International Journal of Gynecology and Obstetrics xxx (2016) xxx–xxx

Contents lists available at ScienceDirect

International Journal of Gynecology and Obstetrics

journal homepage: www.elsevier.com/locate/ijgo

www.figo.org

CLINICAL ARTICLE

Area under curve of temporal estradiol measurements for prediction of the detrimental effect of estrogen exposure on implantation

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ARTICLE INFO

Article history:
Received 6 February 2016
Received in revised form 21 April 2016
Accepted 13 July 2016

Keywords:
Area under the curve of estradiol measurements
Assisted reproductive technology
Clinical pregnancy
Freeze-all policy
Implantation

ABSTRACT

Objective: To assess whether the area under the curve of temporal estradiol measurements (AUCEM) during cycles of assisted reproductive technology (ART) can be used to predict failure of implantation and clinical pregnancy. *Methods:* In a prospective study, women aged 24–39 years undergoing ART at a center in Turkey were enrolled between January and December 2014. Eligible patients had a regular menstrual cycle, normal levels of serum prolactin, and no hormone treatment within the past 3 months. The area under the curve of the time course of estradiol measurements was calculated for each participant, and assessed for its ability to predict successful implantation. *Results:* Among 282 participants, 109 (38.6%) women had successful implantation. There was a significant difference between the two groups of women in AUCEM, estradiol per day (AUCEM divided by duration of stimulation), and endometrial thickness on the day of human chorionic gonadotropin administration ($P < 0.05$ for all). *Conclusion:* The area under the curve of estradiol measurements during ART cycles might be useful for predicting failure of implantation and clinical pregnancy.

1. Introduction

The blastocyst-stage embryo implants into the endometrium, which supports fetal growth by supplying oxygen and nutrients. The endometrium also plays a part in the protection of the growing embryo against microbial invasion. The mid-secretory phase of the endometrium when embryo implantation occurs is defined as the "implantation window." The embryo and the endometrium must be synchronized for successful implantation. Several morphological and functional differentiations occur in the endometrium during the follicular phase, and the subsequent decidualization results in endometrial sensitization. All these changes in the endometrium have been shown to be the result of the balanced effects of estrogen and progesterone [1].

Progesterone and estrogen receptors are expressed in the human endometrium in both the epithelial and stromal compartment [2]. Estrogen and progesterone act on the endometrium through various growth factors, cytokines, lipid mediators, homeobox transcription factors, and morphogens [3]. Strict regulation is needed among the different maternal hormones to obtain synchronization between the blastocyst and a receptive state of the uterine endometrium [4–6].

The endometrium is resistant to implantation during the entire reproductive cycle except for the implantation window. A previous study of mice [7] showed that low levels of exogenous estradiol can maintain the endometrium in a responsive state for a long period of time; however, high doses of estradiol lead to the development of refractory endometrium. The authors concluded that maintaining a narrow range of estradiol is a key factor throughout the implantation window for uterine receptivity in mice. Additionally, the study suggested that the window of receptivity might be manipulated by different doses of estradiol [7].

Implantation plays a key part in the success of assisted reproductive technology (ART). Despite optimal conditions—including the quality of the embryo, the endometrial thickness, and the transfer technique—implantation can fail in 50% of cases [8]. Although several tools to predict endometrial receptivity have been introduced, none has provided accurate predictions or is easily applied to routine use [9,10].

A critical range of estradiol exposure seems to have some importance for receptive endometrium and embryo implantation. A noninvasive tool for prediction of the receptivity of the endometrium could help to guide clinicians to select cases in which to postpone embryo transfer and freeze all the embryos for the next cycle. The aim of the present study was to assess the ability of the area under the curve of temporal estradiol measurements (AUCEM) during cycles of ART to predict failure of implantation and clinical pregnancy.

