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Prediction of pregnancy after frozen-thawed embryo transfer via in vivo intrauterine oxidation-reduction potential measurements: a pilot study

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Abstract

Purpose: During the implantation period, the uterus goes through many complex, orchestrated changes, including alterations of the glycocalyx that are due to sialylation, sulfation, and fucosylation. A previous mouse study showed that the in vivo intrauterine oxidation-reduction potential (ORP) aided in determining the alterations in the uterine endometrium that are suitable for implantation and for evaluating prospective uterine receptivity, while the in vivo intrauterine pH did not. It was assessed if the in vivo intrauterine ORP could be a useful parameter to predict pregnancy in women.

Methods: A prospective cohort study was conducted for patients who had received a frozen-thawed single embryo transfer in a programmed, hormonally controlled cycle. The in vivo intrauterine ORP was measured 3 times during the treatment cycle, at cycle days 9-10, 1 day before progesterone administration and immediately before the embryo transfer.

Results: The amount of in vivo intrauterine ORP at 9-10 days after the start of menstrual bleeding was significantly lower in the pregnant group than in the non-pregnant group. A receiver-operator characteristic curve analysis of the intrauterine ORP as a predictor of non-conception showed an area under the curve of 0.80.

Conclusion: The in vivo intrauterine ORP could be a useful parameter to predict pregnancy for the frozen-thawed embryo transfer treatment cycle.

KEYWORDS
assisted reproductive technology treatment, implantation, infertility, oxidation-reduction potential, uterine receptivity

1 | INTRODUCTION

Infertility affects 10%-18% of couples and >5 million babies have been born by using assisted reproductive technology (ART) treatment since 1978.[1] In ART treatment, the eggs are fertilized in vitro and the appropriately developed embryos are subsequently transferred into the uterine cavity when the serum progesterone level and ultrasonographic endometrial thickness are deemed to be sufficient. However, overall pregnancy success rates remain low.

Implantation failure has long been considered to be a major problem of ART treatment.

Uterine receptivity is associated with various glycosylation changes, including changes in the composition of glycoproteins, proteoglycans, and glycolipids in the endometrial epithelium[2-6] and in the secretion profile of glycoproteins and proteoglycans in the luminal fluid.[3,7] Glycocalyx alterations in the entire uterus involve shifts in sialylation and sulfation. Generally, such changes are known to alter the pH level. This fact, in turn, raises a question as to whether uterine receptivity could be evaluated electrophysiologically.

The most popular medical equipment to measure in vivo pH is oesophageal pH monitoring, which is used for the diagnosis of gastroesophageal reflux disease. To electrophysiologically evaluate glycocalyx changes in the uterus in vivo, much smaller changes in the uterine cavity than those in the oesophagus must be detected; as mentioned, glycocalyx changes in the uterus are based on sialylation, sulfation, and fucosylation.

The oxidation-reduction potential (ORP) is the activity of oxidizers and reducers, or their strength in relation to their concentration, which is similar to the principle of pH. The ORP decreases with an increasing pH, regardless of the oxidant type or concentration, and increases rapidly with an increasing oxidant dosage, particularly at lower concentrations.[8] Generally, the measurement of the ORP can detect more subtle changes, compared with th pH.[8]

The authors' previous mouse study showed that, between postcoitus days 2 and 6, the in vivo intrauterine ORP was significantly increased, while there was no change in the in vivo intrauterine pH.[9] Moreover, 1 day before implantation began, the intrauterine ORP was significantly decreased in the implantation failure mouse model, compared with the naive and control groups. A receiver-operator characteristic (ROC) curve analysis of the intrauterine ORP as a predictor of non-conception (vs the control group) showed an area under the ROC curve of 0.96 (95% CI: 0.92-1.00).[9] Can the intrauterine ORP prospectively evaluate uterine receptivity in humans also?

In this prospective cohort study, in order to investigate whether the intrauterine ORP measurement in vivo could help to predict pregnancy, patients who were undergoing frozen-thawed embryo transfer (ET) in a programmed, hormonally controlled cycle were evaluated.

2 | MATERIALS AND METHODS

2.1 | Ethical approval

This study was approved by the Institutional Ethics Board of Osaka University Medical School Hospital and the Taniguchi Hospital Ethics Committees (clinical trial No. 813; No. 2013-06-1). A written informed consent form was obtained from all the patients.

