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Image Processing-Based Automation of Blastocoel Expansion Grading for Improved IVF Embryo Evaluation

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Peer Review Information	Abstract
<p><i>Submission: 08 Dec 2025</i></p>	<p>To increase implantation success rates, in vitro fertilization (IVF) depends on precise evaluation of blastocyst quality, with blastocoel growth, one of the blastocyst's characteristics. Blastocoel expansion being a critical evaluation parameter in IVF. In order to objectively measure blastocoel expansion from human blastocyst, this work presents an automated image analysis method. Preprocessing the image to improve quality, segmenting the blastocyst and blastocoel sections using image processing techniques, estimating the area of each region, and computing the ratio of blastocoel to blastocyst area are all phases in the proposed method. Expansion grades are assigned using these quantitative measurements, which aid in the selection of embryos during IVF operations. According to experimental data, the results offer objective, and consistent evaluation standards while lowering the subjective variability present in manual assessments. The automation of blastocoel assessment by the system provides reliable information to embryologists, enabling them to make more informed clinical decisions and perhaps increasing the success rate of embryo implantation during IVF treatments.</p>
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<p><i>IVF, ART, Blastocoel, Embryo Grading, Blastocyst, Embryo Selection</i></p>	

Introduction

The inability of a couple to conceive after a year of consistent, unprotected sexual activity is known as infertility, and it is a serious reproductive health issue in all over the world [12]. It can be categorized as primary (when a couple has never conceived) or secondary (when there has been at least one previous successful conception) and affects both men and women [12]. Hormonal imbalances, physical abnormalities, genetic factors, lifestyle choices, and environmental exposures are some of the many contributing reasons to infertility. Improving results in reproductive medicine requires an understanding of its underlying causes as well as the development of efficient diagnostic and treatment methods.

The term Assisted Reproductive Technology (ART) refers to medical treatments that handles sperm and eggs to promote fertilization and pregnancy in order to treat infertility. Infertility is a combination of various causes that prevent pregnancy. In-vitro fertilization (IVF), Intra Cytoplasmic Sperm Injection (ICSI), and Intrauterine Insemination (IUI) are common ART techniques to overcome infertility [9].

The embryo grading system is a technique for choosing embryos based on their quality. One of the measures of a healthy pregnancy result is embryo grading. This examination of the preimplantation embryo is very critical. The highest quality embryo must be chosen for ART in order to have a successful pregnancy. The frequency of transferred embryos and multiple pregnancies is decreased when the best embryo

is chosen [8]. In case of IVF, embryo selection plays an important role. Embryo selection of day-5 embryo i.e. blastocyst, is evaluated by embryologists manually, by observing blastocyst and grade them by using Gardner's grading system. But it is subjective and it depends on mainly three factors: Expansion state of Blastocoel, Inner Cell Mass (ICM) and Trophectoderm (TE) [10]. Conventionally, grading of embryo may perform manually or by using time lapse system. Following figure shows a microscopic image of blastocyst showing its components ZP, TE, BL and ICM.

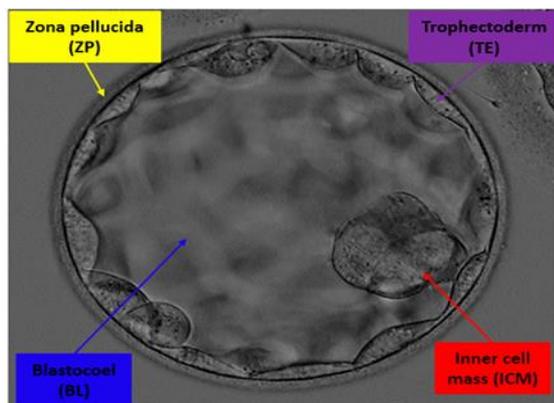


Figure 1. A Blastocyst Microscopic Image Showing The Components ZP, TE, BL And ICM [6]

In this study we emphasize on atomization of blastocyst grading using one factor that is blastocoel. Manual grading may sometimes subjective, leading to inconsistent outcomes, it may have some misleading output and IVF success rate becomes low and may lead to multiple pregnancy [5]. So, this study aims to automation of grading of blastocyst i.e. day-5 embryo on the basis of one factor i.e. blastocoels. Following figure shows a diagram of a blastocyst and microscopic image of blastocyst component.

