Fetal sex specific differences in gestational age at delivery in preeclampsia: a meta-analysis

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Key message

This study highlights the importance of fetal sex during pregnancy showing fetal sex specific differences in preeclampsia with a female dominance among preterm pregnancies complicated by pregnancy.

Short version of title

Sex difference and preeclampsia

Abstract*:*

BACKGROUND Preeclampsia (PE) is a major pregnancy disorder complicating up to 8% of pregnancies. Increasing evidence indicates a sex specific interplay between the mother, placenta and fetus. This may lead to different adaptive mechanisms during pregnancy.

METHODS We performed an individual participant data meta-analysis to determine associations of fetal sex and PE, with specific focus on gestational age at delivery in PE. This was done on 219,575 independent live born singleton pregnancies, with a gestational age at birth between 22.0-43.0 weeks gestation, from 11 studies participating in a worldwide consortium of international research groups focusing on pregnancy.

RESULTS Of the women 9,033 (4.1%) experienced PE in their pregnancy and 48.8% of the fetuses were female versus 51.2% male. No differences in the female/male distribution were observed with respect to term PE (delivered ≥37 weeks). Preterm PE (delivered <37 weeks) was slightly more prevalent among pregnancies with a female fetus than in pregnancies with a male fetus (OR 1.11, 95% CI 1.02-1.21). Very preterm PE (delivered <34 weeks) was even more prevalent among pregnancies with a female fetus as compared to pregnancies with a male fetus (OR 1.36, 95% CI 1.17-1.59).

CONCLUSIONS Sexual dimorphic differences in the occurrence of PE exist with preterm PE being more prevalent among pregnancies with a female fetus as compared to pregnancies with a male fetus and with no differences with respect to term PE.

KEY WORDS sexual dimorphism, preeclampsia, placenta, sex ratio, ALSPAC

Introduction

There are known large sex differences in disease incidence, presentation, diagnosis and outcome to treatment.[1] During past years attention has focused on the female / male distribution during pregnancy and its interaction with maternal health. Apparently, maternal physiological functions are influenced in a fetal sex-specific manner during pregnancy.[2] Preeclampsia (PE) is a major pregnancy disorder complicating up to 8% of pregnancies in some countries. PE is an important contributor to maternal and perinatal morbidity and mortality worldwide.[3] Preeclamptic women as well as their children have an increased risk to develop cardiovascular disease and stroke later in life.[4] A previous study indicated that fetal sex influenced gestational age at delivery in a Norwegian population from up to 50 years ago with female fetuses predominating in preeclamptic pregnancies ending before 37 weeks.[5] Gestational age has been suggested as an indicator of subsets of PE with a different pathophysiology and with different acute and long range outcomes for both mother and baby. Therefore, in this study we sought to confirm and extend these prior findings to very preterm pregnancies in a more diverse and contemporary pregnancy population. To assess sex-specific differences in gestational age at delivery in preeclamptic pregnancies we conducted a meta-analyses of individual data from 219,575 pregnant women participating in 11 studies from several European, Oceanian and US centers.

Material and methods

Inclusion criteria and participating cohorts

In 2011 the Global Pregnancy Collaboration (CoLab) was established to facilitate data and sample sharing between research groups studying PE and other pregnancy disorders (pre-empt.cfri.ca/Collaboration/global-pregnancy-Collaboration). CoLab is a consortium of international research groups with data and biological samples from women prior to, during and in some cases, long after pregnancy. Information on clinical data and samples is offered in a membership-wide shared database and available to CoLab members and to investigators sponsored by CoLab members.[6] In 2012 we invited principal investigators of international research groups active in CoLab to participate in the current study. Studies participated if they included pregnant women with available information on the occurrence of PE. Information on gestational age at birth and fetal sex also had to be available. Only live born singleton pregnancies with a gestational age at birth between 22.0-43.0 weeks gestation were included. Both nulliparous and multiparous women could participate. Eleven studies agreed to participate, comprising 219,575 independent singleton pregnancies that met the inclusion criteria.[7-16] The studies varied in sample size as well as study design, including both low and high risk pregnancies. Study-specific information with references to detailed information about each individual study is show in table 1. All studies were approved by the national, regional and local relevant research review boards. Regarding the ALSPAC study, ethical approval for the study was obtained from the ALSPAC Ethics and Law Committee and the Local Research Ethics Committees. All participants provided written informed consent for using their data. Anonymized data sets were stored on a single central secured data

server with access for the main analysts only. MOOSE guidelines for reporting a meta-analysis were followed.

