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THE USE OF NEURAL IMAGE ANALYSIS IN THE IDENTIFICATION OF INFORMATION ENCODED IN A GRAPHICAL FORM

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ABSTRACT

Numerous scientific and research centres are searching for solutions concerning the problem of quality classification of animal oocytes. Conducting such studies is purposeful, particularly in the context of constant attempts to improve the quality of food products, which depends on the breeding value of livestock. Therefore, searching for methods of stimulation of proper development of a larger number of animal oocytes, particularly in extracorporeal conditions, gains special importance. An increasing interest in assisted reproduction techniques resulted in searching for new, increasingly effective methods of quality assessment of mammalian gametes and embryos. The expected progress in the production of animal embryos *in vitro* is largely dependent on proper classification of obtained oocytes. The aim of this work was to develop a non-invasive method for the quality assessment of oocytes, performed on the basis of graphic information encoded in the form of monochromatic digital images obtained via microscopy techniques. The classification process was conducted based on the information presented in the form of microphotography pictures of domestic pig oocytes, using advanced methods of neural image analysis.

Introduction

The issues related to the quality classification of animal oocytes are studied in numerous scientific and research centres in Poland and abroad (Boniecki et al., 2014; Piekarska-Boniecka et al., 2008). The studies are of current and particular importance, especially in view of constant attempts to improve the breeding value of livestock. Methods resulting in the proper development of an increased number of fertilised animal oocytes in extracorporeal conditions attain priority. An increasing interest in assisted reproduction techniques has resulted in a considerable development of new methods for quality assessment of mammalian gametes and embryos (Kempisty et al., 2011). The observed progress in the *in vitro* production of animal embryos is partially due to efficient and objective classification of obtained oocytes. Mammalian reproductive cells (oocytes) are the fundamental stage of the oogenesis process, in which female gametes form, mature and develop. The importance of these cells in procreation is closely correlated with the dynamics of genome transmission to

subsequent generations. Extracorporeal fertilisation of domestic animals is used, for instance, to accelerate the breeding progress, the purpose of which is to obtain in a short period of time a large number of offspring from genetically valuable parents. Increased effectiveness, and precision in particular, of the quality assessment of oocytes significantly improves the chance of selecting the proper material for laboratory cultures (Coticchio et al., 2004).

Due to the adopted criterion of non-disturbance of the cell's homeostasis, this work focuses on non-invasive methods for oocyte quality assessment. The most frequently used method of non-invasive quality classification of oocytes is the so-called morphological evaluation, performed using microscopy techniques. It consists in allocating the oocytes to appropriate quality classes on the basis of visual observation. For numerous reasons, the assessment is subjective, and thus associated with the risk of error. Fig. 1 presents a morphological structure of an oocyte, and certain representative features which are analysed in the process of classical morphological assessment of oocytes.

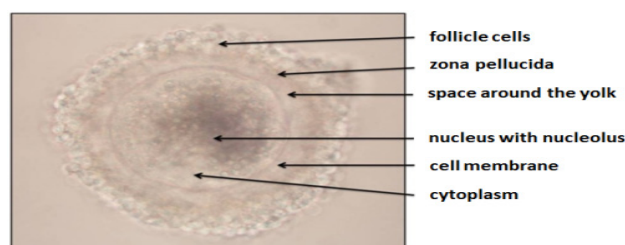


Figure 1. Schematic structure of a domestic pig oocyte. (Institute of Veterinary Medicine, Faculty of Animal Breeding and Biology, Poznan University of Life Sciences)

