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ORIGINAL ARTICLE

# Predicting serum hormone concentration by estimation of urinary hormones through a home-use device

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**STUDY QUESTION:** Can a home-use device be used to predict serum hormone levels?

**SUMMARY ANSWER:** A home-use device can predict urinary hormone values which are well-correlated to serum concentrations of respective hormones and hence can be used as a proxy for serum measurements.

**WHAT IS KNOWN ALREADY:** Home-use devices that predict ovulation are calibrated against the actual day of ovulation. However, the correlation of any quantitative system to serum hormone concentrations has not been established.

**STUDY DESIGN, SIZE, DURATION:** A total of 73 data points obtained from 20 participants across different phases of the menstrual cycle, i.e. bleeding days, follicular phase and luteal phase were used to establish the correlation between serum hormones and urinary metabolite values. Single data points from 20 random users were used to assess the correlation established.

**PARTICIPANTS/MATERIALS, SETTING, METHODS:** Participants were women in the fertile age groups and only current users of the home-use device. Selection was done based on inclusion and exclusion criteria. Blood hormones were tested using chemiluminescent immunoassays and urinary measurements were taken on the home-use device at home.

**MAIN RESULTS AND THE ROLE OF CHANCE:** Serum estradiol (E2), progesterone (P4) and LH were correlated with urinary estrone-3-glucuronide (E3G), pregnanediol glucuronide (PdG) and LH with an $R^2$ of 0.96, 0.95 and 0.98, respectively. Repredicted serum concentration obtained by using the correlation equation had a correlation of 0.92, 0.94 and 0.93 in unknown samples.

**LIMITATIONS, REASONS FOR CAUTION:** The study was designed to include women who have normal cycle lengths regularly; therefore, the values obtained were in the normal range. Certain infertility conditions may cause the values to be higher and correlation in such cases needs to be established.

**WIDER IMPLICATIONS OF THE FINDINGS:** The results of this study imply a new tool that can be used by fertility specialists as a proxy for blood tests whenever required. Extended study on this system can enable its use in assisted reproductive techniques as well.

**STUDY FUNDING/COMPETING INTEREST(S):** No funding was received for this study. S.P. and D.D. are employees of the research and development division of Samplytics Technologies Pvt. Ltd. which is a forwarder for Inito Inc., USA. A.R. and V.A.V. are co-founders of Inito Inc., USA.

**TRIAL REGISTRATION NUMBER:** The trial was registered at the International Standard Randomised Controlled Trial Number (ISRCTN) registry (Identifier: ISRCTN15534557).

**Key words:** Inito Fertility Monitor / quantitative home-use system / serum hormones / urinary hormones / LH / E3G / PdG

## WHAT DOES THIS MEAN FOR PATIENTS?

Many home-use fertility monitors work on the principle of measuring urinary hormones. Semi-quantitative (conveying ranges) and qualitative (yes or no) home-use fertility monitors have been on the market for a long time. However, the lack of a laboratory-grade quantitative system prevents visualization of hormone concentration trends throughout the menstrual cycle and possible interpretation by doctors to suggest any intervention. In this study, we compared the accuracy of urinary metabolite measurements using an Inito Fertility Monitor (IFM) at home with respect to serum hormone concentrations measured in the laboratory. We found the measurements obtained from the IFM to be well-correlated to serum hormone measurements obtained from the laboratory. Additionally, we showed that the clinical relevance of reporting ovulation based on progesterone values is accurately captured using pregnanediol glucuronide measurement using the IFM. Therefore, we propose that the IFM can not only be used by women to accurately monitor their hormone trends but can also be used by doctors to remotely monitor the effect of their interventions on hormones and hence tailor the remedy accordingly.

# Introduction

Home-use fertility monitoring devices have been widely used to increase the probability of conception (Su *et al.*, 2017). These devices implement principles aimed at measuring physiological changes associated with ovulation such as measuring basal body temperature (Marshall, 1968) and physical examination of vaginal discharge (Guida *et al.*, 1999; Ecochard *et al.*, 2001; Alliende *et al.*, 2005). One such method is the measurement of urinary hormones using lateral flow assays. Measurement of urinary hormones is well-correlated with the ultrasound day of ovulation (US-DO).

