At-home urine estrone-3-glucuronide quantification predicts oocyte retrieval outcomes comparably with serum estradiol

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Objective: To investigate the feasibility of monitoring urine estrone-3-glucuronide (E3G) with an at-home device during gonadotropin stimulation for in vitro fertilization and oocyte cryopreservation.
Design: Prospective, observational cohort study.
Setting: Private fertility clinic.
Patient(s): Thirty patients undergoing stimulation with a gonadotropin-releasing hormone antagonist protocol for in vitro fertilization or oocyte cryopreservation.
Intervention(s): Daily collection of the first urine in the morning during stimulation and analysis performed at home by each patient with the Mira Fertility Tracker.
Main Outcome Measure(s): Primary outcomes were correlation of urine E3G and serum estradiol (E2) concentrations on the day of trigger to the number of total and metaphase 2 oocytes (MII). Secondary outcomes of interest were the correlation of matched E3G and E2 measurements and the daily trends of E3G and E2 during stimulation.
Result(s): Both urine E3G and serum E2 concentrations on the day of trigger significantly correlated with retrieval outcomes to a similar extent, with E3G demonstrating slightly higher correlation to the number of MII oocytes than that demonstrated by E2 ($r =$ 0.485 vs. 0.391, respectively). The Pearson correlation coefficient for matched E3G and E2 levels was 0.761. The correlation coefficients of determination for daily trends of E3G and E2 during stimulation were 0.7066 and 0.6102, respectively.
Conclusion(s): Measured on the day of trigger, urine E3G monitoring during gonadotropin stimulation was comparable with serum E2 for predicting oocyte retrieval outcomes. Matched daily samples confirmed good correlation of urine E3G and serum E2. The option of at-home estrogen monitoring with devices such as Mira offers an alternative to traditional serum monitoring that may improve patient experience.
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Key Words: Home urine test, IVF, telehealth, estrone-3-glucuronide, immunoassay

Monitoring ovarian response to gonadotropin stimulation is an important aspect of patient management during the process of in vitro fertilization (IVF). Endeavoring to retrieve approximately 15 oocytes has been suggested as ideal to maximize the cumulative live birth rate while avoiding ovarian hyperstimulation (1). Conversely, anticipation of suboptimal responses during stimulation may help manage expectations and provide the option of cycle cancellation to minimize futile procedures. In addition to serial follicular measurement with transvaginal sonography, monitoring of serum estradiol (E2) concentrations is considered an important indicator of the efficacy and safety of ovarian response (2).

Circulating E2 levels are the product of the cumulative granulosa cell mass from multifollicular growth in response to exogenous gonadotropins. A reliable method to detect a wide range of E2 concentrations is required given the dynamic variation from undetectable to supraphysiological concentrations during stimulation. Automated immunoanalyzers that use competitive binding and chemiluminescent detection are the most common clinical methods of E2 quantification (2). In addition to the inherent limitations of the clinical E2 assays, 2 other associated practical challenges for

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patients undergoing IVF are the need of frequent clinic visits for monitoring and the requirement for repetitive and potentially painful phlebotomy.

Urine hormone testing has long been considered a possible alternative to serum monitoring. The main urinary metabolite of E2, estrone-3-glucuronide (E3G; alternatively abbreviated as E1G and E1-3G), has been correlated with serum concentrations in natural and stimulated cycles by several investigators (3–7). However, consistent urinary hormone quantification is challenging because of the fluctuations in production volume. Although completely impractical for monitoring IVF, 24-hour collection is considered the most precise method for calculating urinary hormone excretion rates (8). A reliable and convenient method to quantify spot urinary E3G concentrations has remained elusive.

Recently, a device registered with the Food and Drug Administration called the Mira Fertility Tracker ("Mira"; Quanovate Tech Inc., San Ramon, CA) became commercially available directly to consumers as an ovulation monitor. The ability to predict ovulation with an algorithm using quantitative urinary luteinizing hormone and E3G concentrations has been validated in preliminary studies (9,10). The prospect of quantifying urine E3G concentration has also led investigators to suggest that Mira could be used for monitoring patients undergoing IVF (11). However, validation has not been performed in the context of gonadotropin stimulation for IVF, where the rationale for hormone monitoring is to help with the anticipation of follicular response.

