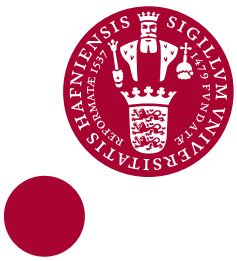

# Early pregnancy reference intervals

## 29 serum analytes from 4 to 12 weeks' gestation in naturally conceived and uncomplicated pregnancies resulting in live births

Friis Petersen, Jesper; Friis-Hansen, Lennart J.; Jensen, Andreas Kryger; Nyboe Andersen, Anders; Løkkegaard, Ellen C.L.

*Published in:*
Clinical Chemistry and Laboratory Medicine

*DOI:*
10.1515/cclm-2019-0495

*Publication date:*
2019

*Document version*
Publisher's PDF, also known as Version of record

Jesper Friis Petersen*, Lennart J. Friis-Hansen, Andreas Kryger Jensen, Anders Nyboe Andersen and Ellen C.L. Løkkegaard

# Early pregnancy reference intervals; 29 serum analytes from 4 to 12 weeks' gestation in naturally conceived and uncomplicated pregnancies resulting in live births

https://doi.org/10.1515/cclm-2019-0495
Received May 14, 2019; accepted June 17, 2019; previously published online July 25, 2019

**Abstract**

**Background:** Pregnancy introduces major physiological changes that also alter biochemical analytes. Maternal and perinatal health can be optimized by early intervention and therefore, pregnancy-specific reference intervals (RIs) for the local population are warranted. While the second and third trimester-specific changes are well described, the first trimester is less well characterized. We therefore wanted to facilitate early detection of abnormalities by generating first trimester reference values for 29 common analytes.
**Methods:** In a prospective early pregnancy (PEP) cohort (2016–2017), 203 pregnant women were recruited from 4 to 8 weeks' gestation. Consecutive blood samples were drawn every 2 weeks until an ongoing second trimester pregnancy (n = 164) or a miscarriage (n = 39) occurred. After exclusion of women with complicated pregnancies or deliveries (n = 42), 122 women were included. The serum samples collected at <6, 6–8, 8–10, 10–12 and >12 weeks' gestation were analyzed for 29 common analytes. Subsequently the RIs were calculated according to the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) recommendations (2.5–97.5th percentiles) and compared with the conventional RIs for non-pregnant women.
**Results:** Human chorionic gonadotropin (hCG), progesterone (P4), estradiol (E2), pregnancy-associated plasma protein A (PAPP-A), cancer antigen 125 (CA125), thyroid stimulating hormone (TSH), creatinine (CREA) and albumin (ALB) showed an early pregnancy-dependent change compared with conventional limits. For ALB the change was seen at 5.5 weeks' gestation.
**Conclusions:** We report gestational age-specific RIs available from the early part of the first trimester applicable to everyday clinical care of pregnant women. Well-known alterations of RIs seen in later trimesters are also observed in the first.

**Keywords:** first trimester; pregnancy; reference interval; reference range; reference value.

***Corresponding author: Jesper Friis Petersen,** MD, Department of Obstetrics and Gynecology, North Zealand Hospital, University of Copenhagen, Dyrehavevej 29, 3400 Hillerød, Denmark, E-mail: jesper.friis.petersen@regionh.dk. https://orcid.org/0000-0002-5619-1809
**Lennart J. Friis-Hansen:** Department of Clinical Biochemistry, North Zealand Hospital, University of Copenhagen, Hillerød, Denmark
**Andreas Kryger Jensen:** Section of Biostatistics, Institute of Public Health, University of Copenhagen, Copenhagen, Denmark; and Department of Clinical Research, North Zealand Hospital, Hillerød, Denmark
**Anders Nyboe Andersen:** The Fertility Clinic, Rigshospitalet, Copenhagen University, Copenhagen, Denmark
**Ellen C.L. Løkkegaard:** Department of Obstetrics and Gynecology, North Zealand Hospital, University of Copenhagen, Hillerød, Denmark

## Introduction

The first trimester has emerged as an increasingly accessible timespan for developing diagnostic algorithms concerning the prevalent diseases of pregnancy, e.g. preeclampsia, small-for-gestational age infants and gestational diabetes mellitus [1–6]. Declared millennium developmental goals (MDG 4 and 5) [7] by the WHO, and the International Federation of Gynecologists and Obstetricians (FIGO) has identified the first trimester as a window of opportunity for optimizing both maternal and fetal health, especially concerning the prediction and prevention of chronic diseases later in life.

Normal pregnancy and the associated surge of reproductive hormones has a major impact on maternal physiology as it increases cardiac output, expands the vascular compartments and increases stress on the liver and kidneys. This marked influence on female physiology also leads to alterations of most commonly used biochemical measurands compared with the conventional non-pregnant reference intervals (RIs) [8–10]. Therefore, the clinical care of pregnant women would benefit from reliable RIs that take demographic and societal factors such as ethnicity, dietary intake and subclinical diseases into account [11]. Modern automated equipment has improved the intra- and interlaboratory assay variations. However,

inter-instrument bias remains a challenging issue in any laboratory and uniform translation across different platforms has yet to be attained. It therefore follows that laboratories offering biochemical analyses of pregnant women ideally should provide gestational age-specified RIs for all relevant analytes across all three trimesters.

Unfortunately, most RIs for pregnant women are available from the late first or early second trimester and onwards [8–10, 12–14] or are extrapolated from a control group of participants in a trial examining a first trimester complication [10]. Accordingly, such values are not ideal for identifying early pregnancy abnormalities and often lack the statistical requirements recommended for establishing RIs by the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) [15].

In response to the increased focus on first-trimester diagnostics, we therefore established gestational age-specific serum-based RIs of 29 commonly used clinical analytes in an early pregnancy cohort of women that had a live birth following an uncomplicated and naturally conceived pregnancy.

# Materials and methods

## Study population

From June 2016 to March 2017, a prospective early pregnancy cohort (the PEP cohort) after natural conception was recruited via online add campaigns and subsequently followed at North Zealand Hospital, Hillerød or Rigshospitalet, Copenhagen, Denmark. Following a positive urinary pregnancy test, 218 women expressed interest in participating and 212 attended the first visit (four lost interest and two were not pregnant). As seen in the Figure 1, 210 were included in the cohort (two had surpassed the inclusion criterion of no more than 55 days' gestation). Participants were at least 18 years of age, able to understand and sign a written consent and had an ultrasonically confirmed pregnancy with one fetus. Women with a history of recurrent pregnancy loss (≥3 consecutive losses), all types of fertility treatments, known or initially diagnosed abnormalities of the uterus or tubes or an ongoing drug abuse were excluded. All participants were followed consecutively with serial blood samples and transvaginal ultrasonography every second week until they either had a miscarriage (n = 37) or they completed the first trimester (n = 166). Also shown in Figure 1, 203 women were included for analyses after exclusion of seven participants due to lost interest (n = 3) and multiple gestation (n = 3). One woman was excluded from the cohort and

