The role of temporal changes in endometrial blood flow in natural and hormone replacement cycles with vitrified-warmed embryo transfer

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Research article

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Abstract

Background Uterine blood flow promotes endometrial development and subsequent implantation of fertilized eggs. We examined the temporal changes in uterine blood flow during natural and hormone replacement (HRT) cycles and clarified the relationship between uterine blood flow and assisted reproductive technology outcomes for vitrified-warmed embryo transfer (ET). Methods This was a retrospective cohort study. A total of 60 patients with vitrified-warmed ET were assigned to two groups according to the endometrial preparation: natural cycle (28 patients) or HRT cycle (32 patients). The uterine endometrial blood flow was evaluated using measurements of the radial artery resistance index (RA-RI) during the early follicular phase, the days of the human chorionic gonadotropin (hCG) trigger during a natural cycle or the start of progesterone administration during the HRT cycle, and the day of ET. We statistically and longitudinally measured and evaluated the RA-RI values of all individual patients. Results During natural cycles, the RA-RI on the day of ET was significantly higher than that during the early follicular phase. During HRT cycles, the RA-RI on the day of ET was also significantly increased compared to that during the early follicular phase. We validated the efficacy of the RA-RI values for predicting the possibility of pregnancy with vitrified-warmed ET. During natural cycles, the area under the receiver-operating characteristic curves (AUCs) for the early follicular phase, the day of hCG trigger, and the day of ET were 0.75 (95% confidence interval [CI], 0.57–0.93), 0.69 (95% CI, 0.49–0.88), and 0.60 (95% CI, 0.36–0.84), respectively. During HRT cycles, AUCs for the early follicular phase, the day of starting progesterone administration, and the day of ET were 0.60 (95% CI, 0.40–0.81), 0.60 (95% CI, 0.39–0.87), and 0.58 (95% CI, 0.37–0.79), respectively. Conclusions The uterine RA-RI increased at approximately the time of the implantation window compared to that of the early follicular phase during both natural and HRT cycles with vitrified-warmed ET. Our findings suggest that RA-RI during the early follicular phase might be effective and useful for deciding whether to choose the natural or HRT cycle for vitrified-warmed ET.

Background

In recent years, there is a trend toward the elective cryopreservation of embryos following in vitro fertilization (IVF) or intracytoplasmic sperm injection [1, 2]. The synchronization of embryo developmental stage with adequate endometrial maturation is required for successful implantation [3]. The vitrified-warmed embryo transfer (ET) is performed in a natural or hormone replacement (HRT) cycle for endometrial preparations [4]. Successful implantation and pregnancy depend on a specific period of endometrial receptivity, so-called implantation window [5].

Uterine blood flow plays a pivotal role in endometrial growth and differentiation during the menstrual cycle [6, 7]. Several reports have demonstrated that there is a close relationship between uterine blood flow and endometrial thickness as a uterine receptivity [8–10]. In fertile women, a uterine artery pulsatility index measured during the period of implantation window correlates inversely with endometrial thickness [11], which is suggested a direct effect of uterine perfusion on endometrial growth [12]. A uterine radial artery, which is a branch of the uterine artery, provides blood flow to the myometrium and functional layer.
of the endometrium of the uterus [8]. The uterine radial artery blood flow is also closely associated with the endometrium thickness and this may predict the implantation success rate [13, 14].

Temporal changes in uterine blood flow have been reported during spontaneous menstrual cycles [15–17]. The temporal changes in uterine blood flow might be a key to successful implantation in both fertile and infertile women. The temporal changes in uterine blood flow during the menstrual period might be dependent on ovarian steroid hormones, such as estrogen and progesterone [18–25]. Because ovarian steroid hormones are necessary for the preparation of endometrium and controlling the implantation window, similar temporal changes in the uterine blood flow might occur during HRT cycles with vitrified-warmed ET. However, no studies have shown the temporal changes in uterine blood flow during the HRT cycle with vitrified-warmed ET.

In the present study, we aimed to examine the temporal changes in uterine blood flow during natural and HRT cycles and clarify the relationship between uterine blood flow and assisted reproductive technology (ART) outcomes in vitrified-warmed ET.