2. Materials and methods

The present prospective study was conducted among women undergoing ART (in vitro fertilization [IVF]/intracytoplasmic sperm

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http://dx.doi.org/10.1016/j.ijgo.2016.04.015
0020-7292/

injection [ICSI]) at the Zeynep Kamil Women and Children's Health Training and Research Hospital, Istanbul, Turkey, between January 1 and December 31, 2014. The inclusion criteria were age 24–39 years, regular menstrual cycle, normal serum prolactin levels, and no hormone treatment within the past 3 months. For all participants, ART was indicated for unexplained infertility, which was diagnosed when a patient was infertile with normal ovulatory and tubal functions, and a normal sperm count for her partner, as determined by menstrual cycle regularity, hysterosalpingography, and semen analysis, respectively. The study was approved by the hospital's ethics committee and all participants provided written informed consent.

For all participants, a gonadotropin-releasing hormone antagonist protocol was used for IVF/ICSI. A regimen of daily recombinant follicle-stimulating hormone (rFSH; Gonal-F, Merck-Serono, Geneva, Switzerland) was started on the second day of the menstrual cycle. The dose used ranged from 150 IU to 300 IU, and was determined by each patient's basal clinical characteristics. Mean follicular growth was monitored every 2–3 days via two-dimensional transvaginal sonography. The daily dose of rFSH was adjusted from day 5 of stimulation according to the ovarian response. The antagonist (Cetrorelix, Merck-Sereno, Geneva, Switzerland) was administered at a dose of 0.25 mg/day when the follicular size reached 12 mm. When the follicular size reached 18 mm, 250 μg recombinant human chorionic gonadotropin (hCG; Ovitrelle, Merck-Sereno, Geneva, Switzerland) was administered subcutaneously, and follicular puncture was performed after 34–36 hours. Next, 8% vaginal progesterone gel (Crinone gel 8%; Merck-Sereno, Geneva, Switzerland) was applied twice daily. ICSI was applied for each oocyte obtained by follicular puncture. Elective transfer of one grade-1 embryo was performed either at cleavage (day 3) or blastocyst (day 5) stage, according to the developmental characteristics of the embryo. Serum levels of the β-subunit of hCG (β-hCG) were measured after 2 weeks. If they were more than or equal to normal levels (5 IU/L) in pregnancy, the patient was considered to have successful implantation, and ultrasonography was performed to detect the pulse of fetus and confirm a clinical pregnancy.

Additionally for each participant, on the fifth day of ovarian stimulation and the following days when follicular growth was monitored, 3–5 mL of venous blood was taken between 8:00 AM and 10:00 AM, and the concentration of estradiol was determined. Estradiol levels were measured using a microparticle enzyme immunoassay, using the ECL2012 system (Siemens, Munich, Germany) in accordance with the manufacturer's protocol.

Age, body mass index, basal hormone levels (estradiol and follicle-stimulating hormone on the third day of the menstrual cycle), estradiol levels during stimulation, duration of stimulation, AUCEM, estradiol level per day during stimulation, total gonadotropin dose, and numbers of total, mature, and fertilized oocytes were recorded for each case.

A curve representing the time course of estradiol measurements was drawn for each patient, with the x-axis representing the day of the menstrual cycle, and the y-axis representing the estradiol level. AUCEM was calculated by adding the area of triangles and rectangles for each time interval, using the following formula reported by Pruessner et al. [11]:

$$\mathrm{AUC} = (m_2 + m_1)t_{1\text{–}2} + (m_3 + m_2)t_{2\text{–}2} + (m_4 + m_3)t_{3\text{–}2} + (m_5 + m_4)t_{4\text{–}2} + (m_n + m_5)t_{n\text{–}2}$$

where m_1 to m_n denotes the single measurements over time, and t_1 to t_n denotes the interval between the measurements. Estradiol per day was calculated by dividing AUCEM by the duration of stimulation.

Data were analyzed using SPSS version 15.0 (SPSS, Chicago, IL, USA). Pearson correlation analysis or Spearman correlation analysis was performed to assess the correlation between different variables and ovarian response, and the correlation between two different variables. The Student *t* test was used to compare continuous variables between women with successful implantation or clinical pregnancy and those without. Multivariate regression analysis was used to assess the adjusted associations. Receiver operator curve (ROC) analysis was used to assess the predictive value of the test and to calculate its sensitivity and specificity. $P < 0.05$ was taken to be statistically significant.

