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RESEARCH ARTICLE

IMPACT OF BLASTOCYST GRADES TO IMPLANTATION AND LIVE-BIRTH RATES IN WOMEN LESS THAN 30 YEARS WITH SINGLE EMBRYO TRANSFERS

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Abstract

Background: Morphological evaluation of blastocysts focusing on key factors such as expansion, inner cell mass (ICM) quality, and trophectoderm (TE) quality have been most widely used for embryo selection. The study emphasizes the importance of detailed blastocyst evaluation, highlighting that morphological variations can have substantial implications for ART outcomes.

Methods:A total of 115women aged less than 30 years undergoing SET from from January 2023 to June 2023 were included in this study**.**The patients were divided into three groups based on their morphologic grading before embryo transfer: good-quality (n = 53), average-quality (n = 52) and poor-quality blastocysts (n = 10). The three morphologic groups' pregnancy outcomes were examined, and the implantation rate was logistically regressed.

Results: Good-quality blastocysts yielded a statistically significantly higher implantation rate (77%) than average / poor-quality blastocysts (44% and 20% respectively). Average-quality blastocysts still yielded in higher implantation rates (44%) compared to poor-quality blastocysts (20%). The baseline characteristics were analysed for significance using the Pearson‘s Chi-squared test. A P-value < 0.05 was considered statistically significant. All the statistical analyses were performed using the social science statistics website with SPSS software.

Conclusions: Higher blastocyst morphologic grading was associated with increased implantation rate for single embryo transfers. These findings suggest that evaluating blastocyst morphology is critical when selecting the best blastocyst.

Introduction:-

In vitro fertilisation (IVF) and assisted reproductive technology (ART) have revolutionised the field of reproductive medicine, offering hope to couples struggling with infertility. IVF involves the fertilisation of an egg outside the body, followed by the transfer of the resulting embryo into the uterus.

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ART encompasses a range of techniques, including IVF, intra-cytoplasmic sperm injection (ICSI), and embryo cryopreservation, aimed at enhancing fertility and increasing the chances of pregnancy. These technologies have significantly improved reproductive outcomes, making them more effective and accessible.

One of the most important goal of ART is to identify a single potential embryo with high rate of implantation. This would result in a single healthy live birth.

The selection of the embryo with the highest implantation potential is therefore a critical stage in an ART treatment. Various approaches can be taken into consideration to achieve this goal. Culturing to the blastocyst stage is one method that enables the self-selection of embryos with microscopic assessment of morphologic parameters linked to enhanced viability, such as trophectoderm, inner cell mass (ICM), and blastocoel expansion. Preimplantation genetic testing for aneuploidy (PGT-A) is another tactic used to lessen the possibility of transferring an aneuploid embryo, which is the main cause of miscarriage, disturbed embryo development, and unsuccessful implantation. When PGT-A was first used, it was used to identify embryos in patients who had experienced repeated in vitro fertilisation (IVF) failure, were elderly, or had experienced repeated pregnancy loss. But embryos might not implant. Failure causes should be investigated. It has long been believed that a healthy embryo is a key indicator of a successful implantation and pregnancy.

In terms of embryo selection, standard morphologic examination has been and continues to be the most used method. Optimal pregnancy outcomes are highly correlated with a greater total blastocyst quality. Therefore, ICM morphologic grades and trophectoderm are probably useful extra factors to consider when choosing embryos.

There are several factors to take into account that may influence clinical embryo selection in euploid blastocysts. Because of this, the purpose of this study is to evaluate how a blastocyst's morphologic features affect its ability to implant.

Methods:-

Study Design

The Banker IVF and women's hospital approved of this study. The data obtained is for 115 patients from January 2023 to June 2023, all undergoing ICSI (intra-cytoplasmic sperm injection) for conception. The criteria for patient data included: age of patients (< 30 years), all undergoing single fresh-embryo transfers (SET). All 115 patients underwent SET.

Ovarian stimulation protocol

The hormones used for ovarian stimulationprotocolweredifferent for every patient depending on their history and profile**.** After undergoing a baseline scan on the second or third day of the menstrual period, hormonal injections were started, the physician adjusted the starting dose according to the patient‘s age, body mass index (BMI) and ovarian reserve. Ovarian follicle development was monitored based on trans-vaginal ultrasonographic measurements. Oocytes were retrieved trans-vaginal 34–36 h after the hormones are administered. The follicles were aspirated using a single-lumen needle attached to a syringe under trans-vaginal ultrasound guidance.

