Am J Clin Exp Obstet Gynecol 2023;9(2):8-12
www.ajceog.us /ISSN:2330-1899/AJCEOG0151912

Original Article

Maternal serum pregnenolone concentrations throughout normal pregnancy

Jacquelyn Shaw[1], Jennifer K Blakemore[1], Isaac J Chamani[2], Ya'el Kramer[1], Cheongeun Oh[3], Ashley S Roman[4], Frederick Licciardi[1]

[1]New York University Langone Fertility Center, Department of Obstetrics and Gynecology, Division of Reproductive Endocrinology and Infertility, New York, NY 10016, USA; [2]Baylor College of Medicine, Department of Obstetrics and Gynecology, Pavilion for Women, 6651 Main Street, Houston, TX 77030, USA; [3]New York University Langone Health, Department of Population Health, New York, NY 10016, USA; [4]New York University Langone Health, Department of Obstetrics and Gynecology, Division of Maternal Fetal Medicine, New York, NY 10016, USA

Received June 25, 2023; Accepted September 20, 2023; Epub October 15, 2023; Published October 30, 2023

Abstract: Objective: Pregnenolone is the only steroid derived directly from cholesterol and is the first and requisite reaction in placental steroidogenesis. A better understanding of pregnenolone production throughout pregnancy may help improve our knowledge of placental function and provide clinical utility. Our objective was to study the relationship between pregnenolone concentrations with gestational age in naturally conceived healthy pregnancies. Methods: We conducted an observational cross-sectional study in an urban, public tertiary care prenatal clinic of low-risk obstetric patients with naturally conceived singleton gestations by comparing gestational age with serum concentration of maternal pregnenolone. Results: Pregnenolone was detected in all specimens collected from 85 patients between 7 4/7 weeks to 38 5/7 weeks gestation with a concentration range of 33 ng/dL to 476 ng/dL. Concentrations increased significantly as gestational age progressed, with a correlation coefficient of 0.789 ($P < 0.001$). There was no association found between patient chronological age and pregnenolone concentration. Conclusion: Maternal serum pregnenolone is measurable throughout pregnancy and is positively correlated with gestational age.

Keywords: Pregnenolone, placenta, pregnancy serum biomarker

Introduction

Production of pregnenolone is the first reaction in the process of placental progesterone steroidogenesis. The conversion takes place in syncytiotrophoblast mitochondrial membranes via cholesterol side-chain cleavage enzyme P450scc in an NADPH dependent reaction [1]. The formation of all other steroid molecules relies on the presence of this obligate precursor. We have previously demonstrated significant stepwise increases in maternal serum pregnenolone concentrations throughout the first trimester of oocyte donation pregnancies. Because these pregnancies advance in the absence of the corpus luteum, increasing pregnenolone must be a product of ongoing trophoblast development [2]. A more thorough understanding of the changes in pregnenolone production throughout the entire duration of pregnancy may help expand our knowledge of placental development and serve as an indicator of placental function and sufficiency, thereby also giving insight into the metabolic status of the fetus. In this study, we sought to determine 1) whether pregnenolone production is measurable in maternal serum throughout the term of a gestation, and 2) if pregnenolone production increases with gestational age.

Material and methods

This is an observational cross-sectional study of low risk, naturally conceived pregnancies. Institutional Review Board (IRB) approval was obtained by the [institution removed for blinded review] (IRB #16-00024). After obtaining informed consent, serum samples were collected from patients undergoing routine phlebotomy during scheduled prenatal care clinic visits

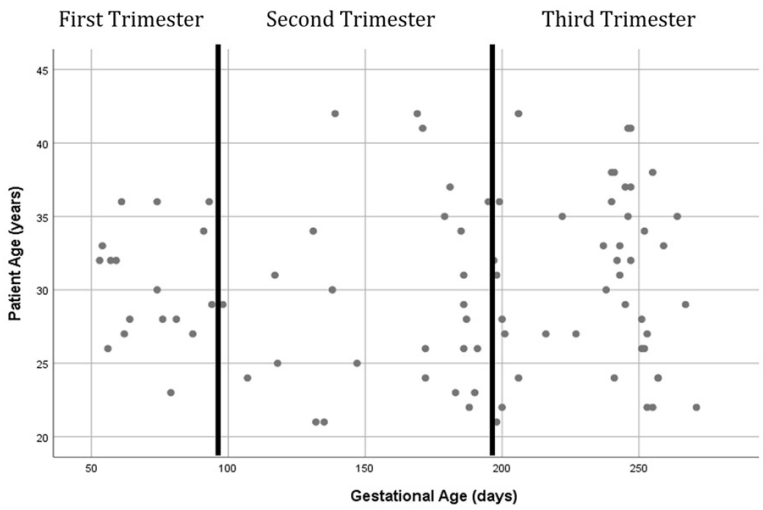

Figure 1. Distribution of patient age according to gestational age.

that occurred from October through December 2017. Inclusion criteria were women between 21 to 55 years of age with naturally conceived singleton gestations. Exclusion criteria were hypertension, diabetes, other major medical comorbidities, and abnormal fetal growth parameters. The majority of patients were recruited from a midwifery clinic at an urban, public, tertiary care center.

