

# Correlation of Blastocyst Quality and Pregnancy Outcome during Freeze - Thaw Cycle

Xiaoguang Yu<sup>1</sup>, Yuanyuan Zhao<sup>2</sup>, Shaohua Huang<sup>2</sup> and Yuzhen Wang<sup>1</sup>

<sup>1</sup>Department of Reproductive Medicine, Baoding Maternal and Child Health Hospital, Baoding, China

<sup>2</sup>Department of Anesthesiology Operating Room, Baoding Maternal and Child Health Hospital, Baoding, China

## ABSTRACT

**Objective:** To explore the relationship between the blastocyst quality and biochemical pregnancy or early embryonic cessation of development during the freeze-thaw cycle of *in vitro* fertilisation embryo transfer (IVF-ET).

**Study Design:** Descriptive study.

**Place and Duration of Study:** Reproductive Centre of Baoding Maternal and Child Health Hospital, from January 2014 to August 2019.

**Methodology:** Eight hundred and twenty-nine embryos, treated by frozen-thawed blastocyst transfer, were analysed, retrospectively. These included 232 embryos in inner cell mass (ICM) Grade C, 272 embryos with trophoderm (TE) Grade C, and 325 embryos with Grades excluding C; ICM Grade and TE Grade were A or B. The pregnancy rate, rate of early embryonic cessation of development, and biochemical pregnancy rate were compared among the three groups after transfer.

**Results:** Compared with embryos with Grades excluding C in the score (with 55.7% in clinical pregnancy rate, 6.5% in biochemical pregnancy rate, and 5.2% in early embryonic development arrest rate), the embryos with ICM Grade C has lower clinical pregnancy rate (43.5%), higher biochemical pregnancy rate (15.1%), and rate of early embryonic cessation of development (19.8%), while the embryos with TE Grade C has lower pregnancy rate (41.2%) and higher biochemical pregnancy rate (14.3%). The differences were statistically significant (all  $p \leq 0.05$ ). There was no significant difference about the above indicators between the ICM Grade C and TE Grade C groups ( $p > 0.05$ ).

**Conclusion:** Embryos with Grades excluding C in the score had better developmental potential and better prognosis. The rate of early embryonic development arrest in the ICM Grade C group was higher than that in the TE Grade C group.

**Key Words:** Embryo quality and score, Blastocyst, Biochemical pregnancy, Early embryonic cessation of development.

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## INTRODUCTION

With the maturity of *in vitro* fertilisation embryo transfer (IVF-ET) technology, the clinical pregnancy rate is gradually increasing, and it has been widely used as an effective means of treating infertility. To this day, how to increase the clinical pregnancy rate of IVF embryo transfer is still the goal pursued by various reproductive centres. The maturity and wide application of vitrification technology and the development of freeze-thaw blastocyst transplantation have made the clinical pregnancy rate as high as over 50%.<sup>1</sup>

It saves more chances of conception for patients, greatly reduces the pain and pressure of patients, so that they no longer undergo repeated superovulation treatments and egg retrieval operations, and also gives doctors more options for treatment. With the application become wider, it attracts more attention and there are more researches on it.

Clinically,  $\beta$ -HCG is one of the important indicators to evaluate early pregnancy,<sup>2</sup> which is usually detected at 7 days after transplantation to preliminarily judge the pregnancy status.<sup>3</sup> However, biochemical pregnancy and early embryonic arrest not only seriously affect the outcome of IVF, but also bring great physical and mental pain to patients. There are many related factors, which may be related to embryo quality, endometrial condition and immune regulation.<sup>4,5</sup> Therefore, it is very important to select high-quality blastocysts with developmental potential to improve pregnancy outcome in laboratory work. Morphological scoring (Gardner scoring) is the most commonly used blastocyst grading method at present.<sup>6</sup> Since blastocyst development is a multi-stage continuous process, pregnancy

Correspondence to: Dr. Xiaoguang Yu, Department of Reproductive Medicine, Baoding Maternal and Child Health Hospital, Baoding, China  
E-mail: luscijhr4431@163.com

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outcomes often fail to meet expectations when evaluating its developmental potential only by blastocyst stage morphology. It is of great clinical guiding significance to continuously explore the relationship between blastocyst level and pregnancy outcomes by using existing data.

The study was conducted to explore the relationship between the morphological score of blastocysts and the outcome after transfer retrospectively combined with the actual data of this laboratory.

## METHODOLOGY

The study was approved by the Institutional Ethics Committee of Baoding Maternal and Child Health Hospital, and written informed consents were obtained from all participants. Clinical data of 829 patients with frozen-thawed blastocyst transplantation from January 2014 to August 2019 in the Reproductive Centre of Baoding Maternal and Child Health Hospital, were retrospectively analysed. The inclusion criteria include infertility in the fresh cycle caused by fallopian tube factors, male factors, ovulation disorders or other unknown reasons. The selected thawing cycles are all artificial cycles. Exclusion criteria were: mother's age over 40 year, a history of habitual miscarriage, uterine diseases such as uterine malformations, history of genetic or familial diseases in the parents.

