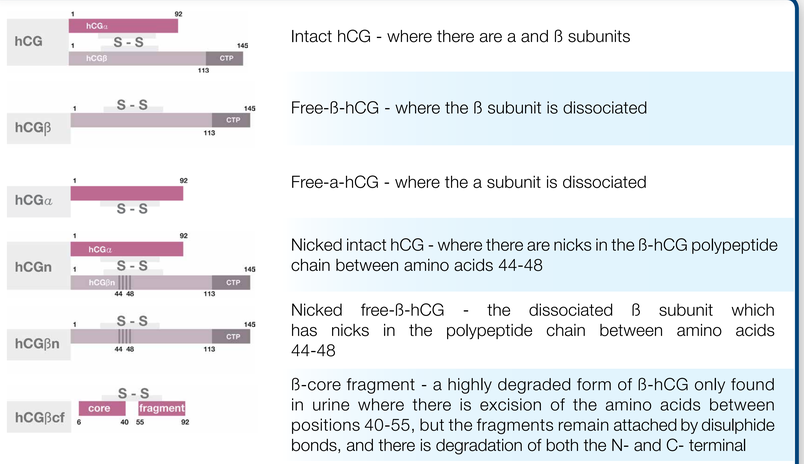
Urinary levels of intact, free beta and beta core fragment of human chorionic gonadotrophin (hCG) in early pregnancy

John Walter Larsen[1], Saji Eapen[2], Sarah Johnson[2], Lorrae Marriott[2], Michael J. Zinaman[3]

1 The George Washington University, Washington, District of Columbia, USA. 2 SPD Development Company Ltd., Bedford, UK. 3 Albert Einstein College of Medicine, Jacobi Medical Center, Bronx, New York, USA.

Introduction

- Home and laboratory pregnancy testing relies on the detection of human chorionic gonadotrophin (hCG),[1] a glycoprotein with two non-covalently linked subunits: alpha (hCG-α) and beta (hCG-ß)
- Multiple forms of hCG are present in the serum and urine of pregnant women: as shown in Figure 1
- The degradation product, ß-core-hCG is only present in urine and becomes the predominate form detectable in later pregnancy.



Intact hCG - where there are a and ß subunits

Free-ß-hCG - where the ß subunit is dissociated

Free-a-hCG - where the a subunit is dissociated

Nicked intact hCG - where there are nicks in the ß-hCG polypeptide chain between amino acids 44-48

Nicked free-ß-hCG - the dissociated ß subunit which has nicks in the polypeptide chain between amino acids 44-48

ß-core fragment - a highly degraded form of ß-hCG only found in urine where there is excision of the amino acids between positions 40-55, but the fragments remain attached by disulphide bonds, and there is degradation of both the N- and C- terminal

Figure 1: Structure of the different forms of hCG[2]

- Reference ranges have been published for intact urinary hCG, but not for other forms present in the urine of pregnant women[1,3]
- Free-ß-hCG and ß-core-hCG have a different profile of daily rise compared with intact hCG
- Very high levels of ß-core-hCG, that can occur in later pregnancy, have caused false negative point-of-care pregnancy test results which can have serious clinical consequences
- Although laboratory testing for ß-core-hCG interference has been conducted on both home and point-of-care pregnancy tests, there is some debate as to what the most appropriate testing methodology should be[4,5]
- The ratio of intact: free-ß-hCG has been related to pregnancy viability[6]
- Therefore, it is important to obtain further understanding of the ranges of these forms of hCG in pregnancy and their relationship with the total level of hCG.

Purpose of the study

- This study sought to improve the understanding of the levels of free-ß and ß-core-hCG in viable pregnancies.

