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Trophectoderm morphology is a better predictor of clinical pregnancy in blastocyst transfers

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ABSTRACT

In assisted reproduction, a successful outcome is closely associated with the selection of potentially implantable. Blastocyst culture has its own merits that it can possibly eliminate the selection of non-viable embryos, can mimic physiology with better embryo-uterine synchrony and the extended culture can also assess the embryos post-embryonic genomic activation. Scoring of blastocyst characteristics can also aid in single embryo transfers (SET), reducing the risks of multiple pregnancies. The morphological assessment can serve as a non-invasive tool for better prediction of the outcome in IVF. The two important morphological parameters of blastocyst grading the inner cell mass (ICM) and the trophectoderm(TE) other than the expansion of the blastocoel cavity. Various groups reported the importance of either characteristic predicting implantation, pregnancy and live birth. In this retrospective study, we have evaluated which one of these parameters possess the predictive ability of implantation and pregnancy. The embryos frozen as blastocysts following morphological scoring in the stimulated cycles were replaced in subsequent cycles. The transferred blastocysts with known implantation were grouped into four different combinations of grades of ICM and TE and analyzed for the association with the clinical pregnancy and the findings were statistically validated. This study demonstrates there is a significant correlation between the grades of TE cells and the clinical pregnancy, so TE grade possibly is a better predictor of in blastocyst transfers. The trophoectoderm grades of embryo can be used as a selection tool for a better IVF outcome.

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INTRODUCTION

In ART (Assisted Reproductive Technologies), there has been an immense research directed towards the prediction of most viable embryo(s) for replacement from the cohort of embryos from patients for a successful outcome (Ahlstrom *et al.*, 2011; Whitney and Anderson, 2015). Advancements in recent decades in IVF such as improved culture compositions, the extended culture of embryos, non-invasive time-lapse imaging and invasive tools such as preimplantation genetic testing of all the chromosomes of the embryos for selection of a most viable embryo enhancing the possibilities of single embryo trans-

fers (Almagor *et al.*, 2016).

There have been various studies that propose the advantages of blastocyst transfer to achieve a higher rate of implantation, pregnancy and live birth (Gardner *et al.*, 1998a; Blake *et al.*, 2007; Papanikolaou *et al.*, 2008). The laboratory-grown blastocysts, when transferred, may demonstrate a greater extent of an embryo - uterine synchronicity as compared to cleavage stage embryos. This could possibly explain the improvement in the implantation rate from blastocyst transfers (Ahlstrom *et al.*, 2011; Subira *et al.*, 2016; Chen *et al.*, 2014).

Current IVF research is directed towards the development of reliable methods to suggest biomarkers of implantation. Despite all these emerging advanced methods, conventional blastocyst grading remains the universal method of embryo selection for replacement (Zhao *et al.*, 2018). Blastocyst grading proposed and validated by Gardner *et al.* (1998b) has been used as the most accepted system to identify the embryos with higher implantation potential (Gardner *et al.*, 2004, 2000; Balaban *et al.*, 2000). This includes three parameters- the expansion of the blastocoel, the grade inner cell mass (ICM) and the trophectoderm (TE).

It is necessary to understand the contribution of the parameters of blastocyst morphology to the implantation potential. There have been various studies that suggest that the transfer of blastocysts with higher grades of both ICM and TE is associated with better outcomes in terms of implantation and live birth (Licciardi *et al.*, 2015; Honnma *et al.*, 2012; Hill *et al.*, 2013; Irani *et al.*, 2017). The Istanbul consensus suggested the ICM as a better predictor of viability (Alpha Scientists in Reproductive Medicine and ESHRE Special Interest Group of Embryology, 2011). Some other groups explained the association of the TE grades with implantation. The current study was done to identify which morphological characteristic out of these two could be the potential marker of a successful pregnancy.

MATERIALS AND METHODS

Study group, period, inclusion and exclusion

This retrospective study includes a total of 550 donor/ non-donor cycles for the period from January to October 2018. The ART center database documented patients' age, body mass index, sperm DNA fragmentation levels, thyroid status, hysteroscopy and ultrasound findings, days of gonadotropin ovarian stimulation, the protocol followed oocytes maturity and the embryo development information. Inclusion criteria were patients age 25-37 years, metaphase 2 oocytes more than 6, less than 30% sperm DNA fragmentation, step down gonadotropin stimulation with fixed antagonist protocol for 11-13 days. Patients with BMI>28, polycystic ovaries, hypo or hyperthyroidism, diabetes, endometriosis, low AMH< 1.5, and with evidence of ovarian hyperstimulation were excluded from the study.

