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Home Monitoring of Estrone-3-Glucuronide (E1-3G) Levels in Two Different Ovarian Stimulation Protocols: A Pilot Study

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Abstract

Background: Studies have shown a strong correlation between the growth of E2 in serum and estrone-3-glucuronide (E1-3G) in urine during ovarian stimulation. Thus, we developed theoretical models for using urinary E1-3G in ovarian stimulation and focused on their experimental verification and analysis. **Methods:** A prospective, observational pilot study was conducted involving 54 patients who underwent 54 cycles of ovarian stimulation. The goal was to establish the growth rate of urinary E1-3G during the course of stimulation and to determine the daily upper and lower limits of growth rates at which stimulation is appropriate and safe. Controlled ovarian stimulation was performed using two different stimulation protocols—an antagonist protocol in 25 cases and a progestin-primed ovarian stimulation protocol (PPOS) in 29 cases, with fixed doses of gonadotropins. From the second day of stimulation, patients self-measured their daily urine E1-3G levels at home using a portable analyzer. In parallel, a standard ultrasound follow-up protocol accompanied by a determination of E2, LH, and P levels was applied to optimally control stimulation. **Results:** The average daily growth rates in both groups were about 50%. The daily increase in E1-3G for the antagonist protocol ranged from 14% to 79%, while they were 28% to 79% for the PPOS protocol. **Conclusion:** This is the first study to analyze the dynamics of E1-3G in two different protocols and to estimate the limits of its increase during the entire course of the stimulation. The results confirm our theoretical model for the viability of using urinary E1-3G for monitoring ovarian stimulation.

Keywords

Ovarian Stimulation Monitoring, E1-3G, Antagonist Protocol, PPOS Protocol, IVF, ART

1. Introduction

Ovarian stimulation is an essential part of IVF treatment and aims to form more follicles, respectively, more eggs and embryos, thereby increasing the success rate of infertility treatment through in vitro technologies. Stimulation is done with different combinations of drugs, known as a "protocol". Currently, many protocols are applied, but based on the drugs that suppress the premature rise of the LH hormone during stimulation, the protocols are conditionally divided into GnRH agonist, GnRH antagonist, and PPOS protocols.

Stimulation control is carried out through regular ultrasound examinations and determination of the levels of estradiol LH and progesterone in the serum. Some practices exclude hormonal studies in stimulation monitoring, but in most IVF clinics, stimulation monitoring is accompanied by the determination of serum E2 levels. The frequency of its determination depends on follicular growth. It shows that E2 levels predict ovarian response to exogenous gonadotropins. This allows individualization of treatment protocols, optimization of results, and reduction of the risk of complications.

It is known that the formed in the ovary E2 is metabolized, becoming sulfated or transformed into glucuronidated conjugates. Uridine diphosphoglucuronosyl transferases (UGTs) are enzymes required for the glucuronidation process that occurs in the liver. Steroid conjugation can also take place in other organs such as the kidneys, biliary epithelium, or brain. Urinary steroid excretion is directly related to enzyme activity and the patient's age. Insufficient or excessive excretion suggests abnormal UGT and β-glucuronidase enzyme activities. Reactions of glucuronidation/deglucuronidation can be reversed under certain conditions in the human body. Steroid glucuronides can be transformed into their active free forms so they can perform their respective biological functions. In these cases, an important place is occupied by the metabolism of drugs and the determination of glucuronide metabolites [1].

Estrogen glucuronide conjugates are hydrophilic and readily excreted in urine and bile [2]. Because of the water solubility of the glucuronic acid functional group, concentrations of ovarian steroid metabolites in urine are higher than ovarian steroid concentrations in serum [3]. A lag in urine excretion of ovarian steroids between 12 - 24 hours has been found, but the overall correlation between the blood and urine profile is extremely high [4] [5]. Algorithms based on ovarian metabolites in urine measurements have been developed to identify the day of ovulation [6] [7] and the ovulatory phase of the cycle [8]. These studies have made it possible to develop a method of analysis and home monitoring of hormones in

urine for the purpose of detecting ovulation [9]-[11].

Estrone-3-glucuronide is one of the major metabolites of estradiol in urine and can be determined by quantitative analysis [12]. A significant correlation has been found between serum E2 and first-morning urine E1-3G concentrations in follicular growth [13] [14], ovarian estradiol production rate, and urinary estrogen excretion rate [15] [16].

Using laboratory analyzers, different authors have established a significant correlation between the growth of estrone-3-glucuronide (E1-3G) urinary levels and the growth of estradiol levels during stimulation [17]-[21].

Theoretical models were developed using urinary estradiol derivatives, respectively E1-3G, as a marker to control ovarian stimulation.

Vladimirov *et al.* [22] developed a theoretical model for monitoring ovarian stimulation by autonomous determination of E1-3G levels in urine from patients at home. Another theoretical model was published in which the authors additionally included an analysis of pregnanediol and LH in urine [23].

The aim of this study is to determine the dynamics of E1-3G growth during stimulation in two different protocols—antagonist and progestin priming ovarian stimulation (PPOS) protocols.

2. Materials and Methods

2.1. Patient Selection Criteria

All patients (n = 54) underwent COH and IVF/ICSI procedures at the clinic (Sofia IVF clinic, SBALAGRM-SOFIA, Sofia, Bulgaria) during the period from June 2022 to March 2023. Inclusion criteria were women patients of age < 40 years, regular menstruation cycle (21 - 35 days), and ovulatory cycle with AMH ≥ 1.1 ng/mL. Exclusion criteria: patients with a severe male factor (spermatozoa < 2 million/ml) and endometriosis.

