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Characterizing urinary hCGβcf patterns during pregnancy

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ABSTRACT

Objective: Elevated concentrations of hCG beta core fragment (hCGβcf) are known to cause false-negative results in qualitative urine pregnancy test devices, but the pattern of urinary hCGβcf during normal pregnancy has not been well characterized. Here, we evaluate the relationship between urine hCG, hCGβcf, and hCG free β subunit (hCGβ) during pregnancy.

Design and methods: Banked second trimester urine specimens from 100 pregnant women were screened for high concentrations of hCGβcf using a qualitative point-of-care device known to demonstrate false-negative results in the presence of elevated hCGβcf concentrations. Additional first and third trimester specimens from the same pregnancy were obtained from 10 women who generated negative/faint positive results, 5 women who generated intermediate positive results, and 10 women who generated strong positive results on the point-of-care device. Intact hCG, hCGβcf, hCGβ, and specific gravity were quantified in these 75 specimens.

Results: Urinary hCGβcf concentrations were greater than intact hCG concentrations at all times. A strong correlation ($r^2 = 0.70$) was observed between urine intact hCG and hCGβcf concentrations. A poor correlation was observed between specific gravity and intact hCG ($r^2 = 0.32$), hCGβ ($r^2 = 0.32$), and hCGβcf ($r^2 = 0.32$). The highest hCGβcf concentrations were observed between 10 and 16 weeks gestation but individual women demonstrated very different patterns of hCGβcf excretion.

Conclusions: Urine specimens with elevated hCGβcf are frequently encountered during pregnancy but hCGβcf excretion patterns are unpredictable. Manufacturers and clinicians must appreciate that hCGβcf is the major immunoreactive component in urine during pregnancy and must design and interpret qualitative urine hCG test results accordingly.

1. Introduction

Human chorionic gonadotropin (hCG) is a well-characterized glycoprotein hormone secreted by the trophoblast cells of the placenta that maintains the corpus luteum and supports fetal growth [1]. Several hCG variants, including intact hCG, nicked hCG (hCGn), free β subunit (hCGβ), and nicked hCGβ (hCGβn), can be detected in both serum and urine while the core fragment of hCGβ (hCGβcf) is detected only in urine [1,2]. Importantly, the concentrations and relative proportions of these variants change throughout pregnancy [2,3].

High concentrations of hCGβcf (≥500,000 pmol/L) have been shown to cause false-negative results in certain pregnancy tests i.e. qualitative point-of-care (POC) hCG devices [4–6]. These pregnancy tests are often used to exclude pregnancy in women subjected to administration of radioactive isotopes for therapeutic or diagnostic purposes. Recent work has demonstrated that the majority of qualitative POC hCG devices are susceptible to false negatives due to high concentrations of hCGβcf [7]. Unfortunately, efforts to predict which women are likely to generate elevated hCGβcf concentrations have been largely unsuccessful [8].

While previous publications have reported mean urinary intact hCG, hCGβcf, and hCGβ concentrations from multiple women during early pregnancy [2] or hCG and hCGβcf concentrations from a single woman throughout pregnancy [1], little has been published about the individual excretion patterns of urinary intact hCG, hCGβcf, and hCGβ in large numbers of women at multiple time points throughout pregnancy. It is unknown whether all women display similar excretion patterns of hCG variants or if the changes in the absolute and relative concentrations of these immunoreactive forms are specific to each woman.

Here, we characterize the relationship between urinary intact hCG, hCGβcf, and hCGβ in first, second, and third trimester specimens from 25 women.

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[2] AMG has served as a consultant and expert witness to Church and Dwight and has received income from Church & Dwight for work that is not a part of this study.

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2. Materials and methods

2.1. Patient samples

One hundred randomly selected second trimester urine specimens were obtained through the Washington University Women and Infant's Health Specimen Consortium (WIHSC), a biobank of specimens from pregnant women. Additional first and third trimester specimens were obtained from 25 of these patients for subsequent analysis. All samples were stripped of identifiers and coded by the WIHSC. Urine specimens were collected during routine physician office visits and were not necessarily first morning collections. Samples were refrigerated within 4 h of collection, aliquoted and frozen within 12 h of collection, and were stored for up to 4 years at −80 °C. Institutional review board approval was obtained for this study.