Stimulation Protocols and Oocyte Retrieval

All patients had controlled ovarian hyperstimulation (150-300 IU recombinant FSH with or without HMG) for 11-13 days with recombinant HCG (Ovitrelle)/GnRH agonist (Luprolide acetate) trigger when dominant follicle reached 22-24mm diameter. Transvaginal oocyte retrieval was carried out after 36 hours of a trigger.

Laboratory Culture

Oocyte cumulus complexes were collected in HEPES buffered medium and cultured in Vitromed single-step medium until denudation. Collected oocytes were exposed to Hyaluronidase enzyme (80IU, Vitromed) for a maximum of 30 seconds and micro pipetting to remove the cumulus complexes. Oocyte maturity and morphological grading were assessed. ICSI was performed under an inverted microscope (Olympus) with a micromanipulator (Narshige) within 4 hours of oocyte retrieval in HEPES buffered medium overlaid with oil(Vitromed). Oocytes following ICSI were washed and cultured into 20 microliter droplets of pre-equilibrated single-step medium (Vitromed) overlaid with oil (Vitromed). Fertilization assessment was performed at 16-18 hours after injection; embryos were examined and graded again at 40-42 hours for cleavage. Embryos were cultured till Day 6 and optimal blastocysts were vitrified using Kitazato medium on both days 5 and 6 of culture.

Blastocyst grading and Embryo Transfer

In our center, the morphological grading of embryos was based on David Gardner Schoolcraft's embryo grading system and the Istanbul consensus on Embryo assessment. Scoring of expansion of the blastocoel cavity is listed in Table 1, (Hardarson *et al.*, 2012). ICM and TE morphology were scored as good(A), fair (B), and poor(C), as shown in Figure 1. Blastocysts were thawed using a vitrification warming kit (Kitazato) and transferred as single or double to the patients.

All blastocysts were expanded at the time of transfer procedure and the grades of ICM and TE were AA, AB, BA and BB, respectively. Transfers with hatched blastocysts or with poor ICM/TE were excluded from this study. In the scenario of transfer of two blastocysts with a singleton pregnancy were again

Table 1: Stages of development, ICM & TE

	Grade	Rating	Description
Stages of Development	1		Early Blastocyst
	2		Blastocyst
	3		Expanding Blastocyst
	4		Expanded Blastocyst
	5		Hatching Blastocyst
	6		Hatched Blastocyst
ICM	1	Good	Prominent, easily discernible, with many cells. That is compacted and tightly adhered together.
	2	Fair	Easily discernible, with many cells that are Loosely grouped together.
	3	Poor	Difficult to discern, with few cells
TE	1	Good	Many cells forming a cohesive epithelium
	2	Fair	Few cells forming a loose epithelium
	3	Poor	Very few cells

The Scoring system of Blastocyst is a combination based on the Embryo grading system proposed by Gardner *et al.* (1998b) and Istanbul Consensus on embryo assessment

Table 2: Calculation of Odds Ratio

Grade (n)	Number of embryos replaced	Clinical Pregnancy	Percentage implantation (%)	Odds Ratio (Confidence Interval)	Z statistic	P-value
4AA(n1)	500	140	28	1.8949(1.4193-2.5298)	4.335	<0.0001
4AB(n2)	388	62	16	0.5549(0.4031-0.7638)	3.613	0.0003
4BA(n3)	46	12	26	8.5433(4.0674-17.9445)	5.665	<0.0001
4BB(n4)	165	28	17	0.6876(0.4454-1.0615)	1.691	0.0909
Total	1099	242	100			