2.2. Methods

Controlled ovarian stimulation was performed using two different stimulation protocols—an antagonist protocol in 25 cases and a progestin-primed ovarian stimulation protocol (PPOS) in 29 cases, with fixed doses of gonadotropins. The starting dose of gonadotropins was determined based on a preliminary assessment of ovarian reserve, including FSH and AMH levels, AFC, BMI, age, and history of smoking [24]. Control of ovarian stimulation was carried out through regular ultrasound examinations by physicians in the clinic, accompanied by determination of E2, LH, and Progesterone. In the PPOS protocol, medroxyprogesterone 10 mg daily (Progevera) was administered from the second day of stimulation until the day of GnRH agonist administration (Decapeptyl) as an ovulation trigger. In the antagonist protocol, from the 6th day of stimulation, the antagonist (Cetrotide) was administered until the day of the GnRH agonist administration. If at least three follicles were >17 mm in diameter, the ovulation trigger was administrated. In both protocols, a similar strategy was carried out after the aspirating of the

oocytes, namely fertilization by ICSI procedure, the culture of the embryos to the blastocyst stage and freezing of all embryos.

Criteria for termination of stimulation: In the case of unsatisfactory ovarian response with the formation of 3 or less mature follicles (size between 16 - 20 mm), or cases with risk of Ovarian hyperstimulation syndrome (OHSS), respectively formation of more than 20 mature follicles.

From the second day of stimulation, urinary E1-3G levels were determined daily using a small portable analyzer (MIRA), which is easy to use and takes a short time to determine E1-3G levels in the sample. The obtained result was sent to the attending physician through an information technology platform created for this purpose.

We analyzed 383 urinary samples, and the quantitative analysis was performed according to the fluorescent immunoassay method. Estradiol levels during stimulations were measured in 203 samples on an automated Electrochemiluminescence Immunoassay analyzer (COBAS 8000, Roche) in an accredited clinical laboratory.

An E1-3G portable urine analyzer and test strips were provided to patients free of charge, and patients were not compensated for participation. All patients gave informed consent to participate in the program. The study was approved by the ethics committee and carried out in accordance with the regulations.

2.2. Statistical Analysis

We estimated median and quantile growth rates for each day during the stimulation. For our statistical model of growth rates, we made some simplifying assumptions to avoid statistical overfitting, given our relatively small statistical sample. Specifically, we assumed a constant daily growth rate (individual-specific) across stimulation days. Individual-specific starting levels and growth rates were allowed in order to accommodate unmeasured factors related to, e.g., genetic background, age, existing chronic conditions and lifestyle. These considerations were captured in a log-linear mixed-effects model, with random effects for the slope and intercept capturing the individual-specific variability. The model was estimated by maximum likelihood.

3. Results

The average female age was 33.7 years (± 4.5 years), BMI 22.8 kg/m^2 (± 4.0 kg/m^2), AMH 3.5 ng/ml (±2.3 ng/ml), stimulation days 9.9 (± 1.2 days), collected oocytes 11.0 (± 9.2), MII oocytes 9.2 (± 6.5), fertilization rate 80.7% (± 22.1%), blastocyst formation 56.1% (± 29.3%), good quality blastocysts 27.8% (± 24.9%) (**Table 1**).

Table 1. Results in 54 patients included average age, BMI, AMH, days of stimulation, oocytes collected, MII oocytes, fertilization rate and blastocyst formation.

	Age	BMI %	AMH	Day st.	Ret. eggs	MII	Fert. %	BI. %	A% + B%
Average	33.74	22.81	3.51	9.89	11.02	9.18	80.67	56.07	27.81
Stdev	4.4545	4.0358	2.2812	1.1601	9.198405	6.536	22.102	29.265	24.896

The average daily growth rates in both groups were about 50% and the daily increase in E1-3G in the antagonist protocol ranged from 14% to 79%, respectively 28% and 79% in the PPOS protocol (**Figure 1**).

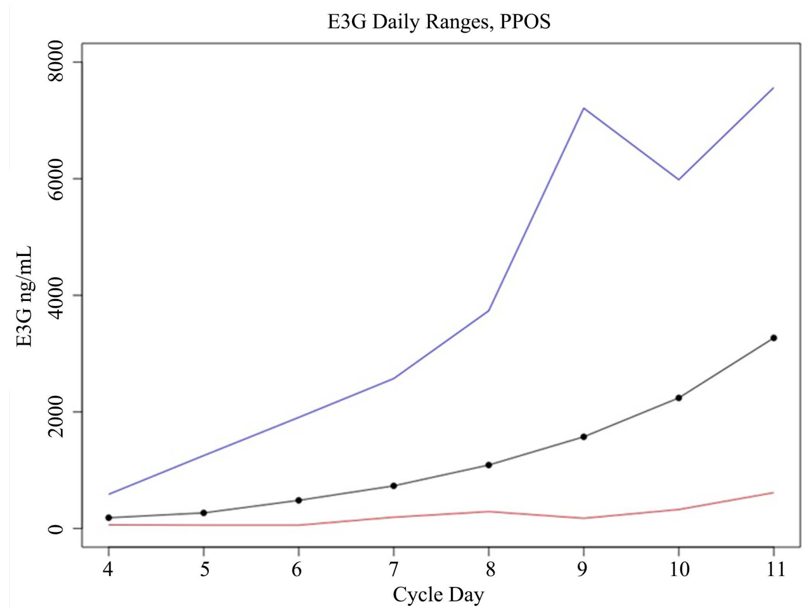

Figure 1. Graph of average daily growth rates in both groups of E1-3G. The daily increase, upper (blue color) and lower (red color), limits the growth of E1-3G in the antagonist protocol and the PPOS protocols.