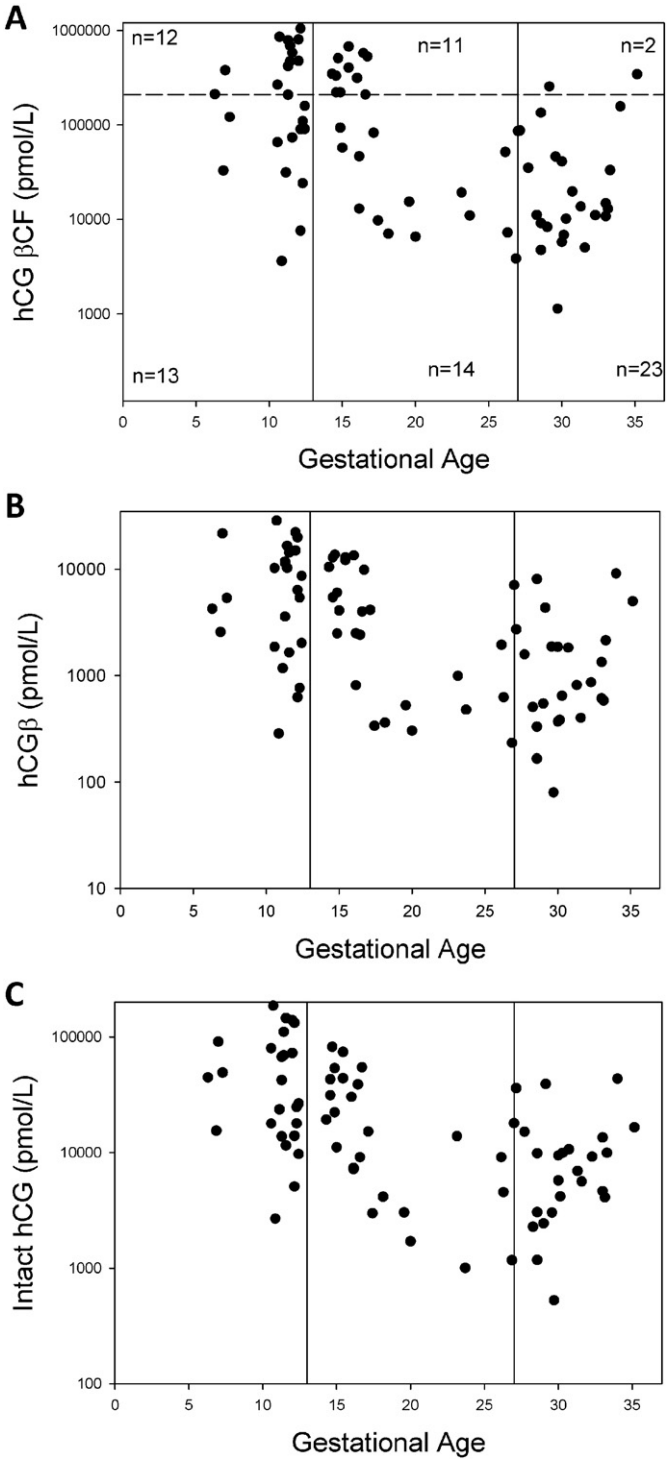

Fig. 1. Urine hCGβcf (A), hCGβ (B) and hCG (C) concentrations from 25 women during pregnancy. Samples were collected during the first, second, and third trimesters. Solid vertical lines represent the trimester cutoffs of 13 weeks and 26 weeks. Dotted horizontal line represents hCGβcf concentration of 209,000 pmol/L. False-negative/low-positive results were observed using the OSOM device at this concentration and above.

2.2. Qualitative hCG measurement

The OSOM POC device (OSOM hCG Combo Test, Genzyme Diagnostics) was used (according to the manufacturer's instructions) as a crude screening device for elevated concentrations of hCGβcf. This device has previously been shown to produce false-negative results at hCGβcf concentrations >500,000 pmol/L [4,7]. One hundred second trimester urine samples were screened and ten urine samples that showed negative/weak positive, five urine samples that showed intermediate positive and ten samples that showed strong positive results were selected for further analysis. This approach was taken to ensure the widest range of hCGβcf concentrations.

