Cytokine 136 (2020) 155290

Contents lists available at ScienceDirect

Cytokine

journal homepage: www.elsevier.com/locate/cytokine

Male fetal sex is associated with low maternal plasma anti-inflammatory cytokine profile in the first trimester of healthy pregnancies

David Ramiro-Cortijo[a], María de la Calle[b], Rainer Böger[c], Juliane Hannemann[c], Nicole Lüneburg[d], María Rosario López-Giménez[e], Pilar Rodríguez-Rodríguez[a], María Ángeles Martín-Cabrejas[f], Vanesa Benítez[f], Ángel Luis López de Pablo[a], María del Carmen González[a], Silvia M. Arribas[a,*]

[a] Department of Physiology, Faculty of Medicine, Universidad Autónoma de Madrid, Madrid, Spain
[b] Obstetrics and Gynecology Service, La Paz University Hospital, Madrid, Spain
[c] Department of Clinical Pharmacology and Toxicology, University Medical Center Hamburg-Eppendorf, Hamburg, Germany
[d] Hospital Pharmacy, University Medical Center Hamburg Eppendorf, Hamburg, Germany
[e] Department of Preventive Medicine, Public Health & Microbiology, Faculty of Medicine, Universidad Autónoma de Madrid, Madrid, Spain
[f] Institute of Food Science Research (CIAL) & Faculty of Sciences, Universidad Autónoma de Madrid, Madrid, Spain

ARTICLE INFO

Keywords:
ADMA
Cytokines
Fetus
Plasma
Sex
Nitric oxide
Pregnancy
First trimester

ABSTRACT

Male fetal sex associates with higher rates of materno-fetal complications. Inflammation and inadequate vasoactive responses are mechanisms implicated in obstetric complications, and alterations in maternal plasma cytokine profile and nitric oxide (NO) metabolites are potential predictive biomarkers. We aimed to assess if these parameters are influenced by fetal sex. A prospective, observational study was carried out in 85 healthy pregnant women with singleton pregnancies in the first trimester of gestation. A blood sample was extracted at the tenth week of gestation. In plasma, we assessed: 1) cytokines (micro-array): pro-inflammatory (IL1α, IL1 β, IL6, TNFα), anti-inflammatory (IL4, IL10, IL13), and chemoattractant (IL8, MCP1, IFNγ), and 2) NO metabolites (liquid chromatography–tandem mass spectrometry and Griess reaction): L-arginine, ADMA, SDMA, nitrates (NOx). Women with a male fetus (n = 50) exhibited, compared with those with a female (n = 35): higher IL1β (OR = 1.09 with 95% CI: 0.97–1.28), and lower IL13 (OR = 0.93 with 95% CI: 0.87–0.99), and higher plasma NOx (OR = 1.14 with 95% CI: 1.03–1.31). Our data suggest that fetal sex influences maternal plasma cytokine profile and NO in early pregnancy. Women with a male fetus may have a worse capacity to counteract an inflammatory response. They may have better vasodilator capacity, but in the presence of an oxidative environment, a higher nitrosative damage may occur. These data reinforce the need to include sex as variable in predictive models.

1. Introduction

Adaptations to the intrauterine environment differ in relation to fetal sex, particularly under adverse conditions, with short and long-term consequences for health. Several lines of evidence indicate that male sex is associated with higher risk of perinatal morbidities such as those related to prematurity [1] or small-for-gestational age [2]. Systematic reviews have also evidenced that women carrying a male fetus have a higher risk to develop gestational diabetes [3] and preeclampsia [4]. The mechanisms mediating this so-called male disadvantage remain unclear and may include different sensitivity to inflammatory mediators or endocrine responses [5].

Cytokines are key factors at all stages of pregnancy, with multiple roles such as regulation of inflammatory response and angiogenesis, and dysregulation can be a contributing factor to pregnancy complications. This is evidenced by the pro-inflammatory cytokine pattern found in serum from women with gestational diabetes [6] and preeclampsia [7]. The placenta exhibits the same sex of the fetus, and the higher prevalence of materno-fetal complications in women carrying a male has been associated with sex differences in placental cytokine expression [8,9]. However, studies in maternal plasma have provided inconsistent results. Some studies found higher levels of various pro-inflammatory cytokines in women with male fetuses [10,11], while others reported no differences [12].

* Corresponding author at: C/ Arzobispo Morcillo, 2, 28029 Madrid Spain.
E-mail address: silvia.arribas@uam.es (S.M. Arribas).

https://doi.org/10.1016/j.cyto.2020.155290
Received 15 June 2020; Received in revised form 19 August 2020; Accepted 6 September 2020

Nitric oxide (NO) is another key factor in pregnancy with multiple roles, including cytotrophoblast invasion, angiogenesis, and spiral artery remodeling. NO is the main vasodilator in the placenta, being responsible for the decrease in vascular resistance in early pregnancy [13]. Therefore, it is not surprising that preeclampsia is associated with alterations in maternal NO metabolism, including low availability of the precursor L-arginine (L-Arg) [14] and increased asymmetric dimethylarginine (ADMA), an endogenous NO synthase inhibitor [15–17]. Sex is known to be an important factor in vascular physiology [18]. However, to the best of our knowledge, the influence of fetal sex on maternal NO metabolism in normal pregnancy has not been explored.

