Maternal Vascular Health in Pregnancy and Postpartum After Assisted Reproduction

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Abstract—Although most pregnancies after assisted reproduction are associated with a favorable outcome for the mother and infant, reports of abnormal vascular adaptation in early pregnancy and emerging maternal and perinatal pathology warrant further investigations. Herein we extended our previous work and further examined whether perturbations of blood pressure and endothelial function during the first trimester in conceptions with nonphysiological corpus luteum (CL) numbers would persist through the third trimester of pregnancy and into the postpartum period. We investigated both maternal and perinatal outcomes. Participants were grouped according to CL number and method of conception: 0 CL (programmed autologous frozen-thawed embryo transfer, N=10–18); 1 CL (spontaneous conception [N=16] and natural cycle frozen-thawed embryo transfer [N=12]); or >3 CL associated with autologous fresh embryo transfer [N=8–12]. Augmentation index was higher during the third trimester in the absence of a CL compared to 1 CL (P=0.03) and in frozen-thawed embryo transfer in a programmed compared to a natural cycle (P=0.02). Moreover, baseline pulse-wave amplitude was higher in >3 CL conceptions at all time points (all P<0.05). The incidence of preeclampsia and preeclampsia with severe features was significantly higher in the absence of a CL compared to the presence of one or >3 CL (P=0.045 and P=0.03). Infants from conceptions with >3 CL had lower birth weights (P=0.02) and a higher rate of low birth weight offspring (P=0.008). Deficient vascular adaptation during early gestation in conceptions with nonphysiological CL numbers might predispose women to adverse pregnancy outcomes, for example, preeclampsia. **(*Hypertension*. 2020;75:549-560. DOI: 10.1161/HYPERTENSIONAHA.119.13779.)** • **Online Data Supplement**

Key Words: blood pressure ■ corpus luteum ■ preeclampsia ■ pregnancy ■ reproduction

With the increasing utilization of assisted reproductive technology, which has resulted in the birth of over 8 million babies worldwide, our understanding of the potential adverse risks is steadily expanding.[1] Many of the reported adverse maternal and perinatal outcomes are associated with placental defects, including small for gestational age infants, preeclampsia, placental abruption, placenta accreta, and previa which likely have their origin in early gestation.[2]

Recent data suggest an increased rate of hypertensive disorders of pregnancy especially in donor egg recipients[3–5] and after frozen-thawed embryo transfer (FET) cycles.[4,6–13] The underlying mechanisms are not yet fully understood, but recent studies suggest a contribution of the treatment protocol to maternal adaptation to pregnancy and preeclampsia risk.[13–16] Pregnancies after spontaneous conceptions and fertility treatments performed in a women's natural cycle start in a physiological endocrine environment as there is one corpus luteum (CL). The CL is the major source of reproductive hormones, for example, estrogen, progesterone, relaxin, and others in early pregnancy.[17–22] Certain protocols used in assisted reproduction affect the CL number and disrupt this maternal hormonal milieu. On the one hand, FET cycles performed in a programmed setting lack a CL due to suppression of the hypothalamic-pituitary axis by estradiol intake. On the other hand, controlled ovarian stimulation in in vitro fertilization (IVF) cycles with fresh embryo transfer leads to the development of multiple CL. These 2 scenarios are associated with extremes of CL hormone concentrations in early pregnancy—absent or very low versus supraphysiologic levels (the latter in many but not all women). While estrogen and progesterone are usually replaced as part of the treatment and are ultimately secreted by the developing placenta, other potentially important CL hormones are not.

In a prospective cohort, we recently showed an increased risk for the development of preeclampsia and preeclampsia with severe features in women who conceived in the absence

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of a CL in programmed FET cycles compared to women with one or multiple CL.[14] In an ancillary study, women with no CL lacked the physiological drop in mean arterial pressure (MAP) in first trimester and also showed impaired vascular function, including reduced endothelial responsiveness and increased arterial stiffness.[15] Angiogenic and nonangiogenic circulating endothelial progenitor cells were significantly reduced, suggesting an impact on endothelial homeostasis and repair capacity.[15] In this study, we present longitudinal follow-on data to clarify whether CL number and method of conception impact various aspects of maternal vascular health throughout pregnancy and postpartum.

Methods

All data that support the findings of this study are available within the article and its online-only Data Supplement.

Patients

Patients with viable, sonographically confirmed, singleton intrauterine pregnancies from autologous oocytes were recruited at 6 to 8 weeks' gestation at the Stanford University School of Medicine, Division of Reproductive Endocrinology and Infertility from June 2015 until March 2018 after providing written informed consent for the collection of demographic and clinical data, as well as participation in physiological assessments and blood draw. The longitudinal cohort study approved by the Institutional Review Board at Stanford University, adhered to the principles of the Declaration of Helsinki and Title 45, US Code of Federal Regulations and follows procedures in accordance with institutional guidelines. Subjects were studied 2× during pregnancy (11 to 14 weeks and 35 to 37 weeks' gestation) and one time postpartum (6 to 12 weeks).

Exclusion criteria were age >50 years, body mass index (BMI) >30 kg/m^2, latex allergy, known vascular disease (ie, chronic hypertension, lupus erythematosus, rheumatoid disease, etc), steroid or heparin intake, donor egg transfer, or gestational carrier pregnancies. Three groups with different CL status at conception were included in this study: (1) 0 CL from FET in a programmed cycle; (2) 1 CL (FET in a natural cycle or from spontaneous conception after infertility); or (3) >3 CL associated with IVF and fresh embryo transfer. An overview about enrollment numbers and study participation at each time point can be found in Figure S1 in the online-only Data Supplement.

Measurement of Blood Pressure and Endothelial Function

Following an overnight fast and avoidance of caffeinated products, alcohol, and pain medications during the preceding 24 hours, the participant's weight and height were measured on testing day (Scale-Tronix scale, White Plains, NY; Seca stadiometer, Columbia, MD).[15] After at least 5 minutes of rest, 4 sets of resting blood pressures (BPs) were taken on the testing arm in a sitting position using the oscillometric method (Connex Vital Signs Monitor, Welch Allyn, Beaverton, OR). The average of the last 3 BP measurements was used as per laboratory protocol. MAP was calculated using the following formula: (MAP=[systolic BP (SBP) + 2×diastolic BP (DBP)]/3). The preconception BPs and BMI were measured before the start of fertility treatment at a clinical visit and obtained from the medical record.

