Circulating pregnancy hormone relaxin as a first trimester biomarker for preeclampsia

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ABSTRACT

Objective: Preeclampsia, a multi-system hypertensive disorder, is associated with perturbations in the maternal cardiovascular system during early pregnancy. The corpus luteal hormone relaxin, a potent vasodilator, may contribute to physiological circulatory changes especially in early gestation when circulating levels are highest. This study investigated whether first trimester circulating relaxin may be a suitable biomarker for the early prediction of preeclampsia.

Methods: Relaxin was initially measured in first-trimester samples of women who developed late-onset pre-eclamptic (LO-PE; delivery $\geq$ 34 weeks; n = 33) and uncomplicated pregnancies (n = 25) in Pittsburgh, USA. Subsequently, to expand the group numbers, relaxin was measured in women who developed LO-PE (n = 95), early-onset preeclamptic (EO-PE; delivery $<$ 34 weeks; n = 57), and uncomplicated pregnancies (n = 469) in Utrecht, the Netherlands.

Results: In the Pittsburgh subjects, low relaxin levels (lowest centile: $<$ p10) showed an adjusted odds ratio (OR) of 5.29 (95%CI 1.10–25.5) for LO-PE. In the Utrecht population, low relaxin levels ($<$ p10) demonstrated adjusted ORs of 1.45 (95%CI 0.54–3.90) and 2.03 (95%CI 1.06–3.88) for EO-PE and LO-PE respectively, the latter increasing to an adjusted OR of 3.18 (95%CI 1.41–7.20) when newborn weight was $<$ 10%. Serum relaxin concentrations slightly improved the detection rate of a previously derived prediction model for LO-PE from 42.5% to 45.1% at a fixed 10% false-positive rate.

Conclusion: Relaxin shows little improvement in the performance of first trimester prediction models, which does not support its clinical implementation as a biomarker. Although this study was only correlational, the results point to a possible pathophysiologic role for low relaxin levels in pregnancies that later develop LO-PE.

1. Introduction

Preeclampsia, a gestation-specific hypertensive syndrome affecting 3–5% of all pregnant women, is a leading cause of maternal and perinatal morbidity and mortality [1]. The exact etiology of preeclampsia remains to be elucidated, although it is widely believed that preeclampsia is a consequence of an inadequate maternal vascular response to placentation during early pregnancy [2–5]. Due to impaired placental function, women with preeclampsia often give birth to a small for gestational age (SGA) infant [6,7]. In most cases ($>$ 75%) preeclampsia symptoms have a late-onset, developing after 34 weeks of gestation (LO-PE) [8]. Early-onset preeclampsia (EO-PE) is associated with more severe placental pathology [9,10], and a higher rate of fetal growth restriction [11,12]. Both LO- and EO-PE confer an increased risk

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Table 1

Study population characteristics in unaffected and preeclampsia pregnancies of the Pittsburgh and Dutch group. Values are presented as median (IQR) or number (%).

Characteristics	Pittsburgh group		Dutch group		
	Controls	LO-PE	Controls	EO-PE	LO-PE[$]
	n = 25	n = 33	n = 469	n = 57	n = 95
Pregnancy characteristics:					
Maternal age (y)	25.5 (20–32)	23.2 (20–31)	33 (30–35)	33 (30–36)	32 (30–36)
Maternal BMI at sampling (kg/m^2)	23.1 (21.3–26.1)	26.5 (22.6–31.0)	22.8 (20.9–24.9)	**24.7** (21.9–28.9)*	**23.6** (21.2–26.3)*
Nulliparity	20 (80.0%)	25 (75.8%)	217 (46.3%)	**46** (80.7%)*	**69** (72.6%)*
Gestation at sampling (d)	52 (48–69)	61 (49–71)	88 (85–91)	**85** (77–89)*	**85** (81–89)*
First trimester systolic blood pressure (mmHg)	120 (112–125)	**148** (143–156)*	110 (100–120)	**120** (110–130)*	**118** (110–122)*
First trimester diastolic blood pressure (mmHg)	71 (68–76)	**90** (83–94)*	65 (60–70)	**70** (70–80)*	**70** (66–78)*
First trimester MAP (mmHg)	80 (77.0–86.3)	**87** (82.7–90.3)*	80 (73.3–86.7)	**88.2** (83.3–96.7)*	**86.7** (81.8–91.7)*
Smoking	7 (28%)	12 (36.4%)	19 (4.1%)	4 (7.0%)	6 (6.3%)
Birth characteristics:					
Gestation at delivery (d)	272 (267–277)	272 (260–279)	283 (277–288)	**222** (213–230)*	**265** (256–275)*
Sex, n female (%)	8 (32%)	15 (45%)	238 (50.7%)	29 (50.9%)	44 (46.3%)
Birthweight (gr)	3164 (2979–3413)	2948 (2638–3590)	3560 (3250–3820)	**1300** (1040–1610)*	**2650** (2155–3065)*
Birthweight centile	55.5 (25.2–76.5)	36.8 (19.0–68.2)	57.9 (33.7–79.1)	**26.4** (11.9–52.8)*	**13.8** (4.1–45.6)*
Birthweight < p3 (%)	0 (0%)	0 (0%)	9 (1.9%)	3 (5.3%)	**20** (21.1%)*
Birthweight < p10 (%)	1 (4.0%)	3 (9.1%)	44 (9.4%)	**12** (21.1%)*	**47** (49.5%)*