The median growth curve of E1-3G in the two protocols is different. In the PPOS protocol, the median E1-3G levels were higher compared to the antagonist protocol, but there is no statistical significance (**Figure 2**).

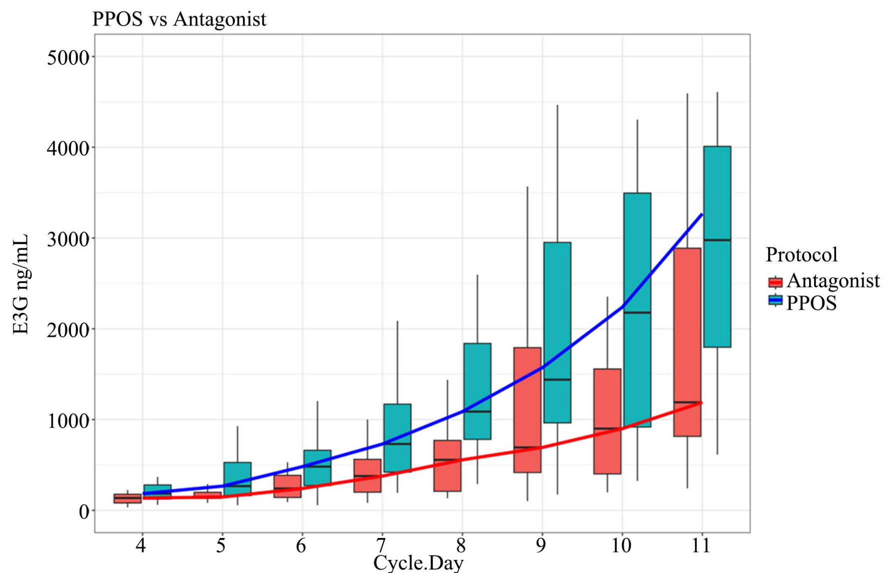

Figure 2. Graph of the median growth curves of E1-3G in two stimulation protocols: antagonist and PPOS protocols.

The growth rate and levels of E1-3G followed the growth rate and levels of E2. At the end of stimulation, on the trigger day, median serum E2 was 2220 ng/ml in the antagonist protocol and 4177 ng/ml in PPOS protocol. Correspondingly, median urine E1-3G levels were 3427 ng/ml in antagonist protocol and 5770 ng/ml in PPOS. The growth rate and levels of E1-3G followed the growth rate and levels of E2.

Additionally, patients were divided into two subgroups, where the constraint was AMH values between 1.1 ng/ml and 3.4 ng/ml, with 15 cases for the antagonist protocol and 15 cases for the progestin-primed ovarian stimulation protocol (PPOS). No statistical difference was found in the two protocols when comparing average age, BMI, AMH, days of stimulation, oocytes collected, MII oocytes, fertilization rate and blastocyst formation. (Table 2 and Table 3).

Table 2. Results in 15 patients with antagonist protocol and AMH between 1.1 - 3.4 ng/ml, including criteria are average age, BMI, AMH, days of stimulation, oocytes collected, MII oocytes, fertilization rate and blastocyst formation.

	Age	BMI %	AMH	Day st.	Ret. eggs	MII	Fert. %	BI. %	A%	B%
Average	36.44	23.05	2.43	9.28	7.50	6.13	69.99	37.63	5.00	10.38
Stdev	2.6838	3.8339	0.9144	1.0741	4.857983	4.193	31.310	28.501	12.5831	17.6141

Table 3. Results in 15 patients with PPOS protocol and AMH between 1.1 - 3.4 ng/ml, including criteria are average age, BMI, AMH, days of stimulation, oocytes collected, MII oocytes, fertilization rate and blastocyst formation.

	Age	BMI %	AMH	Day st.	Ret. eggs	MII	Fert. %	BI. %	A%	B%
Average	34.86	23.88	2.30	10.29	8.92	7.08	84.08	58.14	7.69	16.15
Stdev	3.4162	5.2583	1.0786	1.1387	3.882901	2.871	18.968	31.6267	15.8923	20.40317

Here, we found that the median growth curves of E1-3G in the two protocols had similar growth rates with a very slim narrowing distance (Figure 3).

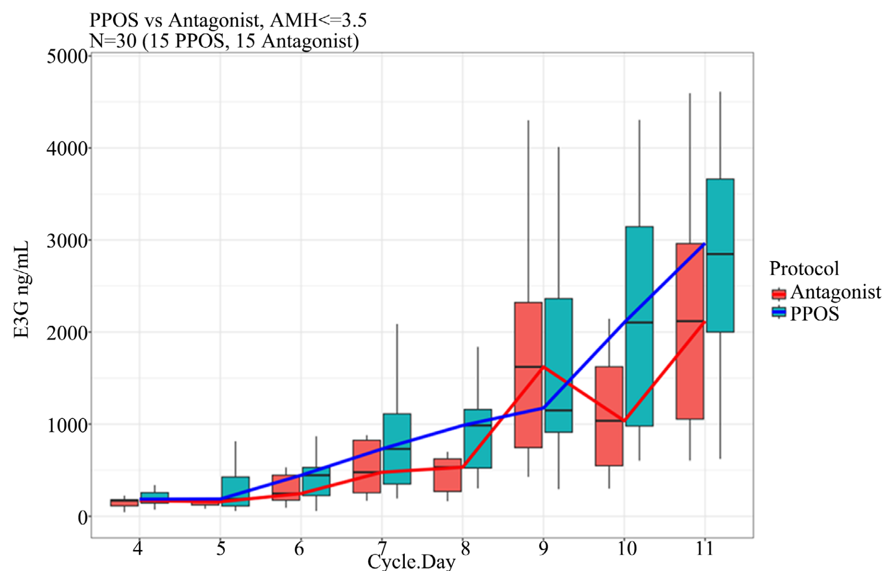

Figure 3. The median growth curves of E1-3G in antagonist and PPOS protocols in cases with AMH between 1.1 - 3.4 ng/ml.

