Journal of Developmental Origins of Health and Disease

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Intergenerational effects of prenatal hypoxia exposure on uterine artery adaptations to pregnancies in the female offspring

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Brief Report

Cite this article: Wooldridge AL, Hula N, Kirschenman R, Spaans F, Cooke C-LM, and Davidge ST. (2022) Intergenerational effects of prenatal hypoxia exposure on uterine artery adaptations to pregnancies in the female offspring. *Journal of Developmental Origins of Health and Disease* **13**: 794–799. doi: 10.1017/S2040174422000216

Received: 24 November 2021
Revised: 19 March 2022
Accepted: 24 March 2022
First published online: 26 May 2022

Keywords:
Prenatal hypoxia; F1 offspring; developmental programming; uterine artery; vascular endothelial function

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Abstract

Prenatal hypoxia is a common complication of pregnancy and is associated with detrimental health outcomes, such as impaired cardiac and vascular function, in adult offspring. Exposure to prenatal hypoxia reportedly impacts the reproductive system of female offspring. Whether exposure to prenatal hypoxia influences pregnancy adaptations and outcomes in these female offspring is unknown. We hypothesised that prenatal hypoxia impairs uterine artery adaptations in pregnancies of the adult offspring. Pregnancy outcomes and uterine artery function were assessed in 14–16 weeks old non-pregnant and late pregnant (gestational day 20; term = 22 days) adult female offspring born to rats exposed to prenatal normoxia (21% oxygen) or hypoxia (11% oxygen, between days 15–21 of gestation). Compared with normoxia controls, prenatal hypoxia was associated with pregnant adult offspring having reduced placental weights in their litters, and uterine artery circumferential stress that increased with pregnancy. Overall, prenatal hypoxia adversely, albeit mildly, compromised pregnancies of adult offspring.

Introduction

Prenatal hypoxia represents an adverse intrauterine environment, and occurs with many pregnancy complications. Prenatal hypoxia is a major cause of intrauterine growth restriction and contributes to adverse developmental programming,[1] such as impaired cardiovascular function in the adult offspring.[2,3] The reproductive system of female offspring was also reported to be adversely affected[4–6]; however, it is unknown whether this extends to the arteries supplying the reproductive system.

Pregnancy requires substantial maternal haemodynamic adaptations, including increased blood volume, cardiac output and decreased peripheral vascular resistance.[7] Extensive remodelling of the uteroplacental vasculature is critical to increase artery diameter, which facilitates sufficient blood supply to transport nutrients and oxygen to the placenta and developing fetus.[8] Maternal vascular function during pregnancy appears to be altered with maternal hypoxia. For instance, pregnant mice exposed to hypoxic conditions (chronic intermittent hypoxia during the light cycle of 12 h, 12% oxygen) throughout pregnancy from gestational day (GD) 0.5 had reduced maximal uterine artery endothelium-dependent vasodilation on GD14.5 (term = 19 days) compared to normoxia-exposed (21% oxygen) pregnant mice.[9] Pregnant rats exposed to hypoxia (11% oxygen, continuously) from GD6–20 (term = 22 days) had uterine arteries with impaired endothelium-dependent vasodilation compared to uterine arteries from control pregnant rats.[10] Moreover, at GD45 (term = 63 days), guinea pigs directly exposed to chronic hypoxia (continuously, via a simulated altitude of 3960 m that resulted in an inspired pO_2 of 90 Torr) from within 3 days of conception had uterine arteries with greater distensibility, decreased stiffness, and lower elastin levels than normoxia-exposed controls (continuously, via the laboratory altitude of 1600 m that resulted in an inspired pO_2 of 125 Torr).[11]

In addition to the maternal impacts, prenatal exposure to hypoxia clearly affects progeny cardiovascular health. For instance, we showed that imposing maternal hypoxia during late pregnancy (11.5% oxygen, continuously from GD15 to term) in rats reduced offspring tolerance to ischaemia-reperfusion injury and reduced cardiac efficiency.[2] In the same model, we showed that nitric oxide (NO)-dependent relaxation in mesenteric and gastrocnemius muscle arteries was impaired in female offspring.[3] Moreover, adult rat offspring exposed to maternal hypoxia from early pregnancy (GD6) to term had markedly impaired NO-dependent relaxation in femoral resistance arteries and aortic thickening with increased peroxynitrite generation, which can lead to endothelial dysfunction.[6] Vascular endothelial dysfunction in adult offspring prenatally exposed to hypoxic conditions has also been shown in mice, guinea pigs, chickens, sheep and humans (reviewed in Giussani[12]). Despite this evidence of widespread effects of prenatal hypoxia on endothelium-dependent arterial function in adult offspring, the effects on reproductive