2.3. Quantitative hCG, hCGβcf, and hCGβ measurement

Samples were shipped on dry ice and stored frozen at −80 °C for quantitative measurement of intact hCG, hCGβ, and hCGβcf by time-resolved immunofluorometric assays as described previously [9,10]. Briefly, 25 μl of urine sample was incubated with 200 μl assay buffer

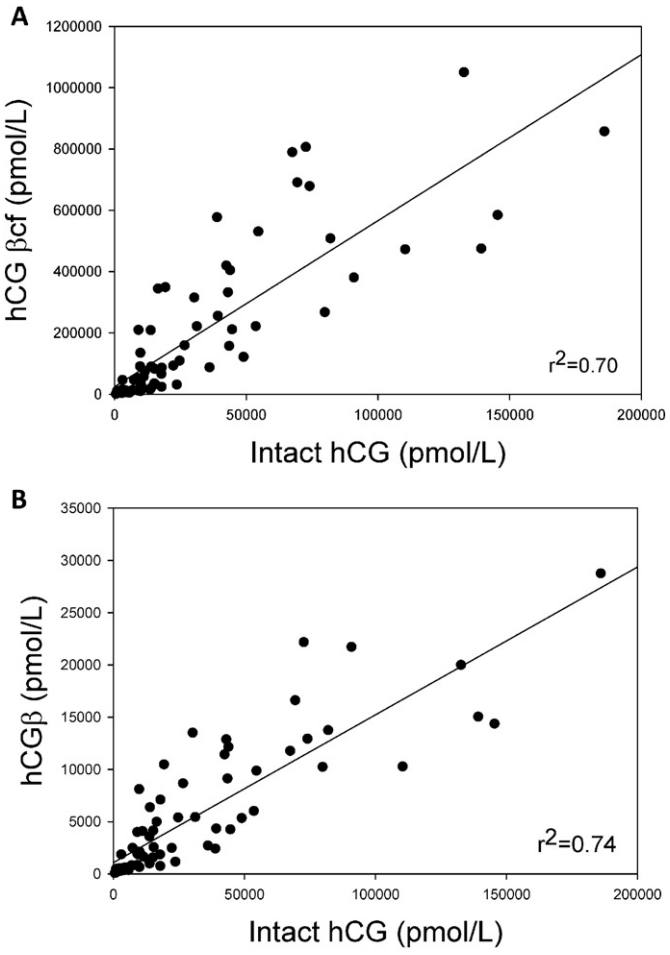

Fig. 2. Correlation between urine hCG and hCGβcf (A) and hCGβ (B) concentrations in 75 samples from 25 pregnant women. Solid line represents line of regression.

for 1 h in antibody-coated microtitration vessels. After washing, 100 ng of Eu-labeled detector antibody was added and incubation continued for 30 min. Time-resolved fluorescence was measured after addition of enhancement solution in a Victor fluorometer [10].

2.4. Measurement of specific gravity

Urine-specific gravity was measured with an ATAGO UG-1 refractometer.

