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Research Article

Association between Available Blastocyst Formation Rate and Pregnancy Outcome Following Fresh Embryo Transfer

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ABSTRACT

Background: Embryo quality usually has been regarded as a key predictor of successful implantation and pregnancy potential. The identification of embryos that have the capacity to implant and result in a healthy pregnancy is a crucial part of In-Vitro Fertilization (IVF). Usually, the morphologically high quality embryos are chosen for embryo transfer in IVF treatment. The aim of this study was to assess the association between available blastocyst formation rate with pregnancy outcome following first fresh embryo transfer cycles. Our objective was to predict pregnancy outcome according to embryonic development potential, and provide a systematical individual treatment to adjust endometrial receptivity for the next transfer cycle.

Methods: This retrospective, single-center study including 512 fresh embryo transfers conducted between 11/2019-08/2021, which consisted of 385 cleavage-stage (day 3) and 127 blastocyst-stage (day 5) transfers. The two groups were divided into clinical pregnancy group and non-clinical pregnancy group respectively for comparison. The association with available blastocyst formation rate and the clinical pregnancy rate between days 3 or day 5 transfer groups were concerned.

Results: In the day 3 group, there were 275 clinical pregnancies, and the clinical pregnancy rate was 71.43%. Although the 2 Pronuclei (PN) oocytes rate and available embryo rate at day 3 were significantly higher in clinical pregnancy group compared with non-clinical pregnancy group ($P<0.05$), the blastocyst formation rate and the available blastocyst formation rate had no significant differences between the clinical pregnancy group and non-clinical pregnancy group ($P>0.05$).

In the day 5 group, there were 81 clinical pregnancies, and the clinical pregnancy rate was 63.78%. All the baseline characteristics had no obvious differences between the clinical pregnancy group and non-clinical pregnancy group ($P>0.05$). The blastocyst formation rate in the non-clinical pregnancy group was higher than that in the clinical pregnancy group, but the difference was not statistically significant (81.06% *vs.* 77.03%, $P=0.083$). Interestingly, the available blastocyst formation rate was significantly higher than the clinical pregnancy group (66.19% *vs.* 60.79%, $P=0.014$).

Conclusion: In fresh cycles, available blastocyst formation rate was not associated with pregnancy outcome with a day 3 embryo transfer. However, available blastocyst formation rate was negatively associated with pregnancy outcome with a day 5 embryo transfer.

Keywords: Available blastocyst formation rate; Pregnancy outcome; Fresh embryo transfer; Clinical pregnancy

INTRODUCTION

Along with the development of Assisted Reproductive Technology (ART), there is a significant improvement in successful pregnancies [1]. Embryos created with Assisted Reproductive Technology (ART, or IVF) can be transferred into a woman's uterus at either the cleavage (day 3) or the blastocyst stage (day 5-7). Advance in the embryo culture up to the blastocyst stage enables a better selection of embryos with a superior developmental capacity and consequently a higher implantation potential [2,3]. The rationale for blastocyst transfer is to improve both uterine and embryonic

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synchronicity and enable self-selection of available embryos, thus resulting in better live birth rates [4]. Embryo transfer at the blastocyst stage increases the pregnancy rate per embryo transferred, and this is especially important in the context of Single Embryo Transfer (SET) policies, intending to reduce multiple gestations [5,6]. However, it is possible that culture of embryos to the blastocyst stage in the laboratory leads to the loss of some embryos that may have survive inside the uterus. Thus, at many IVF centers, cleavage-stage transfers are performed in patients with few available embryos to reduce the incidence of cycle cancellation if no embryo reaches the blastocyst stage and women with previous failed blastocyst transfers are referred to day 3 fresh transfers [7]. Most notably, blastocyst transfer policy does not appear to increase the Cumulative Live Birth Rate (CLBR) compared with cleavage-stage transfer [8].

The blastocyst participates in the first physical and physiological interaction with the maternal endometrium to initiate implantation, which is a complex process involving both the blastocyst and the maternal endometrium, which is open for 48 hours 7-10 days after ovulation [9]. Interaction between the uterus and the blastocyst can only occur during a limited defined period, known as the "Window Of Implantation" (WOI), during which the maternal endometrium undergoes dramatic changes [10]. Successful implantation requires a receptive endometrium, a functional embryo and a synchronized dialogue between them, which interact in a highly synchronized fashion [9,11]. The ability of the endometrium to allow normal implantation is termed receptivity, and optimal receptivity leads to normal implantation process that serves as a foundation for a healthy pregnancy [12]. However, luteal phase defect and a lack of synchrony in the development of different cellular compartments of the endometrium could decrease embryo implantation synchronized.