outcomes in female offspring require further study. Specifically, it remains unknown whether exposure of dams (F0) to hypoxia also impairs endothelium-dependent vascular function of uterine arteries of the female progeny (F1), and whether this affects pregnancy-induced adaptations. We hypothesised that female rat offspring exposed *in utero* to maternal hypoxia have impaired vascular adaptations to pregnancy, leading to adverse pregnancy outcomes. To test this, we used a rat model of prenatal hypoxia and assessed endothelium-dependent vasodilation and passive circumferential stress–strain properties of uterine arteries from first generation non-pregnant and pregnant rats exposed to normoxia or hypoxia prenatally.

Methods

Animal model

This study took into account guidelines on designing DOHaD studies.[13] Sprague Dawley rats were housed in the Animal Care Facility at the University of Alberta, which maintains an ambient temperature of 22 ± 1°C and a 14:10 h (light:dark) lighting schedule. The rats had *ad libitum* access to standard rat chow and water. All experiments were done in the female offspring (F1) of dams (F0) that had been exposed to hypoxia during late pregnancy, an established model of fetal hypoxia.[14] For the F0 rats, 14–16 week-old females (Charles River, Canada) were mated overnight. Pregnancy was confirmed the next morning by the presence of a plug or sperm in a vaginal smear (designated as GD0), and pregnant dams were single-housed. From GD15, the F0 dams were then exposed to hypoxia (11% O_2) by placing their cage into a hypoxic chamber, or they continued to be housed in standard atmospheric oxygen conditions (21% O_2) as normoxia controls. Dams were removed from the hypoxic chamber on GD21 (term = GD22) and delivered naturally at atmospheric oxygen conditions. Upon delivery, litter size was reduced to 8 pups/litter (four males and four females) to standardise postnatal conditions. Male F1 offspring were used in another study,[15] while the female F1 offspring (F1 rats that had been prenatally exposed to either normoxia or hypoxia, respectively, and then studied as adults, termed F1-pNormoxia and F1-pHypoxia, respectively) were used in the current study. The F1 females were weaned on postnatal day 21 and were housed in pairs until 14–16 weeks of age. At 14–16 weeks of age, the F1 females were randomly allocated to either non-pregnant or pregnant groups. Rats allocated to the pregnant group were mated, as described above. Pregnant F1 females at GD20 and age-matched, non-pregnant F1 females were humanely killed via exsanguination under isoflurane-induced anaesthesia. The uterine vasculature was excised and placed into ice-cold HEPES-buffered physiological saline solution (PSS; in mmol/L: 142 NaCl, 4.7 KCl, 1.17 $MgSO_4$, 4.7 $CaCl_2$, 1.18 K_2PO_4, 10 HEPES, 5.5 glucose, pH 7.4) for vascular experiments. Pregnancy outcomes of the F1 dams (litter size, numbers of reabsorptions, placental weight, and fetal sex, weight, crown-rump length and abdominal girth) were recorded.

Ex vivo *vascular function*

A segment of the right main uterine artery was isolated from adipose tissue and mounted on two glass cannulas in a pressure myograph (Living Systems, Burlington, US) containing PSS at 37°C. Intravascular pressure was controlled throughout the experiment using a pressure servo control PS/200 and perfusion pressure monitor. Vessel and wall diameters were measured using a digital filar eyepiece (Lasico, model 1602E-10) and processor, attached to a stereo microscope (Olympus, model SZH10). Arteries were equilibrated for 40 min, during which they were exposed to a stepwise increase in pressure from 60 to 80 mmHg.[16] The intraluminal pressure was then adjusted to 50 mmHg for a further 10 min prior to cumulative concentration response testing. For this, vessels were pre-constricted by adding an ~pEC_{80} dose (the mean effective concentration that produces 80% of the maximal response) of phenylephrine (PE, Millipore Sigma #P6126) to the bath. Arteries were pre-constricted for at least 5 min (with stable internal diameter), after which a cumulative concentration response curve to superfused methylcholine (MCh; range 0.0001–100 µm; Millipore Sigma #A2251) was generated. Passive artery characteristics were assessed in relaxed arteries, which was done using Ca^{2+}-free solution containing EGTA (in mmol/L: 142 NaCl, 4.7 KCl, 1.17 $MgSO_4$, 1.18 KH_2PO_4, 10 HEPES, 2 EGTA). The myograph bath solution was changed at least four times before the addition of superfused papaverine (0.1 µmol/l; Sigma). Following a 20 min equilibration at 50 mmHg, passive characteristics (arterial diameters and wall thicknesses) were assessed across 4–160 mmHg, with intervals of ≥ 1 min between each step.

