

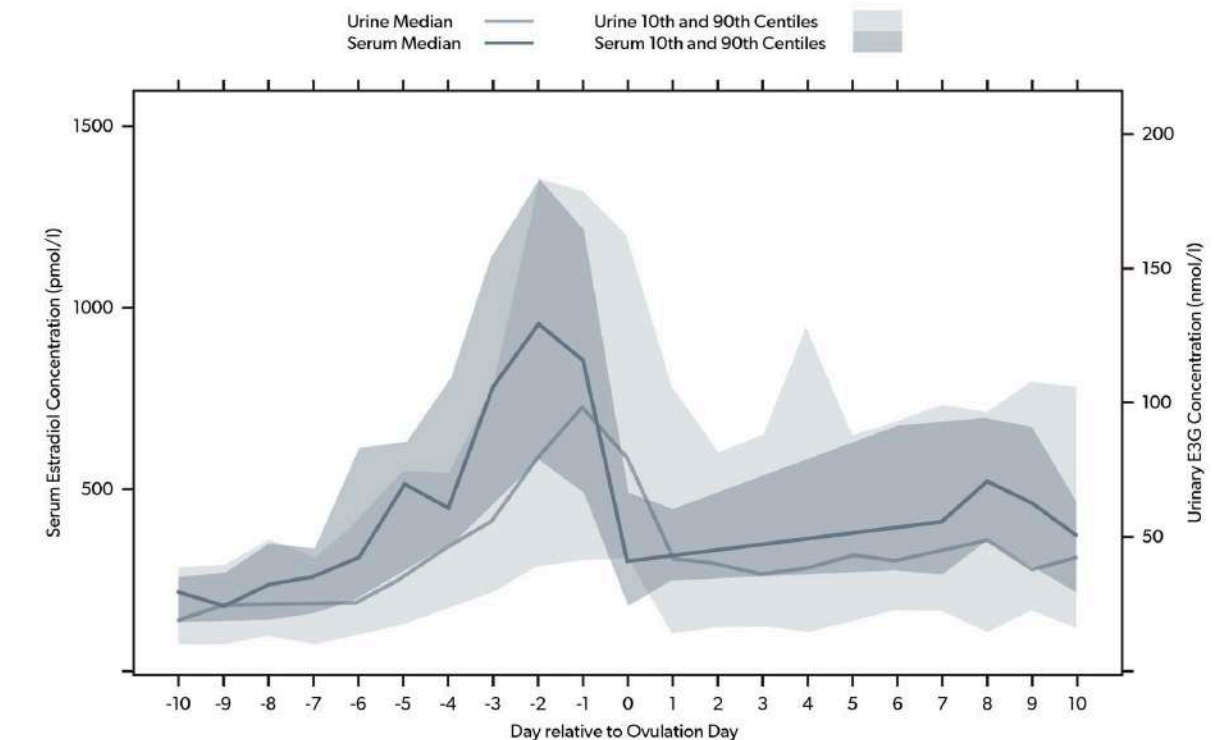
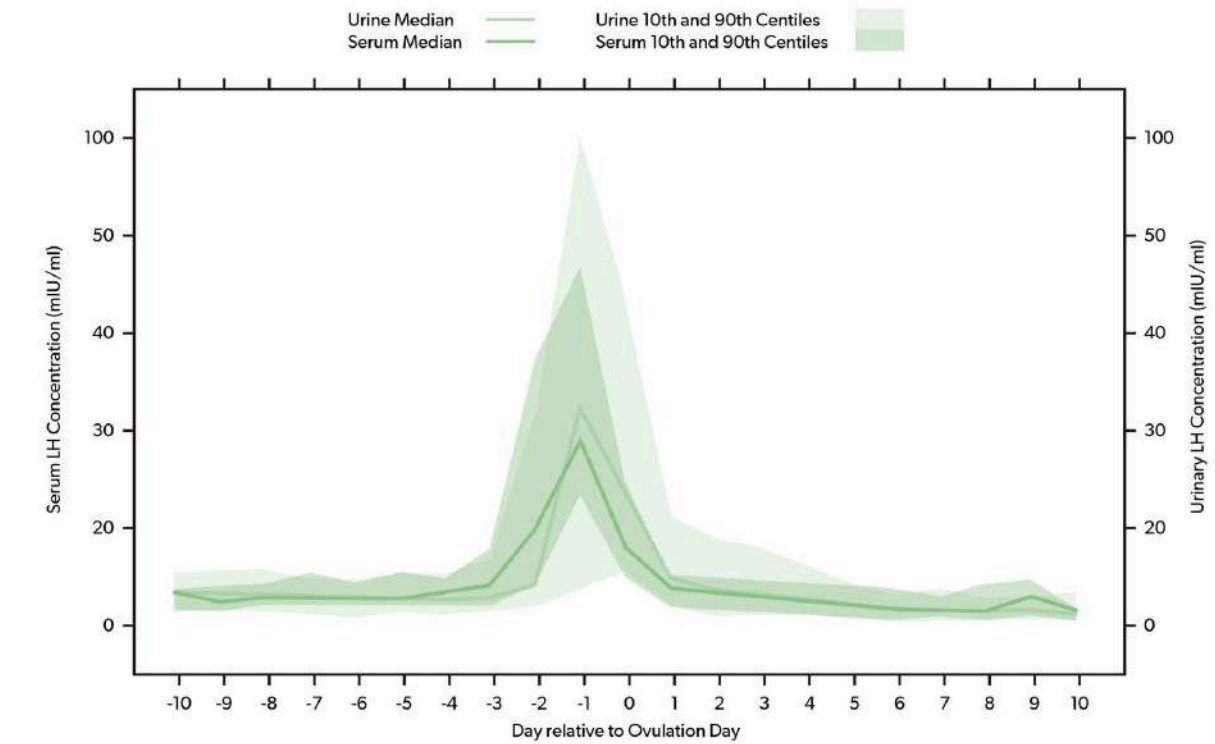
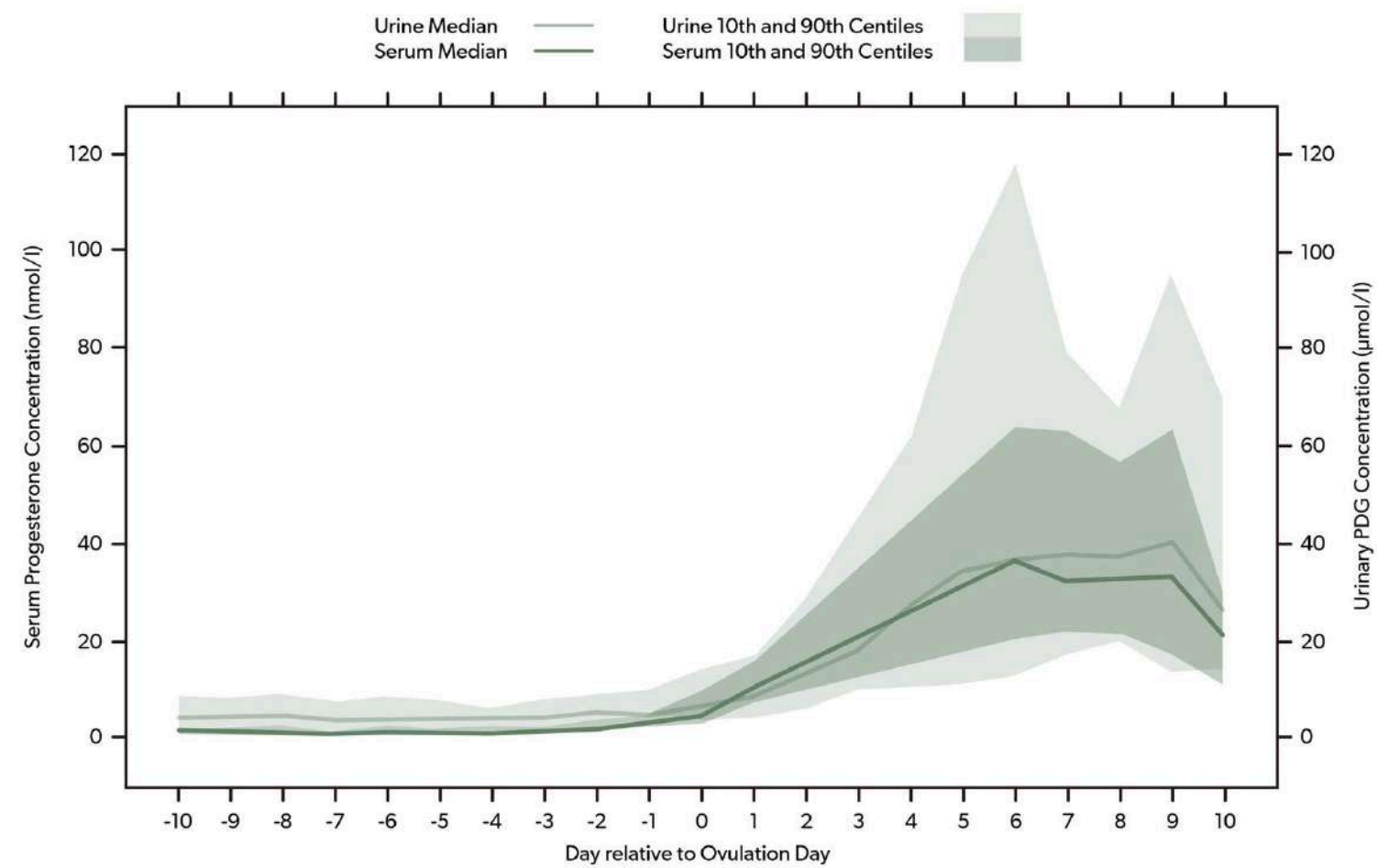
mira