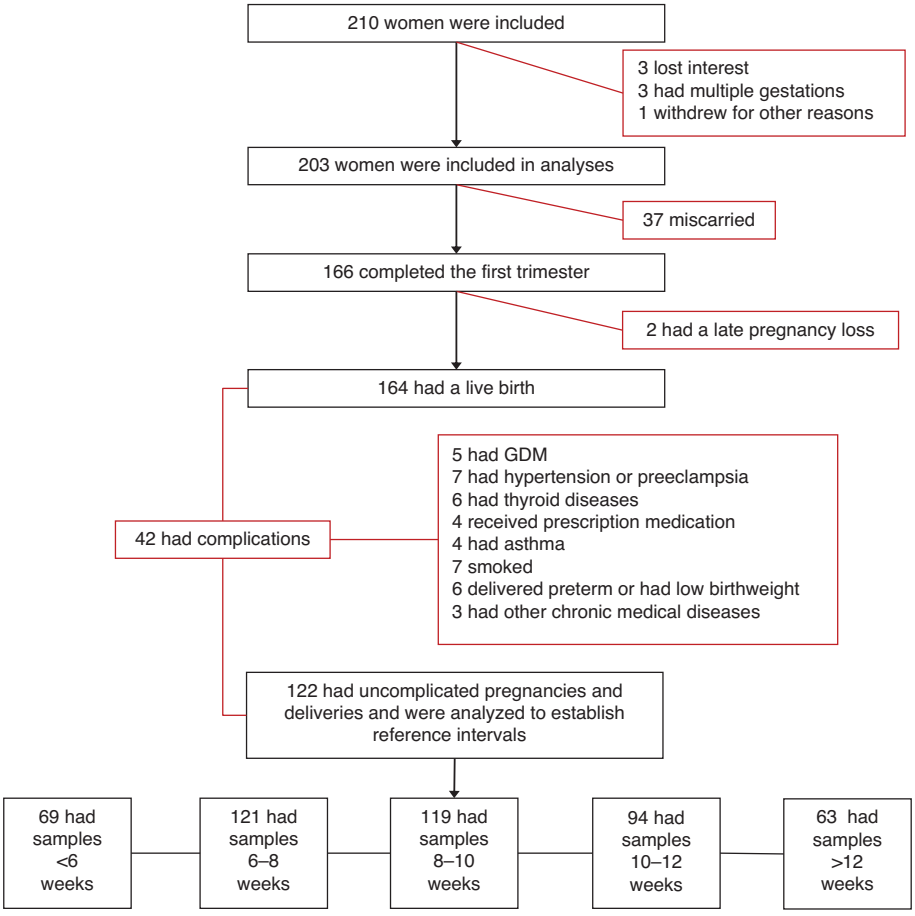


**Figure 1:** Flow chart of the selection of participants.

further blood sampling because her pregnancy developed ultrasonically resembling an early pregnancy loss. However, continued clinical follow-up later proved a healthy pregnancy.

A pregnancy follow-up of all participants in 2018 recorded 164 live births (two had late pregnancy loss). Within this group, RIs were based on 466 blood samples from 122 women, who fulfilled the criteria of an uncomplicated pregnancy and delivery. As described in Figure 1, exclusion criteria for this study comprised gestational diabetes mellitus, hypertension or preeclampsia, thyroid disease, prescription medication, asthma, other chronic medical diseases, smoking, preterm or low birthweight infants and 5 min Apgar scores (<7).

## Gestational age estimation

All participants had a transvaginal ultrasonography (GE Voluson i BT14, GE Healthcare, Solingen, Germany) at each visit. Gestational age was determined when a crown-rump-length (CRL) could be measured [16]. Due to early inclusion, some participants did not have a CRL-measurable fetus from their initial scan, thus their gestational age at visit 1 was calculated and registered after visit 2. Therefore, a visit 2 with CRL showing 50 days' gestation meant that a previous visit 1, 14 days earlier, without CRL estimation, was set at 36 days' gestation.

## Blood samples

Each participant had an 18 mL peripheral blood sample drawn from the veins of the antecubital fossa using the Vacutainer serum separator tubes (BD Diagnostics, Franklin Lakes, NJ, USA). Fasting was not required as it was considered intolerable due to frequent nausea in the first trimester. All samples were done from 8 a.m. to 7 p.m. To allow clotting, the blood was stored at room temperature for 15 min before 10 min of 3500 *g* centrifuge at 5 °C (Hettich Rotina 380 R, Andreas Hettich GmbH, Tuttlingen, Germany). The separated components were finally aliquoted to polypropylene RNase-, DNase-free microcentrifuge tubes and stored at −80 °C. The samples were thawed and analyzed 8 months after the last inclusion to allow for appropriate follow up to be completed.

**Table 1:** Biochemical details of serum analytes.

| Analyte (abbreviation) | CV% | Traceability | NPU code |
|---|---|---|---|
| Siemens Dimension Vista 1500 (Siemens Healthcare Diagnostics Inc., Newark, DE, USA) | | | |
| Albumin (ALB) | 5.8 | ERM-DA470k/IFCC | NPU19673 |
| Alkaline phosphatase (ALP) | 5.6 | IFCC acc. Schumann Gen 2. | NPU27783 |
| Alanine aminotransferase (ALT) | 3.8 | IFCC | NPU19651 |
| Aspartate aminotransferase (AST) | 5.7 | IFCC | NPU19654 |
| Lactate dehydrogenase (LDH) | 6.7 | IFCC 2002 | NPU19658 |
| Bilirubin, total (BIL) | 8.5 | Doumas candidate reference method | NPU01370 |
| Iron, free (IRON) | 4.2 | SRM 937 | NPU02508 |
| Ferritin (FTL) | 4.4 | NIBSC standard 80/602 | NPU19763 |
| Cholesterol (CHOL) | 4.2 | ID-MS | NPU01566 |
| High-density lipoprotein (HDL) | 5.4 | Internal | NPU01567 |
| Low-density lipoprotein (LDL) | 4.0 | Internal | NPU01568 |
| Triglyceride (TRIG) | 5.5 | ID-MS | NPU04094 |
| Blood urea nitrogen (BUN) | 5.5 | ID-MS | NPU01459 |
| Creatinine (CREA) | 4.7 | ID-MS | NPU04998 |
| Sodium (Na) | 1.5 | NIST SRM929A | NPU03429 |
| Potassium (K) | 3.0 | NIST SRM928A | EPC00026 |
| Uric acid (URIC) | 4.9 | ID-MS | NPU03688 |
| Thyroid stimulating hormone (TSH) | 6.1 | WHO 80/558 2nd IRP | NPU03577 |
| C-reactive protein (CRP) | 10.3 | Internal method traceable to CRM 470 | NPU19748 |
| Human chorionic gonadotropin, total (hCG) | 5.7 | IS 75/589; proc | NPU01580 |
| Progesterone, total (P4) | 14.5 | ID-GC/MS-method | NPU03242 |
| Estradiol (E2) | 7.6 | ID-GC/MS-method | NPU01972 |
| Siemens Centaur XP (Siemens Healthcare Diagnostics Inc., Tarrytown, NY, USA) | | | |
| Triiodothyronine, total (T3) | 3.4 | Derived from USP material | NPU03625 |
| Triiodothyronine, free (fT3) | 4.4 | Derived from USP material | NPU03625 |
| Thyroxine, total (T4) | 4.4 | Derived from USP material | NPU03578 |
| Thyroxine, free (fT4) | 4.5 | Derived from USP material | NPU03579 |
| Cancer antigen 125 (CA125) | 4.6 | Derived from an internal standard | NPU01448 |
| Kryptor Compact Plus (Thermo Fisher, Nimes, France) | | | |
| Human chorionic gonadotropin, free (fhCG) | 5 | 1.st IRP WHO 75/551 | NPU01580 |
| Pregnancy-associated plasma protein A (PAPP-A) | 5 | Internal reference | NPU22157 |

CV, coefficient of variation; NPU, nomenclature for properties and units.

### Laboratory analysis

All samples were examined at the Department of Clinical Biochemistry, North Zealand Hospital. The analytes examined, equipment manufacturers and assay coefficients of variation, traceability and nomenclature for properties and units (NPU Codes) are listed in Table 1.

Albumin (ALB), alkaline phosphatase (ALP), alanine aminotransferase (ALT), aspartate aminotransferase (AST), lactate dehydrogenase (LDH), total bilirubin (BIL), free iron (IRON), ferritin (FTL), cholesterol (CHOL), high-density lipoprotein (HDL), low-density lipoprotein (LDL), triglyceride (TRIG), blood urea nitrogen (BUN), creatinine (CREA), sodium (Na), potassium (K), uric acid, (URIC), thyroid stimulating hormone (TSH), C-reactive protein (CRP), total human chorionic gonadotropin (hCG), total progesterone (P4) and estradiol (E2) were analyzed using Siemens Dimension Vista 1500. Free and total triiodothyronine (fT3 and T3) and thyroxine (fT4 and T4) as well as cancer antigen 125 (CA125) were measured by Siemens Centaur XP and pregnancy-associated plasma protein A (PAPP-A) and the free beta human chorionic gonadotropin (fhCG) using the Kryptor Compact Plus.