Initially, urinary LH tests to predict ovulation were routinely used at home. While these tests were good enough to implicate the time of ovulation in a 12 h interval, a major disadvantage was the ambiguity of timing the tests. Roos *et al.* (2015) inferred that measuring urinary estrone-3-glucuronide (E3G) in addition to urinary LH improved the probability of conception. Recently, a few home tests have been developed to measure urinary pregnanediol glucuronide (PdG) to confirm ovulation (Bouchard *et al.*, 2019). The accuracy of these tests concerning the actual day of ovulation has been established. These home-use tests mostly implement lateral flow assays to estimate the levels of hormones. This is an indirect prediction method since the concentration is predicted based on test and control line intensities in an absolute way or a ratio of them. Therefore, the question remains whether a correlation with serum hormones does apply. In addition, establishing such a correlation will enable clinicians to monitor serum hormones in patients using these devices.

The Inito Fertility Monitor (IFM) is a quantitative home-use system for measuring E3G, PdG and LH by quantifying lateral flow assays. A combination of these hormones enables women to predict their fertile window in the menstrual cycle as well as confirm ovulation, making this a tool that women could use during the entire menstrual cycle. The IFM uses a mobile camera to quantify the intensities of test and control lines on a lateral flow assay and derive the concentration from the ratio of the test and control lines' intensities. A previous study has substantiated the accuracy and reproducibility of the IFM concerning test strips as well as camera variation (Thakur *et al.*, 2020). Here, we show that values of urinary metabolites predicted by the IFM are well-correlated with serum hormone concentrations. We also show that the correlation equation can be used to predict serum concentrations of hormones in unknown samples accurately.

# Materials and methods

## Study participants

The study design was approved by the Institutional Review Board (IRB) of Sparsh Hospital (EC approval number: CLIN/INI/001). All women selected for the experimental cohort were new users of the IFM who were testing for the first cycle at home (retrospectively selected). Women were included in the study if their age was between 21 and 45 years and with an average cycle length between 23 and 45 days. Women were excluded if:

a. They were on infertility medications or hormone replacement therapy containing hCG or LH.
b. They were using hormonal contraceptives, including oral, emergency oral, implants, patches, transdermal injections, vaginal ring and progesterone intrauterine systems.
c. They were taking clomiphene citrate or other ovulation induction drugs.
d. They had recently been pregnant, miscarried or breastfeeding.
e. They had irregular cycle lengths.
f. They had missed more than two out of four assigned tests.

The inclusion and exclusion criteria applied to recruit women for the experimental cohort and verification cohort were the same. However, the recruitment of the verification cohort was done after the analysis with the experimental cohort was completed.

## Study design

The IFM, Inito fertility test strips and a customized clip to attach the fertility monitor to mobile phones were shipped to all participants for testing at home. Testing days were assigned to recruited participants such that the days were from different phases of the menstrual cycle:

(1) Early follicular phase: cycle day 5–7.
(2) Late follicular phase: cycle days 9–15.
(3) Luteal phase: cycle day 17 or above.

A total of 18 data points were collected in the early follicular phase, 36 data points in the late follicular phase and 19 data points were collected in the luteal phase.

On assigned days for testing, 2 ml of venous blood samples were collected in EDTA-coated BD vacutainer® (Becton Dickinson and Co., Mississauga, ON, Canada) by a phlebotomist at home and samples were transported in the collection tubes to the laboratory for testing. Serum estradiol (E2), progesterone (P4) and LH were measured. All participants tested with the first urine of the day (morning) on the IFM at home. The urine testing was performed by the women only after the venous blood samples were collected by the phlebotomist. Test timings were recorded on the back. Subjects were asked to maintain a fasting period of 10–12 h before sample collection in order to prevent the effect of any food consumed on the hormone readings. Serum E2 and P4 were measured using a chemiluminescent microparticle immunoassay, and serum LH was measured using a chemiluminescent immunoassay on an Abbott ARCHITECT i2000SR immunoanalyzer (Abbott Laboratories, Chicago, IL, USA).