Subjects who met the inclusion criteria were contacted and signed an informed consent.

Biochemical pregnancy was defined as  $\beta$ -HCG exceeded 5 IU/L, but it did not increase obviously or it decreased, or the transvaginal ultrasonography showed no gestational sac 28 days after transfer.

Early embryonic cessation of development was defined as four weeks after transfer, the examination result of HCG was positive, and intrauterine pregnancy sac and embryo were detected by B-ultrasound, but no cardiac pulsation was found. There was no cardiac pulsation for two consecutive times in examination or cardiac pulsation was seen 4 weeks after transfer, and the gestational sac did not develop; and cardiac pulsation disappeared in twice reexaminations within nine weeks of pregnancy.

Clinical pregnancy was defined as B-ultrasound detected intrauterine pregnancy sac, yolk sac and heart tube pulsation 4 weeks after transfer, which remained unchanged until the 12th week.

On the 5th to 6th day after insemination (D5/6), the blastocysts were graded by Gardner scoring system. First of all, the stage was divided according to the degree of capsular expansion. The first stage was called the early blastocyst, when the capsule volume did not reach 50% of the embryo. The second stage was the blastocyst formation stage, when the capsular volume exceeded 50% of the embryo. The third stage was the embryo sac stage, when the whole embryo was filled with the capsular. In the fourth stage, the transparent band of the expanded blastocyst became thinner. In the fifth stage, the blastocyst was in a

hatching state. At the sixth stage, the blastocyst hatched completely. Secondly, the embryo structure was evaluated, and the ICM score and TE score were respectively performed according to the arrangement and number of cells in the inner cell mass and trophoctoderm. A large number of cells that were tightly arranged were scored as A, a small number of cells that were loosely arranged were scored as B, while the number of cells was rarely scored as C.

In this study, blastocysts of Stage 4 and above with ICM or TE grades not both C, were selected and frozen. All embryos were vitrified and frozen and resuscitated rapidly.<sup>7,8</sup> Embryos re-expanded after 2 hours of embryo culture after resuscitation were transferred.

SPSS16.0 software was used for statistical analysis. The qualitative data were expressed as frequencies and percentages, while quantitative data as mean  $\pm$  S.D. Univariate analysis of variance was used for general clinical data. Chi-square test was used for embryo quality and clinical outcome. A p value  $< 0.05$  was considered as significant difference.

## RESULTS

Comparing the thawed and transferred blastocysts, namely, Group ICM Grade C, Group TE Grade C, and Group Grades excluding C, there was no significant difference among the three groups in women's age, men's age, infertility years, endometrial thickness on FET day and estradiol level on the transfer day ( $p > 0.05$ , Table I).

According to the comparison of data (Table II), we found that compared with embryos with Grades not excluding C in the score, the pregnancy rate of embryos with ICM Grade C was lower ( $p = 0.005$ ), the biochemical pregnancy rate and rate of early embryonic cessation of development were higher, and the differences were statistically significant ( $p = 0.001$  and  $p < 0.001$ ). Compared with embryos with TE Grade C, there was no difference between pregnancy rate ( $p = 0.593$ ) and biochemical pregnancy rate ( $p = 0.813$ ), but the rate of early embryonic cessation of development was higher with significant difference ( $p = 0.005$ ). Compared with embryos with Grades excluding C in the score, embryos with TE Grade C had lower pregnancy rate ( $p < 0.001$ ) and higher biochemical pregnancy rate ( $p = 0.001$ ), with significant difference and statistical significance. There was no difference in the rate of early embryonic cessation of development ( $p = 0.428$ ,  $p < 0.05$ , Table II).

## DISCUSSION

With the gradual optimisation and maturity of embryo culture system *in vitro*, the blastocyst formation rate of each reproductive centre is increasing day-by-day, and the application of vitrified freezing technology makes it possible that blastocyst transfer becomes a routine transfer technology, and blastocyst transfer is supported by more and more technologies.<sup>9</sup> The use of blastocyst culture technology has an additional optimisation process, which eliminates embryos with poor developmental potential and defective genes naturally.

**Table I: Comparison of general clinical data.**

Project	Group ICM grade C	Group TE grade C	Group grades AB	p-value
Number of embryos transferred (pieces)	232	272	325	
Woman's age	31.2±3.4	31.5±2.9	30.8±3.4	0.733
Man's age	31.8±3.4	32.3±3.2	32.5±3.0	0.769
Infertility years	4.6±1.7	4.5±1.4	4.0±1.6	0.355
Endometrial thickness on FET day	10.6±1.6	11.2±1.6	11.1±1.7	0.465
Estradiol level on the transfer day (pmol/L)	520.0±41.2	512.5±38.8	510.6±31.8	0.688
<i>p</i> > 0.05				

