

ENDOCRINOLOGY AND METABOLISM

A Single Serum Test for Measuring Early
Pregnancy Outcome with High Predictive
Value

*Jaime M. Sutton-Riley**, Sarah A. Khanlian,
Francis W. Byrn and Laurence A. Cole

Department of Obstetrics and Gynecology
University of New Mexico
Albuquerque, New Mexico 87131

* Address correspondence to
Jaime M Riley
University of Missouri – Columbia
463c Life Sciences Center
1201 Rollins Road
Columbia, MO 65211-7310

Abstract

Objectives

Current testing to determine a failing pregnancy requires two separate clinic visits to measure the hCG doubling rate. Diagnosing a failing pregnancy is often done in emergency departments where simplified and accelerated testing methods are needed. Here we investigated hyperglycosylated hCG (hCG-H) for predicting pregnancy failure.

Design and Methods

We studied two independent sets of patient samples collected in the early weeks of gestation. One set was urine samples and the other was serum samples. In all cases, hCG and hCG-H were measured using automated chemiluminescence immunoassays. Concentrations of hCG and hCG-H were plotted on a scattergram and levels in failing pregnancies were compared to those in continuing pregnancies.

Results

Data indicated that a threshold level of hCG-H (13ug/L) in both serum and urine samples defined the concentration below which pregnancies were likely to fail. This cut-off corresponded to 73% detection of failures at a 2.9% false positive rate using serum, and 75% detection at a 15% false positive rate using urine. Using an hCG cut-off that corresponded to the same false positive rates, hCG detected only 42% of failures using serum and 43% of failures using urine.

Conclusions

Our data indicates that hCG-H provides a much more accurate single test than hCG for assessing pregnancy outcome. Compatible with the use of serum or urine samples, a single hCG-H test might provide simpler, faster, and more accurate results for predicting the progress of a pregnancy than standard hCG testing.

Abbreviations

hCG = Human chorionic gonadotropin

hCG-H = Hyperglycosylated human chorionic gonadotropin

ROC = Receiver operating characteristics

POC = Point of care

Introduction

Human chorionic gonadotropin (hCG), a glycoprotein hormone, is detectable during pregnancy in urine and serum as early as the week following implantation (1-6). Maternal concentrations of hCG are commonly used to distinguish between normal and abnormal pregnancies. The concentration of maternal hCG reportedly doubles approximately every 2 days in the first 2 months of a favourable outcome gestation (7-9). Falling serum hCG levels or concentrations that do not double within two days are used to indicate a failing pregnancy, either a spontaneous abortion or an ectopic pregnancy, in the first trimester. hCG levels that at least double over a 2 day period are favourable and indicate a pregnancy that likely will go to term (9).

The reported risks of spontaneous abortion and ectopic pregnancy in the USA are 16% and 2%, respectively (12-15). Currently, for patients at high risk for spontaneous abortion or ectopic pregnancy, physicians use the hCG doubling rate to monitor gestational progress. Early identification of a failing pregnancy, particularly in the case of an ectopic pregnancy, ensures proper care and management of the mother and maximizes the chance of preserving fertility.

The hCG doubling test is burdensome because it requires two visits to the physician's clinic or laboratory within a very specific time window. Additionally, this test has limited sensitivity (reports vary from 62-78%) and a high false-positive rate (26-40%) (10-11). Given the prevalence of failing pregnancies in the USA and the risks associated with ectopic pregnancy, it is important that more accurate and easier to use tests become available to assess the risk of pregnancy failure.

Hyperglycosylated hCG (hCG-H) is an hCG molecule with additional sugar residues on its oligosaccharide side chains (16-18). hCG-H has been shown by multiple authors to be the predominant form of hCG present in serum and urine samples in early pregnancy, during the time when implantation is occurring and the month that follows (19-23). It has been previously shown that significantly lower proportions of hCG-H (vs. hCG) are found in spontaneously aborting and ectopic pregnancies (22-23). Considering the lower concentrations of hCG in

failing pregnancies (7, 8, 10, 11), and the lower proportions of hCG-H (22-23), measurement of hCG-H concentrations may provide an amplified means of detecting failing pregnancies. However, no parameters have been described for measuring hCG-H to differentiate between favourable outcome and pregnancy failures.

