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ARTICLE

First-trimester maternal renin–angiotensin–aldosterone system activation and fetal growth and birthweight: the Rotterdam Periconceptional Cohort

BIOGRAPHY

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KEY MESSAGE

Increased first-trimester prorenin concentrations are associated with decreased late fetal growth and birthweight, and an increased risk of small for gestational age (SGA) infants. In contrast, aldosterone concentrations (relative to renin) are negatively associated with the risk of SGA infants, probably due to the volume-regulating effects of aldosterone.

ABSTRACT

Research question: Does first-trimester maternal renin–angiotensin–aldosterone system (RAAS) activity determine early and late fetal growth and birthweight?

Design: A total of 201 ongoing pregnancies, of which 104 were conceived naturally, seven following single intrauterine insemination (IUI), eight after IUI with ovulation induction and 82 after IVF or intracytoplasmic sperm injection treatment were selected from the Rotterdam Periconceptional Cohort. Renin, prorenin and aldosterone concentrations were determined in blood plasma at 9 and 11 weeks of gestational age. Serial crown–rump length and embryonic volume at 7, 9 and 11 weeks of gestational age were measured using virtual reality software to assess early fetal growth. Estimated fetal weight at 22 and 32 weeks of gestational age and birthweight were measured to assess late fetal growth trajectories. Pregnancy outcomes, small for gestational age (SGA) (<p10) and birthweight were retrieved from medical records.

Results: Prorenin concentrations at 11 weeks of gestation showed significant negative associations with late fetal growth (beta −0.07, 95% CI −0.11 to −0.03, $P < 0.001$) and birthweight ($beta_{z\text{-scores}}$ −0.28, 95% CI −0.55 to −0.01, $P=0.03$), and an increased risk of SGA (<p10) (OR 10.47, 95% CI 2.49 to 53.21, $P=0.002$). The aldosterone/renin ratio associated negatively with the risk of SGA (OR 0.38, 95% CI 0.15 to 0.91, $P=0.03$). In addition, adjusted linear regression models using the total Rotterdam Periconceptional Cohort ($n=1401$) confirmed decreased birthweight percentiles in pregnancies with >1 corpus luteum versus pregnancies with 1 corpus luteum ($beta_{z\text{-scores}}$ −0.17, 95% CI −0.32 to −0.01, $P=0.04$).

Conclusion: This study shows that excessive first-trimester prorenin release, resulting in angiotensin up-regulation, can explain why pregnancies with >1 corpus luteum are at risk for offspring with decreased birthweight and SGA. Simultaneously, high concentrations of aldosterone, independent of renin, seem to have a beneficial influence.

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KEYWORDS

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Renin–angiotensin–aldosterone system
Small for gestational age

INTRODUCTION

Suboptimal maternal conditions in early pregnancy compromise placenta development, thereby resulting in adverse birth outcomes (*Burton et al., 2009*; *Steegers-Theunissen et al., 2013*). An important determinant of these conditions is the hyperdynamic circulatory state of pregnancy, which requires major endocrine, cardiovascular and renal adaptations (*Chapman et al., 1998*). This process is different after IVF treatment, including ovarian stimulation treatment and fresh embryo transfer with supraphysiological hormone concentrations (*Conrad et al., 2019b*). Indeed, such pregnancies display a greater risk for low birthweight (<2500 g) and small for gestational age (SGA) infants (*Conrad et al., 2019b*; *Maheshwari et al., 2012*). The most likely explanations are the altered periconceptional hormonal environment (*Conrad and Baker, 2013*; *Kalra et al., 2011*) affecting the receptivity of the endometrium (*Macklon and Brosens, 2014*), the underlying cause of subfertility, and different laboratory cell culture techniques.

During the first trimester of pregnancy the corpus luteum is the major source of reproductive hormones (*Conrad and Baker, 2013*; *Conrad et al., 2019a*). While normally the follicle is transformed into a single corpus luteum, pregnancies conceived after ovarian stimulation treatment either show a suppression of the pituitary–ovarian axis and absence of the corpus luteum, or superovulation resulting in the presence of >1 corpus luteum. In conceptions without a corpus luteum, the systemic and renal adaptations in the first weeks of pregnancy are attenuated (*Conrad et al., 2019b*; *von Versen-Hoynck et al., 2019*). Conversely, a higher number of retrieved oocytes is negatively associated with birthweight (*Baker et al., 2015*). These observations suggest that the role of corpus luteum-related factors in the maternal haemodynamic adaptation in early gestation is critical.

In addition to steroids, the corpus luteum releases prorenin (*Conrad et al., 2019a*; *Derkx et al., 1987*; *Sealey et al., 1986*; *Wiegel et al., 2020a*). Prorenin is the inactive precursor of renin, and contributes to activation of the renin–angiotensin–aldosterone system (RAAS) present in early pregnancy. This involves an increase in renin from the kidneys that is related to the fall in systemic vascular tone. A concurrent increase in circulating angiotensinogen occurs, due to the stimulation of hepatic angiotensinogen synthesis by oestrogen (*Immonen et al., 1983*). Together, renin and angiotensinogen generate angiotensin (Ang) I, which is subsequently converted to the active end-product of the cascade, Ang II, by angiotensin-converting enzyme (ACE). Ang II is not only a potent vasoconstrictor, it also stimulates the release of aldosterone from the adrenals. Aldosterone induces water and salt retention, and thus is a major determinant of the 30–40% increase in plasma volume that occurs during pregnancy (*Chapman et al., 1998*). Interestingly, inadequate volume expansion is associated with low birthweight, potentially because it diminishes utero-placental blood flow (*Gibson, 1973*; *Rosso et al., 1993*; *Salas et al., 2006*; *Steegers et al., 2010*). Simultaneously, pregnant women are relatively resistant to the pressor response of Ang II, to avoid the rise in blood pressure that normally accompanies RAAS activation. Nevertheless, excessive concentrations of Ang II are still detrimental (*Haase et al., 2020*).

From this background, it was postulated that a high corpus luteum number induces an excessive RAAS activation that compromises haemodynamic adaptation during early pregnancy, thereby subsequently reducing fetal growth and birthweight. Hence, the first aim of this study was to investigate first-trimester maternal concentrations of RAAS determinants in association with both early and late fetal growth trajectories and birthweight. To achieve this goal, three-dimensional ultrasound and virtual reality techniques were used. Secondly, the association between corpus luteum number and birthweight was studied in a large, prospective, periconception cohort study.

MATERIALS AND METHODS

Study population

This study was embedded in the Rotterdam Periconceptional Cohort (Predict Study), an ongoing, prospective, tertiary hospital-based cohort at the outpatient clinic of the Department of Obstetrics and Gynecology of the Erasmus University Medical Center, Rotterdam, the Netherlands. The design of the cohort has been published previously (*Steegers-Theunissen et al., 2016*). The study was designed to identify determinants of periconception health and early reproductive performance to embryonic and fetal growth trajectories and development, the infant's health at birth and the first year of life. A sub-cohort of 241 pregnancies was investigated between January 2017 and March 2019 with women who were enrolled before 10^0 weeks of pregnancy. Women with a minimum age of 18 years, with an ongoing intrauterine singleton pregnancy and who were familiar with the spoken and written Dutch language were eligible for participation. For the current analysis, naturally conceived pregnancies, pregnancies following ovulation induction with intrauterine insemination (IUI) or single IUI, or pregnancies after IVF treatment with or without intracytoplasmic sperm injection (ICSI), were selected. Naturally conceived pregnancies were dated based on the first day of the last menstrual period with a regular cycle between >25 and <35 days. The insemination date was used to calculate gestational age in pregnancies conceived through IUI. In IVF/ICSI pregnancies, gestational age was calculated from oocyte retrieval day plus 14 days or, for cryopreserved embryo transfer, from the transfer day plus 19 days. Gestational age was estimated using crown–rump length (CRL) in pregnancies with irregular cycles, when the last menstrual period was unknown, or when gestational age based on last menstrual period differed by more than 6 days from the calculated gestational age based on CRL.