**Methods**

**Study design and setting**

This study is designed as a single-center retrospective cohort study. This study was approved by the institutional review board of the Fukushima Medical University (the committee's reference number: 30123). It was conducted at a reproductive center in Nasu Red Cross Hospital, Tochigi, Japan. Women who underwent vitrified-warmed ET cycle participated in the study from April 2016 to August 2017. Inclusion criteria were as follows: at least two cleavage-stage embryos in storage, less than two previous vitrified-warmed ET cycles, women aged < 40 years with the regular menstrual cycle, and a day 3 follicle stimulating hormone level <10 mIU/mL. Those with uterine factor infertility, a history of uterine surgery and/or apparent endometrial pathology and clinically relevant systemic diseases such as hypertension, diabetes mellitus, ulcerative colitis, Crohn's disease, and connective tissue diseases, were excluded.

We planned a study of a continuous response variable from matched pairs of study subjects. Because there have not been studies on the temporal change in uterine blood flow during HRT cycles with vitrified-warmed ET, preliminary data regarding that during the natural cycle were collected. The preliminary data of uterine radial artery resistance index (RA-RI) in the natural cycle indicate that the difference between RIs of follicular phase and luteal phase in the response of matched pairs is normally distributed with standard deviation 0.07. If the true difference in the mean response of matched pairs is 0.04, we will need to study 27 pairs of subjects to be able to reject the null hypothesis that this response difference is zero with probability (power) 0.8. The Type I error probability associated with this test of this null hypothesis is 0.05. In the present study, 60 women, 28 of natural and 32 of HRT cycles, have been recruited.
Study Procedure

Programs of vitrified-warmed ET cycle

All patients visited hospital between days 2 and 3 of their monthly cycle for a baseline scan. At this visit, they could wish to select either natural or HRT cycle with vitrified-warm ET program. Participants in the natural cycle were prepared for endometrium as follows. Follicular growth was monitored by measuring the levels of serum hormones and performing a transvaginal ultrasound (Toshiba Viamo, Japan, vaginal probe 7.5 MHz) from day 10 of the cycle. When the diameter of the dominant follicle was >18 mm and the endometrial thickness was >8 mm, human chorionic gonadotropin (hCG, Gonatropin; Asuka Pharmaceutical, Tokyo, Japan) trigger was needed due to the patient’s luteinizing hormone (LH) value. If the level of LH was <15 mIU/ml, 5000 IU of hCG was administered to trigger ovulation, and the transfer of cleavage embryos was performed 4 days later. If the level of LH was >15 mIU/ml, 5000 IU hCG was injected immediately, and the ET was conducted 3 days later. Participants in the HRT cycle were prepared for endometrium as follows. Patients started transdermal administration of 2.88 mg/day estradiol (Estrana TAPE; Hisamitsu Pharmaceutical, Tokyo, Japan) every other day for endometrial preparation from days 2 and 3 of their monthly cycle. Twelve days later, the endometrium thickness was evaluated by transvaginal ultrasound as ready for the ET procedure. If the endometrial thickness less than 8 mm, transdermal estradiol administration continued and monitoring with ultrasound was undertaken to confirm further endometrial thickening. When the endometrial thickness was > 8 mm, participants began to use vaginal administration of progesterone (LUTEUM; Asuka Pharmaceutical, Tokyo, Japan) 400 mg twice daily as luteal support and determining the proposed day of ET. In both protocols, one or two vitrified-warmed cleavage embryos were transferred under the ultrasound monitoring. The pregnancy test was performed 14 days after the ET. When the pregnancy test was positive, intramuscular depot medroxyprogesteroneacetate (Proge Depot 125 mg; Mochida, Tokyo, Japan) 125 mg was injected once a week in a natural cycle until 8 weeks of gestation, while transdermal estradiol and vaginal progesterone supplementation were continued until 8 weeks of gestation in HRT cycle. Then, following up were continued until confirmation of viable clinical pregnancy with fetal heart activity.