Clinical, research and  
case studies using Mira

Relevant research studies  
comparing hormone levels in urine  
and serum

# Comparison of urinary and serum reproductive hormones referenced to true ovulation (n=40)

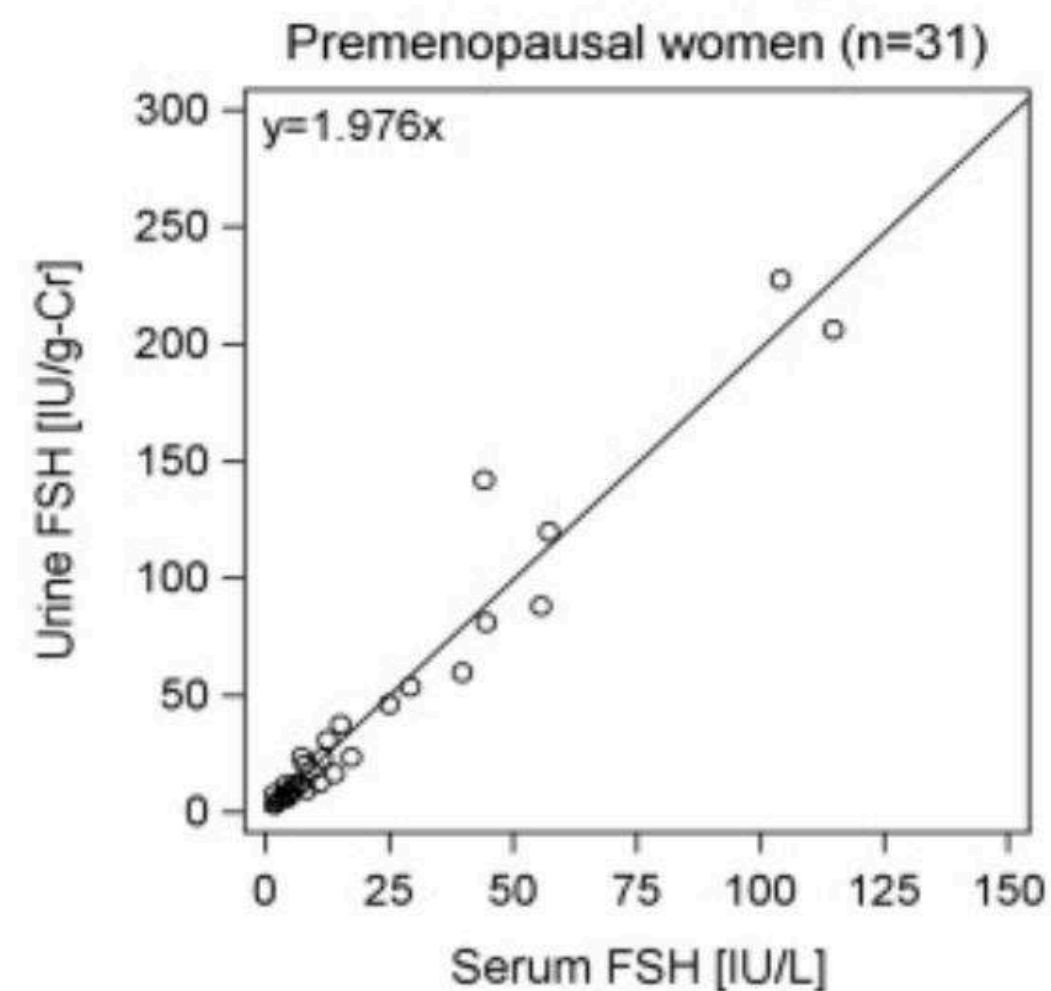
Urinary and serum reproductive hormones showed excellent agreement and may be used interchangeably. The beginning of the surge in serum and urinary LH was an excellent predictor of ovulation. The rise in progesterone and P3G above baseline was a consistent marker of luteinisation confirming ovulation. Both LH and progesterone surges delivered clear, sharp signals in all volunteers, allowing reliable detection and confirmation of ovulation.