To this end, we performed a pilot study to explore the feasibility of using Mira for at-home monitoring of urine E3G as an alternative to the current standard of serum E2 testing. Primary outcomes of interests were to compare the correlation of serum E2 and urine E3G concentrations on the day of trigger with the number of total and metaphase 2 (MII) oocytes retrieved. The secondary outcomes of interest were the correlations of paired serum E2 and urine E3G concentrations and the daily trends of E2 and E3G during stimulation.

MATERIALS AND METHODS

A prospective observational study was conducted at a private fertility clinic. A cohort of 30 patients was recruited as a pilot population. Patients were eligible for enrollment if they were undergoing stimulation with gonadotropins for IVF or oocyte cryopreservation with a gonadotropin-releasing hormone (GnRH) antagonist protocol, had an antimüllerian hormone level between 1 and 3.5 ng/mL within one year of enrollment, and were able to collect their first urine in the morning during their stimulation cycle. The exclusion criteria included the use of aromatase inhibitors and the inability to have routine phlebotomy performed during the stimulation cycle. Mira devices and testing wands were given to recruited patients after informed consent was obtained. Patients did not receive any compensation for participation. The protocol was institutional review board–approved (Advarra Pro00057795) and was registered with clinicaltrials.gov (NCT05493202).

Stimulation and Monitoring

Gonadotropin dosage and choice of specific gonadotropins were determined as per physicians' discretion on the basis of parameters such as ovarian reserve testing and clinical history. The protocol for cycle monitoring typically involved an initial serum E2 drawn on day 6, at which time daily GnRH antagonist (Cetrotide, Ferring, Saint-Prex, Switzerland) was routinely started. Subsequent visits and dosage adjustments were determined on the basis of the initial E2 concentration, per clinical routine. Each subsequent visit after day 6 included a repeat E2 measurement and follicular sonography. Final oocyte maturation was typically induced when most of the lead follicles exceeded 17 mm using either human chorionic gonadotropin, GnRH agonist (GnRH-a), or a combination of both.

All management decisions were based on serum E2 and follicular sonography per routine. Urinary E3G concentrations were collected for observational purposes only.

Estrogen Analysis

Phlebotomy was performed between 7:30 and 9:30 AM, with samples collected in a serum separator tube with gel and clot activator and then centrifuged at room temperature at 1,200 RCF for 10 minutes. Estradiol concentrations were analyzed on an automated chemiluminescent immunoanalyzer (Access 2, Beckman-Coulter Brea, California) in an accredited clinical laboratory. Internal quality control was verified daily, and an external quality assurance program was completed monthly. The assay time was approximately 30 minutes. According to the product literature, assay characteristics are as follows:

Limit of quantification (<20% coefficient of variation [CV]): 10.4–15.1 pg/mL

Limit of detection: 9.4–12.4 pg/mL

Reportable range: 15–5,200 pg/mL

In addition, each patient was provided with a Mira Fertility Tracker and test wands for at-home use on each day of gonadotropin stimulation (Supplemental Fig. 1, available online). Per manufacturer's literature, patients were instructed to dip the wand into a sample of the first urine in the morning for 15 seconds and then insert the wand into the device.

Mira uses a fluorescent lateral flow immunoassay design. The concentration of accumulated fluorescent-labeled antibodies triggered by the presence of the analyte on the test wand correlates with the fluorescent light intensity, thereby generating a quantitative value calibrated to a standard curve. The assay time is approximately 16 minutes, with results transferred via Bluetooth from the Mira device to the associated application on the patients' smartphone and subsequently to the clinical research portal.

Because of the supraphysiological levels of E3G expected with gonadotropin stimulation, a higher range assay wand was developed with the following characteristics:

Limit of detection (<20% CV): 10–20 ng/mL
Limit of quantification: 20–40 ng/mL
Reportable range: 40–4,000 ng/mL

Precision was defined with a CV of ≤ 20%; the tested CVs at 250 ng/mL and 1000 ng/mL were 16.6% and 18.1%, respectively.