The Predict Study was conducted in accordance with the ethical principles for medical research set out in the Declaration of Helsinki and was approved by the Medical Ethics Committee of the Erasmus University Medical Center (15 October 2009; MEC-2004-227). Written informed consent was obtained from all study participants at enrolment.

Renin, prorenin and aldosterone measurement

Non-fasting venous blood samples were taken twice between 7^0 and 11^6 weeks of gestation at the Erasmus Medical Center. Blood was collected in 10 ml Vacutainer ethylenediaminetetraacetic acid (EDTA) tubes and centrifuged (2000*g* for 10 min), and plasma was stored at –80°C

in macro-tubes until analysis. Renin and prorenin concentrations were measured by an immunoradiometric assay (Cisbio, Saclay, France) using an active site-directed radiolabelled antibody that only recognizes renin (sensitivity 1 pg/ml, interassay variability 4%). Prorenin concentrations were calculated by subtracting renin from total renin measured after activating prorenin with aliskiren (*Batenburg et al., 2008*). Aldosterone concentrations were measured by solid-phase radioimmunoassay (Demeditec Diagnostics, Kiel, Germany; sensitivity 12 pg/ml, interassay variability 5%).

Embryonic and fetal growth and birthweight

Embryonic growth (representing early fetal growth) was estimated by three-dimensional (3D) ultrasound data obtained during serial transvaginal ultrasound scans, at 7, 9 and 11 weeks of gestational age, performed by experienced sonographers with a 6–12 MHz transvaginal transducer using a Voluson Expert E8 or E10 system and 4D View software (General Electric Medical Systems, Zipf, Australia). As previously described by *Rousian et al. (2010)*, to visualize the 3D obtained data sets, the images were transferred to the Barco I-Space (a Cave Automatic Virtual Environment-like virtual reality system), at the Department of Bioinformatics, Erasmus University Medical Center. The use of 3D ultrasound in combination with an interactive virtual reality hologram enabled us to create depth perception and the assessment of volumetric measurements. Offline CRL measurements using the I-Space and V-scope software were performed three times by the same researcher and the mean of these three measurements was used for analysis. The embryonic volume measurements were performed once, by using a semi-automatic segmentation algorithm to calculate the volumes, based on grey-scale differences, previously validated by *Rousian et al. (2010)*. The reliability and accuracy of this technique have been described before (*Verwoerd-Dikkeboom et al., 2008*).

Fetal weight measurements (representing late fetal growth) were performed by the same experienced and trained sonographers using transabdominal ultrasonography with an abdominal rm6c transducer in the second trimester (median 22.4 weeks, 90% range 22.0–23.3) and third trimester (median 32.4 weeks, 90% range 31.9–33.0). Estimated fetal weight (EFW) was calculated using the Hadlock formula: $\log_{10}$ (EFW) = 1.3596– (0.00386 × AC × FL) + (0.0064 × HC) + (0.00061 × BPD × AC) + (0.0424 × AC) + (0.174 × FL), where BPD = biparietal diameter, HC = head circumference, AC = abdominal circumference and FL = femur length in centimetres (*Hadlock et al., 1985*).

Birth outcomes

Information on birthweight, date of birth and fetal sex at birth was obtained from questionnaires that were filled out post-partum, and were verified with medical records. SGA was defined as a standardized birthweight (adjusted for fetal sex and gestational age at birth) lower than the 10th percentile of the study population (*Zeve et al., 2016*) and large for gestational age (LGA) was defined as standardized birthweight above the 90th percentile. Fetal growth restriction (FGR) was defined as fetal abdominal circumference and/or EFW below the 10th percentile according to Hadlock curves, or a more than 20th percentile decrease on the growth curve, compared with previous measurements with an interval of at least 2 weeks (*Gordijn et al., 2016*; *Verfaille et al., 2017*). Preterm birth (PTB) was defined as gestational age at delivery <37+0 weeks. Pregnancy-induced hypertension (PIH) was defined as a systolic blood pressure above 140 mmHg or a diastolic blood pressure above 90 mmHg after 20 weeks of gestation without signs of hypertension prior to pregnancy or presence of proteinuria, and pre-eclampsia was defined as hypertension after 20 weeks of gestation and presence of more than 300 mg proteinuria in a 24 h period (*ACOG Practice Bulletin No. 202, 2019*).

Classification of the corpus luteum groups

Given the fact that the corpus luteum is the major source of prorenin in pregnancy (*Derkx et al., 1987*; *Sealey et al., 1986*; *Wiegel et al., 2020a*), the population was grouped according to corpus luteum number at conception: 0 corpus luteum (programmed cycle frozen embryo transfer [FET]), 1 corpus luteum (natural conceptions, conceptions following IUI without hormonal treatment or FET in a natural ovulatory cycle) and >1 corpus luteum (ovarian stimulation and fresh embryo transfer). The corpus luteum status at conception in pregnancies conceived by fresh embryo transfer is based on number of retrieved eggs during ovum retrieval (median 11.0; interquartile range 7–16).

Covariates

Participants completed a self-administered questionnaire regarding baseline characteristics and medical and obstetric history. At study entry, data were verified and anthropometrics were measured by a research nurse. Smoking and alcohol use were defined as any consumption during the periconception period (from 14 weeks prior to 10 weeks after conception). Information regarding subfertility diagnosis, ovarian stimulation treatment and IVF protocol were retrieved from electronic patient files. Pregnancies conceived by ovarian stimulation treatment and fresh embryo transfer were stimulated with either Menopur or rFSH (Bemfola, Gonal-F or Rekovelle). The assisted reproduction protocols have been described previously (*Wiegel et al., 2020a*).

Birthweight in the prospective cohort: the Rotterdam Periconceptional Cohort (Predict Study)

To investigate whether the pregnancies in this cohort conceived in the presence of multiple corpus luteum have a lower birthweight and are at increased risk of SGA, birth outcomes were analysed according to corpus luteum number in participants included between November 2010 and December 2018. Post-partum completed questionnaires were checked with medical records. Subsequently, an analysis was carried out of whether the number of follicles, number of retrieved oocytes and total follicle diameter (specified on the day of ovulation triggering, and representing the sum of the diameters of all follicles) determined birthweight.