### Statistical analysis

Conditional quantile regression according to the LMS method [17] was performed using R [18] and the package gamlss [19]. The degrees of freedom for the spline expansions and a choice between the Box-Cox t distribution and the Box-Cox normal distribution were selected according to the AIC criterion. A non-parametric bootstrap procedure with 25,000 resamples was used to estimate 90% overall confidence envelopes for the 2.5% and 97.5% conditional quantile functions [20]. The envelopes were compared with the corresponding 90% confidence intervals (CIs) of the conventional RI to decide whether and, if so, when the pregnancy-dependent interval became approximately significantly different. The Reference Intervals, software package and an IFCC-recommended approach [15, 21] was used to calculate RI including 90% CI for the limits every second week of the observational period. Outliers were detected according to Horn's method [22] and evaluated with the patient chart. If a value was biologically unexplainable, suggesting measurement failure, it was removed. All missing data were assumed to be missing complete at random (MCAR) [23] due to laboratory processing glitches. Irrespective, all values were reported from a complete case analysis as RIs are usually not derived from imputed data. When the concentration was equal to or below the lower detectable limit of the assay, the value of the detection limit was used as a test result. Student's t-test and the chi-squared test were used to test for differences in means or proportions, respectively, and p-values below 5% were considered statistically significant.

### Ethics

All participants received oral and written information about the study prior to participation and signed an informed consent according to the standards of the Helsinki declaration. The study was approved by the local Regional Ethical Committee (H15018059) as well as the Danish Data Protection Agency (NOH-2015-042) and was published at clinicaltrials.org prior to first patient enrollment (NCT02761772).

## Results

All participants delivered a healthy singleton neonate during 2017. Table 2 shows the study population demography compared with the 2017 nationwide Danish birth data [24]. Participants were younger (mean ± SD; 29 ± 4 vs. 30 ± 5 years, $p = 0.01$) due to 62% being <30 years of age (46% nationwide, $p < 0.001$). Participants were less likely to be nulliparous (27% vs. 39% nationwide, $p = 0.02$) and birthweights were marginally higher than the nationwide mean (3592 ± 457 vs. 3474 ± 609 g, $p = 0.005$). All participants were Caucasian.

Table 3 shows RIs (2.5–97.5th percentiles) and the 90% CI for these limits per 2 weeks' gestation of each analyte. As also shown in Figure 2: ALB, hCG, P4, E2, PAPP-A, CA125, TSH and CREA, already exhibited a notable change during the earliest part of the first trimester. ALB values reduced almost linearly from a starting range (36–48 g/L) equal to the conventional RI (37–48 g/L) and finished with the median below the 2.5 percentile of the conventional (>12 weeks' RI 31–42 g/L). The pregnancy-dependent markers of hCG, fhCG and PAPP-A developed expectedly showing surges around 6–9 weeks' gestation. hCG plateaus about 9 weeks' gestation before decreasing in the remaining period. The P4 and E2 97.5th percentile surpassed the luteal phase-based conventional equivalent throughout the observation and were both significantly increased after 4.5 weeks' gestation. Additionally, the E2 2.5th percentile exceeded the conventional 97.5th at about 8 weeks' and became significantly higher after 11 weeks' gestation. CA125 levels developed a peak at 6 weeks' gestation (<6 weeks RI 7–170 U/mL) with the 97.5th percentile significantly higher already after 4.5 weeks. Levels decreased thereafter and at 11 weeks' gestation the 97.5th percentile were within conventional limits. TSH described a U-shaped curve with a minimum at 8–10 weeks' gestation (0.10–4.1 mU/L) resembling the conventional range (0.30–4.0 mU/L). About half of CRP values (279/463, 60%) were at the lowest limit of assay detection, although a tendency of higher values with gestation was seen. Early CREA were marginally higher than values by 9 weeks' gestation (<6 weeks: 42–68 vs. 8–10 weeks: 39–66 μmol/L) with an almost steady curve thereon. Compared with conventional limits, the 97.5th percentile was reduced throughout the observation and the 2.5th became significant after 4.5 weeks' gestation.

**Table 2:** Cohort demographics at the time of inclusion compared with the 2017 nationwide Danish birth data.

| | Study population | | Danish 2017 births | | p-Value |
|---|---|---|---|---|---|
| n | 122 | | 60,871 | | |
| Age, years, mean, SD[a] | 29.3 | (4.1) | 30.3 | (5.0) | 0.01 |
| Age groups, n (%)[b] | | | | | |
| < 30 years | 76 | (62.3) | 27,694 | (45.5) | <0.001 |
| 30–35 years | 33 | (27.0) | 23,547 | (38.7) | |
| > 35 years | 13 | (10.7) | 9630 | (15.8) | |
| BMI, kg/m$^2$, mean, SD[a] | 24.1 | (4.4) | 24.8 | (8.2) | 0.07 |
| BMI groups, n (%)[b] | | | | | |
| Normal weight | 76 | (62.3) | 35,189 | (57.8) | 0.08 |
| Underweight | 3 | (2.5) | 2579 | (4.2) | |
| Pre-obese | 34 | (27.9) | 12,683 | (20.8) | |
| Obese | 9 | (7.4) | 8009 | (13.2) | |
| Missing | NA | | 2411 | (4.0) | |
| Former pregnancies, n (%)[b] | | | | | |
| 0 | 33 | (27.0) | 23,637 | (38.8) | 0.02 |
| 1 | 42 | (34.4) | 19,395 | (31.9) | |
| 2+ | 47 | (38.5) | 17,839 | (29.3) | |
| Parity, n (%)[b] | | | | | |
| 0 | 51 | (41.8) | 29,490 | (48.4) | 0.1 |
| 1 | 53 | (43.4) | 20,913 | (34.4) | |
| 2+ | 18 | (14.8) | 9532 | (15.7) | |
| Gestational age at delivery, mean (SD) | 280 | (7.70) | NA | | |
| Method of delivery, n (%)[b] | | | | | |
| Acute CS | 9 | (7.4) | 6520 | (10.7) | 0.4 |
| Planned CS | 14 | (11.5) | 5548 | (9.1) | |
| Vacuum extraction[c] | 3 | (2.5) | NA | | |
| Vaginal | 96 | (78.7) | 48,803 | (80.2) | |
| Infant sex, n (%)[b] | | | | | |
| Boy | 63 | (51.6) | 31,166 | (51.2) | 0.9 |
| Girl | 59 | (48.4) | 29,705 | (48.8) | |
| Infant weight, g, mean, SD[a] | 3592 | (457) | 3474 | (609) | 0.005 |
| Apgar <7 at 5 min, n (%) | 0 | (100.0) | NA | | |

[a]T-test, [b]chi-squared test, [c]censored from test. SD, standard deviation; n (%), column % per level; NA, not applicable.

As shown in Supplementary Figure 1, other markers were either aligned with the conventional RI or rather were unchanged during the entire first trimester.

# Discussion

This study successfully established detailed RIs for 29 common analytes in the first trimester of women who had a naturally conceived and uncomplicated pregnancy with the delivery of a healthy singleton neonate. Primarily, the study provided novel and detailed data from 4 to 8 weeks' gestation. This allowed for an assessment of when these analytes deviated from the RIs of non-pregnant women and hence could become clinically important. Furthermore, by determining the RIs for 29 biomarkers of endocrine, renal, liver and inflammatory function the study provided an overview of the many-facetted early reprograming of body physiology induced by pregnancy.