The state of knowledge and conclusions derived from practical experience indicate the presence of a stochastic correlation between the quality class of animal oocytes and their further *in vitro* development (Bilodeau – Goeseels and Panich, 2002; Pujol et al., 2004). Taking this fact into consideration, the authors used advanced methods of neural modelling for the quality classification of oocytes, assisted by the techniques of computer image analysis. Artificial neural networks are among the most popular artificial intelligence tools, effectively used in many scientific areas. The techniques are successfully applied in various areas of scientific research, where they are employed primarily to solve classification or regression problems. They are increasingly often used to create neural classification models, dedicated to support the processes of identification of information encoded in a graphic form (Boniecki et al., 2015; Koszela et al., 2013; Przybył et al., 2014). Therefore, the authors formulated a hypothesis stating that it is possible to perform neural quality classification of domestic pig oocytes on the basis of information obtained using image analysis methods. Verification of this hypothesis required solving a scientific problem, formulated as the question: is there a neural classifier which enables effective quality assessment of domestic pig oocytes, performed on the basis of information encoded in the form of their digital images? The aim of the paper was to develop an original, non-invasive method for oocyte quality assessment, applied with the use of knowledge on their morphological structure, presented in the form of digital microscopy images. The quality classification process

was conducted based on the information presented in the form of microphotography pictures of domestic pig oocytes, using advanced methods of neural image analysis. In order to do that, the discriminative features of oocytes, presented in the digital photographs, were identified and extracted. This was necessary to create empirical training sets required in the process of generating neural classifiers.

Materials and methods

The empirical material used to achieve the aim of the work consisted of domestic pig oocytes obtained using laboratory methods. Next to cattle and poultry, pigs play an important role in the animal production in Poland. The Polish White Landrace was selected to participate in the study, as it is the most common breed in the Polish farming. To obtain sow oocytes, it was necessary to perform works, which were divided into the following stages:

- obtaining (collecting) ovaries,
- obtaining the follicular fluid by aspiration,
- microscopy evaluation of the follicular fluid to locate the oocytes.

The oocytes were collected in the laboratories of the Institute of Veterinary Medicine, Poznan University of Life Sciences. Fig. 2 presents a schematic collection of the experimental material.

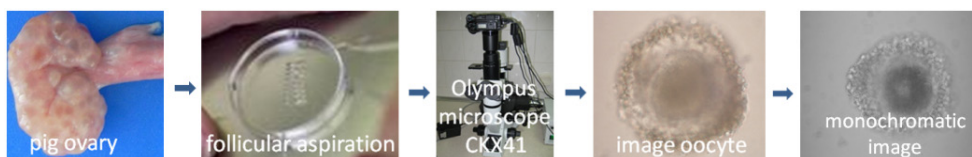


Figure 2. Schematic collection of the empirical material

The empirical data recorded directly from the light-sensitive matrix of a digital camera contained images in *raw* format. This format includes information on the record of light intensity registered by the matrix during exposition. The total number of recorded digital images used in the study was 1,154. The photographs were analysed by an expert, and on the basis of the analysis, 248 photographs were selected as class **I**, 276 photographs as class **II**, 416 photographs as class **III**, and 214 photographs as class **IV**. The examples of oocytes assigned to individual classes are presented in Fig. 3.

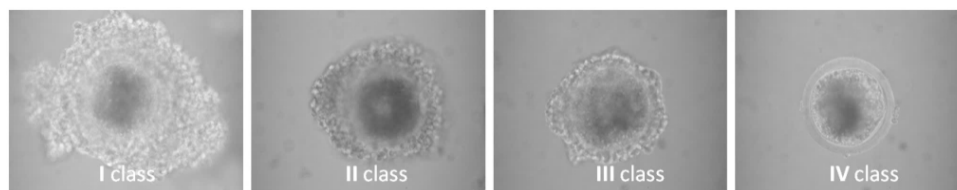


Figure 3. Four qualitative classes of domestic pig oocytes

The abovementioned 4 qualitative classes represented the quality of oocytes defined in the following manner (Jackowska et al., 2009); (Pujol et al., 2004);

- **I** class of oocytes of very good quality, with homogenous, clear and transparent ooplasm, thicker than three layers, and intact zona pellucida;
- **II** class of oocytes of good quality, characterised by compact cumulus consisting of one to two cellular layers, with homogenous ooplasm with porous, rough appearance, dark area in the outer part of the oocyte and intact zona pellucida;
- **III** class of oocytes of satisfactory quality, characterised by less compact cumulus, with irregular colouring of the ooplasm containing dark clusters;
- **IV** class of oocytes of insufficient quality, characterised by absence of cumulus, or expanding cumulus, irregular, dark ooplasm with compact, dark cellular clusters, and damaged or absent zona pellicula; deformations of oocyte may be evident.