## Results

A total of 73 data points were obtained from 20 participants for establishing the correlation between serum hormones and respective urinary metabolites. We found that serum concentrations of E2, P4 and LH were well-correlated with IFM-predicted concentrations of urinary E3G, PdG and LH, respectively (Fig. 1a–c). While E3G and PdG correlated linearly with serum E2 and P4, urinary LH and serum LH were correlated by quadratic regression. We wanted to further delve into the reason for this non-linear correlation between urinary LH and serum LH. Therefore, we decided to look at the correlation in different ranges of LH. We found that at serum LH $< 8$ mIU/ml, the linear correlation coefficient was 0.372 with a slope of 0.0841 indicating that the urine values did not change significantly in this range of serum LH (Supplementary Fig. S1a). However, at serum LH $> 8$ mIU/ml, the linear correlation coefficient was 0.957 with a slope of 0.305 which would indicate that, in this range, the values are well-correlated (Supplementary Fig. S1b).

Interestingly, we found that the first-morning urinary metabolite concentrations had a better correlation with their respective serum hormones compared to the creatinine normalized values (Fig. 1d–f), which may indicate that a creatinine-correction may not be required for predicting serum hormone levels from urine metabolite concentrations.

Furthermore, using the correlation equation, we wondered if we could predict the serum hormone concentrations from urinary metabolite measurements. We recruited 20 new users of the IFM and collected their blood samples following the same protocol as the primary cohort. This served as the verification cohort. The samples were collected on random cycle days without any specific

