Indian J. Anim. Res., 51 (2) 2017 : 322-326
Print ISSN:0367-6722 / Online ISSN:0976-0555

AGRICULTURAL RESEARCH COMMUNICATION CENTRE
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A study regarding the fertility discrimination of eggs by using ultrasound

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Received: 24-04-2016 Accepted: 16-08-2016 DOI:10.18805/ijar.v0iOF.4561

ABSTRACT

The aim of this research was to track the growth of chicken eggs, and make a decision as to whether the egg was fertilized or not. A digital imaging system has been developed in order to take an image from six different points without damaging the egg shell. All the images were transferred to a PC and turned into binary images. All the images were reduced to 1024 pixels and fed directly into the classification algorithm. The logistic regression method was used to discriminate the fertility of the eggs. Python programming language and the scikit-learn machine learning library was used to carry out the classifications. True positive, true negative, wrong positive, and wrong negative detection numbers in the trials were 350, 344, 56, and 50, respectively. Negative indicates the egg was infertile, and positive indicated that the egg was fertilized. The model accuracy was measured as 0.8675.

Key words: Fertility, Poultry egg, Ultrasound.

INTRODUCTION

Millions of chicken eggs are produced each year in poultry farms worldwide. After hatching, the chickens have to be separated by their genders. The breeding of the male layer chickens is considered to be highly uneconomical. The female chickens are kept for the production of eggs, and the male chickens are disposed of. Therefore, it is necessary to develop methods for sexing chickens early in the embryonic development, preferably before incubation (Burkhardt *et al*., 2011). The morphological features, behavior, acoustic, laparoscopy, laparotomy, cloacal examination, and examinations of the fecal steroid hormones, are widely used methods for discrimination, but they are all time consuming, expensive and can sometimes be detrimental (Morinha *et al*., 2012).

It is only possible to incubate female chickens if the gender discrimination could be determined at the early stage of the embryo. The detection of the infertile eggs prior to incubation, and the removal of them will increase the production cost efficiency and quality of the chickens. Furthermore, it is important for lower pathogen contamination (Liu and Ngadi, 2013).

The aim of this study was to develop an image-processing system which could monitor the fertilization of the eggs and different stages of growth. Ultrasonographic images were collected and examined for this purpose.

MATERIALS AND METHODS

Ovoscope and ultrasonographic imaging techniques were used to monitor and record both the growth of the embryo and detect special regions and organs.

The eggs were kept in an incubator under 38°C and 70% humidity. All the implementations were made with three repetitions and with nine eggs for both techniques. Totally, 189 eggs were used for the trials, and 1000 ultrasonographic images were obtained.

The images of the embryo were taken with a probe which was connected to an ultrasound device. These probes emit ultrasound signals to the region of interest,which are then passed through the tissues. The depth of access depends on the features of the tissues. The shape of the object was detected by sensing the reflected ultrasound signals, and then this shape can be monitored on the screen. This is called as ultrasonographic image. Ultrasonographic images can be printed directly or a recorded on a CD or loaded on to a USB stick. For this purpose, an ultrasound device and a phased probe were used. The measurements were made between 3-10 MHz.The images were taken from 6 different points on the eggs. However, the ultrasound signals were not able to pass the calcium surface. A hole was opened from the air cell by the help of an ovoscope, and thus, the measurement could be carried out after (Fig. 1).

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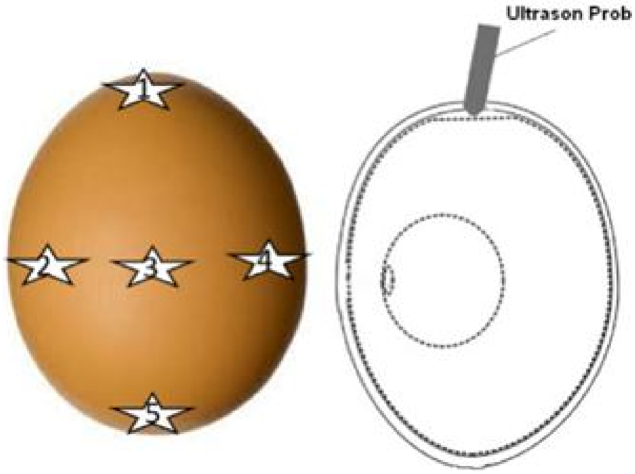

Fig 1: Ultrasound measurement points and the probe position

The hole was closed by using sterile tape, and then the eggs were put back in order to continue their growth in an incubation device (Steiner *et al*., 2011). Figure 2 shows the structure of the fertilized egg.