# Urine estrone-3-glucuronide (E3G) assay: is there any place during ovarian stimulation for IVF cycles?

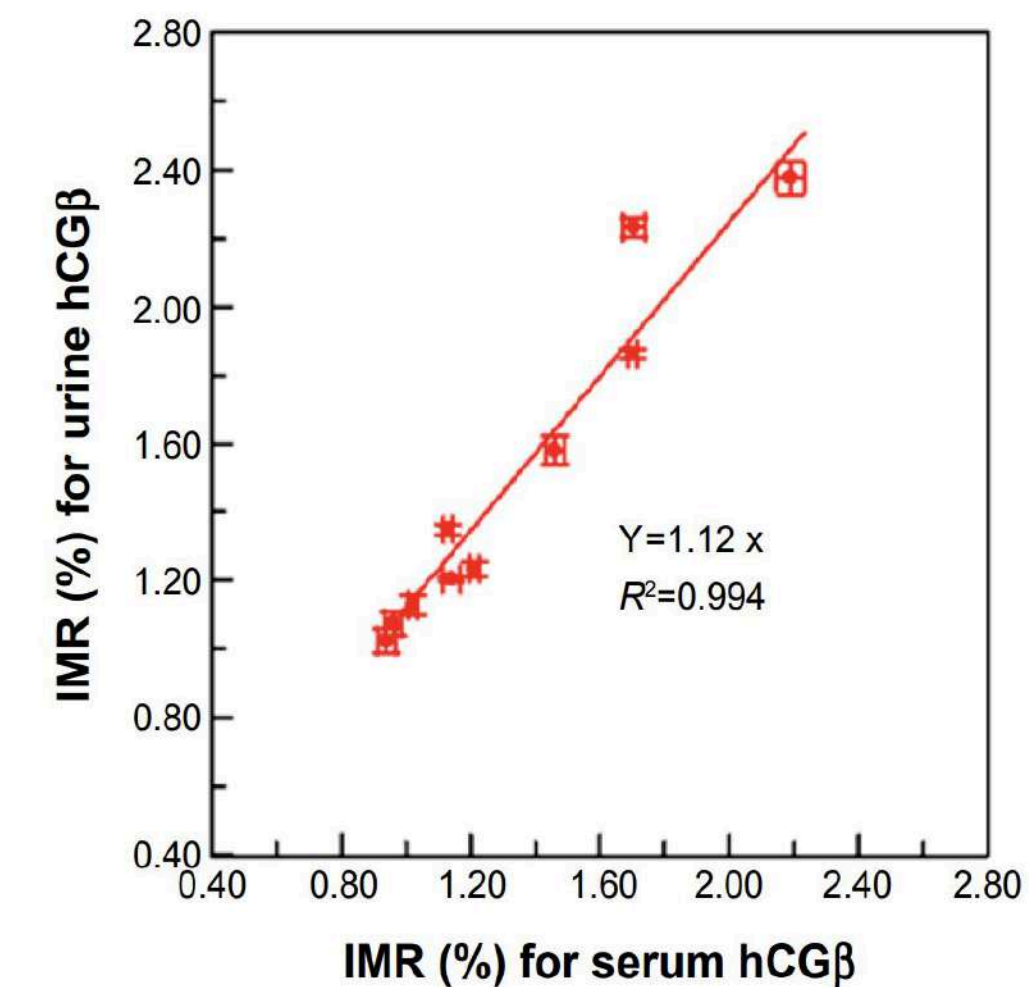
## Association between FSH levels in urine and serum in premenopausal women

Urine FSH strongly correlated with FSH in serum in premenopausal ( $R^2 = 0.98$ ) women.



## Quantitative analysis of total $\beta$ -subunit of hCG concentration in urine

An excellent correlation of total hCG $\beta$  IMR signals between urine and serum was noted ( $R^2=0.994$ ).



# Mira vs. Laboratory Readers

# Quantifiable Concentration Ranges

## Dynamic range of Mira Tests

Hormone changes during Menstrual cycle	LH	E2 or E3G	hCG	Progesterone or PdG
Serum	0-200 mIU/ml	0-2000 pg/ml	0-300000 mIU/ml	0-20 ng/ml
Urine	0-200 mIU/ml	0-4000 ng/ml	0-300000 mIU/ml	0-15 µg/ml
Mira test	0-400 mIU/ml, customizable to 0-1000 mIU/ml	0-640 ng/ml, customizable to 0-4000 ng/ml	0-100000 mIU/ml	0-30 µg/ml

# Quantifiable Concentration Ranges

## Precision (Coefficient of Variation)

Hormone changes during Menstrual cycle	LH wand	E3G wand	hCG wand	Industry Standart
Intra-Lot	10%	15%	10%	15%
Inter-Lot	15%	20%	15%	20%

## Recovery rate

Hormone changes during Menstrual cycle	LH wand	E3G wand	hCG wand	Industry Standart
Recovery rate	10%	15%	10%	15%