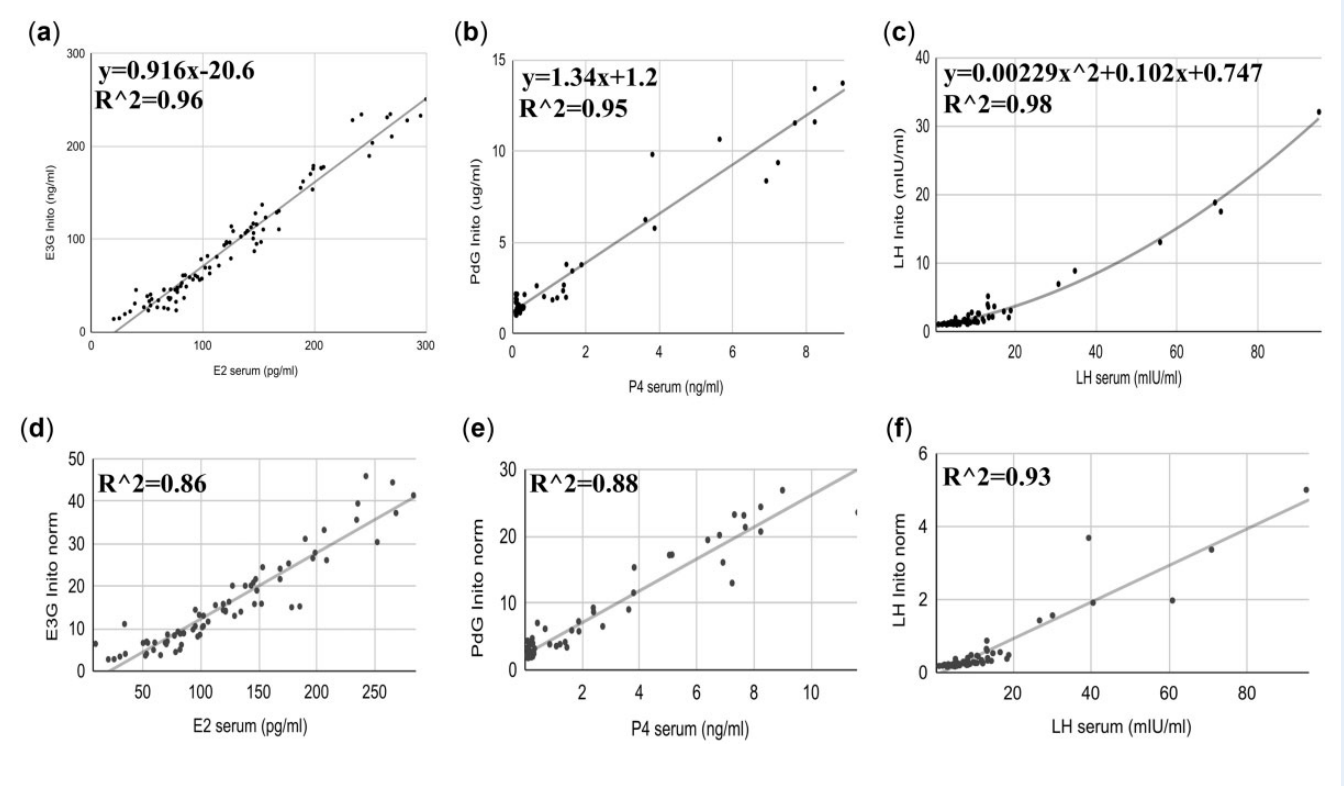


**Figure 1. Correlation between urinary measurements by the Inito Fertility Monitor (Inito) and serum hormone concentrations (experimental cohort).** (**a**) Linear correlation between urinary estrone-3-glucuronide (E3G) and serum estradiol (E2). (**b**) Linear correlation between urinary pregnanediol glucuronide (PdG) and serum progesterone (P4). (**c**) Quadratic correlation between urinary LH and serum LH. (**d**–**f**) Correlation between creatinine-corrected urinary E3G, PdG and LH as measured by IFM, and serum E2 (d), P4 (e) and LH (f).

preference toward a particular phase of the menstrual cycle. Applying the equation to this new dataset, we found that the predicted serum concentrations were highly correlated to the actual serum concentrations (Fig. 2a–c).

In addition, to show that the clinical significance of results based on these hormones is maintained across both the methods, we used the prediction of the ovulatory status of the menstrual cycle as the parameter. Typically, a mid-luteal phase serum progesterone value of >3 ng/ml was used to confirm ovulation. Recently, it has been shown that mid-luteal phase measurement of urinary PdG (a threshold value of >5 μg/ml) has a good correlation with serum P4 behavior and can also be used to confirm ovulation (Ecochard *et al.*, 2013; Leiva *et al.*, 2019). Therefore, we compared the data points where serum value was >3 ng/ml to see its correlation with the occurrence of urinary PdG >5 μg/ml measured by the IFM. We found that in all 11 data points where serum values confirmed ovulation, urinary PdG values also confirmed ovulation indicating that the clinical relevance of measurements persist in the IFM (Table I).

# Discussion

The accuracy and scope of point-of-care (POC) devices have long been questioned in the fertility space due to the unsatisfactory correlation between the results provided by POC devices and laboratory values. For monitoring the effects of interventions on hormones, blood tests are prescribed, which require an invasive procedure to be performed along with a delay in results. Moreover, since cycle lengths and hormonal patterns may vary from one individual to another (Grieger and Norman, 2020), the number of such tests cannot be accurately predicted, therefore demanding the need for a POC device that could aid testing at home with good correlation to laboratory values. While most POC devices measure urinary metabolites of fertility hormones to provide a putative fertile window, the underlying assumption is that the laboratory correlation will hold good in an indirect measurement as well. However, this has not been proven so far.

We show that the IFM could reproduce laboratory-level correlations with serum hormones and that the correlation equation could in