2.2 | In vivo intrauterine oxidation-reduction potential measurement

The intrauterine ORP measurements were performed by using a 2.1 mm diameter pH catheter for the digestive tract (Multi-use pH catheter, 819205; Synectics Medical, Lda., Queluz, Portugal) and a pH/mV digital meter (CL-9D02; Chemical Instruments, Tokyo, Japan). The accuracy of the measurements that were made with the CL-9D02 meter is ±0.01 pH or ±1 mV and its power supply is AC 100 V (50/60 Hz). Prior to use, the electrical safety was assessed by the Medical Engineering Centre of Osaka University Medical School Hospital. The catheter, which has an antimony electrode on the tip and was intended for single use only, was sterilized using ethylene oxide gas and was inserted into the uterine cavity by using a vaginal speculum. A separate skin reference electrode (ER-240P; SK Medical Electronics Company, Ltd., Shiga, Japan) was placed on the skin above the umbilicus. One minute after the uterine catheter insertion, the intrauterine ORP was measured 3 times in the first 3-seconds and was measured a further 3 times in 5-second intervals. The average of 6 measurements in each time frame was used in a comparison of the pregnant and non-pregnant groups. The serum estrogen and progesterone levels and the ultrasonographic endometrial thickness were measured on the day of the intrauterine ORP measurement.

2.3 | Participants and study design

In total, 52 patients who were scheduled to undergo frozen-thawed ET treatment in Osaka University Medical School Hospital and Taniguchi Hospital from July, 2013 to November, 2014 were enrolled in this study (Figure 1). The participants were aged 20-45 years old and were married. Decisions to cancel ET were made according to general clinical criteria.

The patients who were set to receive frozen-thawed ET were administered a daily gonadotropin-releasing hormone (GnRH) agonist, buserelin acetate (Suprecur® nasal solution; Mochida Pharmaceutical Company, Ltd., Tokyo, Japan) in the mid-luteal phase to achieve pituitary desensitization. At cycle day (CD) 3, all the patients began a transdermal estradiol supplement (Estrana® tape; Hisamitsu Pharmaceutical Company, Inc., Saga, Japan) to induce endometrial proliferation, while suppressing the development of the dominant follicle.

The in vivo intrauterine ORP was measured at CD 9-10 (Time [T] 1), 1 day before progesterone administration (600 mg/d, Utrogestan® capsules; Vifor SA, Villars-sur-Glâne, Switzerland) (T2), and immediately before the ET (T3).