Although the live birth rate per transfer cycle is generally used as a measure of treatment outcome in ART, it is not a good indicator of the biological efficacy of oocytes or embryos. Culture to blastocyst stage can further eliminate part of the embryos with chromosomal abnormalities or no development potential, so we believe that the available blastocyst formation rate may be used to assess the development potential of oocytes and embryos more truly and accurately. Then we aimed to investigate whether the available blastocyst formation rate could be used to predict the pregnancy outcome in patients who have undergone IVF/Intracytoplasmic Sperm Injection (ICSI) cycles. In present study, 512 infertile couples receiving fresh IVF treatment in Reproductive Medicine Center of our hospital between 11/2019-08/2021 were retrospectively studied. We investigated the relationship between the clinical pregnancy rate after day 3 or day 5 fresh embryo transfers and the available blastocyst formation rate, and provided information for the clinical of *In Vitro* Fertilization-Embryo Transfer (IVF-ET) based on blastocyst culture.

MATERIALS AND METHODS

Patients and study design

This was a retrospective cohort study of women undergoing day 3 or day 5 fresh embryo transfers at the department of reproductive medicine, xiamen maternity and child health hospital from November 1, 2019, to August 31, 2021. Eligible patients were females younger than 35 years of age, who were undergoing their first fresh IVF cycle using their own oocytes. The number of retrieved oocytes was no less than 5, and the proportion of mature oocytes in the day of oocytes recovery ≥ 60%. Patients who had the history of recurrent pregnancy loss (RPL) (≥ 2 spontaneous abortions) or had underlying uterine malformation, chromosomal abnormalities, abnormal oocytes and cycles involving donor oocytes or embryos were excluded from the study. Patients were divided into two groups: day 3 transfer group (after day 3 transfer, all the remaining cleavage embryos were cultured to blastocyst stage); day 5 transfer group (day 5 blastocyst transfer was performed after all day 3 cleavage embryos were cultured to blastocyst stage). All data were extracted from our electronic medical record system, thus informed consent was not required.

Embryo culture and assessment

Ovarian stimulation was carried out using standard protocols according to female age, basal hormone levels, basal ovarian reserve and Body Mass Index (BMI). Ovulation was triggered mainly by human chorionic gonadotropin (hCG, aizer, Switzerland Merck serono) after dominant follicles reached a diameter of ≥ 18 mm, and oocyte retrieval was scheduled 36 h later under the guidance of vaginal ultrasound. IVF/ICSI was selected for insemination on the basis of the semen quality. Oocyte maturity was assessed after granulosa cells were stripped and observing the oocyte for the presence of a polar body on an inverted microscope. Embryos were cultured individually in micro drops (25 μl) in IVF sequential culture medium (CM/BM media; COOK, Australia) throughout the entire developmental stage and incubated under mineral oil (Vitrolife, Sweden) in a low-oxygen atmosphere (6% CO_2, 5% O_2 and 89% N_2) at 37°C. Embryo morphology was assessed and recorded on the day 3 and day 5 post-fertilization. Cleavage stage embryos were evaluated on the basis of the cell number, symmetry, fragmentation rate and presence of multinucleated blastomeres [13]. According to the Istanbul consensus [14], a high-quality embryo on day 3 was defined as follows: 7-9 blastomeres with less than 15% fragmentation and no vacuoles or multinucleation. Day 3 available embryos were 6-12 blastomeres with less than 30% fragmentation. Blastocysts were assessed according to the Gardner and Schoolcraft blastocyst scoring system [15,16], which based on the blastocyst expansion grades from 1 to 6, the number and cohesiveness of the ICM and TE organization scores A, B, or C. Blastocyst outcome (transfer, freezing and discarding) were based on morphological parameters. Available blastocysts were defined as those that met the following criteria: the blastocyst expanded up to 3 stage (cavity completely filling the embryo), International Confederation of Midwives (ICM) and the Time of Expiration (TE) were scored as AA, AB, BA, BB, AC and BC. The remaining blastocysts were excluded in this study.

The blastocyst formation rate=Total blastocysts formed on day 5 and day 6/the number of embryos performed blastocyst culture at day 3 × 100%.

The available blastocyst formation rate=Number of available blastocysts on day 5 and day 6/the number of embryos performed blastocyst culture at day 3 × 100%.

Embryo transfer and clinical outcome

All embryo transfers were under the guidance of abdominal ultrasound, and the vaginal progesterone sustained-release vaginal gel (Snoton, Merck Serono) was given 90 mg/d for luteal support immediately after transfer. The serum β-HCG was measured on the 14th day after embryo transfer. The outcome of the study was clinical pregnancy rate (CPR), clinical pregnancy was confirmed by the visualization of a gestational sac on the transvaginal ultrasound scan.