Maternal plasma alterations in cytokines or NO metabolites can be used as biomarkers of obstetric complications, being an early detection a critical factor. The influence of fetal sex on their levels in early normal pregnancy, without the confounding factors of an obstetric complication, is currently lacking. This was the aim of the present study, where we have analyzed in the first trimester of uncomplicated singleton pregnancies, differences in maternal plasma cytokine profile and NO metabolism according to fetal sex.

2. Material and methods

2.1. Study cohort

This is a prospective single center observational study, which includes healthy pregnant women, attended at the Obstetrics and Gynecology Service from Hospital Universitario La Paz (HULP, Madrid; Spain). Recruitment was performed in the ninth week of gestation. Inclusion criteria were healthy women. Women who accepted to participate in the study had a medical evaluation, assessing their health status, and filled a questionnaire including maternal age, nationality, civil status, university studies and lifestyle (use of abuse drugs or tobacco prior or during pregnancy). Women with previous history of hypertension, diabetes mellitus, obesity, inflammatory-related disorders and those using drugs or smokers were excluded. Women included in the study voluntarily signed an informed consent and were appointed for the following week to provide a blood sample. Women were followed-up until delivery, recording the development of obstetric complications, type of gestation (singleton or multiple), gestational age (completed weeks), the sex of the infant and birth weight. Data from women with twin pregnancies, or who developed preeclampsia or Gestational diabetes were also excluded from the study. From the initial cohort, plasma from 85 women with term labor were analyzed.

2.2. Ethical approval

The study design was approved by the Ethics Review Board of Universidad Autónoma de Madrid (CEMU, 2013-10; Spain) and by the Ethical Committee of Human Research of HULP (PI-1490; Spain) and the work was conformed to the standards set by the Declaration of Helsinki (modified in 2013).

2.3. Blood sample extraction

Blood samples were collected at the tenth week of gestation between 8:00 and 9:00 am, at the Laboratory Medicine of HULP. Extraction, under fasting conditions, was performed by a nurse by venipuncture in Vacutainer® tubes containing lithium heparin (Becton Dickinson comp., Madrid; Spain). A 6 mL plasma volume was collected to assess maternal hematological and biochemical routine parameters at the Laboratory Medicine Service. A 5 mL volume was collected to obtain plasma by centrifugation (2100 rpm, 15 min at 4 °C), within a maximum of 2 h after extraction. Plasma was immediately aliquoted and stored at −80 °C until use.

2.4. Plasma cytokines assay

Plasma cytokines (IL1α, IL1 β, IL4, IL6, IL8, IL10, IL13, MCP1, TNFα and IFNγ) were measured by microarray assay according to manufacturer's instructions (Coefficient of variation < 20%; Quantibody Human Inflammation Array, RayBiotech, GA; USA). Cytokine levels were expressed as pg/mL. Cytokines were classified according to their main roles as pro-inflammatory, anti-inflammatory, or chemoattractant.

2.5. Plasma NO-related metabolites

Plasma levels of L-arginine, ADMA and symmetric dimethylarginine (SDMA) were determined by liquid chromatography–tandem mass spectrometry (LC–MS/MS) using a validated high-throughput LC–MS/MS assay [19]. Briefly, proteins were precipitated with methanol using 96-well 0.20 μm-pore-size microfiltration plates pre-coated with L-[2H7]arginine, [2H6]ADMA and [2H6]SDMA as internal standards. After centrifugation, the microfiltrates were dried and analytes were converted into their butyl ester derivatives. Subsequent analyses were performed using a Chirobiotic T, 20 mm (long) × 1.0 mm (internal diameter), microbore guard column (Astec, Whippany, NJ; USA) connected to a Varian 1200L Triple Quadrupole MS instrument (Varian, Walnut Creak, CA; USA) in the ESI + (positive electrospray ionization) mode. The sample run time was 1.6 min with intra-assay and inter-assay precisions of 2.2% and 4.7%, respectively.

To assess plasma nitrates, Griess reaction modified to microplate reader was used. This method has been regarded a reliable and quantitative estimate of NO output (NOx) [20,21]. Briefly, 100 μL of plasma reacted with 10 μL of N-ethylmaleimide 150 mM (Thermo Fisher Scientific, MA; USA) and 110 μL of trichloroacetic acid 20% w/v. The reaction was centrifuged (11440 rpm, 5 min at 4 °C) and 40 μL of supernatant was charged in a microplate. In the microplate, the supernatant reacted with 40 μL of vanadium (III) chloride (saturated with hydrochloric acid 1 M), 20 μL of sulfanilamide 2% (w/v diluted in hydrochloric acid 5% v/v; Thermo Fisher Scientific, MA; USA), 20 μL of naphthyl-ethylenediamine dihydrochloride 0.1% (w/v diluted in H2O-Q; Thermo Fisher Scientific, MA; USA). The microplate was incubated (1 h at 37 °C) and the absorbance was read at 540 nm. Nitrates were expressed as μM.

2.6. Statistical analysis

Statistical analysis was performed using R software (version 3.6.0, 2018, R Core Team, Vienna; Austria) within R Studio interface using ggplot2, ggpubr, dplyr, devtools, BBmisc, arsenal, nlme and questionr packages. Data were expressed as median and interquartile range (IQR, Q1; Q3). Wilcoxon rank-sum was applied to test differences between groups. Rho-Spearman test (ρ) was used to explore correlations between quantitative variables. A logistic regression model was used to determine the possible association between maternal plasma parameters and fetal sex. The adjusted model considered all maternal plasma variables that were significant in the univariate analysis. In the models, female sex was considered as reference category, and reported odds ratios with 95% of confidence interval. Statistical significance was assumed for p-values < 0.05.

