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Original Article

Differences in Implantation Timing After Embryo Transfer Based on Embryo Characteristics

Yihsien Enatsu[1], Noritoshi Enatsu[1*], Junko Otsuki[1,2], Eri Okamoto[1], Shoji Kokeguchi[1], Masahide Shiotani[1]

[1]Hanabusa Women's Clinic, Kobe, Hyogo, Japan

[2]Assisted Reproductive Technology Center, Okayama University, Okayama, Japan

[*]**Corresponding author:** Noritoshi Enatsu, Hanabusa Women's Clinic, Kobe, Hyogo, Japan.

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Abstract

Objective: To evaluate the differences in implantation timing after Embryo Transfer (ET) based on embryo characteristics. **Patients:** This retrospective study evaluated cases of 7,269 frozen-thawed ET cycles using single blastocysts that resulted in single live birth. The implantation timings after ET were speculated by a calculation formula made for the dynamics of serum human chorionic gonadotropin (HCG) changes. The patients were divided into two groups based on the initial serum HCG (10 days after ET): high-HCG group (HCG > 800) and low-HCG group (HCG < 50). **Results:** The average estimated implantation timing (HCG = 0) was 3.3 days after ET. The serum HCG levels after logarithmic conversion (logHCG) changed in parallel regardless of the starting HCG levels. The estimated implantation timing varied approximately five days (2.5–7.5 days after ET) based on the starting HCG levels. The ratios of matured blastocysts (3 or higher in Gardner's grading scale) were significantly higher in the high-HCG group (90.9%) than in the low-HCG group (47.4%). Similarly, the average grade of blastocysts was higher in the high-HCG group than in the low-HCG group (4.72 vs. 3.15, $p < 0.01$). **Conclusions:** The implantation timing after ET varies based on embryo characteristics.

Keywords: *In Vitro* Fertilization; Embryo transfer; Implantation timing; Endometrial Receptivity

Introduction

Despite its many advances and achievements, *In- Vitro* Fertilization (IVF) has yet often resulted in failure in daily clinical application. In recent years, the spread of Pre-Implantation Genetic Testing (PGT) has known to increase live birth rate per ET by avoiding transferring an embryo with chromosomal aneuploidies that would result in implantation failure or pregnancy loss. However, many studies reported that although the use of PGT improves success rate, only 45 to 60 % per ET has been reported to have resulted in live birth [1-3]. Therefore, the development of methods for improving IVF outcomes has been driven from embryo factor to non-embryo factor. Endometrial factor has been considered the primary cause of implantation failure which has been evaluated by many researchers. Numerous studies have reported a certain period of endometrial maturation called the Window Of Implantation (WOI), which starts on Day 19 or 20 of the menstrual period and lasts 4–5 days. During the WOI the blastocyst can attach to the endometrial epithelial cells and subsequently proceed to invade the endometrial stroma; this process is called endometrial receptivity [4,5]. In recent years, the optimal timing of the WOI can be determined through Endometrial Receptivity Analysis (ERA). The ERA evaluates the gene expression profile to identify the specific transcriptomic signature, providing a more precise timing for Personalized Embryo Transfer (pET) [6]. Until recently, many studies have investigated the effectiveness of ERA and reported that pET improves IVF success rate [7-9]. On the other hand, some studies have reported that ERA did not improve the ongoing pregnancy rate in good prognosis patients or in PGT cycles [10,11]. Similarly, a recent meta-analysis reported that an ERA showed no significant improvement in the IVF outcomes

except in the LBR for patients undergoing the first IVF cycle [12]. Therefore, the clinical effectiveness of ERA in IVF cycles needs to be investigated further.

The reason why pET does not have a good success rate is still unclear. Some researchers suggested that it is due to the unreliability of ERA, indicating that the result of ERA changes in different cycles [13]. Another factor is that the implantation timing after ET could vary in each embryo. Although it is generally considered that it will take about 48 to 72 hours for embryos to implant after ET, further research is needed to confirm this. Until recently, some researchers have reported cases of delayed implantation after ET, in which the embryo becomes temporarily arrested at the blastocyst stage of development [14,15]. Therefore, we hypothesized that the implantation timing after ET may differ depending on the embryo characteristics. The aim of this study is to evaluate the implantation timing after ET based on embryo characteristics.