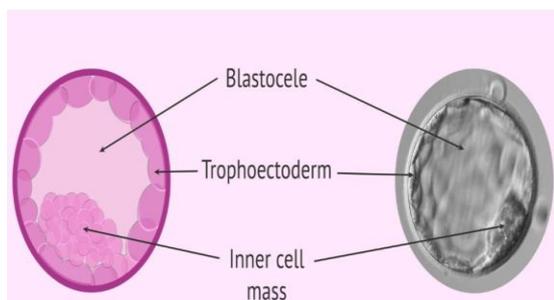


Figure 2. Structure of a Human Blastocyst

Literature Survey

In this study, they applied deep learning to 11,275 multifocal images from 1,025 normally fertilized blastocysts to automatically assess embryo quality. Images captured 116 hours

post-insemination were labeled as good or poor. In this study three CNN-based architectures were compared, with VGG-16 performing best (AUC 0.936). By stacking images from multiple focal depths as separate channels, the model utilized richer morphological information, improving classification accuracy. Grad-CAM visualization showed that the Trophectoderm (TE), Inner Cell Mass (ICM), and Zona Pellucida (ZP) were important factors affecting predictions. This method shows that blastocyst quality assessment may be done accurately and interpretably using multichannel, multifocal deep learning models [10].

A large retrospective study analyzed 10,482 frozen-thawed single blastocyst transfer cycles to evaluate the predictive value of morphological parameters on pregnancy outcomes. Blastocysts were scored by expansion stage, Inner Cell Mass (ICM), and Trophectoderm (TE) quality. Clinical pregnancy and live birth rates increased with higher ICM and TE grades, with ICM grade showing the strongest correlation to live birth. Stage 3 blastocysts had lower pregnancy and live birth rates compared to stages 4–5. Miscarriage rates were lowest in ICM grade A embryos. The study suggest that blastocysts with stage 4–5, ICM grade A, and TE grade A/B should be prioritized for single embryo transfer [4].

In this review paper they summarized that infertile couples can conceive with In Vitro Fertilization (IVF), however the success rate is still low i.e. approximately 38%. The quality of the embryo is crucial, and morphology is usually used to choose the most viable embryo. In traditional assessment, embryologists use pre-established grading systems to evaluate embryos, while time-lapse photography enables continuous monitoring without interfering with culture. Because both approaches are still subjective, selection or grading of embryo may vary. IVF is increasingly using machine learning to increase accuracy by examining morphological and developmental characteristics to produce more partial and predictive assessments. Traditional, time-lapse, and machine learning methods for morphology-based embryo selection are all summarized in this paper [14].

In this research they assess the predictive significance of morphological features on pregnancy outcomes. A retrospective study included 914 frozen euploid single blastocyst transfer cycles. Based on their Trophectoderm (TE), Inner Cell Mass (ICM), and growth, blastocysts were categorized as exceptional, good, medium, or poor. Overall grades were associated with greater clinical pregnancy and

live birth rates; the highest associations with successful outcomes were found with A- or B-grade TE and A-grade ICM. The expansion stage had no appreciable impact. According to the results, TE and ICM morphology are good indicators of the success of euploid blastocyst transfers, which supports their use as selection criteria for embryos in order to improve IVF results [13].

They study some recent applications of machine learning (ML) have improved clinical decision-making and predicted IVF success. One study used clinical and patient demographic data, including as age, BMI, semen characteristics and infertility causes, to create models. Out of the four algorithms that were assessed, gradient boosting, logistic regression, random forest, and support vector machine, achieved the maximum

accuracy of 87%. Embryo quality, age, and retrieved oocytes were important prognostic factors. The study shows that machine learning (ML) can improve IVF result predictions; nevertheless, additional validation with larger and more varied datasets is required to guarantee clinical relevance and generalizability [7].