Preeclampsia

Information on PE per study was obtained per participating center by using measurements, medical registries and hospital records, and/or specific questionnaires. Gestational hypertension was defined as a blood pressure >140 mmHg systolic or >90 mmHg diastolic in a woman who was normotensive before 20 weeks' gestation without concurrent new onset proteinuria. In all studies participating in CoLab and in this study PE is defined according to former International Society for the Study of Hypertension in Pregnancy criteria (de novo gestational hypertension with concurrent new onset proteinuria [≥0.3 g protein in a 24-hour specimen, correlating with ≥30 mg/dL (≥1+ reading on dipstick) in a random urine determination with no evidence of urinary tract infection]).[17] Superimposed PE was defined as chronic hypertension diagnosed prior to pregnancy or in first 20 weeks of pregnancy, complicated by de novo proteinuria occurring after gestational week 20, in the absence of renal disease and urinary tract infection. As PE is a syndrome that does not necessarily present as de novo hypertension and proteinuria the same day, and as routine antenatal follow-up schedules differ between countries and pregnancies, the time of PE diagnosis is difficult to define precisely. Instead, gestational age at delivery was used as a proxy for the onset of disease. Women with a very early onset of PE (prior to gestational week 34) often present with combined intrauterine growth restriction (IUGR) or rapidly increasing maternal symptoms and rarely remain undelivered for many days or weeks. Women with term PE (from gestational week 37+0) are likely to be induced (provided vaginal delivery is

feasible and clinically justified) and delivered shortly after diagnosis complying with current international clinical PE guidelines. As gestational age at delivery was reliably registered in the centers that were included in this analysis this was used as a proxy to distinguish between term, preterm and very preterm PE (i.e. delivery ≥37+0 weeks of gestation, < 37 weeks of gestation and <34 weeks of gestation). This distinction between early and very early versus term "onset" of PE is a commonly used categorization in PE studies.

Covariates

Information about maternal characteristics (maternal age, parity, body mass index and the presence of chronic hypertension) and birth characteristics (gestational age at birth, offspring birth weight, and fetal sex) in each study was obtained per participating center by using measurements, medical registries and hospital records, and/or specific questionnaires.

Statistical analyses

Individual datasets were integrated into one central database. For the cleaning of the central database the following criteria were used: values had to be within three standard deviations at either side of the mean and/or values had to be clinically reasonable. Random-effects models as proposed by DerSimonian and Laird were used to take the potential between-study variation next to the within-study variation into account.[18, 19] In this model, the inverse of standard errors from the individual studies combined with the between study variation were used as weights. Heterogeneity was assessed by the I^2 index. The I^2 index describes the proportion of total variation in the effect sizes that is due to heterogeneity between studies.

To determine the influence of any particular cohort on overall results, we repeated each meta-analysis, leaving out one cohort at a time (leave-one-out methodology). The overall effects are presented as forest plots with the pooled odds ratios from the random-effect models with 95% confidence intervals (CI). Statistical analyses were performed with SAS 9.2 software (SAS Institute, Cary, NC) and Comprehensive Meta-Analysis 2.0 (Biostat, Englewood, USA).

Results

Subject characteristics

Study specific information about maternal and birth characteristics is shown in Table 2. The overall distribution of female and male fetuses was 48.8% versus 51.2%. The overall prevalences of gestational hypertension and PE were 2.9% and 4.1% (n=6,150 and n=9,033), respectively. Of the preeclamptic women 6.4% had superimposed PE (n=575). Of the remaining 8,458 de novo preeclamptic women, 15.4% were diagnosed with very preterm PE (< 34 weeks of gestation, n=1,306).

Preeclampsia and fetal sex

In this meta-analysis we observed no differences in the distribution of female versus male fetuses in the overall occurrence of PE (Figure 1.). Furthermore no differences in the distribution of female versus male fetuses with respect to de novo PE, superimposed PE or gestational hypertension were observed. We observed no differences in the female/male distribution with respect to term de novo PE (i.e. ≥37 weeks of gestation) (Figure 2.). After stratification into preterm and very preterm de novo PE (i.e. < 37 weeks of gestation and <34 weeks of gestation), differences in the distribution of female versus male fetuses in the occurrence of PE were observed. Female preterm PE was more prevalent than male preterm PE in pregnancies going beyond 22.0 weeks (OR 1.11, 95% CI 1.02-1.21, I^2 = 32.7%) (Figure 3.). These results did not change after applying the leave-one-out method nor did restriction of these analyses to nulliparous women change the results. Very preterm PE was even more

prevalent among pregnancies with a female fetus as compared to pregnancies with a male fetus (OR 1.36, 95% CI 1.17-1.59, I^2 = 21.0%) (Figure 4.). Applying the leave-one-out method did not change the results nor did restriction of these analyses to nulliparous women (Supplemental Figure 1-2.). Finally, no differences in the female / male distribution with respect to de novo PE between 34-37 weeks of gestation were observed. This suggests that the effects with respect to preterm PE are majorly determined by effects in the distribution of female versus male fetuses in very preterm PE (Supplemental Figure 3.).