The obtained images were transferred to a computer program and evaluated. All the images were obtained from the eggs from the same period. All the 1000 ultrasonographic images were reduced to a 32x32 pixel size (all images consist of 1024 pixels) for using in the machine learning algorithm.

Each of the images which were formed on the 1024 pixels were directly fed into a classification algorithm. Logistic regression was used to classify the fertilization of the eggs.

Logistic regression is also known as logit regression or the logit model in statistics (Freedman, 2009). It is the regression model which is used in cases where the dependent variable is categorical. Logistic regression was developed by David Cox in 1958 (Cox, 1958; Walker and Duncan, 1962). It is used to find the probability of the binary classification depending on one or more dependent variable. Logistic regression was performed calculating this relationship by using the logit function (Fig. 3). It can create probability values between 0-1 related to the t independent variable.

RESULTS AND DISCUSSION

The eggs were examined by using ultrasonographic imaging, and the manual ovoscop technique (Fig. 4) for tracking the growth level of the embryo and for detecting the special regions or organs. The flow chart of the classification system can be seen in Figure 5.

Totally, 1000 ultrasonographic images were used at classification, and this dataset was divided by 80% for training and 20% for the validation subsets. The logistic regression classification method was used to decide as to whether the eggs were fertilized or not. The accuracy of the classification was determined by the confusion matrix (Table 1).

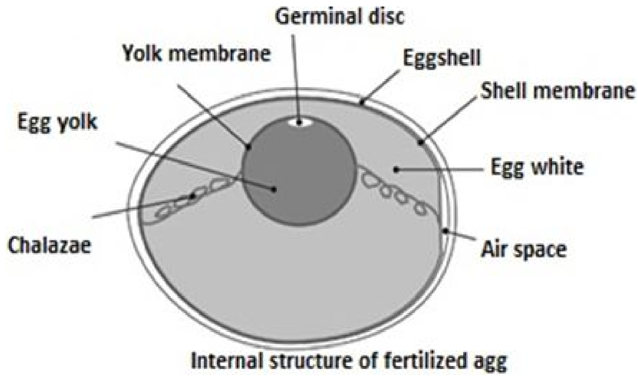

Fig 2: Structure of fertilized egg

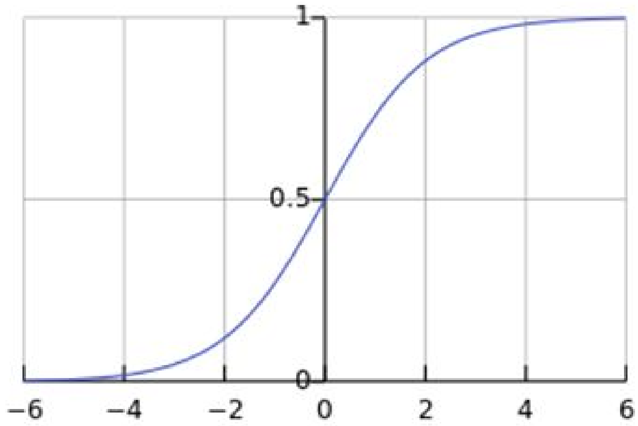

Fig 3: Standard logit function$^{\sigma(t)}$; for all t$^{\sigma(t)\varepsilon(0,1)}$

$$\sigma(t) = \frac{1}{1-e^{-t}} \qquad (1)$$

The Python programming language and scikit-learn machine learning library was used to do the classification (Pedregosa *et al*., 2011). The generic program is below.

```
//All the required libraries are imported
fromIPython.display import Image
fromsklearnimport datasets
fromsklearn.linear_model import Logistic Regression
fromsklearnimport metrics
fromsklearn.cross_validation import train_test_split
```

//1000 ultrasonographic image is used with 32x32px size.

```
X, y =datasets.make_classification(n_samples=1000, n_features=1024)
```

Table 1: Fertilized / infertile logistic regression classification

		Predicted Class	
		Fertilized	**Infertile**
Real Class	Fertilized	350	50
	Infertile	56	344

Fig 4: Images from ovoscope technique

//Ultrasonographic image dataset are divided in to training and validation subsets.