Statistical analysis

Baseline characteristics of study participants, stratified for corpus luteum number, are presented as mean and SD for normally distributed data and as median and interquartile ranges (IQR) for non-parametrically divided data. Categorical data are presented as number of individuals with percentage. To test for difference among the corpus luteum groups, Student's *t*-test, Kruskal–Wallis test or the chi-squared test, respectively, were used. Spearman's rank correlation coefficients (*R*) were calculated for the entire group of

participants to assess the correlation of the renin, prorenin and aldosterone concentrations at 9 and 11 weeks of gestational age. RAAS determinants were log-transformed to obtain a normal distribution before analysis. Square root transformation of CRL data, third root transformation of embryonic volume data and log transformation of EFW were applied for the same reason.

The associations between RAAS component concentrations and embryonic growth (CRL and embryonic volume) in a subgroup of pregnancies were investigated, excluding pregnancies that were dated based on CRL, to minimize confounding by gestational age. Linear mixed models were used to determine the association between maternal RAAS component concentrations and embryonic growth trajectories, assessed by longitudinal CRL and embryonic volume measurements between 7 and 12 weeks of gestational age. The association with late fetal growth throughout gestation was also investigated by using linear mixed models. This was done to analyse unbalanced repeated measurements, for which the outcome consisted of standardized EFW in the second and third trimester and standardized birthweight. These models account for the correlation between the repeated measurements within the same pregnancy that would make ordinary linear regression invalid. Multiple linear regression was used to analyse the associations with birthweight within the total study population. Given the differences in RAAS component concentrations among the different corpus luteum groups, the analyses were stratified by corpus luteum number during conception (0 corpus luteum, 1 corpus luteum and >1 corpus luteum). Multiple logistic regression models were used to study the association of RAAS component concentrations with the risk of SGA. The first model adjusted for gestational age only; in the second model, additional adjustment was made for the maternal covariates age, body mass index (BMI), smoking, parity, mode of conception, fetal sex and the presence of polycystic ovary syndrome (PCOS). These confounders were selected based on the characteristics of the study population, previous data and literature (*Danser et al., 1998*; *Pringle et al., 2015*; *van Uitert et al., 2013*; *Wiegel et al., 2020a*).

Finally, given the fact that renin and prorenin are highly correlated, it was decided to use the prorenin concentrations in the current models to evaluate associations mediated via the Ang II pathway. Here, the consideration was that prorenin concentrations are higher than renin concentrations and so are less likely to be affected by the modest changes that might occur during storage, i.e. the so-called cold-induced prorenin 'activation'. This results in a small percentage of prorenin being recognized as renin (*Schalekamp et al., 2008*). Given that prorenin concentrations are >10-fold higher than renin concentrations, such activation would affect renin more strongly than prorenin. It should be stressed here that when performing the analysis with total renin (i.e. renin + prorenin), results were the same (data not shown). Secondly, to evaluate associations mediated via aldosterone, independently of the Ang II pathway, the aldosterone/renin ratio was used.

Birthweight and SGA were analysed by corpus luteum number and compared by the Kruskal–Wallis test for non-normally distributed variables and by the chi-squared test for categorical variables. To adjust for fetal sex and gestational age at delivery, birthweight was expressed in percentiles based on Dutch reference curves for birthweight (*Hoftiezer et al., 2016*). Multivariate linear regression was used with standardized birthweight percentiles (*z*-scores) as outcome variable, and corpus luteum number and fertility parameters as predictor. The model was adjusted for maternal age, BMI, parity, smoking, alcohol consumption, folic acid use and geographic origin. All analyses were performed in R (R for Windows, version 3.5; R Core Team). *P*-values <0.05 were considered statistically significant.

RESULTS

RAAS and birthweight

This sub-cohort of the Predict Study included 241 pregnancies. Forty pregnancies were excluded due to miscarriage (n = 22), oocyte donation (n = 4), withdrawal (n = 1), missing blood samples (n = 5) or fetuses/neonates with congenital malformations (n = 8). Second- and third-trimester ultrasounds were available for 190 of the 201 remaining pregnancies.

TABLE 1 shows the maternal and fetal characteristics of the sub-cohort. Mean maternal age was 32.2 ± 4.5 years, 79.2% of the population was of Dutch geographic origin, and 82 women conceived through IVF/ICSI treatment (51 by ovarian stimulation followed by fresh embryo transfer, in the presence of multiple corpus luteum, and 23 and eight by FET in a natural cycle [1 corpus luteum] or programmed cycle [0 corpus luteum], respectively). Of the other 119 pregnancies, seven occurred after single IUI, eight occurred after IUI with ovulation induction and the rest were naturally conceived. At study entry, all pregnant women were normotensive, and 82.1% used folic acid periconceptionally. Parity was evenly divided, with 115 (57.2%) women being nulliparous. In the total study population of 201 pregnancies, 50 (24.9%) women developed placenta-related complications including maternal complications (4.5% PIH and 3.5% pre-eclampsia) and had adverse birth outcomes (7.0% FGR, 9.5% SGA and 10.4% PTB). From the total live births, 48.3% of the newborns were female. Differences in maternal characteristics between groups according to corpus luteum number have been published previously (*Wiegel et al., 2020a*), and are shown in TABLE 1. Pregnancies in the 1 corpus luteum group were less often nulliparous and strictly dated and PCOS was less common compared with the 0 corpus luteum and >1 corpus luteum groups.

RAAS determinant measurements

In this study, 111 and 192 blood samples were available at 9 and 11 weeks of gestation, respectively. The maternal plasma concentrations of renin, prorenin and aldosterone at 9 and 11 weeks of gestational age have been reported previously (*Wiegel et al., 2020a*) and are summarized in FIGURE 1 and TABLE 1. RAAS concentrations were significantly different among the corpus luteum groups, with higher corpus luteum numbers showing increased renin, prorenin and aldosterone concentrations. All RAAS component concentrations at 9 weeks correlated significantly with their concomitant concentrations measured at 11 weeks of gestational age (FIGURE 1). Because both measurements were highly correlated and did not differ significantly between week 9 and week 11, and because of the larger number of available measurements, it was decided to perform the analyses with the RAAS determinants measured at 11 weeks.