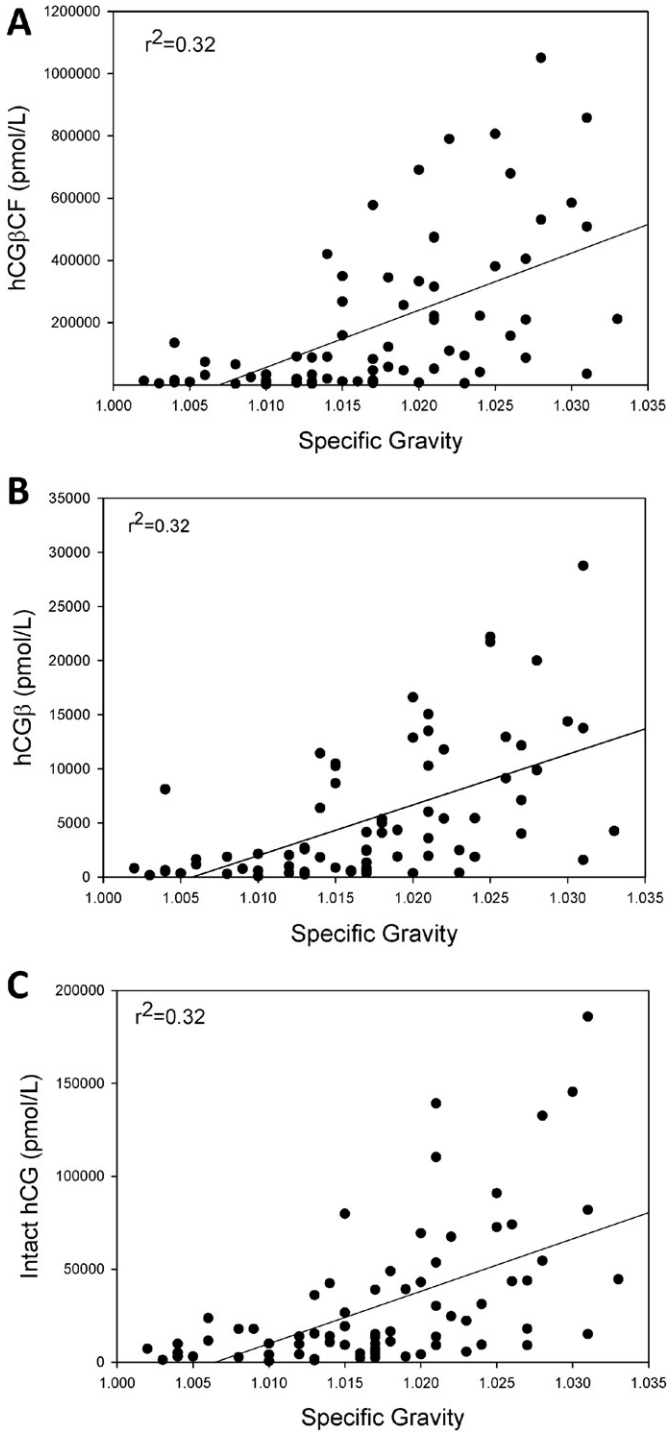

Fig. 3. Correlation between specific gravity and urine hCGβcf (A) hCGβ (B) and hCG (C) concentrations in 75 samples from 25 pregnant women. Solid line represents line of regression.

3. Results

In order to identify urine specimens with a diverse range of hCGβcf concentrations, 100 randomly selected second trimester urine specimens were screened with a qualitative POC hCG device known to be susceptible to false-negative results caused by hCGβcf [4,7]. Of these 100 specimens, the results were as follows: 18 test band darker than control band, 24 test band equivalent to control band, 53 test band lighter than control band, and 5 test band nearly invisible. Ten specimens that generated the most negative/faint positive results, ten specimens that generated the strongest positive results, and five specimens that generated intermediate positive results were selected for further analysis. The most negative/faint positive results were those in which the test band was barely visible but could be detected. Intermediate positive results were clearly positive bands (2 test band equivalent to control band; 3 test band lighter than control band). Strong positives constituted the darkest and thickest test bands out of the 100 specimens screened. For each of these 25 second trimester specimens, additional first and third trimester specimens from the same pregnancy were obtained, and intact hCG, hCGβcf, and free hCGβ subunit concentrations were measured by immunoassay in all three specimens (first, second, and third trimester) for all 25 women (75 specimens total). In the specimens that generated the most negative/faint positive results using the