Methodology

Proposed method

The goal of this research study is to develop a system that can automatically score blastocysts' blastocoel characteristic. In addition to making the grading process quicker and more reliable, the technology also reduces subjectivity and helps embryologists to observe by hand.

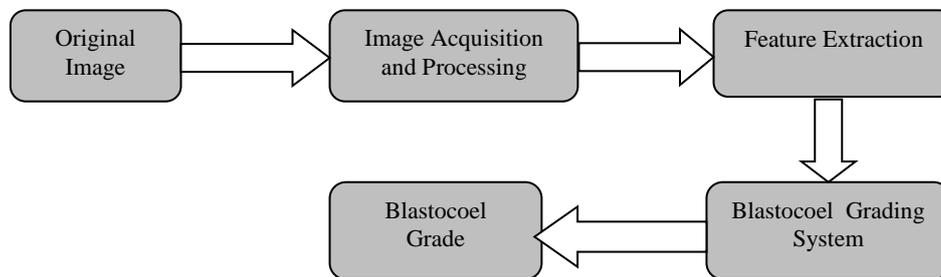


Figure 3 : Proposed Methodology Diagram

Data Collection

This research uses blastocyst images collected from standard dataset Kaggle. This blastocyst images were saved in Portable Network Graphics (PNG) format with a resolution of 384 x 512 pixels (height x width). The images are essentially grayscale even though the files are packed in RGB color format (8 bits per channel). Each image has a file size roughly 200–300 KB. The bright-field microscopy was used to take these pictures.

In this research, the focus is to develop a image based analysis technique to objectively evaluate the blastocoel expansion stage of blastocysts according to Gardner's grading system. The methodology involves several stages of image preprocessing techniques like segmentation, and quantitative analysis.

Image Acquisition and Preprocessing

Images of blastocysts are used in this research were taken from a dataset of day-5 IVF. The PNG-formatted high-resolution images in the dataset showed the morphological characteristics of growing blastocysts. To establish consistency and remove noise, preprocessing was used because samples differed in terms of image quality and backdrop conditions. Initially, the RGB raw images were

transformed to grayscale in order to make analysis easier and helps to find its structural boundaries. Then, to reduce background noise while keeping important embryonic details, a Gaussian filter was used. The blastocoel cavity and other interior characteristics were made more visible by using contrast enhancement techniques. The accuracy of automated feature extraction and grading was increased by these pretreatment procedures.

To save processing time and remove unnecessary color information while keeping crucial intensity data, the RGB blastocyst images were first converted to grayscale. Because grayscale conversion guarantees consistency across all images, subsequent processing becomes more reliable. To improve visual clarity and reduce noise, a Gaussian filter ($\text{fspecial('gaussian', [5 5], 2)}$) was used. The Inner Cell Mass (ICM) and blastocoel, two crucial structural boundaries, are not obscured while the filter smoothes high-frequency fluctuations. A clearer image is produced, acquisition artifacts are decreased, and biological components essential for precise segmentation, feature extraction, and blastocyst quality evaluation are preserved throughout this preprocessing stage.

Blastocoel Segmentation

Adaptive thresholding was used to segment the blastocoel cavity, which manifested as a darker area inside the blastocyst. To guarantee that the cavity was completely captured, the first binary mask was refined by morphological opening to eliminate minor noise and artifacts, and then hole filling. Concurrently, global thresholding was used to segment the blastocyst border, and the greatest linked area was taken out as the embryo mask. The improved, cropped image made it easier to measure the blastocyst and blastocoel areas precisely. This allowed the blastocoel-to-blastocyst ratio to be calculated for later grading using Gardner's expansion scale.

