Association Between Urine and Serum Estradiol Levels in *In Vitro* Fertilization Cycles

Chokchai Chotboon

Khon Kaen University

Lingling Salang (✉ slingling@kku.ac.th)

Khon Kaen University

Pranom Buppasiri

Khon Kaen University

Siriruthai Amnatbuddee

Khon Kaen University

Nuntasiri Eamudomkarn

Khon Kaen University

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Abstract

Objectives

To study the correlation between urine and serum estradiol (E2) controlled ovarian hyperstimulation (COH)

Methods

This is a cross-sectional analytical study that was conducted in a tertiary care hospital. Seventy-seven urine and blood samplings were collected from infertile women who were treated with COH. An electrochemiluminescent immunoassay was performed to evaluate E2 levels between urine and serum samples on the 6th day and the day of ovarian trigger. In addition, the correlations were evaluated between urine E2 level and number of follicles, retrieved, metaphase II oocytes, and fertilization rate. A sub-analysis was performed for age, responding status and BMI.

Results

Seventy-seven infertile women were recruited. The medians of serum and urine E2 level (E2/creatinine) levels on the day 6th of ovarian stimulation were 833.20 pg/ml (IQR; 516.90–1,371.00) and 3.67 (IQR; 2.84–4.81), respectively. On the day of ovarian trigger, the median of serum E2 level was 2,113.00 pg/ml (IQR; 1,382.00–3,885.00) and urine E2 level (E2/creatinine) was 6.84 (IQR; 5.34–8.70). The correlation between serum and urine E2 level on day 6th was 0.53 and the day of ovarian trigger was 0.59, $p < 0.001$. Moreover, the correlations of urine E2 level on the day of ovarian trigger to number of follicles, number of oocytes retrieved, metaphase II oocytes and fertilization rate were 0.57, 0.58, 0.61, and 0.64 ($P < 0.001$).

Conclusion

The urine E2 level was moderately correlated to serum E2, number of follicles growth, oocytes retrieved and also fertilization rate.

1. Introduction

Controlled ovarian hyperstimulation (COH) involves the administration of hormone to stimulate follicular growth in expectation of multiple oocytes collection for *In Vitro* Fertilization (IVF) or intracytoplasmic sperm injection (ICSI), for the propose of increasing the live birth rate of infertile couples¹. Currently, transvaginal sonography, serum estradiol (E2), follicle stimulation hormone (FSH) and progesterone are used for monitoring ovarian responses in the COH cycle. Serum E2 level is not only used to predict the ovarian response but also to predict the ovarian hyperstimulation syndrome (OHSS)². The OHSS is the

major complication of ovarian hyperstimulation due to over-response to medication. The severity varies from mild to severe. The mild form of OHSS is nausea and vomiting. The severe form such as pleural effusion, massive ascites and thrombosis^{3,4} occurs in 0.2-1%⁵. An existing guideline suggested that women with > 25 developed follicles, > 24 retrieved oocytes or a serum E2 level > 3,500 pg/ml are at a much higher risk for OHSS development⁴.

Although there is not any the benefit of additional serum E2 monitoring to transvaginal ultrasonography for ovarian monitoring, in order to prevent OHSS and oocyte retrieval prediction, the included studies were of low quality¹. Until the safety concerns about OHSS and thromboembolic risk in supraphysiologic estrogenemic conditions were evident, adding serum E2 monitoring was acceptable⁶. The serum E2 monitoring is also necessary for measuring ovarian stimulation in estrogen sensitive cancer patients. The high level of estrogen can promote some types of cancer cell growth⁷. The ovarian stimulation might be safer with added estrogen-lowering medicine such as letrozole and monitoring serum E2 until the decrease is in the normal range, for these patients^{8,9}.

There are many estrogenic metabolites secreted via urine, such as urinary estrone metabolites, estriol, 2-hydroxyestrone, 2-methylestrone, 2-methoxyestrone, 2-hydroxyestrone-3-methyl ether, 4-hydroxyestrone, 4-methoxyestrone, 16 α -hydroxyestrone and urinary E2 metabolites. The urine E2 level is the most relevant to serum E2 level^{10,11}. Recently, many studies have been reported that the serum E2 level is moderately correlated to urine E2 level in pre- and postmenopausal women¹²⁻¹⁶. Unfortunately, the data for the COH cycle are scarce. Therefore, for this study it was hypothesized that the urine E2 level might be correlated to the serum E2 level in the COH cycle. The objectives of this study were to study the correlation between urine and serum E2 in women treated with COH and to evaluate the correlation between urine E2 level to the number of follicles, number of retrieved oocytes, number of metaphase II oocytes, and percentage of fertilization.

