



# Classification of Bovine Cumulus-Oocyte Complexes with Convolutional Neural Networks

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## Abstract

**Aim:** Determining oocyte quality is crucial for successful fertilization and embryonic development, and there is a serious correlation between live birth rates and oocyte quality. Parameters such as the regular/irregular formation of the cumulus cell layer around the oocyte, the number of cumulus cell layers and the homogeneity of the appearance of the ooplasm are used to determine the quality of the oocytes to be used in in vitro fertilization (IVF) and intracytoplasmic sperm injection (ICSI) methods.

**Material and Methods:** In this study, classification processes have been carried out using convolutional neural networks (CNN), a deep learning method, on the images of the cumulus-oocyte complex selected based on the theoretical knowledge and professional experience of embryologists. A convolutional neural network with a depth of 4 is used. In each depth level, one convolution, one ReLU and one max-pooling layer are included. The designed network architecture is trained using the Adam optimization algorithm. The cumulus-oocyte complexes (n=400) used in the study were obtained by using the oocyte aspiration method from the ovaries of the bovine slaughtered at the slaughterhouse.

**Results:** The CNN-based classification model developed in this study showed promising results in classifying three-class image data in terms of cumulus-oocyte complex classification. The classification model achieved high accuracy, precision, and sensitivity values on the test dataset.

**Conclusion:** Continuous research and optimization of the model can further improve its performance and benefit the field of cumulus-oocyte complexes classification and oocyte quality assessment.

**Keywords:** Cumulus-oocyte complexes, convolutional neural networks, classification, oocyte quality

## INTRODUCTION

Infertility is a reproductive issue affecting millions of people worldwide (1). IVF and ICSI methods, which are applied in the centers of Assisted Reproductive Technologies (ART) are among the most common treatment methods for infertility today. These methods also used in animal breeding and biotechnology centers. After ovulation induction under clinician supervision, oocyte aspiration is performed on the day determined by the clinician for obtaining oocytes to be used in IVF and ICSI methods. The collected oocyte(s) are examined microscopically by the embryologist. High-quality oocytes are selected for

fertilization based on various criteria and morphological classifications. Oocyte quality is crucial for successful fertilization and embryonic development (2). There is a serious correlation between oocyte quality and live birth rates. In order to select high-quality oocytes to improve the ability to select the best single embryo with the highest implantation potential, minimize the likelihood of multiple pregnancies due to multiple embryo transfers, increase pregnancy rates, and achieve an increase in live birth rates, oocyte classification must be done without causing doubt. In determining the quality of oocytes to be used in IVF and ICSI methods, morphological parameters related to

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the cumulus-oocyte complex structure, oocyte cytoplasm, polar body, meiotic spindle properties, perivitelline space, and zona pellucida can be used (3).

Cumulus cells are critical for oocyte maturation, ovulation, and fertilization (4). Many studies have shown that the presence of cumulus cells is a requirement for oocytes to gain developmental abilities in vitro. Cumulus cells also support energy production in the cumulus-oocyte complex (5) and play a role in protecting oocytes from damage that reactive oxygen species (ROS) can cause (6). Recent studies suggest that the mitochondrial function of cumulus cells can directly affect the reproductive capacity (7,8). The number of cumulus cell layers surrounding the oocyte is an essential factor in determining oocyte quality (4). Oocyte quality is generally evaluated based on the structure of cumulus-oocyte complexes. This method is simple and provides information about oocyte quality. Embryologists and researchers evaluate the cumulus-oocyte complex by looking at parameters such as the regular/irregular formation of the cumulus cell layer around the oocyte, the number of cumulus cell layers, and the homogeneity of the ooplasm's appearance (9,10). They use their theoretical knowledge and professional experience to select the most ideal oocytes.