Specific antibodies have been generated against hCG-H from a purified urine preparation that was 100% hyperglycosylated (24). This antibody has been highly characterized and has <1% cross reactivity with hCG (25-26). This monoclonal antibody, named B152, is the foundation for all hCG-H assays. An FDA-approved automated chemiluminescence immunoassay for hCG-H is currently available internationally marketed under the name "ITA" for Invasive Trophoblast Antigen (27). This same test should be available in the near future in the USA, marketed under the name "Hyperglycosylated hCG."

This study aimed to establish improved means for evaluating pregnancy outcome. We hypothesized that a quantitative measurement of hCG-H in serum or urine could provide an improved pregnancy outcome test. Using quantitative levels of total hCG and hCG-H in both a collection of early pregnancy urine samples and in a separate collection of early pregnancy serum samples, we compared a new method using a single point cut-off of hCG-H versus hCG. Direct comparison of both methods using identical sample sets allowed us to assess sensitivity, specificity, predictive values, and diagnostic accuracy for this newly proposed method using either hCG-H or hCG.

Methods

Patient Samples, University of New Mexico

This study was approved, and is being actively monitored, by the University of New Mexico Human Research Review Committee (protocol 03-146). All volunteers were patients attending clinics in the Department of Obstetrics and Gynecology at the University of New Mexico Health Science Center, September 2003 through September 2004. In all cases, once a positive pregnancy test was shown (from natural conception), an extra tube of blood was collected at the time of phlebotomy. In this case, pregnancy was confirmed using a point-of-care serum pregnancy test.

Inclusion criteria required patients to be in the 3rd to 8th weeks of gestation (3 weeks 0 days to 8 weeks 6 days since the start of their last menstrual period) on the day of sample collection. All cases were recruited blindly with no selection or elimination of cases. Pregnancies that reach the end of the first trimester will very likely lead to a normal-term delivery, for this study such a pregnancy was considered to be a favourable outcome. Pregnancy failure was characterized either as an ectopic pregnancy or a spontaneous abortion, which are likely identified before the end of the first trimester (7-11). Our study population had no reported pregnancy failures beyond the first trimester.

We examined 120 serum samples from continuously recruited patients attending the clinic. Eighty-seven serum samples were collected from patients having uneventful pregnancies as of the end of the first trimester of gestation. Twenty-nine serum samples were from pregnancies terminating in spontaneous abortion before their 8th week of gestation and 4 samples were from those having ectopic pregnancies. Blood samples (red top vacutainer tubes) were separated after phlebotomy using a desk-top centrifuge (2,000 x g). Serum was collected, placed into a sterile tube, and stored in a -80°C freezer until it was assayed for total hCG and hCG-H.

Patient Samples, Yale University

This study was originally approved by the Yale University Human Investigations Committee (protocol #8340) and then later approved by the University of New Mexico Human Research Review Committee (protocols 99-349 and 03-146). All volunteers were patients attending clinics in the Department of Obstetrics and Gynaecology at Yale University in 1999. At Yale University, once an enrolled patient had a positive pregnancy test (from natural conception) a tube of urine was collected at the clinic, whenever possible, from the 4th to the 7th weeks of pregnancy (4 weeks 0 days to 7 weeks 6 days since the start of their last menstrual period). Pregnancy was confirmed in all cases using serum hCG point-of-care tests. All cases were recruited blindly with no selection or elimination of cases. A total of 167 cases were recruited, of which 138 had favourable outcomes (reaching term deliveries). Twenty cases had spontaneous abortion in the first trimester, and 8 were ectopic pregnancies.

Urine samples were frozen at -20 °C following collection and transferred daily to a -80 °C freezer. Samples were transferred on dry ice from Yale to the University of New Mexico where they were further stored at -80 °C. Samples were thawed for the first time and assayed for total hCG and hCG-H at the University of New Mexico for this study.

Laboratory Tests

All laboratory testing was performed in the USA hCG Reference Service Laboratory at the University of New Mexico Health Science Center in 2004. This laboratory is certified by the Department of Health and Human Services for running clinical tests (CLIA ID# 32D0972561). The consistency of laboratory tests is monitored by the College of American Pathologists (CAP #7176750-01). The hCG-H test used was an immunometric assay produced by Nichols Institute Diagnostics (NID), a division of Quest Diagnostics, (San Clemente, CA) for their chemiluminescence Nichols Advantage automated platform. The total hCG assay used is produced by Diagnostic Products Corporation (DPC; Los Angeles, CA) for

their series of chemiluminescence Immulite automated platforms. The DPC Immulite total hCG assay detects hCG, hCG-H and free β -subunits on an equal basis (18). As previously published, both tests can be used with serum and urine samples without a matrix effect (26-27).