At the end of stimulation, on the trigger day, median serum E2 was 995 ng/ml in the antagonist protocol and 1017 ng/ml in PPOS protocol. Respectively, median urine E1-3G levels were 1870 ng/ml in the antagonist protocol and 2143 ng/ml in PPOS protocol (Figure 4).

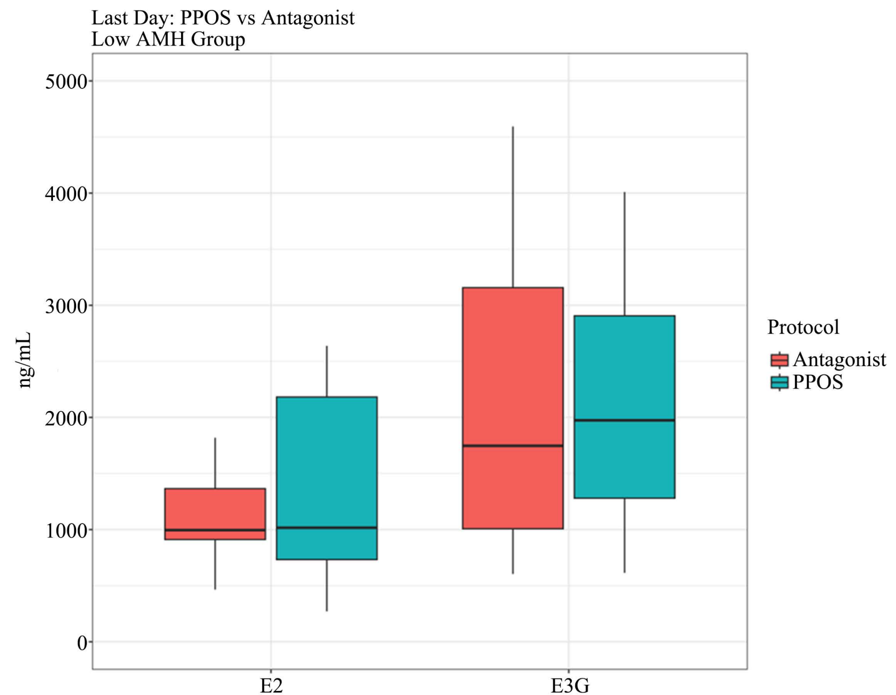

Figure 4. Median serum E2 and urine E1-3G levels in antagonist and PPOS protocols on the ovulation trigger day.

From the analysis of the provided evaluation questionnaire, patients declared the new method of ovarian stimulation monitoring with E1-3G in urine to be easy to perform in 32% of cases and very easy to perform in 68% of cases. Patients preferred our new method of stimulation monitoring in 87% of cases, compared to the standard method, which includes regular ultrasound examinations and determination of serum hormone levels. Patients required 2 - 3 hours to travel 24% for each visit to the clinic for follow-up of ovarian stimulation, and for 37% of patients, more than 4 hours were needed. (Appendix)

4. Discussion

The topic of using urinary sex hormone derivatives as a marker in clinical practice is not new. A number of studies have evaluated the use of urine analysis to monitor ovulation induction. The first successful ovulation induction resulting in pregnancy and birth was reported by Lunenfeld *et al.* in the early 1960s. In that study, only urine-based hormone monitoring was used for cycle monitoring [25].

Later, Maclean *et al.* developed a direct RIA for the determination of E1-3G in urine with the aim of using this marker to monitor ovulation induction in women undergoing infertility treatment. The authors conducted a study concluding that

urine hormone analysis is a reliable method for monitoring ovarian function in patients undergoing ovulation induction [26]. Another study supports these results by further establishing a correlation between changes in urinary hormones and follicle size, as determined by ultrasound in a group of 31 women undergoing ovulation induction [17].

Rapi *et al.* [19] also used urine analysis to track ovulation and assess the correlation with follicular growth in ovarian stimulation patients, with the aim of the women undergoing an in vitro procedure. Based on the results obtained, the authors determined that the analysis of E1-3G in urine is a reliable method for detecting the optimal day for the administration of HCG as an ovulation trigger.

A study by Sallam [27] supported these results for the successful use of E1-3G in clinical practice as an objective marker to control ovarian stimulation. The authors studied the rate of increase in the concentration of the three metabolites of estrogens in first morning urine, with the best time regression with log10 concentration being found for estrone-3-glucuronide (E3G) ($R = 0.97$). According to them, ultrasound scanning of follicles, combined with the determination of estrogen derivatives in morning urine samples, is an ideal combination of analyzing a biophysical parameter and an easy-to-determine biochemical parameter for monitoring gonadotropin therapy. Based on this, they create a chart combining the two parameters to determine the best time to administer HCG as an ovulation trigger. An additional contribution of this study is the correlation found between the concentration of total estrogens in a 24-hour urine collection and the concentration of each of the metabolites in early morning urine samples ($R = 0.89$ for E1G, $R = 0.83$ for E2G, and $R = 0.80$ for E3G, respectfully).

The main disadvantage of these studies and their application in clinical practice is that the analysis of estrogens in urine must be carried out in laboratory conditions. For this reason, the patient will have to find time to visit the laboratory or send in the sample, which will delay the preparation of the sample and its analysis.

