# AUTOENCODER-BASED ANOMALY DETECTION FOR ANALYZING FERTILIZATION ABNORMALITIES IN SERC OOCYTES

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**Abstract:**

**In infertility treatment, smooth endoplasmic reticulum clusters (sERCs) are occasionally observed in oocytes. The permissibility of transplanting sERC embryos is still under discussion, and its impact on pregnancy remains unclear. This study aims to clarify the relationship between sERC and abnormal fertilization. We investigate the differences between normally fertilized and abnormally fertilized oocytes containing sERC. If the differences are observed, it suggests that factors other than sERC may be related to abnormal fertilization. In this study, we conducted experiments using an AutoEncoder model. Images of oocytes taken during intracytoplasmic sperm injection (ICSI) were used and categorized into three stages: "not touch", "touch", and "puncture". The AutoEncoder model was trained on images of normally fertilized oocytes, while input images consisted of oocytes that resulted in abnormal fertilization. We calculated the brightness values of the difference images and performed a Wilcoxon rank-sum test. In the "not touch" stages, significant differences were confirmed. These findings indicate that morphological differences may exist prior to or during injection, suggesting the possibility of identifying oocytes with abnormal fertilization potential even before ICSI is performed.**

**Keywords:**

**AutoEncoder; Image analysis; Infertility treatment; Intracytoplasmic sperm injection; Statistical analysis; Smooth endoplasmic reticulum clusters; Wilcoxon rank-sum test**

## 1. Introduction

In recent years, the number of people concerned about infertility and those who have undergone infertility testing or treatment has been increasing. According to National Fertility Survey conducted by the National Institute of Population and Social Security Research, 22.7% of couples reported having undergone infertility testing or treatment in 2021 [1]. Approximately one in 4.4 couples experienced infertility testing or treatment. Since the success rate of infertility treatment directly affects birth rates, its significance has been increasing.

One method of infertility treatment is ICSI, in which a single sperm is directly injected into an oocyte. During this procedure, a vacuole-like structure known as a sERC is sometimes observed in the oocyte. Figure 1 presents an example of an sERC in an oocyte, where the sERC region is enclosed by a red line. The sERC is considered one of the major morphological abnormalities in human oocytes. It has attracted attention due to its potential impact on fertilization and embryonic development. The smooth endoplasmic reticulum is a cellular organelle consisting of membranous tubules that lack ribosomes on their surface. It normally forms a continuous network with the rough endoplasmic reticulum. Transmission electron microscopy has revealed that sERCs are aggregates of the smooth endoplasmic reticulum, appearing as translucent clusters primarily in metaphase II (MII) oocytes. Unlike general vacuoles that may appear at various developmental stages, sERCs are predominantly observed in MII-stage oocytes and are structurally distinct.

In 2004, a study reported that pregnancy rates decreased in cycles where sERC occurred [2]. Some studies reported that oocytes with sERC had lower fertilization and pregnancy rates and delayed embryo development [3]. However, a recent study reported that healthy children have been born following the transplantation of embryos derived from oocytes in which sERC was observed [4]. Some studies even suggest that the presence of sERC does not affect childbirth at the very least [5]. Currently, the permissibility of sERC embryo transplantation remains under debate. The impact of sERC on pregnancy is not yet fully understood. Due to these uncertainties and potential clinical implications, sERC is regarded as an important factor in reproductive medicine.

AI-based analysis allows for the extraction of features

that are imperceptible to the human eye. In our previous work, we analyzed uterine and oocyte characteristics in patients with infertility [6, 7]. In the present study, we employ a deep learning model called an AutoEncoder to analyze oocytes in which sERC. The application of AutoEncoder has been reported in reproductive medicine [8]. Our objective is to investigate the differences between sERC oocytes that result in normal fertilization and those leading to abnormal fertilization. By applying anomaly detection with an AutoEncoder, we aim to clarify the relationship between sERC and abnormal fertilization. Identifying morphological features associated with abnormal outcomes may help in selecting suitable oocytes for ICSI. This could lead to higher success rates and more efficient infertility treatment.