Vascular endothelial function was evaluated noninvasively using the EndoPAT 2000 device (Itamar Medical, Caesarea, Israel) in accordance with the manufacturer's recommendations and in line with the published literature.[15,23–26] Outcome variables included (1) peripheral endothelial function using the reactive hyperemia index (RHI, arbitrary units) to assess endothelial function by reactive hyperemia, (2) augmentation index (AI) normalized to heart rate of 75 (AI75) to reflect arterial stiffness, and (3) baseline pulse-wave amplitude (BPWA) to reflect arterial tone. The delta of outcome variables between time points was calculated as percent change ([time point A value–time point B value])/time point A value×100).

Quantification of Lipid Levels

Plasma fasting levels of total cholesterol, triglycerides, LDL (low-density lipoprotein), HDL (high-density lipoprotein), and non-HDL cholesterol were measured using automated methods in the clinical laboratory at Stanford Medical Center at time of blood draw.

Statistical Analysis

The primary outcome of interest was impact of CL on maternal peripheral endothelial function measured as RHI over time and involved the comparison by CL status regardless of method of conception. Secondary outcomes of interested included change in MAP, AI75, and BPWA. The 1 CL group served as the control and 0 CL and >3 CL as exposure groups. To disregard the impact of embryo treatment in culture and cryopreservation, secondary analyses included the comparisons of conception methods, for example, natural cycle FET (CL=1) versus programmed cycle FET (CL=0) or spontaneous conceptions after a period of infertility versus natural cycle FET (both CL=1). A sample size calculation using published data on differences in RHI (α=0.05, power=0.8, effect size 10%) revealed a goal of 12 to 15 participants to finish all 3 visits per group and an initial enrollment target was set for a total of 50 participants assuming a modest withdrawal/drop-out rate.[25,27] Given a higher than expected withdrawal and drop-out rate over time (60%), we increased enrollment to greater than 80 subjects. Study data were managed using REDCap (Research Electronic Data Capture).[28]

Normality of the data distribution was evaluated by the Shapiro-Wilk test with P<0.05 indicating no normal distribution. Continuous data at each visit are summarized by mean and SD (normality distribution) or median and interquartile range (non-normality distribution). Categorical variables are expressed as frequencies and percentages. Group comparisons were performed using t test to compare means or the Mann-Whitney test to compare ranks. The reference group of the pairwise comparisons was either the 1 CL or the natural cycle FET group. For the major end points, SBP, DBP, MAP, RHI, AI75, and BPWA, linear mixed models were used to model the trajectories of the respective data at preconception (SBP, DBP, MAP only) in first trimester, third trimester, and postpartum. Major end points were analyzed using a restricted maximum likelihood-based repeated measures approach in combination with the Newton-Raphson algorithm. Analyses included the fixed categorical effects of CL group/method of conception, time, and CL group/method of conception-by-time interaction. An unstructured (co)variance structure was used to model the within-subject errors and implicitly handle missing data. Significance tests were based on type III F-test using a 2-sided α=0.05. For all analyses, the statistical software JMP Pro 14 software (SAS Institute Inc, Cary, NC), Prism 7 (GraphPad Software Inc, San Diego, CA), or SAS (SAS Institute Inc, Cary, NC) were used.

Results

Participant Characteristics and Infertility Diagnoses

Participant age, ethnicity, parity, gravidity, and maternal smoking were comparable among the 3 cohorts who conceived with 0, 1, or >3 CL (Tables 1 and 2). There were more Asian women in the 0 CL and more white women in the >3CL group when compared to each other. There was a higher rate of aspirin intake until 10 weeks' gestation in the 0 and >3 CL groups. Diminished ovarian reserve was more frequent in the >3 CL group while male factor infertility was more often an infertility diagnosis in the 0 CL and >3 CL cohorts compared with the 1 CL group. When comparing the demographic data by method of conception, more Asian women received a programmed FET and more white women conceived spontaneously. Aspirin use and male factor infertility were more frequent in both FET protocols compared with spontaneous conceptions. Diminished ovarian reserve and male factor infertility were more often reported as reason for infertility

Table 1. General Characteristics of the Study Population by CL Number

CL Number			
Characteristic	0 CL (N=18)	1 CL (N=28)	>3 CL (N=12)
Participant age, y	35.6±3.9	35.6±3.4	36.3±3.4
Race			
Asian	11 (61.1)	13 (46.4)	4 (33.0)
White	6 (33.3)	13 (46.4)	8 (66.7)
Black	0	0	0
Other	1 (5.6)	2 (7.1)	0
Ethnicity			
Hispanic/Latino	0	2 (7.1)	1 (8.3)
Non-Hispanic/Non-Latino	18 (100)	26 (92.9)	11 (91.7)
Smoking	1 (5.6)	1 (3.7)	0
Gravidity	2 (0–2.0)	1 (0–2.0)	1.5 (0–2.8)
Parity	0 (0–0.3)	0 (0–0)	0 (0–0.8)
Aspirin up to 10 wk	17 (94.4)	11 (39.3)	12 (100.0)
Reason for treatment			
Age	0	0	0
Diminished ovarian reserve	6 (33.3)	4 (14.3)	6 (50.0)
Male factor	11 (61.1)	5 (17.9)	5 (41.7)
PCOS	1 (5.6)	1 (3.6)	1 (8.3)
Ovulatory disorder	1 (5.6)	1 (3.6)	0
Tubal	1 (5.6)	4 (14.3)	2 (16.7)
Uterine	2 (11.1)	4 (14.3)	1 (8.3)
Endometriosis	1 (5.6)	3 (10.7)	1 (8.3)
Recurrent pregnancy loss	2 (11.1)	6 (21.4)	2 (16.7)
Single gene disorder	2 (11.1)	3 (10.7)	0
Same sex partner	0	1 (3.6)	0
Unexplained	1 (5.6)	4 (14.3)	2 (16.6)
Other	0	1 (3.6)	1 (8.3)

Mean value ± SD, median with interquartile range or number (% of total) are shown. CL indicates corpus luteum; and PCOS, polycystic ovary syndrome.