Thanks to the recent developments in technology, this problem has been overcome. It is now possible for patients to independently examine urine hormone levels, respectively E1-3G, through a small portable analyzer at home, and the results are sent to the attending physician for analysis.

This is the first study to establish the utility of quantifying urinary E1-3G by patients alone at home under two different stimulation protocols. The first comparative analysis of the rate of increase of urinary E1-3G was performed in different stimulation protocols, estimating the lower and upper limit of daily increase of E1-3G, for which the ovarian response to stimulation was deemed adequate and safe. As another innovation, the dynamics of urinary E1-3G increase were investigated and analyzed in normal responder and high responder groups of patients in PPOS and antagonist protocols.

With the development of IV technologies, it became possible to develop and manufacture portable analyzers for the quantitative analysis of E1-3G in urine, which makes it easier for patients to measure themselves and also feasible to

perform the test at home. This new approach makes it possible to facilitate the ovarian stimulation monitoring process.

The first study establishing the utility of quantitative measurement of urinary E3G using a portable fluorescent immunoassay (Mira Fertility Plus®) during stimulation for IVF with an antagonist protocol was performed [28]), with the authors establishing a correlation of R = 0.81 in the quantitative analysis with an increase in serum E2 and urinary E1-3G, as well as correlation in the quantitative increase in urinary E1-3G with follicular growth. The study was carried out in 20 women and 56 early morning urine samples and E2 were examined on different days of stimulation.

Another study by Nakhuda *et al.* [29], using the same analyzer, found a correlation coefficient of 0.76 for matched E3G and E2 levels and when measured on the ovulation trigger day, urine E1-3G determination during ovarian stimulation is comparable to serum E2 for prognosing oocytes retrieval results.

The study applied an antagonist stimulation protocol to 30 ovulatory, normal responder women with AMH levels between 1 - 3.5 ng/ml. 270 early morning urine samples and 90 serum E2 samples were tested. Other studies also found a high correlation coefficient, within R = 0.92, between E2 and E1-3G in urine [29]. Also, a study using a small portable analyzer from another manufacturer found a high correlation coefficient of R = 0.91 [14].

Other researchers [30] have found a lower correlation between serum and urine E2 level on day 6: R = 0.53, and the day of ovarian trigger: R = 0.59, in 77 stimulated patients using an antagonist protocol.

Correlation between E2 and E1-3G is evident from the figure showing the daily levels for the two different protocols for stimulation. Comparing the median curves for E2 and E1-3G in the subgroup with low AMH (1 - 3.5 ng/ml), we observed that the PPOS and Antagonist curves are similar. Moreover, the levels E2 and E1-3G on the trigger day also are close (not shown). However, in the full sample, for the PPOS protocol, the median E1-3G levels and E2 serum levels were higher compared to the Antagonist protocol. The higher E1-3G levels in the PPOS protocol are a consequence of the higher E2 levels respectively. Comparing the median curves for E2 and E1-3G in the full sample, we observed that the PPOS and Antagonist curves appear to diverge, however, this difference does not attain statistical significance, likely due to our small statistical sample. Further investigation of any potential difference is warranted in larger samples, and the same applies to comparisons on the day of the ovulation trigger.

In the comparative analysis, we found that when the growth dynamics of E1-3G were estimated with a log-linear mixed effect model (LLMEM) the average daily growth rates in both groups were around 50%, which resembles the average growth rate of estradiol in the serum. The daily increase in E1-3G in the antagonist protocol ranged (2 standard deviations) from 14% to 79% and 28% and 79% in the PPOS protocol, respectively. No statistically significant difference was found between the two different protocols.

Another study carried out by our team, using a similar log-linear mixed effect model (LLMEM), found similar results in the rate of rise in urinary E1-3G and the lower and upper limits of daily rise in E1-3G [31]. We also found a close correlation between the increase in serum E2 and urinary E1-3G during stimulation using a progestin priming ovarian stimulation (PPOS) protocol. The authors also used a portable fluorescence immunoanalyzer (Mira) to determine urinary E1-3G levels. They found a correlation of R = 0.83 in the quantitative analysis of increases in serum E2 and urinary E1-3G. The study included 28 women aged < 40 years, with regular menstrual periods (21 - 35 days) and AMH ≥ 1.1 ng/mL, respectively normal responders and high responders. In this study, for the first time, the average daily growth rates of E1-3G during stimulation were determined, as well as the upper and lower limits of growth at which stimulation can be considered adequate and safe, as well as daily growth of E1-3G between 25% and 77%. [32]

We have established that estrone-3-glucuronide concentrations in urine on the trigger ovulation day are higher than those of estradiol in serum. Respectively, median serum E2 was 995 ng/ml, and E1-3G levels were 1870 ng/ml in the antagonist protocol, median serum E2 was 1017 ng/ml, and E1-3G levels were 2143 ng/ml in PPOS protocol. This correlates with the lab analysis results of Falk *et al*. [3].

Patient attitude towards E1-3G use in ovarian stimulation monitoring is positive, with all patients describing the use of the small portable analyzer as ‘easy’ or ‘very easy’. Due to the advantages of this new method of monitoring ovarian stimulation, most patients (87% of those asked) prefer it compared to the classic method, which involves regular ultrasound monitoring and determination of serum hormone levels. From the analysis, it is clear that the new approach to stimulation monitoring saves the time needed for regular visits to the clinic, which is one of the main reasons for the positive attitude towards the new method.

This study has a number of limitations. First of all, the study does not cover all stimulation protocols, nor does it answer which protocol is most appropriate to monitor, using urinary E1-3G as a marker of dynamic follicular growth. Second, the patient groups in the studied protocols were small in number, and a prospective study with a larger group of women is needed.