Statistical analysis was performed using SPSS 27 (IBM, Armonk, New York). Descriptive statistics were represented as median, interquartile range, and minimum and maximum values. The Wilcoxon's rank sum test was used for statistical comparisons between groups. Pearson's correlation coefficient (r) was calculated for correlations of interest in the primary analysis and the correlation of matched E3G and E2 samples in the secondary analysis; the correlation of determination (R^2) was calculated for the trends of daily E3G and E2 in the secondary analysis.

RESULTS

Thirty patients were recruited and completed the study. Patient and cycle characteristics are summarized in Table 1. Nineteen (63.4%) patients were pursuing IVF for primary or secondary infertility, whereas 11 (36.6%) patients were pursuing IVF for elective oocyte cryopreservation.

For gonadotropin dosing, 19 patients received follitropin delta (Rekovelle, Ferring) in combination with menotropin (Menopur, Ferring) and 11 patients received follitropin alfa (Gonal-f, Merck, Darmstadt, Germany) in combination with menotropin. No statistical differences in patient characteristics or cycle outcomes were apparent when stratified by follitropin type including total and MII oocytes and trigger E2 and E3G concentrations.

Final oocyte maturation was induced with 0.2-mg GnRH-a (Decapeptyl, Ferring) in 12 patients and a combination of 0.2-mg GnRH-a and 10,000-IU human chorionic gonadotropin (Pregnyl, Merck) in 18 patients. Patients who received GnRH-a alone had significantly more total oocytes retrieved (12 ± 1.4 vs. 9 ± 0.95; $P = .023$); however, the number of MII oocytes recovered was not statistically different (8.5 ± 0.98 vs. 8 ± 0.79, $P = .119$). All other parameters, including trigger-day E2 and E3G concentrations were statistically similar when stratified by trigger type.

Primary Analysis

On the trigger day, median serum E2 and urine E3G concentrations were 1,775 ± 947 pg/mL (770–4,559) and 1,599 ± 1102 ng/mL (283–4,000), respectively. Pearson's correlation coefficients for the relationship between serum E2 and urine E3G on the day of trigger and the number of total and M2 oocytes retrieved are presented in Table 2. Correlations were found to be statistically significant in all cases.

Secondary Analysis

Overall correlations of serum E2 and urine E3G are represented in Figure 1 with a Pearson r of 0.761 ($P < .001$). The trend of daily serum E2 and urine E3G over the course of stimulation in the study cohort is illustrated in a box plot in Figure 2. The R^2 values for the trend lines of E2 and E3G were 0.6102 and 0.7066, respectively. As noted in Table 1, most patients were triggered on day 9 ± 2. Only two patients had a 12-day duration of stimulation, which likely accounts for the idiosyncratic dispersions of E2 and E3G on that day. If the day 12 measurements were excluded for those two patients, the R^2 for the trend lines of E2 and E3G would have changed to 0.4637 and 0.9107, respectively.

TABLE 1

Patient and cycle characteristics.

Variables	Result
Age (y)	37 ± 5; 29–43
BMI (kg/m^2)	22.7 ± 7.69; 16.36–36.9
AMH (ng/mL)	1.98 ± 1.09; 1–3.4
Duration of stimulation	9 ± 2; 8–12
Cumulative follitropin delta (μg) (n = 19)	108 ± 21; 70–144
Cumulative follitropin alfa (IU) (n = 11)	2700 ± 750; 2,025–3,600
Cumulative menotropin (IU) (n = 30)	1650 ± 713; 675–2,475
Number of E2 measurements	3 ± 0; 2–4
Number of E3 measurements	9 ± 2; 5–11
Number of total oocytes	10 ± 7; 3–23
Number of MII oocytes	8 ± 4; 2–15

Note: Data are represented as median ± IQR; range. AMH = antimüllerian hormone; BMI = body mass index; E2 = estradiol; E3 = estriol; IQR = interquartile range; MII = metaphase 2.