3. Results

3.1. Cohort characteristics

In the cohort, 91.5% (78/85) of the women were of Spanish origin, the rest being from South America or North African origin. Regarding socioeconomic characteristics, 65.5% (56/85) were married and 69.7% (59/85) had university education. With respect to maternal and neonatal characteristics, 58.8% (50/85) of the women had a male and

Table 1
Maternal and neonatal characteristics according to fetal sex.

	Male (n = 50)	Female (n = 35)	p-value
Maternal age[1] (years)	33.5 (30.7; 37.0)	33.0 (31.0; 35.0)	0.73
Maternal weight[1] (kg)	60.0 (54.8; 67.0)	58.0 (53.5; 60.0)	0.15
Gestational age[2] (weeks)	39.0 (38.0; 40.0)	39.5 (38.0; 40.0)	0.42
Birth weight[2] (g)	3165 (2855; 3492)	3160 (2805; 3395)	0.63

Data show median and interquartile range (Q1; Q3) and p-value was reported by Wilcoxon rank-sum test. [1]At the beginning of pregnancy; [2]At labor.

Table 2
Maternal blood parameters according to fetal sex.

	Ref.	Male (n = 50)	Female (n = 35)	p-value
RBC (10^6/mL)	≥3.5	4.35 (4.23; 4.55)	4.27 (4.09; 4.51)	0.29
Hemoglobin (g/dL)	≥11	13.7 (13.0; 17.5)	13.8 (13.2; 24.1)	0.42
Hematocrit (%)	≥33	39.8 (38.3; 41.4)	39.5 (37.5; 40.9)	0.48
Platelets (10^3/mL)	≥240	237.0 (202.8; 268.0)	235.5 (199.3; 264.3)	0.80
Leukocytes (10^6/mL)	5–10	7.2 (6.2; 8.4)	7.2 (5.9; 8.5)	0.88
Glucose (mg/dL)	68–93	86.0 (79.0; 91.8)	84.0 (79.3; 90.3)	0.52
Cholesterol (mg/dL)	140–220	170.5 (154.8; 187.0)	165.5 (148.3; 178.0)	0.30
Triglycerides (mg/dL)	< 100	76.0 (59.0; 96.0)	73.5 (59.0; 89.0)	0.56

Data show median and interquartile range (Q1; Q3) and p-value was reported by Wilcoxon rank-sum test. Ref., reference range at the beginning of pregnancy; RBC, Red blood cells.

41.2% (35/85) a female infant. No significant differences in maternal age, weight, gestational age, or birth weight were detected between women with male or female fetuses (Table 1).

3.2. Hematological and biochemical maternal variables

All blood parameters evaluated at the tenth week of gestation were in the normal range. We did not find statistically differences in maternal hematological or biochemical variables between women with male or female fetuses (Table 2).

3.3. Maternal plasma cytokines

Regarding maternal plasma pro-inflammatory cytokines, we only found a significant difference in IL1 β, which was higher in women with a male compared to women with a female fetus (Fig. 1A). The levels of individual anti-inflammatory cytokines were not significantly different between sexes, except IL13, which was significantly lower in women with a male compared to a female fetus (Fig. 1B). Chemoattractant cytokines were not significantly different between sexes (Fig. 1C).

We did not detect statistical correlation between birth weight and any of the cytokines analyzed (Table 3).

3.4. Maternal plasma NO-related parameters

Maternal plasma level of nitrates was significantly higher in women with a male fetus than female (male = 5.6 (2.4; 9.3) μM; female = 2.8 (1.8; 5.5) μM; p-value = 0.019). No significant differences between sexes were found in L-arginine, ADMA or SDMA plasma levels (Fig. 2).

We did not detect statistical correlation between birth weight and NO-related parameters (L-Arginine: ρ = 0.26, p-value = 0.32; Nitrates: ρ = −0.09, p-value = 0.48; ADMA: ρ = 0.26, p-value = 0.28; SDMA: ρ = −0.32, p-value = 0.21).

3.5. Logistic regression model associated with fetal sex

The logistic regression model showed associations between maternal plasma IL13 and nitrates, with fetal sex. Women with a male fetus had 0.94-times lower IL13 and 1.14-times higher nitrate levels in plasma than women with a female fetus (Table 4).

4. Discussion

The present study aimed to assess if maternal plasma cytokines and NO metabolites, which have been proposed as biomarkers of obstetric complications, were influenced by fetal sex in uncomplicated singleton pregnancies in the first trimester of gestation. We provide evidence of the modulation of systemic levels of IL1β, IL13 and NOx by fetal sex.

Successful pregnancy requires coordinated communications between mother and fetus, and immune cells and their cytokine signaling pathways play key roles. In the first trimester of pregnancy, an inflammatory microenvironment is required for successful implantation and placentation. Pro-inflammatory cytokines and chemokines participate during these processes [22], and increased levels have been demonstrated in first trimester compared to non-pregnant women [23]. However, excessive inflammation is at the basis of many obstetric complications, and a delicate balance between pro- and anti-inflammatory cytokines is critical for successful pregnancy. Placental immune response is different between sexes. The male feto-placental unit is more sensitive to maternal inflammation, which has been associated with the risk to develop mental disorders later in life [24]. In addition, male placentae from preterm delivery also exhibit a higher level of inflammation, possibly because of a more robust maternal immune response to a male fetus [25]. Maternal systemic inflammatory status is likely to mirror placental levels, being cytokine levels potential biomarkers to diagnose the initiation of an obstetric complication. Therefore, we assessed if fetal sex had an influence on early maternal plasma concentrations of several types of cytokines.