5. Conclusions

This is the first pilot study that establishes the rate of increase in urinary E1-3G, as well as the lower and upper limits of daily rise in E1-3G at which the ovarian response to stimulation is adequate and safe. The study found differences in the median growth curve of E1-3G, as well as the lower limits of daily increase in the two protocols for E1-3G, but these were not statistically different, and further investigation in larger groups of patients is needed.

For the first time, the dynamics of urinary E1-3G increase have been investigated and analyzed in normal responder and high responder groups of patients, in two different stimulation protocols (PPOS and antagonist).

Using the dynamics of E1-3G change during stimulation, we can achieve a reduction in patient visits to the clinic, which would also lead to:

Reduced costs of regular ultrasound and hormone tests;

Reduced stress of frequent blood draws to determine serum hormone levels;

Reduced time the patient wastes for frequent clinic visits and reduced travel time;

Reduced direct non-medical expenses related to the use of car, bus, train, hotel accommodation, and food;

Reduced patient risk of infection in situations similar to COVID-19.

The consequences would be lower prices for infertility treatment, using IVF technologies as the main method. Treatment would become cheaper and more available for most patients. This would further simplify the implementation of family-planning policies and help countries deal with their decreasing total fertility rates and the demographic implications connected to that [33].

Author Contributions

I.K.V.: Design, conception, analysis and interpretation of data; drafting of the article; writing—review & editing, supervision. D.T.: Design; drafting of the article; funding acquisition. M.R.: Acquisition of data, design. E.G.: Analysis and interpretation of data, writing—review & editing, visualization. M.V.: Conception, writing—review & editing, visualization. All authors approved the final manuscript. All authors contributed to the article and approved the submitted version.

Institutional Review Board Statement

The study was conducted in accordance with the Declaration of Helsinki and approved by the Institutional Review Board of SBALAGRM-SOFIA, Sofia (protocol code 045/2021 date of approval 29 December 2021). Informed consent was obtained from all subjects involved in the study.

Data Availability Statement

The data are available upon reasonable request to the corresponding author.

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Conflicts of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

References

[1] Wang, R., Hartmann, M.F. and Wudy, S.A. (2021) Targeted LC-MS/MS Analysis of

Steroid Glucuronides in Human Urine. *The Journal of Steroid Biochemistry and Molecular Biology*, **205**, Article ID: 105774.
https://doi.org/10.1016/j.jsbmb.2020.105774

[2] Ebner, T., Remmel, R.P. and Burchell, B. (1993) Human Bilirubin UDP-Glucuronosyl Transferase Catalyzes the Glucuronidation of Ethinylestradiol. *Molecular Pharmacology*, **43**, 649-654.

[3] Falk, R.T., Gail, M.H., Fears, T.R., Rossi, S.C., Stanczyk, F., Adlercreutz, H., Kiura, P., *et al*. (1999) Reproducibility and Validity of Radioimmunoassays for Urinary Hormones and Metabolites in Pre- and Postmenopausal Women. *Cancer Epidemiology, Biomarkers & Prevention*, **8**, 567-577.

[4] Cekan, S.Z., Beksac, M.S., Wang, E., Shi, S., Masironi, B., Landgren, B., *et al*. (1986) The Prediction and/or Detection of Ovulation by Means of Urinary Steroid Assays. *Contraception*, **33**, 327-345. https://doi.org/10.1016/0010-7824(86)90095-8

[5] Munro, C.J., Stabenfeldt, G.H., Cragun, J.R., Addiego, L.A., Overstreet, J.W. and Lasley, B.L. (1991) Relationship of Serum Estradiol and Progesterone Concentrations to the Excretion Profiles of Their Major Urinary Metabolites as Measured by Enzyme Immunoassay and Radioimmunoassay. *Clinical Chemistry*, **37**, 838-844.
https://doi.org/10.1093/clinchem/37.6.838

[6] Baker, T.S., Jennison, K. and Kellie, A.E. (1980) A Possible Method for the Detection of Ovulation and the Determination of the Duration of the Fertile Period. *Journal of Steroid Biochemistry*, **12**, 411-415. https://doi.org/10.1016/0022-4731(80)90300-3

[7] Baird, D.D., Weinberg, C.R., Wilcox, A.J., McConnaughey, D.R. and Musey, P.I. (1991) Using the Ratio of Urinary Oestrogen and Progesterone Metabolites to Estimate Day of Ovulation. *Statistics in Medicine*, **10**, 255-266.
https://doi.org/10.1002/sim.4780100209

[8] O'Connor, K.A., Brindle, E., Miller, R.C., Shofer, J.B., Ferrell, R.J., Klein, N.A., *et al*. (2006) Ovulation Detection Methods for Urinary Hormones: Precision, Daily and Intermittent Sampling and a Combined Hierarchical Method. *Human Reproduction*, **21**, 1442-1452. https://doi.org/10.1093/humrep/dei497

[9] Thornton, S.J., Pepperell, R.J. and Brown, J.B. (1990) Home Monitoring of Gonadotropin Ovulation Induction Using the Ovarian Monitor. *Fertility and Sterility*, **54**, 1076-1082. https://doi.org/10.1016/s0015-0282(16)54008-4

[10] Behre, H.M., Kuhlage, J., Gaβner, C., Sonntag, B., Schem, C., Schneider, H.P.G., *et al*. (2000) Prediction of Ovulation by Urinary Hormone Measurements with the Home Use Clearplan® Fertility Monitor: Comparison with Transvaginal Ultrasound Scans and Serum Hormone Measurements. *Human Reproduction*, **15**, 2478-2482.
https://doi.org/10.1093/humrep/15.12.2478