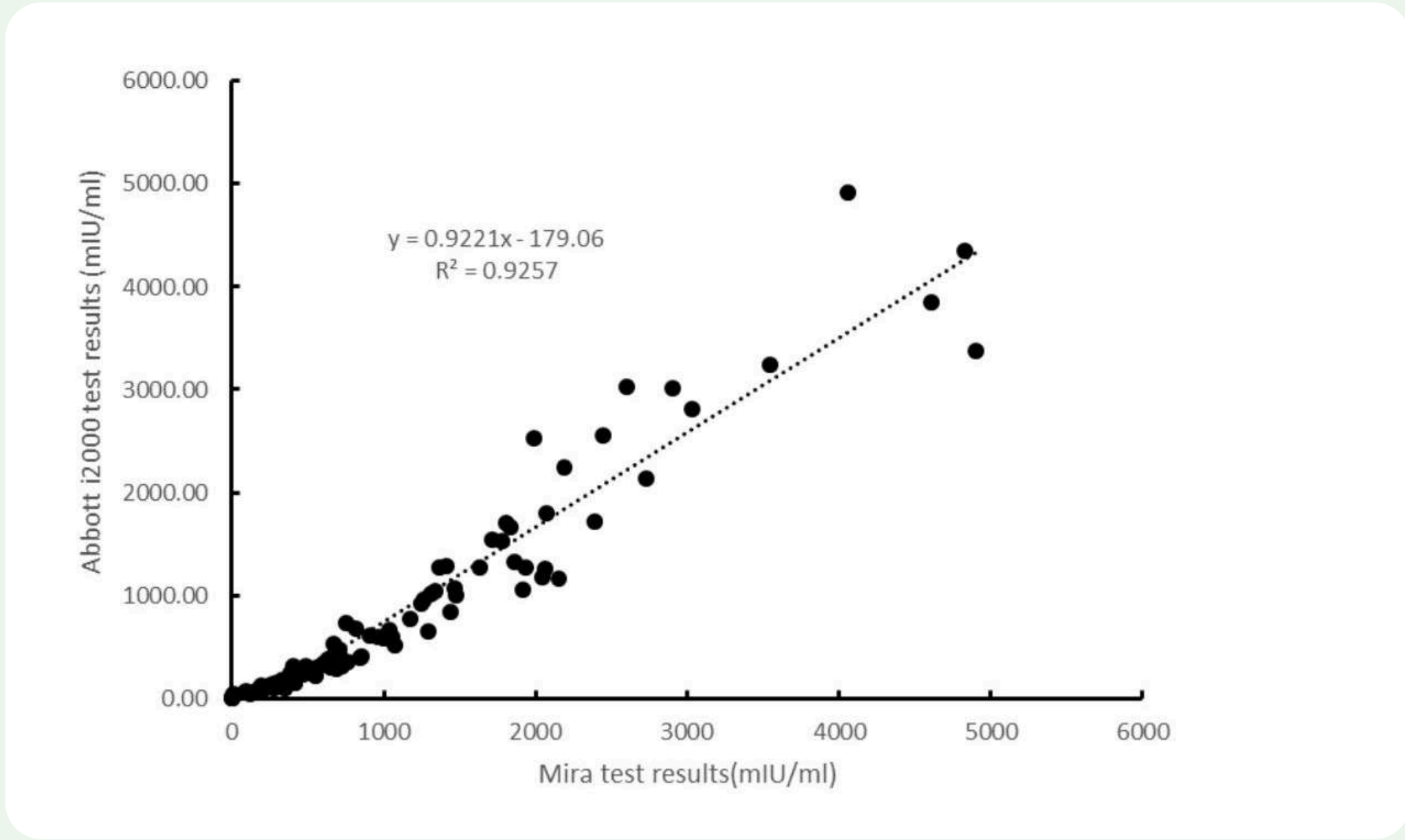
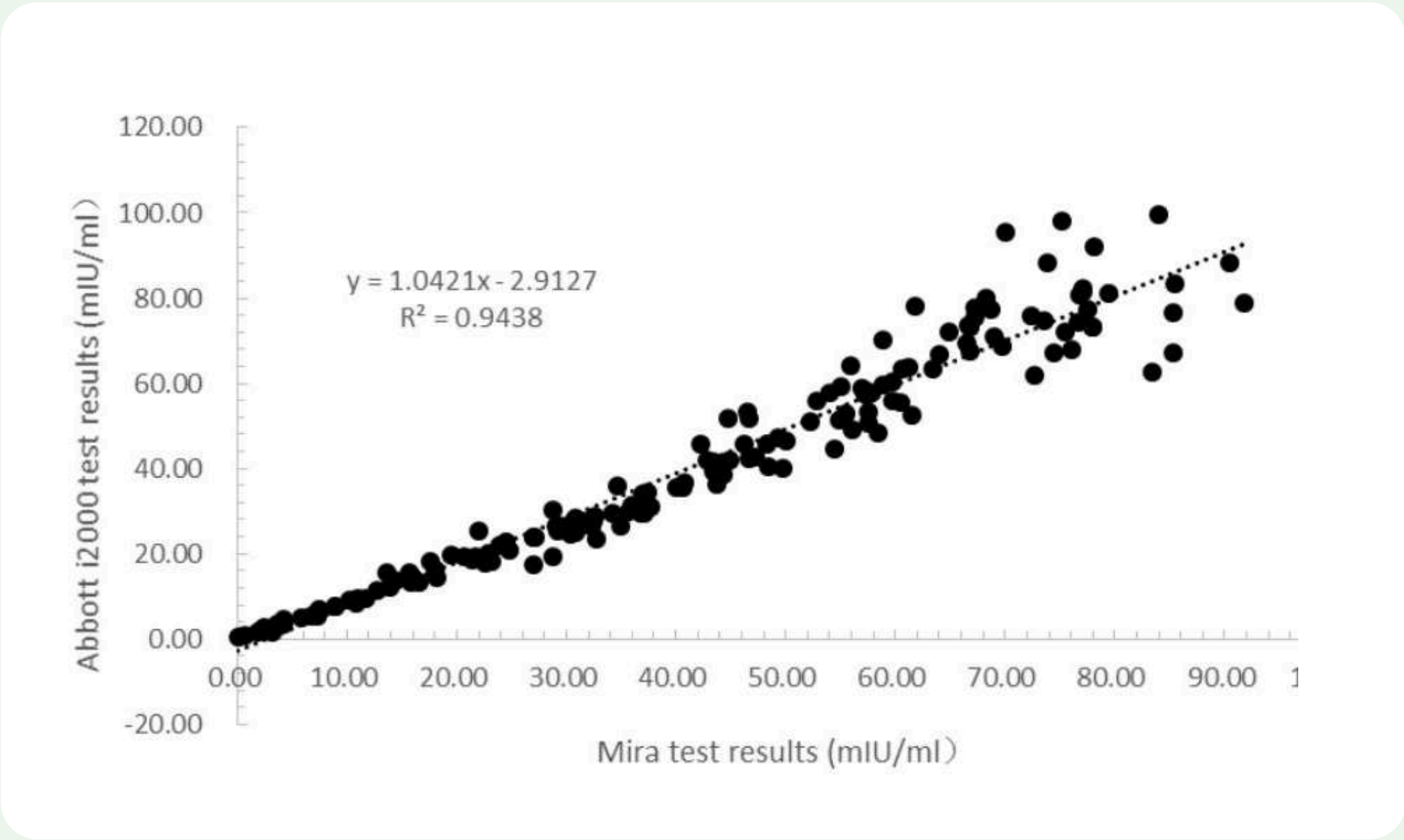


# Mira's technology is based on the immunofluorescence method with accuracy equivalent to laboratory readers

The Mira LH and hCG measurements strongly correlated with Abbott ARCHITECT i2000SR. A good linear correlation ( $R^2 > 0.9$ ) was displayed by The Mira Analyzer and Abbott ARCHITECT i2000SR for both LH ( $R^2 = 0.944$ ; slope = 1.042) and hCG ( $R^2 = 0.926$ ; slope = 0.922)\*.