Chemokines are cytokines that cause migration and activation of leukocytes. MCP1, IL8 and INFγ are pro-inflammatory chemokines, known to participate in pathology of adverse pregnancy outcomes, such as those associated with infections [26], gestational hypertension [27], and gestational diabetes [26]. However, these chemokines are detectable in maternal plasma in the first trimester of normal pregnancies and have physiological roles. For example, IL8 promotes angiogenesis, MCP1 is implicated in recruitment of Th2 regulatory T cells [28], important to avoid rejection of the fetus [29], and INFγ is implicated in spiral artery remodeling [30]. In the present study, at tenth week of pregnancy, the concentrations of these chemokines in our population were similar to those found in the above-mentioned studies in control pregnancies. We failed to detect sex differences in the levels of MCP1, IL8 or INFγ. Instead, Taylor and co-workers reported lower first trimester levels of INFγ in plasma from women carrying a female fetus [11], and the study of Chow and co-workers no significant differences were found in the second trimester [28]. Given the dual role of some chemokines as pro-inflammatory molecules and important physiological mediators, further studies are needed to clarify their relationship with fetal sex and the development of obstetric complications. On one hand, it would be important to explore in detail evolution of these molecules in different stages of pregnancy. On the other hand, it would be interesting to analyze the relationship between chemoattractant cytokine levels, the populations of leukocytes being recruited and the impact of sex on Th1/Th2 balance. In this context, it is relevant to note that Eninnga and co-workers found, in early pregnancy, that maternal plasma levels of Th1-type cytokines were greater in women with male fetus, while those with a female had higher levels of Th2-type [10].

Regarding the pro-inflammatory cytokines assessed in our study, we found similar values compared to those previously reported in the first trimester [23,27], and lower compared to those found in the second trimester [28]. We only found significant differences in the levels of IL1 β, which were higher in plasma from women with a male fetus. Elevation of IL1 β in early pregnancy has been consistently found in women who developed obstetric complications, such as gestational