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DISCUSSION

Although several studies have investigated the use of home monitoring of urinary hormones for ovulation detection and correlation with serum levels and gonadotropin response, to the best of our knowledge, this is the first study to investigate the utility of quantitative urinary E3G measurement with an at-home device during stimulation for IVF and egg freezing relative to oocyte yield (5,12). The primary analysis demonstrated that trigger-day concentrations of both serum E2 and urine E3G were similarly correlated to the number of total and MII oocytes retrieved to a statistically significant degree. As illustrated in Table 2, the extent of correlation was comparable for both sets of relationships. As a predictor of oocyte yield from the day of trigger, these data suggest that urine E3G concentrations quantified by the Mira device are comparable to serum E2 concentrations measured on a laboratory grade immunoanalyzer.

TABLE 2

Pearson correlation of peak estrogen levels and oocytes retrieved.

Oocytes retrieved	Serum E2	Urine E3G
Total Oocytes	0.518 (0.004; 0.187–0.744)	0.402 (0.038; 0.026–0.678)
MII Oocytes	0.391 (0.036; 0.028–0.662)	0.485 (0.010; 0.128–0.730)

Note: Data are represented as Pearson's r coefficient (*P* value; 95% confidence interval). E3G = estrone-3-glucuronide; E2 = estradiol; MII = metaphase 2.

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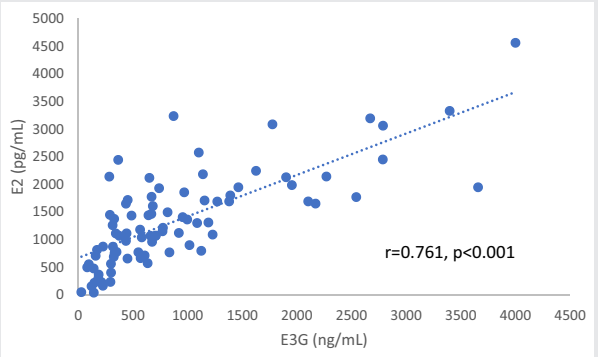
FIGURE 1



Matched urine estrone-3-glucuronide (E3G) and serum estradiol (E2).

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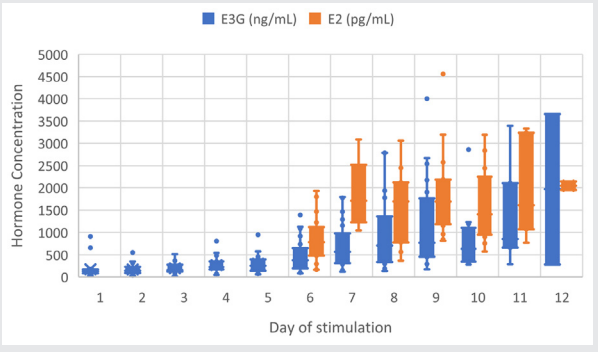
The secondary analysis, illustrated in Figure 1, demonstrates the correlation between matched samples of serum E2 and urine E3G in the study population, with a correlation coefficient of 0.761 ($P<.001$). Although the degree of correlation may be considered large by statistical standards, it is more relevant to compare the predictive value of each method to the clinical outcome of interest as performed in the primary analysis rather than simply indexing to another surrogate marker (13). In addition, Figure 2 illustrates the trend in daily serum E2 and urine E3G concentrations over the course of stimulation in the study cohort, with urine E3G demonstrating greater linearity according to the coefficient of determination.

The study of urinary estrogen metabolites during gonadotropin stimulation has a long historical precedent (14–17). More recently, Chotboon et al (18) compared serum and urine concentrations on the same automated electrochemiluminescent immunoassay with antibodies directed against 17β E2 during stimulation for IVF and reported a correlation coefficient of 0.59, less than the level of correlation found in our current data ($r = 0.761$). Similar to our primary analysis, they also calculated correlation coefficients of urine and serum E2 concentrations on trigger day with the number of total and MII oocytes, which were similar to those found in our study when confidence intervals are considered. Of note, urine hormone determinations were corrected by creatinine concentration in their study, a method that has been proposed to minimize urine production variability (8). The method used in the current data of first urine sampling in the morning has also been validated to demonstrate good correlation with serum levels with the benefit of simplicity (6).

FIGURE 2



Box plot of daily serum estradiol (E2) and urine estrone-3-glucuronide (E3G) over the stimulation course.

