# Comparison of reference measures of gestational age with urinary hCG Michael Zinaman MD<sup>1</sup>, Phillip Buchanan PhD<sup>2</sup>, Sarah Johnson PhD<sup>3</sup>, John Larsen MD<sup>2</sup>, Sonya Godbert CStat<sup>3</sup>

## 1Tufts University School of Medicine, Boston, USA; 2 The George Washington, DC and Fetal Medicine Foundation USA, Dayton, Ohio, USA; 3 SPD Development Company Limited, Bedford, UK.

## ABSTRACT

Introduction: The daily levels of early pregnancy urinary human chorionic gonadotrophin (hCG) could provide information on pregnancy progression; however, most published studies suffer from using last menstrual period (LMP) as the reference for dating of pregnancy, leading to broad value ranges. We studied daily urinary hCG levels stratified by two accurate references for pregnancy duration; first trimester Crown Rump Length (CRL) measurement and luteinizing hormone (LH) surge as a marker for ovulation, and LMP.

Methods: Women were recruited preconception, in five US sites, to collect daily urine samples. The LH surge day was determined. Gestational age was also calculated by ultrasound at 11<sup>+0</sup> to 13<sup>+6</sup> weeks for CRL measurement, using standardized methodology. Urine hCG values from 153 single, viable pregnancies were evaluated.

**Results:** There was a highly consistent rise in daily hCG concentration. Extent of the 10<sup>th</sup>-90<sup>th</sup> centile range had least variability using LH surge as reference, and most variability when LMP was used. Median daily concentrations were very similar when LH surge and LMP were used as reference. With ultrasound as a reference, median hCG concentration was lower on each day. This was due to pregnancy duration being on average 3 days more when calculated by ultrasound compared to LH

**Conclusions:** Daily urinary hCG is highly consistent between women, when day of pregnancy is referenced appropriately; thus it can be considered an independent measure of pregnancy duration. Pregnancy duration by LH surge and ultrasound correlate well, but are not identical. LMP is a poor

## INTRODUCTION

Accurate information on gestational age (GA) is of great importance to clinical care.

- Last menstrual period (LMP) is usually the only information available, but is frequently inaccurate because:
- o It assumes ovulation on day 14, which is not true for most women; ovulation leading to conception has been seen on days 9-30<sup>1</sup>
- o Many women have poor recollection of their LMP, especially in unplanned pregnancy<sup>2</sup>
- o Early pregnancy bleeding, recent hormonal contraception usage, breast-feeding, or recent miscarriage/pregnancy make LMP unreliable
- Day of fertilization provides an accurate GA assessment. Ovulation usually occurs 24-36 hours after the luteinizing hormone (LH) surge<sup>3</sup> and as human eggs survive <1 day, LH surge +1 day can be assumed as day of fertilization. Unfortunately, such information is not usually available in natural conceptions
- Later in pregnancy, ultrasound can provide a reliable GA estimate, e.g. Crown Rump Length (CRL) measured by standardized methodology can provide an estimate within  $\pm 5$  days<sup>4</sup>
- Human chorionic gonadotrophin (hCG) levels rise exponentially and predictably during early pregnancy and appear to relate to pregnancy duration;<sup>5</sup> however, evidence on the utility of hCG to provide a GA estimate has often been confounded by the use of LMP as GA reference.

No direct comparison of these different methods for estimating GA has been published in a single study, nor have these methodologies been used to examine the utility of urinary hCG in dating pregnancy.

## METHODS

This was a multi-center (Chicago, Atlanta, Minneapolis, San Antonio, Dallas), prospective study, approved by Quorum Review Institutional Review Board (clinical trial: NCT01077583). Women aged 18-45 years were recruited preconception. They were required to:

- Keep a daily menstrual cycle diary
- Collect daily urine samples from LMP until 30 days after their expected period (EP) if pregnant (volunteers who did not become pregnant continued to collect for up to three menstrual cycles)
- Have a scan at 11<sup>+0</sup>-13<sup>+6</sup> weeks after their LMP to measure fetal CRL under controlled conditions,<sup>6</sup> with central reader verification of scan quality.

Quantitative LH (to determine surge day) and hCG testing was conducted using a validated quantitative automated immunoassay system (Auto DELFIA, Perkin Elmer).<sup>7</sup> The day of pregnancy was determined using three reference methods, on the date each sample was provided (aligned using day of EP). For LMP, day of EP was calculated as LMP + normal cycle length and for ovulation day it was calculated as LH surge +15 days. To calculate the EP date using ultrasound, the GA by Hadlock formula<sup>4</sup> (-14 days) was extrapolated back to the sample date. The median and 10<sup>th</sup> and 90<sup>th</sup>centiles of daily urinary hCG levels were calculated for each day of pregnancy with respect to EP, using the three reference methods. SAS version 9.2 was used for the statistical analysis.

## RESULTS

Cycle samples were available for analysis from 153 pregnancies. The key demographics of these volunteers are summarized in Table 1.

