RELATIONSHIP BETWEEN MORPHOLOGIC GRADING AND CLINICAL PREGNANCY RATES AFTER SINGLE EUPLOID BLASTOCYST TRANSFER

Nguyen Thi Cam Van✉, Le Thi Phuong Lan

The ART Center of Vinmec International Hospital

This retrospective study aimed to evaluate the relationship between the morphologic grading of euploid blastocyst and the clinical pregnancy rate after single frozen-thawed blastocyst transfer. All patients in this study were performed preimplantation genetic testing for aneuploidy (PGT-A) and they were transferred single euploid blastocyst in the frozen-thawed embryo transfer cycles. The results figured out that clinical pregnancy rates in the embryo transfer groups of good inner cell mas (ICM) and trophectoderm (TE) morphologic grading were significantly higher than those of groups without good morphologic grading blastocyst. And the ICM and TE morphologic grading had the same value in prognosis of clinical pregnancy. Hatching status of the transferred blastocysts could result in different prognosis of clinical pregnancy in cases of poor-quality embryo transfer.

Key words: blastocyst morphologic grading, clinical pregnancy, preimplantation genetic testing for aneuploidy, inner cell mass, trophectoderm.

I. INTRODUCTION

Selecting the most viable embryo to transfer has always been important in an in vitro fertilization attempt. The current methods are available to select the embryos are as follows: morphologic grading, preimplantation genetic testing for aneuploidy (PGT-A), and embryo morphokinetics based on time-lapse observation. All these methods aim to evaluate embryos with the highest implantation potential to transfer into the maternal uterus. Preimplantation genetic testing for aneuploidy analyzes all 24 chromosomes by comprehensive chromosomal screening with different techniques such as KaryoLite-Bobs, array comparative genomic hybridization (aCGH), and next generation sequencing (NGS) to select euploid embryos that will most likely result in pregnancy. Transferring single euploid embryos significantly improves in vitro fertilization implantation and delivery rates.[1,2] PGT-A is indicated for patients with advanced maternal age, recurrent implantation failure, recurrent miscarriage, family history of aneuploidy conditions such as trisomy 21 (Down syndrome), trisomy 13 (Patau syndrome), trisomy 18 (Edward syndrome), and sex chromosome aneuploidies such as Turner syndrome and Klinefelter syndrome.

However, there are patients who still fail to get pregnant after euploid embryos transfer and many factors contribute to this result. One of the most important factors is embryo morphologic grading.

This study reviewed the correlation between morphologic grading and clinical pregnancy rates after single euploid blastocyst transfer in vitro fertilization preimplantation genetic screening.

Corresponding author: Nguyen Thi Cam Van,
The ART Center of Vinmec International Hospital,
Email: nguyencamvan.art@gmail.com.
Received: 24/02/2020
Accepted: 26/03/2020

II. METHODS

1. Patient details

This retrospective study analyzed the IVF attempts with PGT-A conducted in the Assisted Reproductive Technologies Center of Vinmec International Hospital and the Center of IVF and Tissue Engineering - Hanoi Medical University Hospital between October 2017 and December 2019. The patients had no history of PGT-A embryo transfer before and they were transferred one frozen-thawed euploid blastocyst. The patients were included in the study if they had at least one characteristic as follows:

- Age ≥ 35
- Miscarriages/stillbirth ≥ 2
- Failed embryo transfer ≥ 2
- History of birth defect delivery

This study excluded the following cases:

- IVF cycles with PGT-A performed on cleavage embryos.
- Number of transferred embryos ≥ 2.
- Sperm/oocyte/embryo donation.