However, the professional experience required for oocyte selection, which is heavily based on subjective opinions, is a challenging and time-consuming process. Although the observational experiences accumulated cumulatively over the years are precious, human error margins, time-consuming protocols, and high-cost equipment encountered in every traditional method cannot be ignored. ART methods have significantly improved over the past 30 years but success rates have not reached desired levels and remain relatively low. Minimizing human intervention in oocytes and embryos, increases the viability of these highly sensitive cells and reduces the rate of human errors.

In recent years, machine learning (ML) methods have been widely used in biomedical imaging. ML is the field of study that gives computers the ability to learn from data without explicitly writing code (11). ML gives computers the ability to "learn from experience," a trait naturally found in humans. Machine learning algorithms use computational methods to "learn" information directly from data without relying on a predetermined equation model. As the number of samples available for learning increases, the algorithms adaptively improve their performance. ML is fundamentally about predicting the future based on past experience (12,13).

Convolutional Neural Network (CNN) is a specialized form of ML methods, is a very important tool for medical image classification. This method is widely used for classifying images obtained from various medical imaging techniques, such as computed tomography (CT), magnetic resonance imaging (MRI), ultrasonography (USG), and microscopic imaging. By its nature, CNN has image-shaped inputs and automatically performs feature extraction from images (11,14). Upon reviewing these studies, almost all of them focus on segmenting oocytes, and the studies addressing

the classification problem using CNN are quite insufficient.

The classification of medical images with highly detailed structures is often a time-consuming and high-attention task, making it prone to human errors. The classification success may vary depending on the workload and experience of the staff performing the classification. However, artificial intelligence methods like CNN, which can learn from data, can perform classification tasks faster and with higher accuracy rates. We believe that an artificial intelligence approach trained on hundreds of oocytes can reliably predict classification of cumulus-oocyte complex and oocyte quality without human intervention. In our literature review, we have not come across a study classifying the cumulus-oocyte complex, an important criterion in determining high-quality oocytes to be applied in ART procedures, using artificial intelligence methods.

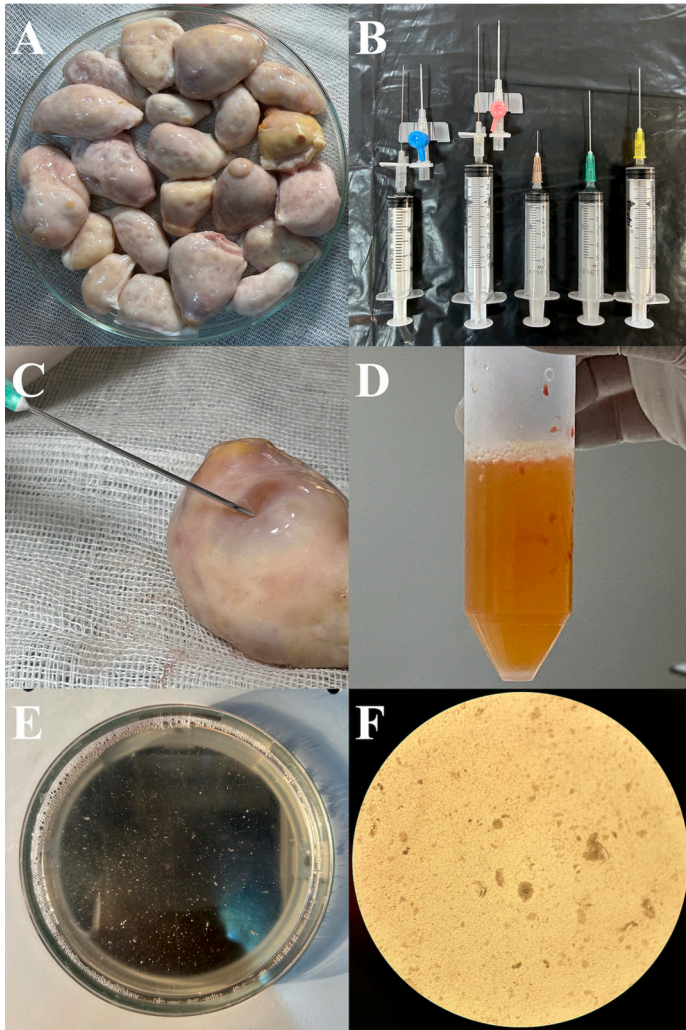
This study is expected to be a pioneering study in the literature and lay the groundwork for the integration of artificial intelligence into ART. The image data obtained of this study will be useful in increasing the originality and sensitivity of future studies on this subject and will shed light on scientific research.