### Table 1. Study population demographics.

Age, years	
Mean (SD)	30.4 (4.0)
Median (range)	30.0 (20.0-40.0)
Ethnicity, n (%)	
White	131 (85.6)
Hispanic or Latino (subset of white)	12 (7.8)
Asian	7 (4.6)
Black or African American	10 (6.5)
Native Hawaiian/other Pacific Islander	0 (0.0)
American Indian or Alaska Native	2 (1.3)
Mixed	3 (2.0)
Self-reported menstrual cycle length (days)	
Mean (SD)	29.6 (2.9)
Range	22-39
Previous pregnancies, n (%)	
0	60 (39.2)
1	55 (35.9)
2	27 (17.6)
≥3	11 (7.2)

#### SD, standard deviation

- There was excellent correlation between GA determined by ultrasound (CRL measurement using the Hadlock formula) and ovulation day (LH surge +1 day; Figure 1)
- An approximately normal distribution is observed when the number of days difference between the two methods calculated for an individual woman are examined, centered around the day of most agreement, with a range of  $\pm 6$  days. However, GA estimate was on average 3 days more by ultrasound
- The relationship between both ovulation day and ultrasound to LMP was more variable. The spread in range of days difference was  $\pm 9$  days for LMP compared with ovulation day (agreement centered on 0 days difference) and  $\pm 11$  days for ultrasound; with ultrasound, again, providing estimates that were on average 3 days more.



Figure 1. Plot of difference in estimation of gestational age by (a) ultrasound vs. ovulation day, (b) ultrasound vs. LMP and (c) LMP vs. ovulation day.

- There was a consistent rise in daily hCG concentration when levels of hCG were compared with GA using all three reference methods (Table 2, Figure 2)
- The extent of the 10<sup>th</sup>-90<sup>th</sup> centile range varied; least variability was apparent when using ovulation day, and most variability when using LMP, as reference methods
- Median daily concentrations of hCG were very similar when ovulation day and LMP were used as reference estimates of GA
- When ultrasound was the reference, median hCG concentration was lower on each day, due to GA being on average 3 days more when calculated by ultrasound, compared with ovulation day
- No differences in hCG profiles were observed in results obtained for women from different ethnic groups.

Table 2. Levels of hCG in relation to duration of pregnancy estimated by LH surge, LMP and ultrasound (CRL measurement).

Pregnancy duration	Median hCG (mIU/mL) (10 <sup>th</sup> , 90 <sup>th</sup> centile) relative to reference methods		
(from EP)	LH Surge	LMP	Ultrasound
-2	38 (12, 97)	42 (1, 356)	6 (0.4, 30)
0	123 (44, 324)	145 (5, 805)	20 (3, 85)
2	328 (120, 781)	268 (11, 1682)	55 (8, 201)
4	820 (277, 1711)	660 (47, 3559)	167 (44, 637)
6	1511 (567, 5673)	1450 (119, 5769)	490 (105, 1243)
10	6397 (2227, 16585)	5820 (1092, 20218)	3122 (1051, 8082)

CRL, crown rump length; EP, estimated period; hCG, human chorionic gonadotrophin; LH, luteinizing hormone; LMP, last menstrual period.



Figure 2. Level of urine hCG vs. duration of pregnancy (time since fertilization) as determined by (a) day of ovulation (LH surge), (b) LMP and (c) ultrasound (CRL measurement using the Hadlock formula).

The hCG concentrations related to median levels at the week boundaries (9, 150 and 2600 mIU/mI) were used to classify pregnancy duration into weeks categories, 1-2, 2-3 and 3+ weeks (since ovulation). Beyond 3 weeks, the hCG trajectory begins to plateau; therefore further classifications are inaccurate.

Using these boundaries, a modeling exercise was conducted to determine whether pregnancy duration category of the samples had been correctly assigned, when compared with the GA reference using ovulation day. The following percentage correct classification of results by week was seen:

- 95.9% of 1-2 weeks
- 93.4% of 2-3 weeks
- 95.2% of 3+ weeks.

This illustrates a good base ability of urinary hCG to provide an estimate of pregnancy duration.

## CONCLUSIONS

- Daily urinary hCG is highly consistent between women, when day of pregnancy is referenced appropriately
- Therefore hCG can be considered an early, accurate measure of pregnancy duration
- Pregnancy duration by LH surge and ultrasound correlate well, but are not identical due to a systematic bias<sup>8</sup> in the Hadlock formula
- The range in agreement between LH surge and ultrasound indicate that the  $\pm 5$  days variability associated with ultrasound is appropriate
- LMP is a poor reference of gestational age, as highlighted by the increased variability seen when using it as a reference
- hCG can provide an estimate of pregnancy duration in weeks (1-2, 2-3 and 3+ since ovulation) with a high level of accuracy.

## REFERENCES

- 1. Johnson SR et al. CMRO 2009;25:741-8.
- 2. Gardosi J. Ultrasound Obstet Gynecol 1997;9:367-8.
- 3. Behre HM, et al Hum Reprod 2000;15:2478-82.
- 4. Hadlock FP et al. Radiology 1992;182:501-5.
- 5. Johnson SR *et al*. CMRO 2011;27:393-401.
- 6. NHS Fetal Anomaly Screening Program Manual for Ultrasound Practitioners July 2012.
- 7. Johnson SR. Clin Chem 2011 57;10 Suppl. A188.
- 8. Pexsters A et al. Ultrasound Obstet Gynecol 2010;35:650-5.

## **DECLARATION OF INTEREST**

This study was funded by SPD Development Company Ltd., a wholly owned subsidiary of SPD Swiss Precision Diagnostics GmbH. Sarah Johnson and Sonya Godbert are employees of SPD Development Company Ltd., and Michael Zinaman, Phillip Buchanan and John Larsen have received honoraria from SPD Development Company Ltd. for their contribution to this research.





![](_page_0_Picture_70.jpeg)