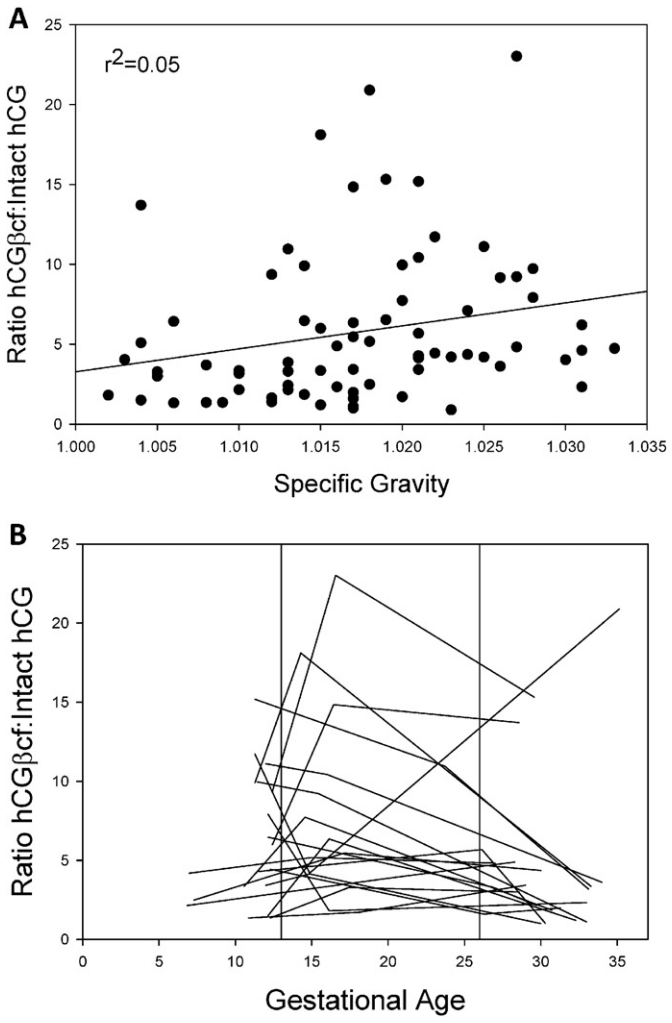

Fig. 4. Correlation between specific gravity and the ratio of urine hCGβcf:hCG concentrations (A) and relationship between gestational age and the ratio of urine hCGβcf:hCG concentrations (B) in 75 samples from 25 pregnant women. Solid line in A represents line of regression. Solid vertical lines in B represent the trimester cutoffs of 13 weeks and 26 weeks.

POC device, hCGβcf concentrations ranged from 209,800 pmol/L to 679,000 pmol/L. Out of all 75 specimens, 12/25 first trimester specimens, 11/25 second trimester specimens, and 2/25 third trimester specimens contained hCGβcf concentrations above 209,000 pmol/L (Fig. 1A). Urine specimens with the highest hCGβcf concentrations were observed between weeks 8 and 17, which is consistent with the well-characterized peak in serum hCG concentrations during weeks 8–12 of pregnancy [1,2]. Urine specimens with the highest hCGβ and intact hCG concentrations were also observed during this time frame (Fig. 1B and C). Correlation between intact hCG and hCGβcf was $r^2 = 0.70$ (Fig. 2A). Correlation between intact hCG and hCGβ was $r^2 = 0.74$ (Fig. 2B).

The concentration of urine analytes is dependent on the highly variable urine excretion rate. Therefore, specific gravity was measured in all 75 specimens. A poor correlation was observed between specific gravity and hCGβcf ($r^2 = 0.32$ Fig. 3A), hCGβcf ($r^2 = 0.32$ Fig. 3B), and intact hCG ($r^2 = 0.32$, Fig. 3C), demonstrating that elevated hCG and hCGβcf concentrations are encountered more frequently with increasing urine density, but clearly specific gravity is not the only factor impacting concentration of these hCG variants. Interestingly, there was no correlation between the specific gravity and the intact hCG:hCGβcf ratio ($r^2 = 0.05$, Fig. 4A), and the intact hCG:hCGβcf ratio was not consistent across gestational age in individual women (Fig. 4B). Therefore, other factors in addition to specific gravity must influence the urinary concentrations of intact hCG and hCGβcf and the ratio between the two variants.