Protocols for vitrification and warming of embryos were as follows: cleavage-stage embryos of 3 days after fertilization were vitrified using the CRYOTOP® (Kitazato, Shizuoka, Japan), and the embryos were storage into liquid nitrogen. The storage embryos were subjected to warming according to the manufacturer’s protocol. The warmed-embryos were assessed the quality and the one or two good quality embryos were transferred.

Measurement of uterine radial artery blood flow impedance by ultrasonography
Blood flow impedance of uterine radial arteries was evaluated with the utilization of transvaginal ultrasonography with an integrated pulsed Doppler scanner (Toshiba Viamo, Japan, vaginal probe 7.5 MHz) in the early follicular phase (days 2–3 of the menstrual cycle), the days of the hCG trigger in natural cycle or starting with progesterone administration in HRT cycle, and the day of the ET (Fig. 1). The details of the measurement of blood flow impedance as described below. After the areas of maximum color intensity were selected, a Doppler range gate was applied and the pulsed Doppler function was activated to assess blood flow velocity. After confirming that waveforms were continuous, an average of three to five cardiac cycles was selected for calculation of the RI, the difference between maximal systolic blood flow velocities and minimal diastolic flow velocities divided by the peak systolic flow velocities. The RIs of uterine radial arteries were used to evaluate the characteristics of endometrial blood flow [13, 14]. The mean and median uterine radial artery RIs at the specific points were used for statistical analyses.

Statistical analysis

All statistical analyses were performed with EZR (Saitama Medical Center, Jichi Medical University, Saitama, Japan), which is a graphical user interface for R (The R Foundation for Statistical Computing, Vienna, Austria) [26]. All values in the text, tables, and figures are presented as means (SD) unless otherwise stated. Student's t-test was used to compare the groups with respect to normally distributed variables, and non-parametric analysis was used for other variables. The χ² test was used to compare categorical variables. The RI values of each were compared with a paired nonparametric Friedman test followed by Bonferroni-corrected Wilcoxon-signed ranks test among three measurement phases. Correlation analysis was used to assess the RI and endometrial thickness. Receiver-operating characteristic (ROC) analysis was used to determine the cut-off value of RI with respect to pregnancy in natural and HRT cycles. The area under the curve (AUC) and their 95% confidence interval (CI) were calculated. Differences were considered with the two-tailed test with an alpha level of 0.05.

Results

Patient characteristics and pregnancy outcomes of vitrified-warmed ET in natural and HRT cycles are shown in Table 1. There were no significant differences of variables, such as age, number of previous treatment cycles, endometrial thickness, pregnancy and ongoing rates, in both groups.

Changes in uterine radial artery blood flow impedance in natural and HRT cycles

We examined whether the RA-RI changes during natural and HRT cycles. We measured the RA-RIs of individual patients longitudinally in natural and HRT cycles. In the natural cycles, although the RA-RIs on the day of hCG trigger was not significantly changed compared to those in the early follicular phase, the RA-RIs on the day of ET was significantly higher than those in the early follicular phase and on the day of
hCG trigger (Fig. 2, left panel). On the other hand, in the HRT cycles, the RA-RIs on the day of hCG trigger were significantly increased compared to those in the early follicular phase, while the RA-RIs on the day of ET were significantly increased compared to those in the early follicular phase and on the day of starting with progesterone administration (Fig. 2, right panel). Next, we compared the RA-RIs at each measurement point between natural and HRT cycles. There were no significant differences in the median values of RA-RIs at each measurement point between natural and HRT cycles (data not shown).

**The significance of uterine radial artery blood flow impedance to the clinical outcomes of vitrified-warmed ET cycles**

We evaluated the significance of RA-RI to the clinical outcomes of vitrified-warmed ET in natural and HRT cycles. The values of RA-RI at each measurement point were compared between pregnant and non-pregnant groups in natural and HRT cycles (Table 2). In the natural cycles, although the RA-RIs in the early follicular phase in the pregnant group were significantly lower compared to those in the non-pregnant group, the RA-RIs on the day of hCG trigger and the day of ET were not significantly different between pregnant and non-pregnant groups. In the HRT cycles, there were no significant differences in RA-RI between pregnant and non-pregnant groups.