Laboratory Protocol

The oocytes were then inseminated via ICSI approximately 4 h after retrieval. Embryos were placed into the incubator (Benchtop) and cultured at6% CO2, 5% O2, 89% N2 and 37 °C. Fertilisation checks are carried out after the 19th hour of ICSI. Normal fertilised oocytes should have 2 polar bodies, 2 pronuclei and 3-7 precursor bodies. Day 3 (pro nucleate stage to day 3,4,5) and blastocyst stage.Embryologists graded the blastocysts on the degree of expansion and the morphology of ICM and TE according to the classification devised by the ASEBIR scoring system. This includes four categories (A, B, C, D) for blastocyst grading. The key parameters for the score are degree of expansion, trophectoderm quality and ICM quality. The trophectoderm condition is the main parameter for the blastocyst quality.

The trophectoderm was graded as follows:

The blastocoel is found in the trophectoderm, the embryo's outermost layer of cells. Blastocyst grading into the four ASEBIR categories is made possible by the evaluation of cell quantity, shape, and cohesiveness. The best trophectoderm has elliptic cells and a homogeneous epithelium (A). The poorest quality has few cells, uneven

epithelium, and degenerative symptoms (D). Higher implantation and pregnancy rates are associated with high-quality trophectoderm.

The following grades were part of the degree of expansion:
The degree of blastocoel expansion is influenced by blastocyst collapse and is strongly dependent on the observation time. In a short time, the blastocoel may enlarge significantly, causing the zona pellucida to thin and eventually cause hatching to begin. increased blastocyst expansion level.

The following was the ICM's grade:
Oval in shape, the inner cell mass should have compacted cells. ICM mass should be over 1900 mm2 according to the ASEBIR score. It will be scored A or B depending on compaction, C if the size is below 1900 mm2, and D mass if degeneration is evident.

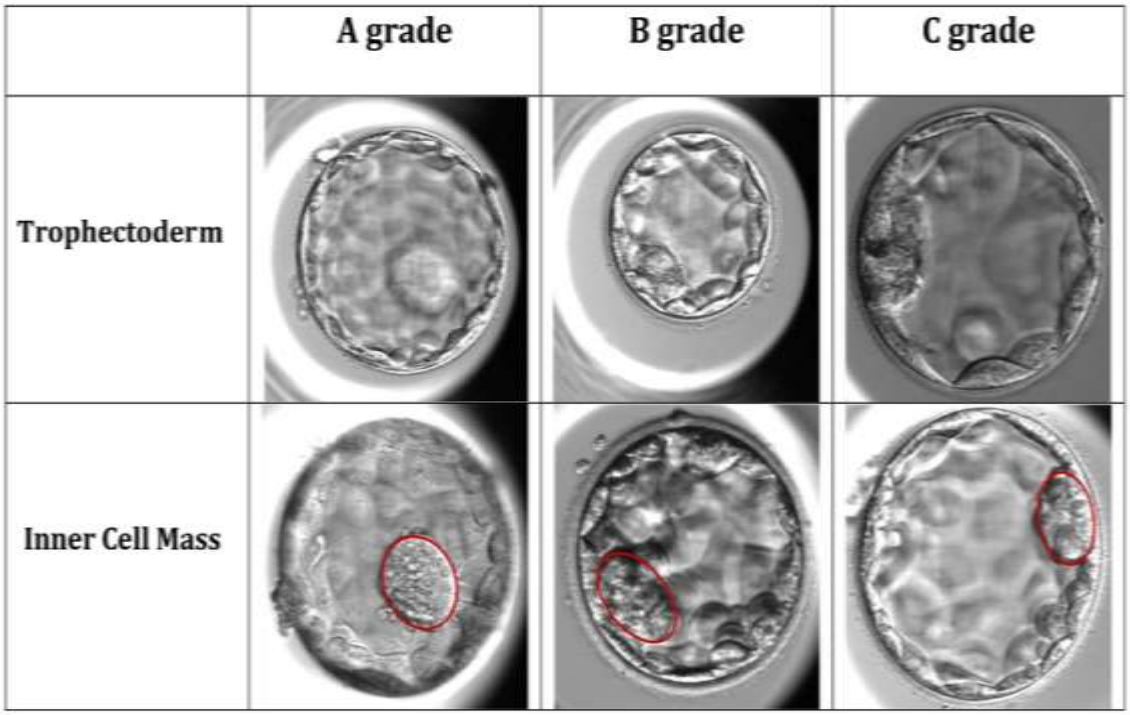


FIGURE 1 Images of grading trophectoderm and inner cell mass using the 2015 ASEBIR scoring system.