All quality control upkeep was performed by the laboratory technicians who performed the actual assays at the University of New Mexico center. The DPC Immulite has an intra-assay precision of approximately 4.7% CV at a mean concentration of 7.7 $\mu\text{g/L}$; while the NID Advantage has an intra-assay precision of approximately 5.7% CV at a mean concentration of 17.6 $\mu\text{g/L}$ (values published in user manuals and confirmed by in-house QC maintenance routines).

Samples were retrieved from the freezer and thawed for the first time in a water bath. Serum and urine samples were both diluted, when necessary, with the provided DPC and NID assay diluents. All testing was carried out on the automated assays using pre-formulated reagent packs and following manufacturers' guidelines. We then calculated hCG-H as a proportion of total hCG using the following formula: ($\mu\text{g/L hCG-H} \div \mu\text{g/L hCG}$).

Data Analysis

Total hCG and hCG-H data for all samples was entered into a Microsoft Excel 2003 spreadsheet (Microsoft Inc., Redmond WA). True positive and corresponding false positive rates for specific cut-off values were calculated. Predictive values were calculated in Microsoft Excel assuming that 16% of clinically-recognized pregnancies end in spontaneous abortion and 2% in ectopic pregnancy in the USA (12-15). Receiver operating characteristics (ROC) curves were plotted and the area under the curve, 95% confidence intervals, standard errors (SE), and multiple ROC curve structural components (significant statistics) were calculated using AccuROC software, version 2.4 (Accumetric Corp., Montreal, QC). To illustrate the diagnostic efficiency of a 13 $\mu\text{g/L}$ cut-off, scattergrams of hCG-H concentrations against gestational age were plotted using Sigma Plot software, version 9.0 (Systat Inc., Richmond CA)

Results

As shown in Table 1, serum and urine hCG concentrations increased exponentially with increasing gestational age. Median serum hCG results were consistently higher than median urine hCG results, while the percent hCG-H in urine samples was consistently higher than that in serum samples. No correlation was observed between rising hCG and hCG-H levels ($r^2 = 0.19$). However a correlation did exist, from the 4th – 7th weeks of gestation, between logarithms of hCG results in serum and in urine ($r^2 = 0.95$). A linear correlation was found between serum and urine hCG-H levels ($r^2 = 0.97$). The median hCG concentration increased an average of 9.1-fold per week in serum and 8.2-fold per week in urine samples. In comparison, the median hCG-H concentration increased by an average of 2.3-fold per week in serum and 1.6-fold per week in urine.

Serum Samples

Individual serum hCG-H concentrations from patients between the 5th- 8th complete weeks of gestation were plotted by gestational age (Figure 1). A pivotal point is visually apparent in the scattergram at 13 $\mu\text{g/L}$ (Figure 1). Using 13 $\mu\text{g/L}$ as a cut-off, the hCG-H test detected 73% of pregnancy failures at a 2.9% false positive rate (Table 2). This corresponded to a predictive value positive (prediction of failures) of 85%.

We examined hCG concentrations in the serum samples from the same patients. We compared the hCG data with the hCG-H results using the same false positive rate (2.9%). At this false positive rate hCG detected only 42% of failures; the predictive value positive was 76% (Table 2). An ROC curve was plotted comparing all combinations of detection rates and false positive rates for hCG-H and hCG. The area under the ROC curve for hCG-H was 0.88 ± 0.003 (mean \pm SE) and 0.71 ± 0.006 for hCG (Table 2). The accuracy from measuring serum hCG-H was significantly different than the accuracy from measuring serum hCG, and it offered a significant improvement for detecting failures ($P < 0.00005$).