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A notable distinction of the current data is that sample processing was performed by patients at home with a commercially available device that can relay the data automatically to a clinical portal. The frequency of clinic visits and blood tests are among the many reported sources of stress for patients undergoing IVF (19). A recent survey reported that compared with hospital-based monitoring, patients felt more empowered and preferred the discreteness of at-home monitoring (20). Thus, even if standard clinical assays offered a modicum of better analytic accuracy, patient preference may justify considering an at-home alternative if overall care and safety are not compromised.

It is worth noting that quantifying E2 concentrations by any method is confounded by numerous limitations, including internal and external interferences, with the potential for significant variation between platforms (21). The current gold standard is direct extraction with high performance liquid chromatography and mass spectrometry; however, this method is still impractical for routine clinical use and also has intrinsic limitations. The most commonly used clinical approach of automated chemiluminescent direct immunoassay has variable consistency with mass spectrometry (22–24). Known interferences include preanalytical confounders related to how samples are collected, handled, and stored; biological variations associated with issues such as age and pathology; cross-reactivity with drugs or other circulating molecules and heterophilic antibodies (21,25). Common endogenous and exogenous substances and their respective concentrations at or below which no measurable interference was detected against the Mira E3G are listed in Supplemental Table 1(available online).

The option of at-home testing may conceivably improve access to care. Patients who live far from the clinic may have the burden of treatment reduced with remote monitoring. Capital and operational costs associated with IVF clinics may be lower if the expenses for equipment, consumables, space requirements, and trained laboratory staff required to process serum specimens are obviated. Integration into electronic medical records and automated digitized communication with patients in real time as their results are reported are other possible benefits. Furthermore, if the barriers for testing are reduced, the accrual of daily data may contribute to machine learning models and artificial intelligence algorithms to optimize management of controlled ovarian stimulation cycles (26).

The need to validate options for remote monitoring during IVF has been addressed recently (27). In a 2018 review, Blackwell et al. presciently surmised, "With current technological advances, we expect remote cycle monitoring through a device such as a smart phone will become the new reality" (28). Self-operated endovaginal telemonitoring has also been explored as an option (29). Urine E3G monitoring with at-home devices, such as Mira, may be an incremental step toward the possibility of remote monitoring in the near future.

Mira is a device for ovulation tracking registered with the Food and Drug Administration and has been validated during natural cycles. The algorithm for ovulation detection was based on retrospective analysis of patients' cycle characteristics and luteinizing hormone detection results (9). Bouchard et al. (10) compared Mira to a qualitative ovulation monitor, the Clear Blue Fertility Monitor (Swiss Precision Diagnostics, Geneva, Switzerland), and concluded that Mira was useful for identifying the fertile window, with the added potential of a quantitative assessment that might provide future insight into understanding physiological and pathophysiological hormonal variations during the menstrual cycle. The use-case for at-home testing with remote monitoring of estrogen response during gonadotropin stimulation for IVF was suggested by Vladimirov et al. (11); however, the authors did not present any data to support its efficacy. Our current data suggest that patient-performed at-home testing of E3G with Mira during IVF may be feasible but requires further characterization and refinement.

The limited sample size is a weakness of this pilot study. Broader inclusion criteria are required to determine whether urine E3G testing with Mira is comparable to serum E2 for management of the extremes of ovarian response and with more varied stimulation protocols. For example, sample dilution may be required at high hormone levels; conversely, sensitivity may be challenged when suppression is anticipated. The data do not address whether Mira can be used to monitor safety during the IVF cycle or help avoid complications such as ovarian hyperstimulation syndrome. In addition, given the challenges of quantifying urine concentrations of hormone metabolites, it is also worth considering whether alternative preanalytical steps, such as correcting for creatinine concentration or dilution to standardize the production rate, would optimize the performance of the current E3G assay (28).

CONCLUSION

At-home monitoring of urine E3G may be a feasible alternative to traditional serum E2 monitoring during IVF. Our data demonstrate a similar correlation with serum E2 to the number of total and M2 oocytes retrieved, and the correlation between paired serum E2 and urine E3G samples is consistent with previous studies. If clinical reliability is confirmed by further studies, the practical advantages of remote monitoring with devices such as Mira are promising for improving both the patient experience and the efficient delivery of care.

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