Materials and Methods

Study population

This retrospective study included 7,269 cases of frozen-thawed ET cycles using five-day-old single blastocysts that resulted in single live birth. All procedures were performed at our institution between 2009 and 2021. The blastocysts were graded based on Gardner's classification, and ET was performed with hormone replacement cycles. Hormone replacement cycle was performed after ovarian suppression using a similar method as reported previously [16]. Briefly, both the transdermal and oral Estradiol (E2) administration were started from Cycle Day (CD) 2, following the additional use of transvaginal and oral progesterone from CD 15. Frozen-thawed ET was performed at 4 to 6 days after progesterone administration (5 days in most cases), while HCG was evaluated 9 to 12 days after ET (10 days in most cases) following 3 to 8 days of follow-ups after the initial HCG evaluation. The subgroup analysis was conducted based on the ET date after progesterone administration and the HCG dynamics of day-5 ET (n=5263) and day-6 ET (n=875) were compared. The cases with the progesterone initiation of different timing (day-5.5 ET or day-4.5 ET) were excluded from this study.

This study was approved by the Institutional Review Board (IRB) of Hanabusa Women's Clinic composed of members chosen by our institute and third-party institute (approval number 2022-05). All patients were well informed, and written informed consent was obtained prior to the treatment period before ET.

Calculation formula to estimate implantation timing

The serum HCG levels were converted in a logarithmic manner to evaluate the dynamics of HCG. Time of implantation was estimated by using the following formula: $Y = A*\log e(X) - B$, where Y represents log10HCG and X is time after ET in days and "e" indicates Euler's number based on natural logarithm and is about 2.71827.

Group separation and blastocyst classification

HCG dynamics were compared between the high-HCG group and low-HCG group. The high-HCG group comprised patients whose serum HCG was more than 800 IU/ml at initial HCG detection on 10 days after ET, while the low-HCG group comprised patients whose serum HCG was lower than 50 IU/ml at initial detection. The cases without HCG analysis at 10 days after ET were excluded in the group definition.

The degree of maturity was evaluated by embryologists based on Gardner's classification [17] and a Gardner grade of 3 or more was defined as matured.

Statistical analysis

All statistical data were calculated using Student's t-test or chi-square test and analyzed using Excel (Office 365, Microsoft USA) and EZR (Saitama Medical Center), which is a graphical user interface for R (The R Foundation for Statistical Computing). Moreover, p-values of <0.01 were considered statistically significant.

Results

The serum HCG levels increased nearly exponentially during the first five days of the observation period and then plateaued after seven to eight days. Figure 1 showed the dynamics of HCG increase after logarithmic conversion, indicating that the serum HCG levels follow the following formula: $Y = 2.1*\log e(x) - 2.5$. This formula revealed that the estimated time of implantation was 3.3 days after ET. The subgroup analysis based on the transferred date (5 days or 6 days after initial progesterone administration) revealed that there is 0.4 days delay of implantation in the group of day-6 ET. However, the HCG dynamics after the implantation was parallel between two groups (Figure 2).