Feature Extraction

To improve contrast and highlight structural differences, the cropped blastocyst image was treated to intensity modification using MATLAB's (`\texttt{imadjust}`) function. Within the blastocyst, this procedure enhances the appearance of minute variations in intensity, especially between the Inner Cell Mass (ICM), the Trophectoderm, and the blastocoel cavity. By dispersing pixel intensities and extending the picture histogram, (`\texttt{imadjust}`) draws attention to internal boundaries that are frequently hidden in raw photos. More precise segmentation of physiologically significant features is made possible by the improved representation, which also eliminates uncertainty in feature extraction and guarantees correct identification of morphological traits necessary for objective blastocyst grading and quality evaluation.

Blastocoel Detection

To precisely separate the blastocoel from the surrounding structures, an adaptive thresholding technique was used, as this area usually shows as a darker cavity in blastocyst images. By adjusting the threshold locally, this method improves segmentation accuracy over global methods by taking into account fluctuations in intensity throughout the image. Morphological opening was used to further

refine the detected region by eliminating tiny, superfluous noise particles while maintaining the cavity's structure. The blastocoel was then continuously and completely represented by morphological hole filling, which produced a strong segmentation appropriate for feature extraction and blastocyst quality assessment.

Feature Extraction

The IVF dataset was used to obtain day-5 blastocyst images, which were then transformed to grayscale to make intensity-based processing easier. Gaussian filtering was used to smooth edges and minimize noise. The largest linked region was taken as the blastocyst mask after the blastocyst boundary was segmented using global thresholding (Otsu's method). Internal structures were highlighted by cropping the image around this area and using histogram adjustment to increase contrast.

Using adaptive thresholding, the blastocoel cavity—which appears as a darker region—was divided into segments. A morphological aperture was added to the mask to eliminate minor noise, and the hole was filled to guarantee full cavity segmentation. The blastocoel-to-blastocyst area ratio was computed by measuring the blastocyst and blastocoel areas from their corresponding masks. Following formula is used to calculate blastocoels-blastocyst ratio.

$$Ratio = \frac{\text{Blastocoel Area}}{\text{Blastocysts Area}} \quad [3]$$

Grading of Blastocoel Expansion

To identify the expansion stage, the blastocoel-to-blastocyst area ratio was computed for every embryo and compared to the Gardner and Schoolcraft (1999) grading scheme. Grade 1 denotes an early blastocyst with the cavity occupying less than half of the embryo, and Grade 6 denotes a completely hatched blastocyst. The grading requirements are summed up in Table 1. By using a quantitative and repeatable approach to evaluate blastocyst expansion, this ratio-based comparison enables consistent developmental stage classification.

Table -1 Experimental Results

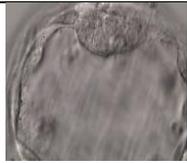
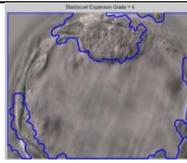
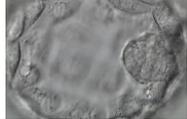
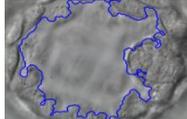
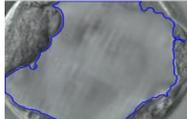
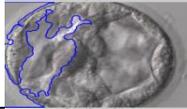
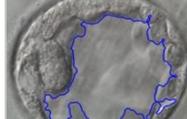
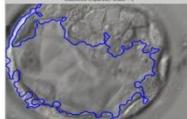
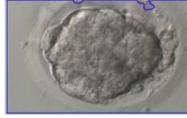
Grade	Description	Ratio Criterion
1	Early blastocyst – cavity < half embryo	Ratio < 0.5
2	Blastocyst – cavity > half embryo	$0.5 \leq \text{Ratio} < 0.9$
3	Full blastocyst – cavity fills embryo	$0.9 \leq \text{Ratio} \approx 1.0$
4	Expanded blastocyst – cavity > embryo, thinning zona	$1.0 \leq \text{Ratio} < 1.2$
5	Hatching blastocyst – trophectoderm herniation	$1.2 \leq \text{Ratio} < 1.5$
6	Hatched blastocyst – fully escaped from zona	Ratio ≥ 1.5