Statistical analysis

Statistical Package for the Social Sciences (SPSS) 25.0 software was used for statistical analysis. Comparisons were made using Chi-square or Fisher's exact tests for categorical variables. Continuous variables were expressed as mean ± Standard Deviation (SD) or median (P25, P75), which based on the data distribution. All tests were two-sided, and P-value<0.05 indicated that the differences were statistically significant.

RESULTS

After exclusions, a total of 512 women were included for analysis. Figure 1 depicted the flow of study participants. The baseline characteristics and laboratory data of these couples were summarized respectively in Tables 1 and 2.

The baseline characteristics and laboratory data of day 3 transfer couples were shown in Table 1. Compared with the non-pregnancy group, women with clinical pregnancy had higher 2PN oocytes rate (69.32% *vs.* 66.05%, P=0.041), available embryo rate at day 3 (82.44% *vs.* 78.49%, P=0.016) and more embryos transfer, but the blastocyst formation rate and available blastocyst formation rate had no significant differences between the clinical pregnancy group and non-clinical pregnancy group (P>0.05), and the remaining results were also no significant differences between the two groups (P>0.05). These results suggested that the remaining cleavage embryos with comparative development potential to reach the blastocyst stage between the two groups with day 3 transplantation, day 3 transfer with two fresh embryos could improve the clinical pregnancy rate (Figure 1).

First IVF cycle patients meeting inclusion criteria were collected in the study, patients meeting the exclusion criteria were excluded. According to the embryo transfer development days, eligible patient's undergone routine IVF cycles were divided into day 3 and day 5 transfer two groups. The baseline characteristics and laboratory data of the two groups were compared respectively according to clinical outcome (Table 1).

The baseline characteristics and laboratory data of day 5 transfer couples were shown in Table 2. Compared with the non-pregnancy group, women with clinical pregnancy had lower blastocyst formation rate, but the difference was not statistically significant (77.03% *vs.* 81.06%, P=0.083). Similarly, women with clinical pregnancy had lower available blastocyst formation rate, and the difference was statistically significant (60.79% *vs.* 66.19%, P=0.014), the remaining results were of no significant differences between the two groups (P>0.05) (Table 2 and Figure 2).

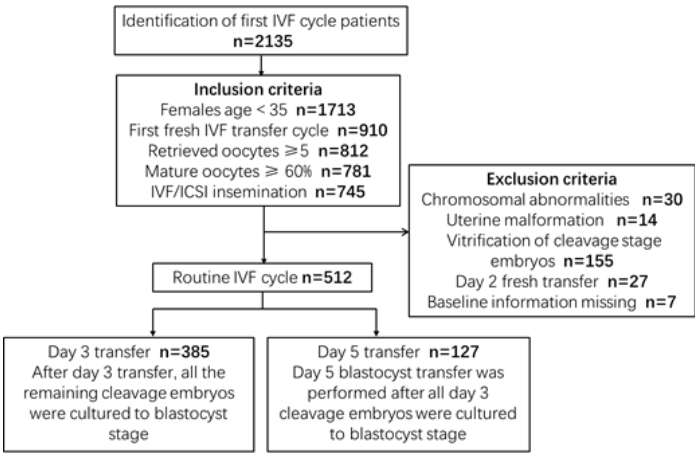

Figure 1: Study design flow diagram.

Table 1: The baseline characteristics and laboratory data of day 3 transfer (women with clinical pregnancy) and control groups.

Characteristics	Clinical pregnancy	No clinical pregnancy	P-value
Number of cases	275	110	
Female Age (years)	29.71 ± 2.74	30.02 ± 2.71	0.322
Male Age (years)	31.31 ± 3.58	31.34 ± 3.50	0.953
Female BMI (kg/m^2)	21.55 ± 2.51	21.43 ± 2.38	0.647
Infertility duration (years)	3.34 ± 2.14	3.28 ± 2.13	0.776
bFSH	7.72 ± 2.39	7.41 ± 2.06	0.229
Gn dosage (IU/L)	2350.32 ± 650.17	2351.36 ± 615.55	0.988
Oocytes retrieved (n)	11.05 ± 3.94	11.04 ± 4.15	0.974
Fertilization model (%)			0.899
IVF	226(82.2)	91(82.7)	