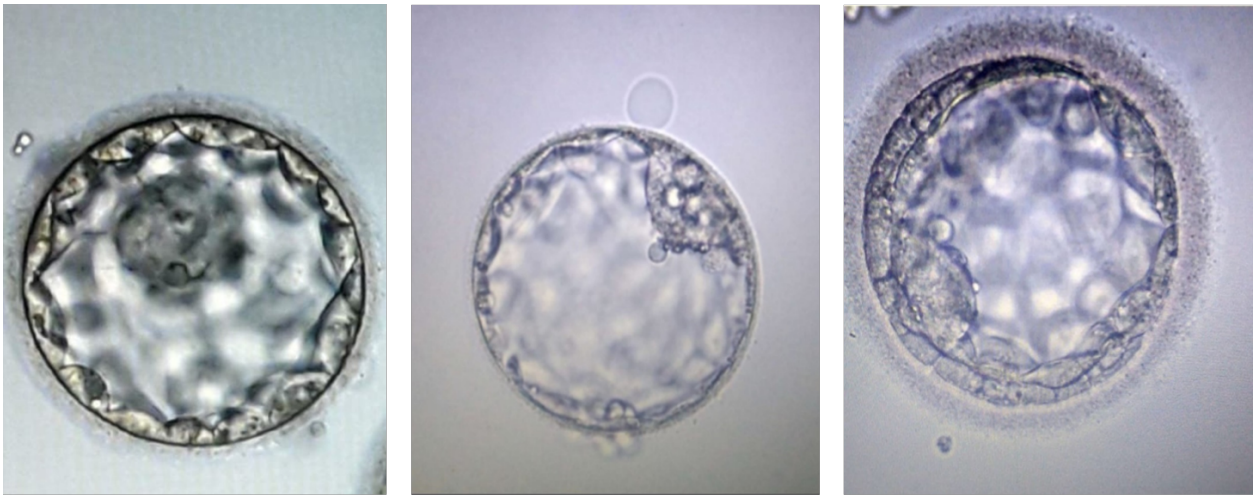

Figure 1: Blastocyst ICM and TE

excluded from the study to avoid any bias. The grades of blastocysts and implantation rates subjected to statistical validation.

Primary and secondary outcome measures

The primary outcome was the clinical pregnancy rate, defined as a pregnancy visualized by ultrasound and demonstrating a normal fetal heart rate (number of clinical pregnancy/number of embryo transfers). The biochemical pregnancy and miscarriage rate (number of miscarriages/number of clinical pregnancies) and the live birth rate (number of live births/number of embryo transfers) remain the secondary measures of outcome.

Statistical Analysis

Descriptive and inferential statistics were used to analyze the data (Table 2). Comparisons of the 4 embryo grades were performed with the use of chi-square tests, Fischer's exact tests, Wilcoxon rank-sum test, or Mantel–Haenszel tests. Overall implantation rates were expressed as percentages. Fischer exact test was used to analyze embryo grade variables. Differences were considered significant when $p< 0.05$. Logistic regression was used to obtain the odds ratio (OR) and 95% CI. Statistical analysis was done using MEDCALC (Belgium).

RESULTS AND DISCUSSION

A total of 576 patients had embryo transfer of 988 blastocysts with A and B grades of ICM and TE mixed.161(27.9%) patients showed positive pregnancy and 415(72.1%) were negative. The biochemical pregnancies (1.2%) and missed abortions (3.3%) were excluded from the analysis. 242 embryos with known implantation were retrospectively analyzed for morphological scores. The implanted embryos were divided into 4 groups with respective ICM and TE grading - AA, AB, BA, BB. In the AA and BA groups,140(28%) and 12(16%) achieved clinical pregnancy, respectively. Similarly, in the AB and BB groups, there were clinical pregnancies from 62(26%) and 28(17%) embryos, respectively.

Our study demonstrated a strong association of TE cells and clinical pregnancy. The main finding of this work is the characterization of TE cells as the most significant morphological marker of implantation and ICM possibly is contributing less.

We must understand the foetus develops from the ICM, but TE cells develop to the placenta, This theory could possibly explain the finding of our current study that the clinical pregnancy rate lowered with a lower grade of TE where the ICM remained good. The proposed mechanisms are the improved function of TE cells at the initiation of implantation could enhance the biochemical and molecular processes for hatching out of zona pellucid and invasion through the endometrium (Norwitz *et al.*, 2001). Unless TE cells establish implantation, ICM may not continue the development.

The previous studies suggested the importance of ICM grades in implantation. Interestingly the recent reports are supporting the TE cells to explain the implantation better (Thompson *et al.*, 2013; Zhang *et al.*, 2016). The strength of this study is the corresponding KID (known as implantation data). The main limitation of this study is there was no other invasive or noninvasive selection method applied to validate the findings. Blastocysts with only good and average grades of ICM and TE were included. The CPR would have been higher if included all implanted embryos from the heterogeneous group.

Though most of the patients had good grades transferred, there is still a need to analyze the negative pregnancies.

Limitations

The main limitation of this study is there was no other invasive or noninvasive selection method applied to the conventional morphological assessment to validate the findings. Blastocysts with only good and average grades of ICM and TE were included. The CPR would have been higher if included all implanted embryos from the heterogeneous group.

CONCLUSION

From this study, we conclude that the grade of trophectoderm than the grade of ICM of blastocysts is positively correlated with clinical pregnancy rate (CPR), and it can be used as a morphological marker in embryo selection and achievement of live birth from an IVF program. Single embryo transfers can be encouraged. This can be expanded to a prospective randomized study to truly determine the current findings to endow a simple and economic model of selection and deselection of embryos for better success in an IVF program.

Ethical Approval

All collected data were examined and approved by the appropriate ethics committee (Ethical Code: SMC/IEC/2016/01/241) and have therefore been performed in accordance with the ethical standards laid down in the Updated Revised Declaration of Helsinki (2008).

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