LH

hCG



\* Under editor's review

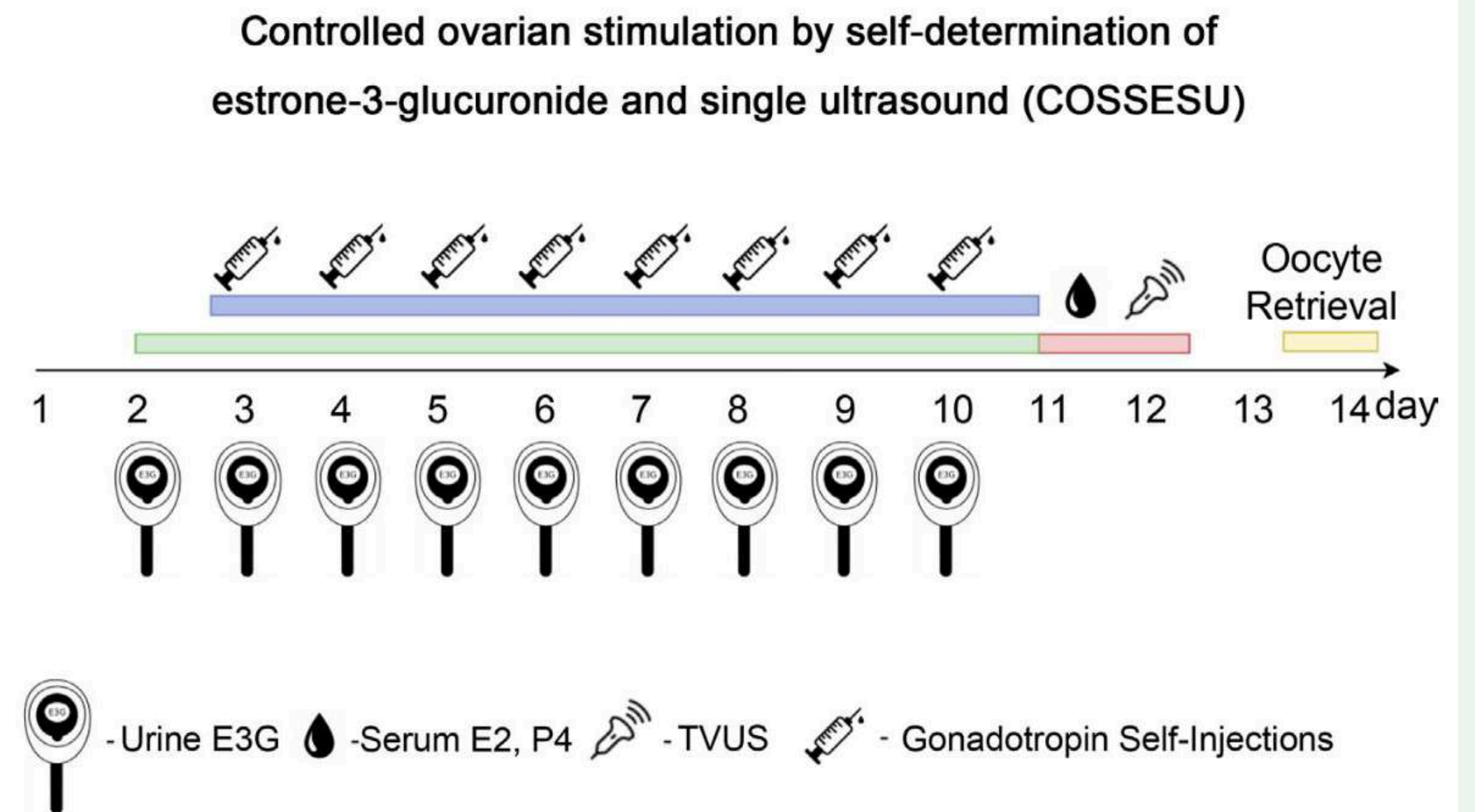


# Mira E3G vs. Serum E2

# Urine estrone-3-glucuronide (E3G) assay: is there any place during ovarian stimulation for IVF cycles?

## Key Findings:

Serum E2 values were assessed routinely, while E3G values were measured and validated using a fluorescent immunoassay Mira Fertility Plus® analyzer. The urine E3G of the assay was validated for intra and inter-assay variability with a coefficient of variation of < 20%. It was also validated for analytical and functional sensitivity and sample stability. Linear regression of serum E2 and E3G values of 56 early morning urine samples who had evaluated between days 4 and 13 of the menstruation cycle provided an  $r = 0,81$ . Urine E3G values also correlated to follicle growth. Patient survey results showed that urine sampling was the preferred method of analysis. Urine E3G testing correlates well to serum E2 assessment in COH. Urine E3G assay provides an alternative to serum-based assessment.



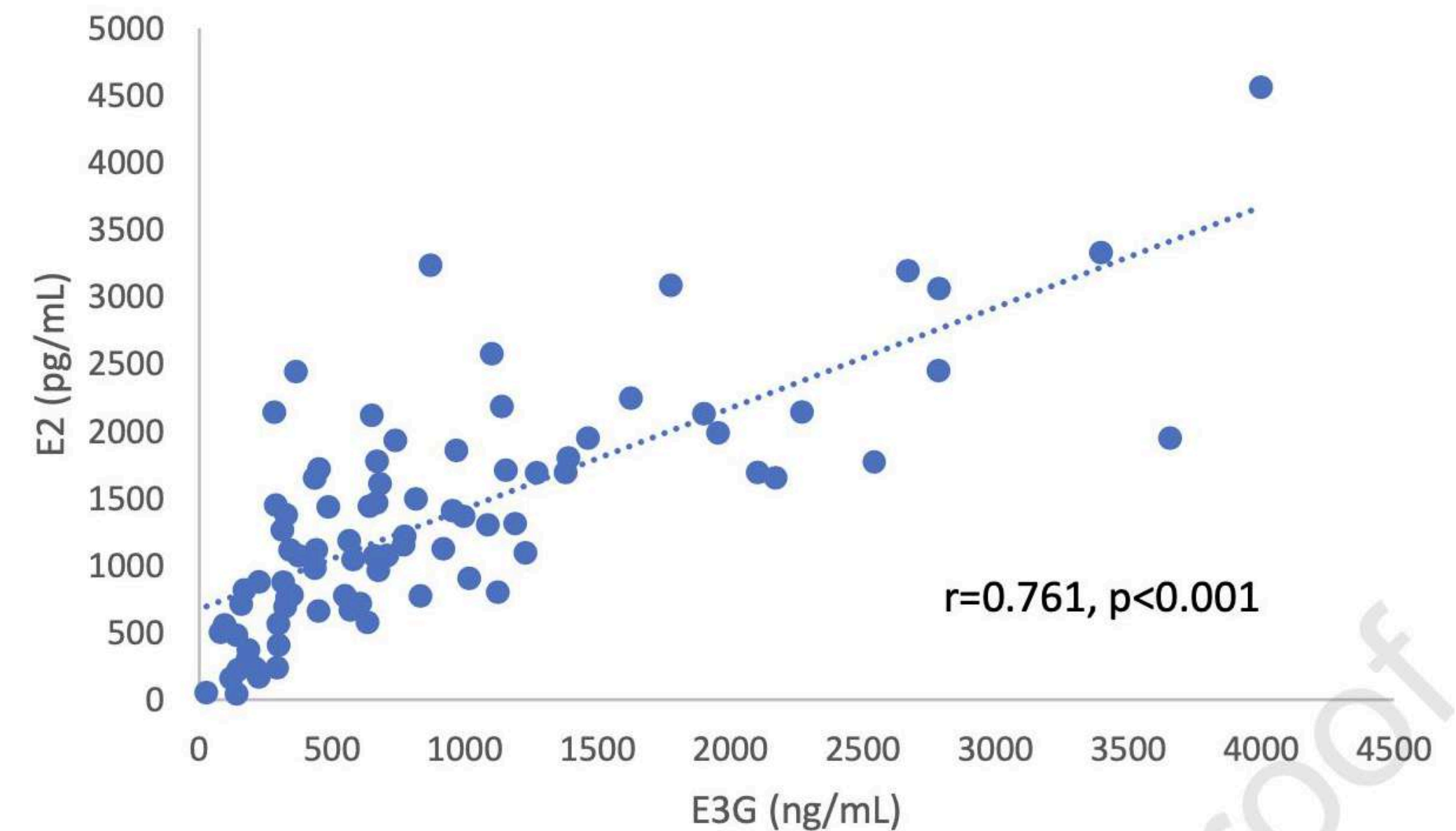
I Vladimirov, V Martin, T Desislava, P-670 Urine estrone-3-glucuronide (E3G) assay: is there any place during ovarian stimulation for IVF cycles?, Human Reproduction, Volume 36, Issue Supplement\_1, July 2021, deab130.669, <https://doi.org/10.1093/humrep/deab130.669>;

Vladimirov, I. , 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. doi: [10.4236/ojog.2021.119115](https://doi.org/10.4236/ojog.2021.119115).