TABLE 1 DESCRIPTIVE CHARACTERISTICS OF THE SUB-COHORT OF THE ROTTERDAM PERICONCEPTIONAL COHORT

Characteristic	Total study population (*n* = 201)	0 CL (*n* = 8)	1 CL (*n* = 142)	>1 CL (*n* = 51)	*P*-value
Maternal age, years	32.2 ± 4.5	30.5 ± 3.9	32.0 ± 4.4	33.0 ± 4.7	0.25
Nulliparous	115 (57.2)	6 (75.0)	67 (47.2)	42 (82.4)	<0.001
Geographic origin					0.24
Dutch	156 (79.2)	6 (75.0)	104 (75.4)	46 (90.2)	
Western other	6 (3.0)	0 (0.0)	5 (3.6)	1 (2.0)	
Non-Western	35 (17.8)	2 (25.0)	29 (21.0)	4 (7.8)	
IVF/ICSI	82 (40.8)				
Frozen ET, programmed cycle	8 (9.8)				
Frozen ET, natural cycle	23 (28.0)				
Fresh ET	51 (62.2)				
Strictly dated pregnancies	151 (75.1)	8 (100)	92 (64.8)	51 (100.0)	<0.001
BMI, at study entry (kg/m^2)	24.9 [22.2, 28.5]	21.4 [20.9, 22.8]	25.2 [22.6, 29.2]	23.9 [21.8, 27.4]	0.02
MAP, at study entry (mmHg)	82.5 ± 7.9	80.8 ± 12.1	80.7 ± 6.8	79.6 ± 7.1	0.74
Cause of subfertility: PCOS	41 (20.4)	5 (62.5)	21 (14.8)	15 (29.4)	0.001
Lifestyle					
Folic acid supplement use	198 (98.5)	8 (100.0)	139 (97.9)	51 (100.0)	1.00
Preconception initiation	165 (82.1)	8 (100.0)	106 (74.6)	51 (100.0)	0.001
Alcohol consumption	57 (28.4)	5 (62.5)	42 (29.6)	10 (19.6)	0.04
Smoking	27 (13.4)	1 (12.5)	21 (14.8)	5 (9.8)	0.68
Multivitamin supplement use	118 (58.7)	4 (50.0)	83 (58.5)	31 (60.8)	0.16
Blood measurements					
GA 9 weeks blood drawn, days, *n* = 111	65.0 [64.0, 68.0]	67.5 [65.5, 68.3]	65.0 [64.0, 67.5]	66.0 [65.0, 67.0]	0.14
Renin, pg/ml	25 [18, 33]	10 [9, 14]	25 [18, 32]	30 [23, 38]	<0.001
Prorenin, pg/ml	277 [201, 380]	107 [75, 134]	264 [191, 365]	373 [270, 443]	<0.001
Aldosterone, pg/ml	357 [216, 571]	323 [214, 469]	297 [185, 496]	533 [348, 866]	<0.001
GA 11 weeks blood drawn, days, *n* = 192	79.5 [78.0, 82.0]	81.5 [79.5, 82.3]	79.0 [78.0, 82.0]	79.0 [78.0, 82.0]	0.39
Renin, pg/ml	25 [17, 32]	12 [11, 17]	23 [17, 31]	27 [23, 34]	0.001
Prorenin, pg/ml	266 [206, 386]	129 [109, 152]	245 [204, 340]	351 [278, 465]	<0.001
Aldosterone, pg/ml	314 [222, 465]	360 [266, 411]	288 [209, 398]	454 [301, 727]	0.002
Fetal outcomes					
GA at birth, weeks	39.1 [38.0, 40.0]	39^{+0} [38^{+6}, 40^{+0}]	39^{+0} [37^{+6}, 39^{+5}]	39^{+2} [37^{+3}, 40^{+4}]	0.19
Birthweight, g	3330 [2975, 3570]	3490 [3243, 3530]	3305 [2939, 3562]	3335 [3045, 3682]	0.70
Birthweight, percentiles	46.5 [22.0, 71.8]	58.0 [35.3, 66.3]	50.4 [25.1, 70.5]	47.6 [31.6, 75.5]	0.82
Fetal sex, female	97 (48.3)	3 (37.5)	67 (47.2)	27 (52.9)	0.91
Pregnancy complications	50 (24.9)	1 (12.5)	39 (27.5)	10 (19.6)	0.38
PE	7 (3.5)	0 (0.0)	6 (4.2)	1 (2.0)	0.65
PIH	9 (4.5)	0 (0.0)	7 (4.9)	2 (3.9)	0.79
FGR	14 (7.0)	0 (0.0)	11 (7.7)	3 (5.9)	0.67
SGA	19 (9.5)	0 (0.0)	16 (11.3)	3 (5.9)	0.35
PTB	21 (10.4)	1 (12.5)	17 (12.0)	3 (5.9)	0.47
GDM	24 (11.9)	0 (0.0)	15 (10.6)	9 (17.6)	0.28

Table is partially based on previously published data (*Wiegel et al., 2020a*).

Data are presented as mean ± SD, median [IQR], or number of individuals (%).

BMI = body mass index; CL = corpus luteum; ET = embryo transfer; FGR = fetal growth restriction; GA = gestational age; GDM = gestational diabetes mellitus; ICSI = intracytoplasmic sperm injection; MAP = mean arterial pressure; PCOS = polycystic ovary syndrome; PE = pre-eclampsia; PIH = pregnancy-induced hypertension; PTB = preterm birth; SGA (<p10) = small for gestational age.

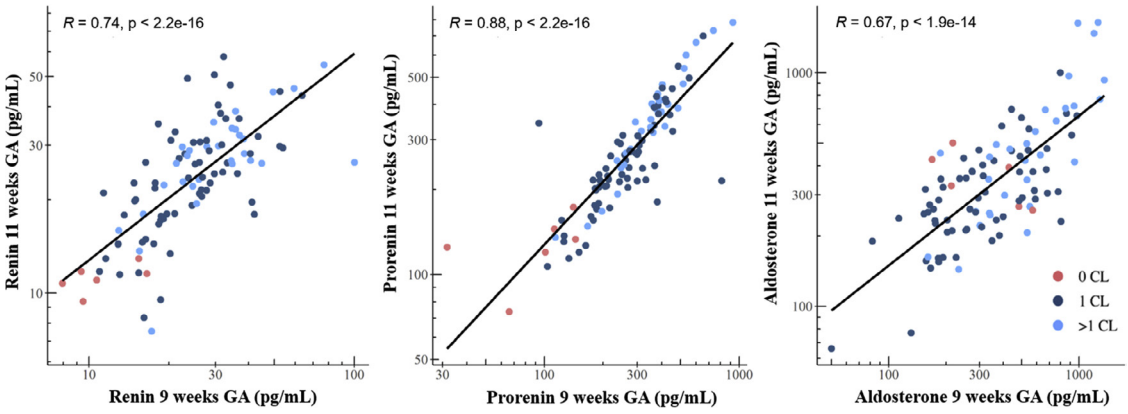

FIGURE 1 Correlations by Spearman (*R*) rank test between renin, prorenin and aldosterone concentrations in plasma samples at 9 and 11 weeks of gestation in women who conceived in the presence of different numbers of corpora lutea, and median and ranges of maternal RAAS determinants. CL = corpus luteum; GA = gestational age; RAAS = renin–angiotensin–aldosterone system.

Maternal RAAS determinants and embryonic growth

Only strictly dated pregnancies (n = 151) were included in the embryonic growth analysis. In total, 315 and 280 ultrasounds of sufficient quality were available for the CRL and embryonic volume measurements, respectively. Neither the negative association between CRL or embryonic volume and prorenin, nor the positive association between CRL or embryonic volume and the aldosterone/renin ratio (TABLE 2) was significant.

Maternal RAAS determinants and fetal growth

In the total study population, maternal prorenin associated negatively with EFW both in model 1 and model 2 (P = 0.009 and P < 0.001, respectively) (TABLE 2). Exclusion of pregnancies complicated by hypertension (pre-eclampsia and PIH) or PTB did not alter this outcome (beta = –0.05, 95% CI –0.10 to –0.007, P < 0.001), nor did subdividing according to corpus luteum number reveal any major differences between the three groups (TABLE 2). The fetal growth trajectories are visualized in FIGURE 2 in the original scale for higher (+2SD) and lower (–2SD) prorenin concentrations, including the absolute and relative differences compared with mean values. Retransformation of the betas to the original values showed that pregnancies with +2SD increase in prorenin concentrations are on average 109 g smaller (decrease of 6.7%) at 32 weeks of gestation compared to those with mean prorenin concentrations.