The serum β-human chorionic gonadotropin (hCG) levels were measured 10-14 days after the ET. A pregnancy test was defined as positive if the serum β-hCG levels were >5 mIU/mL. Clinical pregnancy was defined as a viable intrauterine pregnancy, confirmed via transvaginal ultrasonography at gestational weeks 5-6.
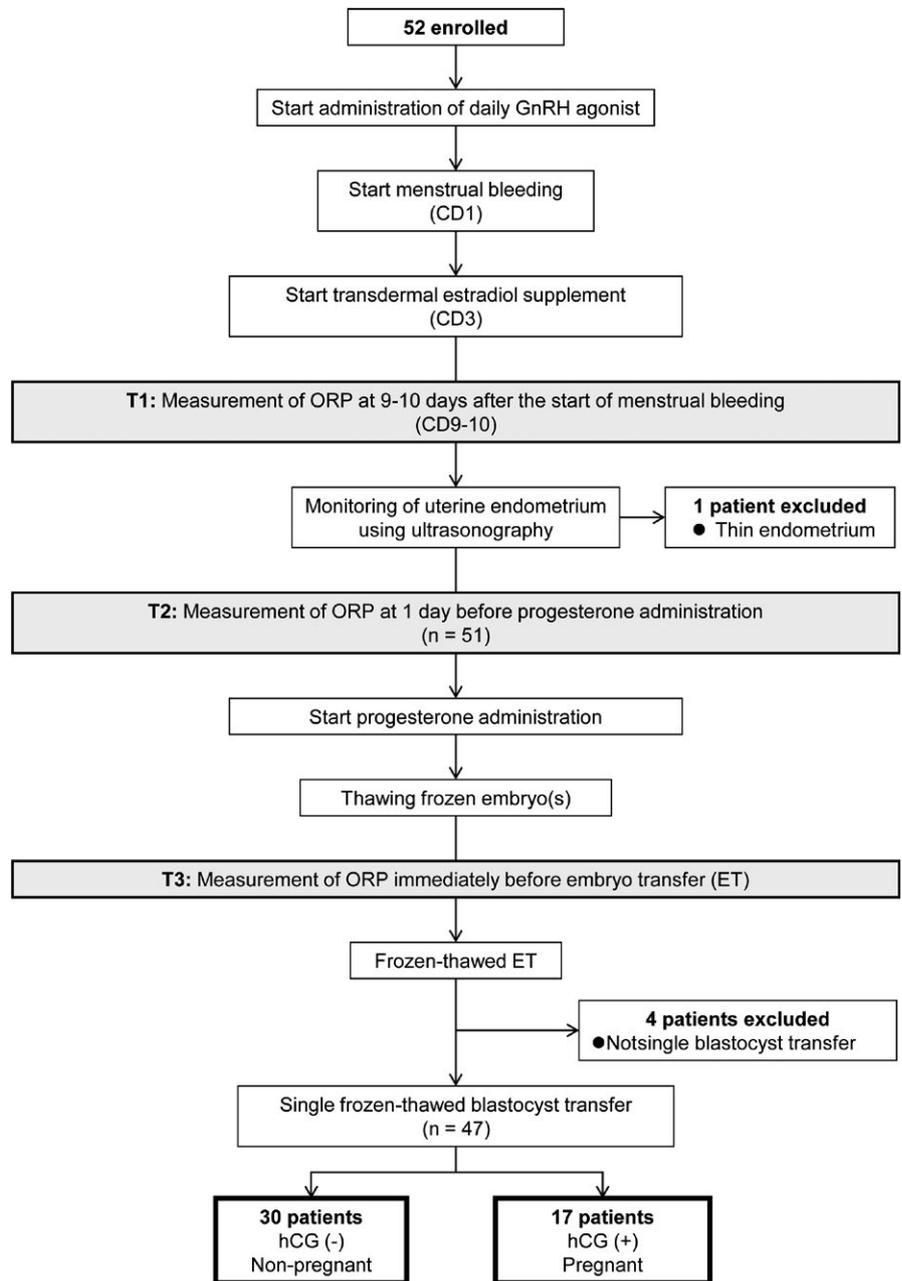
FIGURE 1 Study patient enrollment. CD, cycle day; GnRH, gonadotropin-releasing hormone; hCG, human chorionic gonadotropin; ORP, oxidation-reduction potential

TABLE 1 Patients' characteristics

Characteristic	Age (years) Mean ± SD (range)	BMI (kg/m^2) Mean ± SD (range)	Nulligravida N (%)
Not pregnant after frozen-thawed ET (n = 30)	36.1 ± 5.1 (25-45)	21.1 ± 2.7 (16.7-28.7)	23 (76.7)
Pregnant after frozen-thawed ET (n = 17)	35.6 ± 3.9 (31-44)	22.2 ± 2.8 (17.1-27.4)	10 (58.8)
P-value	.889[a]	.163[a]	.320[b]

BMI, body mass index; ET, embryo transfer.
[a]Student's *t* test.
[b]Two-sided *P*-value by Fisher's exact test.

2.4 | Adverse events

No adverse event, such as uterine bleeding or pelvic pain, was observed after the intrauterine ORP measurements.

2.5 | Statistical analysis

The experimental results were expressed as the mean ± standard error of the mean. The data were analyzed with SigmaPlot® software

FIGURE 2 Measurement of the current parameters and the in vivo uterine oxidation-reduction potential. The data (mean ± standard error of the mean) were evaluated at Time (T) 1 (CD 9-10), T2 (one day before progesterone administration), and T3 (immediately before the embryo transfer) for all patients by using the Shapiro-Wilk Normality test and Student's t test (*P < .001). CD, cycle day

v. 10.01 (Systat Software, Inc., San Jose, CA, USA). A Student's t test or Wilcoxon's rank-sum test with the Shapiro-Wilk normality test were used for the comparison of the groups and differences with a P-value of <.05 were considered statistically significant. The ORP values were analyzed in order to determine whether the ORP is predictive of non-pregnancy by using a ROC analysis to identify the optimal cut-off point.