Fisher's exact tests and Mann-Whitney U tests, both with *post hoc* Bonferroni correction were used for statistical analysis. BMI: Body Mass Index; MAP: Mean Arterial Pressure; CS: Caesarean Section; < p3: below 3rd percentile; < p10: below 10th percentile; EO-PE: early-onset preeclampsia; LO-PE: late-onset preeclampsia. * p < 0.05. [$] The LO-PE group was selected to have a small for gestational age (SGA) infant in half of the cases.

calculated using multivariate logistic regression analysis. In the Dutch group, the LO-PE group was selected to have a SGA infant in half of the cases. Again, multivariate logistic regression analysis was used to study associations between relaxin and preeclampsia among these subgroups.

Based on the predefined cut-off point of relaxin < p10, detection rates (sensitivity) were calculated for relaxin as a single biomarker, as well as in combination with maternal characteristics (prior risk based on: age, BMI, nulliparity) and MAP.

Statistical analyses were performed using SPSS (release 20.0; Chicago, IL, USA) and SAS software package (release 9.2; SAS Institute, Cary, NC, USA).

3. Results

Demographic and clinical characteristics of the study populations are presented in Table 1. Data from a total of 25 control pregnant and 33 LO-PE women were analyzed in the Pittsburgh group. The control group consisted of 16 (64%) Caucasian and 9 (36%) African-American; the LO-PE group 23 (70%) Caucasian and 10 (30%) African-American. The Dutch group consisted of 469 control pregnant, 57 EO-PE and 95

LO-PE women. Data on ethnicity were not collected in the Dutch group, but the make-up of the Dutch pregnant population is reported to be largely Caucasian (84%) [40]. Women who developed preeclampsia had a significantly higher MAP in both groups. In the Pittsburgh group, other characteristics did not reach statistical significance. In the Dutch group there were several statistically significant differences between the study groups. Preeclamptic women had a higher BMI and were more often nulliparous. Furthermore, preeclamptic women had more often a history of hypertensive pregnancy disorders. There was no significant difference in smoking status between study groups. Preeclamptic women delivered earlier, their infants had a lower birth weight, and were smaller for gestational age.