2. Methods

IVF cycles with PGT-A protocol

This study was performed using the medical records of 178 patients who underwent IVF procedures with PGT-A on blastocyst stage. The patients were indicated gonadotropin-releasing hormone (GnRH) antagonist protocol. Gonadotropin doses were formulated according to the patient's antral follicular count, anti-müllerian hormone levels, and previous response to stimulation. Transvaginal ultrasound was performed to monitor follicular response to stimulation and gonadotropin doses were adjusted accordingly. Final oocyte maturation was triggered with hCG when the mean diameter of one follicle ≥ 18 mm or ≥ 2 follicles sized ≥ 17 mm (Ovitrell; Industria Farmaceutica Serono S.P.A, Italy). Ultrasound-guided oocyte-retrieval under conscious sedation was performed 36 hours after the trigger. All mature oocytes were fertilized by ICSI procedure. After ICSI, the oocytes were cultured in G1 plus medium covered with Ovoil oil, and placed the cultured dish into a benchtop incubator (37ºC, 6% CO_2 and 5% O_2) (Vitrolife, Denmark). On day 3, the embryos underwent laser-assisted hatching and transferred into G2 plus medium (Vitrolife, Denmark) covered with Ovoil oil. On day 5, embryo quality was scored and blastocysts were selected for biopsy. The trophectoderm biopsy was only performed on stages from full blastocysts and beyond. The early blastocysts were cultured until day 6 and if the requirements were met, trophectoderm biopsy would be performed. After biopsy, the blastocysts were cryopreserved by vitrification technique with Cryotech vitrification medium (Cryotech, Japan). The trophectoderm cell samples were analyzed by aCGH technique to screen aneuploidies.

In the frozen-thawed embryo transfer cycles, the endometrium was prepared by a natural cycle, a hormone replacement cycle, or a stimulated cycle. The blastocyst was transferred when the endometrial thickness was ≥ 7 mm. In the morning of embryo transfer day, the euploid blastocyst was selected and thawed. The thawed blastocyst was then cultured in sterile oil-cover media at 37ºC, 6% CO_2 and 5% O_2 for at least 2 hours before transferring. Before loading the transferred blastocyst into the catheter, we graded the embryo quality under an inverted microscope and used this score for analysis. After embryo transfer, luteal phase support was provided either with progesterone vaginal suppositories, such as Utrogestan (Besins Healthcare, Paris, France), Cyclogest (Actavis UK Limited, UK), Crinone

gel 8% (Merck) or intramuscular injections. A blood test was scheduled 12 days after embryo transfer to detect and measure βhCG level. If the βhCG level was < 25 IU/mL the patient was categorized as non-pregnant. The patient was categorized as pregnant if their βhCG level was ≥ 25 IU/mL; a vaginal ultrasound was indicated 7 – 10 days later. Clinical pregnancy was defined as the presence of one gestational sac on ultrasound.

Blastocyst morphologic grading

In this study, to score the quality of blastocyst stage embryos, we used a scoring system which is deduced from the grading system of Gardner and Schoolcraft.[3] Three different blastocyst parameters were assessed. First, the developmental stage of the blastocyst was evaluated. Second, the quality of the inner cell mass was scored, and finally, the quality of the trophectoderm cells was graded.

According to the type of blastocoel formation and grade of expansion of the blastocyst, the following blastocyst stages could be distinguished and categorized from early to advanced developmental stage:

BL1: Early blastocyst in which the volume of the blastocoel was less than half the volume of the embryo. Embryos in which a cavity was starting to form (indicated by the presence of sickle-shaped cells) were also considered as BL1 blastocysts.

BL2: Early blastocyst in which the cavity was larger than half the volume of the embryo.

BL3: Full blastocyst: the blastocoel filled the embryo completely. In this stage of blastocyst formation the inner cell mass and trophectoderm can be distinguished.

BL4: Expanded blastocyst: the volume of the blastocoel was larger than the initial volume of the embryo. The zona pellucida had also significantly thinned.

BL5: Hatching blastocyst: the trophectoderm cells were expelled from the zona pellucida.

BL6: Hatched blastocyst: the blastocyst has completely escaped from the zona pellucida.

BL7: Blastocyst that was artificially hatching through the hole of the embryo biopsy in case of preimplantation genetic diagnosis. Additionally, the zona pellucida is not thin.