Blood samples (5-8 mL) were collected in a serum separator tube (SST) and spun down to separate blood cells from serum. Serum was extracted and stored at -80 Celsius until measurement at the Endocrine Sciences Laboratory, LabCorp. There, stable labeled isotopic pregnenolone was added as an internal standard to serum aliquots. Samples were then extracted, and the extract was purified, evaporated, and reconstituted. An MDS-Sciex API5000 triple quadrupole mass spectrometer, operating in positive ion atmospheric pressure chemical ionization (APCI) mode, was used for detection. Quantification of analyte and the internal standard was performed in selected reaction monitoring mode (SRM). The back-calculated amount of pregnenolone in each sample was determined from duplicate calibration curves generated by spiking known amounts of purified pregnenolone into charcoal stripped human serum from 10 to 2000 ng/dL. Pregnenolone was cleanly separated from progesterone based on differences in masses: there was no cross-reactivity with progesterone or pregnenolone sulfate (preg-s), the neuroactive metabolite of pregnenolone.

The primary outcome variable was maternal serum pregnenolone quantity. A correlation coefficient was used to measure its association with gestational age. The only patient data points that were collected were patient age, gestational age, and pregnenolone concentrations. Patients were not followed serially, and no additional information was collected. Statistical analysis included Spearman's rank correlation coefficients for continuous variables and Kruskal-Wallis for continuous non-parametric analysis. Statistical values of $P < 0.05$ were considered statistically significant.

Results

Samples from eighty-five unique patients were collected and evaluated. Individual demographic data was not collected, as per the study protocol. The patients were recruited from a population that is 60% Hispanic, 15% Non-Hispanic Black, and 25% Asian, Non-Hispanic White, or unspecified, with 90% of patients on Medicaid or Medicaid-based HMOs. Patient ages ranged from 21 to 42 years, with a mean age of 30.2 years (± 5.7 years) (**Figure 1**; **Table 1**).

Gestational ages ranged from 7 4/7 weeks to 38 5/7 weeks with 20% of data points occurring in the first trimester (less than 14 weeks), 31% in the second trimester (14 to 27 6/7 weeks) and 49% in the third trimester (28 weeks and above). Pregnenolone was detected in all specimens with a range of 33 ng/dL to 476 ng/dL. The mean pregnenolone concentration was 67.8 ng/dL (± 24 ng/dL) for the first trimester, 125.8 ng/dL (± 52 ng/dL) for the second trimester, and 227.2 ng/dL (± 82 ng/dL) for the third trimester. Concentrations increased significantly as gestational age progressed, with a correlation coefficient of 0.789 ($P < 0.001$) (**Figure 2**). Maternal age did not correlate with pregnenolone concentrations (correla-

Table 1. Patient age by trimester

	Trimester 1	Trimester 2	Trimester 3
N	17	26	42
Mean Age	30.41176	29.42308	30.69048
Standard deviation	3.857765	6.463269	5.882956