3.1. Pilot study in 'Pittsburgh group'

In the Pittsburgh group relaxin concentrations did not prove to be significantly different in LO-PE from controls (median with IQR for controls 802 [571–966] pg/mL and LO-PE 783 [452–1081] pg/mL; p = 0.561). Moreover, relaxin concentrations showed no correlation with gestational age in the control group (Fig. 1). However,
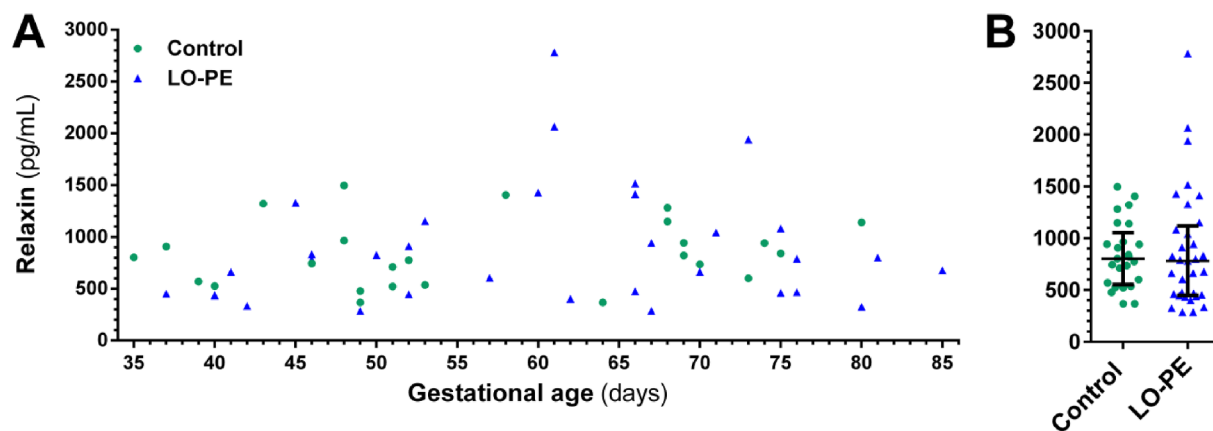
Fig. 1. Distribution of relaxin concentrations in unaffected (green dots) and LO-PE pregnancies (blue triangles) in the Pittsburgh group. (A) Presented as a function of gestational age. (B) Summary plot per outcome group, indicated are median concentrations with interquartile range. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 2
Association of relaxin concentration with EO-PE and LO-PE at different cut-off values (p25, p10 of the control population) in the Pittsburgh and Dutch group.

Pittsburgh group - LO-PE			Unadjusted			Adjusted		
	controls (n = 25)	cases (n = 33)	OR	95% CI	*p*-value	OR	95% CI	*p*-value
n < p25 (< 570.8 pg/mL)	6	14	1.81	0.57–5.77	0.316	2.12	0.58–7.77	0.157
n < p10 (< 495.4 pg/mL)	3	13	4.19	1.03–17.0	**0.045**	5.29	1.10–25.5	**0.038**
Dutch group - EO-PE			Unadjusted			Adjusted		
	controls (n = 469)	cases (n = 57)	OR	95% CI	*p*-value	OR	95% CI	*p*-value
n < p25 (< 1342 pg/mL)	117	14	0.98	0.52–1.89	0.949	0.79	0.36–1.76	0.567
n < p10 (< 1054 pg/mL)	47	8	1.47	0.66–3.28	0.352	1.45	0.54–3.90	0.465
Dutch group - LO-PE			Unadjusted			Adjusted		
	controls (n = 469)	cases (n = 95)	OR	95% CI	*p*-value	OR	95% CI	*p*-value
n < p25 (< 1342 pg/mL)	117	33	1.60	1.00–2.57	**0.05**	1.50	0.90–2.52	0.124
n < p10 (< 1054 pg/mL)	47	18	2.10	1.16–3.81	**0.015**	2.03	1.06–3.88	**0.032**

Odds ratios were adjusted for gestational age at sample collection, Body Mass Index (BMI) and smoking. < p25: below 25th percentile; < p10: below 10th percentile; OR: Odds ratio; CI: confidence interval; EO-PE: early-onset preeclampsia; LO-PE: late-onset preeclampsia.

significantly more women in the LO-PE group had a relaxin concentration below the 10th centile (p10; < 495.4 pg/mL) of control pregnancies, OR 4.19 (1.03–17.0) (Table 2). When the OR was adjusted for gestational age at sampling, BMI and smoking it increased to 5.29 (1.10–25.5). A cut-off value at the p25 of the control group (< 570.8 pg/mL) showed a similar trend, but did not reach statistical significance (OR unadjusted 1.81 and adjusted 2.12, respectively).

3.2. Validation in 'Dutch group'

The same analysis strategy was applied for the independent data of the Dutch group. Relaxin concentrations were not different between EO-PE, LO-PE and controls (median with IQR for controls 1663 [1342–2061] pg/mL, EO-PE 1739 [1398–2230] pg/mL and LO-PE 1604 [1198–2198] pg/mL; p = 0.543 for EO-PE and p = 0.456 for LO-PE). Again there was no change in relaxin concentration with gestational age (Fig. 2). Relaxin concentrations were higher in the Dutch group compared to the Pittsburgh group; the latter was sampled on average 4–5 weeks earlier in pregnancy. As in the Pittsburgh group, a relaxin concentration below p10 (< 1054.4 pg/mL) was associated with LO-PE: OR 2.1 (p = 0.015) and adjusted OR 2.03 (p = 0.032) (Table 2). A cut-off value at p25 (< 1342 pg/mL) resulted in an OR of 1.60 (p = 0.05), which was not significant after adjustment (OR 1.50; p = 0.124). There was no significant association with EO-PE.