2. Material And Methods

2.1 Study participant and study design

A cross-sectional study was conducted. The serum and urine samples were obtained in the infertility clinic between April 2020 and November 2020 from infertile women treated with controlled ovarian hyperstimulation for IVF or ICSI at Srinagarind Hospital, Khon Kaen University. The inclusion criteria were women aged > 20 years-old without contraindication for hormonal treatment. After receiving written informed consents of the participants, urine specimens were collected at the same time of routine blood collections in the morning on the 6th day and the last day of ovarian stimulation. Other information such as the cause of infertility, body mass index (BMI), type of hormone, and follicular sizes were recorded, and the follicular sizes were measured by 2 dimensional (2D) transvaginal ultrasonography. This research was approved by the Khon Kaen University Ethics Committee on Human Research (HE621557).

2.2 Controlled ovarian hyperstimulation protocol

Transvaginal ultrasound was used for all participants on the second or third day of menstruation in those whose ultrasound was normal with a normal uterus and ovaries without cysts. Daily gonadotropin injection treatment, the HMG (Menopur®, Ferring AG, Germany) or rFSH (Gonal-F®, Merk, France) was started on the 2nd or 3rd day of menstruation. Long-acting rFSH treatment at a subcutaneous dose (100 - 150 mcg) of corifollitropin alpha (Elonva®, MSD, USA) was injected on the second or third day of menstruation. The treatment continued from day eight of stimulation with rFSH and the dose was based on ultrasound findings. The GnRH antagonist (Orgalutran®, MSD, USA) was used daily until triggering occurred when the leading follicles reached a mean diameter of 14 mm. The ovulation was triggered by the GnRH agonist or human chorionic gonadotropin (HCG) in case of > 2 dominant follicles of >17 mm.

2.3 Estradiol analysis

Both urine and serum E2 levels were analyzed by an Electrochemiluminescent immunoassay (ECLIA) within 1 hour after collection. The ECLIA at the KKU center is Elecsys Estradiol III Cobase 801® that employs a competitive test principle using two monoclonal antibodies specifically directed against 17-β E2. The duration of the automated assay was 18 minutes, calibrated with an internal quality control daily and external quality control monthly. Urine E2 was corrected by urine creatinine as the urine E2-creatinine ratio (E2/creatinine) to correct the glomerular infiltration rate.

Serum and urine collection: On the 6th day of stimulation and day of trigger ovulation, serum and urine E2 levels were analyzed.

2.4 Data analysis

Based on the result of Maskarinec G and et al, 2015¹⁶, with a 10% dropout rate, a 77 patient sample size was needed. The general appearances of participants were analyzed by SPSS V.23 in terms of mean and standard deviation (SD). The correlations between urine E2 to serum E2, ultrasonographic follicles, retrieved oocytes, metaphase II oocytes and fertilization rate were analyzed by Spearman's correlation. A p-value <0.05 indicated statistical significance.

3. Results

A total of 77 infertile women were included. Their mean age was 35.81 ± 4.49 years, BMI was normal (22.63 ± 3.54 kg/m²) and most of them were primarily infertile (89.61%). The three main causes of infertility were unexplained 35.6%, male factor 29.8% and tubal factor 23.4% (Table 1). The most common type of gonadotropin injection was daily human menopausal gonadotropin (HMG) 36.36%, followed by recombinant FSH (rFSH) 35.07% and combined long acting rFSH (corrifollitropin alpha) followed by rFHS or HMG 28.57%. The average day of gonadotropins injection was 9 days, and the average of the gonadotropin injection was 2,495.91 units. In gonadotropin releasing hormone (GnRH) antagonist types, half of the participants (51.95%) were given the dual trigger, the combined GnRH antagonist with HCG while 36.36% and 11.69% were triggered by GnRH-antagonist alone or HCG alone. The median of retrieved oocytes and metaphase II oocytes were 9 oocytes (IQR; 5–18) and 7 oocytes

(IQR; 4–15). The numbers of 2PN were 5 (IQR; 3–10), cleavage 5 (IQR; 2–9, and blastocyst stages 4 (IQR; 1–6).