It is assumed that the oocytes in the two first qualitative classes, i.e. **I** and **II** are the most desired ones for *in vitro* cultures.

To achieve the aim of the work, the following tests and analyses were required:

- acquisition of digital images of domestic pig oocytes,
- defining the characteristics of the oocytes presented in the form of digital images,
- development of a computer method for identification and extraction of the representative features,
- transformation of the obtained empirical data to a form accepted by the ANN simulator,
- creation of training files for the development of neural models,
- generating a neural models' file and their preliminary assessment,
- identification of an optimal model,
- validation and testing of the generated neural model,
- analysis of exploitation aspects of the generated neural classifier.

In order to perform qualitative identification of the oocytes, advanced methods of neural analysis of graphic data were applied (Boniecki et al., 2014; Koszela et al., 2014). Artificial neural networks (ANN) are currently acknowledged classifiers of data in different forms, also in a graphic form, e.g. digital images (Zaborowicz et al., 2014). A digital image which may be introduced to the generated ANN needs to be expressed in the form of a sequence of numbers included in the multidimensional vector structure.

Identification, extraction and selection of the representative features of the images, best suited to distinguish and classify the images (the so-called discriminative features), and to determine their numeric descriptors, were important stages in the creation of training files necessary to generate ANN. To achieve this aim the micrographic photographs of oocytes were transformed into monochromatic images. To analyse and transform the digital images of oocytes, the MatLab programming environment by MathWorks was used. The choice of this programming software was dictated by its high functionality, especially regarding the application of inbuilt methods of image processing, and the ability to use a wide range of statistic modules. The MatLab library – Image Processing Toolbox (IPT) – was used in the work. It is a programme tool containing a set of specialised functions to process and analyse images. The library functions enabled the following: to perform geometric transformations, use filters, transform images, improve the image quality and analyse and process the images. Representative variables of the analysed oocyte image (referred to as object) necessary to build a training file dedicated to the created neural model, were selected on the basis of literature references (Rudnicki et al., 2002) and the analysis of the task's problem

domain. The characteristics were divided into 3 qualitative groups, covering the representative parameters obtained on the basis of:

- the analysis of the histogram of the original monochromatic image,
 - the analysis of the amplitude spectrum of the monochromatic image,
 - the selected coefficients of the size of the objects (oocytes) presented in avgraphic form.
- a) The first group of characteristics included 11 parameters, selected with the use of probabilistic techniques. The adopted representative features were statistical parameters characterising histograms of the monochromatic images of the oocytes. A histogram is a simple, global description of a digital image. It is described by a vector: $H = [H_0, H_1, \dots, H_g, \dots, H_{L_g-1}]$, whose H_g components provide for each level of grey g ($0 \leq g \leq L_g$) a number of image pixels containing this level (Rudnicki et al., 2002).

The following statistical parameters describing the distribution of the “greyness intensity” were adopted as the representative features of the histogram of the monochromatic image of an oocyte:

[1] standard deviation, [2] variance, [3] skewness, [4] kurtosis, [5]... [11] quantiles (7 percentiles: 35%; 45%; 55%; 65%; 75%; 85%; 95%).

- b) The second set of representative features included 5 texture indicators obtained on the basis of the analysis of the amplitude spectrum of the Fourier transform of the original photographs of domestic pig oocytes (Haralick et al., 1973). Therefore, two-dimensional Fourier transforms were determined for the digital images of oocytes, following the formula (1) (Rudnicki et al., 2002):

$$FFT \rightarrow F(u, v) = \frac{1}{N^2} \sum_{x=0}^{N-1} \sum_{y=0}^{N-1} f(x, y) \exp\left(-2\pi j \frac{xu+yv}{NN}\right) \quad (1)$$

where:

- $f(x, y)$ – digital image in the spatial domain (original’s domain),
- $F(u, v)$ – image in the frequency domain (image domain),
- N – number of digital signal samples,
- j – imaginary unit ($j^2 = -1$).