## MATERIAL AND METHOD

### Oocyte Aspiration and Collection of Cumulus-Oocyte Complexes

This study was conducted with the approval of the Ege University Animal Experiments Local Ethics Committee, numbered 2021-045, stating that ethical committee approval is not required according to the Ministry regulations. Ovaries were obtained as waste material from animals slaughtered for human consumption at various commercial slaughterhouses in accordance with international meat production guidelines.

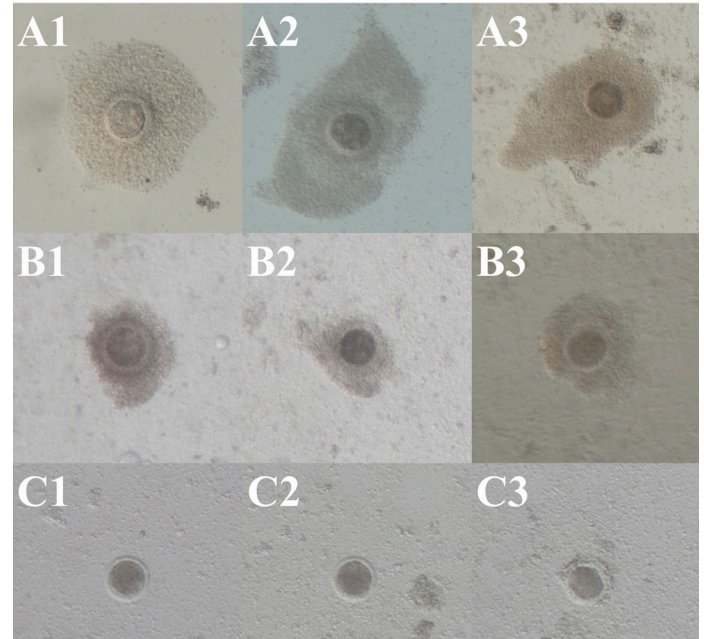
Ovaries collected at different times were placed in thermos flasks containing a transport medium immediately after slaughter and brought to the laboratory within a maximum of 3 hours. Sterile PBS, adjusted to a temperature of 20-25°C and supplemented with Penicillin (50-100 IU/ml) and Amphotericin-B (50 ng/ml), was used as the transport medium. The transport medium was freshly prepared for each study. The ovaries brought to the laboratory were washed twice with 0.9% NaCl to remove blood and transport medium and then dried with a drying paper before proceeding to the aspiration process. Follicles with a diameter of 2-10 mm in the ovaries were aspirated with 5-10 ml sterile needle-tipped syringes varying in thickness from 26-20-Gauge (Ayset, Adana, Türkiye; Berika, Konya, Türkiye; Beybi, İstanbul, Türkiye). The follicular fluid obtained by aspiration was collected in 50 ml Falcon tubes. The tubes containing the collected follicular fluid were left at room temperature for 20 minutes to allow the cumulus-oocyte complexes to settle at the bottom. At the end of this period, the cumulus-oocyte complexes that settled at the bottom of the tube in the follicular fluid were taken with the help of a Pasteur pipette and transferred to 90x15 mm Petri dishes (Figure 1).