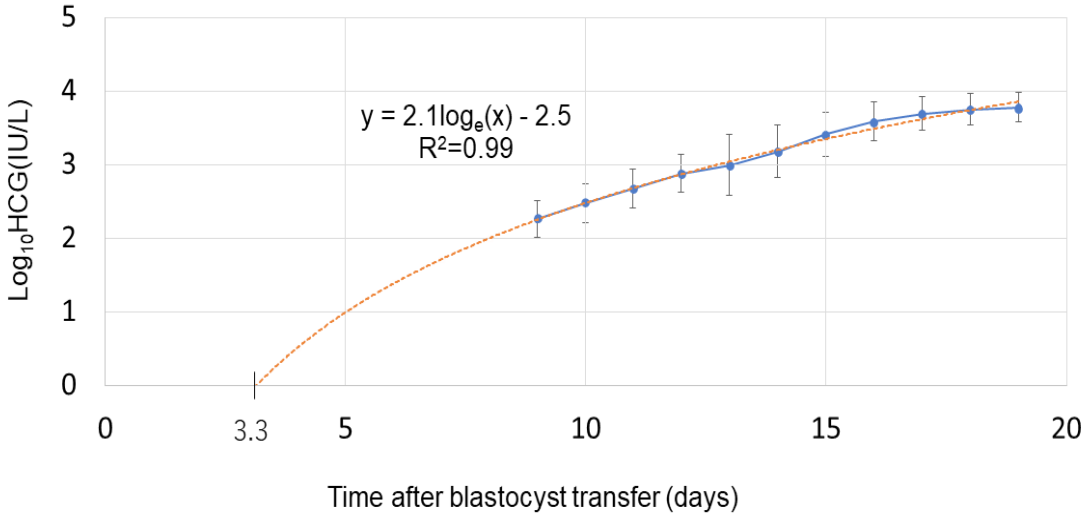

Figure 1: The average values (±95% confidence limits) of log HCG and the logarithmic curves fitted to HCG dynamics on Day 9 to 19 after ET resulted in single live birth. The estimated times of implantation can be read on the x-axis (3.3 days after ET).

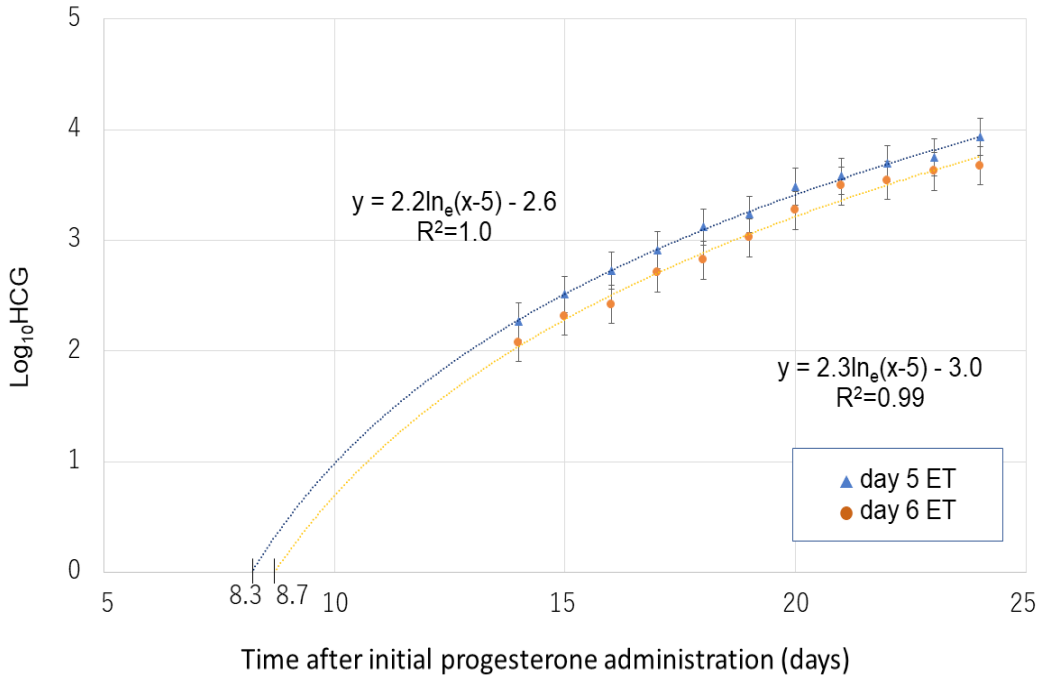

Figure 2: Comparison of the serum HCG dynamics based on the ET date. The HCG dynamics of day-5 ET (n=5263) was 0.4 days earlier than that of day-6 ET (n=875), and the estimated time of implantation was 8.3 days and 8.7 days after initial progesterone administration (3.3 days and 2.7 days after ET) respectively.