Table 2. General Characteristics of the Study Population by Method of Conception

Characteristics	Spontaneous Conception (N=16)	Natural Cycle FET (N=12)	Programmed Cycle FET (N=18)
Participant age, y	35.7±3.3	34.2±3.4	35.6±3.9
Race			
Asian	6 (37.5)	7 (58.3)	11 (61.1)
White	9 (56.3)	4 (33.3)	6 (33.3)
Black	0	0	0
Other	1 (6.3)	1 (8.3)	1 (5.6)
Ethnicity			
Hispanic/Latino	1 (6.3)	1 (8.3)	0
Non-Hispanic/Non-Latino	15 (93.8)	11 (91.7)	18 (100.0)
Smoking	1 (6.3)	0	1 (5.6)
Gravidity	1 (0–2.0)	1 (0–2.0)	2 (0–2.0)
Parity	0 (0–0)	0 (0–0)	0 (0–0.3)
Aspirin up to 10 wk	3 (18.8)	8 (66.7)	17 (94.4)
Reason for treatment			
Age	0	0	0
Diminished ovarian reserve	3 (18.8)	1 (8.3)	6 (33.3)
Male factor	0	5 (41.6)	11 (61.1)
PCOS	1 (6.3)	0	1 (5.6)
Ovulatory disorder	1 (6.3)	0	1 (5.6)
Tubal	3 (18.8)	1 (8.3)	1 (5.6)
Uterine	2 (12.5)	2 (16.7)	2 (11.1)
Endometriosis	1 (6.3)	2 (16.7)	1 (5.6)
Recurrent pregnancy loss	5 (31.3)	1 (8.3)	2 (11.1)
Single gene disorder	1 (6.3)	2 (16.7)	2 (11.1)
Same sex partner	0	1 (8.3)	0
Unexplained	2 (12.5)	2 (16.7)	1 (5.6)
Other	1 (6.3)	0	0

Mean value ± SD, median with interquartile range or number (% of total) are shown. FET indicates frozen-thawed embryo transfer; and PCOS, polycystic ovary syndrome.

in women who received a programmed FET compared with women with a natural cycle FET.

Body Mass Index and Blood Pressure

Maternal BMI was comparable among the 0 CL, 1 CL, and >3 CL cohorts at first trimester and postpartum. Maternal BMI was 8.3% lower in third trimester of pregnancy in 0 CL conceptions compared to 1 CL (Tables 3 and 4).

BP parameters were analyzed across the cohorts at the 3 different times points. Using type III F-test for the fixed effects in the mixed model, the CL group by time interaction for DBP, SBP, and MAP was not significant, but there was a significant effect of time on DBP (P=0.03; Table S1). Preconception BP levels were not different among CL groups.[15] While we have shown before that women without a CL lacked the normal physiological drop in BP in the first trimester[15] compared to 1 CL and >3 CL conceptions, BP levels in the 0 CL group remained highest throughout pregnancy and postpartum (Figure 1A, Table 3).

There was a significant effect of method of conception and time for SBP (P=0.01), DBP (P=0.045), and MAP (P=0.02) when comparing results of type III F-test (Table S1). While we have reported differences in preconception and first trimester BP before[15] diastolic BP of women with conceptions in a natural cycle FET rose more between first and third trimester of pregnancy (7.5% versus 0.8%; P=0.03), but there was a greater decline of diastolic BP from third trimester to postpartum compared with spontaneous conceptions (−6.3% versus 2.9%; P=0.005; Figure 2A, Table 4).

Endothelial Function

Endothelial function parameters are presented in Figures 1 and 2, Tables 5 and 6, and Table S1.

Table 3. BMI and BP of the Study Population by CL Number

CL Number					
Characteristic	0 CL	1 CL	>3 CL	*P* Value, 1 CL vs 0 CL	*P* Value, 1 CL vs >3 CL
BMI, kg/m^2					
Preconception	22.11±2.74	23.27±2.97	23.88±2.17	0.17	0.52
First trimester	22.48±2.77	23.77±3.30	24.46±3.07	0.17	0.52
Third trimester	26.09±3.13	28.44±3.81	29.22±1.92	0.049	0.54
Postpartum	22.33±2.69	24.81±4.20	26.04±1.72	0.07	0.40
SBP, mm Hg					
Preconception	106.50 (101.50 to 117.30)	110.00 (102.00 to 120.80)	105.00 (102.00 to 114.50)	0.35	0.31
First trimester	108.50 (103.50 to 115.00)	103.50 (99.25 to 110.75)	106.00 (102.25 to 112.00)	0.06	0.23
Third trimester	112.00 (105.50 to 118.00)	110.00 (103.00 to 114.50)	109.50 (103.50 to 109.50)	0.45	0.63
Postpartum	109.60±9.08	107.11±7.25	109.00±6.44	0.69	1.00
DBP, mm Hg					
Preconception	69.22±8.29	70.71±7.68	66.92±10.47	1.00	0.40
First trimester	72.50 (67.00 to 77.00)	68.50 (64.35 to 73.75)	67.00 (65.25 to 73.50)	0.07	0.87
Third trimester	74.69±5.15	71.67±6.23	68.50±7.25	0.14	0.42
Postpartum	71.90±4.86	69.78±6.41	69.43±7.30	1.00	1.00
MAP, mm Hg					
Preconception	80.34 (76.00 to 87.67)	84.34 (78.33 to 90.08)	77.00 (72.33 to 89.50)	0.34	0.14
First trimester	84.67 (79.17 to 90.00)	80.33 (76.33 to 86.08)	79.00 (78.08 to 86.50)	0.06	0.64
Third trimester	87.26±6.07	84.14±6.42	82.53±4.92	0.15	0.49
Postpartum	84.47±6.01	82.22±6.57	82.00±6.58	1.00	1.00
Percent change BMI					
Preconception—first trimester	1.74±4.00	2.09±3.98	2.15±4.90	0.79	0.96
First trimester—third trimester	15.74±5.73	20.68±7.70	16.88±9.35	0.09	0.21
First trimester—postpartum	−1.76±5.14	6.38±7.14	2.69±8.05	0.07	0.20
Third trimester—postpartum	−12.28±2.01	−11.91±4.08	−10.97±4.88	0.81	0.57
Preconception—postpartum	2.93±5.82	7.78±7.14	6.19±6.04	0.07	0.57
Percent change SBP					
Preconception—first trimester	0.18±7.37	−5.61±10.03	0.56±5.55	0.03	0.09
First trimester—third trimester	0 (−10.67 to 3.50)	1.82 (−7.99 to 2.23)	0.44 (−8.01 to 4.90)	0.83	0.45
First trimester—postpartum	0.43±5.37	0.96±4.82	1.07±4.42	0.48	0.96
Third trimester—postpartum	−3.64 (−1.44 to 5.53)	−2.47 (−2.46 to 6.44)	0.05 (−6.34 to 6.09)	0.94	0.39
Preconception—postpartum	2.00 (−5.98 to 2.60)	-4.77 (−1.86 to 13.24)	0.50 (−4.95 to 5.77)	0.13	0.17
Percent change DBP					
Preconception—first trimester	6.14±10.93	−0.97±11.77	4.81±14.57	0.06	0.17
First trimester—third trimester	0 (−11.61 to 0)	1.37 (−8.14 to 4.60)	0 (−9.16 to 6.21)	0.71	0.72
First trimester—postpartum	−1.57±3.79	0.17±7.52	1.16±8.23	0.61	0.74
Third trimester—postpartum	−4.23 (1.42 to 5.29)	−0.71 (2.91 to 8.03)	4.51 (−6.41 to 12.46)	0.53	0.64
Preconception—postpartum	−28.28 (−39.16 to −14.39)	−15.95 (−28.99 to −6.05)	−16.93 (−34.83 to −4.29)	0.06	0.60
Percent change MAP					
Preconception—first trimester	3.39±8.86	−3.28±9.73	2.16±9.84	0.02	0.10
First trimester—third trimester	0 (−10.08 to 1.38)	1.57 (−8.99 to 3.43)	1.07 (−6.43 to 4.63)	0.89	0.77
First trimester—postpartum	−1.09±3.89	0.29±6.00	0.19±6.09	0.53	0.84
Third trimester—postpartum	−2.45±5.04	−1.52±7.17	−1.12±9.34	0.75	0.89
Preconception—postpartum	7.02 (−11.75 to 2.70)	−3.29 (−5.61 to 10.46)	1.32 (−10.01 to 10.50)	0.10	0.64