ICSI	49(17. 2)	19(17.3)	
Endometrial thickness on ET day (cm)	11.76 ± 6.28	11.04 ± 2.11	0.24
No. of embryos transferred, n (%)			0.016*
1	21/529(4.0)	17/203(8.4)	
2	508/529(96.0)	186/203(91.6)	
MII oocytes rate (%)	88.48%(2689/3039)	87.81%(1066/1214)	0.537
2PN oocytes rate (%)	69.32%(2054/2963)	66.05%(782/1184)	0.041*
Cleavage rate of 2PN oocytes (%)	98.20%(2017/2054)	98.08%(767/782)	0.836
Available embryo rate at day 3 (%)	82.44%(1663/2017)	78.49%(602/767)	0.016*
High-quality embryo rate at day 3 (%)	27.12%(547/2107)	27.77%(213/767)	0.331
Blastocyst formation rate (%)	66.71%(1118/1676)	66.51%(419/630)	0.928
Available blastocyst formation rate (%)	48.45%(812/1676)	48.73%(307/630)	0.904

Note: Values are presented as mean ± Standard Deviation (SD) or number (%); BMI: Body mass index; bFSH: Basal follicle-stimulating hormone; Gn: Gonadotropin; 2PN: Two pronucleus; *P<0.05 was considered statistically significant.

Table 2: The baseline characteristics and laboratory data of day 5 transfer (women with clinical pregnancy) and control groups.

Characteristics	Clinical pregnancy	No clinical pregnancy	P-value
Number of cases	81	46	
Female Age (years)	29.33 ± 2.69	29.93 ± 2.71	0.23
Male Age (years)	30.86 ± 3.23	31.93 ± 4.25	0.113
Female BMI (kg/m^2)	21.65 ± 2.63	21.55 ± 3.04	0.857
Infertility duration (years)	3.50 ± 1.96	3.33 ± 2.55	0.676
bFSH	7.53 ± 2.77	8.15 ± 4.04	0.305
Gn dosage (IU/L)	2251.24 ± 545.51	2281.79 ± 699.25	0.799
Oocytes retrieved (n)	13.59 ± 3.48	13.39 ± 3.44	0.753
Fertilization model (%)			0.255
IVF	74(91.4)	39(84.8)	
ICSI	7(8.6)	7(15.2)	
Endometrial thickness on ET day (cm)	11.57 ± 2.12	11.76 ± 1.97	0.629
No. of embryos transferred, n (%)			0.745
1	80/82(97.6)	46/46(100.0)	
2	2/82(2.4)	0	
MII oocytes rate (%)	92.01%(1013/1101)	93.18%(574/616)	0.378
2PN oocytes rate (%)	74.24%(804/1083)	76.19%(464/609)	0.374
Cleavage rate of 2PN oocytes (%)	98.89%(795/804)	98.92%(459/464)	0.945
Available embryo rate at day 3 (%)	89.69%(713/795)	88.02%(404/459)	0.362
High-quality embryo rate at day 3 (%)	41.89%(333/795)	42.48%(195/459)	0.837
Blastocyst formation rate (%)	77.03%(664/862)	81.06%(398/491)	0.083
Available blastocyst formation rate (%)	60.79%(524/862)	66.19%(325/491)	0.014*

Note: Values are presented as mean ± standard deviation (SD) or number (%); BMI: Body mass index; bFSH: Basal follicle-stimulating hormone; Gn: Gonadotropin; 2PN: Two pronucleus; *P<0.05 was considered statistically significant.

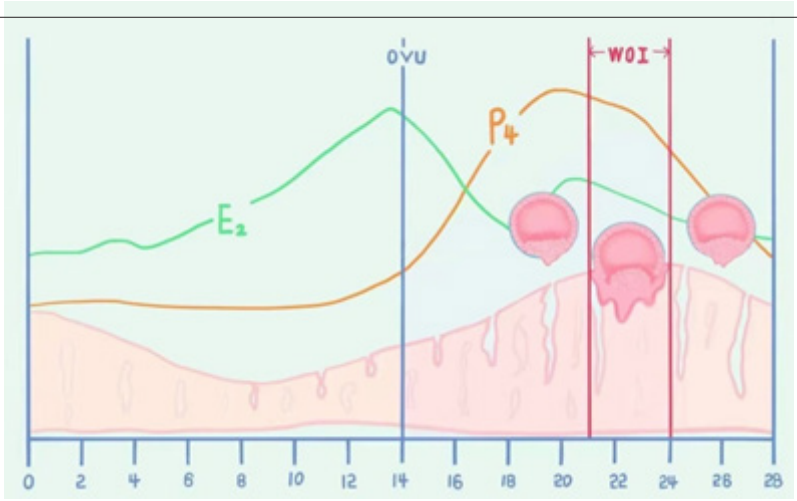

Figure 2: The baseline characteristics and laboratory data of day 5 transfer (women with clinical pregnancy) and control groups.