## Endocrine

The production of hCG has been detected in plasma as early as 6–14 days after fertilization [25]. In our assay, hCG and fhCG were primarily increased after placentation (week 6), as shown in Figure 2. Hereafter, the production increased markedly until week 9. PAPP-A is a protease that cleaves IGFBP4 causing the release of IGF1 and IGF2. PAPP-A, like hCG, are synthesized by the syncytiotrophoblast and secreted into the maternal circulation in increasing concentrations until term [26]. Low levels of PAPP-A are therefore associated with restricted fetal growth as IGF markedly influence fetal intrauterine

**Table 3:** Reference intervals per 2 weeks' gestation.

| Analyte (abbreviation), conventional range, unit, outliers | Weeks' gestation | n | Reference interval | | 90% CI for the reference interval percentiles | | | |
|---|---|---|---|---|---|---|---|---|
| | | | 2.5 percentile | 97.5 percentile | 2.5 percentile | | 97.5 percentile | |
| Human chorionic gonadotropin (hCG) total, U/L | <6 | 69 | 226 | 32,660 | 0 | 364 | 21,755 | 48,387 |
| | 6–8 | 120 | 10,622 | 161,144 | 5422 | 15,571 | 124,479 | 200,000 |
| | 8–10 | 117 | 45,180 | 200,000 | 36,685 | 68,721 | 200,000 | 207,915 |
| | 10–12 | 93 | 36,709 | 171,645 | 34,758 | 38,585 | 143,290 | 194,872 |
| | >12 | 63 | 22,314 | 143,568 | 14,363 | 26,780 | 118,943 | 181,980 |
| Human chorionic gonadotropin (fhCG) free, U/L | <6 | 70 | 0.1 | 27.1 | 0.0 | 0.2 | 26.0 | 32.8 |
| | 6–8 | 121 | 7.9 | 155.3 | 3.8 | 11.2 | 127.3 | 206.9 |
| | 8–10 | 118 | 20.5 | 223.8 | 10.8 | 31.0 | 120.6 | 257.1 |
| | 10–12 | 93 | 21.0 | 211.6 | 18.9 | 25.7 | 201.0 | 265.3 |
| | >12 | 63 | 11.4 | 118.4 | 7.8 | 13.5 | 86.5 | 155.0 |
| Pregnancy-associated plasma protein A (PAPP-A), U/L | <6 | 70 | 0.00 | 0.24 | 0.00 | 0.01 | 0.00 | 0.46 |
| | 6–8 | 121 | 0.02 | 0.46 | 0.01 | 0.02 | 0.36 | 3.48 |
| | 8–10 | 118 | 0.13 | 1.87 | 0.12 | 0.19 | 0.00 | 2.19 |
| | 10–12 | 93 | 0.46 | 5.79 | 0.33 | 0.54 | 0.34 | 6.60 |
| | >12 | 63 | 0.90 | 15 | 0.45 | 0.96 | 13 | 19 |
| Estradiol (E2) 0.25–1.2 nmol/L (luteal phase) | <6 | 69 | 0.4 | 3.0 | 0.3 | 0.5 | 2.4 | 3.6 |
| | 6–8 | 120 | 0.8 | 5.8 | 0.7 | 0.9 | 5.1 | 6.3 |
| | 8–10 | 119 | 1.0 | 8.6 | 0.4 | 1.3 | 7.1 | 10 |
| | 10–12 | 90 | 1.5 | 10 | 0.9 | 2.0 | 9.6 | 12 |
| | >12 | 54 | 2.2 | 10 | 1.5 | 2.4 | 9.7 | 11 |
| Progesterone (P4), total 20–100 nmol/L (luteal phase) | <6 | 70 | 21 | 138 | 13 | 26 | 123 | 152 |
| | 6–8 | 120 | 27 | 147 | 20 | 30 | 118 | 154 |
| | 8–10 | 118 | 28 | 156 | 23 | 31 | 144 | 185 |
| | 10–12 | 92 | 38 | 177 | 28 | 48 | 173 | 206 |
| | >12 | 59 | 53 | 172 | 48 | 54 | 161 | 187 |
| Cancer antigen 125 (CA125) <35 U/mL | <6 | 70 | 7 | 170 | 4 | 7 | 131 | 254 |
| | 6–8 | 120 | 8 | 98 | 4 | 14 | 82 | 119 |
| | 8–10 | 118 | 8 | 69 | 5 | 11 | 32 | 76 |
| | 10–12 | 94 | 6 | 50 | 4 | 8 | 32 | 59 |
| | >12 | 63 | 6 | 47 | 4 | 7 | 38 | 62 |
| Albumin (ALB) 37–48 g/L | <6 | 70 | 36 | 48 | 34 | 36 | 47 | 50 |
| | 6–8 | 119 | 36 | 46 | 35 | 37 | 44 | 46 |
| | 8–10 | 118 | 34 | 45 | 33 | 35 | 43 | 46 |
| | 10–12 | 94 | 32 | 43 | 31 | 33 | 43 | 44 |
| | >12 | 63 | 31 | 42 | 29 | 31 | 42 | 43 |
| Alkaline phosphatase (ALP) 37–106 U/L | <6 | 69 | 33 | 108 | 25 | 43 | 106 | 125 |
| | 6–8 | 118 | 33 | 96 | 29 | 39 | 79 | 111 |
| | 8–10 | 118 | 32 | 117 | 29 | 37 | 108 | 148 |
| | 10–12 | 94 | 32 | 101 | 29 | 33 | 90 | 124 |
| | >12 | 63 | 28 | 127 | 23 | 28 | 117 | 164 |
| Alanine transaminase (ALT) 8–46 U/L | <6 | 69 | 10 | 49 | 9 | 10 | 30 | 63 |
| | 6–8 | 117 | 8 | 43 | 7 | 10 | 31 | 53 |
| | 8–10 | 118 | 7 | 53 | 6 | 8 | 40 | 70 |
| | 10–12 | 94 | 7 | 38 | 6 | 7 | 28 | 50 |
| | >12 | 63 | 6 | 28 | 5 | 7 | 27 | 31 |
| Aspartate transaminase (AST) 13–37 U/L | <6 | 70 | 9 | 33 | 8 | 10 | 31 | 42 |
| | 6–8 | 118 | 9 | 24 | 9 | 10 | 20 | 27 |
| | 8–10 | 118 | 8 | 26 | 7 | 9 | 24 | 30 |
| | 10–12 | 94 | 8 | 26 | 7 | 10 | 21 | 31 |
| | >12 | 63 | 9 | 19 | 8 | 9 | 19 | 21 |
| Lactate dehydrogenase (LDH) 103–204 U/L One outlier removed | <6 | 69 | 109 | 205 | 103 | 118 | 136 | 235 |
| | 6–8 | 118 | 109 | 175 | 104 | 114 | 152 | 187 |
| | 8–10 | 118 | 105 | 172 | 100 | 136 | 164 | 176 |
| | 10–12 | 94 | 98 | 184 | 83 | 102 | 175 | 202 |
| | >12 | 63 | 83 | 186 | 57 | 110 | 185 | 208 |