3 | RESULTS

3.1 | Patient characteristics and pregnancy

In total, 52 patients were enrolled in this study (Figure 1). All of them had at least 1 failed attempt to conceive with ART treatment. At CD 16, the treatment cycle for 1 patient was cancelled because of a thin endometrium (<6 mm). All were to undergo frozen-thawed ET using their own oocytes in a programmed, hormonally controlled cycle using sequentially administered exogenous estrogen and progesterone to induce synchronization between the embryonic and endometrial development. Four patients who had 1 or 2 cleavage-stage embryos transferred were excluded from the analysis. Of the 52 patients, a total of 47 who received a single blastocyst transfer was analyzed. The pregnancy tests were positive in 17 of the 47 patients. Gestational sacs were observed in all 17 patients (clinical pregnancy rate: 36.2%). There was no twin pregnancy.

A woman's age is one of the most significant factors in fecundability,[10] while obesity is considered to have a possible association with suboptimal reproductive performance.[11] Nevertheless, no statistically significant difference in age or Body Mass Index between the pregnant and non-pregnant groups was found (Table 1). Moreover, nulligravid women might encounter some innate difficulties in becoming pregnant, while women with a history of pregnancy, including miscarriages, might have incurred damage to their uterus. The number of nulligravid women in both groups was analyzed by using a 2-sided Fisher's exact test (Table 1). The factor of nulligravida did not affect the pregnancy outcomes in this study.

3.2 | Current clinical parameters to evaluate uterine receptivity

The serum estradiol and progesterone levels and ultrasonographic endometrial thickness were compared between the pregnant and the non-pregnant women. There was no significant difference for these parameters between the 2 groups at T1, T2, or T3 (Figure 2).
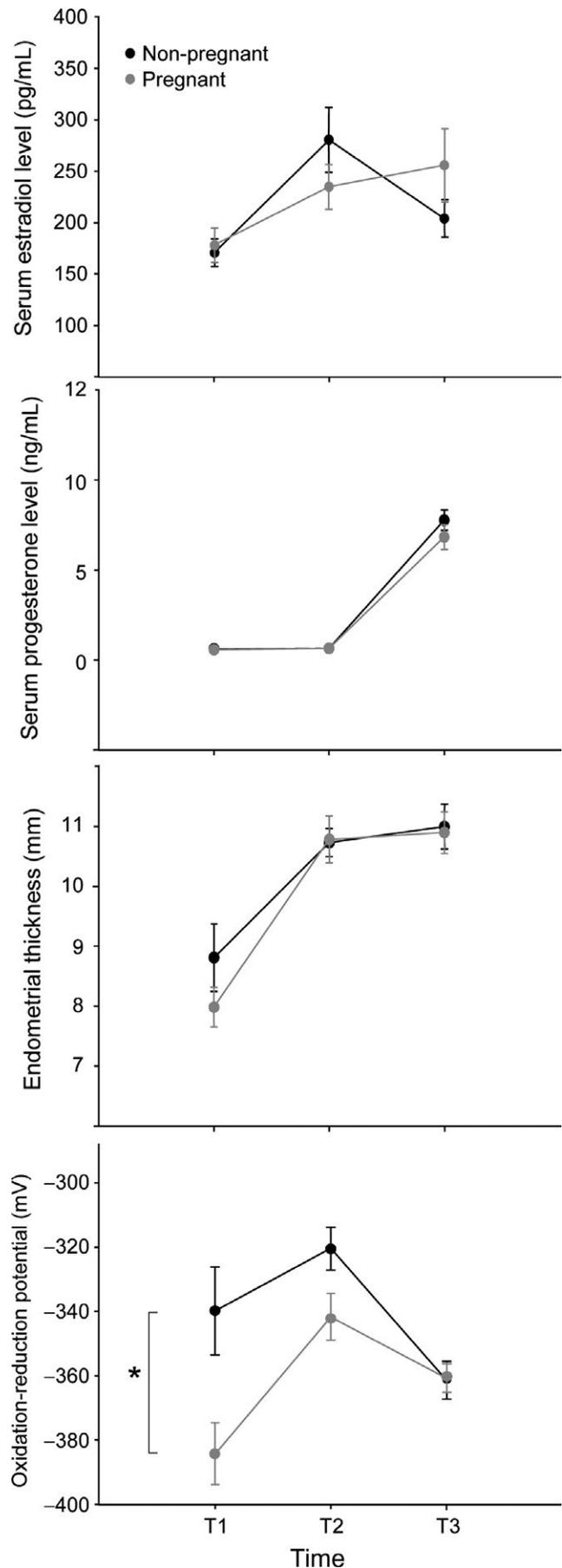
3.3 | Intrauterine oxidation-reduction potential as a parameter for evaluating uterine receptivity