**Table II: Embryo quality and its clinical outcome.**

Groups	Pregnancy numbers n (%)	Biochemical pregnancy numbers n (%)	Early embryonic cessation of development n (%)
Group ICM grade C (CA,CB)	101( 43.5)	35 (15.1)	25 (19.8)
Group Grade A or B (AA,AB,BA,BB)	181 (55.7)	21 (6.5)	10 (5.2)
<i>P</i>	0.005	0.001	<0.001
Group TE grade C (AC,BC)	112 (41.2)	39 (14.3)	9 (7.4)
Group grade A or B (AA,AB,BA,BB)	181 (55.7)	21 (6.5)	10 (5.2)
<i>P</i>	<0.001	0.001	0.428
Group ICM grade C(CA,CB)	101 (43.5)	35 (15.1)	25 (19.8)
Group TE grade C (AC,BC)	112 (41.2)	39 (14.3)	9 (7.4)
<i>P</i>	0.593	0.813	0.005

At the same time, blastocyst transfer has more advantages in improving the synchronisation between embryos and endometrium, and reducing ectopic pregnancy. Studies have shown that the speed of embryo development to the blastocyst stage and the evaluation of blastocyst morphology are the key to selecting the best embryo.<sup>10</sup> In the daily work of embryo culture room, the most commonly used method to evaluate blastocyst quality is morphology. There are many limitations (personal subjective factors), but its advantages (non-invasive, intuitive and simple) make this method still play an important guiding role in the daily work of laboratory. ICM and TE grading is the best method to select embryos with normal morphology, cell number, and cell distribution as viable embryo candidates for transplantation.<sup>11</sup> The results of this study are consistent with this approach, suggesting that ICM and TE grades are important in predicting clinical pregnancy outcomes.

It has also been found that the incidence of aneuploidy in trophoblast cells is obviously related to its grade. Compared with trophoblast cells with Grade A, the incidence of aneuploidy in trophoblast cells with Grade C is much higher.<sup>12</sup> Early studies have confirmed that  $\beta$ -HCG is secreted very early during embryonic development, and its expression level steadily increases with the speed of embryonic development.<sup>13</sup> The production of HCG is directly related to the number and development of trophoblast cells, so it is recognised as one of the most valuable biochemical indicators for early prediction of pregnancy outcome. Moreover, with the increase of the number and further development of embryonic trophoblast cells, its ability to secrete  $\beta$ -HCG is significantly enhanced.<sup>14</sup>  $\beta$ -HCG can stimulate the synthesis and secretion of progesterone in luteum and regulate the receptivity of endometrium to the embryo. Therefore, more and more attention has been paid to the trophoblastic layer in blastocyst freezing selection. At the

same time, some research results show that ICM score is more relevant to the pregnancy outcome in the single blastocyst transfer in the resuscitation cycle.<sup>15</sup> Biochemical pregnancy occurs mainly because the embryo implantation process has started but failed to complete, and its mechanism is still unclear.<sup>16</sup>

In this study, comparing embryos with TE Grade C with high-quality blastocysts, the pregnancy rate of the former was significantly lower, and there was no difference in the rate of early embryonic cessation of development ( $p > 0.05$ ), while the biochemical pregnancy rate of the former was significantly higher. Compared with embryos with ICM Grade C in terms of blastocyst transfer results, their pregnancy rate and biochemical pregnancy rate showed no difference. It shows that the quality of blastocyst TE cannot be regarded as the main cause of biochemical pregnancy alone, and the quality of ICM also has certain influence.

It is generally considered that comprehensive analysis of ICM and TE can predict the pregnancy outcome in a better way. The development and progress of ICM, as a part to develop into a fetus in the future, is complex and critical. Its dysplasia can still lead to biochemical pregnancy.<sup>17</sup> Studies have found that the quality of ICM is positively correlated with the pregnancy outcome; it is considered that ICM is more important to the pregnancy outcome.<sup>18,19</sup> In this study, compared with high-quality blastocyst group, the pregnancy rate of blastocyst group with ICM Grade C was lower, and the biochemical pregnancy rate and rate of early embryonic cessation of development were higher. Compared with the group with TE Grade C, the rate of early embryonic cessation of development was higher in group with ICM Grade C and the difference was significant. It showed that with the same level of trophoblast cells, the better ICM had a better development potential,<sup>20</sup> which

was positively correlated with the pregnancy outcome. Blastocysts with ICM with better quality should be preferred in the transfer and selection of frozen-thawed blastocysts, and ICM with better quality is more important than the TE grading.

## CONCLUSION

Embryos with Grades excluding C in the score had better developmental potential and better prognosis. The rate of early embryonic development arrest in the ICM Grade C group was higher than that in the TE Grade C group.

## CONFLICT OF INTEREST:

The authors declared no conflict of interest.

## AUTHORS' CONTRIBUTION:

XY, YZ: Designed study and prepared manuscript, and responsible and accountable for the accuracy or integrity of the work.

SH: Collected and analysed clinical data.

YW: Significantly revised this manuscript.

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