Figure 3 represents the comparison of the serum HCG dynamics after ET between the high-HCG group and low-HCG group at the initial detection of serum HCG. The high-HCG group included 47 patients whose serum HCG was >800 IU/ml at 10 days after ET, while the low-HCG group included 70 patients whose serum HCG was <50 IU/ml at 10 days after ET. This graph shows that the HCG dynamics of the low-HCG groups had a delay of about 5 days compared with that of the high-HCG group.

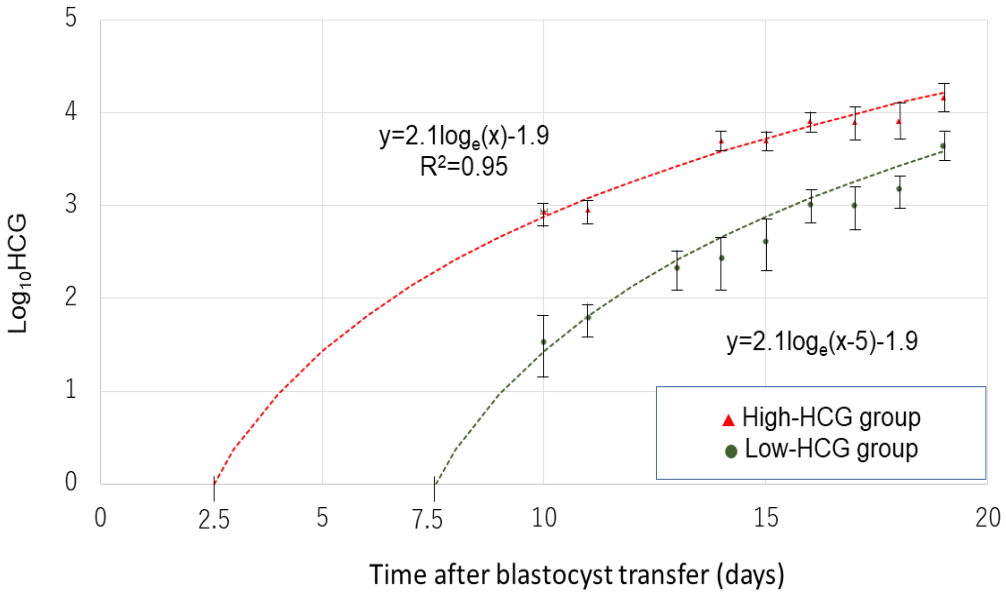

Figure 3: Comparison of the serum HCG dynamics after ET between the high-HCG group and low-HCG group at the initial detection of serum HCG. The high-HCG group included 47 patients whose serum HCG was >800 IU/ml at 10 days after ET, while the low-HCG group included 70 patients whose serum HCG was <50 IU/ml at 10 days after ET.

Table 1 represents the comparison of blastocyst characteristics between the two groups. The ratio of matured blastocysts was significantly higher in the high-HCG group (91.4%) than that of the low-HCG group (47.1%). Similarly, the grade of blastocysts was higher in high the HCG group (4.72 in average) than that of the low-HCG group (3.15 in average).

	High-HCG group		Low-HCG group		
	Number	Ratio (%)	Number	Ratio (%)	p-value
Matured blastocyst	43	91.4	33	47.1	<.01
Immature blastocyst	4	8.5	37	52.8	
Average grade	4.72		3.15		<.01

Table 1: The comparison of blastocyst characteristics between the high-HCG group and low-HCG group. Matured blastocysts indicated a Gardner grade of ≥ 3, while immature blastocysts indicate a Gardner grade of < 3. *p-value was calculated by chi-square test. **p-value was calculated by Student's t-test.