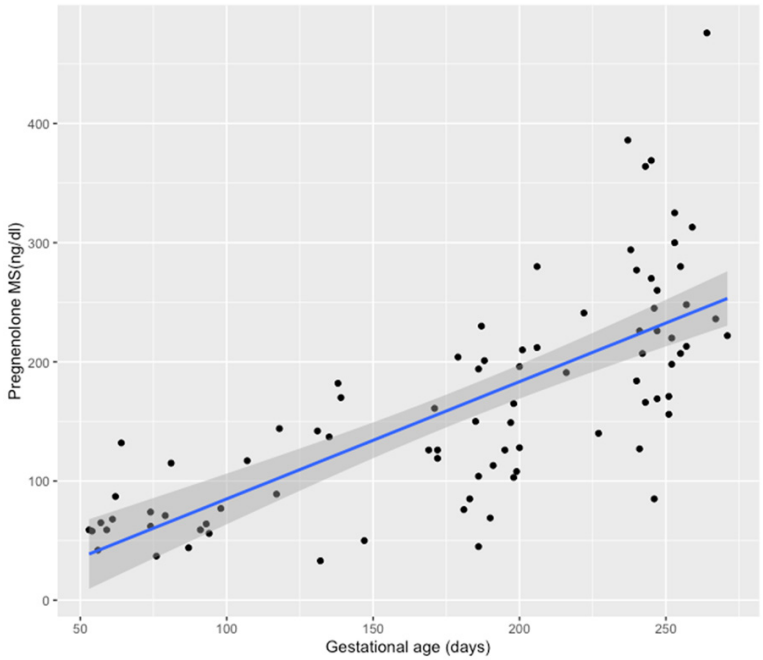

Figure 2. Positive correlation seen with pregnenolone levels and increaasing gestational age, correlation coefficient of 0.789.

tion coefficient of 0.110, P = 0.316), nor with the initial volume of serum (correlation coefficient of 0.126, P = 0.249).

Discussion

This report demonstrates a successful measurement of maternal serum pregnenolone throughout pregnancy, with results showing a significant positive correlation with gestational age. Previous work examining the relationship between pregnenolone concentrations and gestational age has been conflicting, leaving this question open to additional investigation. Early reports from Little et al. in 1971 indicated pregnenolone concentrations do increase with gestational term during pregnancy, but they utilized a small sample population and used a technically challenging assay that required the injection of radiolabeled isotopes to measure plasma concentrations [3]. These characteristics of their study make their results hard to apply to broader practice. Two additional studies, both reported in Japanese but with English abstracts, reported conflicting outcomes regarding whether pregnenolone concentrations increase with gestational term in pregnancy, leaving this question open to additional investigation [4, 5].

In this study, we utilized a much larger sample size, and more easily and precisely assessed pregnenolone concentrations using mass spectrometry. This improved assay increases reporting accuracy by reducing cross-reactivity and allows for substrate detection in smaller sample volumes. We believe that these improvements in our study design allow more seamless transference of our results to the clinical treatment of patients.

There exist several important potential clinical applications that give practical relevance to our results. Prior studies demonstrate an increased concentration of pregnenolone in the *serum* of preeclamptic women at term, without measurement of prior concentrations in the pregnancy [6]. Other studies suggest a decrease in *placental* pregnenolone concentrations in preeclamptic patients [7]. Finally, a possible link between pregnenolone concentrations and intrauterine growth restriction (IUGR) has been suggested [8]. These diagnoses can be diagnostically challenging when confounded by lack of accurate dating or medical co-morbidities, and an additional objective clinical biomarker would prove to be invaluable in those situations.

Previously documented non-pregnancy pregnenolone values range from 33-248 ng/dl according to the Mayo Clinic Laboratories [9]. While there appears to be some overlap between extreme pregnancy and non-pregnancy pregnenolone concentrations, we show a clear linear trend that occurs with increasing

gestational age. The potential clinical utility of this assay, then, will lie in comparing the absolute value as well as the trend in multiple discrete data points over a patient's gestation.

The most intuitive theoretical explanation for the elevation in serum pregnenolone concentration with gestational age is that it is a result of the increased metabolic output produced by the growing placenta as pregnancy progresses [10]. In addition, it has been shown that the P450 system becomes more efficient as pregnancy progresses [11], and that uterine artery blood flow increases linearly throughout gestation which increases the delivery of substrates, including maternal cholesterol [12]. While the conversion of fetal pregnenolone sulfate to maternal pregnenolone in the placenta is possible [13, 14], this would be expected to have a negligible effect on the concentrations we obtained [15].

There are limitations and potential for expansion with our work. All of our samples were collected from unique patients, limiting our ability to trend patient-specific pregnenolone concentrations across an individual pregnancy. The lack of more comprehensive patient demographics and characteristics also prevents correlation of other factors (such as race or BMI) with pregnenolone. Further research is necessary to quantify the rate of pregnenolone concentration rise in relation to both of these variables. There are also several areas of clinical importance, as we have discussed previously, that likely are related to pregnenolone concentrations and would benefit for additional research. Broadly speaking, our findings call for the further study of pregnenolone in normal and abnormal pregnancies.

In conclusion, maternal serum pregnenolone, the first reaction in placental steroidogenesis, is easily measurable and rises with advancing gestational age. This trend suggests further study of pregnenolone as a marker of placental competency and fetal well-being.