The features of the amplitude spectrum were selected on the basis of the characteristics of the transform’s image texture. To achieve this aim, the analysis of RLM (Run-Length Matrix) was performed, frequently used to identify the nature of anisotropy of a digital image texture. The matrix contained information on the number of pixel sequences of identical brightness and a given length (the length was the number of the column, and brightness was the number of the row). RLM was described by: L_g – number of levels of grey, and L_r – maximum run length (expressed in pixels). On the basis of RLM, 5 representative features of the transforms of the monochromatic images of oocytes were selected, presented in Table 1.

Table 1
Representative features of digital image calculated with the use of RLM (Rudnicki et al., 2002)

Representative feature	Formula
[12] Short Run Emphasis inverse moment	$SRE = \left(\sum_{g=0}^{Ng-1} \sum_{j=1}^{Nr} \frac{R_{g,j}}{j^2} \right) / c$
[13] Long Run Emphasis Moment	$LngREmph = \left(\sum_{g=0}^{Ng-1} \sum_{j=1}^{Nr} j^2 \cdot R_{g,j} \right) / c$
[14] Run Length Nonuniformity	$RLNonUni = \left(\sum_{j=1}^{Nr} \left(\sum_{g=0}^{Ng} R_{g,j} \right)^2 \right) / c$
[15] Fraction of Image In Runs	$FIR = c / \left(\sum_{g=0}^{Ng-1} \sum_{j=1}^{Nr} j \cdot R_{g,j} \right)$
[16] Grey Level Nonuniformity	$GLevNonUni = \left(\sum_{g=0}^{Ng-1} \left(\sum_{j=1}^{Nr} R_{g,j} \right)^2 \right) / c$

where:

- $R_{g,j}$ – contains information about the occurrence rate of a pixel run with colour g and length j ,
- N – number of pixels in the image,

$$c = \sum_{g=0}^{Ng-1} \sum_{j=1}^{Nr} R_{g,j}$$

- c) The third group of features included 6 selected parameters characterising the shape of the oocytes. It consisted of adequate parameters in the form of dimensionless shape coefficients, used as a standard in digital image analysis. The coefficients are characterised by sensitivity to changes in the shape of the object analysed, and simultaneous insensitivity (invariance) to changes in the manner of presentation of a figure in the image. Selected shape coefficients chosen to assess the quality of the oocytes are presented in Table 2.

The generated training file, containing in its structure the mentioned representative features, was used in the process of creating the classification neural models. The training file structure consisted of 22 input variables and 1 nominal (four-state) output variable. The file contained 1,154 training examples, representing the measurements of the 22 representative features mentioned above, typically divided at a 2:1:1 ratio, to the respective subsets: a training file, validation file and test file (which did not participate in the process of generating classification neural models).

Table 2
Selected shape coefficients

	Name of the coefficient	Formula
[17]	R_S - dimensionless shape coefficient, for quantitative characterisation of the shape of particles	$R_S = \frac{L^2}{4\pi S}$
[18]	R_{C1} - circularity coefficient, describing the diameter of a circle, whose area equals the area of the object analysed	$R_{C1} = 2 \sqrt{\frac{S}{\pi}}$
[19]	R_{C2} - circularity coefficient, describing the diameter of a circle, whose perimeter equals that of the object analysed	$R_{C2} = \frac{L}{\pi}$
[20]	R_M - Malinowska's coefficient	$R_M = \frac{L}{2\sqrt{\pi S}} - 1$
[21]	R_E - ellipse coefficient, describing the quotient of the shorter circumellipse of the object to the longer one	$R_E = \frac{d_k}{d_d}$
[22]	R_Z - content coefficient, describing the quotient of the square of the object's perimeter to its area	$R_Z = \frac{L^2}{S}$

where:

- L – object's perimeter,
- S – surface area of the whole object,
- d_k – length of the shorter axis of circumellipse of the object,
- d_d – length of the longer axis of circumellipse of the object.