Implantation is a complex process involving both the blastocyst and the maternal endometrium, interaction between them can only occur during a limited defined period, known as the WOI. Well-developed blastocysts adhere ahead or delayed WOI time, and then miss the WOI.

DISCUSSION

Cleavage stage embryo transfer is often carried out in certain patients [2], such as, patients' cleavage stage embryos with lower ability to reach an available blastocyst to reduce the risk of cycle cancellation. Beyond that, in order to overcome deficiencies in embryo viability assessment, many IVF centers worldwide choose to transfer more than one embryo in some cycles [17]. In this study, in the day 3 transfer group, compared with the non-pregnancy group, women with clinical pregnancy had higher 2PN oocytes rate and available embryo rate at day 3, but the day 3 high-quality embryos rate, blastocyst formation rate and available blastocyst formation rate almost had no differences between the two groups. The reason for these results may be the clinical pregnancy group used more day 3 fresh available embryos for transplantation than the control group.

Embryo morphology is currently the most commonly used method of selecting embryos worldwide. Blastocyst morphologic grading is associated with implantation rate and live birth rate after fresh and frozen embryo transfer [18]. Higher implantation rates were observed with high-graded blastocysts [19]. Interestingly, present findings revealed that in the day 5 transfer group, the blastocyst formation rate in the non-pregnancy group was higher than that in the clinical pregnancy group, and the available blastocyst formation rate was significantly higher than that in the clinical pregnancy group. The high percentage of available blastocyst formation rate indicated that the overall embryo developmental potential was good, but why IVF treatments were not successful when high-quality embryos or even euploid embryos were transferred into the endometrial cavity, which may be due to the endometrial factor [20,21]. It is well known that implantation is a critical step in human reproduction [9,11]. The success of ET relies on synchronization between the embryo and endometrium so that the endometrium is optimally receptive for the embryo to implant [9,22]. Previous studies had investigated that women with repeated implantation failure (RIF), personalized timing for transfer resulted in a higher clinical pregnancy rate compared with routine protocol [23-25]. Thus, although there is a benefit favoring blastocyst transfer in fresh cycle, it remains unclear whether the day of transfer impacts on pregnancy rate [4]. In a 5-year multi-centre, international, Randomized Controlled Trial (RCT), Carlos suggested statistically significant improvement in pregnancy, implantation and cumulative live birth rates in Personalized Embryo Transfer (PET) compared with frozen embryo transfer (FET) and fresh embryo transfer arms, indicating the potential utility of PET guided by the Endometrial Receptivity Analysis (ERA) test at the first appointment [26].

In human, the WOI period corresponds to the mid-secretory phase, occurring between the 20th and the 24th day of the menstrual cycle, or 6-10 days after the Luteinizing Hormone (LH) peak [27] (Figure 2). But, lower growing embryos are intrinsically different and require a longer duration of progesterone exposure for optimal synchronization with the endometrium [28]. We postulated that in our study well-developed blastocysts adhered ahead or delayed WOI time, then missed the WOI (Figure 2).

CONCLUSION

Overall, embryo quality was a key factor in determining pregnancy, other factors, including receptive endometrium, were also considered to be predictive. High-quality embryos and the appropriate endometrial preparation protocol both had great significance for improving the ET pregnancy rate. Thus, further study is warranted to explore personalized treatment, regarding transfer timing, would improve pregnancy outcomes in ET cycle. In conclusion, our results suggested that available blastocyst formation rate was not associated with pregnancy outcome with a day 3 fresh embryo transfer, and available blastocyst formation rate was negatively associated with pregnancy outcome with a day 5 fresh embryo transfer. Based on these finding, we further confirmed that, for a successful clinical pregnancy in artificial cycles in women undergoing fresh embryo transfer, a competent blastocyst synchronizing with receptive endometrium was pre-requested, which provided a hint for more work needed to improve clinical implantation by personalizing, diagnosing and

synchronizing the endometrial factor.

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AUTHOR'S CONTRIBUTION

- Yufei Jiang, Longmei Wang and Pingping Qiu are acknowledged regarding conceived and designed the study.
- Lizhi Jiang is acknowledged regarding drew the model.
- Longmei Wang is acknowledged regards analyzing data.
- Longmei Wang, Yufei Jiang and Ping Li are acknowledged regards writing the manuscript.

All the authors contributed to revising the manuscript and approved the final version for publication.

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AVAILABILITY OF DATA AND MATERIALS

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

CONSENT FOR PUBLICATION

All authors approved the final manuscript for publication.

COMPETING INTERESTS

The authors declare no conflict of interest.

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