Acknowledgements

The authors would like to acknowledge Endocrine Sciences Laboratory, LabCorp and the Midwifery service at our institution for assisting with recruitment.

Disclosure of conflict of interest

None.

Address correspondence to: Dr. Jacquelyn Shaw, New York University Langone Fertility Center, Department of Obstetrics and Gynecology, Division of Reproductive Endocrinology and Infertility, 660 First Avenue, 5th Floor, New York, NY 10016, USA. Tel: 212-263-0039; E-mail: jacquelyn.shaw@nyulangone.org

References

[1] Fraichard C, Bonnet F, Garnier A, Hebert-Schuster M, Bouzerara A, Gerbaud P, Ferecatu I, Fournier T, Hernandez I, Trabado S and Guibourdenche J. Placental production of progestins is fully effective in villous cytotrophoblasts and increases with the syncytiotrophoblast formation. Mol Cell Endocrinol 2020; 499: 110586.

[2] Licciardi F, Tan O and Oh C. Using the oocyte donation model to identify early trophoblast pregnenolone production. J Assist Reprod Genet 2013; 30: 699-702.

[3] Little B, Billiar RB, Halla M, Heinsons A, Jassani M, Kline IT and Purdy RH. Pregnenolone production in pregnancy. Am J Obstet Gynecol 1971; 111: 505-514.

[4] Kawamura E, Hashino M, Akiyama T, Kosaki T, Yanaihara T, Nakayama T, Mori H and Yamaguchi T. Serum concentrations of delta 5C21 steroids during pregnancy and at delivery. Nihon Naibunpi Gakkai Zasshi 1989; 65: 42-54.

[5] Suzuki H, Hashino M, Yanaihara T and Nakayama T. Changes in maternal plasma levels of C21 and C19 steroid hormones during pregnancy. Nihon Sanka Fujinka Gakkai Zasshi 1988; 40: 575-582.

[6] Moon JY, Moon MH, Kim KT, Jeong DH, Kim YN, Chung BC and Choi MH. Cytochrome P450-mediated metabolic alterations in preeclampsia evaluated by quantitative steroid signatures. J Steroid Biochem Mol Biol 2014; 139: 182-191.

[7] Shin YY, Jeong JS, Park MN, Lee JE, An SM, Cho WS, Kim SC, An BS and Lee KS. Regulation of steroid hormones in the placenta and serum of women with preeclampsia. Mol Med Rep 2018; 17: 2681-2688.

[8] Maruyama S, Suzuki T, Yanaihara T, Araki H and Nakayawa T. C21-steroid profiles in pregnancy associated with intrauterine growth retardation. Nihon Sanka Fujinka Gakkai Zasshi 1986; 38: 513-519.

[9] Laboratories MC. Pregnenolone, Serum. mayocliniclabs.com: Mayo Clinic Laboratories; 2023.

[10] Leon RL, Li KT and Brown BP. A retrospective segmentation analysis of placental volume by magnetic resonance imaging from first trimester to term gestation. Pediatr Radiol 2018; 48: 1936-1944.
[11] Simpson ER and Miller DA. Cholesterol side-chain cleavage, cytochrome P450, and iron-sulfur protein in human placental mitochondria. Arch Biochem Biophys 1978; 190: 800-808.
[12] Konje JC, Kaufmann P, Bell SC and Taylor DJ. A longitudinal study of quantitative uterine blood flow with the use of color power angiography in appropriate for gestational age pregnancies. Am J Obstet Gynecol 2001; 185: 608-613.
[13] Pasqualini JR. Enzymes involved in the formation and transformation of steroid hormones in the fetal and placental compartments. J Steroid Biochem Mol Biol 2005; 97: 401-415.
[14] Mickan H and Zander J. Pregnanolones, pregnenolone and progesterone in the human feto-placental circulation at term of pregnancy. J Steroid Biochem 1979; 11: 1461-1466.
[15] Tal R and Taylor HS. Endocrinology of Pregnancy. In: Feingold KR, Anawalt B, Blackman MR, Boyce A, Chrousos G, Corpas E, de Herder WW, Dhatariya K, Dungan K, Hofland J, Kalra S, Kaltsas G, Kapoor N, Koch C, Kopp P, Korbonits M, Kovacs CS, Kuohung W, Laferrère B, Levy M, McGee EA, McLachlan R, New M, Purnell J, Sahay R, Shah AS, Singer F, Sperling MA, Stratakis CA, Trence DL, Wilson DP, editors. Endotext. South Dartmouth (MA): MDText.com, Inc. Copyright © 2000-2023, MDText.com, Inc.; 2000.