[11] Blackwell, L.F., Brown, J.B., Vigil, P., Gross, B., Sufi, S. and d'Arcangues, C. (2003) Hormonal Monitoring of Ovarian Activity Using the Ovarian Monitor, Part I. Validation of Home and Laboratory Results Obtained during Ovulatory Cycles by Comparison with Radioimmunoassay. *Steroids*, **68**, 465-476.
https://doi.org/10.1016/s0039-128x(03)00049-7

[12] Collins, W.P., Branch, C.M. and Collins, P.O. (1981) Ovulation Prediction and Detection by the Measurement of Steroid Glucuronides. In: Cortes-Prieto, J., Campos de Paz, A. and Neves-e-Castro, M., Eds., *Research in Fertility and Sterility*, MTP Ltd., 19-33.

[13] Branch, C.M., Collins, P.O. and Collins, W.P. (1982) Ovulation Prediction: Changes in the Concentrations of Urinary Estrone-3-Glucuronide, Estradiol-17β-Glucuronide and Estriol-16α-Glucuronide during Conceptional Cycles. *Journal of Steroid Biochemistry*, **16**, 345-347. https://doi.org/10.1016/0022-4731(82)90189-3

[14] Catalan, R., Castellanos, J.M., Palomino, T., Senti, M., Antolin, M. and Galard, R.M. (1989) Correlation between Plasma Estradiol and Estrone-3-Glucuronide in Urine during the Monitoring of Ovarian Induction Therapy. *International Journal of Fertility*, **34**, 271-275.

[15] Franco, J.G., Baruffi, R.L.R., Vagnini, L. and Oliveira, J.B.A. (1993) Calculation of Plasma Estradiol Levels by Analysis of Number and Size of Follicles Measured by Ultrasound. *International Journal of Gynecology & Obstetrics*, **41**, 261-264. https://doi.org/10.1016/0020-7292(93)90553-9

[16] Cahill, D.J., Wardle, P.G., Harlow, C.R., Hunt, L.P. and Hull, M.G.R. (2000) Expected Contribution to Serum Oestradiol from Individual Ovarian Follicles in Unstimulated Cycles. *Human Reproduction*, **15**, 1909-1912. https://doi.org/10.1093/humrep/15.9.1909

[17] Lessing, J.B., Reuben Peyser, M., Gilad, S., Amit, A., Kogosowski, A., Yovel, I., *et al*. (1987) Estrone-3-Glucuronide Chemiluminescence Immunoassay: An Alternative Method for Monitoring Induction of Ovulation with Human Menopausal Gonadotropin in an *in Vitro* Fertilization Program. *Fertility and Sterility*, **48**, 450-453. https://doi.org/10.1016/s0015-0282(16)59416-3

[18] Brown, J.B., Blackwell, L.F., Cox, R.I., Holmes, J.M. and Smith, M.A. (1988) Chemical and Homogeneous Enzyme Immunoassay Methods for the Measurement of Estrogens and Pregnanediol and Their Glucuronides in Urine. *Progress in Clinical and Biological Research*, **285**, 119-138.

[19] Rapi, S., Fuzzi, B., Mannelli, M., Pratesi, S., Criscuoli, L., Pellegrini, S., *et al*. (1992) Estrone 3-Glucuronide Chemiluminescence Immunoassay (LIA) and 17β Estradiol radio-Immunoassay (RIA) in the Monitoring of Superovulation for *in Vitro* Fertilization (IVF): Correlation with Follicular Parameters and Oocyte Maturity. *Acta Europaea Fertilitatis*, **23**, 63-68.

[20] Kesner, J.S., Knecht, E.A., Krieg, E.F., Barnard, G., Mikola, H.J., Kohen, F., *et al*. (1994) Validations of Time-Resolved Fluoroimmunoassays for Urinary Estrone 3-Glucuronide and Pregnanediol 3-Glucuronide. *Steroids*, **59**, 205-211. https://doi.org/10.1016/0039-128x(94)90029-9

[21] Alper, M.M., Halvorson, L., Lasley, B. and Mortola, J. (1994) Relationship between Urinary Estrone Conjugates as Measured by Enzyme Immunoassay and Serum Estradiol in Women Receiving Gonadotropins for *in Vitro* Fertilization. *Journal of Assisted Reproduction and Genetics*, **11**, 405-408. https://doi.org/10.1007/bf02211727

[22] Vladimirov, I.K., Vladimirov, M. and Tacheva, D. (2021) A New Protocol for Controlled Ovarian Stimulation Monitoring by Self-Determination of Estrone-3-Glucuronide and Single Ultrasound (COSSESU). *Open Journal of Obstetrics and Gynecology*, **11**, 1217-1228. https://doi.org/10.4236/ojog.2021.119115

[23] Hart, R.J., D'Hooghe, T., Dancet, E.A.F., Aurell, R., Lunenfeld, B., Orvieto, R., *et al*. (2021) Self-Monitoring of Urinary Hormones in Combination with Telemedicine—A Timely Review and Opinion Piece in Medically Assisted Reproduction. *Reproductive Sciences*, **29**, 3147-3160. https://doi.org/10.1007/s43032-021-00754-5

[24] Yovich, J., Alsbjerg, B., Conceicao, J., Hinchliffe, P. and Keane, K. (2016) PIVET rFSH Dosing Algorithms for Individualized Controlled Ovarian Stimulation Enables Optimized Pregnancy Productivity Rates and Avoidance of Ovarian Hyperstimulation Syndrome. *Drug Design*, *Development and Therapy*, **10**, 2561-2573. https://doi.org/10.2147/dddt.s104104