TABLE 2 ASSOCIATIONS BETWEEN FIRST-TRIMESTER MATERNAL LOG-TRANSFORMED PRORENIN AND ALDOSTERONE/RENIN RATIO CONCENTRATIONS AT 11 WEEKS OF GESTATION AND FIRST-TRIMESTER EMBRYONIC GROWTH AND DEVELOPMENT AND FETAL GROWTH THROUGHOUT THE SECOND AND THIRD TRIMESTERS

	Early fetal growth								Late fetal growth			
	CRL trajectory (√mm)				EV trajectory (^{3}√cm^3)				Fetal growth trajectory (g)			
	Model 1		Model 2		Model 1		Model 2		Model 1		Model 2	
	Beta (95% CI)	*P*-value	Beta (95% CI)	*P*-value	Beta (95% CI)	*P*-value	Beta (95% CI)	*P*-value	Beta (95% CI)	*P*-value	Beta (95% CI)	*P*-value
Log [prorenin (pg/ml)]	–0.001 (–0.10, 0.09)	0.98	–0.06 (–0.19, 0.07)	0.38	0.01 (–0.05, 0.06)	0.86	–0.02 (–0.09, 0.06)	0.65	–0.05 (–0.08, –0.01)	**0.009**	–0.07 (–0.11, –0.03)	**<0.001**
Log [prorenin (pg/ml)], stratified per CL group												
0 CL (n = 8)									–0.20 (–0.47, 0.06)	0.10	–0.19 (–0.67, 0.32)	0.27
1 CL (n = 142)									–0.06 (–0.11, –0.01)	**0.02**	–0.06 (–0.12, –0.01)	**0.02**
>1 CL[a] (n = 51)									–0.05 (–0.14, 0.03)	0.18	–0.06 (–0.15, 0.04)	0.22
Log [aldosterone/renin ratio]	0.02 (–0.05, 0.09)	0.57	0.03 (–0.05, 0.11)	0.48	–0.002 (–0.04, 0.04)	0.94	0.02 (–0.04, 0.08)	0.57	0.01 (–0.02, 0.04)	0.46	0.01 (–0.02, 0.04)	0.49

Table shows effect estimates of linear mixed model for the associations between total prorenin and aldosterone/renin ratio concentrations and embryonic growth (CRL and EV measured using transvaginal ultrasound at 7, 9 and 11 weeks of gestation) and log-transformed fetal growth (fetal weight measured using ultrasound in the second (20–25 weeks) and third trimester (29–35 weeks) of pregnancy and birthweight).

Model 1: adjusted for gestational age. Model 2: adjusted model for gestational age, mode of conception, maternal age, maternal smoking, BMI, parity, fetal sex and PCOS.

BMI = body mass index; CI = confidence interval; CL = corpus luteum; CRL = crown–rump length (√mm); EV = embryonic volume (^{3}√cm^3).

[a] Additionally adjusted for stimulation method and dose.

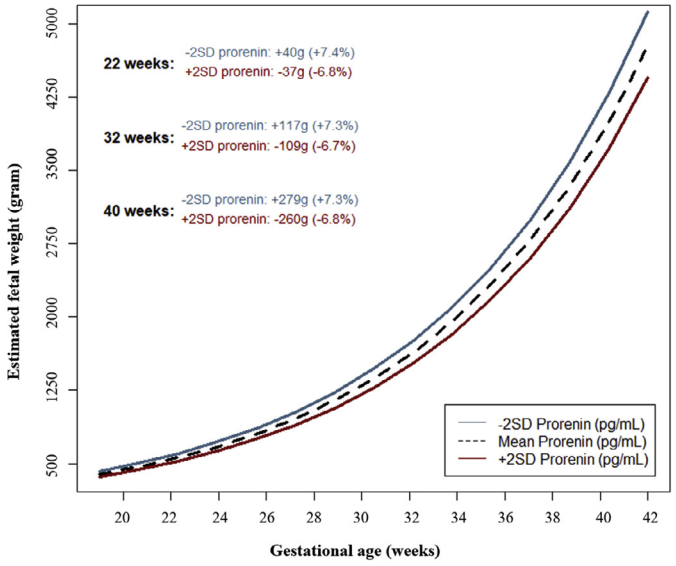

FIGURE 2 Fetal growth trajectories of pregnancies presented with higher (mean +2SD) and lower (mean –2SD) concentrations of prorenin in pg/ml ($P < 0.001$) in the total study population at 11 weeks of gestation. Fetal growth is depicted as fetal weight measured using ultrasound in mid-pregnancy (20–25 weeks), late pregnancy (29–35 weeks) and birthweight.

The aldosterone/renin ratio associated positively with fetal growth trajectories, but this was not statistically significant.

Maternal RAAS components and birthweight

In the total study population maternal prorenin associated negatively with birthweight both in model 1 and model 2 ($P = 0.04$ and $P = 0.03$, respectively), and positively with the risk of SGA ($P = 0.02$ and $P = 0.002$, respectively) (TABLE 3 and FIGURE 3). Additionally adjusting for aldosterone did not alter this outcome (model 2: birthweight $P = 0.04$ and SGA $P = 0.002$), nor did exclusion of pregnancies complicated by hypertension (pre-eclampsia and PIH) and PTB (birthweight: beta = –0.28, 95% CI –0.54 to –0.02, $P = 0.03$ and SGA: OR 6.06, 95% CI 1.30 to 33.8, $P = 0.03$). Subdividing according to corpus luteum number revealed no major differences between the corpus luteum groups (TABLE 3), although *P*-values were often no longer significant due to low values of *n*. The aldosterone/renin ratio was positively associated with birthweight, but this relationship was not statistically significant (beta = 0.08, 95% CI –0.10 to 0.25, $P = 0.19$), and was negatively associated with the risk of SGA (OR 0.38, 95% CI 0.15 to 0.91, $P = 0.03$) (TABLE 3 and FIGURE 3).

Corpus luteum number and birthweight

Of the 1401 singleton live birth pregnancies that were included between November 2010 and December 2018 in the Predict Study, 32 pregnancies were conceived in the absence of a corpus luteum, 1078 with a single corpus luteum, of which 102 were FET in a natural cycle, 869 were natural, 65 after IUI with ovulation induction and 42 after single IUI, and 291 with >1 corpus luteum. Women who conceived after fresh embryo transfer (>1 corpus luteum) were older and more often nulliparous (TABLE 4). Pregnancies conceived with 1 corpus luteum were characterized by a higher BMI (median 24.7, IQR 22.2–28.7), a higher percentage of non-Western origin and smoking cigarettes, and less adequate use of folic acid versus women becoming pregnant after fresh embryo transfer or FET in a programmed cycle. Furthermore, alcohol consumption and the incidence of PCOS were higher in women who conceived in the absence of a corpus luteum. Level of education and fetal sex were not different among the study groups.