**Table 3** (continued)

| Analyte (abbreviation), conventional range, unit, outliers | Weeks' gestation | n | Reference interval | | 90% CI for the reference interval percentiles | | | |
|---|---|---|---|---|---|---|---|---|
| | | | 2.5 percentile | 97.5 percentile | 2.5 percentile | | 97.5 percentile | |
| Bilirubin (BIL) total | <6 | 70 | 4 | 29 | 4 | 4 | 29 | 39 |
| 5–24 µmol/L | 6–8 | 118 | 4 | 20 | 3 | 4 | 11 | 24 |
| | 8–10 | 118 | 3 | 20 | 3 | 4 | 12 | 24 |
| | 10–12 | 94 | 3 | 21 | 2 | 3 | 19 | 25 |
| | >12 | 63 | 3 | 16 | 3 | 3 | 12 | 21 |
| Cholesterol (CHOL) total | <6 | 70 | 2.6 | 6.6 | 2.2 | 2.8 | 5.9 | 7.7 |
| 3.4–6.9 mmol/L | 6–8 | 117 | 2.8 | 5.5 | 2.7 | 3.4 | 5.4 | 5.8 |
| | 8–10 | 118 | 2.9 | 5.9 | 2.6 | 3.3 | 3.9 | 6.2 |
| | 10–12 | 94 | 3.2 | 6.4 | 3.0 | 3.4 | 4.9 | 6.9 |
| | >12 | 63 | 3.0 | 8.0 | 2.4 | 3.1 | 7.7 | 9.9 |
| Low-density lipoprotein (LDL) | <6 | 70 | 1.1 | 4.9 | 0.8 | 1.5 | 4.3 | 5.7 |
| 1.4–4.7 mmol/L | 6–8 | 117 | 1.0 | 3.9 | 0.5 | 1.5 | 3.8 | 4.4 |
| | 8–10 | 118 | 1.1 | 4.2 | 0.9 | 1.5 | 2.6 | 4.7 |
| | 10–12 | 94 | 1.5 | 4.3 | 1.3 | 1.7 | 2.6 | 4.5 |
| | >12 | 63 | 1.1 | 6.1 | 0.5 | 1.7 | 6.0 | 8.1 |
| High-density lipoprotein (HDL) | <6 | 70 | 1.1 | 2.3 | 1.0 | 1.1 | 2.1 | 2.5 |
| 1.0–2.6 mmol/L | 6–8 | 118 | 1.0 | 2.5 | 0.9 | 1.2 | 1.9 | 2.8 |
| | 8–10 | 118 | 1.1 | 2.6 | 1.0 | 1.2 | 1.6 | 2.8 |
| | 10–12 | 94 | 1.1 | 3.0 | 0.9 | 1.2 | 2.4 | 3.3 |
| | >12 | 63 | 1.2 | 3.2 | 1.0 | 1.3 | 2.6 | 3.8 |
| Triglyceride (TRIG) | <6 | 70 | 0.42 | 2.3 | 0.40 | 0.44 | 1.5 | 3.0 |
| 0.47–2.6 mmol/L | 6–8 | 118 | 0.43 | 2.3 | 0.39 | 0.50 | 1.8 | 2.7 |
| | 8–10 | 118 | 0.46 | 3.1 | 0.33 | 0.59 | 3.0 | 3.8 |
| | 10–12 | 94 | 0.56 | 2.4 | 0.49 | 0.60 | 2.4 | 2.7 |
| | >12 | 63 | 0.70 | 2.9 | 0.64 | 0.70 | 2.5 | 3.5 |
| Sodium (Na) | <6 | 70 | 132 | 143 | 130 | 135 | 140 | 150 |
| 137–145 mmol/L | 6–8 | 120 | 134 | 141 | 133 | 135 | 140 | 141 |
| | 8–10 | 117 | 133 | 141 | 132 | 134 | 140 | 141 |
| | 10–12 | 94 | 134 | 141 | 134 | 135 | 140 | 141 |
| | >12 | 63 | 135 | 142 | 133 | 134 | 139 | 146 |
| Potassium (K) | <6 | 69 | 3.3 | 4.4 | 3.1 | 3.3 | 4.1 | 4.6 |
| 3.6–4.6 mmol/L | 6–8 | 120 | 3.6 | 4.3 | 3.4 | 3.6 | 4.2 | 4.5 |
| | 8–10 | 117 | 3.5 | 4.4 | 3.4 | 3.6 | 4.3 | 4.5 |
| | 10–12 | 94 | 3.5 | 4.4 | 3.5 | 3.6 | 4.3 | 4.5 |
| | >12 | 63 | 3.4 | 4.3 | 3.3 | 3.4 | 4.2 | 4.4 |
| Creatinine (CREA) | <6 | 70 | 42 | 68 | 37 | 43 | 67 | 71 |
| 51–84 µmol/L | 6–8 | 118 | 43 | 67 | 42 | 48 | 61 | 69 |
| | 8–10 | 118 | 39 | 66 | 37 | 43 | 60 | 70 |
| | 10–12 | 94 | 40 | 64 | 40 | 41 | 62 | 67 |
| | >12 | 63 | 39 | 64 | 36 | 40 | 59 | 68 |
| Urea nitrogen (BUN) | <6 | 70 | 2.1 | 6.3 | 1.8 | 2.4 | 5.7 | 6.6 |
| 2.7–6.4 mmol/L | 6–8 | 118 | 2.2 | 5.7 | 2.0 | 2.2 | 5.3 | 6.0 |
| | 8–10 | 118 | 2.1 | 5.6 | 1.9 | 2.5 | 5.3 | 6.1 |
| | 10–12 | 94 | 2.0 | 5.5 | 1.8 | 2.0 | 4.2 | 5.6 |
| | >12 | 63 | 1.9 | 5.2 | 1.6 | 2.1 | 4.9 | 5.6 |
| Uric acid (URIC) | <6 | 70 | 0.12 | 0.30 | 0.10 | 0.12 | 0.27 | 0.34 |
| 0.15–0.35 mmol/L | 6–8 | 118 | 0.10 | 0.27 | 0.08 | 0.10 | 0.24 | 0.29 |
| | 8–10 | 118 | 0.10 | 0.26 | 0.09 | 0.12 | 0.19 | 0.28 |
| | 10–12 | 94 | 0.09 | 0.25 | 0.08 | 0.10 | 0.22 | 0.27 |
| | >12 | 63 | 0.10 | 0.26 | 0.09 | 0.10 | 0.24 | 0.28 |
| Thyroxine (T4) total | <6 | 70 | 67 | 118 | 62 | 70 | 118 | 124 |
| 60–140 nmol/L | 6–8 | 120 | 71 | 142 | 66 | 77 | 126 | 166 |
| | 8–10 | 118 | 79 | 151 | 71 | 86 | 150 | 159 |
| | 10–12 | 94 | 77 | 164 | 61 | 80 | 144 | 174 |
| | >12 | 63 | 80 | 167 | 66 | 89 | 164 | 182 |

**Table 3** (continued)

| Analyte (abbreviation), conventional range, unit, outliers | Weeks' gestation | n | Reference interval | | 90% CI for the reference interval percentiles | | | |
|---|---|---|---|---|---|---|---|---|
| | | | 2.5 percentile | 97.5 percentile | 2.5 percentile | | 97.5 percentile | |
| Thyroxine (fT4) free | <6 | 67 | 11 | 17 | 11 | 12 | 17 | 18 |
| 12–21 pmol/L | 6–8 | 112 | 11 | 19 | 11 | 12 | 18 | 20 |
| | 8–10 | 114 | 11 | 18 | 10 | 12 | 15 | 18 |
| | 10–12 | 90 | 11 | 18 | 11 | 11 | 18 | 20 |
| | >12 | 63 | 11 | 17 | 10 | 11 | 17 | 18 |
| Triiodothyronine (T3) | <6 | 70 | 1.3 | 2.6 | 1.2 | 1.3 | 2.4 | 3.2 |
| 1.1–2.5 nmol/L | 6–8 | 120 | 1.3 | 2.4 | 1.3 | 1.4 | 2.4 | 2.5 |
| | 8–10 | 118 | 1.5 | 2.9 | 1.4 | 1.6 | 2.7 | 3.3 |
| | 10–12 | 94 | 1.5 | 2.9 | 1.4 | 1.6 | 2.7 | 3.2 |
| | >12 | 63 | 1.5 | 3.0 | 1.3 | 1.5 | 3.0 | 3.3 |
| Triiodothyronine (fT3), free | <6 | 67 | 3.6 | 6.2 | 3.2 | 3.8 | 6.2 | 6.6 |
| 3.9–6.8 pmol/L | 6–8 | 112 | 3.7 | 6.0 | 3.3 | 3.9 | 5.7 | 6.4 |
| | 8–10 | 113 | 3.5 | 5.6 | 3.2 | 3.7 | 5.6 | 5.9 |
| | 10–12 | 90 | 3.8 | 5.4 | 3.7 | 4.1 | 4.9 | 5.5 |
| | >12 | 63 | 3.7 | 5.2 | 3.4 | 3.7 | 5.2 | 5.4 |
| Thyroid stimulating hormone (TSH) | <6 | 70 | 0.68 | 5.29 | 0.34 | 0.86 | 4.72 | 6.42 |
| 0.30–4.00 mU/L | 6–8 | 117 | 0.35 | 4.05 | 0.30 | 0.40 | 1.82 | 4.55 |
| | 8–10 | 117 | 0.10 | 4.14 | 0.03 | 0.14 | 3.56 | 5.00 |
| | 10–12 | 94 | 0.08 | 5.76 | 0.00 | 0.12 | 4.28 | 8.17 |
| | >12 | 63 | 0.37 | 7.41 | 0.22 | 0.48 | 3.89 | 11.5 |
| Iron (IRON), free | <6 | 69 | 5 | 26 | 3 | 7 | 25 | 29 |
| 9–34 μmol/L | 6–8 | 118 | 7 | 30 | 4 | 9 | 22 | 32 |
| 1 outlier removed | 8–10 | 118 | 4 | 33 | 3 | 6 | 32 | 38 |
| | 10–12 | 94 | 5 | 34 | 3 | 6 | 28 | 40 |
| | >12 | 63 | 4 | 29 | 1 | 4 | 27 | 32 |
| Ferritin (FTL) | <6 | 70 | 13 | 141 | 7 | 21 | 124 | 154 |
| 15–200 μg/L | 6–8 | 120 | 17 | 146 | 6 | 20 | 124 | 171 |
| | 8–10 | 118 | 14 | 149 | 9 | 15 | 103 | 172 |
| | 10–12 | 94 | 12 | 161 | 6 | 14 | 153 | 179 |
| | >12 | 63 | 9 | 109 | 1 | 9 | 76 | 138 |
| C-reactive protein (CRP) | <6 | 70 | 2.9 | 19 | 2.9 | 2.9 | 12 | 22 |
| <3 mg/L | 6–8 | 118 | 2.9 | 18 | 2.9 | 2.9 | 8.5 | 30 |
| | 8–10 | 118 | 2.9 | 22 | 2.9 | 2.9 | 14 | 44 |
| | 10–12 | 94 | 2.9 | 17 | 2.9 | 2.9 | 13 | 34 |
| | >12 | 63 | 2.9 | 31 | 2.9 | 2.9 | 16 | 32 |