[25] Lunenfeld, B. (2011) Gonadotropin Stimulation: Past, Present and Future. *Reproductive*

Medicine and Biology, **11**, 11-25. https://doi.org/10.1007/s12522-011-0097-2

[26] Maclean, A.R., Outch, K.H., Russell, J.M., Brown, J.B. and Dennis, P.M. (1981) Monitoring Induction of Ovulation by Rapid Radioimmunoassays of Oestrogen and Pregnanediol Glucuronides. *Annals of Clinical Biochemistry*: *International Journal of Laboratory Medicine*, **18**, 343-349. https://doi.org/10.1177/000456328101800604

[27] Sallam, H.N. and Sallam, A.N. (2002) Monitoring Gonadotropin Therapy with Ultrasound and Urinary Oestrogen Metabolites. *Fertility and Sterility*, **78**, S217-S218. https://doi.org/10.1016/s0015-0282(02)04020-7

[28] Vladimirov, I., Martin, V. and Desislava, T. (2021) P-670 Urine Estrone-3-Glucuronide (E3G) Assay: Is There Any Place during Ovarian Stimulation for IVF Cycles? *Human Reproduction*, **36**, deab130.669. https://doi.org/10.1093/humrep/deab130.669

[29] Nakhuda, G.S., Li, N., Yang, Z. and Kang, S. (2023) At-Home Urine Estrone-3-Glucuronide Quantification Predicts Oocyte Retrieval Outcomes Comparably with Serum Estradiol. *F&S Reports*, **4**, 43-48. https://doi.org/10.1016/j.xfre.2023.01.006

[30] Pattnaik, S., Das, D., Venkatesan, V.A. and Rai, A. (2022) Predicting Serum Hormone Concentration by Estimation of Urinary Hormones through a Home-Use Device. *Human Reproduction Open*, **2023**, hoac058. https://doi.org/10.1093/hropen/hoac058

[31] Chotboon, C., Salang, L., Buppasiri, P., Amnatbuddee, S. and Eamudomkarn, N. (2022) Association between Urine and Serum Estradiol Levels in *in Vitro* Fertilization Cycles. *Scientific Reports*, **12**, Article No. 4393. https://doi.org/10.1038/s41598-022-08292-z

[32] Vladimirov, I.K., Tacheva, D., Gatev, I., Rangelova, M. and Vladimirov, M. (2023) P-603 Viability of Home Monitoring of Estrone-3-Glucuronide (E1-3G) Urine Levels in Controlled Ovarian Stimulation: A Pilot Study. *Human Reproduction*, **38**, dead093.932. https://doi.org/10.1093/humrep/dead093.932

[33] Fauser, B.C.J.M., Adamson, G.D., Boivin, J., Chambers, G.M., de Geyter, C., Dyer, S., *et al*. (2024) Declining Global Fertility Rates and the Implications for Family Planning and Family Building: An IFFS Consensus Document Based on a Narrative Review of the Literature. *Human Reproduction Update*, **30**, 153-173. https://doi.org/10.1093/humupd/dmad028

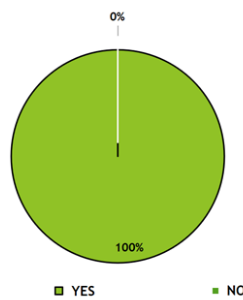
Appendix

Patient Questionnaire

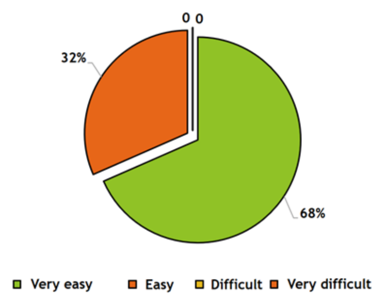
Ovarian Stimulation Protocol COSSESU

E3G

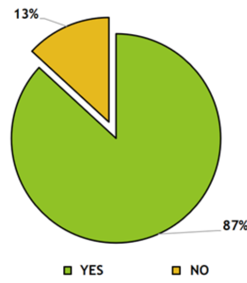
Is this method easy and convenient for you to apply at home?



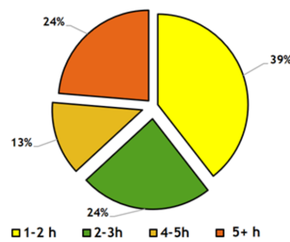
How would you rate it?



Is this method of tracking your ovarian stimulation more convenient for you, or do you prefer a regular visit to the clinic for an ultrasound and blood tests for hormones?

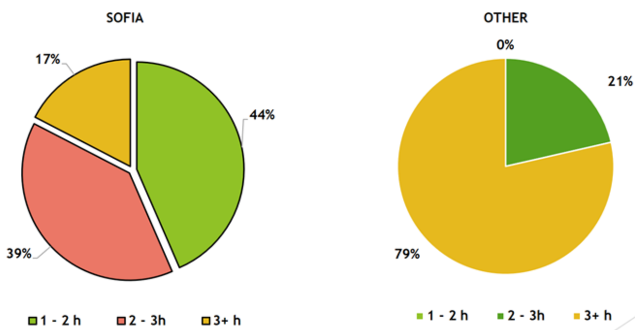


How long does one clinic visit take for you, including time for transportation, the ultrasound examination, the blood draw, the appointment and the administration of the relevant medications included in your treatment?



- These data include all patients.

How long does one clinic visit take for you, including time for transportation, ultrasound examination, blood draw, appointment, and administration of the relevant medications included in your treatment?



- The data are divided: the capital Sofia and other settlements.