Table 1
Baseline characteristics

Baseline cycle characteristics (mean ± SD)	
- Age (years)	35.81 ± 4.49
- BMI (kg/m ²) ^a	22.00 (17.26–37.13)
Parity (%)	69 (89.61)
• Primary infertility	8 (10.39)
• Secondary infertility	
Cause of infertility (%)	27 (35.06)
• -Unexplained	23 (29.87)
• -Male factors	18 (23.38)
• -Tubal factors	6 (7.79)
• -Ovulation disorders	3 (3.90)
• -Uterine factors	
AFC ^a	7 (2–25)
Gonadotropin types (%)	28 (36.36)
-HMG	27 (35.07)
-rFSH	22 (28.57)
-Corifollitropin alpha followed by rFSH or HMG	
Days of gonadotropin injection (days) ^a	10 (9–10)
Gonadotropin (mean ± SD)	2,495.91 ± 600.81
-Daily gonadotropin; HMG or rFSH (IU)	127.27 ± 25.48
-Long-acting gonadotropin (Corrifollitropin alpha) (mcg)	
Days of GnRH antagonist injection (days) ^a	4 (3–5)

Note ^a Data presented as median and interquartile range (IQR)

Abbreviation; rFSH = recombinant follicle stimulating hormone, HMG = human menopausal gonadotrophin, HCG = Human Chorionic Gonadotropin

Baseline cycle characteristics (mean ± SD)	
Hormone for trigger ovulation (%)	40 (51.95)
-Combined GnRH agonist and HCG	28 (36.36)
-GnRH agonist	9 (11.69)
-HCG	
Number of retrieved oocytes ^a	9 (5–18)
Number of metaphase II oocytes ^a	7 (4–15)
Fertilization rate ^a	5 (3–10)
-Number of 2PN stage	5 (2–9)
-Number of cleavage stage	4 (1–6)
-Number of blastocyst stage	
Note ^a Data presented as median and interquartile range (IQR)	
Abbreviation; rFSH = recombinant follicle stimulating hormone, HMG = human menopausal gonadotrophin, HCG = Human Chorionic Gonadotropin	

The median serum E2 levels were 833.20 pg/ml (IQR; 516.90–1,371.00) on the 6th day and 2,113.00 pg/ml (IQR; 1,382.00–3,885.00) and on the last day of ovarian stimulation. While the median urine E2 levels (E2/creatinine) were 3.67 (IQR; 2.84–4.81) on the 6th day and 6.84 IQR; (5.34–8.70) on the last day of ovarian stimulation (Table 2). In addition, the urine E2 level was correlated with serum E2 level at 0.53 ($p < 0.001$) on the 6th day of ovarian stimulation and 0.59 ($p < 0.001$) on the day of ovarian trigger. Furthermore, Fig. 1 demonstrates the linear correlations between urine and serum E2 level on the 6th day of stimulation and on the day of ovarian trigger.

Table 2
Data distribution of urine and serum E2

E2 level	Median (IQR)
Serum E2 (pg/ml)	833.20 (516.90–1,371.00)
- On 6th day of stimulation	2,113.00 (1,382.00–3,885.00)
- On the day of ovarian trigger	
Urine E2 (E2/creatinine)	3.67 (2.84–4.81)
- On 6th day of stimulation	6.84 (5.34–8.70)
- On the day of ovarian trigger	

In the secondary outcomes, on the 6th day of stimulation, the urine E2 was correlated as Spearman's correlation coefficient rate 0.39 ($p = 0.001$), to sonographic follicles (≥ 10 mm), retrieved oocytes 0.42 ($p < 0.001$), metaphase II oocytes 0.44 ($p < 0.001$), 2PN rate 0.47 ($p < 0.001$), cleavage rate, 0.43 ($p < 0.001$) and blastocyst 0.48 ($p < 0.001$). The day of ovarian trigger showed higher correlations than the 6th day of urine E2 to sonographic follicles (≥ 14 mm) 0.57, retrieved oocytes 0.58, metaphase II oocytes 0.61, 2PN rate 0.64, cleavage rate 0.62 and blastocyst rate 0.57 ($p < 0.001$) as Spearman's correlation coefficients (Table 3).