growth restriction. However, our data display great variability in what was needed to maintain the pregnancy, demonstrated by one participant who had all three proteins consistently below the 2.5th percentile limit throughout the first trimester. Despite this, the pregnancy was uneventful and ended up in the delivery of a healthy and normal-weight neonate. This illustrates the difference between RI and establishing clinically applicable decision limits, for example, "lowest values associated with live birth". Accordingly, diagnostics based on our reported limits should be cautiously done and serial measurements are warranted [27].

As expected, total T4 and T3 increased while the levels of the free hormones were unchanged. Increased total concentrations are caused by the well-known increase of thyroglobulin binding protein (TBG) beginning at the time of placentation [28–30]. The TSH concentration was inversely linked to hCG attributed to their structural similarities; they share the same alpha subunit causing cross reactivity at the TSH receptor. Therefore, hCG, when in abundance, acts in a thyrotrophic manner in the pituitary and produces a negative feedback [31].

Another feature of Figure 2 is the luteoplacental shift traditionally defined as the onset of placental steroidogenesis of P4 and E2. This juncture is clinically important, especially in reproductive endocrine therapy of infertile women seeking fertility treatment. Theoretically, the onset of endogenous placental sex steroid production is the earliest time when exogenous steroid administration may be withdrawn. The exact timing of this shift has been

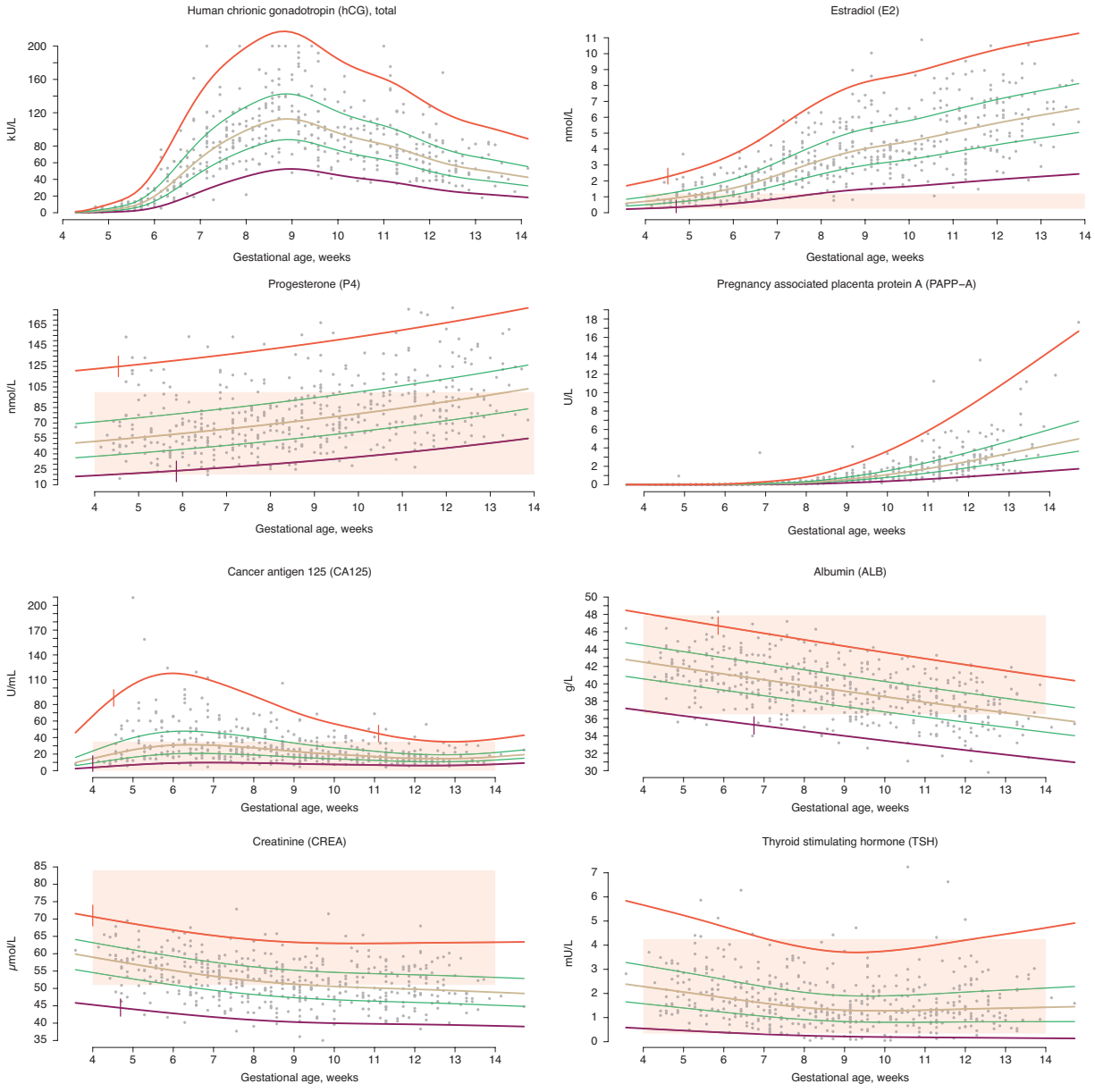


**Figure 2:** Scatter plot of the eight analytes with the most prominent changes early in pregnancy.
Every observed value and smoothed percentiles (red: 97.5th; maroon: 2.5th; green: 25–75th and yellow: the median) are plotted to the gestational age in weeks 4–14. Vertical lines mark the timing of the 2.5th (maroon) and the 97.5th (red) centile of the calculated reference interval becoming statistically significantly different from the conventional limits if available (orange boxes).

debated. In 1990, Devroey et al. [32] reported a significant E2 and P4 rise by 7 and 9 weeks' gestation, respectively, examining 18 pregnancies from oocyte donation in agonal women. One year later, Scott et al. [33] performed a similar study and observed similar elevations already at 6 and 7 weeks' gestation, respectively. These studies provided a near-optimal *in vivo* model of placental steroidogenesis as the endogenous contribution from the corpus luteum was unavailable. Nonetheless, as the developing conceptus is dependent on the corpus luteum, these protocols had to supply exogenous sex steroids that potentially disturb the estimation of onset timing. Conversely, our setup displayed the continuum of E2 and P4 levels attributed by both the corpus luteum and the developing placenta in a non-supplemented pregnancy. Therefore, the exact origin of steroid production cannot be determined. As shown in Figure 2, P4 increased almost linearly while E2 elevated more rapidly from 6 to 8 weeks' gestation. We decided to define statistical significance as the time when 90% CIs of the calculated RI limits (2.5th or 97.5th) were decreased or increased compared with the non-pregnant equivalents. Therefore, our results support the notion of increased sex

steroid production around week 6 (P4 97.5th and 2.5th significantly increased by 4.5 and 5.9 weeks' gestation, respectively), probably attributed by the placental onset and seemingly more pronounced in E2 (E2 97.5th and 2.5th were significantly increased by 4.5 and 4.7 weeks' gestation, respectively).