Urine Samples

Individual hCG-H concentrations were also compared in urine samples from patients between the 4th - 7th weeks of gestation. Figure 2 is a scattergram of this data, with urine hCG-H results plotted according to gestational age. An ROC curve was plotted comparing all combinations of detection and false positive rates. The 13µg/L cut off used for serum was also a clear discriminatory point in the urine hCG-H scattergram (Figure 2). Considering the close correlation between concentrations of hCG-H in serum and urine (Table 1), the same cut-off point seemed appropriate. Using 13 µg/L as a cut-off, the hCG-H test detected 75% of pregnancy failures at a 15% false positive rate. This corresponded to a predictive value positive (prediction of failures) of 52%. The area under the ROC curve was 0.83 ± 0.049 (Table 2).

We examined hCG concentrations in these same urine samples. We compared hCG-H data with hCG results using the same false positive rate (15%). At this false positive rate, hCG detected 43% of failures; the predictive value positive was 39% (Table 2). The area under the ROC curve was 0.74 ± 0.047 (Table 2). Again, a significant improvement in accuracy was observed by measuring hCG-H rather than hCG for the detection of failures, $P=0.00005$.

Discussion

Approximately 16% of clinically-recognized pregnancies end in spontaneous abortion and 2% end in ectopic pregnancy in the USA each year (12-15). Early identification of pregnancy failure, as well as identification of patients who are at high risk for pregnancy failure, is important in order to provide appropriate clinical care and counselling. Currently, the primary biochemical test used to predict or identify pregnancy failure is the hCG two-day doubling test. Not only does this test literally take days to produce results, it also has a high false positive rate and a predictive value of only 35% for detecting pregnancy failure (10-11). A reliable biochemical test that could identify a high risk pregnancy in the presence of an identifiable uterine foetal sac would prepare patients and clinicians for a possible spontaneous abortion. Perhaps more importantly, a reliable biochemical test that could identify a high risk pregnancy in the absence of an identifiable uterine foetal sac could predict ectopic pregnancy and early therapy could be administered preventing an emergency situation, possible life-threatening peritonitis, and preserving fertility.

As shown by our data, hCG values increase exponentially in the 5th-8th weeks of gestation. The widely changing values make it difficult to identify a specific cut-off for hCG for detecting failing pregnancies during this period, thus the development of the 2 day doubling test (7, 9, 13, 15). This test, however, is a cumbersome test, requiring multiple visits to the physician's clinic and laboratory. Previous studies have evaluated alternate methods for using hCG to predict pregnancy outcome with only small improvements in accuracy and ease-of-use (28).

We questioned the relationship between serum and urine results. Previous reports show that serum and urine concentrations of hCG and hCG-H change proportionally with advancing gestational age, and serum and urine concentrations closely correlate with each other in normal and abnormal pregnancies (19-23). Results presented here show a clear correlation between early pregnancy serum

and urine concentrations of both hCG and hCG-H. This allowed us to confidently continue our analysis using both serum and urine samples

We investigated hCG-H in serum and urine and compared hCG-H results with hCG values in the same samples. As shown, hCG-H concentrations increase slowly with advancing gestation relative to exponentially rising hCG concentrations. hCG-H concentrations in serum and urine approximately double every week compared to doubling every 2 days. As such hCG-H results are much more stable with advancing gestation. We investigated the use of a single serum sample and a single urine sample hCG-H measurement as markers for pregnancy outcome.

hCG-H results were compared to hCG values using the same samples at the same false positive rates. The area under and ROC curve is an independent measure which tests the accuracy of a diagnostic application. Examining the data for regular hCG, the ROC area under the curve values were 0.71 ± 0.006 and 0.74 ± 0.047 for serum and urine, respectively. Using hCG-H, however, the ROC area under the curve values were 0.88 ± 0.003 ($P < 0.0005$) and 0.83 ± 0.049 ($P < 0.0005$) for serum and urine, respectively. Clearly, hCG-H provides a much more accurate single test than hCG for assessing pregnancy outcome.

Using serum samples and an hCG-H cut-off of $13\mu\text{g/L}$, 73% of failing pregnancies were detected at a 5% false positive rate. Using hCG and a cut-off corresponding to this same false positive rate, only 42% of failures were detected. Using urine samples and the hCG-H $13\mu\text{g/L}$ cut-off concentration, 75% of failing pregnancies were detected at a 15% false positive rate. Using hCG and a cut-off corresponding to this same false positive rate, only 43% of failures were detected. Using an arbitrary cut-off concentration and fixed false positive rates, we confirm the greater accuracy and utility of hCG-H measurements for predicting pregnancy outcome.