BL8: collapsed blastocyst. This stage indicated that the blastocyst did not hatch after it has expanded, but had collapsed instead. However, it was possible that the blastocyst re-expands afterwards and transforms into a blastocyst with a blastocoel.

From the full blastocyst stage onwards (BL3), the inner cell mass and trophectoderm could be scored.

The type of inner cell mass (ICM) was recorded as the first digit after the blastocyst stage. Four ICM types could be distinguished:

• A: ICM cells were tightly packed, many cells were present

• B: The ICM cells were loosely grouped, several cells are present

• C: Very few ICM cells were visible

• D: No cells were visible, the ICM was not present or was degenerative

The type of trophectoderm (TE) was recorded as the second digit after the blastocyst stage. Four types of trophectoderm qualities were possible:

• A: Many cells forming a cohesive epithelium

• B: Few cells forming a loose epithelium

• C: Very few large cells were visible

• D: The trophectoderm was degenerative or abnormal with no cells visible.

Based on the expansion of the blastocyst, the quality of the inner cell mass and the quality of the trophectoderm cells, the blastocyst morphology was graded in the following way:

• Grade 1 (top quality): blastocysts scored

as ≥ 3AA or ≥ 3AB.

• Grade 2 (good quality): blastocysts scored as

- ≥ 3BA or ≥ 3BB

- The expansion of the blastocyst was scored as BL1/BL2 (early blastocyst)

• Grade 3 (poor quality): blastocysts scored as:

- ≥ 3AC, ≥ 3AD

- ≥ 3BC, ≥ 3BD

- ≥ 3CA, ≥ 3CB, ≥ 3CC, ≥ 3CD.

• Grade 4 (bad quality): when the inner cell mass score was D and/or the trophectoderm cells score was D.

3. Statistical analysis

Continuous data were expressed as mean ± standard deviation, and categorical variables were in absolute and percentage frequency. Chi-square test, Fisher's exact test, student's t-test were used for statistical analysis. A P value < 0.05 was considered statistically significant and the statistical analysis was performed with SPSS ver.20 (IBM, Armonk, NY, USA).

4. Ethics statement

This study was an observational study, all the treatment results were not affected by the investigating process. The consent to participate was waived due to its retrospective nature and medical records were only used in this analysis. All the results and personal data was treated as strictly confidential and used only for scientific purposes.

III. RESULTS

1. Patient Demographics and Embryo characteristics

Table 1. Baseline characteristics of patients and embryo transfer cycle.

Total number of patients		178
Age		33.2 ± 4.4
Years of infertility		3.3 ± 1.9
Infertility classification	Primary	10 (5.7%)
	Secondary	168 (94.3%)
History of miscarriage/stillbirth	Yes	127 (72.3%)
	No	51 (28.7%)
History of failed embryo transfer	Yes	42 (23.6%)
	No	136 (76.4%)
C-section scar	Yes	74 (41.6%)
	No	104 (55.6%)
Endometrial thickness (mm)		8.5 ±1.4
Progesterone level on day of embryo transfer (ng/ml)		12.0 ± 10.2
Estradiol level on day of embryo transfer (pg/ml)		411.3 ± 621.4
Clinical pregnancy after embryo transfer	Yes	99 (55.6%)
	No	79 (44.4%)

Total number of single euploid blastocyst transfers in this study was 178 and the clinical pregnancy rate was 56.7%.

2. Relationship between blastocyst morphologic grading and clinical pregnancy rate after single euploid blastocyst transfer

Table 2. Blastocyst grading and clinical pregnancy rate after single euploid blastocyst transfer.