**Figure 1.** Obtaining cumulus-oocyte complexes from bovine ovaries with photographs from current study **A.** Specimens from bovine ovaries **B.** Injectors with various needle sizes **C.** Aspiration process **D.** Aspirated material in Falcon tube **E.** Examination under a stereo microscope **F.** Imaging and analysis

### Cumulus-Oocyte Complex Classification

A total of 400 cumulus-oocyte complexes were classified under the Olympus SC50 digital camera attached to the Olympus SZ61 Stereo Microscope with WHSZ10X-H/22 eyepieces. The classification was performed by embryologists on the research team, considering the number and appearance of cumulus cell layers surrounding the oocyte and the characteristics of the oocyte and oocyte cytoplasm, based on the features described in the literature (15-17). Accordingly: Cumulus-oocyte complexes surrounded by at least five layers of compact cumulus cells, with transparent, homogeneous, and bright cytoplasm were classified as Good Quality (Category A) (n=100). Cumulus-oocyte complexes surrounded by 3-5 layers of compact cumulus cells, with slightly granular, dark-colored cytoplasm were classified as Medium Quality (Category B) (n=100). Cumulus-oocyte complexes surrounded by 1-2 layers of sparse irregular cumulus cells, with granular, dark-colored cytoplasm were classified as Poor Quality (Category C) (n=100) (Figure 2).



**Figure 2.** The classification of cumulus-oocyte complexes with representative images from the training set **A1-3.** Good quality (category A) **B1-3.** Medium quality (category B) **C1-3.** Poor quality (category C)

### Data Set

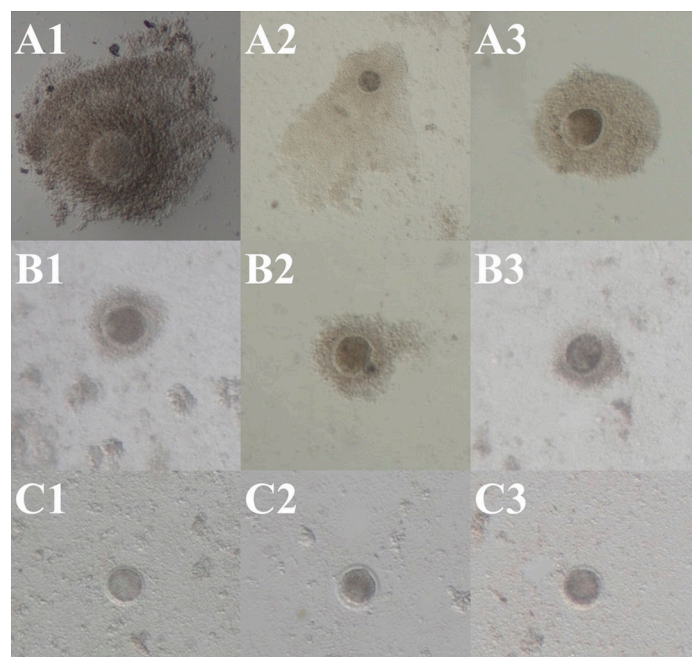
For the preparation of data sets to be used in artificial intelligence training, digital images of each cumulus-oocyte complex in categories A, B, and C were obtained with one hundred images per category. To test and evaluate the performance of the deep learning method, 100 cumulus-oocyte complexes, which were never used in artificial intelligence training, were classified by embryologists on the research team, considering the features described in the literature (15-17), and their digital images were obtained from the images taken with the the stereo microscope.

### Cumulus-Oocyte Complex Classification with Convolutional Neural Networks

In this study, the classification process was performed using convolutional neural networks on images divided into 3 different classes. 75% of the 400 images from different classes were used for the training of the designed CNN model, while 25% were used for testing. In the training set, there were 100 images for each of the classes A, B, and C, while in the test set, there were 34 images for class A and 33 images for classes B and C. Sample images from different classes are given in Figure 3 below.