Mean ± SD or median with interquartile range are reported. Data are statistically significant at P<0.05. BMI indicates body mass index; BP, blood pressure; CL, corpus luteum; DBP, diastolic blood pressure; FET, frozen-thawed embryo transfer; MAP, mean arterial pressure; and SBP, systolic blood pressure.

Table 4. BMI and BP of the Study Population by Method of Conception

Characteristic	Spontaneous Conception (N=16)	Natural Cycle FET (n=12)	Programmed Cycle FET (n=18)	*P* Value, Spontaneous vs Natural FET	*P* Value, Natural FET vs Programmed FET
BMI, kg/m^2					
Preconception	23.26±2.95	23.29±3.13	22.11±2.74	0.98	0.29
First trimester	23.65±3.35	23.94±3.37	22.48±2.77	0.81	0.22
Third trimester	28.21±4.27	28.70±3.44	26.09±3.13	0.76	0.10
Postpartum	24.14±3.97	25.48±4.55	22.33±2.69	0.46	0.08
SBP, mm Hg					
Preconception	116.56±11.05	106.33±8.61	109.72±10.57	0.01	0.06
First trimester	103.50 (99.25 to 111.00)	103.50 (99.25 to 105.00)	108.50 (103.50 to 115. 00)	0.76	0.06
Third trimester	108.00±7.87	110.30±6.73	112.39±8.46	0.50	0.53
Postpartum	109.00 (105.00 to 115.00)	105.00 (99.50 to 109.5)	109.00 (102.00 to 115.25)	0.21	0.20
DBP, mm Hg					
Preconception	71.56±8.49	69.58±6.65	69.22±8.29	0.50	0.88
First trimester	70.00±5.93	68.50±5.00	72.89±6.31	0.51	0.05
Third trimester	70.36±6.44	73.10±6.00	74.69±5.15	0.29	0.52
Postpartum	71.33±5.94	68.22±6.83	71.90±4.86	0.27	0.19
MAP, mm Hg					
Preconception	86.56±7.61	81.83±6.85	82.72±8.43	0.12	0.76
First trimester	80.84 (76.42 to 87.34)	79.17 (76.09 to 82.59)	84.67 (79.17 to 90.00)	0.53	0.06
Third trimester	82.91±6.86	85.50±5.96	87.26±6.07	0.35	0.51
Postpartum	84.00±6.20	80.44±6.79	84.47±6.01	0.24	0.18
Percent change BMI					
Preconception—first trimester	1.59±4.57	2.75±3.10	1.74±4.00	0.45	0.50
First trimester—third trimester	18.37±4.88	23.22±9.57	15.74±5.73	0.12	0.01
First trimester—postpartum	4.33±3.15	8.44±9.43	−1.76±5.14	0.19	0.03
Third trimester—postpartum	−12.44±2.95	−11.37±5.11	−12.28±2.01	0.53	0.58
Preconception—postpartum	4.12±5.30	11.44±7.09	2.93±5.82	0.02	0.006
Percent change SBP					
Preconception—first trimester	−8.64±9.89	−1.57±9.07	−0.18±7.37	0.04	0.60
First trimester—third trimester	1.22±6.81	6.18±7.45	3.95±9.45	0.17	0.52
First trimester—postpartum	−1.73±4.34	−0.18±5.39	−0.43±5.37	0.52	0.80
Third trimester—postpartum	2.03±6.13	−4.50±4.01	−1.41±6.38	0.02	0.25
Preconception—postpartum	−6.90±13.78	−2.24±9.10	0.75±9.86	0.38	0.56
Percent change DBP					
Preconception—first trimester	−1.11±12.67	−0.79±11.00	6.14±10.93	0.94	0.12
First trimester—third trimester	0.80±7.91	7.51±9.45	3.98±8.44	0.03	0.33
First trimester—postpartum	−0.11±6.30	−0.45±8.97	−1.57±3.79	0.86	0.71
Third trimester—postpartum	2.90±7.79	−6.30±6.43	−3.22±4.31	0.005	0.29
Preconception—postpartum	0.52±15.05	2.50±11.53	8.19±10.41	0.74	0.07
Percent change MAP					
Preconception—first trimester	−4.82±9.94	−1.23±9.46	3.39±8.86	0.32	0.19
First trimester—third trimester	0.07±7.40	6.92±8.35	3.95±8.64	0.06	0.25
First trimester—postpartum	0.80±5.36	0.23±6.87	−1.09±3.89	0.69	0.73
Third trimester—postpartum	−2.51±6.96	−5.60±4.90	−2.45±5.04	0.006	0.24
Preconception—postpartum	−3.74±13.06	−2.54±9.24	4.81±9.78	0.82	0.15

Mean ± SD or median with interquartile range are reported. Data are statistically significant at P<0.05. BMI indicates body mass index; BP, blood pressure; CL, corpus luteum; DBP, diastolic blood pressure; FET, frozen-thawed embryo transfer; MAP, mean arterial pressure; and SBP, systolic blood pressure.