Discussion

The present study revealed that the HCG dynamics in the day-5 ET and day-6 ET were parallel, as well as in high-HCG group and low-HCG group, indicating that the increase rate of serum HCG is the same regardless of the initial HCG level in cases with pregnancies that resulted in single live birth. In the clinical setting, it is well known that the ongoing pregnancy rate after ET is highly related to the initial serum HCG level, which is more likely to result in success in cases with a high initial HCG level. This finding is due to the differences in the ratio of serum HCG wherein the increase in the speed of HCG delays or even decreases in cases with abnormal pregnancy, including spontaneous abortion or ectopic pregnancy [18]. Therefore, most physicians give more importance on the dynamics of serum HCG than the initial serum HCG level to speculate the condition whether or not it results in favorable pregnancy outcomes.

Based on these characteristics, we speculated the implantation timing after ET in cases that resulted in single live birth.

It is reported that in the case of IVF, human blastocysts normally hatch out of their shells and start to implant about one to five days (two to four days in most cases) after the day of the IVF-ET [18,19]. It is similar to the results shown in this present study, in which we calculated the average timing of implantation as 3.3 days after ET. During this period, blastocysts are considered to go through the process of hatching, apposition, adhesion, and invasion before the first detection of HCG. The interesting finding of the present study is that the implantation timing after ET changes within a maximum of five days. This fact indicates that the WOI was less considered than expected. As mentioned in the Introduction section, the clinical effectiveness of ERA in IVF cycles needs to be investigated further. One factor that should be considered is that the implantation timing after ET could vary in each embryo. For example, when we undergo ET according to the results of ERA, it will not work if the implantation timing of the embryo differs as we have expected.

It is well known that in some mammalian species, early embryos have the ability to pause their development until the uterus conditions are right. This phenomenon is called "Embryonic Diapause" and documented in 2% of mammals but never properly investigated in primates. Ptak et al. reported the induction of embryonic diapause in non-diapausing ovine embryos, suggesting that all mammals may have the ability to diapause their embryos [20]. It has also been reported that delayed implantation sometimes occurs in humans and is related to psychological stress and smoking marijuana or nicotine [21]. The longest delay reported was the case of implantation after seven weeks from IVF-ET [14]. Apart from this extreme example, Wilcox et al. reported that the implantation occurs anywhere between 6 and 12 days after ovulation [22]. Based on these findings, some researchers have suggested that minor diapause is frequent in humans [23].

This then leads to the following question: Is it possible to estimate the best timing of ET according to the embryo status. The present study indicated that the immature blastocysts tend to be implanted later than mature blastocysts. This fact indicates that the detailed observation of the embryo may provide further information related to implantation timing. Thus, further research using time-lapse imaging or DNA profiling similar to PGT or ERA may solve the question in future.

However, this study has several limitations. Firstly, since the implantation timing in this study was estimated using the dynamics of serum HCG, the exact timing of implantation may vary. However, considering the parallel increase of serum HCG, it must be true that the implantation timing differs up to five days according to the embryo characteristics. Secondly, this study was a non-randomized, retrospective study with a limited sample size. Therefore, a larger study would be needed to develop a better approach to ET.

In conclusion, we revealed that the implantation timing after ET varies based on embryo characteristics. Matured blastocysts tend to be implanted earlier than immature blastocysts. This fact implies that we need to think about not only the endometrial receptivity but also the duration to implantation after ET based on embryo characteristics.

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Disclosures

Conflict of interest: Yihsien Enatsu, Noritoshi Enatsu, Junko Otsuki, Eri Okamoto, Shoji Kokeguchi and Masahide Shiotani declare that they have no conflict of interest.

Human rights statement and informed consent: All patients were well informed, and written informed consent was obtained prior to the treatment period. This is a retrospective study and the treatments has established as standard assisted reproductive technology.

Animal studies: This article contains no studies with animal subjects performed by any of the authors.

Approval by Ethics Committee: This study was approved by the Institutional Review Board (IRB) of Hanabusa Women's Clinic composed of members chosen by our institute and third-party institute (approval number 2022-05).

Data Availability Statement: Data supporting the study results can be provided followed by request sent to the corresponding author's e-mail.

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