Comment

Results from this large-scale meta-analysis of individual participants' data show sexual dimorphic differences in the rates of PE subgroups with preterm and very preterm PE being more prevalent among pregnancies with a female fetus as compared to pregnancies with a male fetus and with no differences with respect to term PE. No differences in female / male distribution are observed in the overall risk of PE.

Comparison with earlier studies and interpretation of main findings

PE has a deleterious impact on maternal and fetal morbidity, mortality and future health. It is a heterogeneous disorder with a complex etiology and pathogenesis. Progress in the understanding of the disorder would be assisted greatly if subtypes could be characterized.[20] Despite increasing evidence that maternal physiological functions are influenced in a fetal sex-specific manner during pregnancy, in most studies that assess potential pathophysiologic mechanisms of PE fetal sex has not been taken into account.

Previously, a large Norwegian population-based data study suggested that the sex ratio in PE displays a pattern strongly dependent on length of gestation.[5] They showed that female babies were more frequent in PE with preterm delivery, whereas PE with term delivery was dominated by male offspring. Interestingly, when only assessing normotensive pregnancies opposite results were observed with a male predominance in preterm births.[5] Our results on PE are in line with theirs indicating that fetal sex influences gestational age at delivery in preeclamptic pregnancies. These results are further supported by a recent study by Broere-Brown et al.[21] showing fetal sex specific differences in maternal vascular adaptation to

pregnancy. They observed sex specific differences in Doppler measurements of the uterine artery and sex specific differences in both systolic and diastolic blood pressure patterns throughout pregnancy. Interestingly, differential effects according to the presence or absence of the placental syndromes, encompassing PE, IUGR and preterm birth, were observed. In pregnancies complicated by the placental syndromes women pregnant with a female fetus showed a higher blood pressure compared to women with a male fetus at the beginning of pregnancy. In contrast, by the end of the second trimester a shift in the male blood pressure pattern and female blood pressure pattern was observed. This resulted in a higher blood pressure for women with a male fetus compared to women with a female fetus at the end of pregnancy.[21]

Gestational age has been suggested as an indicator of subsets of PE with a different pathophysiology and with different acute and long range outcomes for both mother and baby. We hypothesize that perhaps we might be looking at a biological phenomenon in which the observed sex specific differences reflect a functional placental difference and subsequent response by the mother between the sexes with differential PE phenotypes as a result.

So what underlies the sexual dimorphism in PE? According to the two-stage model, impaired placentation including dysfunctional remodeling of the uteroplacental arteries has been considered as powerful predisposing step in the etiology of PE. This has especially been suggested for the early-onset subtype of PE.[3, 22] The first decidua-associated-remodeling step should be initiated around implantation. Exposures at this stage might influence the risk of PE. Previously, it was hypothesized by Vatten et al. that a sex specific susceptibility to the process of embryonic implantation could partly explain sexual dimorphic differences in PE.[5]

The so-called “cross-over” in the sex ratio of PE was interpreted as an indication for the existence of two separate pathogenetic entities. The first pathogenetic entity would be associated with IUGR. Unfortunately, we did not have information available on the occurrence of IUGR to test this. The other pathogenetic entity proposed was that late onset disease originated from abnormal implantation. Male embryos would be more susceptible to suboptimal implantation or abnormal placental development.[23] This might imply that those pregnancies with a male embryo that are susceptible to develop PE due to impaired placentation may already have miscarried in the first trimester. The male fetuses that survive the period of placentation will thereby represent a relatively healthy group of fetuses leading to a female biased prevalence of PE. Orzsack et al.[24] showed higher first trimester male miscarriage rates.[24] Furthermore, lower first trimester human chorionic gonadotrophin hormone concentrations (hCG) have been described for pregnancies with a male fetus compared to pregnancies with a female fetus.[25] Since progesterone levels are higher in male fetuses and exert an inhibitory effect on hCG production, this may result in a lower hCG production by the male placenta and thereby results in a differential endometrial receptivity.[26] HCG is proposed to promote angiogenesis in the uterine vasculature and to block any immunological action by the mother on foreign invading placental cells.[27] This might also be related to earlier reported observations on a positive correlation between hCG levels, hyperemesis gravidarum and early onset PE and fetal sex. Hyperemesis gravidarum is associated with higher levels of hCG and with an increased risk of early onset PE.[28-30] The presence of a female fetus is associated with hyperemesis.