# At-home urine estrone-3-glucuronide quantification predicts oocyte retrieval outcomes comparably to serum estradiol.

## Key Findings:

Both urine E3G (measured by fluorescent immunoassay Mira Fertility Plus® analyzer) and serum E2 concentrations on day of trigger significantly correlated with retrieval outcomes to a similar extent, with E3G demonstrating slightly higher correlation to the number of M2 oocytes compared to E2 ( $r = 0.485$  vs  $0.391$ , respectively). The Pearson correlation coefficient for matched E3G and E2 levels was  $0.761$  ( $p < .001$ ). The correlations of determination for daily trend of E3G and E2 during stimulation were  $0.7066$  and  $0.6102$ , respectively. Measured on the day of trigger, urine E3G monitoring during gonadotropin stimulation is comparable to serum E2 for predicting oocyte retrieval outcomes. Matched daily samples confirm 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 the patient experience.





# Viability of home monitoring of estrone-3-glucuronide (E3G) urine levels in controlled ovarian stimulation: A pilot study

## Key Findings:

The average female age was 32,1 years ( $\pm 4.4$ ), BMI 22,9 kg/m<sup>2</sup> ( $\pm 4,3$ ), AMH 3,9 ng/ml ( $\pm 2,7$ ), stimulation days 10,1 ( $\pm 1,2$ ), collected oocytes 12,6 ( $\pm 8,5$ ), MII oocytes 10,8 ( $\pm 7,9$ ), fertilization rate 83,4% ( $\pm 22,7$ ), blastocyst formation 66,9% ( $\pm 28,6$ ), good quality blastocysts 31,1% ( $\pm 16,6$ ).

The log-linear mixed effect model (LLMM) estimation produced reasonable estimates of 49% average day-to-day growth rates (slope fixed effect), with one standard deviation (SD) range of 25% to 77% across patients (SD of the slope random effect).

Moreover, there was a comparatively high correlation of 0.76 between the individual growth rates of E3G estimated over days 3-6 (the slope random effects of the LLMM model) and the E3G levels at day 10. In this way, the estimated slope random effects appear to have a prognostic value and may potentially have therapeutic implications, for example, adjustment of the stimulation dose. Moreover, the Spearman correlation between Estradiol and E3G was 0.83

After analyzing interviews and questionnaires, patients evaluated the applied method as easy and convenient, with 97% of them preferring OS monitoring to be performed in this manner compared to the standard method, which includes regular ultrasound examinations and determination of serum hormone levels.

# Mira vs. Qualitative Solutions

# Quantitative Versus Qualitative Estrogen and Luteinizing Hormone Testing for Personal Fertility Monitoring (Natural Family Planning — Marquette Method)

## Key Findings:

The study established that due to the quantitative measurement of LH the peaks that indicates the most fertile period of the menstrual cycle by Mira monitor throughout 3 menstrual cycles, only 2% of LH peaks have been missed in comparison to 5% of peaks missed when using qualitative measurement with ClearBlue Fertility Monitor.