The CNN architecture used in the study for classifying images is given in Figure 4 below. The designed architecture has an input size of 128x128x3 and an output consisting of 3 neurons. The Rectified Linear Unit (ReLU) activation function was used as the activation function for each convolutional layer in the architecture, which consists of 4 convolutional layers. A max-pooling layer was used after each convolutional layer in the architecture, and a fully connected layer consisting of 128 neurons was placed between the last convolutional layer and the output layer. The CNN model training was carried out with the ADAM

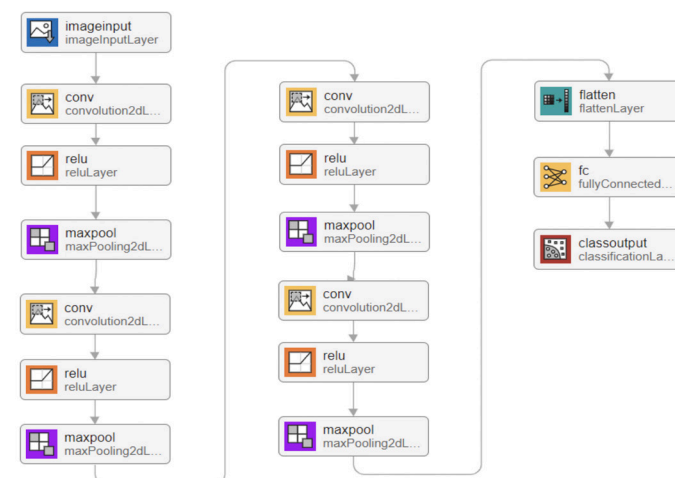
optimization algorithm in 200 epochs, and the batch size was set to 64.



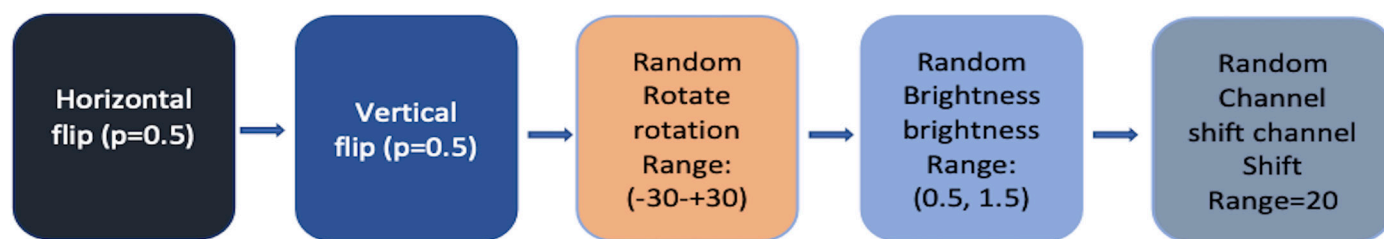
**Figure 3.** Sample images from the test set representing the classification of cumulus-oocyte complexes as good quality (A1-3), medium quality (B1-3), and low quality (C1-3)

In this study, data augmentation was performed on the training set to prevent overfitting and achieve better performance by increasing the relatively small number of data for deep CNN model training. For the test set,

only original images were used. The data augmentation process was carried out within the flow shown in Figure 5 below. Each image follows the process shown in Figure 5 before being applied to the network for training. During the augmentation process, horizontal and vertical flip operations are applied to the images with a probability of  $p=0.5$ . Then, after performing a rotation operation with a random angle between  $-30$  and  $+30$  degrees, the brightness of the image is changed with a random coefficient between  $0.5$  and  $1.5$ . Finally, each color channel of the images is shifted randomly between  $-20$  and  $20$ . This process can help the model generalize more broadly against color changes by altering the color distributions of the images.



**Figure 4.** Proposed CNN model



**Figure 5.** Data augmentation process

## RESULTS

The confusion matrices obtained for the test set are given in Table 1. According to the confusion matrix, the model made 32 correct predictions for Class A, with only 2 incorrect predictions. For Class B, 24 correct predictions were made, while 7 and 2 incorrect predictions were made for Class A and C, respectively. Finally, 32 correct predictions were obtained for Class C, and only 1 incorrect prediction was made for Class B.