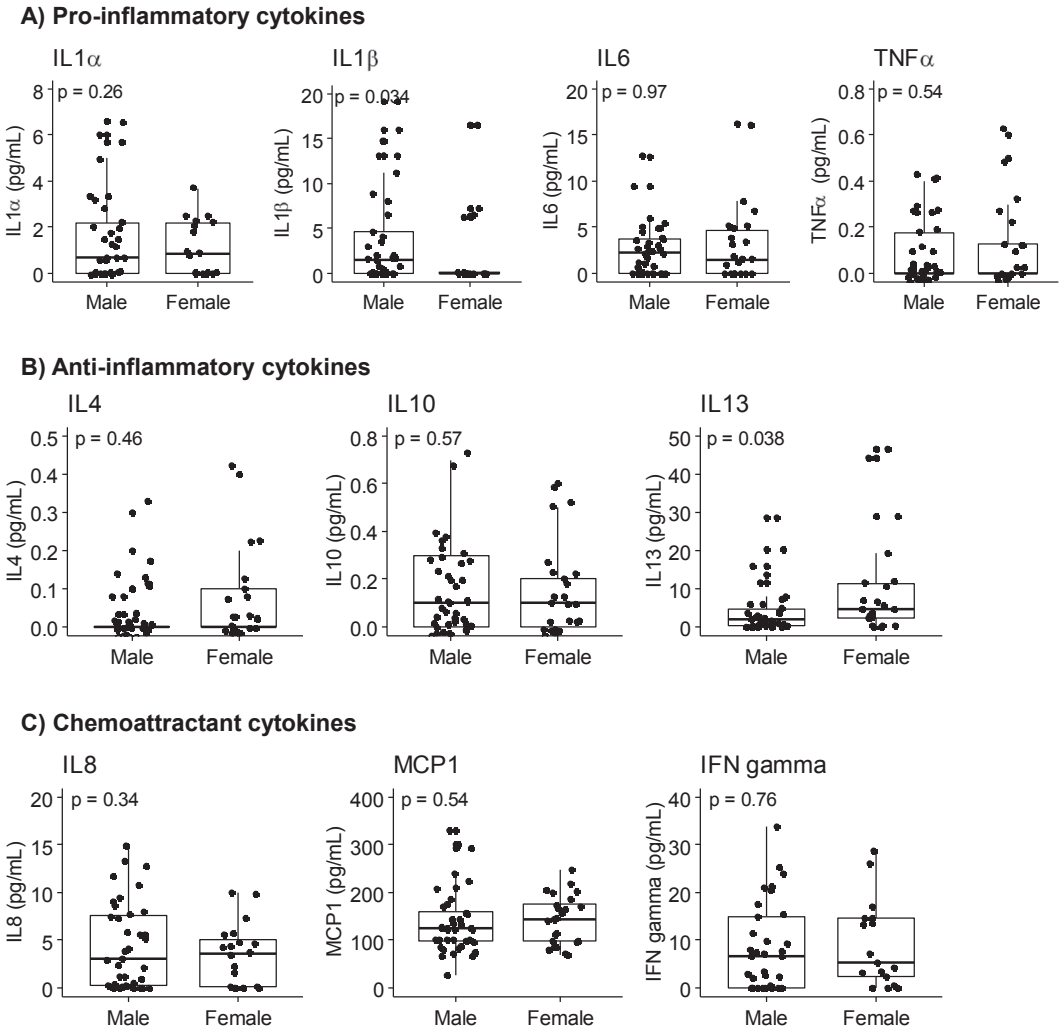

Fig. 1. Maternal plasma pro-inflammatory (A), anti-inflammatory (B) and chemoattractant (C) cytokines according to fetal sex. Male = 50, female = 35. Data show median and interquartile range (IQR) and p-value was reported by Wilcoxon rank-sum test. IL1α, Interleukin 1 alpha; IL1 β, Interleukin 1 beta; IL6, Interleukin 6; TNFα; Tumor Necrosis Factor alpha; IL4, Interleukin 4; IL10, Interleukin 10; IL13; Interleukin 13; IL8, Interleukin 8; MCP1, Macrophage Chemoattractant protein 1; IFNγ, Interferon gamma.

Table 3
Relationship between birth weight and maternal plasma cytokines.

Pro-inflammatory			Anti-inflammatory			Chemoattractant		
	ρ	p-value		ρ	p-value		ρ	p-value
IL1α	−0.03	0.85	IL4	−0.10	0.47	IL8	−0.10	0.48
IL1 β	0.03	0.82	IL10	−0.25	0.051	MCP1	−0.07	0.60
IL6	−0.10	0.45	IL13	0.00	0.99	IFNγ	−0.13	0.39
TNFα	−0.07	0.64						

Rho-Spearman (ρ) correlation and associated p-value. IL1α, Interleukin 1 alpha; IL1β, Interleukin 1 beta; IL6, Interleukin 6; TNFα; Tumor Necrosis Factor alpha; IL4, Interleukin 4; IL10, Interleukin 10; IL13, Interleukin 13; IL8, Interleukin 8; MCP1, Macrophage Chemoattractant protein 1; IFNγ, Interferon gamma.

hypertension [27] and preeclampsia [31,32]. In the present study, we did not assess the relationship between complications and fetal sex. Confirmation of early IL1β elevations in relation to male sex and the development of obstetric complications would be an interesting study, but would require a large cohort. Some studies have found a concomitant increase in pro-inflammatory cytokines and anti-inflammatory counterparts IL4 or IL10 and IL13 [27], which may represent an early compensatory mechanism. In our study, we did not find such elevation in women with male fetus; instead we detected a lower level of IL13. We also noticed that IL13 was the cytokine with largest concentration in maternal plasma, suggesting an important physiological role. In agreement with our results, higher levels of the anti-inflammatory cytokine IL5 [28], and IL1-ra (the natural antagonist of IL1 β) [33], have also been found in maternal plasma from women carrying a female. This pattern of compensatory mechanism was also evidenced in the second trimester in the study of Taylor, who found higher levels of IL1 β and TNF β, but a parallel elevation of anti-inflammatory molecules [11]. We did not assess if the more favorable cytokine pattern observed in women with a female fetus was maintained along pregnancy, which would add strength to the hypothesis of their advantage. However, taken together, our results and previous reports, we suggest that women with a female fetus might have a better capacity to resolve inflammation, compared to those with a male. A higher compensatory anti-inflammatory response could be beneficial around the tenth week of gestation, coincident with the entry of maternal oxygenated blood leading to a rise in reactive oxygen species (ROS), which is associated with pro-inflammatory cytokines in the placenta [23].

We did not evidence a relationship between any of the cytokines assessed and birth weight. However, we cannot discard an association at later stages of pregnancy, which were not evaluated in the present study. Besides, in our cohort, we did not have low birth weight infants (< 1500 g). It is possible that a relationship could be found analyzing

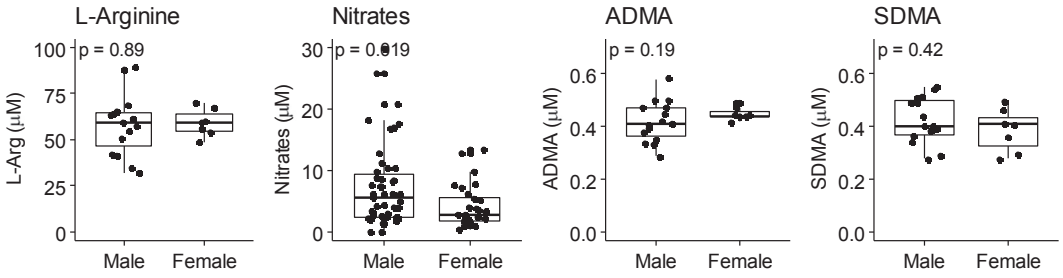

Fig. 2. Maternal plasma NO-related metabolites according to fetal sex. Male = 50, female = 35. Data show median and interquartile range (IQR) and p-value was reported by Wilcoxon rank-sum test. ADMA, Asymmetric dimethylarginine; SDMA, symmetric dimethylarginine.

Table 4
Logistic regression models associated with fetal sex.

	Odd ratio [95% CI]	p-value
IL1 β	1.09 [0.97; 1.28]	0.21
IL13	0.94 [0.87; 0.99]	0.049
Nitrates	1.14 [1.03; 1.31]	0.032

Data show odd ratios with 95% confidence interval (CI). Female sex was considered as a reference in the models.

plasma from mothers with premature and small for gestational age infants. Since male sex is associated with higher risk of prematurity and low birth weight and related pathologies [1,2], it would be interesting to follow a large population of women to assess the possible relationship between sex, pro-inflammatory cytokines and birth weight.