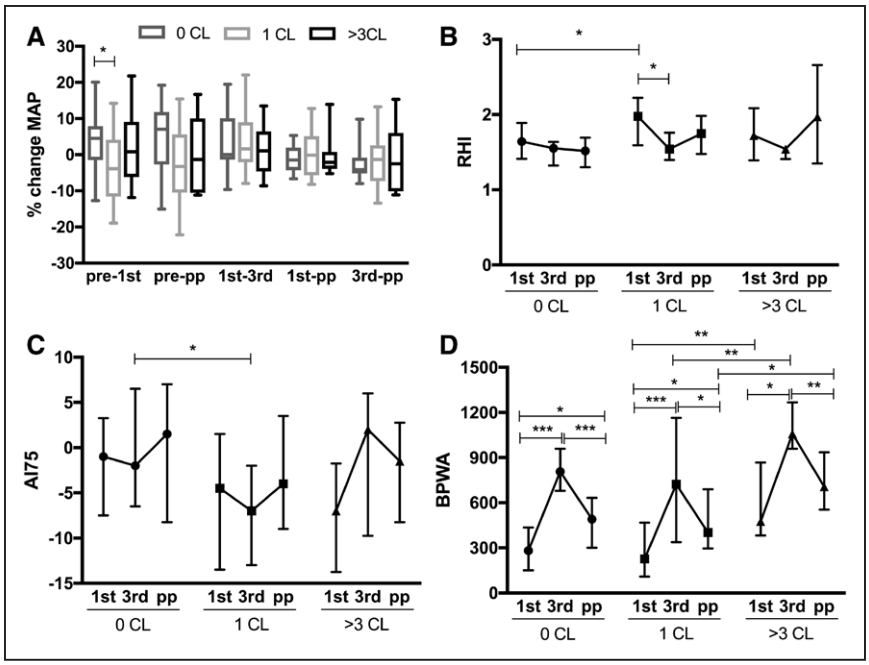

Figure 1. Vascular parameters measured in groups with different numbers of CL (0 CL, 1 CL, or >3 CL). **A**, Percent change in mean arterial pressure (MAP), (**B**) reactive hyperemia index (RHI), (**C**) augmentation index at heart rate 75 (AI75), and (**D**) baseline pulse wave amplitude (BPWA). Within group and between group (1 CL vs. 0 CL or >3 CL) comparison are shown. **A** represents box plots with medians, interquartile ranges and 10th and 90th percentiles; **B**–**D** are medians and interquartile ranges. *P <0.05; **P<0.01; ***P<0.001. 1st indicates first trimester; 3rd, third trimester; CL, corpus luteum; pp, postpartum; and pre, preconception.

Using type III F-test, the group by time interaction for RHI, a measure of endothelial function, was not significant (P=0.09) for CL number comparisons. However, there was a significant effect of time (RHI; P=0.0002; Table S1). As reported before RHI was different during the first trimester[15] and dropped by 22.2% between first and third trimester in 1 CL conceptions (P=0.04). RHI was not different between CL groups in late pregnancy and postpartum. In general, in the 1 CL and >3 CL groups RHI declined during pregnancy (−22.2% and −10.5%) and there was an overall rise in RHI postpartum (13.6% and 27.9%) compared to late pregnancy while RHI has hardly changed in the 0 CL group in late pregnancy (−5.5%) and postpartum (−1.9%; Figure 1B, Table 5).

AI75 was 4.7-fold higher in the third trimester of pregnancy in 0 CL conceptions compared to 1 CL conceptions (P=0.03, Figure 1C) but not different in the mixed model.

There was no significant group by time interaction for BPWA in the mixed model (P=0.91). However, there was an effect of time (P<0.0001) and CL number (P=0.002; Table S1). BPWA rose from first to third trimester and fell after pregnancy within all groups (P<0.05). BPWA in women who conceived with >3CL compared to 1 CL was 209.5% higher in first trimester (P=0.009),[15] 151.2% higher in third trimester (1 CL versus >3CL: P=0.006) and 152.0% higher postpartum (P=0.03; Figure 1D, Table 5).

For the comparisons of conception method, there was a significant effect of time in the mixed model for RHI (P=0.0003) and BPWA (P<0.0001; Table S1). While differences in RHI in first trimester have been shown before,[15] RHI dropped significantly between first and third trimester especially in natural cycle FET conceptions (−22.7%; P=0.006). Postpartum, RHI in women with spontaneous conceptions reached 20.7% higher levels compared to FETs in a programmed cycle (P=0.05; Figure 2B). AI75 was 6.4-fold higher in late pregnancy (P=0.02) in programmed cycle FETs compared to natural cycle FETs (Figure 2C). BPWA rose between first and third trimester and dropped postpartum and was not different

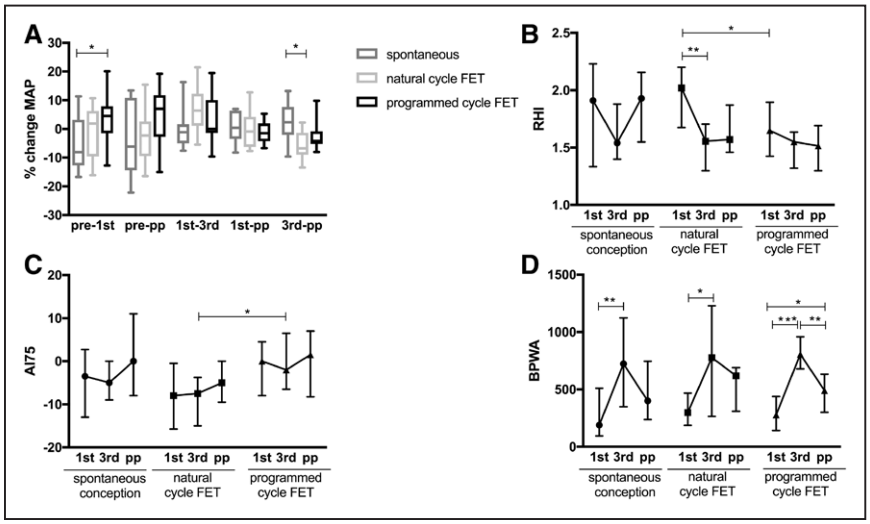

Figure 2. Vascular parameters measured in spontaneous conceptions, frozen-thawed embryo transfer (FET) in a natural or programmed cycle. **A**, Percent change in mean arterial pressure (MAP), (**B**) reactive hyperemia index (RHI), (**C**) augmentation index at heart rate 75 (AI75), and (**D**) baseline pulse wave amplitude (BPWA). Within group and between group comparison (natural cycle FET vs. spontaneous or programmed FET) are shown. **A** represents box plots with medians, interquartile ranges and 10th and 90th percentiles; **B**–**D** are medians and interquartile ranges. *P <0.05; **P<0.01; ***P<0.001. 1st indicates first trimester; 3rd, third trimester; pp, postpartum; and pre, preconception.