Results and discussion

The following types of neural networks were tested: linear networks, MLP networks (Multi Layer Perceptron), RBF networks (Radial Basis Function), PNN networks (Probabilistic Neural Network). 100 different ANN typologies were generated, out of which the best network was selected. The optimal neural topology was MLP-ANN, with a 22:22-54-4:1 structure having 22 neurons in the input layer, 54 neurons in the hidden layer and 4 neurons in the output layer. The network was trained with a hybrid algorithm, using standard algorithms dedicated for optimisation of the weights of MLP neural models. The BP (Back Propagation) algorithm was used for implementation of 5,500 epochs, and the network was trained with the CG (Conjugate Grading) algorithm for 800 epochs.

The structure of the generated MLP neural network: 22:22-54-4:1 is presented in Fig. 4.

MLP artificial neural networks are among the best studied network topologies, the most frequently used in practice. Multilayer perceptron represents the class of parametric neural models. It is characterised for instance by a significantly lower number of neurons in its structure compared to the number of cases in the training file.

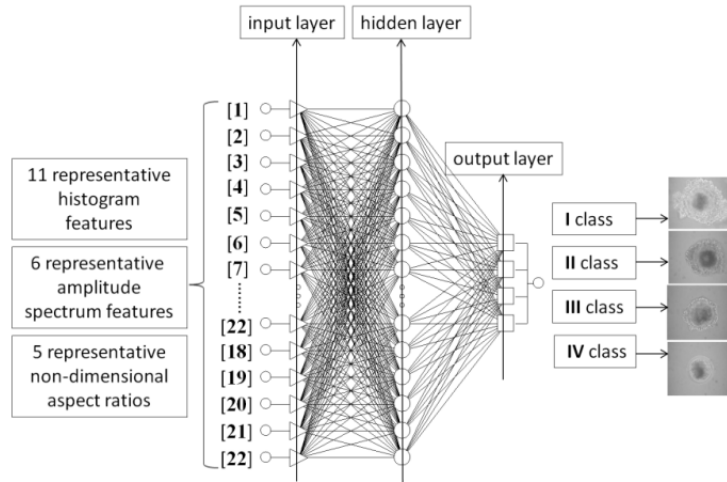


Figure 4. The structure of the optimal MLP neural network: 22:22-54-4:1

The standard measure of correctness of the generated ANN classification is RMS (Root Mean Square). This measure is defined as a total error made by the network on a data file (training, test and validation data). It is derived from the formula (2):

$$RMS = \sqrt{\frac{\sum_{i=1}^n (y_i - z_i)^2}{n}} \quad (2)$$

where:

- n – number of cases,
- y_i – real values,
- z_i – values determined with the use of the network.

It is therefore determined by a sum of squares of individual errors, and then the obtained sum is divided by the number of values (cases) considered, and a square root of the resulting quotient is derived. RMS error is an easy to interpret, single number, describing the total error made by the ANN during its exploitation. For the developed optimal MLP model: 22:22-54-4:1, the RMS error was:

- 0.090716 for the training file,
- 0.1030141 for the validation file,
- 0.1168181 for the test file.

A low and similar RMS error value for the training, validation and test files indicates good generalisation properties of the generated ANN. It also indicates a good classification ability of the proposed model during the presentation of new input data to the network. Standard classification statistics for the training, validation and test files are presented in Table 3.