Increased plasma NOx were found in women carrying a male fetus. Nitrates represent the final products of NO oxidation, and plasma concentrations can be taken as an index of NO output [20,21]. Besides, nitrates are stable if the sample is processed within 48 h [34]. Since no differences between sexes were found in L-arginine (NO precursor) or in ADMA (main eNOS inhibitor), the larger NOx concentration in males may be attributed to a higher eNOS activity. One plausible mechanism may be the effect of sex steroids. Both, estrogens and androgens are known to activate eNOS through e-NOS phosphorylation and several molecular pathways [18]. Estrogens and progesterone rise throughout pregnancy, being responsible for systemic vasodilation and reduction in total peripheral resistance. Placental trophoblast has also the biosynthetic machinery to produce androgens and their circulating levels increase throughout gestation, being three-fold higher by the third trimester in pregnant compared to non-pregnant women. In normal pregnancies, plasma levels of estradiol, estrone and progesterone do not differ by fetal sex [10]. Instead, testosterone concentrations are higher in males, being important for sex determination, sex differences in fetal growth, and possibly, response to adverse factors [35]. We did not assess the levels of sex hormones in the present study. However, taken together the above-mentioned evidence, we suggest that the presence of testosterone in women carrying a male fetus may contribute to a higher eNOS activity under physiological conditions. However, this interpretation remains speculative and deserves further investigation. We would like to mention the potential influence of diet in NOx levels, an aspect that was not controlled in the present study. Foods like cured meats, cheese, and some vegetables (such as spinach and beetroot) are rich in nitrates and nitrites and can influence plasma NOx [36].

NO plays a key role during gestation, being the main vasodilator in the placenta and responsible for the decrease in vascular resistance in early pregnancy [13]. The higher NOx observed in women carrying a male infant, suggests that male fetus could provide an advantage regarding vascular adaptations in pregnancy. However, it is also important to consider that, under inflammatory and pro-oxidant environments, NO and ROS would generate peroxynitrite leading to damage. Nitrosative damage related to higher NO production has been observed in placenta infected with *Trypanosoma cruzi* [37], and could also take place under situations of placental hypoxia or deficient antioxidant defenses.

The present study has some limitations. We focused on the first trimester of pregnancy, where biomarker detection is more important for early diagnoses. We are aware of the importance to obtain a time-course development of the studied parameters, which may help to draw conclusions on the influence of fetal sex along gestation. A larger sample size would also be desirable to add robustness to the study. In addition, it would have been interesting to explore other well-known inflammation-related molecules, such as C-reactive protein (CRP) and their association with fetal sex. CRP increases along gestation concomitantly with IL-6, being higher in women developing preeclampsia [38], and in obese pregnant woman [39]. Besides, exposure to infection and inflammation during the fetal period is associated with offspring neuropsychiatric disorders, and sex-dependent alterations in maternal CRP levels have been reported [40]. Finally, our population was very homogeneous in terms of ethnicity and sociocultural parameters. It would be interesting to obtain data in pregnant women of different ethnic, social background with diverse lifestyles, such as diet, which may have an influence on the results.

5. Conclusions

The present study provides evidence of the influence of fetal sex on maternal systemic levels of cytokines and NO metabolites in uncomplicated pregnancies. Our data suggest that women with a male fetus may be endowed with a lower capacity to counteract an inflammatory environment. Instead, male sex is associated with better NO availability; this may help to respond better to a vascular compromise, but may be detrimental under a high ROS environment. These data may help to understand the influence of sex on the development of obstetric complications, and reinforce the need to include sex as variable in predictive models.

Funding

This research was funded by Universidad Autónoma de Madrid; Multidisciplinary Research Project (CEMU, 2013-10; Spain), Ministerio de Economía y Competitividad (FEM2015-63631-R; Spain) and Ministerio de Ciencia y Universidades (RTI2018-097504-B-I00; Spain), co-founded by FEDER funds.

CRediT authorship contribution statement

David Ramiro-Cortijo: Data curation, Formal analysis, Investigation, Methodology, Software, Visualization, Writing - original draft, Writing - review & editing. **María de la Calle:** Conceptualization, Supervision, Writing - review & editing. **Rainer Böger:** Formal analysis, Supervision, Writing - review & editing. **Juliane Hannemann:** Writing - review & editing. **Nicole Lüneburg:** Formal analysis, Methodology, Writing - review & editing. **María Rosario López-Giménez:** Data curation, Software. **Pilar Rodríguez-Rodríguez:** Methodology. **María**

Ángeles Martín-Cabrejas: Conceptualization, Funding acquisition, Supervision, Writing - review & editing. **Vanesa Benítez:** Methodology, Writing - review & editing. **Ángel Luis López de Pablo:** Writing - review & editing. **María del Carmen González:** Writing - review & editing. **Silvia M. Arribas:** Conceptualization, Funding acquisition, Investigation, Supervision, Writing - original draft, Writing - review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

We would like to acknowledge all the women who participated in the study and the Laboratory Medicine Service from Hospital Universitario La Paz (Madrid; Spain).