CA125 is usually associated with ovarian cancer diagnostics. In pregnancy, CA125 is expressed by the decidual cells and secreted to circulation in the first trimester due to decidual invasion by the trophoblast during placentation [34]. Therefore, CA125 becomes a marker of placentation also seen in Figure 2, as the values from 4 to 8 weeks' gestation exceeds the ovarian cancer diagnostic cutoff of 35 U/mL. Interestingly, the upper limit was significantly increased already at 4.5 weeks' but returned to non-pregnant limits after 11 weeks. This finding warrants clinical vigilance towards interpretation of CA125 in the early first trimester and support the previously shown potential of CA125 as a first trimester biomarker [35]. As expected, the theoretical lower limit of 0 in the CA125 non-pregnant RIs was exceeded in all observations.

## Renal

Pregnancy has a major impact on kidney function and fluid/salt balance and the biomarkers Na, K, URIC, CREA and BUN reflect these physiologically changes in the pregnant body throughout the first trimester. Following progesterone and relaxin-mediated decreased systemic vascular resistance from the corpus luteum, also renal vasculature is relaxed and allows increased blood flow and glomerular filtration rate (GFR) [36, 37]. Consequently, as seen in Figure 2 and Supplementary Figure 1, CREA (97.5th and 2.5th percentile significantly decreased throughout the observation and by 4.5 weeks' gestation, respectively), URIC and BUN started at a low value and decreased with gestation [8–10]. Furthermore, the hypervolemic-hypoosmolar-characteristic state of pregnancy [36, 37] was seen by the stable and unchanged RIs of sodium and potassium – both with at least the 2.5th percentile at a lower level compared with the non-pregnant references.

## Liver

There was a steady increase in serum lipids throughout the first trimester. As the fetus develops, the triglycerides increase as metabolic changes prioritize the development of fatty stores during the first trimester to satisfy maternal energy needs later in pregnancy. Additionally, the increased production of sex steroids necessitates increased CHOL, HDL and LDL levels as precursor substrates [37, 38]. Compared with conventional limits the differences were marginal. Nonetheless, our data clearly illustrate that the well-known gestational changes already can be visualized from the beginning of the pregnancy. Also evident at this stage, was the well-known reduction in albumin during the first trimester [9, 10]. The albumin RIs were initially within conventional limits [39], but already from about 5.5 weeks' gestation the 2.5th percentile was lower than the conventional equivalent (Figure 2) and significantly different at 6.8 weeks. This decrease continued throughout the first trimester before ending up in a range >12 weeks (31–42 g/L) comparable to the work by Klajnbard et al. [8] who reported an RI of 32–43 g/L by 13–20 weeks' gestation. Although their work constitutes the most thorough investigation of the Danish population and serves as the reference for pregnant women in most Danish obstetric consultations, the authors were not able to sample individuals from the first trimester and an RI is therefore unavailable. In another report from the Scandinavian population, Larsson et al. [9] also found ALB values from 7 to17 weeks' gestation of 32–43 g/L. We argue, that this 10-week period probably contained a higher and non-pregnant-like ALB RI in the earliest part of the first trimester and illustrate the added precision that may arise from scrutinizing references in smaller intervals.

Multiple mechanisms such as hemodilution [9], increased catabolism [8] and maximized pregnancy-dependent reprogramed liver synthesis [40] have been suggested to cause the decrease in albumin concentration with gestation. Our early declining values support the theory of albumin being increasingly catabolized for the development of the fetal unit at a rate that exceeds the normal capacity for liver synthesis. From gestational week 5.5 some hemodilution is possible, although this traditionally has not been observed before at least 6 weeks' gestation [41]. Additionally, bigger molecules such as HDL are unchanged or slightly increasing within the period as also shown by Klajnbard et al. [8]. Supporting the view that these changes are caused by reprograming of liver synthesis during pregnancy is the fact that liver cell turnover, as evaluated by the ALT, AST and LDH, was stable and unaffected (Supplementary Figure 1). Conjugation and excretion of bilirubin also seemed unaffected (Supplementary Figure 1). The lower 2.5th percentile reference limit of AST, ALP and BIL was interpreted as a difference between the age of our study population (22–38 years) and the conventional value population. These mostly originated from the Nordic Reference Interval Project (NORIP) [39] providing

comparable non-pregnant intervals specified by female gender, ethnicity and societal factors such as the dietary intake in the Nordic countries, but pooled values from participants from 18 to 49 years of age.

### Inflammation

CRP has been intensively researched in pregnancy with a general tendency of increased values by gestation [8, 42, 43]. Supported by our findings, values vary immensely, and any meaningful interpretation needs to be closely correlated to clinical development of symptoms.

### Strengths and limitations

The study population was a well-characterized and homogenous cohort of Caucasian women with complete follow-up until delivery. Hence, the reported RIs give a solid base for clinical application in populations of this demography. As seen in Table 2, compared with the Danish birth cohort of 2017, our sample represents the nationwide numbers rather accurately with minor differences of negligible significance. All reported RIs follow the recommendations of the IFCC [11, 21], albeit with few samples in the extreme <6 and >12 weeks' gestational intervals. These values should therefore be cautiously interpreted, while they simultaneously provide a rare view at naturally conceived pregnancies <6 weeks' gestation. The prospective design allowed for CRL-determined gestational age even at the earliest available samples. All analyzed samples were handled by the same investigator and followed the directions of the UK biobank [44], optimizing the integrity and precision of the reported values. Ideally, we would have provided a complete first trimester material of all parameters investigated by Klajnbard et al. [8] Unfortunately, our laboratory setup was unable to do full-blood analyses, for example, hematological analytes. Future research might focus on procuring these missing quantities in the early part of the first trimester.

## Conclusions

Collectively, this study reports gestational age-specific RIs from 4 to 12 weeks' gestation in 29 commonly used analytes applicable to clinicians caring for pregnant women. Our findings confirm the well-known fact that pregnancy alter non-pregnant RI. This should be considered already in the first trimester.

**Acknowledgments:** This study received invaluable practical assistance from the staff of the Departments of Clinical Biochemistry and Clinical Research at North Zealand Hospital, and from the data management of Steen Rasmussen, MSC.

**Author contributions:** All the authors have accepted responsibility for the entire content of this submitted manuscript and approved submission.

**Research funding:** Grants from the North Zealand Hospital Research Council, the Gangsted Foundation, the Foundation for Development of Danish Private Practice, the Tvergaard Foundation, the AP Møller Foundation, the Foundation from Danish Doctors Pension and Copenhagen University made this research possible. No funders were involved in the design, acquisition, analysis or interpretation of data prior to submission.

**Employment or leadership:** None declared.

**Honorarium:** None declared.

**Competing interests:** The funding organization(s) played no role in the study design; in the collection, analysis, and interpretation of data; in the writing of the report; or in the decision to submit the report for publication.