As endometrial thickness affects the successful implantation and subsequent pregnancy in frozen-thawed ET cycles [27, 28], we analyzed the correlation between RA-RIs and endometrial thickness in natural and HRT cycles (Table 3). In the natural cycle, the weak negative correlation, although not significant, between RA-RIs at any measurement point and endometrial thickness was observed. On the other hand, in the HRT cycles, there were no correlations between RA-RIs at either measurement point.

We validated the efficacy of the levels of RA-RI in predicting the possibility of pregnancy in vitrified-warmed ET. Figure 3 shows the ROCs of RA-RI for the prediction of pregnancy in natural and HRT cycles. In the natural cycles, the AUCs for early follicular phase, the day of hCG trigger, and the day of ET were 0.75 (95% CI 0.57–0.93), 0.69 (95% CI 0.49–0.88), and 0.60 (95% CI 0.36–0.84), respectively (Fig. 3, upper lane). In the HRT cycles, AUCs for early follicular phase, the day of starting with progesterone administration, and the day of ET were 0.60 (95% CI 0.40–0.81), 0.60 (95% CI 0.39–0.87), and 0.58 (95% CI 0.37–0.79), respectively (Fig. 3, lower lane). Table 4 summarizes the sensitivities, specificities, AUCs, and positive and negative predictive values (PPV and NPV) for predicting pregnancy at various thresholds for RA-RI at each measurement point in natural and HRT cycles.

**Discussion**

In the present study, we showed that the RA-RIs were changed during the natural and HRT cycles with vitrified-warmed ET. We also showed that the value of RA-RI in the early follicular phase might predict
pregnancy in the natural cycle with vitrified-warmed ET.

Firstly, we demonstrated that the RA-RI values at specific measuring points were changed during the natural and HRT cycles with vitrified-warmed ET. This result suggests that the uterine blood flow might be regulated by ovarian steroids, such as estrogen and progesterone. The conflicting results have been reported about the uterine blood flow impedance in a natural menstrual cycle. Achiron et al. reported that the RA-RI decreases during the follicular phase, while that increases in the luteal phase [18]. On the other hand, Miwa et al. reported that there were not significantly different of RA-RI during the menstrual period in the normal endometrial thickness [14]. Miwa et al. compared the value of RA-RI at each point of the menstrual cycle by a non-paired nonparametric test. In the present study, we compared the data of RA-RI by a paired nonparametric test in individual patients. The difference in the result may be due to the use of different statistical methods.

Similar changes in the natural cycle were observed in women with premature ovarian failure and postmenopausal women receiving hormone replacement therapy [18, 23]. These results suggest that ovarian steroids, such as estrogen which has the effect of the uterine vasodilator and progesterone which antagonized the effect of estrogen, have an opposite role for uterine blood flow during the menstrual cycle [15]. These phenomena might be reasonable for accepting implantation of the embryo and subsequent maintenance of pregnancy.

In the present study, the RA-RI at the starting with progesterone administration was higher than that in the early follicular phase. Moreover, we showed the RA-RI at ET was higher than that in the early follicular phase and starting with progesterone administration. This increased event might be due to progesterone effects. However, in the natural cycle, this increase was not observed. We speculate the difference of changes in RA-RI between HRT and natural cycles might be dependent on the levels of estrogen. However, we did not measure the hormonal levels, such as estradiol and progesterone in both cycles. Regarding this point, further study is necessary for clarifying the effects of estrogen on the uterine blood flow.

Secondly, we showed that the RA-RI in the early follicular phase might raise the possibility of predicting pregnancy in vitrified-warmed ET. The RA-RI in the early follicular phase showed the best predicting efficacy of pregnancy in the natural cycle but not in the HRT cycle. This result might show some advantages for the patients with the entry of vitrified-warmed ET. Clinically, if the patient had more than 0.68 of RA-RI in the early follicular phase, the patient would choose to undergo the HRT cycle or cancel the treatment. In fact, in the present study, the number of pregnant patients with more than 0.68 at RA-RI in the early follicular phase in the natural cycle was zero out of 11 and the negative predictive value was 0.92.