No significant difference in the intrauterine ORP was observed between the pregnant and non-pregnant groups at either T2 or T3. At T1, the average ORP in the pregnant group was significantly lower than that in the non-pregnant group (P < .001, Student's t test) (Figure 2).
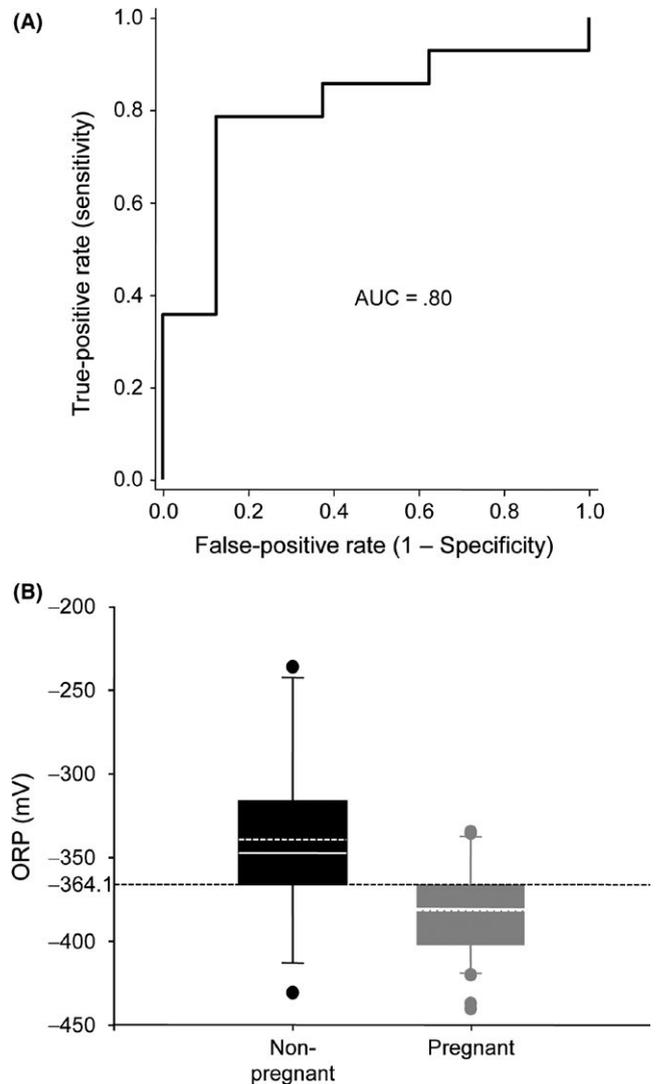
The negative predictive value of the ORP was assessed regarding conception in the frozen-thawed ET group. The area under the ROC curve was .80 (95% CI: 0.61-1.00) at CD 9-10 for the intrauterine ORP (Figure 3A). The optimal cut-off value of −364.1 mV for the intrauterine ORP had a sensitivity of 78.6% and a specificity (non-conception) of 87.5% (Figure 3B).