Dose-response data were analysed as pEC_{75} (75% of the dose required to produce the maximum vasodilation response to MCh) and E_{max} (the maximum vessel response to MCh) using Prism (v9.0.0, GraphPad Software, San Diego, US). Circumferential wall stress and wall strain were calculated as described previously[17] using the initial vessel diameter measured at 4 mmHg. Circumferential stress-strain data were analysed as area under the curve (AUC).

Statistical Analyses

Data were analysed using GraphPad Prism (v9.2.0, San Diego, US). The effect of F0 dam exposure on F1 dam pregnancy outcomes was analysed by t-test (or Mann Whitney U-test for reabsorptions), pregnancy outcomes were assessed by two-way ANOVA with Sidak post-hoc test (prenatal hypoxia and fetal sex as the two factors) and vascular outcomes were assessed by two-way ANOVA with Sidak post-hoc test (prenatal hypoxia and F1 pregnancy status as the two factors). Data are mean ± SEM; $p < 0.05$ was considered statistically significant; n = number of F1 female offspring, 1–2 offspring was used per F0 dam, with n = 4–6 F0 dams per group represented in each outcome.

Results

Pregnancy outcomes (F1)

F1-pNormoxia and F1-pHypoxia dams at GD20 had similar body weights, litter sizes, reabsorptions, fetal weights (Table 1) and fetal sex ratios (data not shown). The F1-pHypoxia dams had litters with lower placental weights than F1-pNormoxia dams ($p = 0.042$, Table 1). Litters from F1-pNormoxia and F1-pHypoxia dams had similar placental efficiency (fetal:placental weight ratio), fetal abdominal girth, fetal crown-rump length, and fetal crown-rump length:abdominal girth ratio (Table 1), none of which were affected by fetal sex (Table 1).

Uterine artery endothelial function and mechanical properties

Arteries from non-pregnant F1-pHypoxia offspring were less sensitive to MCh than those from non-pregnant F1-pNormoxia rats

https://doi.org/10.1017/S2040174422000216 Published online by Cambridge University Press

Table 1. Pregnancy outcomes of F1 dams

	Pregnancies of F1 offspring exposed to prenatal *normoxia* ($n = 6$ *dams*)		Pregnancies of F1 offspring exposed to prenatal *hypoxia* ($n = 5$ *dams*)	
Dam weight at gestational day 20 (g)	453 ± 9		484 ± 14	
Number of viable fetuses	13.0 ± 0.7		13.0 ± 2.1	
Male fetuses in litter (%)	52 ± 6		57 ± 4	
Number of reabsorptions – median (range)	2 (0–3)		0 (0–1)	
	Male fetuses	Female fetuses	Male fetuses	Female fetuses
Fetal weight (g)	4.25 ± 0.17	3.93 ± 0.20	3.93 ± 0.09	3.76 ± 0.12
Placental weight (mg)	500 ± 20	490 ± 20	480 ± 10*	440 ± 20*
Fetal:placental weight ratio	8.60 ± 0.40	8.22 ± 0.40	8.41 ± 0.34	8.67 ± 0.40
Fetal crown-rump length (CRL, cm)	4.6 ± 0.1	4.5 ± 0.1	4.6 ± 0.1	4.5 ± 0.0
Fetal abdominal girth (AG, cm)	4.1 ± 0.1	4.0 ± 0.1	4.0 ± 0.1	3.9 ± 0.1
Fetal CRL:AG ratio	1.1 ± 0.0	1.1 ± 0.0	1.2 ± 0.0	1.2 ± 0.0

Number of viable fetuses and reabsorptions, and body weight, placental weight, fetal:placental weight ratio, abdominal girth, and crown-rump length on gestational day 20 from adult F1 dams that were exposed before birth to maternal normoxia or hypoxia. Data presented as mean ± SEM unless otherwise stated; n = represents litter average; n = 5–6 F1 dams/group. Data from F1 pregnancies are sex-specific means of litter outcomes. Number of viable fetuses were compared using a t-test and reabsorptions were compared using a Mann Whitney U-test. Fetal outcomes were compared using a two-way ANOVA (F1 treatment vs. fetal sex) followed by Sidak's post hoc test.
*$p < 0.042$ main effect of pHypoxia vs pNormoxia (sexes combined).