Parallel differences were observed in serum and urine samples, with hCG-H sensitivities of 73% in serum and 75% in urine, and with hCG sensitivities of 42% in serum and 43% in urine. While sensitivities were similar for serum and urine, corresponding false positive rates were approximately 5-fold higher in urine

samples. This is likely due to the wide variation of protein excretion that occurs in urine due to variable liquid intake (5, 19, 29). This variability broadens the range of urine values and subsequently increases the false positive rates. It is very likely that normalizing the urine samples to creatinine concentrations would have decreased the false positive rates associated with the urine samples, possibly making the false positive rates more comparable to that seen with the serum samples.

It should be noted that while the serum samples evaluated here were tested fresh, the urine samples were investigated after 5 years storage at -80°C . We considered the possibility that this compromised the value of the urine results. As published, multiple freezing and thawing can destabilize hCG-H results (30). As also published, storage for multiple years without freezing and thawing does not change hCG or hCG-H results (31). Based on these previous reports, investigating urine samples that were frozen for 5 years and never thawed should not detract from the merit of our results.

We conclude that single point hCG-H determinations are significantly more sensitive than hCG single point measurements for detecting pregnancy outcome. Both serum and urine measurements could be utilized, with serum measurements possibly providing a lower false positive rate and thus a better predictive value. Whether using the serum or urine single-point hCG-H test, both provide a more sensitive and more accurate evaluation of pregnancy progression than a single-point measurement of hCG. While this data seems promising, it should be used as a foundation for other centers to further investigate hCG-H measurements in pregnancy outcome, possibly using the $13\mu\text{g/L}$ cut off used here. It should also be the focus of future studies to determine if this single measurement of hCG-H could replace the currently-used hCG 2-day doubling test. Our data indicates that a single serum measurement of hCG-H could be a vast improvement in simplicity, speed, and predictive accuracy. Undoubtedly, a single-measurement test could be invaluable in emergency clinical situations where patients and clinicians do not have time to wait two days for a predictor of pregnancy failure.

Table 1. Median hCG values (IU/L), median hCG-H values ($\mu\text{g/L}$), and an average of the proportions of total immunoreactivity due to hCG-H ($\%$, $\mu\text{g/L hCG-H} \div \mu\text{g/L hCG}$) in serum and urine samples from different complete weeks of gestation (weeks since last menstrual period).

Week	Serum				Urine			
	Median hCG Values	Median hCG-H Values	Average % hCG-H \pm SD	n	Median hCG Values	Median hCG-H Values	Average % hCG-H \pm SD	n
3 rd	22	6.8	89 \pm 24	n = 5				
4 th	627 ¹	25 ¹	41 \pm 13%	n = 13	529	30 ¹	61 \pm 37%	n = 45
5 th	2816	54	31 \pm 11%	n = 20	2022	54	40 \pm 30%	n = 69
6 th	12144	120	21 \pm 14%	n = 13	2678	58	32 \pm 32%	n = 20
7 th	19690	348	16 \pm 13%	n = 12	13050	130	31 \pm 4%	n = 5
8 th	98615	347	7.0 \pm 5.4%	n = 24				
Total				n = 87				n = 139

¹ Logarithms of urine and serum hCG correlate ($r^2=0.95$), 4th - 7th weeks of gestation. During this same period, serum hCG-H correlates with urine hCG-H ($r^2=0.97$).

Table 2. Utility of hCG (total hCG) and hCG-H for predicting pregnancy failure using 120 serum and 167 urine samples. Arbitrary cut-off values were determined from scattergrams (Figures 1 and 2). False positive rates and detection rates at these cut-off values were calculated and predictive values were determined. ROC statistics were calculated for serum and urine hCG and hCG-H, independent of any cut-off values.