The frequency of SGA was higher, although this did not meet the pre-specified threshold for statistical significance, and birthweight percentiles were lower in pregnancies conceived with >1 corpus luteum compared with 1 corpus luteum ($P = 0.07$ and $P = 0.009$, respectively) (TABLE 5). Also, after excluding SGA pregnancies complicated

TABLE 3 ASSOCIATIONS BETWEEN FIRST-TRIMESTER MATERNAL LOG-TRANSFORMED PRORENIN AND ALDOSTERONE/RENIN RATIO CONCENTRATIONS AT 11 WEEKS OF GESTATION AND BIRTHWEIGHT AND THE RISK OF SGA

	Birthweight (z-scores)				Small for gestational age (<p10)			
	Model 1		Model 2		Model 1		Model 2	
	Beta (95% CI)	*P*-value	Beta (95% CI)	*P*-value	OR (95% CI)	*P*-value	OR (95% CI)	*P*-value
Log [prorenin (pg/ml)]	–0.23 (–0.46, –0.003)	**0.04**	–0.28 (–0.55, –0.01)	**0.03**	3.70 (1.23, 12.14)	**0.02**	10.47 (2.49, 53.21)	**0.002**
Log [prorenin (pg/ml)], stratified per CL group								
0 CL (*n* = 8)	–1.02 (–1.90, –0.13)	**0.03**	–0.88 (–1.70, –0.06)	**0.04**				
1 CL (*n* = 142)	–0.19 (–0.50, 0.12)	0.23	–0.21 (–0.55, 0.12)	0.22				
>1 CL[a] (*n* = 51)	–0.47 (–0.98, 0.04)	0.07	–0.65 (–1.23, –0.06)	**0.03**				
Log [aldosterone/renin ratio]	0.09 (–0.07, 0.25)	0.29	0.08 (–0.10, 0.25)	0.19	0.41 (0.18, 0.89)	**0.03**	0.38 (0.15, 0.91)	**0.03**

Table shows effect estimates of linear model analyses for the association between maternal prorenin concentrations, aldosterone/renin ratio and birthweight.

Model 1: adjusted for gestational age. Model 2: adjusted model for gestational age, mode of conception, maternal age, maternal smoking, BMI, parity, fetal sex and PCOS.

BMI = body mass index; CI = confidence interval; CL = corpus luteum; SGA (<p10) = small for gestational age.

[a] Additionally adjusted for stimulation method and dose.

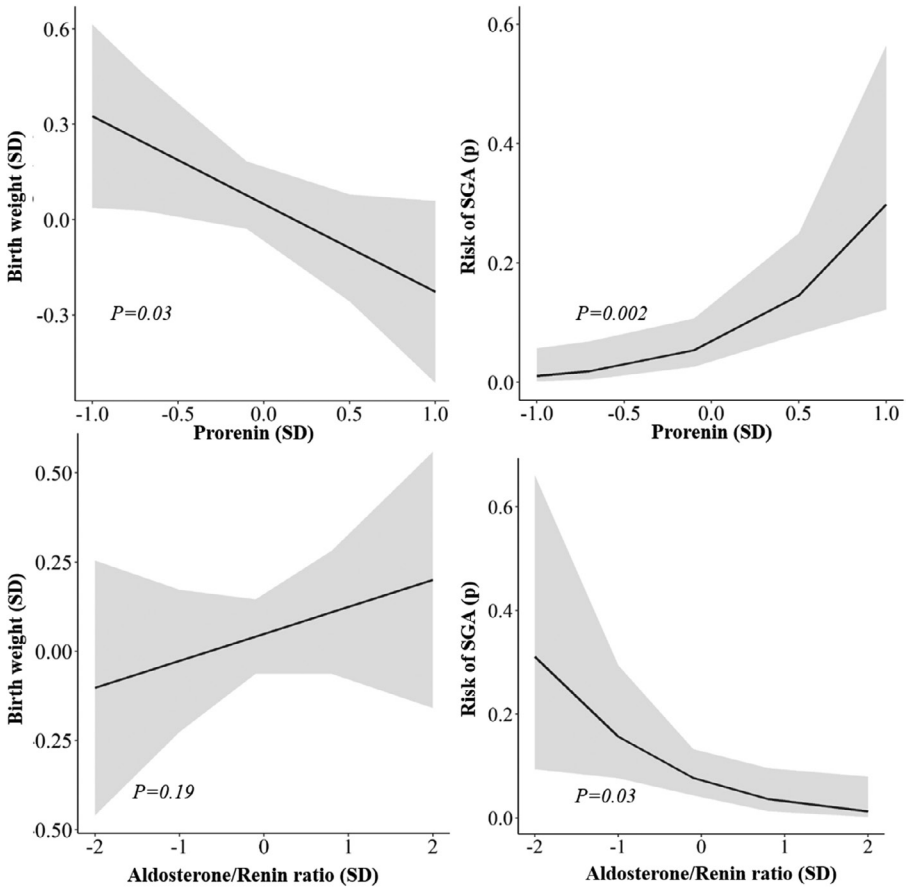

FIGURE 3 Plots show the linear regression model for standardized plasma prorenin and aldosterone/renin ratio at 11 weeks of gestation and birthweight (standardized according to gestational age at birth, *n* = 192), as well as the logistic regression model for prorenin and the risk of SGA (defined as birthweight below 10th percentile when adjusted for gestational age and fetal sex) as predicted mean with 95% CI. Analyses were adjusted for mode of conception, maternal age, smoking, BMI, parity, fetal sex and PCOS. BMI = body mass index; PCOS = polycystic ovary syndrome; SGA (<p10) = small for gestational age.

by pre-eclampsia and/or PTB, the analysis demonstrated a significant difference, with a greater risk of SGA in the >1 corpus luteum group compared with the 1 corpus luteum group (15.5% and 10.7%, respectively, *P* = 0.02). Because the reference group with 1 corpus luteum also contains FET pregnancies which are known to involve larger fetuses, birthweight percentiles were compared between the >1 corpus luteum group with only naturally conceived neonates (*n* = 976). The results showed that neonates from the >1 corpus luteum group still have a relatively lower birthweight compared with naturally conceived pregnancies (birthweight percentiles: 38.0 [18.0–69.0] and 49.0 [23.0–75.0], *P* = 0.004, respectively). The adjusted multivariable linear regression model for birthweight percentiles showed that pregnancies with >1 corpus luteum resulted in offspring with lower birthweight percentiles (beta = –0.17, 95% CI –0.32 to –0.01, *P* = 0.04). In addition, to also minimize confounding by subfertility diagnosis, the group with 1 corpus luteum was restricted to only pregnancies conceived after FET in a natural ovulatory cycle (1 corpus luteum, *n* = 102). Using this method, the >1 corpus luteum group still showed lower birthweight percentiles compared with the 1 corpus luteum group (beta = –0.35, 95% CI –0.62 to –0.08, *P* = 0.011).

The fertility parameters, total follicle diameter (median 164.3, IQR 119.8–212.0), number of follicles (median 8.5, IQR 6–12) and retrieved oocytes (median 7.0, IQR 5.0–10.0) were available in 272 pregnancies conceived after fresh embryo transfer. In the >1 corpus luteum group, after additionally adjusting for stimulation method and dose, total follicle diameter prior to ovum retrieval associated negatively with birthweight percentiles (beta = –0.61, 95% CI –1.01 to –0.20, *P* = 0.004) (TABLE 6). No linear relation was found between the number of follicles or retrieved oocytes and birthweight.