The ASEBIR (Asociación para el Estudio de la Biología de la Reproducción) embryo grading criteria is used to evaluate the quality of embryos in the context of assisted reproductive technologies (ART). The grading system assesses embryos based on their morphological characteristics at different stages of development. Here follows a summary of the ASEBIR blastocyst grading criteria in Table 1.0:

Stage	Grade	Blastomeres	Fragmentation	Zona Pellucida	Cytoplasm	Inner Cell Mass (ICM)	Trophectoderm (TE)
Day 3	**A**	Uniform in size	Less than 10%	Regular	Clear, without vacuoles	N/A	N/A
	B	Slightly uneven in size	10-25%	Slightly irregular	Minor vacuolization	N/A	N/A
	C	Significantly uneven in size	25-50%	Irregular	Presence of vacuoles	N/A	N/A
	D	Severely uneven in size or very few	More than 50%	Very irregular	Numerous vacuoles or other abnormalities	N/A	N/A
Day 5	**A**	N/A	N/A	N/A	N/A	Prominent, many cells, tightly packed	Many cells forming a cohesive layer
	B	N/A	N/A	N/A	N/A	Slightly less prominent, fewer cells, loosely packed	Fewer cells, some loose cells
	C	N/A	N/A	N/A	N/A	Sparse, few cells, very loosely packed	Few cells, scattered
	D	N/A	N/A	N/A	N/A	Very sparse or absent	Very few cells, poorly organized or absent

Table 1.0 - ASEBIR Grading Blastocyst Criteria

Embryo Transfer and Procedure Outcomes:

		Total Patients	Clinical Pregnancy	Live birth
		115	66	44
Grade	***Good** (A + B)*	53	41	29
	***Average** (C)*	52	23	14
	***Poor** (D)*	10	2	1

Table 2.0:- Data Collection for clinical pregnancy and live birth compared to their corresponding blastocyst grades

Statistical Analysis:

The Pearson's Chi-squared test was used to determine whether the baseline features were significant. The degree of correlation between two categorical variables is assessed using this test. Clinical pregnancy and live birth are the two factors being compared to their later embryo morphologic grades. P-values less than 0.05 were regarded as statistically significant. The social science statistics website using SPSS software was used for all statistical studies.

Results:-

The analyses include 115 patients having single embryo transfers. According to morphologic grading, there were the following numbers of cycles divided into three groups: good-quality blastocysts (n = 53), average-quality blastocysts (n = 52), and poor-quality blastocysts (n = 10). The pregnancy and live birth rates in relation to their respective embryo grades are summarised in table 3.0 below.

		Total Patients	**Clinical Pregnancy**	**Pregnancy rate**	**Live birth**	**Live birth rate**
		115	66	57%	44	38%
Grade	**Good** (A + B)	53	41	77%	29	55%
	Average (C)	52	23	44%	14	27%
	Poor (D)	10	2	20%	1	10%

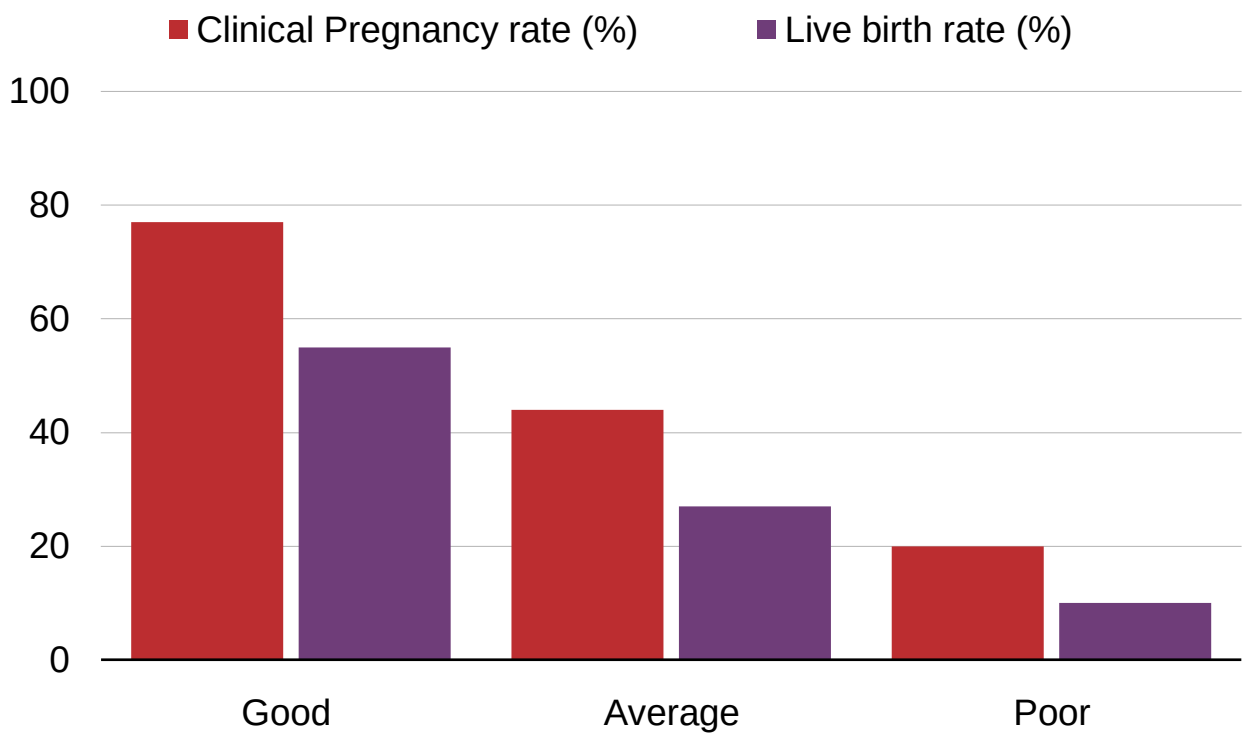
Table 3.0:- Pregnancy rates and live birth rates compared to their corresponding blastocyst grades.