	Serum hCG-H	Serum hCG	Urine hCG-H	Urine hCG
Term outcome pregnancies	n = 87	n = 87	n = 139	n = 139
Cut-off concentration	13 µg/L	125 IU/L	13 µg/L	215 IU/L
Corresponding false positive rate	5%	5%	15.0%	15.0%
Corresponding Detection Rate:	n = 33	n = 33	n = 28	n = 28
a. All failures	24 of 33 (73%)	14 of 33 (42%)	21 of 28 (75%)	12 of 28 (43%)
b. Spontaneous abortions only	20 of 29 (71%)	12 of 29 (41%)	14 of 20 (70%)	9 of 20 (45%)
c. Ectopic pregnancy only	4 of 4 (100%)	2 of 4 (50%)	7 of 8 (88%)	3 of 8 (38%)
Predictive value positive	85%	76%	52%	39%
Area under ROC curve ± SE	0.88 ± 0.003 ¹	0.71 ± 0.006 ¹	0.83 ± 0.049 ²	0.74 ± 0.047 ²
ROC 95% confidence interval	0.83 - 0.99	0.78 - 0.97	0.73 - 0.92	0.65 - 0.83

¹ A significant difference was observed between serum hCG and serum hCG-H area under the ROC curve results (P<0.00005).

² A significant difference was observed between urine hCG and urine hCG-H area under the ROC curve results (P<0.00005).

References

1. Saxena BB, Hasan SH, Haour F, Schmidt-Gollwitzer M. Radioreceptor Assay of human chorionic gonadotropin: Detection of Early Pregnancy. *Science* 1974; 184:793-5.
2. Braunstein GD, Rasor J, Adler D, Danzer H, and Wade ME. Serum human chorionic gonadotropin levels throughout normal pregnancy. *Am J Obstet Gynecol* 1976; 126: 678-681.
3. Gasser RF. 1981. Embryology and fetology. In *Principles and Practice of Obstetrics and Perinatology*, Vol 1, edited by Iffy L, Kaminetzky HA (New York; John Wiley & Sons), pp. 127-80.
4. Lenton EA, Grudzinskas JG, Neal LM, Chard T, Cooke ID. Chorionic gonadotropin concentrations in early human pregnancy: Comparisons of specific and nonspecific assays. *Fertil Steril* 1981; 35: 40-5.
5. Wilcox AJ, Baird DD, Dunson D, McChesney R, Weinberg CR. Natural limits of pregnancy testing in relation to the expected menstrual period. *JAMA* 2001; 286: 1759-61.
6. Wilcox AJ, Weinberg CR, O'Connor JF, Baird DD, Schlatterer JP, Canfield RE, *et al.* Incidence of early loss of pregnancy. *N Engl J Med* 1988; 319: 189-94.
7. Pittaway DE and Wentz AC. Evaluation of early pregnancy by serial chorionic gonadotropin determinations: A comparison of methods by receiver operating characteristic curve analysis. *Fertil Steril* 1985; 43: 529-533.

8. Braunstein GD, Grodin JM, Vaitukaitis J, Ross GT. Secretory rates of human chorionic gonadotropin by normal trophoblast. *Am J Obstet Gynecol* 1973; 115: 447-50.
9. Barnhart K, Sammel MD, Chung K, Zhou L, Hummel AC, Wensheng Gou. Decline of serum human chorionic gonadotropin and spontaneous abortion: Defining the normal curve. *Obstet Gynecol* 2004; 104: 975-81.
10. Liu HC, Davies O, Berkeley A, Graf M, Rosenwaks Z. Late luteal estradiol patterns are a better prognosticator of pregnancy outcome than serial beta-human chorionic gonadotropin concentrations. *Fertil Steril* 1991; 56: 421-6.
11. Cowan BD. Ectopic Pregnancy. *Curr Op Obstet Gynecol* 1993; 5: 328-32.
12. Abrar N. (May 2004) "Miscarriage: The Incidence of miscarriage" Retrieved 12-09-04, from <http://www.ivf-infertility.com>
13. Sepilian V. (November 2004) eMedicine Specialties: Obstetrics and Gynecology. "Ectopic Pregnancy." Retrieved 12-09-04, from <http://www.emedicine.com>.
14. Jones RE. 1991. Pregnancy, Spontaneous Abortion. In *Human Reproductive Biology*, edited by Richard E. Jones. (San Diego, CA, Academic Press, Inc.), pp. 189-228.
15. Heffner LJ. 2001. Pregnancy complications, early pregnancy loss. In *Human Reproduction at a Glance*, edited by Blackwell Sciences editorial offices (Berlin, Germany, Blackwell Science Ltd.). pp. 86-91.