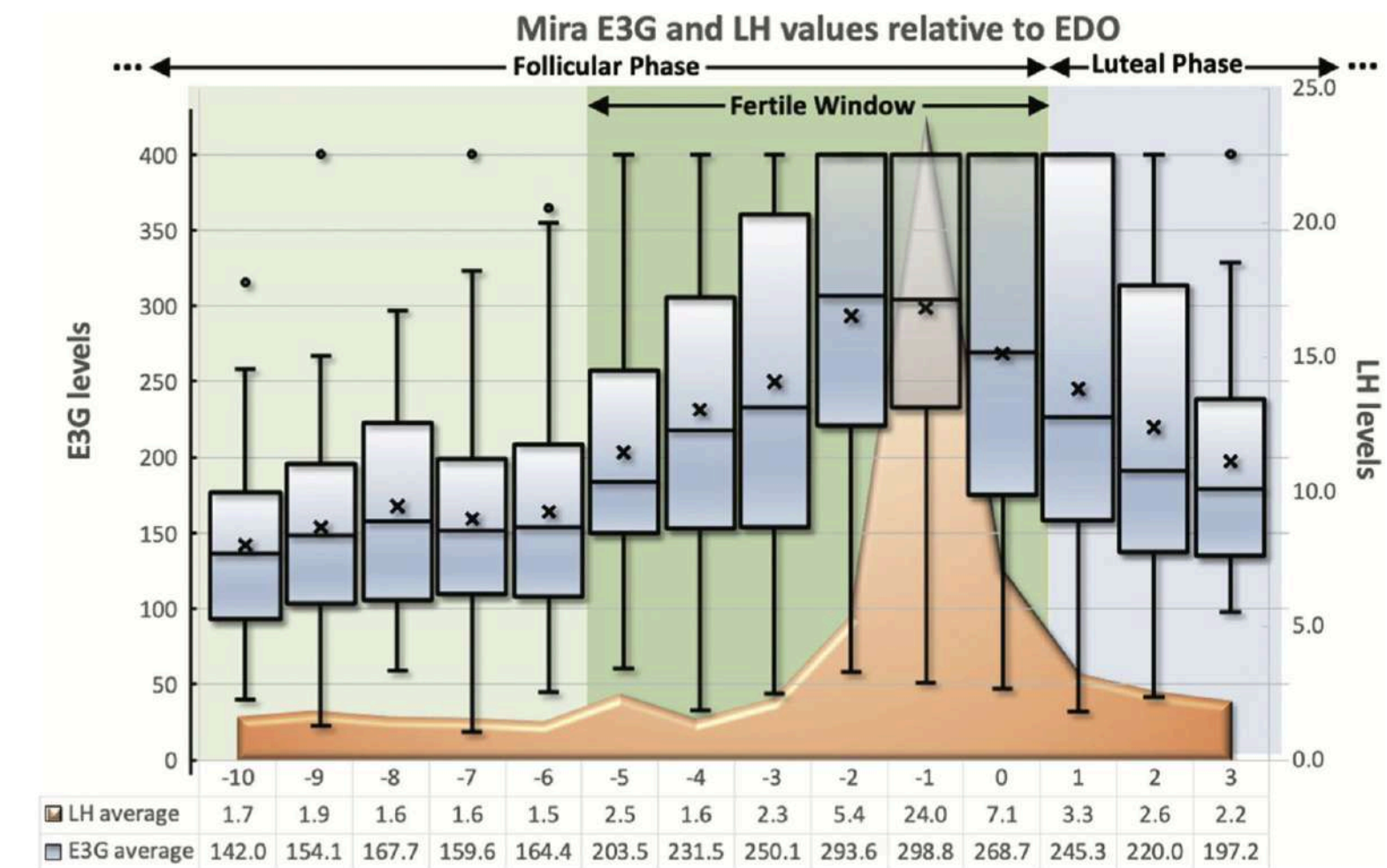


Figure 5. Quantitative Mira levels of E3G (in ng/ml, blue box-and-whisker plots) and LH (in ng/ml, orange mean daily values). The Mira EDO (day 0) was defined as the day after the peak LH day, which is why the LH surge is noted on day -1. LH and E3G mean values are shown in the table below, with peak E3G at 298.8ng/ml and peak LH at 24.0 mIU/ml. E3G values were represented with 25-75 percentile boxplots with min/max whiskers (excluding outliers). The (x) represents the average (mean) for the day, and the horizontal line represents the median for values in the range. The Follicular Phase is defined from the first day of the menstrual period up to and including the day of ovulation. The Fertile Window is defined as the 6-day interval from 5 days before (-5) up to and including the estimated day of ovulation (EDO = 0). The Luteal phase is defined as the day after EDO until the past day of the menstrual cycle.



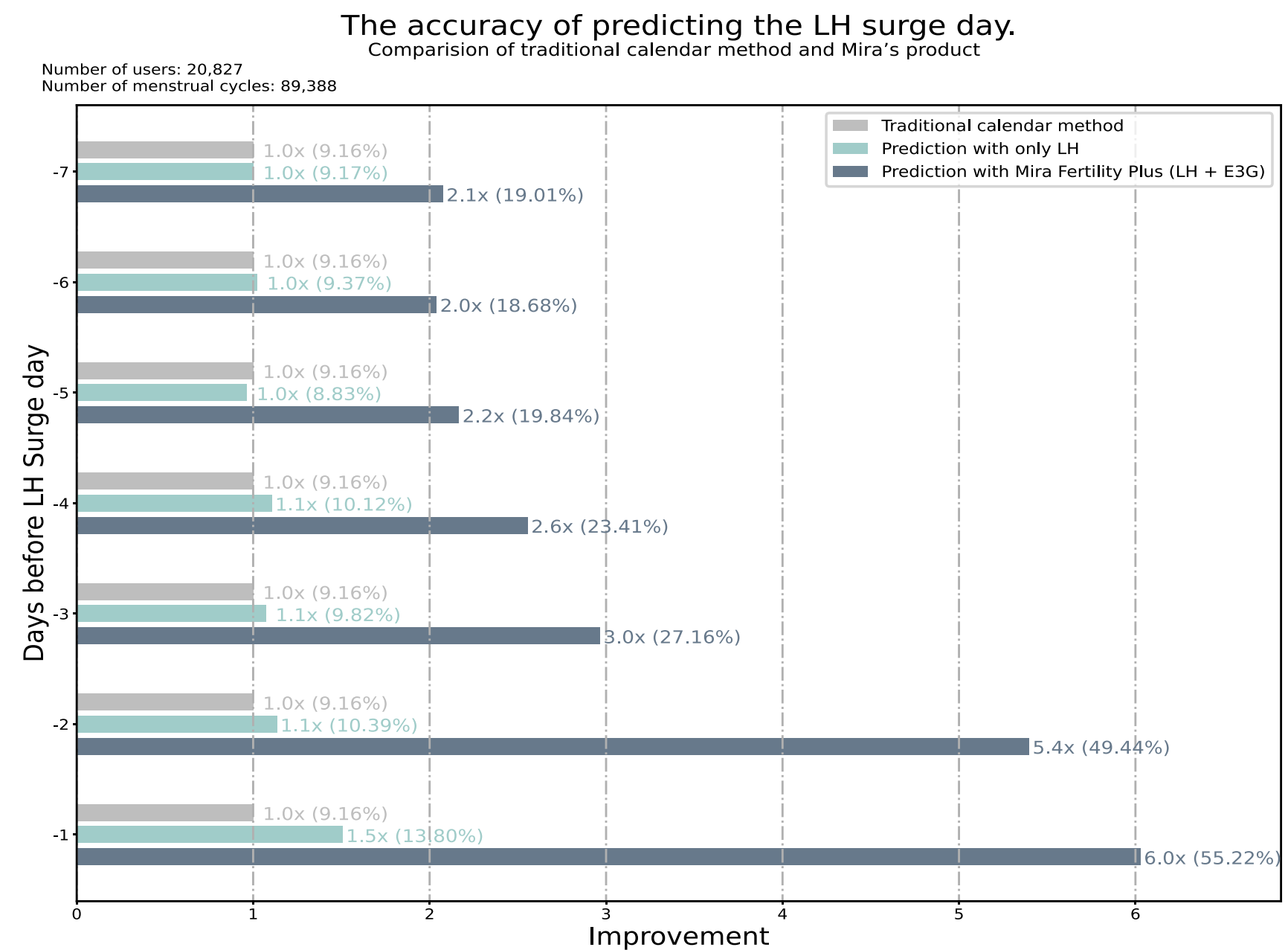
# Mira vs. Calendar Method

# Intelligent algorithms combined with a device that detects luteinizing hormone and E3G levels can significantly improve the prediction accuracy of ovulation day

## Key Findings:

The accuracy of predicting the LH surge day was compared among three solutions: the traditional calendar method, using LH hormone only, and using both LH and E3G hormones together. Calculated in a retrospective analysis, from 1 to 7 days before the actual LH surge day.

Optimized with more than 7M data points and 600K+ menstrual cycles, the Mira algorithm is 6X more accurate than traditional calendar method and 5X more accurate than traditional ovulation prediction solutions offered over the counter.



# Other Case Reports and Studies

# Case Reports from Women Using a Quantitative Hormone Monitor to Track the Perimenopause Transition

## Conclusions:

Tracking fertility during Perimenopause with a quantitative hormonal device is a novel idea. A quantitative hormone monitor allows for the exact measurements of hormones in a woman's cycles and can give more accurate results of cycle patterns, especially as she ages. This study of women in perimenopause has revealed certain cycle characteristics unique to this period, which include cycles with delayed LH surges, quick rises in E3G toward the LH surge, low E3G and LH levels in a cycle, double LH surges in one cycle with corresponding FSH elevation during the highest LH surge, continuous high levels of E3G and LH throughout the cycle, and low PdG levels after an LH surge.

**Figure 10.** Variable cycle with MIRA: 49 yo with late LH peak, no PdG rise, and a 29-day cycle.





# Using Quantitative Hormone Monitoring to Identify the Postpartum Return of Fertility

## Conclusions:

In Cycle 0, higher thresholds of LH are required to trigger ovulation, so a higher threshold of LH (i.e.,  $LH < 15$ ), along with a more conservative E3G threshold (i.e.,  $E3G < 100$ ) to reflect the average E3G levels within the fertile window of Cycles 0–6 postpartum (Table 3), could be used in Cycle 0 for avoiding pregnancy (Table 6). This protocol will be the basis of a larger effectiveness study using the Mira Analyzer for avoiding pregnancy postpartum.