Table 5. Vascular Reactivity Characteristics of the Study Population by CL Number

CL Number					
EndoPAT	0 CL	1 CL	>3 CL	*P* Value, 1 CL vs 0 CL	*P* Value, 1 CL vs >3 CL
RHI (AU)					
First trimester	1.64 (1.41 to 1.89)	1.98 (1.59 to 2.22)	1.72 (1.39 to 2.08)	0.04	0.32
Third trimester	1.55 (1.32 to 1.64)	1.54 (1.40 to 1.76)	1.54 (1.13 to 1.59)	0.72	0.74
Postpartum	1.52 (1.30 to 1.70)	1.75 (1.48 to 1.99)	1.97 (1.35 to 2.66)	0.09	0.78
AI75 (AU)					
First trimester	−1.83±9.20	−6.50±9.99	−7.17±8.37	0.11	0.84
Third trimester	1.31±10.02	−6.14±8.74	−1.00±8.71	0.03	0.15
Postpartum	−0.90±10.48	−0.33±11.94	−2.88±9.39	1.00	0.85
BPWA					
First trimester	281.63 (151.24 to 435.86)	226.86 (109.72 to 468.20)	475.29(383.47 to 867.87)	0.71	0.009
Third trimester	824.31±191.03	744.14±430.43	1125.21±275.42	0.51	0.006
Postpartum	484.47±207.89	498.19±305.30	757.40±250.91	0.90	0.03
Percent change RHI					
First trimester—third trimester	8.03±14.22	14.99±23.39	3.58±22.36	0.35	0.16
First trimester—postpartum	11.71 (−19.71 to 19.42)	7.63 (20.47 to 27.44)	−23.73 (−80.96 to 28.17)	1.00	0.21
Third trimester—postpartum	5.36 (−8.67 to 11.40)	−7.26 (−27.64 to −0.53)	−27.24 (−91.64 to 11.52)	0.06	0.76
Percent change AI75					
First trimester—third trimester	114.29 (22.22 to 400)	37.86 (5.26 to 238.89)	77.50 (10.71 to 170.00)	0.32	0.86
First trimester—postpartum	44.44 (1.50 to 233.33)	33.33 (−83.33 to 78.95)	27.5 (−88.46 to 89.23)	0.55	0.93
Third trimester—postpartum	14.29 (−78.33 to 168.75)	86.67 (−1.39 to 216.67)	0 (−100 to 116.67)	0.26	0.11
Percent change BPWA					
First trimester—third trimester	−280.12 (−458.61 to −64.49)	−176.20 (−309.78 to −62.70)	−67.51 (−144.90 to −38.49)	0.57	0.15
First trimester—postpartum	−75.61 (−180.79 to −1.76)	−90.60 (−226.75 to 2.64)	−15.62 (−64.97 to 24.23)	0.76	0.16
Third trimester—postpartum	37.68 (26.74 to 54.99)	44.08 (−7.84 to 51.47)	27.69 (13.52 to 49.43)	0.72	0.80

AI75 indicates augmentation index normalized to heart rate of 75; AU, arbitrary units; BPWA, baseline pulse-wave amplitude; CL, corpus luteum; FET, frozen-thawed embryo transfer; and RHI, reactive hyperemia index.

at all time points between the method of conception groups (Figure 2D, Table 6).

Lipid Levels

As expected, total cholesterol and triglyceride concentrations rose during pregnancy and dropped after delivery in all groups (Table S2). HDL concentrations were lowest in conceptions with >3 CL at all time points. Aside from higher total cholesterol levels in first trimester in programmed FETs compared to FETs in a natural cycle or spontaneous conceptions,[15] lipid concentrations were comparable among conception groups and time points (data not shown).

Maternal and Neonatal Outcomes

Although the study was not powered for adverse maternal and neonatal outcomes, preeclampsia incidence was significantly higher in conceptions in the absence of a CL compared to the presence of 1 or >3 CL. Furthermore, preeclampsia with severe features only occurred in 0 CL conceptions (Table 7). The birth weight of singleton infant was significantly less and the number of infants with low birth weight (<2500 g) was higher in conceptions with >3 CL compared to the groups with 0 CL and 1 CL. There were no differences in the number of very low birth weight infants (<1500 g), incidence of preterm birth, admission rate to neonatal intensive care unit, Apgar at 1 and 5 minutes, or infant sex. When comparing FET, there was a higher rate for preeclampsia in conceptions occurring after a programmed cycle FET compared to an FET in a natural cycle (Table 8).

Discussion

In this longitudinal follow-on study, impaired maternal vascular health predominated in the first trimester in women who conceived in the absence of a CL. This is reflected by an elevated MAP and impaired endothelial responsiveness while in late pregnancy the AI was higher compared to conceptions with 1 CL or >3 CL. BPWA was higher at all time points in conceptions with supraphysiologic CL numbers.

We previously reported the impaired BP adaptation and reduced endothelial function (RHI) during early pregnancy in our 0 CL cohort.[15] As expected, this finding was corroborated

Table 6. Vascular Reactivity Characteristics of the Study Population by Method of Conception.

	Spontaneous Conception (N=16)	Natural Cycle FET (n=12)	Programmed Cycle FET (n=18)	*P* Value, Spontaneous vs Natural FET	*P* Value, Natural FET vs Programmed FET
RHI (AU)					
First trimester	1.91 (1.34 to 2.23)	2.02 (1.68 to 2.20)	1.64 (1.41 to 1.89)	0.73	0.03
Third trimester	1.54 (1.40 to 1.88)	1.56 (1.30 to 1.71)	1.55 (1.32 to 1.64)	0.72	1.00
Postpartum	1.93 (1.55 to 2.16)	1.57 (1.46 to 1.87)	1.52 (1.30 to 1.69)	0.29	0.37
AI75 (AU)					
First trimester	–5.13±10.99	–8.33±8.60	–1.83±9.20	0.39	0.08
Third trimester	4.09±10.36	–8.40±6.29	1.31±10.02	0.29	0.02
Postpartum	3.11±14.40	3.78±8.30	0.90±10.48	0.21	0.58
BPWA					
First trimester	189.62 (95.86 to 510.99)	298.46 (186.82 to 468.20)	281.63 (151.24 to 435.86)	0.74	0.76
Third trimester	736.23±414.88	752.84±469.34	824.31±191.03	0.40	0.44
Postpartum	505.09±371.24	491.28±245.23	484.47±207.89	0.86	0.65
Percent change RHI					
First trimester—third trimester	–12.19±27.46	–18.06±18.93	–8.03±14.22	0.81	0.20
First trimester—postpartum	3.02±29.47	–5.00±33.29	–6.88±22.20	1.00	0.90
Third trimester—postpartum	3.52 (0.71 to 25.69)	8.92 (–3.99 to 44.41)	–5.36 (–11.40 to 8.67)	1.00	0.15
Percent change AI75					
First trimester—third trimester	172.09±375.14	–45.82±232.81	213.80±258.02	0.49	0.22
First trimester—postpartum	65 (–383.33 to 83.33)	25.76 (–58.15 to 370.83)	44.44 (–233.33 to 1.50)	0.31	0.31
Third trimester—postpartum	–567.64±1205.10	102.23±181.01	–62.14±188.67	0.31	0.60
Percent change BPWA					
First trimester—third trimester	145.58 (91.10 to 270.37)	255.19 (7.47 to 430.95)	280.12 (64.49 to 458.61)	0.65	0.69
First trimester—postpartum	92.36±109.19	151.11±183.12	93.44±109.78	0.72	0.65
Third trimester—postpartum	–44.27 (2.17 to 52.26)	–43.89 (21.76 to 59.05)	–37.68 (26.74 to 55.00)	0.72	0.71

AI75 indicates augmentation index normalized to heart rate of 75; AU, arbitrary units; BPWA, baseline pulse-wave amplitude; CL, corpus luteum; FET, frozen-thawed embryo transfer; and RHI, reactive hyperemia index.

herein, but importantly, these deficiencies recovered by the third trimester and postpartum. However, in the third trimester AI75, a measure of arterial stiffness, was higher in 0 CL compared to 1 CL conception as well as for the comparison of FET in a programmed (CL=0) and natural cycle (CL=1). An association between increased arterial stiffness (reduced arterial compliance) and preeclampsia was previously reported[29–31] and might contribute to the observed increased rate of preeclampsia in our cohort.