Previous studies have emphasized that the uterine blood flow impedance around the peri-implantation period correlates with embryo implantation and pregnancy in the IVF-ET cycle [29–31]. Wang et al. reported that the parameters of endometrial blood flow, but not endometrial thickness, on the day of hCG trigger predict pregnancy in fresh IVF-ET cycles [30]. The concept that the endometrial blood flow may be
necessary for pregnancy outcome is consistent with our results. However, this result was performed in the fresh ET cycle, which is different from vitrified-warmed ET cycles.

However, how the mechanism of the early follicular phase uterine endometrial blood flow with predicting pregnancy remains unknown. The endometrial thickness is an important factor for implantation and is associated with uterine endometrial blood flow. Miwa et al. reported that the high RA-RI was measured in the early follicular phase and remained high throughout the menstrual cycle in patients with a thin endometrium [14]. In this study, the RA-RI in the early follicular phase was negatively correlated with endometrium and predicted the pregnancy outcomes. The uterine endometrial blood flow in the early follicular phase might be necessary for subsequent endometrial growth.

There are several limitations to this study. Because of the observational study, we could not allocate the patients randomly and were unable to adjust for potential confounding variables. Also, in the present study, the transferred embryos were early-cleavage embryos but not blastocyst-stage embryos. The day of ET is determined according to the developing of the embryo stage. If the transferred embryos are blastocysts, the day of ET is scheduled 5 to 6 days after hCG trigger in the natural cycle and starting progesterone administration in the HRT cycle. There is 2 days delay of ET in the blastocyst-stage embryos compared to early-cleavage stage embryos. There could be a possibility of different value of RA-RI at the day of ET in the blastocyst-stage embryos. Further examinations are necessary to elucidate the uterine blood flow during the implantation window.

Conclusions

We firstly reported that the changes in endometrial blood flow during natural and HRT cycles were similar. In the natural cycle, the RA-RI in the early follicular phase might predict pregnancy outcomes by regulating the endometrial thickness. Our findings suggest that RA-RI in the early follicular phase might be an effective and useful tool to decide between natural or HRT cycles with vitrified-warmed ET. Further studies are needed to examine the effectiveness of measuring RA-RI in the early follicular phase in ART treatments.

List Of Abbreviations

IVF: in vitro fertilization; ET: embryo transfer; HRT: hormone replacement; ART: assisted reproductive technology; RA-RI: radial artery resistance index; hCG: human chorionic gonadotropin; LH: luteinizing hormone; ROC: Receiver-operating characteristic; AUC: area under the receiver-operating characteristic curve; CI: confidence interval; PPV: positive predictive value; NPV: negative predictive value

Declarations

Ethics approval and consent to participate
This study was approved by the institutional review board of the Fukushima Medical University (the committee's reference number: 30123). Written informed consent was obtained from all participants.

Consent for publication

Not applicable

Availability of data and materials

The datasets during and/or analysed during the current study available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Authors’ contributions

K. O. and T. T. organized the study, collected the data, analyzed the data, and revised the manuscript. K. O. drafted the manuscript. T.T and H. M. revised the manuscript.

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Authors’ information (optional)

Not applicable

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**Tables**

**Table 1.** Patient characteristics and outcomes in natural and HRT cycles with vitrified-warmed ET.
|                              | Natural cycle | HRT cycle | P-value |
|------------------------------|---------------|-----------|---------|
|                              | (n = 28)      | (n = 32)  |         |
| Age at embryo vitrification (years)* | 35.2 (3.2)    | 36.5 (3.5)| 0.44    |
| Age at the time of the study (years)* | 36.8 (3.8)    | 36.9 (3.6)| 0.22    |
| Endometrium thickness at ET (mm)* | 10.3 (1.3)    | 9.9 (0.8 )| 0.56    |