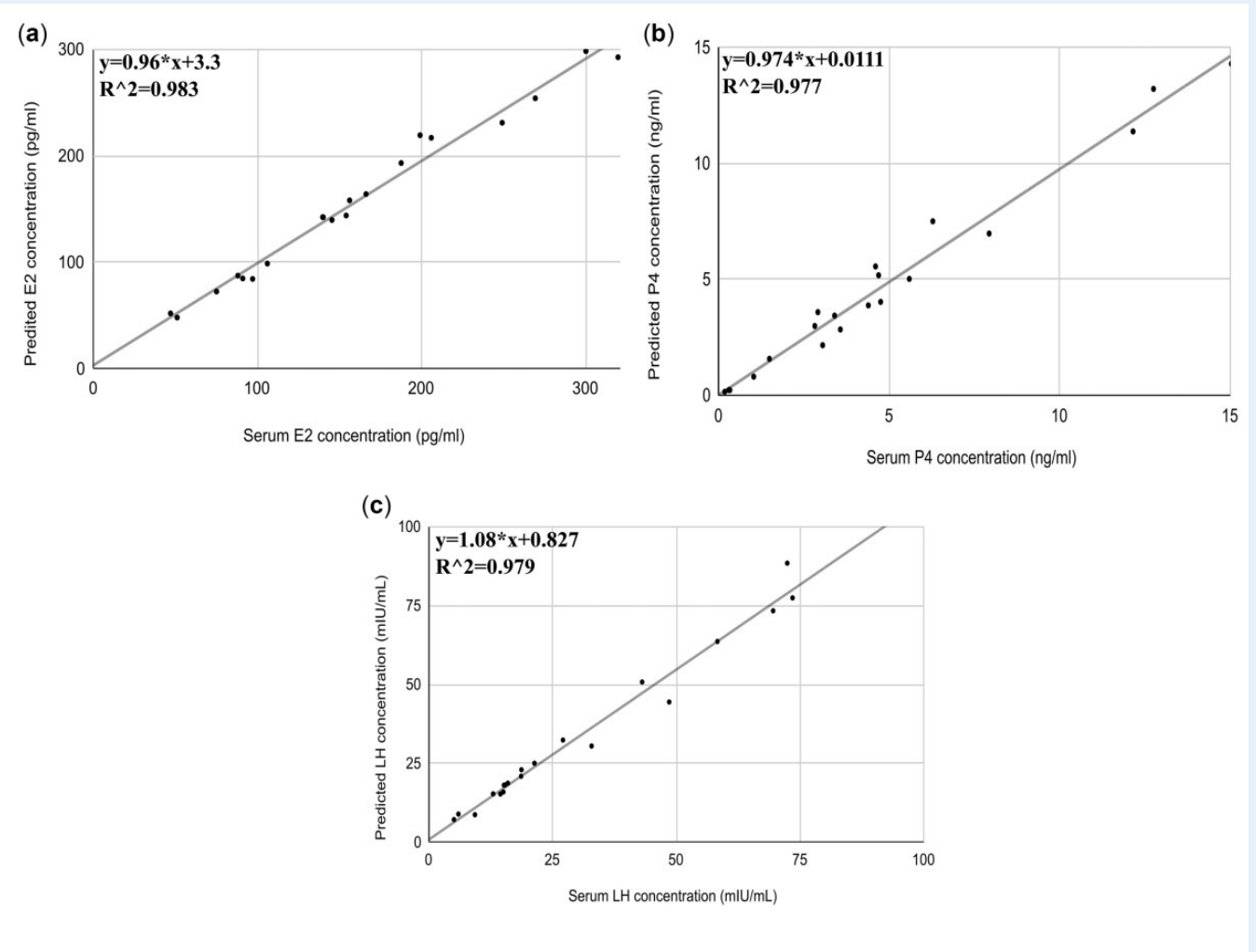


**Figure 2. Correlation between serum concentration predicted by Inito and actual serum concentration**. Linear correlations in the verification cohort between the actual serum concentrations of (**a**) estradiol (E2), (**b**) progesterone (P4) and (**c**) LH and the predicted serum concentrations derived from the equations generated from the experimental cohort based on urinary hormone concentrations obtained from the Inito Fertility Monitor.

**Table I Comparison of serum P4* values and urinary PdG** values for data points based on which a cycle was classified as ovulatory.**

| P4 Serum (ng/ml) | PdG Inito*** (μg/ml) |
|---|---|
| 6.91 | 8.37 |
| 8.23 | 13.42 |
| 3.81 | 9.82 |
| 8.99 | 13.72 |
| 8.23 | 11.60 |
| 7.69 | 11.54 |
| 3.62 | 6.25 |
| 5.64 | 10.65 |
| 7.23 | 9.37 |
| 3.87 | 5.78 |
| 3.13 | 6.38 |

*Progesterone.
**Pregnanediol glucuronide.
***Measurements from the Inito Fertility Monitor.

turn be used to predict serum concentrations with a high correlation coefficient. We predict that such a device could help in predicting the day of ovulation with higher accuracy compared to semi-quantitative or qualitative devices. As a result, this may lead to higher chances of conceiving successfully (Wilcox *et al.*, 1995). Since it has also been suggested that fertilization by older sperm leads to loss of pregnancy, we speculate that predicting the LH surge closer to ovulation may lead to more successful pregnancies (Simpson *et al.*, 1988).