In the LO-PE group we performed a sub-analysis to assess the influence of an SGA infant (Table 3). In LO-PE women who delivered an SGA infant (LO-PE with SGA), the correlation with a relaxin concentration below p25 and p10 was stronger, with adjusted ORs of 2.17 (p = 0.034) and 3.18 (p = 0.005) respectively. In LO-PE women without a SGA infant (LO-PE without SGA), the correlations with a relaxin concentration below p25 and p10 were lost (adjusted OR's of 1.06, p = 0.871; and 1.30, p = 0.582 respectively). We did not include
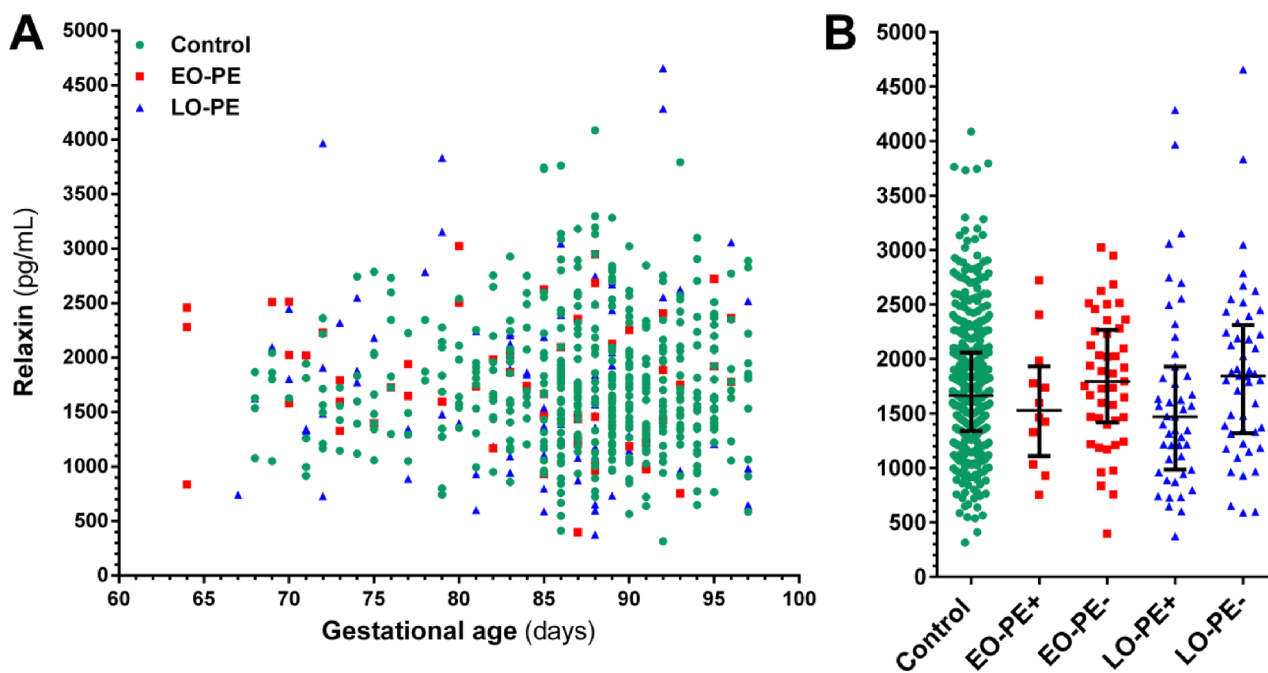
Fig. 2. Distribution of relaxin concentrations in unaffected (green dots), EO-PE (red squares) and LO-PE pregnancies (blue triangles) in the Dutch group. (A) Presented as a function of gestational age. (B) Summary plot per outcome group, (+) with SGA infant (−) without SGA infant. Indicated are median concentrations with interquartile range. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 3
Association of relaxin concentration with LO-PE pregnancies with and without a small for gestational age (SGA) infant at different cut-off values (p25, p10 of the control population) in the Dutch group.