16. Cole LA. The O-Linked Oligosaccharide structure are striking different on pregnancy and choriocarcinoma hCG. *J Clin Endocrinol Metab* 1987; 65: 811-3.
17. Amano J, Nishimura R, Mochizuki M, Kobata A. Comparative study of the mucin-type sugar chains of human chorionic gonadotropin present in the urine of patients with trophoblastic diseases and healthy pregnant women. *J Biol Chem* 1988; 263: 1157-65.
18. Elliott MM, Kardana A, Lustbader JW, Cole LA. Carbohydrate and peptide structure of the alpha- and beta-subunits of human chorionic gonadotropin from normal and aberrant pregnancy and choriocarcinoma. *Endocrine* 1997; 7: 15-32.
19. Cole LA, Khanlian SA, Sutton JM, Davies S, Stephens ND. Hyperglycosylated hCG (invasive trophoblast antigen, ITA) a key antigen for early pregnancy detection. *Clin Biochem* 2003; 36: 647-55.
20. Kovalevskaya G, Birken S, Kakuma T, O'Connor JF. Early pregnancy human chorionic gonadotropin isoforms measured by an immunometric Assay for Choriocarcinoma-like hCG. *J Endocrinol* 1999; 161: 99-106.
21. Kovalevskaya G, Genbacev O, Fisher SJ, Caceres E, O'Connor JF. Trophoblast Origin of hCG Isoforms: Cytotrophoblasts are the primary Source of Choriocarcinoma-like hCG. *Mol Cell Endocrinol* 2002; 194: 147-55.
22. Kovalevskaya G, Birken S, Kakuma T, Osaki N, Sauer M, Lindheim S, *et al.* Differential expression of human chorionic gonadotropin (hCG) Glycosylation Isoforms in failing and continuing Pregnancies: Preliminary characterization of the hyperglycosylated hCG Epitope. *J Endocrinol* 2002; 172: 497-506.

23. O'Connor JF, Elish N, Kakuma T, Schlatterer J, Kovalevskaya G. Differential Urinary gonadotropin profiles in early pregnancy and early pregnancy loss. *Prenat Diagn* 1998; 18: 1232–40.
24. Birken s, Krichevsky A, O'Connor J, Schlattere J, Cole L, Kardana A, Canfield R. Development and characterization of antibodies to a nicked and hyperglycosylated form of hCG from a choriocarcinoma patient: generation of antibodies that differentiate between pregnancy hCG and choriocarcinoma hCG. *Endocrine* 1999; 10: 137-44.
25. Birken S, Yershova O, Myers RV, Bernard MP, Moyle W. Analysis of human choriogonadotropin core 2 o-glycan isoforms. *Mol Cell Endocrinol*; 204: 21-30.
26. Cole LA ,Sutton JM, Higgins TN, Cembrowski GS. Between-method variation in hCG test results, *Clin Chem*, 2004; 50:874-882.
27. Pandian R, Lu J, and Ossolunska-Plewnia J. Fully automated chemiluminometric assay for hyperglycosylated human chorionic gonadotropin (invasive trophoblast antigen). *Clin Chem* 2003; 49: 808-10.
28. Urbancsek J, Hauzman E, Fedorcsak P, Halmos A, Devenyi N, Papp Z. Serum human chorionic gonadotropin measurements may predict pregnancy outcome and multiple gestations after in vitro fertilization. *Fertil Steril* 2002; 78: 540-42
29. Shahabi, S., Rinne, Oz, U.A., Bahado-Singh, R.O., Mahoney, M.J., Omrani, A., Baugarten, A., and Cole, L.A. Serum hyperglycosylated hCG a potential screening test for fetal Down syndrome. *Prenat Diagn*, 1999; 19:488-489.