($p = 0.0391$, Fig. 1A, 1B). Sensitivity to MCh was similar between arteries of non-pregnant and pregnant rats in both groups (Fig. 1A, 1B).

Circumferential stress was similar between arteries of F1-pNormoxia non-pregnant and pregnant rats, but within the F1-pHypoxia rats, arteries from pregnant rats had greater circumferential stress than those from non-pregnant rats (Fig. 1C, 1D, $p = 0.0046$; interaction $p = 0.0431$). Arteries from pregnant rats had greater circumferential strain (Fig. 1C, 1E, $p = 0.0003$) and absolute diameter (AUC Non-pregnant 690 ± 12, Pregnant 1010 ± 17; $p < 0.0001$) than non-pregnant rats, and pHypoxia did not affect circumferential strain or absolute diameter. Pregnancy adaptations of main uterine arteries from F1-pHypoxia offspring did not significantly differ from F1-pNormoxia offspring in circumferential strain (Fig. 1C, 1E) or unconstricted (passive) artery diameters over a pressure range of 4–160 mmHg (data not shown). Wall thickness (AUC, data not shown) did not differ between the groups.

Discussion

Overall, the effects of *in utero* exposure to prenatal hypoxia on adult offspring pregnancies were adverse, albeit mild of the parameters that we assessed. Pregnancies in adult F1-pHypoxia female offspring were not associated with greatly impaired main uterine artery function (endothelium-dependent vasodilation and structural properties) or reduced fetal weights at GD20, although we did observe reduced placental weights. It may be suggested that the impaired endothelium-dependent vasodilation observed in uterine arteries from non-pregnant F1-pHypoxia offspring was able to be negated by GD20 by other, compensatory, adaptations against the effects of *in utero* hypoxia in the pregnant F1-pHypoxia offspring.

Against our expectations, we saw no differences in fetal outcomes as the result of F1 *in utero* exposure to maternal hypoxia; however, the placental weights of litters from F1-pHypoxia dams were significantly lower than those of F1-pNormoxia dams. This did not translate to a difference in placental efficiency, possibly due to low n numbers. Others have reported no difference in placental weight or efficiency in the pregnancies of F1 female rats that had been born to an F0 dam whose pregnancy had been compromised via bilateral uterine vessel ligation on GD18.[18] A smaller placenta may indicate impaired early placental development and growth, and while fetal weights were similar between the groups, the smaller placental size may have had other detrimental effects on fetal development that were not assessed. Additionally, the smaller placentas may not adapt to compounded pregnancy challenges[19] such as advanced maternal age. Moreover, as we only collected data at GD20, it is possible that impaired placental and fetal development may have differed between groups at earlier or later time points that were not assessed in the present study.

Our finding that *in utero* exposure to hypoxia impairs MCh-induced vasodilation of uterine arteries of non-pregnant rats is consistent with reports of impaired MCh-induced relaxation of arteries from different vascular beds of adult rats born to pregnancies compromised by hypoxia.[3,6] Others have reported changes in the reproductive organs of adult females previously exposed *in utero* to maternal hypoxia. Exposure to chronic hypoxia during fetal development resulted in offspring with accelerated biological ageing of the oviduct[4] and ovaries, including a reduced ovarian reserve.[5] The reduced sensitivity to MCh may indicate a more constrictive vascular phenotype. Overall, these data add to the literature on the effects of *in utero* exposure to maternal hypoxia on offspring reproductive health, by confirming that this impaired endothelium-dependent vasodilation exists in another vascular bed, at least in non-pregnant offspring.