**Table 3.** Estrone-3-glucuronide (E3G) values in ng/mL for days leading up to estimated day of ovulation (EDO = 0).

	-6	-5	-4	-3	-2	-1	0	1
Cycle 0 (n = 10)	89 ± 58	105 ± 41	117 ± 63	159 ± 101	216 ± 132	255 ± 103	177 ± 176	129 ± 51
Cycle 1 (n = 10)	133 ± 162	142 ± 125	126 ± 73	148 ± 71	161 ± 76	155 ± 71	239 ± 180	236 ± 190
Cycle 2 (n = 8)	95 ± 55	111 ± 80	129 ± 62	119 ± 50	165 ± 60	216 ± 113	251 ± 191	300 ± 242
Cycle 3 (n = 8)	127 ± 76	110 ± 71	157 ± 97	190 ± 64	284 ± 142	207 ± 81	296 ± 189	197 ± 136
Cycle 4 (n = 5)	100 ± 58	88 ± 64	137 ± 33	133 ± 79	181 ± 106	212 ± 108	252 ± 229	167 ± 65
Cycle 5 (n = 4)	190 ± 170	166 ± 172	138 ± 89	135 ± 117	186 ± 88	232 ± 118	367 ± 108	128 ± 84
Cycle 6 (n = 2)	162 ± 73	116 ± 78	227 ± 249	106 ± 71	199 ± 88	294 ± 143	331 ± 83	175 ± 94

**Table 6.** Revised Cycle 0 protocol with updated thresholds based on the current pilot data. This is based on users testing daily.

	Available Day for Intercourse?
If E3G is $< 100$ today and yesterday AND If LH is $< 15$ today and the last 4 days	Yes
If E3G is $\geq 100$ today or yesterday	No
If LH is $\geq 15$ today or the last 4 days	No

# Establishing a Gold Standard for Quantitative Menstrual Cycle Monitoring

## Upcoming study:

*Background and Objectives:* The Quantum Menstrual Health Monitoring Study will measure four key reproductive hormones in the urine (follicle-stimulating hormone, FSH; estrone-3-glucuronide, E13G; luteinizing hormone, LH; and pregnanediol glucuronide, PDG) to characterize patterns that predict and confirm ovulation, referenced to serum hormones and the gold standard of the ultrasound day of ovulation in participants with regular cycles. These normal cycles will provide a reference for comparison to irregular cycles in subjects with polycystic ovarian syndrome (PCOS) and athletes.

*Hypothesis:* The Mira monitor quantitative urine hormone pattern will accurately correlate with serum hormonal levels and will predict (with LH) and confirm (with PDG) the ultrasound day of ovulation in those with regular cycles as well as those with irregular cycles.

*Rationale:* Once the ultrasound validation is complete, tools like the Mira monitor with a customized app may become a new standard for at-home and remote clinical monitoring of the menstrual cycle without having to use labor-intensive follicular-tracking ultrasound or follow serum hormone changes. *Conclusions:* Precision monitoring of the menstrual cycle is expected to impact individuals who want to increase their menstrual health literacy and guide decisions about fertility.

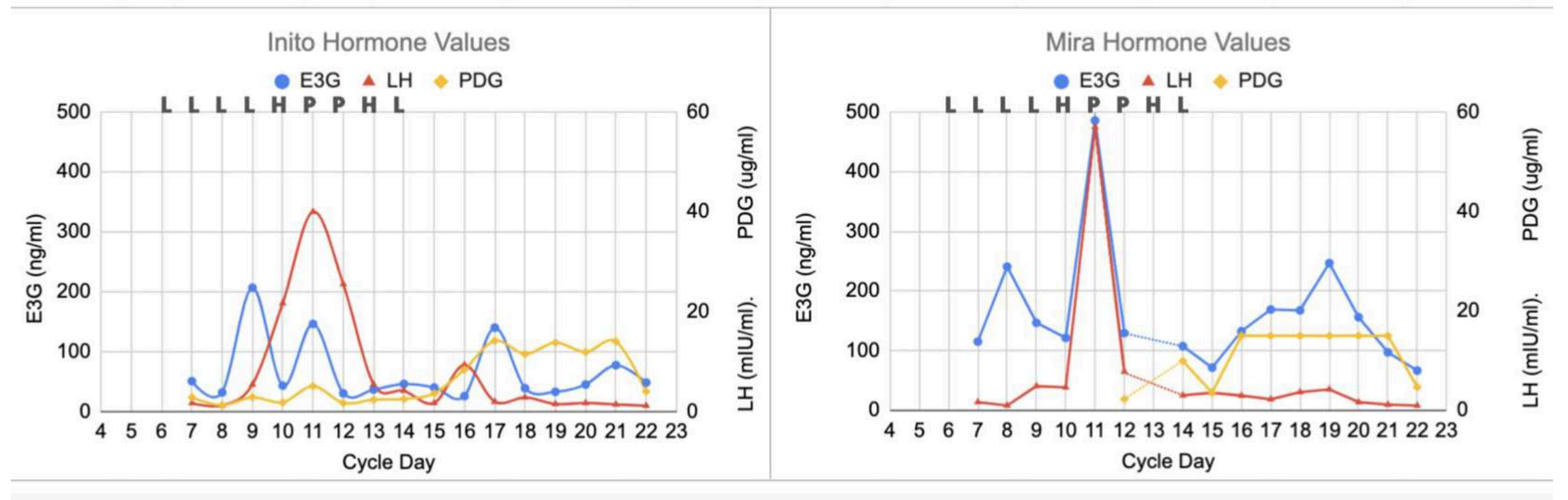


# Using Quantitative Hormonal Fertility Monitors to Evaluate the Luteal Phase: Proof of Concept Case Study

## Abstract:

Several new quantitative fertility monitors are now available for at-home use that measure estrogen, luteinizing hormone (LH), and progesterone (PDG) in urine. This case report compares the Mira and Inito quantitative fertility monitors with the well-established qualitative ClearBlue fertility monitor. Three clinical scenarios were evaluated: a normal cycle, a prolonged luteinization cycle, and an anovulatory cycle. The identification of the luteal phase (or lack thereof in the case of anovulation) and the transition through the three processes of luteinization, progestation, and luteolysis were clearly demarcated with the help of quantitative LH and PDG. Quantitative fertility monitors have the potential to identify details of the luteal phase to help women with regular cycles and abnormal luteal phases to help target interventions for optimizing fertility.

**Figure 1.** Normal cycle showing all three monitors with agreement on the peak day, with the highest LH value on that day (cycle day 11). The ClearBlue results (L = Low, H = High, and P = Peak) are shown above each graph on the respective days. The estimated day of ovulation was day 12 (day after LH peak). The luteal phase was 13 days (cycle length of 25 days). PDG initially rose on day 16 on the Inito monitor and on day 14 on the Mira monitor.



mira

Thank You!

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