To determine whether a characteristic pattern of hCG, hCGβcf, and free β subunit excretion could be identified during early, middle, and late pregnancy, we plotted the concentrations of these three hCG

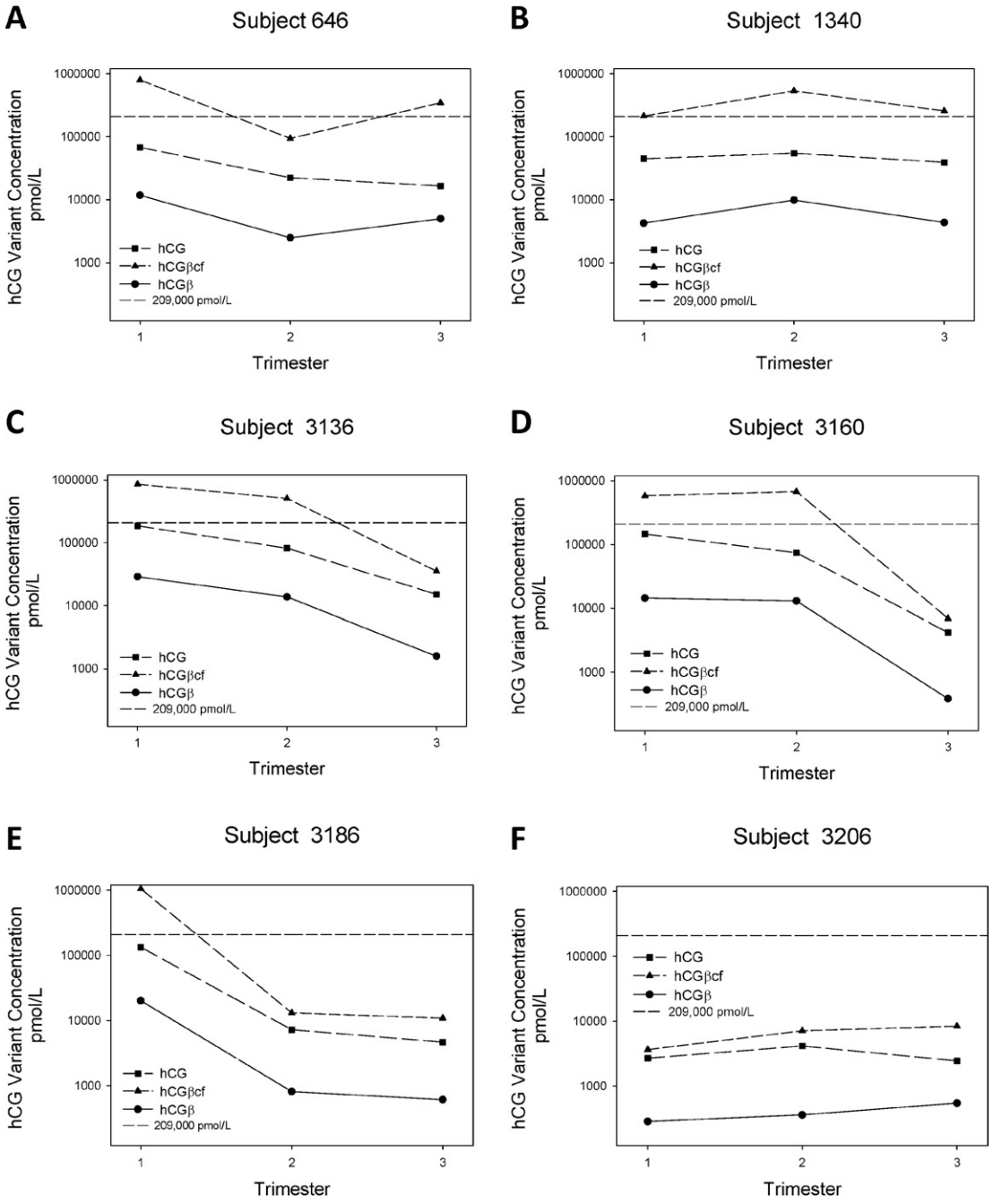

Fig. 5. Urine hCGβcf, hCG, and hCGβ concentrations in six representative women (A–F) during pregnancy. These six women were selected to represent a wide variety of hCG patterns and concentrations. Samples were collected during the first, second, and third trimesters. Dotted horizontal line represents hCGβcf concentration of 209,000 pmol/L. False-negative/low-positive results were observed using the OSOM device at this concentration and above.