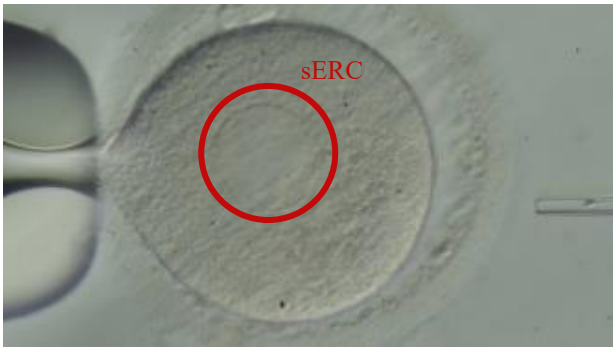



**FIGURE 1.** Example of sERC in oocyte

## 2. Materials and Methods

In normal fertilization, two pronuclei appear, fuse into a single nucleus, and then disappear. Fertilized eggs with two pronuclei are evaluated as 2PN. Fertilized eggs classified as 2PN are considered normally fertilized. In contrast, fertilized eggs showing no pronuclei (0PN), one pronuclei (1PN), or three pronuclei (3PN) are considered abnormally fertilized. Additionally, during the denudation process of cumulus-oocyte complexes after egg collection, oocytes exhibiting cytoplasmic shrinkage or a broken zona pellucida are classified as degenerated (deg). The oocytes are also considered abnormally fertilized, as they cannot be used for fertilization procedures. For this study, we use videos recorded during ICSI procedures as a dataset. The videos were recorded at Reproduction Clinic Tokyo and consist only of sERC oocytes. In this study, only videos in which the entire oocyte is captured are included in the analysis. The dataset consists of 56 cases of normal fertilization and 21 cases of abnormal fertilization. For each case, one image is extracted for each stage: “not touch”, “touch”, and “puncture”. The “not touch” stage indicates that the oocyte remains untouched by the needle. The “touch” stage represents the moment the needle comes into contact with the oocyte. The “puncture” stage indicates the point immediately before the needle punctures the oocyte. An oocyte region is extracted by applying a circular mask to the image. The images are resized to 128×128 pixels and converted to a grayscale to create the dataset. We investigate morphological differences associated with abnormal fertilization by categorizing the dataset in this way, as oocyte morphology can change significantly depending on the timing of ICSI manipulation. Table 1 presents the classification and the number of cases in the dataset.

We employ an AutoEncoder model with the structure shown in Figure 2. Separate models are constructed for the “not touch”, “touch”, and “puncture” image categories. Images classified as 2PN are divided by case into training (70%) and testing (30%) groups, and labeled as 2PN_A and 2PN_B. Images classified as 2PN_A are used as the training dataset for the models. The model train with a learning rate of 0.001, a batch size of 64, a latent dimension (Z_DIM) of 32, 500 training epochs, and a validation split of 0.2. Data augmentation is performed by randomly applying contrast conversion, rotation, and scaling to each batch. The model employs a loss function that combines mean squared error and structural similarity index measure. After training the model, images of abnormally fertilized oocytes are input into the model, and the differences between the input and output images are calculated to investigate characteristics unique to abnormal fertilization. Using the difference images obtained by the model, we calculate the average brightness value as an indicator of reconstruction rate. A Wilcoxon rank-sum test is conducted on the average brightness values between the normal (2PN_B) class and abnormal (0PN, 1PN, 3PN, and deg) class. The significance level ($\alpha$) is set to 0.05.