Methods

- Daily early morning urine samples were collected from women with viable pregnancies throughout early pregnancy
- The samples were collected pre-conception to enable the day of ovulation to be determined for each woman by the detection of the LH (luteinising hormone) surge (AutoDELFIA quantitative LH assay, with ovulation presumed as LH surge + 1 day). This enabled accurate assignment of pregnancy duration for each volunteer
- Intact, free-ß and ß-core-hCG were measured using AutoDELFIA immunoassays, using in-house reagents for ß-core-hCG assays, and the Perkin Elmer assay for intact hCG and free-ß assays.
- Mean and standard deviations (SD) by the day of pregnancy were derived.

Results

- Figure 2 shows the levels of intact hCG, free ß-hCG and ß-core-hCG in the first trimester of pregnancy

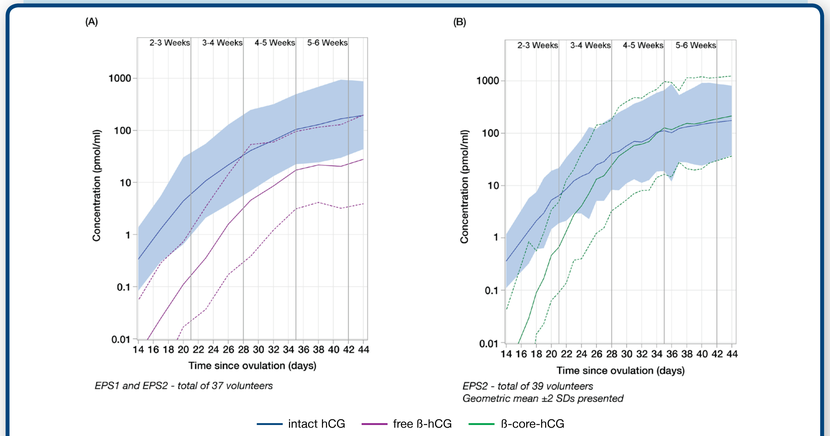


Figure 2: Intact hCG, free ß-hCG (A) and ß-core-hCG (B) concentrations during the first trimester of pregnancy

- As expected, intact hCG was present in the urine of pregnant women 8 days following ovulation, and showed a consistent rise throughout early pregnancy
- However, free ß-hCG was not consistently detectable in urine until day 21
- Free ß-hCG appeared in urine at a constant ratio of approximately 1:100 of intact hCG
- ß-core-hCG had a different profile, appearing in urine later than intact hCG (day 19), yet becoming the predominant form by day 35
- High levels of ß-core-hCG were only present when there was also intact hCG in the sample
- The minimum level of intact hCG in samples with ß-core-hCG >500,000 pmol/l was 10,003 pmol/l (4017 mIU/ml), as shown in Figure 3.

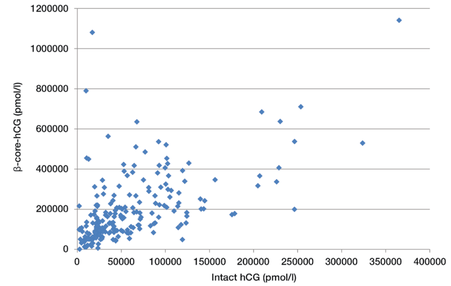


Figure 3: Paired intact hCG: ß-core-hCG measurements in urine samples from women who were 6–12 weeks pregnant

Conclusions

- The urinary ranges of free-ß and ß-core-hCG in viable pregnancies provide a valuable reference tool
- While free-ß hCG appears to be a constant proportion of intact hCG throughout early pregnancy, ß-core-hCG does not have the same direct relationship
- Although urine levels of ß-core-hCG in early pregnancy are negligible, concentrations can reach 500,000 pmol/l by day 28 post ovulation in pregnant women; this level has been shown to cause false negatives in some pregnancy tests
- As these high levels of ß-core-hCG always occur in the presence of total hCG, testing for interference of assays by ß-core-hCG should be conducted using samples that also contain intact hCG
- Only assays that demonstrate they are unaffected by ß-core-hCG interference should be used in later pregnancy.

References

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Declaration of Interest

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