Table 3
Spearman's correlation coefficients for urine E2 level in the infertility treatment outcomes.

Variables	Day 6th of ovarian stimulation	p-value	Day of ovarian trigger	p-value
Number of follicle size ≥ 10 or ≥ 14 mm	0.39	0.001	0.57	< 0.001
Number of oocytes retrieved	0.42	< 0.001	0.58	< 0.001
Number of metaphase II oocytes	0.44	< 0.001	0.61	< 0.001
Fertilization rate	0.47	< 0.001	0.64	< 0.001
- 2PN stage	0.43		0.62	
- Cleavage stage	0.48	< 0.001	0.57	< 0.001
- Blastocyst stage		< 0.001		< 0.001

The sub-analysis presented that on the day 6th of ovarian stimulation, women who had an age ≥ 35 years the urine E2 level 0.72 ($p < 0.001$) was significantly correlated when compared to women with age < 35 years 0.35($p = 0.18$). While on the day of ovarian trigger, the urine E2 levels and serum E2 levels were significantly correlated in both age groups: 0.52 in age < 35 years and 0.60 in age ≥ 35 years. Moreover, the urine E2 level was moderately correlated in a group of women with normal BMI (0.50, $p < 0.001$) and high BMI (0.54, $p < 0.001$) while low the BMI group was lowly correlated (0.13, $p = 0.39$) on day 6. Furthermore, the urine E2 levels in the normal responder group were significantly correlated with serum E2 levels on day 6 of ovarian stimulation (0.82, $p < 0.001$) compared to the poor and high responder groups. In contrast, on the day of ovarian trigger, the poor responding group had a statistically significant difference in the correlation between the urine and serum E2 levels (0.42, $p < 0.05$) when compared to the other groups (Table 4).

Table 4
Spearman correlations of urine and serum E2 in subgroup analysis

Variables	Day 6th of ovarian stimulation	p-value	Day of ovarian trigger	p-value
Age	0.35	0.18	0.52	< 0.05
- Age < 35 years	0.72	< 0.001	0.60	< 0.05
- Age $\geq$ 35 years				
BMI	0.29	0.53	0.79	< 0.05
- Low BMI	0.50	< 0.001	0.59	< 0.001
- Normal BMI	0.54	< 0.05	0.53	< 0.05
- High BMI				
Ovarian response	0.13	0.39	0.42	< 0.05
- Poor response	0.82	< 0.05	0.35	0.28
- Normal response	0.32	0.14	0.11	0.61
- High response				

The correlations between serum E2 at 0.72 and sonographic follicles on the 6th day and the last day of ovarian stimulation were 0.78 ($p < 0.001$), which showed a higher correlation than urine E2 and sonographic follicles.

There were not any COH complications such as OHSS or oocyte retrieval such as bleeding or infection. The specimens were collected and analyzed without any mistakes or complications.

4. Discussion

In this study, it was found that the correlations of the relative levels of serum and urine E2 were valid on both on the 6th day of ovarian stimulation and the day of ovarian trigger in infertile women who had undergone the COH cycle. There was a moderate correlation between serum and urine E2 concentration on the 6th day of ovarian stimulation ($r = 0.53$) and on the day of ovarian trigger ($r = 0.59$). The positive linear correlation in urine E2 levels on the day of ovarian trigger and number of follicle sizes (size ≥ 10 mm $r = 0.57$), number of retrieved oocytes, $r = 0.58$, number of metaphase II oocytes, $r = 0.61$ and rate of fertilization, $r = 0.64$, were relatively significant. Moreover, subgroup analysis was done for evaluation between serum and urine E2 level in factors of IVF success rate (age, BMI, and responding status) and the results showed that there was a significant correlation in women age ≥ 35 year and normal to high BMI, while this correlation was uncertain in the responding factors.