## References

1. Poon LC, McIntyre HD, Hyett JA, da Fonseca EB, Hod M. The first-trimester of pregnancy – a window of opportunity for prediction and prevention of pregnancy complications and future life. Diabetes Res Clin Pract 2018;145:20–30.
2. Rolnik DL, Wright D, Poon LCY, Syngelaki A, O'Gorman N, de Paco Matallana C, et al. ASPRE trial: performance of screening for preterm pre-eclampsia. Ultrasound Obstet Gynecol 2017;50:492–5.
3. Tan MY, Poon LC, Rolnik DL, Syngelaki A, de Paco Matallana C, Akolekar R, et al. Prediction and prevention of small-for-gestational-age neonates: evidence from SPREE and ASPRE. Ultrasound Obstet Gynecol 2018;52:52–9.
4. Halscott TL, Ramsey PS, Reddy UM. First trimester screening cannot predict adverse pregnancy outcomes. Prenat Diagn 2014;34:668–76.
5. Kane SC, Da Silva Costa F, Brennecke S. First trimester biomarkers in the prediction of later pregnancy complications. Biomed Res Int 2014;2014(i).
6. Wortelboer EJ, Koster MP, Kuc S, Eijkemans MJ, Bilardo CM, Schielen PC, et al. Longitudinal trends in fetoplacental biochemical markers, uterine artery pulsatility index and maternal blood pressure during the first trimester of pregnancy. Ultrasound Obstet Gynecol 2011;38:383–8.
7. WHO | Millennium Development Goals (MDGs). WHO [Internet]. 2017 [cited 2019 Feb 27]; Available from: https://www.who.int/topics/millennium_development_goals/en/.
8. Klajnbard A, Szecsi PB, Colov NP, Andersen MR, Jørgensen M, Bjørngaard B, et al. Laboratory reference intervals during pregnancy, delivery and the early postpartum period. Clin Chem Lab Med 2010;48:237–48.

9. Larsson A, Palm M, Hansson L-O, Axelsson O. Reference values for clinical chemistry tests during normal pregnancy. BJOG Int J Obstet Gynaecol 2008;115:874–81.
10. Abbassi-Ghanavati M, Greer LG, Cunningham FG. Pregnancy and laboratory studies: a reference table for clinicians. Obstet Gynecol 2009;114:1326–31.
11. Solberg HE. International Federation of Clinical Chemistry. Scientific committee, clinical section. Expert Panel on Theory of Reference Values and International Committee for Standardization in Haematology, Standing Committee on Reference Values. Approved Recommendation. Ann Biol Clin (Paris) 1987;45:237–41.
12. Jin Y, Lu J, Jin H, Fei C, Xie X, Zhang J. Reference intervals for biochemical, haemostatic and haematological parameters in healthy Chinese women during early and late pregnancy. Clin Chem Lab Med 2018;56:973–9.
13. Dai Y, Liu J, Yuan E, Li Y, Wang Q, Jia L, et al. Gestational age-specific reference intervals for 15 biochemical measurands during normal pregnancy in China. Ann Clin Biochem 2018;55:446–52.
14. Van Buul EJ, Steegers EA, Jongsma HW, Eskes TK, Thomas CM, Hein PR. Haematological and biochemical profile of uncomplicated pregnancy in nulliparous women ; a longitudinal study. Neth J Med 1995;46:73–85.
15. Solberg HE. The theory of reference values Part 5. Statistical treatment of collected reference values. Determination of reference limits. J Clin Chem Clin Biochem 1983;21:749–60.
16. Hadlock FP, Shah YP, Kanon DJ, Lindsey JV. Fetal crown-rump length: reevaluation of relation to menstrual age (5–18 weeks) with high-resolution real-time US. Radiology 1992;182:501–5.
17. Cole TJ, Green PJ. Smoothing reference centile curves: the LMS method and penalized likelihood. Stat Med [Internet] 1992;11:1305–19.
18. R Core Team. R: a language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing; 2017.
19. Rigby RA, Stasinopoulos DM. Generalized Additive Models for Location, Scale and Shape [Internet]. Vol. 54, Journal of the Royal Statistical Society. Series C (Applied Statistics). Wiley Royal Statistical Society; 2005 [cited 2019 May 14]. p. 507–54. Available from: https://www.jstor.org/stable/3592732.
20. Davison AC, Hinkley DV. Bootstrap methods and their application. Cambridge: Cambridge University Press; 1997. 582 p.
21. Solberg HE. The IFCC recommendation on estimation of reference intervals. The RefVal program. Clin Chem Lab Med 2004;42:710–4.
22. Horn PS, Feng L, Li Y, Pesce AJ. Effect of outliers and nonhealthy individuals on reference interval estimation. Clin Chem 2001;47:2137–45.
23. Donders AR, van der Heijden GJ, Stijnen T, Moons KG. Review: a gentle introduction to imputation of missing values. J Clin Epidemiol 2006;59:1087–91.
24. TiGrAB – Danish Early Pregnancy Registry [Internet]. [cited 2019 Mar 4]. Available from: http://tigrab.dk/.
25. Nepomnaschy PA, Weinberg CR, Wilcox AJ, Baird DD. Urinary hCG patterns during the week following implantation. Hum Reprod 2008;23:271–7.
26. Monget P, Oxvig C. PAPP-A and the IGF system. Ann Endocrinol (Paris) [Internet] 2016;77(2):90–6.
27. Seeber BE. What serial hCG can tell you, and cannot tell you, about an early pregnancy. Fertil Steril 2012;98:1074–7.
28. Teng W, Shan Z, Patil-Sisodia K, Cooper DS. Hypothyroidism in pregnancy. Lancet Diabetes Endocrinol 2013;1:228–37.
29. De Leo S, Pearce EN. Autoimmune thyroid disease during pregnancy. Lancet Diabetes Endocrinol 2018;6:575–86.
30. Cooper DS, Laurberg P. Hyperthyroidism in pregnancy. Lancet Diabetes Endocrinol 2013;1:238–49.
31. Lejeune B, Bourdoux P, Lemone M, de Nayer P, Kinthaert J, Steirteghem A Van, et al. Regulation of maternal thyroid during pregnancy. J Clin Endocrinol Metab 2009;71:276–87.
32. Devroey P, Camus M, Palermo G, Smitz J, Van Waesberghe L, Wisanto A, et al. Placental production of estradiol and progesterone after oocyte donation in patients with primary ovarian failure. Am J Obstet Gynecol 1990;162:66–70.
33. Scott R, Navot D, Liu HC, Rosenwaks Z. A human *in vivo* model for the luteoplacental shift. Fertil Steril 1991;56:481–4.
34. Gziri MM, Han SN, Hanssens M, Lotgerink A, Amant F, Van Calsteren K. Physiologic variations of serum tumor markers in gynecological malignancies during pregnancy: a systematic review. BMC Med 2012;10:86.
35. Pillai RN, Konje JC, Tincello DG, Potdar N. Role of serum biomarkers in the prediction of outcome in women with threatened miscarriage: a systematic review and diagnostic accuracy meta-analysis. Hum Reprod Update 2016;22:228–39.
36. Lockitch G. Clinical biochemistry of pregnancy. Crit Rev Clin Lab Sci 1997;34:67–139.
37. Soma-Pillay P, Soma-Pillay P, Nelson-Piercy C, Tolppanen H, Mebazaa A, Mebazaa A. Physiological changes in pregnancy: review articles. Cardiovasc J Afr 2016;27:89–94.
38. Bartels Ä, O'Donoghue K. Cholesterol in pregnancy: a review of knowns and unknowns. Obstet Med 2011;4:147–51.
39. Rustad P, Felding P, Franzson L, Kairisto V, Lahti A, Mårtensson A, et al. The Nordic Reference Interval Project 2000: Recommended reference intervals for 25 common biochemical properties. Scand J Clin Lab Invest 2004;64:271–83.
40. Frederiksen MC. Physiologic changes in pregnancy and their effect on drug disposition. Semin Perinatol 2001;25:120–3.
41. Lund CJ, Donovan JC. Blood volume during pregnancy. Significance of plasma and red cell volumes. Am J Obstet Gynecol 1967;98:393–403.
42. Romem Y, Artal R. C-reactive protein in pregnancy and in the postpartum period. Am J Obstet Gynecol 1985;151:380–3.
43. Belo L, Santos-Silva A, Rocha S, Caslake M, Cooney J, Pereira-Leite L, et al. Fluctuations in C-reactive protein concentration and neutrophil activation during normal human pregnancy. Eur J Obstet Gynecol Reprod Biol 2005;123:46–51.
44. Elliott P, Peakman TC. The UK Biobank sample handling and storage protocol for the collection, processing and archiving of human blood and urine. Int J Epidemiol 2008;37:234–44.


**Supplementary Material:** The online version of this article offers supplementary material (https://doi.org/10.1515/cclm-2019-0495).