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Salivary estradiol as a surrogate marker for serum estradiol in assisted reproduction treatment☆

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ABSTRACT

Introduction: Controlled ovarian hyper-stimulation for in vitro fertilization or intra cytoplasmatic sperm injection necessitates close monitoring using ultrasound and estradiol measurements. Monitoring is also important to prevent or limit the severity of ovarian hyper stimulation syndrome, an iatrogenic and potentially life-threatening complication. Self-operated endovaginal telemonitoring has been shown to offer an attractive and less costly alternative to classic consultation and saliva estradiol measurements could be a stress-free and practical alternative to serial blood determinations. Objectives were to evaluate whether saliva can be a surrogate marker for serum estradiol and its potential applicability in assisted reproduction treatment monitoring.

Material and methods: Serial blood and saliva samples were collected from 31 patients undergoing ovarian hyper-stimulation. All patients were followed-up using in-house serial vaginal sonograms and immunoassay serum estradiol measurements. Afterwards estradiol was determined in saliva and serum by LC-MS/MS. For a subset equilibrium dialysis and measurement of free serum estradiol was performed.

Results: About 1% of estradiol is present in serum in its free, unbound, form. Salivary estradiol correlates well to both serum free estradiol and serum total estradiol ($r = 0.80$). The concentration of salivary estradiol corresponds to the unbound concentration in serum. The dynamics observed in serum monitoring during treatment are closely mimicked in saliva. ROC analysis on the current limited dataset suggested a saliva cut-off of 22 pg/mL (81 pmol/L) could help predict risk for OHSS.

Conclusions: Salivary E2 can be considered a surrogate marker for free serum estradiol and total serum estradiol in assisted reproduction treatment. Additionally there might be a role as a prediction marker for OHSS although this finding has to be validated in larger datasets.

1. Introduction

Controlled ovarian hyper stimulation for in vitro fertilization (IVF) or intra cytoplasmatic sperm injection (ICSI) necessitates close monitoring of follicular growth. Both transvaginal sonography (TVS) and blood hormone measurements are used in conjunction to count the number of growing follicles and measure the increase in 2D size in order to adjust gonadotropin dosage and, at the end of stimulation, for proper timing for the administration of the ovulation trigger. Monitoring is also important to prevent or limit the severity of ovarian hyper stimulation syndrome (OHSS), an iatrogenic and potentially life threatening complication occurring in its severest form in 0.5–1% of IVF treatment attempts. Multiple follicular enlargement, hemoconcentration and intravascular volume depletion leading to the risk for thrombo-embolic complications are key symptoms associated with OHSS. Risk factors include polycystic ovaries, young age, incipient pregnancy as the result of treatment, administration of HCG or a prior history of OHSS although the exact etiology is not completely elucidated [1].

Measurement of serum estradiol (E2) has been demonstrated to be a potential predictor for OHSS and patients with high E2 levels on the day of the ovulation trigger are at particular risk of ovarian hyper response [2,3]. Irrespective of the debatable role for E2 itself in the pathogenesis of OHSS, D'Angelo et al. demonstrated that a serum E2 level of >3500 pg/mL (>12,850 pmol/L) on day 11 of ovarian stimulation predicts the occurrence of OHSS with a sensitivity and specificity of 85% [4].

However, a Cochrane review in 2014 was unable to demonstrate a clear advantage for a combined monitoring strategy based on TVS and serum E2 measurements, usually considered the safest option, compared with either of them alone, although data quality was rated poor [5]. Therefore, although ultrasound monitoring alone is probably

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equally safe, the generally accepted good clinical practice suggests the use of both. Both serial vaginal sonograms and venous blood measurements contribute to patient stress and productivity loss during the course of treatment and contribute significantly to the total healthcare cost of ART procedures. Self-operated endovaginal telemonitoring (SOET) has been shown to offer an attractive and less costly alternative to classic consultation and serial conventional two-dimensional transvaginal sonography [6,7] but this approach is far from general practice. As a potential alternative to blood, saliva has been shown to be a stress-free, non-invasive and practical matrix for measurement of hormones such as cortisol (C) and testosterone (T) [8,9]. In blood steroid hormones such as C and T are strongly bound to cortisol binding globulin (CBG) and sex hormone binding globulin (SHBG) respectively and both are weakly bound to albumin. Only a small free fraction is assumed to be the active hormone and can be measured by reference methods such as equilibrium dialysis (ED) coupled to LC-MS/MS in serum [10]. Total C and T concentrations in saliva are very low compared to serum total C and T. Saliva measurement of T has been demonstrated to correlate well with the free fraction in blood as measured using ED-LC-MS/MS and thus be a plausible surrogate marker for the direct measurement of free T. These results also support the hypothesis that hormones in saliva are largely present in their free, unbound form, even if there has been shown to be some binding to salivary proteins for free T in women [11].

For E2 a similar mechanism based on the Law of Mass Action is suggested. E2 is strongly bound in blood to SHBG (although 2–3× less so than T) and weakly to albumin. In women it is assumed that only about 1% of E2 is actually present as free hormone in blood and as for T some calculations have been suggested for E2 to estimate this free fraction [12]. In order to avoid serial blood collections at home to monitor E2 the use of saliva would be a potentially attractive alternative. Although authors have looked at measuring salivary E2 and have looked at its correlation to fertility cycles [13] these were based on salivary immunoassays. As concentrations for total and free E2 (F-E2) are much lower than total T and F-T the same problems associated with T immunoassay measurement (aspecificity, matrix related problems) are even more important for E2 [14,15]. Up to now there has also been no investigation whether salivary E2 could be a good surrogate marker for serum E2 by LC-MS/MS or how direct measurement of free E2 in serum by ED-LC-MS/MS correlates to salivary E2. The ultimate question is whether salivary E2 could be used as a low cost and stress-free potential surrogate marker for predicting OHSS in woman undergoing ART. This would imply the possibility for women coming to a fertility center for their sonograms to use a saliva sample instead of having to undergo a venapuncture; and for women performing their sonograms at home to determine saliva E2 concentrations in a stress-free homecare environment. The primary objective for this study was to elucidate the exact relation between salivary and serum E2 values and to determine whether saliva could be used as a surrogate marker for serum E2. The secondary objective was to evaluate whether saliva could potentially be useful in a home setting for women undergoing ART.