The second stage of the two-stage model is associated with an exaggerated endothelial activation and a generalized hyperinflammatory state.[3, 31, 32] Episodes of placental hypoxia

or reperfusion result in oxidative stress, subsequent apoptotic and necrotic disruption of syncytial architecture and release of various components from the intervillous space into the maternal circulation that stimulates the production of inflammatory cytokines.[3, 33, 34] Broere-Brown et al.[21] previously showed that the placental release of circulating angiogenic and fibrinolytic factors differs according to fetal sex.[35] They observed higher S-Flt1, PAI-2 and PlGF blood concentrations in case of female as compared to male placentas. In pregnancies complicated by PE, spontaneous preterm birth or IUGR, however, no fetal sex specific differences were observed. From this they concluded that perhaps other mechanisms causing these complications dominated the fetal sex effect.[35] Muralimanoharan et al.[36] also presented evidence of sexual dimorphism in placentas from male fetuses compared to placentas from female fetuses with higher levels of inflammatory, hypoxia and apoptotic molecules in males. This was observed in placental tissue of term preeclamptic pregnancies and consistent with Vatten et al.[5] In addition, they reported that in an obesogenic environment primary trophoblasts derived from placentas of female fetuses have higher sensitivity to inflammatory stress compared with placentas of males. Interestingly, Minghetti et al.[37], when studying preterm births, showed other results with higher umbilical cord blood levels of the oxidative stress biomarker 8-*iso*-$PGF_2\alpha$ in male fetuses compared with female fetuses using a natural twinning model.[37] Isoprostanes are free radical-catalyzed prostaglandin-like products and considered as reliable marker of oxidative stress. In line with this, Yeganegi et al.[38] and Challis et al.[39] also demonstrated greater pro-inflammatory responses with a male fetus versus higher anti-inflammatory responses in pregnancies with a female fetus. They suggested that the male fetus exists in a relatively more "pro-inflammatory environment" than the female fetus. This could account for the increased loss

by miscarriage and spontaneous preterm birth with male fetuses. However, these latter three studies focused on preterm births in non-preeclamptic pregnancies and thereby are not completely pertinent to the distinct and multi-step entity of PE. We hypothesize that differences between pregnancies with male and female fetuses in the first (placental) but also second (systemic maternal) stage predispose to dimorphic differences in PE. Perhaps as previously suggested by Haig[40] PE is a disorder of failed interaction between two genetically different organisms. As PE is associated with long term maternal health and in view of increasing interest in microchimerism (i.e. the long term presence within an individual of a low level of cells derived from a different individual) the observed sexual dimorphic differences in the occurrence of PE might not be pertinent to pregnancy alone but also might have important long-term cardiovascular health implications for the mother.[2, 4]

Strengths and limitations

We performed a large meta-analysis with individual data from 11 studies participating in the CoLab consortium. We did not rely on published data, which limits any potential publication bias. The large number of participants enabled us to assess small effects. We presented results from random-effects models, which allow heterogeneity in the true effect estimates between different populations and take between-study variation into account. By applying the leave-one-out method we were able to determine the influence of any particular cohort on overall results. In agreement with other studies we used the dating of gestational age at delivery as a proxy for the onset of PE, and not the time of first diagnosis. In a small subset of women (n=1,716) however we did have information available on actual gestational age at PE diagnosis. These data were highly correlated with gestational age at birth (r=0.89, p<

0.001). We therefore think it is unlikely that nondifferential misclassification affected our effect estimates greatly.

Finally, we choose to exclude stillbirths since some studies did only include live born infants whereas in other studies the presence of stillbirths could have been under sampled (due to participation bias or loss-to-follow-up bias). Some stillbirths might have occurred before PE has been recognized clinically, or fetal sex may not have been determined in some of the very early stillbirths. Vatten et al.[5] showed an increased risk of perinatal death in preeclamptic pregnancies in case of a male fetus. We had information available on 660 stillbirths. Additional analyses, however, in this subgroup showed no differences in the female/male distribution.

Conclusion

In conclusion we found that there are fetal sex specific differences in the occurrence of PE with a female dominance among preterm, but not term, pregnancies complicated by PE. Our results highlight the importance of fetal sex when studying placenta-mediated-diseases.

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