References

[1] J.L. Peacock, L. Marston, N. Marlow, S.A. Calvert, A. Greenough, Neonatal and infant outcome in boys and girls born very prematurely, Pediatr. Res. 71 (3) (2012) 305–310.
[2] L. Tamayev, L. Schreiber, A. Marciano, J. Bar, M. Kovo, Are there gender-specific differences in pregnancy outcome and placental abnormalities of pregnancies complicated with small for gestational age? Arch. Gynecol. Obstet. 301 (5) (2020) 1147–1151.
[3] D. Jaskolka, R. Retnakaran, B. Zinman, C.K. Kramer, Sex of the baby and risk of gestational diabetes mellitus in the mother: a systematic review and meta-analysis, Diabetologia 58 (11) (2015) 2469–2475.
[4] D. Jaskolka, R. Retnakaran, B. Zinman, C.K. Kramer, Fetal sex and maternal risk of pre-eclampsia/eclampsia: a systematic review and meta-analysis, BJOG 124 (4) (2017) 553–560.
[5] L. Bennet, Sex, drugs and rock and roll: tales from preterm fetal life, J. Physiol. 595 (6) (2017) 1865–1881.
[6] A.S. Morisset, M.C. Dubé, J.A. Côté, J. Robitaille, S.J. Weisnagel, A. Tchernof, Circulating interleukin-6 concentrations during and after gestational diabetes mellitus, Acta Obst. Gynecol. Scand. 90 (5) (2011) 524–530.
[7] J. Valencia-Ortega, A. Zárate, R. Saucedo, M. Hernández-Valencia, J.G. Cruz, E. Puello, Placental proinflammatory state and maternal endothelial dysfunction in preeclampsia, Gynecol. Obstet. Invest. 84 (1) (2019) 12–19.
[8] N.M. Scott, N.A. Hodyl, V.E. Murphy, A. Osei-Kumah, H. Wyper, D.M. Hodgson, R. Smith, V.L. Clifton, Placental cytokine expression covaries with maternal asthma severity and fetal sex, J. Immunol. 182 (3) (2009) 1411–1420.
[9] V.L. Clifton, Review: sex and the human placenta: mediating differential strategies of fetal growth and survival, Placenta 31 (2010) 33–39.
[10] E.A.L. Enninga, W.K. Nevala, D.J. Creedon, S.N. Markovic, S.G. Holtan, Fetal sex-based differences in maternal hormones, angiogenic factors, and immune mediators during pregnancy and the postpartum period, Am. J. Reprod. Immunol. 73 (3) (2015) 251–262.
[11] B.D. Taylor, R.B. Ness, M.A. Klebanoff, G. Tang, J.M. Roberts, D.M. Hougaard, K. Skogstrand, C.L. Haggerty, The impact of female fetal sex on preeclampsia and the maternal immune milieu, Pregnancy Hypertension 12 (2018) 53–57.
[12] A.M. Mitchell, M. Palettas, L.M. Christian, Fetal sex is associated with maternal stimulated cytokine production, but not serum cytokine levels, in human pregnancy, Brain Behav. Immun. 60 (2017) 32–37.
[13] W. Dymara-Konopka, M. Laskowska, The role of Nitric Oxide, ADMA, and Homocysteine in the etiopathogenesis of preeclampsia—review, Int. J. Mol. Sci. 20 (11) (2019) pii: E2757.
[14] H.I. Sirenden, S.AN. Usman, I. Idris, M.A.A. Deficiency of serum l-arginine levels in severe preeclampsia; a consideration for supplementation, Enfermería Clínica 30 (2020) 566–569.
[15] R.H. Böger, A. Diemert, E. Schwedhelm, N. Lüneburg, R. Maas, K. Hecher, The role of nitric oxide synthase inhibition by asymmetric dimethylarginine in the pathophysiology of preeclampsia, Gynecol. Obstet. Invest. 69 (1) (2010) 1–13.
[16] M. Anderssohn, L.M. Maaß, A. Diemert, N. Lüneburg, D. Atzler, K. Hecher, R.H. Böger, Severely decreased activity of placental dimethylarginine dimethylaminohydrolase in pre-eclampsia, Eur. J. Obst. Gynecol. Reprod. Biol. 161 (2) (2012) 152–156.
[17] D. Tsikas, A. Bollenbach, M.D. Savvidou, Inverse correlation between maternal plasma asymmetric dimethylarginine (ADMA) and birthweight percentile in women with impaired placental perfusion: circulating ADMA as an NO-independent indicator of fetal growth restriction? Amino Acids 50 (2) (2018) 341–351.
[18] S. Chaudhari, S.C. Cushen, O. Osikoya, P.A. Jaini, R. Posey, K.W. Mathis, S. Goulopoulou, Mechanisms of sex disparities in cardiovascular function and remodeling, Compr. Physiol. 9 (1) (2018) 375–411.
[19] E. Schwedhelm, R. Maas, J. Tan-Andresen, F. Schulze, U. Riederer, R.H. Böger, High-throughput liquid chromatographic-tandem mass spectrometric determination of arginine and dimethylated arginine derivatives in human and mouse plasma, J. Chromatogr. B 851 (1–2) (2007) 211–219.
[20] K.M. Miranda, M.G. Espey, D.A. Wink, A rapid, simple spectrophotometric method for simultaneous detection of nitrate and nitrite, Nitric Oxide – Biol. Ch 5 (1) (2001) 62–71.