Table 3
Statistics regarding classification problems for the subsets: test file

	Training file				Validation file				Test file			
	I class	II class	III class	IV class	I class	II class	III class	IV class	I class	II class	III class	IV class
Total	196	204	305	161	26	30	61	27	26	42	50	26
Correct	194	196	295	160	26	29	56	27	26	39	45	25
Wrong	2	8	10	1	0	1	5	0	0	3	5	1
Unknow	0	0	0	0	0	0	0	0	0	0	0	0

Conclusions

Using neural image analysis to identify the domestic pig oocytes appeared to be an appropriate classification technique. The proposed method may be a practical way to effectively support the decision processes in the breeding of livestock. Graphic identification of the set of selected discriminative features of the microphotographs of oocytes was best performed by the multi-layer perceptron MLP neural network with the structure: 22:22-54-4:1. The analysis of the problem domain led to the conclusion that proper identification of the oocyte class requires only 22 variables containing information about 11 statistical parameters of the oocyte monochromatic image, 5 RLM matrix parameters which characterise the texture of spectrum images, and values of 6 indicators of the oocyte's shape. It is worth emphasising that using neural image analysis enabled a non-invasive classification of oocytes to 4 predetermined classes, without disturbing the oocyte homeostasis during the identification process.

The suggested method also has a utilitarian value, particularly in the context of automation of the oocytes qualitative assessment, and as a tool supporting the process of qualitative assessment. The generated neural classifier's code may be a nucleus of an IT system dedicated to support the automation process in the qualitative assessment of oocytes. The conducted tests enabled the following conclusions to be drawn:

1. The obtained test results confirmed that using neural image analysis enabled effective identification of domestic pig oocytes, thus supporting the process of their qualitative classification.
2. The qualitative analysis of the generated neural models demonstrated that the best classification ability was obtained by the MLP-type three-layer perceptron neural topology with the structure: 22:22-54-4:1.
3. The analysis of the sensitivity of the generated neural model to the input variables demonstrated that 22 selected parameters characterising the histogram, amplitude spectrum and the shape of oocytes presented on monochromatic images were sufficient for effective classification of the oocytes.
4. The most important representative features were the variables [10], [11] and [16] containing information on the histogram of the oocyte image, and about the grey level non-uniformity of the amplitude spectrum image.
5. The performed tests demonstrate the utilitarian aspect of the model developed, in particular as an information tool in the form of a nucleus of an expert system supporting decision processes in the domestic pig *in vitro* production.

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WYKORZYSTANIE NEURONOWEJ ANALIZY OBRAZÓW W IDENTYFIKACJI INFORMACJI ZAKODOWANEJ W FORMIE GRAFICZNEJ

Streszczenie. Rozwiązaniem problemu klasyfikacji jakościowej oocytów zwierzęcych zajmuje się wiele różnych ośrodków naukowo-badawczych. Celowość prowadzenia takich badań jest uzasadniona szczególnie w kontekście ciągłego dążenia do podnoszenia jakości produktów żywnościowych, która jest pochodną wartości hodowlanej zwierząt gospodarskich. W związku z tym, istotnego znaczenia nabierają poszukiwania metod prowadzących do stymulowania prawidłowego rozwoju większej liczby zapładnianych oocytów zwierzęcych, zwłaszcza realizowanego w warunkach pozaustrojowych. Rosnące zainteresowanie technikami wspomaganego rozrodu stało się przyczyną poszukiwania nowych, coraz bardziej efektywnych metod oceny jakościowej gamet oraz zarodków ssaków. Oczekiwany postęp w produkcji zarodków *in vitro* zwierząt uzależniony jest w istocie od poprawnej klasyfikacji pozyskiwanych oocytów. Celem pracy było opracowanie bezinwazyjnej metody oceny jakościowej oocytów dokonywanej w oparciu o informację graficzną zakodowaną w postaci monochromatycznych obrazów cyfrowych pozyskanych metodą mikroskopową. Proces klasyfikacji zrealizowano w oparciu o informację prezentowaną w formie zdjęć mikrofotograficznych oocytów świni domowej, wykorzystując w tym celu nowoczesne metody neuronowej analizy obrazu.

Słowa kluczowe: identyfikacja klas oocytów, klasyfikacja jakościowa, analiza obrazu, sztuczne sieci neuronowe