Although estrogen assays are known to be inaccurate at lower levels, these levels may not be of significance in the case of female fertility (Rosner *et al.*, 2013). In addition, all data points for E2 were above this threshold and hence these challenges in measuring E2 at lower levels may not be a reason for concern for this study.

While identifying the correlation between serum hormones and urinary metabolites, we found that urinary LH correlated in a quadratic manner with serum LH. There could be two hypotheses which may explain such a non-linear correlation. The curve obtained resembles an ELISA-like antigen-antibody kinetic behavior, which follows a sigmoid curve (Engvall and Perlman, 1971). Such a correlation may imply that the non-linearity could be stemming from the type of anti-LH antibodies used in the assay which may be changing less at lower concentrations and more linearly at higher concentrations of LH. The second hypothesis is based on the kinetics of plasma clearance of LH. A previous study that deciphered the clearance of LH in female rats established a similar clearance curve for LH (Ascoli *et al.*, 1975). It is possible that something similar may be occurring in women where the LH in urine may only be appearing above a certain serum threshold.

We also observed that the creatinine correction showed a reduced correlation coefficient with serum hormone concentration. An important reason for this observation could be the variation in age of the women recruited in the study. Previous studies have observed a similar effect of creatinine correction in case of urinary pregnanediol glucuronide (Zacur *et al.*, 1997; Miro *et al.*, 2004) due to a decrease in excretion of creatinine with aging. However, there may be other factors to consider, such as the fact that women maintained a fasting period that may not always be true in a real-life scenario. Hydration levels may affect the concentration of urinary hormones and a creatinine correction may provide better correlation in such cases. Future studies with the IFM will focus on studying such variations in much more detail.

While we established a good correlation between the methods, there are certain limitations of the current study. Firstly, the study was only performed on normally menstruating women. Therefore, the validity of this correlation in the presence of one or more infertility conditions needs to be studied and would be an interesting subject for future studies with the IFM. Secondly, the study was performed on a smaller group of women and the correlation equation was tested on an even smaller group of women to infer that the equation still applied. While the correlation coefficient may apply in different infertility conditions and different racial settings, the correlation equation between IFM urinary measurements and serum measurements may vary owing to genetic factors, medication status, health, metabolic rates (Spaeth *et al.*, 2015) and other reasons that may affect conversion of estrogen and progesterone to E3G and PdG respectively. For instance, women on progesterone supplements may record consistently high PdG values and the correlation with serum progesterone could be limited by the limits of the assay itself.

Future studies will be aimed at establishing the correlation of these hormones in infertility-related conditions, patients under ovulation induction, and patients undergoing procedures such as IVF where the hormone levels tend to be higher than the normal range; hence, it will be important to establish if the correlation applies even at elevated levels.

## Supplementary data

Supplementary data are available at *Human Reproduction Open* online.

## Data availability

The data underlying this article will be shared on reasonable request to the corresponding author.

## Authors' roles

S.P., D.D. and V.A.V. contributed to the concept and design of the study. S.P. and D.D. contributed to the acquisition of data. S.P., V.A.V. and A.R. contributed to the analysis and interpretation of data. S.P. drafted the article and all authors contributed to the revision of the article. All authors approved the final version of the article to be published.

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No funding was received for this study.

## Conflict of interest

S.P. heads the research and development division of Samplytics Technologies Pvt. Ltd. which is a forwarder for Inito Inc., USA. D.D. is employed as the clinical research scientist at Samplytics Technologies Pvt. Ltd. A.R. and V.A.V. are the co-founders of Inito Inc., USA.

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