Table 1. Confusion matrix for the test set				
		Confusion matrix		
		Predicted		
Class		A	B	C
Actual	A	32	0	2
	B	7	24	2
	C	0	1	32

It can be seen that the CNN model is successful in classifying 3-class image data. High accuracy rates were obtained especially for Class A and C, while an acceptable accuracy rate was achieved for Class B. These results indicate that CNN-based classification methods are reliable and effective tools for oocyte quality and classification.

Table 2 presents the precision, recall (sensitivity), and accuracy metric measurement results calculated using the confusion matrices. In this study, the performance of the classification model on data belonging to three different classes was evaluated. The model's success was measured using accuracy, precision, and sensitivity metrics. The accuracy value obtained on the test set was calculated to be 0.8800.

The precision values calculated for Class A are 0.8205; for Class B, 0.9600; and for Class C, 0.8888. These results show that the model makes predictions with the highest

precision value for Class B and the lowest precision value for Class A. The macro average of precision values was found to be 0.8898.

**Table 2. Metric measurement results**

Metrics	A	B	C	Macro average
Precision	0.8205	0.9600	0.8889	0.8898
Recall	0.9412	0.7273	0.9697	0.8794
Overall accuracy	0.8800			

Sensitivity values were calculated as 0.9412 for Class A, 0.7273 for Class B, and 0.9697 for Class C. The model detects true positives with the highest sensitivity value for Class C and the lowest sensitivity value for Class B. The macro average of sensitivity values is 0.8794.

## DISCUSSION

Human ART and animal reproductive technologies have been developing intensively, especially in recent years. For both species, gamete cells are of particular importance as the focus of reproductive biotechnologies. All processes, from in vivo derivation of cumulus-oocyte complexes to in vitro maturation of oocytes, and from fertilization to live birth were human-dependent (15). The integration of artificial intelligence into ART, where success is highly proportional to the knowledge, experience, and manipulation abilities of human beings, has been developed with innovative results. However, the human dependence persists, and there remains a need to develop artificial intelligence applications in reproductive biotechnologies for both humans and animals. In addition, considering the acceleration of the livestock industry of economically valuable animals or the improvement of their products, and the protection of rare species and animal welfare, the magnitude of the need for artificial intelligence applications to be integrated into animal reproductive technologies can be understood in order to make rapid and effective results commercially viable (18).

The process of distinguishing those with the highest viability among oocytes and embryos is based on the analysis of their morphological criteria. Attempts to characterize morphological features associated with oocyte/embryo quality to produce a full-term pregnancy have long been significant, but limited success due to several reasons remains a major barrier. While cellular and molecular analyzes can provide new clues for defining more objective criteria of quality, many of these approaches are incompatible with cell viability (19).

Artificial intelligence applications that minimize human intervention and have the potential to self-develop the established standards and can be applied directly in vivo or in vitro have become essential for acquiring competent oocytes/embryos. Due to the recent acceleration in the capacity to extract tissue descriptors from a given image, there has been increasing interest in the use of artificial intelligence-based methods that select oocyte/embryo by scoring over digital images. Firuzinia et al. and Targosz et

al. have performed oocyte segmentation using pretrained networks, Resnet and MobileNet (20-21). Similarly, Athanasiou et al. have carried out oocyte segmentation using U-net (22). Raudonis et al. designed a 5-class model using AlexNet and Vgg16 to classify embryos based on the number of cells within the embryo in their study (23). Kragh and colleagues predicted the inner cell mass (ICM) and trophectoderm (TE) grades from a single frame in time-lapse imaging using CNN (24). Monge and Beltran classified seven distinct species of avian Eimeria oocytes using their designed CNN (25).