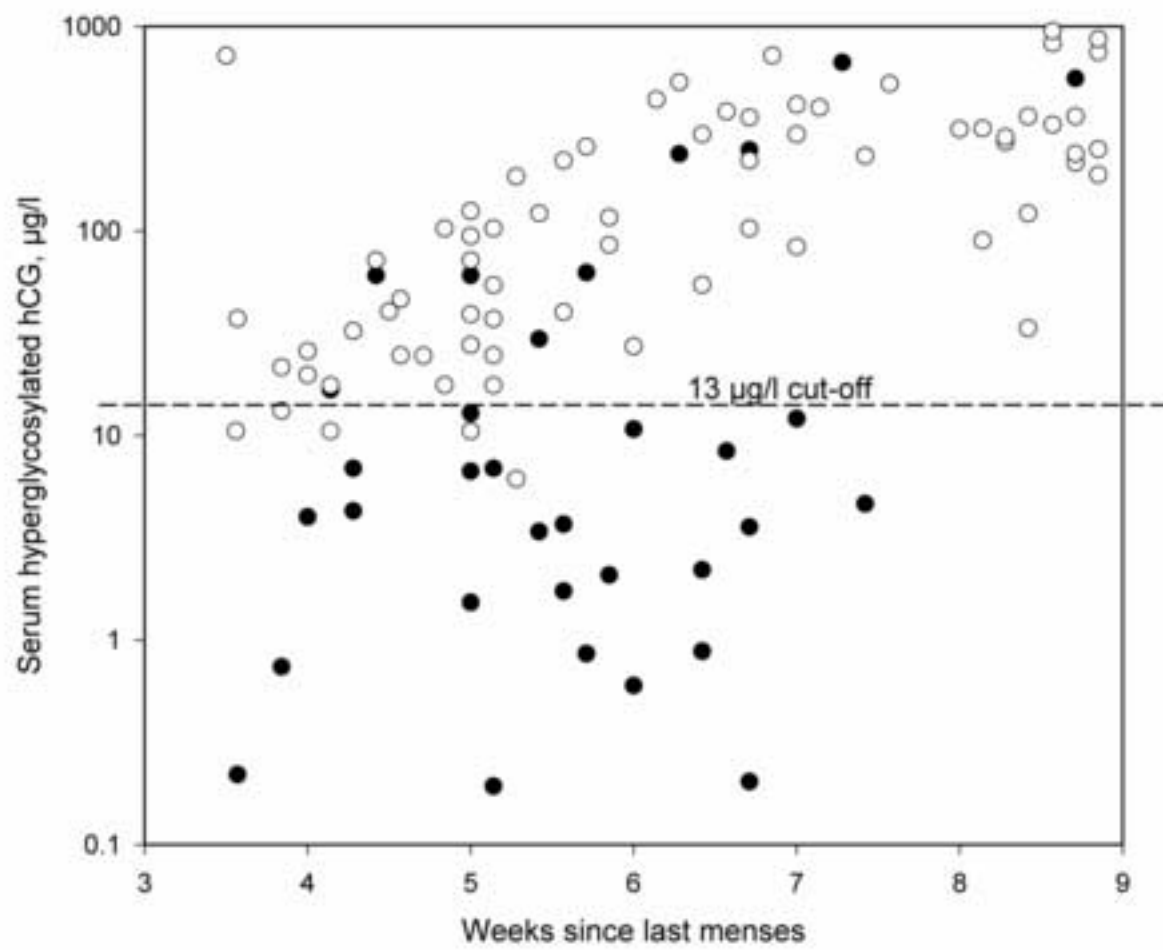
30. Cole LA, Shahabi S, Oz UA, Bahado-Singh RO, Mahoney MJ. Hyperglycosylated hCG (Invasive Trophoblast Antigen) Immunoassay: a New Basis for Gestational Down Syndrome Screening. Clin. Chem. 1999; 45:2109-2119
31. Cole, L.A. Shahabi, S., Rinne, K.M., Oz, U.A., Bahado-Singh, R.O., Mahoney, M.J. Urinary Screening Tests for Fetal Down Syndrome: II. Hyperglycosylated hCG. Prenat Diagn, 1999;19:351-359.

Figure 1. Scatter plot of 120 individual hyperglycosylated hCG serum measurements from normal (○) and failing pregnancies (●) in the 3rd-8th complete weeks of gestation. The horizontal dashed line is drawn at a pivotal point, 13µg/L of hCG-H.

Figure 2. Scatter plot of 167 individual hyperglycosylated hCG urine measurements from normal (○) and failing pregnancies (●) in the 4th-7th complete weeks of gestation. The horizontal dashed line is drawn at 13µg/L of hCG-H.

Figure(s) #1

[Click here to download high resolution image](#)



Figure(s) #2

[Click here to download high resolution image](#)