X_train, X_test, y_train, y_test=train_test_split(X, y, test_size=0.8, random_state=4)

//Logistic model is created and trained

Logistic_model=LogisticRegression()
expected=y_test
Logistic_model.fit(X_train, y_train)

//Validation of the classification model is done on the validation subset.

predicted=Logistic_model.predict(X_test)
accuracy_score=metrics.accuracy_score(expected, predicted)

print"Model accuracy score is {}".format(accuracy_score)
printmetrics.confusion_matrix(expected, predicted)

The confusion matrix of the classification is demonstrated in Table 1.The true positive, true negative, wrong positive, and wrong negative detection numbers in the trials were 350, 344, 56 and 50 respectively. Negative indicates the egg is infertile, and positive indicates that the egg has been fertilized. The model accuracy is measured as 0.8675.

There are numerous studies regarding the instruments and methods for examining the internal quality of the eggs without damage. Bloodstains and other internal disorders were taken into account in these studies (Patel *et al*., 1996; Patel *et al*., 2003; Bartels *et al*., 2008).

It is important to identify the fertility of the eggs at an early stage of incubation. Bartels *et al*. (2008) reported that 3D-X-ray micro computed tomography (3D-CT) has been proven as a suitable method for localizing the germinal disk in the unincubated egg without damaging its shell. On the other hand, optical coherence tomography, acoustic resonance, magnetic resonance imaging, and ultrasonography, are non-commercial research methods for identifying the embryo (Coucke *et al*., 1997; Klein *et al*., 2002; Smith *et al*., 2008).

Ultrasonographic techniques are open to continuous improvement. Ultrasound biomicroscopy is a current development that allows a very high resolution in vivo micro-analysis. The study reported in the embryonic heart can be taken up into the blood stream image in detail. Another technique used in the study regarding the embryo development stage, is to analyze the heart rhythm (Klein *et al*., 2002).

In a recent study, the chicken embryos were examined with RMV 708)Vevo 770 systems (Visualsonics, Inc., Toronto, Canada) which had a 55 MHz transducer and 30 mm axial – 75 mm lateral resolution (Oosterbaan, 2012). The study reported that even the blood stream in the heart could be detected. Examination of the cardiac rhythm is another technique that is used in embryo analyzing.Ontogeny and the development of cardiac rhythm can be monitored by using impedance cardiography (ICG), acoustic cardiography