Prenatal exposure to maternal (F0) hypoxia resulted in greater F1 uterine artery circumferential stress, albeit only in the pregnant state. This was not due to differences in wall thickness, which was not different with pHypoxia and which contributes to the calculation of circumferential stress. The subtle structural differences in the present study suggests that some direct effects of hypoxia that have been reported on F0 uterine arteries, such as (in guinea pigs) a 4-fold greater distensibility, decreased stiffness, and 50% reduction

https://doi.org/10.1017/S2040174422000216 Published online by Cambridge University Press

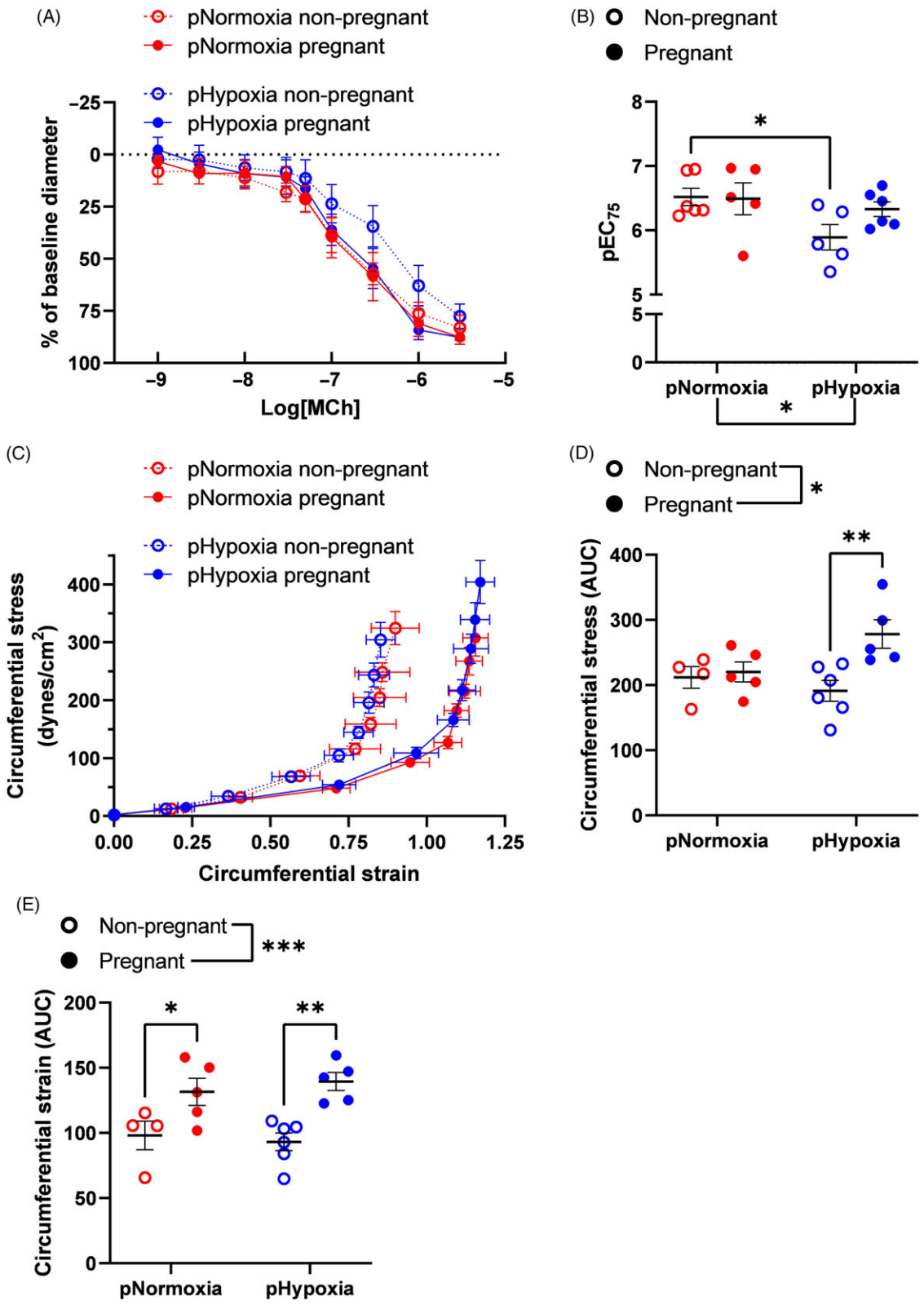

Fig. 1. Uterine artery function and mechanical properties. MCh-induced (*A*) vasodilation responses and (*B*) summary of MCh sensitivity (pEC_{75}), and (*C*) circumferential stress-strain curves, and the summary data (area under the curve; AUC) of circumferential (*D*) stress and (*E*) strain from main uterine arteries of adult pregnant (closed circles) and non-pregnant (open circles) F1 offspring exposed to *in utero* to prenatal normoxia (pNormoxia; red) or hypoxia (pHypoxia; blue). Data are presented as mean ± SEM; n = 4–6 F1 offspring/group; from n = 4–6 F0 dams; 1–2 offspring/dam. All groups were compared using a two-way ANOVA followed by Sidak's post hoc test. *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$.