**Table 1.** Classification of dataset

| Classification | Condition | Number of cases | Remarks |
|---|---|---|---|
| 2PN_A | Normal | 39 | Training |
| 2PN_B | Normal | 17 | Test |
| 0PN | Abnormal | 9 | Test |
| 1PN | Abnormal | 4 | Test |
| 3PN | Abnormal | 6 | Test |
| deg | Abnormal | 2 | Test |
| Total | | 77 | |

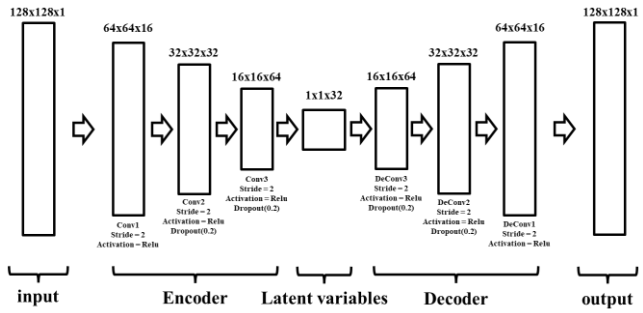



**FIGURE 2.** Architecture of AutoEncoder

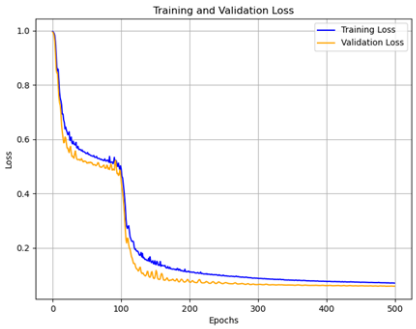
## 3. Results

### 3.1 Abnormality detection by AutoEncoder

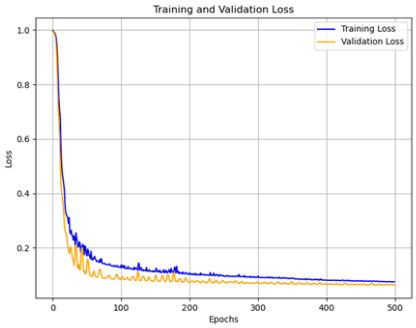
Figure 3 shows the learning curves of the AutoEncoder models for each of the three stages. Figures 3(a), 3(b), and 3(c) correspond to the "not touch", "touch", and "puncture" stages, respectively. The horizontal axis represents the number of epochs, and the vertical axis represents the loss value. The blue and yellow lines indicate the losses for the training and validation data, respectively. No over-learning was observed in any of the models and they were successfully trained. Figure 4 shows sample images generated by the AutoEncoder model trained on the "not touch" stage. Figures 4(a), 4(b), and 4(c) present the input image, output image, and difference images, respectively. In Figure 4(c), the white regions indicate abnormalities that the model failed to reconstruct, highlighting the detected anomalies.

### 3.2 Results of Wilcoxon rank-sum test

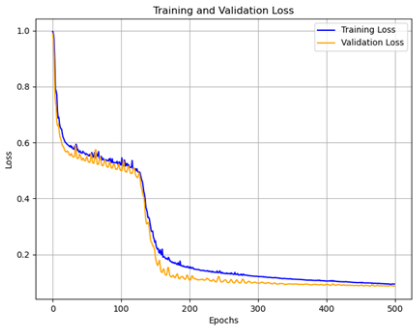
The average brightness value was calculated from the difference images to use it as an indicator. Lower reconstruction rates result in higher average brightness values, which is characteristic when compared to normally fertilized eggs. The Wilcoxon rank-sum test was conducted on the average brightness values between normal and abnormal fertilization cases. The null hypothesis was set as: "There is no significant difference in the average brightness values of difference images between normally fertilized and abnormally fertilized oocytes". The alternative hypothesis was: "There is a significant difference in the average brightness values of difference images between normally fertilized and abnormally fertilized oocytes". The significance level (α) was set to 0.05. Tables 2(a), 2(b), and 2(c) present the mean and standard deviation of the average brightness values, as well as the p-values obtained from the Wilcoxon rank-sum test for the "not touch", "touch", and "puncture" stages, respectively. An asterisk (*) indicates groups for which a statistically significant difference was observed ($p < 0.05$).