This study results are consistent with previous studies. A 1981 reported by Miyakawa I et al. reported that the urinary estradiol-17 beta-glucuronide (E2-17G) measured by direct radioimmunoassay (RIA)

correlation was significantly correlated with serum estrogens in women treated with gonadotropin on the day of ovarian trigger¹⁷. Frenken Y et al., 1985; reported that total the excretion per 24-hours or the concentrations per liter in the 24-hour urine collection of estrogen and estrone-3-glucuronide on the day of prior ovarian trigger were in good correlation with serum E2 levels in women treated with HMG¹⁸. In addition, a 1992 study by Rapi S et al. used a chemiluminescence immunoassay (LIA) method in early morning urine (EMU) samples to evaluate the correlation between serum E2 level and urine E1-3G level on the day of ovarian trigger. They revealed that the urine E1-3G excretions were consistent with serum E2 levels in women treated with COH¹⁹. In the clinical setting, RIA is a complex method that is performed using a 24-hour collection. For simplicity, the ECLIA method was used to evaluate spot urine which is convenient for analyses urine samples. Furthermore, the ECLIA showed good sensitivity in normal and high E2 level women and is used worldwide²⁰⁻²¹.

The present study is the first study that evaluates the correlation between urine E2 levels with the number of follicle growth, number of retrieved oocytes, number of metaphase II oocytes and rate of fertilization with which these data were associated with the IVF outcomes. From these results, urine E2 level on the 6th day of stimulation might be a predictor of the number of oocytes and urine E2 levels and might also predict the numbers of growth pick-ups, numbers of MII oocytes and fertilization rate.

Sub-analysis to investigate the factors that might affect the outcomes especially, age, BMI and responding status were performed and even though the results were uncertain in some factors such as responding status. This might be caused by small sample size. In addition, the participants in this study had an average age of 35 which makes the data more difficult to interpret in high and poor responding group. Therefore, it is suggested to collect a larger and broader sample size to confirm the outcome.

To the best of the authors knowledge, this is the first study that used ECLIA which a good sensitivity method to evaluate the urine E2 level. Using urine samples to monitor E2 level is more convenient and simplifies collection and analysis when compared to serum. In addition, the fact that the serum and urine E levels were collected on the same day reduced possible confounding factors from the fluctuations of hormone levels, moreover, urine E2 levels from collected urine E2 to creatinine ratio reduced the effect of the glomerular infiltration on urine E2 excretion. The limitation of this study, other hormones such follicle stimulating hormone (FSH), luteinizing hormone (LH) and progesterone that might affect to IVF outcomes were not evaluated. Furthermore, some hormones especially LH were unstable and had short half-life in the plasma.

Based on the results of this study, the urine E2 level was correlated with serum E2 level in COH stimulation on the 6th day of ovarian stimulation and the day of ovarian trigger. It may be an advantage to implement this collection method during COH especially in the cases where it is inconvenient to collect serum samples. Further study is needed to confirm the efficacy of this method in women with the risk factor of OHSS.

In conclusion, the urine E2 level was moderately correlated to serum E2, follicle growth, retrieved oocytes and also fertilization rate. After more investigations, the urine E2 might be the alternative test for ovarian stimulation monitoring and OHSS prediction, in cases where blood sampling is not convenient.

Declarations

Acknowledgment

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Ethical consideration

The Human Research Ethics Committee of Khon Kaen University reviewed and approved the study per the Helsinki Declaration and the Good Clinical Practice Guidelines (HE621557).

Consent for publication

All of the authors consent to publishing and hereby grant the Publisher exclusive license of the full copyright.

Disclosures of potential conflicts of interest

Chotboon C. declares that he has no conflict of interest

Salang L. declares that he has no conflict of interest

Buppasiri P. declares that he has no conflict of interest

Amnatbuddee S. declares that he has no conflict of interest

Eamudomkarn N. declares that he has no conflict of interest

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Availability of data and material

Data and materials are available upon request.

Author contributions

CC did the data collection and drafted the manuscript. SL designed the study, proofread, and approved the final manuscript. BP, AS, and EN proofread the manuscript.

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Figures

Urine E2 (E2/creatinine)

6th day of stimulation

Urine E2 (E2/creatinine)

Last day of stimulation

Figure 1

Correlations between urine and serum E2 concentration on 6th day of stimulation and day of ovarian trigger