Primary outcome

The implantation rate of high-quality blastocysts was statistically substantially higher (77%) than that of ordinary or low-quality blastocysts (44% and 20%, respectively). Averagequality blastocysts still yielded in higher implantation rates (44%) compared to poor-quality blastocysts (20%). Graph 1.0 summarises these findings.

Secondary Analyses

Subsequent statistical analysis was performed to measure the extent of association between two categorical variables against their respective blastocyst grades. A chi-squared test for significance was performed at values of $P < 0.05$ being considered significant shown in Table 4.0 and 5.0.



Graph 1.0:- Comparison between Clinical pregnancy rates and Live birth rates.

		Total Patients	**Pregnancy rate**			**P-value**
		115	57%			
Grade	**Good** (A + B)	53	77%		(A+B) vs (C)	0.0846
	Average (C)	52	44%		(A+B) vs (D)	0.0734
	Poor (D)	10	20%		(C) vs (D)	0.3197

Table 4.0:- P-values of Pregnancy rates to their corresponding blastocyst grades.

		Total Patients	**Live Birth rate**			**P-value**
		44	38%			
Grade	**Good** (A + B)	29	55%		(A+B) vs (C)	0.0594
	Average (C)	14	27%		(A+B) vs (D)	0.0800
	Poor (D)	1	10%		(C) vs (D)	0.3473

Table 5.0:- P-values of Live-birth rates to their corresponding blastocyst grades.

Discussion:-

In summary, the relationships between blastocyst morphologic grades and their implantation rates were discussed in this study**.** The primary outcome suggests that good quality (Grade A+B) blastocysts showed higher pregnancy and implantation rates than average (C-grade) or poor quality (D-grade) blastocysts.

Whereas the secondary analyses consisted of statistical analyses performed to measure the extent of association between Clinical pregnancy and Live birth rates against their blastocyst grades. The Chi-squared test conducted determined no statistical significance in the blastocyst grading against pregnancy and implantation rates. The primary criterion for choosing the most viable embryos for transfer was morphologic grading, even if it is graphically clear that this criterion is connected with implantation potential. An age-related decrease in embryo implantation occurs in the older population, despite morphologic grading being one of the most important determinants of cycle outcome. This supports the idea that other factors also contribute to the age-related drop in fertility.

This research has several advantages. To choose the optimal embryo for patients who have experienced several unsuccessful cycles, it was first particularly done to find a linkage. Second, the age range that was selected made it possible to examine the data in detail. Third, we assessed how embryo selection is influenced by blastocyst growth and morphology. Fourth, because only one blastocyst was transferred in this trial. Additionally, the embryologists employed a uniform grading system that included several standardised transfer criteria.

This study includes a number of drawbacks as well. First, the study's sample size was rather modest. Additionally, since the data was gathered from a single location, bias was unavoidable. Secondly, patients older than 30 years were excluded due to the possibility that they would not have access to statistics on live birth rates. This can limit the clinical results' relevance to elderly people whose blastocyst quality is influenced by various circumstances. Despite investigating blastocyst form and development, the study was unable to yield statistically meaningful data on the identification of the most viable embryos for successful implantation. Finally, because this study only looked at single embryo transfers, it cannot be applied to patients having many embryo transfers because it is hard to tell which embryo was implanted.

Conclusions:-

The correlation between implantation potential and embryo morphologic grade is confirmed by this investigation. When choosing an embryo for transfer amongst numerous embryos, morphology should be the primary factor taken into consideration. Nevertheless, it seemed that the relationship between implantation potential and the morphologic grading of blastocyst quality held true only in younger women, not in older ones.

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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 request.

Ethics approval and consent of participation: The study was approved by the Review Board and Ethics Committee of the Banker IVF and Women's Hospital in consideration of the ASEBIR criteria.

Consent for publication: All authors approved final manuscript for publication. All participants involved in this study consented their data to be used.

Competing Interests: The authors declare no competing interests.

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