To the best of our knowledge for the first time in the literature, the images of cumulus-oocyte complexes selected based on the theoretical knowledge and professional experience of embryologists were classified using CNN, a deep learning method, which has 4 depths, has convolution, ReLU and maximum pooling layer at each depth level, and has a designed network architecture trained with Adam optimization algorithm. The results obtained from the classification model developed in this study were aimed to demonstrate the effectiveness of the CNN-based approach in categorizing image data divided into three classes, especially related to oocyte quality and classification.

As shown in Table 1, our results demonstrate the effectiveness of the CNN-based approach for this application, as the model achieved high accuracy rates for Class A and C, and an acceptable accuracy rate for Class B. This indicates that CNN-based classification methods are reliable and effective tools for such applications. Analyzing the performance metrics presented in Table 2, the model's precision and sensitivity values were evaluated for each class. The highest precision value was achieved for Class B, whereas the lowest precision value was observed for Class A. This suggests that the model is more likely to make accurate predictions for Class B, while improvements can be made for Class A. The sensitivity values revealed that the model detects true positives with the highest sensitivity for Class C and the lowest sensitivity for Class B. This indicates that the model can effectively identify true positives for Class C, but has room for improvement in detecting true positives for Class B. Despite the high accuracy, precision, and sensitivity values obtained on the test dataset, the lower sensitivity for Class B and lower precision for Class A indicate that further refinements are required for these classes. Nevertheless, the overall performance of the current model indicates the potential of CNN-based methods as reliable and effective tools for tasks involving oocyte quality assessment and classification.

Due to the limited number of studies, this study is expected to make a significant contribution to the literature. The dataset obtained from this study will fulfill the needs required in this field. However, reviewing the sample size and class balance of the dataset should be considered for future studies to enhance the model's performance. This may involve increasing the number of

samples for underrepresented classes, ensuring a more balanced dataset, and potentially improving classification performance. Additionally, exploring feature engineering methods could lead to more accurate and reliable classification results. Investigating various preprocessing techniques, such as image augmentation, denoising, and normalization, may improve the model's ability to extract relevant features from the image data. Furthermore, the implementation of different CNN architectures or the use of ensemble methods might also enhance the model's performance (26).

In summary, our CNN-based classification model demonstrated promising results in classifying three-class image data related to oocyte quality and classification. While improvements can be made for specific classes, the overall performance of the model indicates that CNN-based approaches are reliable and effective tools for such tasks. Continued research and optimization of the model can further enhance its performance, ultimately benefiting the field of oocyte quality assessment and classification.

## CONCLUSION

The classification model developed in this study achieved high accuracy, precision, and sensitivity values on the test dataset. However, it is important to note that the model shows lower sensitivity for Class B and lower precision for Class A. This indicates that further improvements are needed for these classes. In future studies, reviewing the sample size and class balance of the dataset and considering feature engineering methods might be beneficial to further increase the model's performance.

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**Conflict of Interest:** The authors declare that they have no conflict of interests.

**Ethical approval:** This study was conducted with the approval of the Ege University Animal Experiments Local Ethics Committee, numbered 2021-045, stating that ethical committee approval is not required according to the Ministry regulations.

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**Author's Contribution:** Conception of the study: C.Ş., K.D.K., C.T., A.G., T.Ç. Acquisition of study materials: C.Ş., K.D.K., C.T., A.G. Acquisition of microscopic images: C.Ş., K.D.K., C.T., A.G. Analysis and interpretation of microscopic data: C.Ş., K.D.K., C.T., A.G., T.Ç. Acquisition, analysis and interpretation of computer-based data: E.Ö., O.E., M.K.G. Drafting of the manuscript: A.G., T.Ç., E.Ö., O.E., M.K.G. Revising the manuscript critically for important intellectual content: A.G., T.Ç., O.E., M.K.G. All authors agreed to be

accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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