2. Materials and methods

As part of a randomised prospective interventional investigation serial blood and saliva measurements were collected from patients undergoing ovarian hyperstimulation for ART. All patients were followed-up in the traditional manner, i.e. using in-house serial vaginal sonograms accompanied by measurements of serum estradiol. Blood results were communicated on a daily basis, and the correlation study was carried out afterwards on stored samples of saliva and serum. All participants signed an informed consent and the study was approved by the ethical committee of the University Hospital of Ghent, Belgium (B670201421937) The treatment included seven planned visits during which matching blood and passive drool saliva samples were collected from 31 patients. All samples were stored at −80 °C pending analysis.

17beta estradiol (E2) was obtained from Sigma–Aldrich, 17beta estradiol-d4 (d4-E2) were purchased from CDN Isotopes, Inc. All standards and internal standards were dissolved in methanol. Routinely E2 was determined by immunoassay on an E170 Modular (E2 Gen III, Roche Diagnostics, Germany). For the Roche Gen III E2 immunoassay an inter-assay CV of 3.2% was observed with good linearity and dilutions up to 1/5. LOQ as claimed by Roche is 25 pg/mL (92 pmol/L). Actual inter-assay CV observed at this claimed LOQ level was 9.2% (n = 7). For measurement of E2 by LC-MS/MS (salivary, total and serum free), an AB Sciex 5500 triple-quadrupole mass spectrometer (AB Sciex, Toronto Canada) was used, coupled with an electrospray ionization (ESI) probe on the Turbo-V source and operated in negative ion mode. Saliva was centrifuged at 3000 rpm for 5 min prior to analysis. 500 μL of serum or saliva (1 mL for dialysis) were extracted with 2.5 mL of 9:1 hexane–ethylacetate mixture 25 μL E2-d4 (10 ng/mL in methanol (37 nmol/L)). After mixing for 3 min, samples were frozen and decanted with supernatant collection. With a second extraction, supernatants were combined, dried, washed with 0.5 mL of 9:1 hexane–ethylacetate and dried again to be reconstituted in a final solution of 125 μL methanol of which 100 μL are injected. The liquid chromatography system for 2D-LC operation consisted of a Shimadzu system leveraging four Pump modules LC20AD UFLC and an autosampler SIL20AC (Shimadzu Scientific Instruments, Columbia, MD, USA). As for a first dimension, sample loading and cleaning were carried out on a Supelco Supelguard LC-8-DB (3.0 mm × 20 mm) trapping column (Supelco, St. Louis, MO, USA) meanwhile the chromatographic separation as for the second dimension was performed on a reverse-phase C8 analytical column (Supelco LC-8-DB, 3.3 cm × 2.1 mm, 3 μm particle size). Both columns were kept at room temperature and the built-in switching valve of the 5500 mass spectrometer was used for column switching as previously described [11]. LOQ was defined as CV < 20%. For serum an LOQ of 0.3 pg/mL (1.10 pmol/L) was obtained on a low serum pool (CV at this level was 18.3%, n = 7), with an initially established inter-assay CV of 4% (n = 8) and excellent recovery and dilutions as previously published [11]. Current inter-assay CV on serum is 5.1% (n = 52) with consistently good performance in EQC. For saliva we confirmed the previously obtained LOQ. At 0.5 pg/mL (1.47 pmol/L) a CV of 12.7% was observed (n = 7), at 13.7 pg/mL (50.3 pmol/L) intra-assay CV was 2.3%. Spiking experiments of saliva with 10 pg standard solution yielded good recoveries between 95% and 108% (n = 7). Serum equilibrium dialysis was performed using Fast Micro-Equilibrium dialyzer cartridges and regenerated cellulose 25 KD membranes (Harvard Apparatus; Holliston,

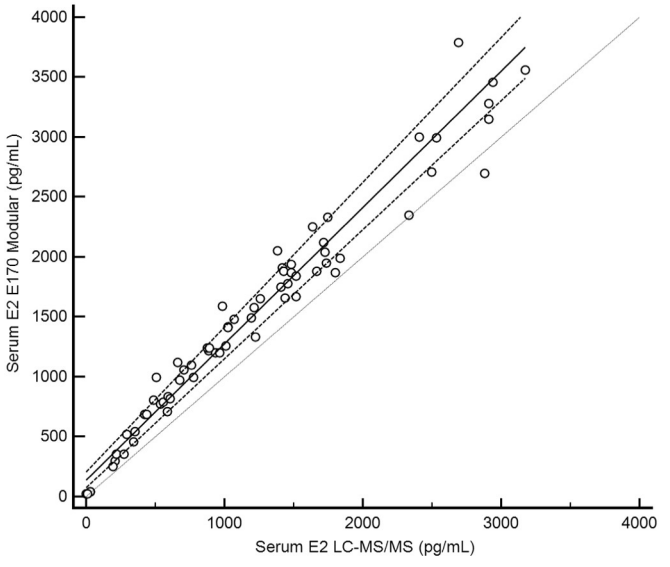

Fig. 1. Passing and Bablock regression of serum E2 by immunoassay (Roche Modular) versus LC-MS/MS.

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