In order to assess the efficacy of the suggested automated blastocoel grading technique, a dataset including 50 Day-5 blastocyst images was used. Prior to segmentation to separate the blastocyst structure and the blastocoel cavity, each image undergoes a variety of preprocessing procedures, such as enhancement and noise reduction, to guarantee accuracy and consistency. Calculating the pixel-based areas of

the blastocyst and blastocoel allowed for the extraction of quantitative features from the segmented regions. A crucial measure of embryonic progress was then determined by calculating the ratio of blastocoel to blastocyst area.

Experimental results of 10 images are shown in following table:

Table-2

Sr. No.	Image No.	Output		Statistical Values
		Original Image	Cropped blastocoel	
1	0003_02.png			Blastocoel Area Ratio = 0.854 Final Gardner Blastocoel Grade = 4
2	0003_03.png			Blastocoel Area Ratio = 0.949 Final Gardner Blastocoel Grade = 4
3	0005_01.png			Blastocoel Area Ratio = 0.815 Final Gardner Blastocoel Grade = 4
4	0005_03.png			Blastocoel Area Ratio = 0.984 Final Gardner Blastocoel Grade = Early Blastocyst OR Compaction
5	0005_04.png			Blastocoel Area Ratio = 0.681 Final Gardner Blastocoel Grade = 4
6	0005_08.png			Blastocoel Area Ratio = 0.893 Final Gardner Blastocoel Grade = 4
7	0007_01.png			Blastocoel Area Ratio = 0.437 Final Gardner Blastocoel Grade = 3
8	0011_03.png			Blastocoel Area Ratio = 0.504 Final Gardner Blastocoel Grade = 4
9	0012_03.png			Blastocoel Area Ratio = 0.518 Final Gardner Blastocoel Grade = 4
10	0013_03.png			Blastocoel Area Ratio = 1.002 Final Gardner Blastocoel Grade = Early Blastocyst OR Compaction

The proposed automated embryo grading was validated by comparing its results to the manual grading performed by the expert embryologists. Basically embryologists independently assessed a blastocyst images using the Gardner's Grading System. The automated system used image processing techniques to access the same images and allocate grades based on its identified morphological features. Proposed system's performance was evaluated by comparing automated grades and the grades assigned by the embryologists.

Validation outcomes showed strong alignment between the two, comparing the proposed system's ability to reliably results. So the proposed system gives 80% accurate results as compared with manual results.

Total number of Images studied = 50
 Number of correctly graded images = 40
 Accuracy = $(40/50) \times 100$
 = 80%

Conclusion

This study presents A MATLAB-based automated image processing pipeline for precise blastocyst expansion, segmentation and grading. The system provides objective and repeatable expansion grades by using the Gardner and Schoolcraft (1999) criteria and calculating the blastocoel-to-blastocyst area ratio. The suggested method's dependability and consistency are demonstrated by the automated results, which closely matches with the manual assessments made by embryologists. This framework has the potential to improve embryo-selection techniques and facilitate better clinical decision-making in IVF procedures because it is a non-invasive, quantitative method. All things considered, the technique helps to raise the success rate of embryo implantation and improves standardization in blastocyst assessment.

Future Scope

Future research might concentrate on expanding the existing MATLAB process to incorporate automated grading of the Trophectoderm (TE) and Inner Cell Mass (ICM), resulting in a comprehensive Gardner-based blastocyst evaluation. By using 3D imaging, it would be possible to determine blastocoel volume precisely instead of 2D area, improving accuracy. Furthermore, machine learning methods have the potential to enhance segmentation precision and facilitate the prediction of embryo viability. Creating an intuitive software interface and testing the technique on bigger, multi-center datasets

would enhance IVF decision-making and encourage clinical use even more.

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