[21] D. Giustarini, R. Rossi, A. Milzani, I. Dalle-Donne, Nitrite and nitrate measurement by Griess reagent in human plasma: evaluation of interferences and standardization, Method Enzymol. 440 (2008) 361–380.
[22] N.J. Hannan, K. Bambang, T.J. Kaitu'u-Lino, J.C. Konje, S. Tong, A bioplex analysis of cytokines and chemokines in first trimester maternal plasma to screen for predictors of miscarriage, PLoS ONE 9(4) (2014) e93320.
[23] J. Calleja-Agius, E. Jauniaux, A.R. Pizzey, S. Muttukrishna, Investigation of systemic inflammatory response in first trimester pregnancy failure, Hum. Reprod. 27 (2) (2012) 349–357.
[24] S.K. Hunter, M.C. Hoffman, A. D'alessandro, K. Noonan, A. Wyrwa, R. Freedman, A. J. Law, Male fetus susceptibility to maternal inflammation: C-reactive protein and brain development, Psychol. Med. (2019) 1–10.
[25] A. Ghidini, C.M. Salafia, Gender differences of placental dysfunction in severe prematurity, BJOG 112 (2) (2005) 140–144.
[26] E. Wender-Ozegowska, G. Michałowska-Wender, A. Zawiejska, M. Pietryga, J. Brazert, M. Wender, Concentration of chemokines in peripheral blood in first trimester of diabetic pregnancy, Acta Obstet. Gyn. Scan 87 (1) (2008) 14–19.
[27] L.H. Tangerås, M. Austdal, R.B. Skråstad, K.A. Salvesen, R. Austgulen, T.F. Bathen, A.C. Iversen, Distinct first trimester cytokine profiles for gestational hypertension and preeclampsia, Arterioscler. Thromb. Vasc. Biol. 35 (11) (2015) 2478–2485.
[28] S.S.W. Chow, M.E. Craig, C.A. Jones, B. Hall, J. Catteau, A.R. Lloyd, W.D. Rawlinson, Differences in amniotic fluid and maternal serum cytokine levels in early midtrimester women without evidence of infection, Cytokine 44 (1) (2008) 78–84.
[29] P. Nancy, A. Erlebacher, T cell behavior at the maternal-fetal interface, Int. J. Dev. Biol. 58 (2–4) (2014).
[30] A.A. Ashkar, J.P. Di Santo, B.A. Croy, Interferon γ contributes to initiation of uterine vascular modification, decidual integrity, and uterine natural killer cell maturation during normal murine pregnancy, J. Exp. Med. 192 (2) (2000) 259–270.
[31] J.E. Siljee, E.J. Wortelboer, M.P.H. Koster, S. Imholz, W. Rodenburg, G.H.A. Visser, A. de Vries, P.C.J.I. Schielen, J.L.A. Pennings, Identification of interleukin-1 beta, but no other inflammatory proteins, as an early onset pre-eclampsia biomarker in first trimester serum by bead-based multiplexed immunoassays, Prenat. Diagn. 33 (12) (2013) 1183–1188.
[32] B.D. Taylor, R.B. Ness, M.A. Klebanoff, R. Zoh, D. Bass, D.M. Hougaard, K. Skogstrand, C.L. Haggerty, First and second trimester immune biomarkers in preeclamptic and normotensive women, Pregnancy Hypertension 6 (4) (2016) 388–393.
[33] R. Romero, R. Gomez, M. Galasso, M. Mazor, S.M. Berry, R.A. Quintero, D.B. Cotton, The natural interleukin-1 receptor antagonist in the fetal, maternal, and amniotic fluid compartments: the effect of gestational age, fetal gender, and intrauterine infection, Am. J. Obstet. Gynecol. 171 (4) (1994) 912–921.
[34] Y. Wang, M.K. Townsend, A.H. Eliassen, T. Wu, Stability and reproducibility of the measurement of plasma nitrate in large epidemiologic studies, N Am. J. Med. Sci. 6 (2) (2013) 82–86.
[35] A.S. Meakin, V.L. Clifton, Review: Understanding the role of androgens and placental AR variants: insight into steroid-dependent fetal-placental growth and development, Placenta 84 (2019) 63–68.
[36] J. Hodžić, S. Izetbegović, B. Muračević, R. Iriškić, H.Š. Jović, Nitric oxide biosynthesis during normal pregnancy and pregnancy complicated by preeclampsia, Med. Glas. 14 (2) (2017) 211–217.
[37] M.F. Triquell, C. Díaz-Luján, M.C. Romanini, J.C. Ramírez, P. Paglini-Oliva, A.G. Schijman, R.E. Fretes, Nitric oxide synthase and oxidative-nitrosative stress play a key role in placental infection by Trypanosoma cruzi, Am. J. Reprod. Immunol. 80 (1) (2018) e12852.
[38] L. Raio, N.A. Bersinger, A. Malek, H. Schneider, F.H. Messerli, H. Hürter, S.F. Rimoldi, M.U. Baumann, Ultra-high sensitive C-reactive protein during normal pregnancy and in preeclampsia: a pilot study, J. Hypertens. 37 (5) (2019) 1012–1017.
[39] P. Pantham, I.L.K.H. Aye, T.L. Powell, Inflammation in maternal obesity and gestational diabetes mellitus, Placenta 36 (7) (2015) 709–715.
[40] S.K. Hunter, M.C. Hoffman, A. D'Alessandro, K. Noonan, A. Wyrwa, R. Freedman, A.J. Law, Male fetus susceptibility to maternal inflammation: C-reactive protein and brain development, Psychol. Med. 2 (2019) 1–10.