Dutch group - LO-PE - SGA+			Unadjusted			Adjusted		
	controls (n = 469)	cases (n = 47)	OR	95% CI	*p*-value	OR	95% CI	*p*-value
n < p25 (< 1342 pg/mL)	117	20	2.23	1.21–4.12	**0.011**	2.17	1.06–4.45	**0.034**
n < p10 (< 1054 pg/mL)	47	12	3.08	1.50–6.34	**0.002**	3.18	1.41–7.20	**0.005**
Dutch group - LO-PE - SGA-			Unadjusted			Adjusted		
	controls (n = 469)	cases (n = 48)	OR	95% CI	*p*-value	OR	95% CI	*p*-value
n < p25 (< 1342 pg/mL)	117	13	1.12	0.57–2.18	0.745	1.06	0.53–2.13	0.871
n < p10 (< 1054 pg/mL)	47	6	1.28	0.52–3.18	0.591	1.30	0.51–3.31	0.582

Odds ratios were adjusted for gestational age at sample collection, Body Mass Index (BMI) and smoking. < p25: below 25th percentile; < p10: below 10th percentile; OR: Odds ratio; CI: confidence interval; LO-PE: late-onset preeclampsia; SGA+: with small for gestational age infant; SGA-: without small for gestational age infant.

a SGA sub-analysis for EO-PE because of low case numbers (12 out of 57 EO-PE cases delivered a SGA infant), and lack of correlation between low relaxin concentrations (< p10) with EO-PE in the initial analysis.

3.3. Screening performance in 'Dutch group'

In the Dutch group, prediction models for the complete LO-PE group (n = 95) and for the subgroup of LO-PE with SGA (n = 47) were fitted based on maternal characteristics (prior risk), MAP and relaxin < p10 (Table 4). Comparison of the performance of these models showed that the highest detection rate (DR) for the complete LO-PE group was obtained for the model combining maternal characteristics, MAP and relaxin < p10, with a DR of 45.1% at a false positive rate (FPR) of 10% (16.6% gain in DR compared to the model using prior risk only; 2.6% gain in DR compared to the model using prior risk + MAP). For the prediction of LO-PE with SGA the best performing model combined maternal characteristics with relaxin < p10 only, with a DR of 37.1% at a FPR of 10% (2.7% gain in DR compared to the model using prior risk only).

3.4. Correlation of relaxin with other circulating biomarkers

We previously reported pregnancy-associated plasma protein A (PAPP-A), free beta human chorionic gonadotrophin (fβ-hCG), A Disintegrin And Metalloprotease 12 (ADAM-12) and placental growth factor (PlGF) concentrations for the study subjects of the Dutch group [18], enabling us to correlate relaxin concentrations with these other circulating biomarkers. There was a statistically significant positive, but low correlation of relaxin with PAPP-A (Spearman's rho 0.098; p = 0.014) and fβ-hCG (Spearman's rho 0.137; p = 0.001). There was no correlation between relaxin and ADAM-12 (Spearman's rho 0.072; p = 0.074) and between relaxin and PlGF (Spearman's rho 0.065; p = 0.111).

4. Discussion

To the best of our knowledge, this two-stage study, in which patients were well-characterized, is the first to investigate relaxin as a predictor for women destined to develop preeclampsia. We observed a clear association of low (lowest centile: < p10) first trimester relaxin concentrations with LO-PE (adjusted OR 2.0–5.3) but not with EO-PE. However, in a multivariate model, a relaxin concentration below the 10th centile only showed a ~2.5% gain in detection rate of prediction models for LO-PE, which combined maternal characteristics (prior risk based on: age, BMI, nulliparity) with or without MAP.

Preeclampsia in combination with a SGA infant is suggestive of a distinct and more severe (placental) pathophysiology [41,42], which generally results in different performance of biomarkers for this subgroup [35,43]. A low relaxin concentration (< p10) indeed showed a stronger association with LO-PE in combination with SGA, compared to LO-PE without SGA. This, as well as the association between low relaxin concentration with LO-PE but not with EO-PE, underscores the heterogenic nature of the preeclampsia syndrome and is in line with accumulating evidence for differences in the underlying etiology. EO-PE is thought to result from poor placental development (placental preeclampsia) [10], whereas LO-PE is more likely to result from poor maternal systemic cardiovascular adaptations to pregnancy (maternal preeclampsia) [44,45]. However, logic dictates that this pathophysiologic division is likely to be graded and not absolute, with no definite gestational age break point. Nevertheless, the association of low relaxin with LO-PE but not EO-PE may indicate that adequate circulating relaxin levels are more important for the maternal *systemic* vascular adaptations to pregnancy, but less so for processes at the maternal-fetal interface that guarantee the establishment of a functional placenta.

