2015.

65. Shah N, Roux F. The relationship of obesity and obstructive sleep apnea. Clin Chest Med, 30: 455-65, 2009.

66. Shi R, Zhao L, Cai W, Wei M, Zhou X, Yang G, Yuan L. Maternal exosomes in diabetes contribute to the cardiac development deficiency. Biochem Biophys Res Commun, 483: 602-608, 2017.

67. Slator PJ, Hutter J, McCabe L, Gomes ADS, Price AN, Panagiotaki E, Rutherford MA, Hajnal JV, Alexander DC. Placenta microstructure and microcirculation imaging with diffusion MRI. Magn Reson Med, 80: 756-766, 2018.

68. Sørensen A, Pedersen M, Tietze A, Ottosen L, Duus L, Uldbjerg N. BOLD MRI in sheep fetuses: a non-invasive method for measuring changes in tissue oxygenation. Ultrasound Obstet Gynecol, 34:687-92, 2009.

69. Telerant A, Dunietz GL, Many A, Tauman R. Mild Maternal Obstructive Sleep Apnea in Non-obese Pregnant Women and Accelerated Fetal Growth. Sci Rep, 8: 10768, 2018.

592 70. Trakada G, Tsapanos V, Spiropoulos K. Normal pregnancy and oxygenation during
593 sleep. Eur J Obstet Gynecol Reprod Biol, 109:128-32, 2003.

594 71. Wilkening RB, Molina RD, Meschia G. Placental oxygen transport in sheep with
595 diferent hemoglobin types. Am J Physiol, 254:R585-9, 1988.

596

FIGURE LEGENDS

Figure 1. (A). Schematic representation of blood flow through the placenta and surrounding tissues. Blue and red arrows show the flow directions of oxygenated (red) and deoxygenated (blue) fetal blood through the placental vasculature. Dashed white arrows show idealized flow lines through intervillous space for maternal blood. Relative oxygenation states shown by the red to blue color gradient. Maternal septa divide vascular spaces into the placental cotyledons. Reproduced from reference (67) under the terms of the Creative Commons Attribution License. (B) Schematic diagram of placental vascular circuit in higher ruminants (sheep and cow). The yellow and gray areas represent the fetus and mother membranes, respectively; the localized areas of formation of the feto-maternal villous units, the placentomes, are indicated (left). The maternal (red) and fetal (blue) vessels are schematized. Detailed scheme of a bovine placentome (right) showing that the placental fetal villi are intimately enmeshed with preformed maternal endometrial crypts. Of note, the placentome organization in the cow and sheep is identical, except that on the fetal side it is convex in the cow and concave in the sheep. Reproduced from reference (17) with permission granted.

Figure 2. Illustrative example of oxygen partial pressure (PO_2) measured in maternal artery blood and in the umbilical venous fetal blood during application of an obstructive apnea mimicking OSA in a pregnant ewe.

Figure 3. (A) Mean (±SE; n=4) of baseline and nadir of oxygen partial pressure (PO_2) measured in maternal and fetal blood during application of obstructive apneas mimicking OSA. (B) Corresponding PO_2 swings in the maternal and fetal blood. *: $p<0.05$.

Figure 4. Dissociation curves of oxygen in adult (black) and fetal (red) ovine blood derived for T=38 ºC and pH=7. Dashed lines indicate how the swings in oxygen partial pressure (PO_2) experienced by both types of circulation during obstructive apneas (Figure 3) translate into swings in oxygen saturation. *In vivo*, these two curves tend to be closer because of the differences of fetal and maternal pH and temperature. See text for further details.

Figure 5. Simplified model of blood hypoxia-reoxygenation dampening in the human placenta. (A). Diagram of the maternal section of the human placenta from a circulatory viewpoint. V' is blood flow entering the intervillous space and V_0 is intervillous space volume. Oxygen diffuses from maternal to fetal blood across the villous membrane (dotted line). (B) In case of a sudden reduction in the oxygen partial pressure (PO_2) of the maternal artery blood, PO_2 in the intervillous space blood decreases exponentially with a time constant $\tau = V_0/V'$. (C) Black line: example of arterial blood PO_2 recorded in an ewe during obstructive apneas. Blue and red lines: result of filtering the original (black) signal with a first order system with a time constant τ of 30 s and 50 s, respectively. See text for detailed explanations.