Interestingly, BPWA, a measure of vascular tone, was significantly higher from early pregnancy to several weeks postpartum in conceptions from fresh IVF. These conceptions start in the presence of multiple CL. Supraphysiologic levels of vasoactive CL hormones, for example, the vasodilator relaxin, might contribute to the enhanced vasodilation that was not associated with adverse maternal outcomes in our cohort although we were not powered to look at adverse outcomes.[15]

In normal human pregnancy maternal systemic vascular resistance decreases and arterial compliance increases reaching a nadir and peak, respectively, by the end of the first or beginning of the second trimester.[32] Other vascular measures, for example, a decrease in AI, correspond with these adaptations.[33] These major physiological adaptations in normal human pregnancy are preceded by a peak of CL product concentrations in the maternal circulation.[17] In first trimester, the CL is a major source of vasoactive hormones such as relaxin, VEGF (vascular endothelial growth factor), and other angiogenic products.[20,34,35] Deficient circulating relaxin is one biologically plausible mediator for any effect of absent CL[36] as relaxin emanates solely from the CL during human pregnancy[18,19] and is a potent vasodilator[37] which mediates circulatory changes and increases arterial compliance in the gravid rat model.[38,39] Other vasoactive products of the CL, such as VEGF, are also not replaced during a programmed cycle FET, and thus, absent CL VEGF could theoretically mediate the impaired circulatory adaptations in women conceiving without a CL. An insufficient vascular adaptation during early pregnancy in 0 CL conceptions was evident from an attenuated decline in central (aortic) pulse-wave velocity and rise in pulse-wave transit time compared to pregnancies occurring in the presence of a CL or multiple CL.[14] Consistent with our findings of impaired BP adaptation and endothelial function,

Table 7. Maternal and Fetal Outcomes by CL Number

CL Number					
Characteristic	0 CL (N=18)	1 CL (N=28)	>3 CL (N=12)	*P* Value, 1 CL vs 0 CL	*P* Value, 1 CL vs >3 CL
Gestational age at delivery, d	270.7±11.9	275.9±10.0	273.8±6.0	0.33	0.65
Infant birth weight, g	3256.8±434.0	3449.5±432.2	3050.6±607.9	0.20	0.02
Low birth weight (<2500 g)	1 (5.9)	0	3 (25.0)	0.17	0.004
Very low birth weight (<1500 g)	0	0	1 (8.4)		0.10
Infant sex male	13 (72.2)	12 (46.2)	7 (58.3)	0.08	0.33
Apgar 1 min	8 (7.5 to 9.0)	8.0 (8.0 to 9.0)	8.0 (7.0 to 8.0)	0.87	0.06
Apgar 5 min	9 (9.0 to 9.0)	9.0 (9.0 to 9.0)	9.0 (9.0 to 9.0)	0.67	0.80
NICU	2 (11.1)	0	0	0.04	0.15
Preterm delivery (<37 wk)	2 (11.1)	1 (3.9)	0	0.34	0.39
Preeclampsia	5 (27.8)	1 (3.9)	2 (16.7)	0.02	0.09
Preeclampsia with severe features	3 (16.8)	0	0	0.02	

Mean value ± SD, median and interquartile range or number (% of total) are shown. CL indicates corpus luteum; and NICU, neonatal intensive care unit.

these vascular deficits in the 0 CL cohort also recovered after the first trimester.[14] In conceptions with multiple CL, for example, in fresh IVF transfers after controlled ovarian hyperstimulation, supraphysiologic concentrations of vasoactive CL products could have contributed to the higher BPWA. One might speculate that the absence of CL products rather than an excess contributes to higher incidence of preeclampsia an preeclampsia with severe features as described herein, and as we previously reported.[14]

Although our study was not powered to detect differences in maternal and neonatal outcomes, some observations deserve further discussion. Specifically, the higher incidence of preeclampsia in conceptions in the absence of a CL compared to the presence of 1 CL or >3 CL or in programmed compared to natural cycle FETs deserves mentioning, particularly given the CL=0 group had lower BMI, which is otherwise associated with a lower preeclampsia risk.[40] An increased risk for the development of preeclampsia is well-known for many years in pregnancies after assisted reproduction compared to spontaneous conceptions of the general population.[4,41,42] More recent data from observational studies report a higher preeclampsia incidence for FET compared to fresh IVF transfers.[4,6–13] A recent population-based study from Sweden[13] and our data from a prospective cohort of 683 deliveries after infertility[14] suggest that absence of CL in programmed FET cycles may be the major contributor to the increased preeclampsia risk reported in FET.

Aspirin is recommended for women at high risk for preeclampsia starting at 12 gestations to reduce the incidence of the disease.[43] The medication is also used during fertility treatment until 10 weeks' gestation to improve implantation and pregnancy rate. Aspirin was discontinued per protocol in our cohort at 10 weeks, which is before the first study visit. Although women with aberrant CL number were more likely to take aspirin, which

Table 8. Maternal and Fetal Outcomes by Method of Conception

Characteristic	Spontaneous Conception (N=16)	Natural Cycle FET (N=12)	Programmed Cycle FET (N=18)	*P* Value, Spontaneous vs Natural FET	*P* Value, Natural FET vs Programmed FET
Gestational age at delivery, d	276.7±11.4	275.0±8.6	270.7±11.9	0.55	0.58
Infant birth weight, g	3436.9±535.5	3464.2±291.8	3256.8±434.0	0.78	0.21
Low birth weight (<2500 g)	0	0	1 (5.9)		0.30
Very low birth weight (<1500 g)	0	0	0		
Infant sex male	6 (42.9)	6 (50.0)	13 (72.2)	0.72	0.22
Apgar 1 min	8.0 (8.0 to 9.0)	8.0 (8.0 to 8.0)	8.0 (7.5 to 9.0)	0.26	0.72
Apgar 5 min	9.0 (9.0 to 9.0)	9.0 (9.0 to 9.0)	9.0 (9.0 to 9.0)	0.48	1.00
NICU	0	0	2 (11.1)	0.95	0.14
Preterm delivery (<37 wk)	1 (6.3)	0	2 (11.1)	0.25	0.14
Preeclampsia	0	1 (8.3)	5 (27.8)	0.19	0.17
Preeclampsia with severe features	0	0	3 (16.7)		0.07

Mean value ± SD, median and interquartile range or number (% of total) are shown. FET indicates frozen-thawed embryo transfer; and NICU, neonatal intensive care unit.

would potential decrease preeclampsia risk, the preeclampsia rate was higher in this group lacking a CL. Therefore, it seems unlikely that short term aspirin use in the first weeks of gestation impacts preeclampsia incidence in the assisted reproductive technology population more than CL status.