https://doi.org/10.1017/S2040174422000216 Published online by Cambridge University Press

in elastin levels[11] appear to be less prominent in the F1 generation. Non-pregnant 18-month-old adult rats that had been growth-restricted *in utero* (bilateral uterine artery and vein ligation from GD18) had uterine arteries with increased arterial wall stiffness and smaller outer diameters over the pressurisation range compared with uterine arteries from rats that were born to sham control dams.[20] It is possible that with age, the effects of an adverse intrauterine environment becomes apparent. Regardless, it would be interesting to assess whether the F2 generation may still display an adverse uterine artery phenotype during their own later pregnancy due to the F0 exposure, as observed with other pregnancy complication models.[21]

The timing of hypoxia exposure within the present study (11% O_2, GD15–21) was focused on the period of most rapid fetal growth (GD13–20),[22] whereas other studies (13% O_2, GD6–20) [4,5] may have exposed dams across a wider range of fetal organ developmental periods. Subsequently, the F1-pHypoxia dams may not have been affected in a manner most likely to adversely affect the subsequent F2 fetuses. Fetal growth restriction of F1 rats can contribute to fetal growth restriction in the F2 generation, as others have shown using a bilateral uterine artery and vein ligation (from GD18) rat model.[23] However, this may have been indirectly caused by an altered F1 adult offspring phenotype that affected the F1 pregnancy, such as impaired glucose regulation, rather than direct programming effects from the F2 (as oocytes) insult of uterine artery and vein ligation.[23]

Prenatal hypoxia can be caused by many pregnancy complications, including placental insufficiency, maternal cardiac disease and preeclampsia. The uterine arteries of non-pregnant female F1 offspring exposed prenatal hypoxia demonstrated impaired endothelium-dependent vasodilation, though these differences were not observed in arteries of F1 pregnant offspring. This implies, at least for young females born to compromised pregnancies themselves, that their pregnancy adaptations may be able to overcome impairments to achieve pregnancies with normal fetal weights. The smaller placental size observed in the pregnancies of F1 offspring exposed to prenatal hypoxia may reflect impaired early placental development and growth, which may affect developmental programming of F2 adult offspring health. Additionally, a smaller placental size in the presence of additional maternal risk factors (such as maternal ageing or obesity) could have a greater impact on pregnancy outcomes for an F1-pHypoxia than an F1-pNormoxia pregnancy. Overall, prenatal hypoxia adversely affects pregnancies in adult offspring. Further studies are needed to assess the full impact of prenatal hypoxia on subsequent pregnancies in the offspring.

Acknowledgements. The authors thank the staff of Health Sciences Laboratory Animal Services for animal care.

Financial support. AW is supported by postdoctoral fellowships from the Canadian Institutes of Health Research (CIHR), Alberta Innovates, and the Women and Children's Health Research Institute (WCHRI). This WCHRI postdoctoral fellowship award is funded by the generosity of the Stollery Children's Hospital Foundation and the Alberta Women's Health Foundation through WCHRI. This work was supported by a CIHR Foundation Grant (FS154313) and by the generosity of the Stollery Children's Hospital Foundation and the Alberta Women's Health Foundation through WCHRI. Dr Sandra Davidge is a former Tier 1 Canada Research Chair in Maternal and Perinatal Cardiovascular Health and a Distinguished University Professor at the University of Alberta.

Conflicts of interest. None.

Ethical standards. The authors assert that all procedures contributing to this work comply with the ethical standards of the relevant national guides on the care and use of laboratory animals (Canadian Council on Animal Care guidelines) and were approved by the institutional committee University of Alberta Health Sciences Animal Policy and Welfare Committee (AUPs #242 and #3693).

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https://doi.org/10.1017/S2040174422000216 Published online by Cambridge University Press

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https://doi.org/10.1017/S2040174422000216 Published online by Cambridge University Press