Grade	Number of pregnant cases	Number of non-pregnant cases	Total	p-value
Grade 1 (n1)	51 (62.2%)	31 (37.8%)	82	$p_{n1n2} = 0.871 > 0.05$ $p_{n1n3} = 0.011 < 0.05$ $p_{n2n3} = 0.029 < 0.05$
Grade 2 (n2)	31 (60.8%)	20 (39.2%)	51	
Grade 3 (n3)	16 (38.1%)	26 (61.9%)	42	
Grade 4	1	2	3	
Total	99	79	178	

There was no significant difference of clinical pregnancy rates between the groups of grade 1 embryo transfer and grade 2 embryo transfer (p = 0.871 > 0.05). Nevertheless, the clinical pregnancy rate in the group of grade 1 embryo transfer was significantly higher than group of grade 3 embryo transfer (p = 0.011). And the clinical pregnancy rate in group of grade 2 embryo transfer was significantly higher than group of grade 3 embryo transfer (p = 0.029).

Table 3. Expansion of the blastocysts and clinical pregnancy rate after single euploid blastocyst transfer.

Expansion	Embryo transfer quality	Number of pregnant cases	Number of non-pregnant cases	Total	p-value
Non-hatching blastocyst	Embryo transfer with good embryo quality (grade 1 or grade 2)	21 (52.5%)	19 (47.5%)	40	p = 0.038
	Embryo transfer without good embryo quality (grade 3 or grade 4)	8 (27.6%)	21 (72.4%)	29	
Hatching blastocyst	Embryo transfer with good embryo quality (grade 1 and grade 2)	61 (65.6%)	32 (34.4%)	93	p = 0.472
	Embryo transfer without good embryo quality (grade 3 or grade 4)	9 (56.3%)	7 (43.4%)	16	
Total		99	79	178	

The expansion of blastocysts has always been a concern of embryo selection for transfer. In the

cases of embryo transfer with non-hatching blastocysts, the clinical pregnancy rate in the group of good embryo quality transfer was significantly higher than group of embryo transfer without good embryo quality (p = 0.038). However, in the cases of embryo transfer with hatching blastocyst, the clinical pregnancy rate in the groups of grade 1 or grade 2 blastocyst transfer was not significantly higher than group of grade 3 blastocyst transfer (p = 0.472).

Table 4. ICM morphologic grading and clinical pregnancy rate after single euploid blastocyst transfer.

ICM morphologic grading	Number of pregnant cases	Number of non-pregnant cases	Total	p-value
Grade A	52 (62.7%)	31 (37.3%)	83	pAB = 0.47
Grade B	34 (56.7%)	26 (43.3%)	60	pAC = 0.015
Grade C	12 (37.5%)	20 (62.5%)	32	pBC = 0.08
Grade D	1	2	3	
Total	99	79	17	

The clinical pregnancy rate in the group of blastocyst transfer with ICM morphology grade A was significantly higher than the clinical pregnancy rate in the group of ICM grade C (p = 0.015). There was no significant difference of clinical pregnancy rates between other groups.

Table 5. TE morphologic grading and clinical pregnancy rate after single euploid blastocyst transfer

TE morphologic grading	Number of pregnant cases	Number of non-pregnant cases	Total	p-value
Grade A	47 (61.8%)	29 (38.2%)	76	pAB = 0.58
Grade B	39 (57.4%)	29 (42.6%)	68	pAC = 0.03
Grade C	13 (41.7%)	20 (58.3%)	33	pBC = 0.09
Grade D	0	1	1	
Total	99	79	178	

The clinical pregnancy rate in the group of blastocyst transfer with TE morphology grade A was significantly higher than clinical pregnancy rate in the group of TE grade C (p = 0.03). There was no significant difference between the other groups.