forms for 6 of the 25 women (Fig. 5A–F). No single pattern of excretion could be identified for all 25 women. Instead, patterns of intact hCG, hCGβcf, and free β subunit were highly variable from woman to woman. In 9 women, the hCGβcf concentrations were below 209,000 pmol/L in all three trimesters (represented in Fig. 5F), and the remaining 16 women had at least one hCGβcf concentration above this threshold. For 16 women, their first trimester specimen contained the highest hCGβcf concentration (represented in Fig. 5A, C, E), but 7 women generated their highest hCGβcf concentration during the second trimester (represented in 5B and 5D) and 2 during the third trimester. hCGβcf concentrations above 209,000 pmol/L were observed in 2 trimesters for 8 women and in all three trimesters for 1 woman.

For all women, the hCGβcf concentration was greater than the intact hCG and free β subunit concentrations in all three trimesters. However, the magnitude of difference varied between women. For 15 women, the magnitude of the difference was greatest in the first trimester, for 8, the greatest difference was observed in the second trimester, and for 2, the greatest difference was in the third trimester.

4. Discussion

Despite increased attention being given to false-negative qualitative POC hCG results caused by elevated concentrations of urine hCGβcf, little is known about why certain women excrete urine with very high hCGβcf concentrations and when these urine specimens are most likely to be encountered in a clinical setting.

High concentrations of hCGβcf (≥500,000 pmol/L) have previously been shown to cause false-negative results in certain qualitative POC hCG devices [4–6]. Previous studies have not carefully examined results from samples with hCGβcf concentrations between 50,000 and 500,000 pmol/L. Results from this study suggest that the threshold associated with false-negative qualitative POC device results is somewhere between 209,000 and 500,000 pmol/L, depending on the device. This cutoff is specific for the OSOM POC device..

Our data indicate that the highest urine concentrations of intact hCG and hCGβcf are most likely to be observed in the late first trimester or early second trimester when serum hCG concentrations reach their peak. However, urine specimens with elevated hCGβcf concentrations were also observed in several third trimester specimens evaluated in our study, indicating that false-negative POC hCG results may be encountered at any point after the 6th–7th week of pregnancy. Both intact hCG and hCGβcf correlated somewhat with specific gravity, providing a potential tool to identify urine specimens that might be more likely to cause false-negative results. However, other and so far unknown factors must influence urine hCGβcf concentrations as some urine specimens with high specific gravity did not contain hCGβcf concentrations above 209,000 pmol/L.

In contrast to previous studies reporting mean intact hCG and hCGβcf concentrations that indicate a controlled, predictable excretion of several hCG variants during early pregnancy [2], our findings suggest that excretion patterns of these hCG forms are quite variable and unpredictable. Of the 25 women included in our study, multiple different patterns of hCG variant excretion were identified, making it impossible to predict the point at which pregnant women are most likely to generate false-negative results.

A limitation of this study was that the samples were stored frozen before analysis. We have found that that this may cause a variable loss of hCG, probably caused by adsorption of all forms of hCG to the sediment formed in urine when frozen [11]. This will cause underestimation of the results in some samples, but it will not change the message of this study.

These data are consistent with previous studies that describe urine hCGβcf concentrations in excess of intact hCG after 5 weeks of pregnancy [1,2]. Likewise, McChesney et al. have described wide day-to-day fluctuations in the concentrations of hCG variants in urine during pregnancy [2]. We observed a wide variation in the urine concentrations of hCG variants between women. The biological mechanism for these differences and the functional significance is unknown at this time. hCGβcf concentrations have been reported to be associated with small for gestational age infants and pre-eclampsia [12,13]; however, medical record review of the patients in this study revealed no common etiologies in women with elevated hCGβcf.

In this study, we confirm that urine specimens with elevated concentrations of hCGβcf occur frequently in a randomly selected population of pregnant women, and we also demonstrate that a large number of these randomly selected women (at least 9 of 100) generated urine with elevated hCGβcf concentrations at multiple points in the same pregnancy. Given that hCGβcf constitutes the major immunoreactive component of hCG in urine during pregnancy, this must be fully appreciated by manufacturers and clinicians in order to properly design new pregnancy test devices and correctly interpret results obtained with different devices.

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