3. Results

During the study period, 282 women were enrolled. Implantation was successful in 109 (38.6%) women, and a fetal heart rate was detected in 92 (32.6%).

There was a significant difference between women with and without implantation in AUCEM, estradiol per day, and endometrial thickness at hCG administration (Table 1). For women with and without positive clinical pregnancy, a significant difference was observed in AUCEM, estradiol per day, and endometrial thickness at hCG administration (Table 2).

Implantation was significantly correlated with AUCEM ($r = 0.173$, $P = 0.004$), estradiol per day ($r = 0.156$, $P = 0.004$), and endometrial thickness at hCG administration ($r = 0.143$, $P = 0.016$). Similarly, clinical pregnancy was significantly correlated with AUCEM ($r = 0.187$, $P = 0.002$), estradiol per day ($r = 0.165$, $P = 0.005$), and endometrial thickness at hCG administration ($r = 0.128$, $P = 0.031$).

Multivariate regression analysis showed a significant association between successful implantation and both AUCEM ($\beta = 0.164$, $P = 0.005$) and endometrial thickness at hCG administration ($\beta = 0.164$, $P = 0.025$). Additionally, multivariate regression analysis showed a significant association between clinical pregnancy and both AUCEM ($\beta = 0.179$, $P = 0.002$) and endometrial thickness at hCG administration ($\beta = 0.117$, $P = 0.047$).

AUCEM was a significant predictor of negative β-hCG (AUC 0.601; $P = 0.004$) (Fig. 1). The optimal cutoff value to predict negative β-hCG was 18 303 pmol/L per stimulation, with 75% sensitivity. The positive predictive value was 61%. AUCEM was a significant predictor of negative fetal heart rate (AUC 0.604; $P = 0.005$) (Fig. 2). The optimal cutoff value to predict negative fetal heart rate was 18 593 pmol/L per stimulation, with 75% sensitivity. The positive predictive value was 68%.

Table 1
Characteristics by implantation success.[a]

Characteristic	Implantation success		*P* value
	Negative (n = 173)	Positive (n = 109)	
Age, y	29.0 ± 3.5	29.3 ± 4.1	0.512
Body mass index[b]	23.8 ± 3.9	24.6 ± 3.5	0.209
Follicle-stimulating hormone, IU/L	4.8 ± 0.7	4.8 ± 0.8	0.889
Day-3 estradiol, pmol/L	206.6 ± 37.8	214.4 ± 38.9	0.116
Duration of infertility, y	5.5 ± 3.1	6.2 ± 3.4	0.138
Total gonadotropin dose, IU	1768.1 ± 703.2	1786.3 ± 661.6	0.829
AUCEM, pmol/L	32 735.7 ± 2236.3	25 440.7 ± 1659.6	0.002
Estradiol level per day, pmol/L[c]	3089.5 ± 225.3	2436 ± 167.7	0.005
Duration of stimulation, d	10.7 ± 1.5	10.5 ± 1.2	0.261
Endometrial thickness at 5th day, mm	7.7 ± 1.5	7.9 ± 1.6	0.378
Estradiol level at hCG administration, pmol/L	8685.9 ± 369.3	7905.8 ± 310.5	0.058
Endometrial thickness at hCG administration, mm	9.7 ± 1.4	10.2 ± 1.6	0.016
No. of total oocytes	10.1 ± 4.7	9.90 ± 4.4	0.763
No. of mature oocytes	7.7 ± 4.0	7.6 ± 3.6	0.709
No. of fertilized oocytes	4.7 ± 3.1	5.08 ± 2.7	0.368

Abbreviations: AUCEM, area under the curve of endometrial measurements; hCG, human chorionic gonadotropin.
[a] Values are given as mean ± SD unless indicated otherwise.
[b] Calculated as weight in kilograms divided by the square of height in meters.
[c] Calculated by dividing AUCEM by the duration of stimulation.

Please cite this article as: Kutlu T, et al, Area under curve of temporal estradiol measurements for prediction of the detrimental effect of estrogen exposure on implantation, Int J Gynecol Obstet (2016), http://dx.doi.org/10.1016/j.ijgo.2016.04.015