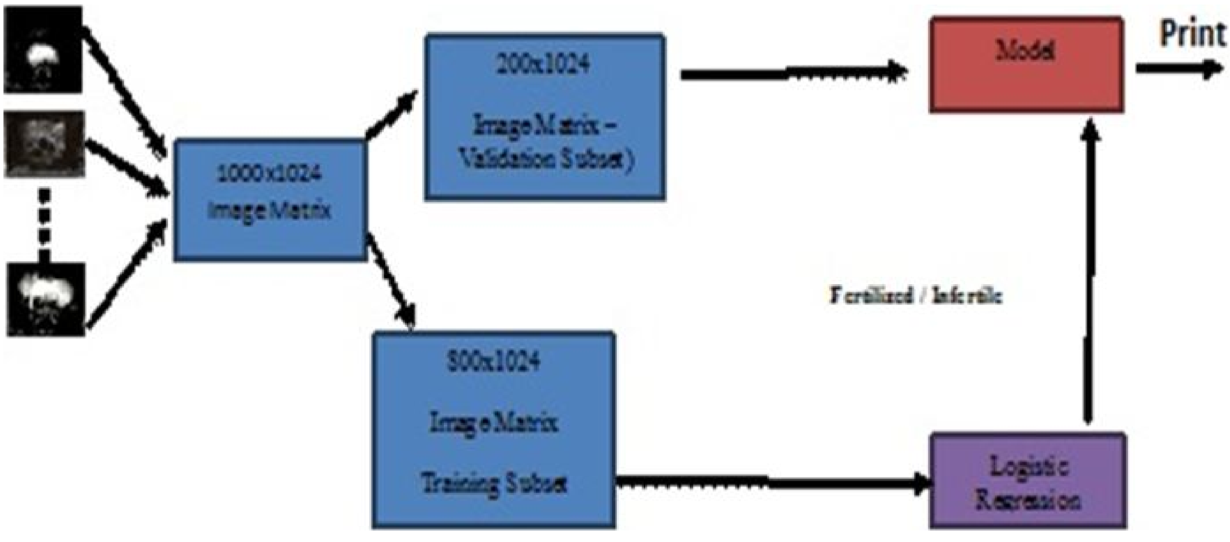

Fig 5: Classification system flow chart

(ACG), allantoic arterial blood pressure, and electrocardiography (ECG) methods (Andrewartha *et al*., 2011). Light transmission and spectral analysis are the basis for the developed commercial systems for examining both the fertility rate and embryo development. Das and Evans (1992a) were able to monitor the fertilized egg development with 88-90% accuracy on the 3rd day of incubation, and 96%-100% accuracy on the 4th day of incubation by using machine vision and histogram analysis. Das and Evans (1992b) used the same imaging system with a neural network classifier. They were able to monitor the fertilized egg development with 67.3% accuracy on the 2ndday of incubation and 93% accuracy on 4th day. In another imaging system, the embryo could be detected on the 4.5-5th days of incubation by using two light waves (Bamelis *et al*., 2002). A patented system was developed for the detection of the embryos at the 18th day of incubation (Chalker *et al*., 2003). A preliminary study showed that hyper spectral imaging can be used to monitor the embryo from the 3rd day of incubation (Smith *et al*., 2005).

Hyper spectral imaging technology was developed to monitor fertility and the growth of the embryo (Smith *et al*., 2005; Smith *et al*., 2008; Liu *et al*., 2013). This imaging technique was reported as inappropriate for broiler chicken eggs. It needs improvements in technique to detect the fertility rate at the early stage of the embryo before the incubation (Smith *et al*., 2008).

Various research studies have been conducted to examine the automatic gender discrimination effects on incubation and chicken quality. During the process, two different places on the egg shell were drilled in order to take allantoic fluid and inoculums. Research showed that closing those holes has no effect on quality (Phelps *et al*., 2003; Chue and Smith, 2011).

Recent studies in commercial hatchery systems focus on the disinfection of the sampling points of the eggs which have been drilled for samples. Studies showed that disinfection prevents the adverse effect of the drill point (Chalker*et al*., 2003; Phelps *et al*., 2003).

The Embrex system is successful at automatic gender discrimination of the eggs. The most serious problem encountered in the system was that discrimination could not be carried out when the allantoic fluid was taken.The automatic system could decide the gender of the eggs successfully. The gender discrimination of 20.000-30.000 eggs/hour can be done by the future development of the system. Hatchery companies will be able to prevent negative criticism concerning animal rights because of the killing of the male chickens (Chalker *et al*., 2003).

In this study, if the egg is fertilized, the fertilization of the eggs and the growth of the embryo are monitored manually by an ovoscope and ultrasonographic imaging. However, ultrasonographic signals are not able to pass through the egg shell, and this situation prevents the ability to take useful images by using the ultrasonographic imaging technique. The egg shell cracks from the air cell and this does not affect the health of egg. Ultrasonographic images can be taken from this hole, and a decision regarding fertilization could be decided.

The software is developed by using the logistic regression model, which is determined by the ultrasonographic images. This software can decide on the fertilization of the egg. Male/female sex discrimination can be possible by the improvement of image quality and this depends on the possible progress of sensor technology. Further research should be conducted for this in the near future.

ACKNOWLEDGEMENT

This research was supported by a grant from Namik Kemal University Scientific Research Project (Project No: NKUBAP.00.24.AR.14.27.)

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