In this study, infants from pregnancies with >3 CL after fresh embryo transfer had on average lower birth weights and a higher rate of low birth weight infants (<2.500 g) when compared to conceptions in the absence of a CL or in the presence of 1 CL. Data from our small cohort, therefore, are consistent with the increased risk for low birth weight in fresh IVF transfer as abundantly documented in the literature.[2,44–46] The number of retrieved oocytes appears to be negatively correlated with birth weight.[47] Several recent studies reported increased birth weights and a higher risk for large for gestational age infants after FET compared to fresh embryo transfers.[48–50] Fetal growth might already be impacted early in pregnancy as a study suggests smaller crown-rump-length at 6 and 8 weeks' gestation after fresh IVF transfers compared to FETs.[51] If these growth differences are already present as early as 6 to 8 weeks as it has been shown before it is likely that periconceptional effects can account for the differences in birth weight.[51]

In our cohort, total cholesterol and triglyceride levels rose as expected in all groups from first trimester to late pregnancy and dropped postpartum. The finding of higher total cholesterol concentrations in first trimester in women lacking a CL compared to 1 CL or >3 CL deserves further discussion. During the first weeks of pregnancy when most organs are formed, the fetus depends largely on maternal cholesterol.[52,53] Conditions affecting maternal cholesterol levels may have an adverse effect on the development and growth of the fetus. Data on the effect of CL or method of conception on lipid parameters are lacking. One recent observational study reported different metabolomic profiles between spontaneous and IVF conceptions, including higher levels of acylcholine derivates, phosphatidylinositols, phosphatidylethanolamines, and diacylglyerols in late first trimester.[54] The authors did not discriminate in their results between fresh embryo transfers and FETs nor FET protocol used. Thus, we can only speculate at this point that in the published cohorts the higher birth weights and a higher rate of large for gestational age infants after FET might be attributable to higher nutrient availability in early gestation in programmed FET cycles with absence of a CL.

Maternal nutrient concentrations and placental nutrient production and transport impact fetal weight and infant outcomes. Pregnancy leads to substantial changes in maternal lipid profiles compared to the nonpregnant state. Serum lipid and lipoprotein levels gradually increase from 12 weeks' gestation, especially in the second and third trimester with greatest elevation in triglycerides.[55,56] These changes reflect a physiological adaptation of the mother including hepatic enzyme activities and placental steroid synthesis to satisfy the energy demand of mothers and the fetus. While it is known from several observational studies that elevated lipid levels during late pregnancy are associated with complications and adverse outcome for both mother[57] and newborn,[58] a disturbed lipid profile during early pregnancy has been shown to have similar negative associations. Specifically, an increase in triglycerides was linearly associated with an increased risk of preeclampsia (odds ratio, 1.69; P=0.018)[59] and a higher rate of large for gestational age infants.[60] How fertility treatment and method of conception affect maternal lipid profiles remains largely unknown.

There are several strengths and limitations to our study that deserve mention. This is the first study to report longitudinal RHI data from conceptions after infertility in pregnancy and postpartum. An advantage of the study is the inclusion of women with different CL numbers and methods of conception along with broad information about demographic, clinical, and outcome data as well as the follow-up throughout pregnancy and postpartum. Although every effort was made to achieve a reasonable sample size at all time points, the nature of the study and the study population left us with decreasing participant numbers over time. Due to the small sample size, true effects might have been masked or overvalued and a correction of data for potential confounders was not possible. Also, preconception data were only available for BMI and BP but not endothelial function or lipid concentrations. Therefore, some women might have already been different before conception.

Conclusions

In this study, we found impaired vascular health parameters in conceptions with nonphysiological CL numbers. These were most dramatic in the first trimester which is the time of greatest CL function, however, later in pregnancy, patients developed stiffer arteries. Despite the lack of persistent differences in endothelial function in later pregnancy, preeclampsia incidence was significantly higher in the group with absence of a CL during early pregnancy, while pregnancies with multiple CL had infants with lower birth weights, suggesting that placental function might be altered in both these types of pregnancies.

Perspective

With the increasing utilization of FET and a reported higher risk for the development of preeclampsia, further studies exploring the impact of different FET protocols on pregnancy physiology as well as randomized controlled trials for FET protocols to investigate maternal and offspring outcomes are needed to provide clinical guidance to maximize maternal and child health following IVF conception.

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Disclosures

K.P. Conrad discloses use patents for relaxin. F. von Versen-Höynck, E.S.S. Tierney, V.L. Baker, and V.D. Winn contributed substantially to study design, supervision of study protocol, and interpretation of data; F. von Versen-Höynck was responsible for data collection and analysis. K.P. Conrad and V.L. Baker conceived of the basic concept of the P01 study. The first draft of the article was written by F. von Versen-Höynck. All co-authors revised the article and approved the final article.

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Novelty and Significance

What Is New?

- Differences in blood pressure and impaired endothelial function previously observed in first trimester in 0 corpus luteum (CL) conceptions do not persist to late pregnancy or postpartum.
- Lack of CL at conception is accompanied by higher augmentation index at heart rate 75 in late pregnancy.
- Baseline pulse-wave amplitude is higher in early and late pregnancy as well as postpartum in conceptions with supraphysiologic CL numbers.
- Preeclampsia incidence is higher in 0 CL conceptions while birth weight is lower in conceptions with >3 CL.

What Is Relevant?

- Impaired vascular health in early pregnancy in women with a lack of CL at conception, might contribute to the risk of preeclampsia later in pregnancy.
- Women with frozen-thawed embryo transfers in a natural cycle have favorable vascular parameters compared to women with frozen-thawed embryo transfers in a programmed cycle.

Summary

After confirmation, preference should be given to frozen-thawed embryo transfers in a natural cycle over frozen-thawed embryo transfers in a programmed cycle.