(a) Not touch




(b) Touch




(c) Puncture

**FIGURE 3.** Learning curve of AutoEncoder model

(a) input image (b) output image (c) difference image

**FIGURE 4.** Example of images (2PN)

**TABLE 2.** Summary of calculation results for average brightness value

(a) not touch

| Classification | AVG | STD | P-value |
|---|---|---|---|
| Normal (2PN_B) | 4.10 | 0.43 | < 0.05* |
| Abnormal (0PN, 1PN, 3PN, deg) | 4.63 | 0.84 | |

(b) touch

| Classification | AVG | STD | P-value |
|---|---|---|---|
| Normal (2PN_B) | 4.30 | 0.51 | 0.25 |
| Abnormal (0PN, 1PN, 3PN, deg) | 4.60 | 0.75 | |

(c) puncture

| Classification | AVG | STD | P-value |
|---|---|---|---|
| Normal (2PN_B) | 5.61 | 0.36 | 0.86 |
| Abnormal (0PN, 1PN, 3PN, deg) | 5.70 | 0.78 | |

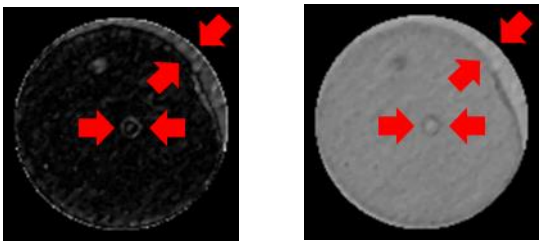
AVG indicates average of brightness value, STD indicates standard deviation of brightness value, and asterisk (*) indicates $p < 0.05$.

## 4. Discussion

A significant difference between normal and abnormal was observed in the stages of “not touch”. These findings suggest that certain morphological differences are present. Figure 5 shows examples of differences and original images. The sample is the 1PN class images selected from the “not touch” stage. The red arrows in Figure 5(a) indicate regions with high brightness, and Figure 5(b) shows the corresponding original image with these arrows superimposed. The region with high brightness values appears as an area showing a significant difference between normal and abnormal fertilization, and corresponds to the cell membrane of the oocyte and the sERC region in the figure. These results show that the shape of the oocyte and the presence of sERC may influence fertilization.

In this experiment, no significant difference was observed in the stages of “touch” and “puncture”. In the stages, the position of the needle and the extension of the oocyte differs from case to case. Therefore, the images from these stages exhibit a high degree of variation in features. In this study, only one image per case was used, which may have limited the model’s ability to fully learn the characteristics. To address this issue, the number of data samples can be increased by incorporating neighboring frames of the currently acquired images. Since the “puncture” process is continuous and the characteristics of the oocyte change gradually over time, applying convolution operations along the temporal axis to a sequence of puncture images may enable the model to capture these dynamic features more effectively.

The significant differences detected at the “not touch” stage suggest that features associated with abnormal fertilization can be identified prior to the initiation of ICSI. This finding suggests the potential to identify unsuitable oocytes prior to injection, which could contribute to improving fertilization success rates. However, the results may be influenced by variations in the number of images, which stem from the small number of cases in certain classes. Therefore, the findings should be interpreted with caution.



(a) Difference image with red arrows (b) Original image with red arrows

**FIGURE 5.** Examples of difference and original images

## 5. Conclusion

This study investigated the relationship between sERC and abnormal fertilization by comparing sERC oocytes that resulted in normal and abnormal fertilization. The results demonstrated that a significant difference between normal and abnormal was observed in the stages of “not touch”. Visual analysis of the difference images revealed the occurrence of high-brightness areas in the oocyte’s cell membrane and the sERC region. These results show that the shape of the oocyte and the presence of sERC may influence fertilization. Notably, differences observed before injection suggest the possibility of identifying oocytes at risk of abnormal fertilization prior to ICSI procedures.

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