The finding of a stronger association between low circulating relaxin and LO-PE in combination with SGA may at first glance seem paradoxical. However, given the higher occurrence of LO-PE compared to EO-PE [8], SGA is more commonly associated with LO-PE. On a

Table 4
Screening performance of relaxin for LO-PE. Model predicted LO-PE detection rate for a false positive rate of 10% with prior risk, MAP and relaxin < p10 in the complete LO-PE group and in the LO-PE group with a SGA infant.

Dutch group	LO-PE - all (n = 95)			LO-PE - SGA+ (n = 47)		
	DR (%)	AUC	95% CI	DR (%)	AUC	95% CI
Prior risk	28.5	0.722	0.661–0.783	34.4	0.765	0.683–0.847
Prior risk + MAP	42.5	0.798	0.742–0.854	31.0	0.751	0.668–0.834
Prior risk + Relaxin < p10	30.9	0.729	0.668–0.790	37.1	0.791	0.712–0.870
Prior risk + MAP + Relaxin < p10	45.1	0.801	0.745–0.857	31.0	0.760	0.678–0.842

Prior risk of preeclampsia was based on: maternal age, BMI and nulliparity. MAP: mean arterial pressure; < p10: below 10th percentile; DR: detection rate; AUC: area under the curve; CI: confidence interval; LO-PE: late-onset preeclampsia; SGA+: with small for gestational age infant.

pathophysiological basis, low circulating relaxin in early human pregnancy may impair maternal vascular adaptation, thereby compromising uterine perfusion and fetal growth [46]. In support of this hypothesis, relaxin was shown to increase uterine blood flow velocity and to increase uterine arterial compliance in conscious rats [47].

There was a low but significant correlation between relaxin and placenta-derived biomarkers measured in the 469 control samples of the Dutch cohort: Pregnancy-associated plasma protein A (PAPP-A) and free beta human chorionic gonadotrophin (fb-hCG). Adding relaxin to a prediction model of PAPP-A and/or fb-hCG (including MAP and prior risk) did not significantly improve the performance of these prediction models to predict EO-PE or LO-PE (data not shown). No correlation was found between concentrations of relaxin with A Disintegrin And Metalloprotease 12 (ADAM-12) and placental growth factor (PlGF) [35]. Relaxin concentrations showed a slight inverse correlation with BMI and MAP (data not shown).

Overall strengths of the present study are the two well-characterized study populations with similar assays run in blinded fashion, and the consistency in resulting findings of both groups adding to the generalizability. The strengths of the Pittsburgh group are the very well characterized population with a jury of investigators reviewing and confirming the diagnosis of preeclampsia. Limitations of the Pittsburgh group include the relatively small sample size, the selection of participants in a tertiary clinical setting and the lack of EO-PE cases. Furthermore, preeclampsia cases in this group had a relatively high BMI adding to the stronger association with low relaxin concentrations than found in the Dutch group. The Dutch group was much larger, includes both EO-PE and LO-PE cases, and comprises an unselected general population, as samples derived from surplus material of the national Dutch first trimester down syndrome screening program. Consequently this population has a relatively high maternal age (~33 years) compared to the Pittsburgh group which may affect study comparison, although both studies were internally controlled. Moreover, the gestational age at sampling was on average 4–5 weeks later in pregnancy in the Dutch group than the Pittsburgh group. This may partly explain differences in absolute relaxin concentrations between these studies, as circulating relaxin concentrations are known to rise after ovulation until the end of the first trimester [18,19]. Furthermore, other differences in study population characteristics of the Pittsburgh compared to the Dutch group (predominately Caucasian) may also have contributed.

In conclusion, relaxin only provides little improvement in performance of prediction models for LO-PE and no improvement for EO-PE, which does not support clinical implementation. The association between low relaxin concentrations (< p10) with LO-PE, particularly when complicated by a SGA infant may indicate a potential pathophysiologic role for deficient circulating relaxin in one subtype of preeclampsia.

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Disclosure of interest

K.P.C. is an inventor or co-inventor of use patents for relaxin, and has served as a paid or unpaid consultant to Connetics, Corthera and Novartis. D.R.S. was a co-founder and employee of Corthera and previously employed by Novartis with equity interest in each. The other authors report no conflict of interest. Portions of this work were presented at the Society for Gynecologic Investigation Annual Meeting 2009 (*Reprod Sci.* 16(3 Suppl): 101A, 2009).

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