IV. DISCUSSIONS

Embryo morphology plays an essential role in selection of best quality embryo for transfer even if the transferred embryos are euploid. Clinical pregnancy rate in the embryo transfer groups of good ICM and TE morphologic grading was significantly higher than other groups. Other authors also figured out similar results with this studies.[4,5] From the findings in this study, the ICM and TE morphologic grading had the same value in prognosis of clinical pregnancy. This issue is still controversial, A. Ahlström[6] and Hill[7] agreed that TE morphology was very valuable in the prediction of live birth in frozen–thawed single blastocyst transfer

cycles. Nevertheless, results from Kovacic[5] and Richter's studies[8] showed that ICM morphology not TE morphology was the prognostic factor of blastocyst implantation potential. In this study, clinical pregnancy rate in groups of good quality blastocyst transfer were significantly higher than group of poor quality blastocyst transfer if the transferred blastocysts were in hatching status. However, there was nonsignificant difference of clinical pregnancy rate in the groups of good quality blastocysts transfer and poor quality blastocyst transfer if the transferred blastocysts were non-hatching. It could be inferred that transferring hatching blastocyst could result in a higher clinical pregnancy rate when the transferred embryo was not good quality. In summary, the findings in this study emphasize the fact that combination between good blastocyst morphologic grading and euploid status of the blastocyst confirmed after preimplantation genetic screening resulted in good prognosis of clinical pregnancy. This retrospective study may be limited by the small sample size of single euploid blastocyst transfers. Further long-term studies including more number of patients should be performed to analyze other factors that contribute to clinical pregnancy rates such as: male factors, previous chromosomopathy, recurrent miscarriage, maternal age, endometrial receptivity, embryo transfer technique, luteal support…

V. CONCLUSIONS

Single euploid blastocyst transfer with good ICM and TE morphologic grading resulted in high clinical pregnancy rate. The implantation rate was also high in the embryo transfers with day 5 and hatching blastocysts. To conclude, combining the morphologic grading and PGT-A technique was a beneficial tool to choose the embryo with the best potential of implantation and reduce the time successful impregnation.

Acknowledgments

This study was conducted in the Assisted Reproductive Technologies Center of Vinmec International Hospital and the Center of IVF and Tissue Engineering - Hanoi Medical University Hospital. We could not express enough thanks to the leaders of two centers for their continued support and encouragement. We would like to offer our sincere appreciation to all staffs and patients who contributed remarkably throughout the entire study period.

REFERENCES

1. Scott RT, Upham KM, Forman EJ, et al. Blastocyst biopsy with comprehensive chromosome screening and fresh embryo transfer significantly increases in vitro fertilization implantation and delivery rates: a randomized controlled trial. *Fertil Steril.* 2013;100(3):697-703. doi: 10.1016/j.fertnstert.2013.04.035.

2. Forman EJ, Hong KH, Ferry KM, et al. In vitro fertilization with single euploid blastocyst transfer: a randomized controlled trial. *Fertil Steril.* 2013;100(1):100-7.e1. doi: 10.1016/j.fertnstert.2013.02.056.

3. Gardner DK, Schoolcraft WB. In vitro culture of human blastocyst. Robert Jansen, David Mortimer (eds). Towards reproductive certainty. *Carnforth, Parthenon Press;* 1999: 378–388.

4. Irani M, Reichman D, Robles A, et al. Morphologic grading of euploid blastocysts influences implantation and ongoing pregnancy rates. *Fertil Steril.* 2017;107(3):664-670. doi: 10.1016/j.fertnstert.2016.11.012.

5. Kovacic B, Vlaisavljevic V, Reljic M, et al. Developmental capacity of different morphological types of day 5 human morulae and blastocysts. *Reprod BioMed Online.* 2004;8(6):687-94.

6. Ahlström A, Westin C, Wikland M, et

al. Prediction of live birth in frozen–thawed single blastocyst transfer cycles by pre-freeze and post-thaw morphology. *Hum Reprod*. 2013 May;28(5):1199-209. doi: 10.1093/humrep/det054.

7. Hill MJ, Richter KS, Heitmann RJ, et al. Trophectoderm grade predicts outcomes of single-blastocyst transfers. *Fertil Steril.* 2013;99(5):1283-1289.e1. doi: 10.1016/j.fertnstert.2012.12.003.

8. Richter KS, Harris DC, Daneshmand ST, et al. Quantitative grading of a human blastocyst: optimal inner cell mass size and shape. *Fertil Steril.* 2001;76(6):1157-67.