DISCUSSION

This study is the first to show a significant association between elevated first-trimester prorenin concentrations and decreased birthweight, as well as an increased risk of SGA. The opposite was observed for elevated first-trimester aldosterone concentrations (relative to renin) and the risk of SGA. A decrease in fetal growth during the second half of pregnancy seems to underlie the associations with prorenin. In agreement with this finding, given that the corpus luteum is the major source of prorenin in early pregnancy (*Derkx et al., 1987*; *Sealey et al., 1986*; *Wiegel et al., 2020a*), pregnancies with >1 corpus luteum display a decreased birthweight and an increased risk for SGA offspring compared with those conceived with 0 or 1 corpus luteum. Furthermore, a

TABLE 4 BASELINE CHARACTERISTICS OF STUDY PARTICIPANTS INCLUDED IN NOVEMBER 2010 TO DECEMBER 2017 IN THE ROTTERDAM PERICONCEPTIONAL COHORT (*N* = 1401), STRATIFIED BY NUMBER OF CORPUS LUTEUM PERICONCEPTIONALLY

	0 CL (*n* = 32)	1 CL (*n* = 1078)	>1 CL (*n* = 291)	*P*-value
Age, years	32 [29, 34]	32 [29, 35]	33 [30, 36]	<0.001
>40 years	0 (0.0)	26 (2.4)	21 (7.2)	0.001
Missing	0	0	0	
Nulliparous	20 (64.5)	456 (42.7)	211 (73.8)	<0.001
Missing	1	9	5	
BMI, kg/m^2	22.8 [21.2, 24.7]	24.7 [22.2, 28.7]	24.3 [21.6, 28.06]	0.004
>30	3 (10.0)	208 (20.1)	42 (15.4)	0.095
Missing	2	43	18	
Geographic origin: Western	29 (90.6)	816 (75.7)	240 (82.5)	0.01
Missing	0	0	0	
Education				0.39
Low	0 (0.0)	82 (8.5)	23 (8.3)	
Middle	14 (45.2)	331 (34.1)	101 (36.5)	
High	17 (54.8)	557 (57.4)	153 (55.2)	
Missing	1	108	14	
Smoking	1 (3.2)	166 (16.9)	35 (12.4)	0.03
Missing	1	93	9	
Alcohol consumption	12 (38.7)	338 (34.3)	55 (19.5)	<0.001
Missing	1	93	9	
Folic acid supplement use	31 (100.0)	726 (73.6)	273 (97.2)	<0.001
Missing	1	91	10	
Fetal sex, female	16 (51.6)	527 (49.9)	139 (48.9)	0.98
Missing	1	22	7	
PCOS	16 (50.0)	82 (7.6)	48 (16.5)	<0.001
Missing	0	0	0	

Data are presented as *n*, median [IQR], or number of individuals, *n* (%).

BMI = body mass index; CL = corpus luteum; PCOS = polycystic ovary syndrome.

sub-analysis of fresh embryo transfers demonstrated that total follicle diameter prior to ovum retrieval was linearly associated with birthweight.

Prorenin is the inactive precursor of renin. It has no known function, although it occurs at concentrations that are easily 10-fold higher than those of renin. It originates from the kidney but also has extrarenal sources, such as the ovary, testis and adrenal glands (Krop *et al.*, 2008). Prorenin consists of a prosegment and a renin moiety. The prosegment covers renin's active site, so that prorenin cannot display enzymatic activity. Yet, depending on pH and temperature, the prosegment may move out of the enzymatic cleft. Under such conditions, prorenin is capable of reacting with angiotensinogen. At pH 7.4 and 37°C this applies to approx. 1% of the prorenin molecules. The percentage of 'open' prorenin molecules increases further at low pH or temperature, or if prorenin binds to a receptor, although the existence of a 'prorenin receptor' (e.g. at tissue sites, for instance in the utero-placental unit) is controversial. Taken together, especially in >1 corpus luteum pregnancies, the excessive amounts of prorenin are probably contributing to angiotensin generation, possibly even to a larger degree than renin (*Wiegel et al., 2020b*). Here it is important to realize that increased RAAS activity is needed in pregnancy to stimulate the synthesis and release of aldosterone, which is responsible for the 30–40% increase in plasma volume in pregnancy. However, given that Ang II is a potent vasoconstrictor, excessive increases are undesirable, because this will reduce placental blood supply (*Glance et al., 1984*). Importantly, the body has developed a mechanism involving progesterone and prostacyclin (*Gant et al., 1980*) to diminish Ang II sensitivity in pregnant women and to avoid such effects (*Verdonk et al., 2014*). Nevertheless, the current data show that RAAS overactivity, reflected by elevated prorenin concentrations, associates with detrimental consequences. This is not due to aldosterone, because aldosterone itself associated positively with growth independently of the renin–angiotensin axis (TABLE 3 and FIGURE 3). Therefore it is most likely that these consequences concern aldosterone-independent effects of Ang II. Apart from a reduction in placental blood flow, this could also involve local effects of Ang II within the placenta. Indeed, given the high density of Ang II type 1 (AT_1) receptors on trophoblasts (*Anton*

TABLE 5 BIRTH OUTCOMES BY CORPUS LUTEUM GROUPS IN THE ROTTERDAM PERICONCEPTIONAL COHORT, NOVEMBER 2010 TO DECEMBER 2018 (*N* = 1401)

Birth outcomes	0 CL (*n* = 32)	1 CL (*n* = 1078)	>1 CL (*n* = 291)	*P*-value
Gestational age at birth, days	277 [272, 285]	273 [265, 280]	276 [270, 283]	<0.001[a,b]
Missing	1	54	26	
Birthweight, g	3490 [3210, 3795]	3345 [2980, 3704]	3315 [2976, 3638]	0.21
<1500 g	0 (0)	14 (1.3)	2 (0.7)	
<2500 g	2 (6.5)	83 (8.0)	21 (7.7)	
>4000 g	2 (6.5)	103 (9.9)	28 (10.2)	
Missing	1	36	17	
Birthweight, percentiles	57.0 [25.8, 79.3]	49.0 [23.0, 75.8]	38.0 [17.8, 69.0]	0.009[b]
Missing	4	244	39	
z-scores, birthweight percentiles	0.32 [−0.72, 1.06]	0.05 [−0.82, 0.94]	−0.32 [−0.99, 0.72]	0.009[b]
LGA	2 (7.1)	91 (10.9)	20 (7.9)	0.34
SGA	1 (3.6)	114 (13.7)	45 (17.9)	0.07
SGA, non-PE/PTB cases	0 (0.0)	89 (10.7)	39 (15.5)	0.02[2]

Data are presented as median [interquartile range] or *n* (%).

CL = corpus luteum; LGA (>p90) = large for gestational age; PE = pre-eclampsia; PTB = preterm birth; SGA (<p10) = small for gestational age.

[a] Statistical difference between 0 CL and 1 CL.

[b] Statistical difference between 1 CL and >1 CL.

For no statistical significant difference, the *P*-value of the three groups compared are shown.

and Brosnihan, 2008), elevated local concentrations of Ang II might result in inadequate placental development, for instance by inducing oxidative stress (*Mistry et al., 2013*). This concept is supported by the observation that the growth-suppressant influences became particularly apparent during late fetal development (and not during early fetal development), i.e. they emerged at the time that the fetal–maternal circulation has been established. If inadequate, this would have consequences for fetal growth. Furthermore, Ang II disturbs amino acid transport across the placenta, by suppressing Na^+-K^+-ATPase in human placental villi via AT_1 receptor stimulation (*Shibata et al., 2006*). This might diminish fetal growth independently of placental development.