No. of previous ET cycles/total cycles

|                              | Natural cycle | HRT cycle | P-value |
|------------------------------|---------------|-----------|---------|
|                              | (n = 28)      | (n = 32)  |         |
| No. of previous ET = 0       | 14/28 (50.0%) | 19/32 (59.4%)| 0.60    |
| No. of previous ET = 1       | 8/28 (28.5%)  | 10/32 (31.3%)| 1.00    |
| No. of previous ET = 2       | 6/28 (21.4%)  | 3/32 (9.3%)  | 0.28    |
| No. of clinical pregnancy cycles/total cycles | 9/28 (32.1%) | 11/32 (34.4%) | 0.83 |
| No. of miscarriage cycles/total pregnancy cycles | 1/9 (11.1%) | 1/11 (9.1%) | 0.83 |
| No. of ongoing pregnancy cycles/total cycles | 8/28 (28.5%) | 10/32 (31.3%) | 0.83 |

*Data are presented as mean (standard deviation) unless otherwise stated. HRT: hormone replacement; ET: embryo transfer.

Table 2. Radial artery resistance index of each measurement point of the nonpregnant and pregnant groups in natural and HRT cycles with vitrified-warmed ET

| Measurement point | Natural cycle (n = 28) | HRT cycle (n = 32) |
|-------------------|------------------------|--------------------|
|                   | Nonpregnant (n = 19)   | Pregnant (n = 9)   |         | Nonpregnant (n = 21) | Pregnant (n = 11) |         |
|                   | P-value                | P-value            |         | P-value                | P-value            |         |
| A                 | 0.69 (0.07)            | 0.63 (0.05)        | 0.04    | 0.68 (0.07)            | 0.66 (0.06)        | 0.34    |
| B                 | 0.68 (0.08)            | 0.65 (0.05)        | 0.34    | 0.69 (0.07)            | 0.66 (0.07)        | 0.35    |
| C                 | 0.72 (0.08)            | 0.70 (0.07)        | 0.61    | 0.70 (0.07)            | 0.68 (0.07)        | 0.46    |

A: early follicular phase; B: day of hCG trigger or progesterone initiation; C: day of embryo transfer.

Data are presented as the mean (standard deviation). ET: embryo transfer; HRT: hormone replacement.

Table 3. Correlation between radial artery impedance and endometrial thickness in natural and HRT cycles with vitrified-warmed ET
A: early follicular phase; B: day of hCG trigger or progesterone initiation; C: day of embryo transfer.

ET: embryo transfer; HRT: hormone replacement; CI: confidence interval; r: Pearson's correlation coefficient.

Table 4. Predictive efficacy of radial artery resistance index for pregnancy outcomes in natural and HRT cycles with vitrified-warmed ET

| Measurement point | Natural cycle (n = 28) | HRT cycle (n = 32) |
|-------------------|-----------------------|--------------------|
|                   | r         | 95% CI | P-value | r         | 95% CI | P-value |
| A                 | -0.32    | -0.62 to 0.06 | 0.09 | -0.06 | -0.40 to 0.30 | 0.76 |
| B                 | -0.20    | -0.53 to 0.19 | 0.32 | 0.03 | -0.32 to 0.37 | 0.88 |
| C                 | -0.21    | -0.54 to 0.17 | 0.27 | 0.02 | -0.33 to 0.37 | 0.89 |

A: early follicular phase; B: day of hCG trigger or progesterone initiation; C: day of embryo transfer.

HRT: hormone replacement; RI: resistance index; AUC: area under the curve; CI: confidence interval; PPV: positive predictive value; NPV: negative predictive value.

Figures
Figure 1

Protocol for the measurement of uterine radial artery resistance index in natural and hormone replacement cycles with vitrified-warmed embryo transfer. A: early follicular phase; B: day of hCG trigger or starting with progesterone administration; C: day of embryo transfer. RA-RI: radial artery resistance index; HRT: hormone replacement.

Figure 2
Temporal changes in uterine radial artery resistance index in natural and hormone replacement cycles with vitrified-warmed embryo transfer. A: early follicular phase; B: day of hCG trigger or starting with progesterone administration; C: day of embryo transfer. RA-RI: radial artery resistance index; HRT: hormone replacement; *, P < 0.05; **, P < 0.01.

Figure 3

ROC analysis of uterine radial artery resistance index for prediction of pregnancy in natural and hormone replacement cycles with vitrified-warmed embryo transfer. A: early follicular phase; B: day of hCG trigger or starting with progesterone administration; C: day of embryo transfer.