4 | DISCUSSION

Many glycocalyx changes in the uterus during implantation have been reported since the 1970s. It recently has been reported that implantation begins with binding between L-selectin on the trophoblasts and selectin oligosaccharide-based ligands, including 6-sulfo sLe^x (Siaα2→3Galβ1→4[Fucα1→3][SO$_3$→6] N-acetylglucosamine), on the uterine endometrial epithelial cells.[12,13] Early histological studies showed a decline in the negative charge of the uterine epithelium during implantation in rats,[14] rabbits,[2] and mice.[15] As in the authors' previous mouse study,[9] it was hypothesized that at T3, the pregnant group would show a significantly higher in vivo intrauterine ORP than the non-pregnant group in this study. However, no significant difference in the in vivo intrauterine ORP was found between the groups at T2 and T3. Surprisingly, a significant difference in the ORP was found at T1 (CD 9-10), suggesting that the ORP could, in fact, be predictive of pregnancy and that the fate of a pregnancy could be determined at days 9-10 after the start of menstrual bleeding.

While the mean duration of menstrual bleeding is approximately 5 days,[16-18] there are individual variations.[16,17,19] In this study, the in vivo intrauterine ORP was measured at CD 9-10, which is considered to be the earliest day of the menstrual cycle that would not be affected by menstrual bleeding. Only the cases with a single blastocyst transfer in a programmed, hormonally controlled cycle using the same dose of sequentially administered exogenous estrogen and progesterone were assessed in order to reduce the variety of factors that could affect the pregnancy outcome.

Preimplantation genetic screening (PGS), which screens embryos with aneuploidy, has been used to select human embryos with the highest developmental potential in order to improve the efficiency of ART treatment.[20] However, PGS could not be performed in this study due to the policies of the Institutional Ethics Committee. The first appearance of hCG occurs 6-12 days after ovulation.[21] However, in this study, the serum hCG levels were assessed 10-14 days after the ET, depending on each patient's cycle,

FIGURE 3 Negative predictive value of the intrauterine oxidation-reduction potential (ORP) regarding conception after a frozen-thawed embryo transfer. A, The receiver-operator characteristic curve analysis of the intrauterine ORP as a predictor of non-conception. B, The box plots show the in vivo intrauterine ORP in the non-pregnant group (black box) and pregnant group (gray box) at CD 9-10. The horizontal line within the box shows the median value and the dotted line within the horizontal box shows the mean value. AUC, area under the curve; CD, cycle day

and therefore the first appearance of hCG could not be analyzed. Thus, to be accurate, the end point of this study was not implantation, but pregnancy. It is required to assess the first appearance of hCG after a single blastocyst transfer with PGS in order to evaluate uterine receptivity.

In mice, the transient increase in the intrauterine ORP was observed on the same day as the preimplantation estrogen surge.[9] In the women in this study, the serum estradiol levels were significantly increased at T2, compared with T1, in the pregnant and non-pregnant groups (Figure 2). In the non-pregnant group, the serum estradiol levels were significantly decreased at T3, compared with T2, but there was no significant difference between T2 and T3 in the pregnant

group. In contrast, the intrauterine ORP was significantly increased at T2, compared with T1, and was significantly decreased at T3, compared with T2, in both groups. However, no correlation between the serum estrogen and the intrauterine ORP at T1 and/or T2 was found in this study (data not shown). The in vivo intrauterine ORP might detect the alteration of progesterone receptor expression or the functional availability of progesterone receptors in the endometrium after estrogen priming. It is not known which molecular regulators directly alter the in vivo intrauterine ORP; these should be evaluated in further studies.

The technique for embryo-freezing and thawing is already well established and has been used in ART treatment worldwide. According to the results of this study, when the intrauterine ORP was above −361.4 mV at CD 9-10, frozen embryos could not be used. A combination of intrauterine ORP measurement and a frozen-thawed ET strategy could improve the efficacy of ART treatment. However, as there was a small sample size in this prospective cohort study, larger cohorts are needed for evaluation, as well as for the assessment of the alteration in intrauterine ORP during the natural spontaneous cycle in order to confirm this study's results.

DISCLOSURES

Conflict of interest: The authors declare no conflict of interest. *Human Rights Statement and Informed Consent*: The procedures were followed in accordance with the ethical standards of the responsible committees on human experimentation (institutional and national) and with the Helsinki Declaration of 1964 and its later amendments. The protocol for the research project that included human participants was approved by the institutional review board or independent ethics committee at each site prior to the study's commencement. Informed consent was obtained from all the patients that were included in the study. This article does not contain any study with animal participants that has been performed by any of the authors. *Animal studies:* This article does not contain any study with animal participants that have been performed by any of the authors.

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