That sufficient plasma volume expansion is required for suitable fetal growth and birthweight is undisputed (*Campbell and MacGillivray, 1972*; *Gibson, 1973*; *Pirani et al., 1973*; *Salas et al., 1993*). Indeed, extensive research has demonstrated the relationship between poor plasma volume expansion in early pregnancy and pregnancy complications (*Duvekot et al., 1995*). The current observation that higher concentrations of aldosterone (relative to renin) reduce the risk of SGA most likely relates to its facilitation of this early volume expansion. In addition, aldosterone has been suggested to induce trophoblast proliferation (*Gennari-Moser et al., 2011*). Hence, the positive association with aldosterone may involve both volume expansion, facilitating placental blood supply, and local growth stimulation

TABLE 6 ASSOCIATIONS BETWEEN CORPUS LUTEUM NUMBER AND FERTILITY PARAMETERS AND BIRTHWEIGHT PERCENTILES, TRANSFORMED TO *Z*-SCORES

	Birthweight percentiles (z-scores)			
	Model 1		Model 2	
	Beta (95% CI)	*P*-value	Beta (95% CI)	*P*-value
Total study group				
Corpus luteum, >1 CL	−0.21 (−0.35, −0.07)	0.004	−0.17 (−0.32, −0.01)	0.04
Fertility parameters in fresh ET group (*n* = 291)[a]				
No. of follicles	0.07 (−0.19, 0.34)	0.58	0.02 (−0.26, 0.29)	0.90
Total follicle diameter	−0.39 (−0.78, −0.003)	0.049	−0.61 (−1.01, −0.20)	0.004
No. of oocytes	−0.004 (−0.24, 0.47)	0.98	−0.003 (−0.24, 0.24)	0.98

The corpus luteum analyses refer to the total study population (0 CL, 1 CL and >1 CL), and fertility parameters refer to pregnancies conceived after fresh ET with multiple corpora lutea. Table shows effect estimates of multivariate regression models for the associations between CL group and birthweight percentiles in the total study population and fertility parameters with birthweight percentiles in the fresh ET group.

Model 1: no adjustments. Model 2: adjusted model for maternal age, BMI, parity, maternal smoking and alcohol use, geographic origin, folic acid use and PCOS.

[a] Additionally adjusted for stimulation method and dose.

BMI = body mass index; CI = confidence interval; CL = corpus luteum; ET = embryo transfer; PCOS = polycystic ovary syndrome.

in the placenta, which result in optimal (vascular) development of the placenta. Normally, Ang II is a major determinant of aldosterone synthesis, and it is therefore not surprising that a previous study observed the highest aldosterone concentrations and the strongest correlation between renin and aldosterone in pregnancies with >1 corpus luteum, where (pro) renin concentrations are highest (*Wiegel et al., 2020a*). This group also displays the highest progesterone concentrations (*Conrad et al., 2019a*). Because progesterone antagonizes the mineralocorticoid receptor (*Persson, 2003*), the balance between beneficial effects of aldosterone and deleterious effects of Ang II may tip to the latter in the >1 corpus luteum group. Moreover, this group also displays the highest circulating concentrations of the corpus luteum-derived compound relaxin (*Conrad et al., 2019a*). Because relaxin may increase sodium excretion in an aldosterone-independent manner (*Alsadek et al., 2005*), it may antagonize volume expansion even further.

The Predict cohort data in the present study are in agreement with previous reports showing that pregnancies conceived with >1 corpus luteum are more disposed to developing SGA and a lower birthweight (*Ginod et al., 2018*; *Nakashima et al., 2013*; *Spijkers et al., 2017*; *van Duijn et al., 2021*; *Vidal et al., 2017*). Obviously, prorenin is just one factor that is increased in such pregnancies, and thus it is still possible that the relationship between prorenin and growth is not causal. Sub-analysis of pregnancies with >1 corpus luteum showed that total follicle diameter prior to ovum retrieval linearly associated with birthweight percentiles. A larger diameter is suggestive for an increased contribution of theca cells, which normally invade the vascular granulosa layer to proliferate to luteinized cells and to form the corpus luteum. Because theca cells are the source of elevated prorenin concentrations in pregnancy, this finding again (albeit indirectly) supports a role for prorenin. Earlier studies were unable to consistently link the number of oocytes after ovarian stimulation to birthweight (*Baker et al., 2015*; *Griesinger et al., 2008*; *Sazonova et al., 2011*; *Shih et al., 2008*), and in the current study also no such relationship was found. This may be due to a variation in definitions and the low number of observations available to establish this correlation, combined with the fact that there was a low percentage of cycles with retrieval of ≥15 oocytes. To put the associations with prorenin into perspective, we also calculated (data not shown) the association with smoking, a well-known determinant of birthweight (*van Uitert et al., 2013*). Its beta value in the current population was –0.12, i.e. about half that of prorenin. This illustrates the influence of ovarian stimulation, and the potential consequences of an increased corpus luteum number.

Strengths of the current study are the original data, size, prospective and longitudinal design and analysis, and its single-centre setting, applying identical, standardized procedures, thereby limiting variability. The availability of birthweight *z*-scores allowed a careful adjustment for gestational age and fetal sex. Yet, given that participants recruited from a tertiary hospital are at increased risk for adverse birth outcomes, the generalizability of the results is limited. This sub-cohort was not powered to detect associations between RAAS component concentrations and pregnancy complications. It is interesting to note here that pre-eclampsia occurs more often in the 0 corpus luteum group, which displays the lowest degree of RAAS activation. In fact, this disorder is characterized by low RAAS activity and volume expansion, and its treatment with mineralocorticoid receptor agonists has already been suggested (*Birukov et al., 2019*). Future studies should now investigate first-trimester RAAS component alterations combined with detailed serial measurements of haemodynamic adaptation, corpus luteum number and volume, and placenta growth and development. Other corpus luteum products (e.g. relaxin) may be evaluated simultaneously, to verify whether RAAS activation truly is the culprit. Given the multiple effects of steroid hormones on the RAAS, the impact of the IVF protocol itself should also be taken into consideration in such studies. Finally, it is likely that excessive early maternal RAAS activation has long-term health consequences for mother and offspring, even far beyond a reduction of birthweight. For instance, it might increase the risk of hypertension, ischaemic heart disease and diabetes mellitus via epigenetic pathways. Investigating this would require an epidemiological approach linking RAAS component concentrations (or their epigenetic consequences) to observations in the offspring in later life.

This study strongly suggests that increased first-trimester prorenin release, potentially resulting in angiotensin up-regulation, is associated with reduced late fetal growth trajectories and birthweight and an increased risk of SGA, whereas aldosterone, when adjusted for renin, is negatively associated with the risk of SGA. Additionally, using the total Rotterdam Periconceptional Cohort, pregnancies with multiple corpus luteum display decreased birthweight and an increased risk for SGA offspring versus pregnancies with 0 or 1 corpus luteum. These data underline the important contribution of the RAAS in early pregnancy adaptation, fetal growth and pregnancy outcomes, while any derangement after ovarian stimulation might predispose to impaired placental development and maternal maladaptation.

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