



Automatic Estrus Cycle Identification System on Female Dogs Based on Deep Learning

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Abstract. Vaginal cytology is a complementary economic method and of simple realization, an indicative to determine in which stage of the estrous cycle the dog is, to achieve a higher fertility and fertility rate. This method is based on determining the type and quantity of cells of the different stages of the estrous cycle, since the hormonal changes that the vaginal mucosa undergoes during the estrous cycle are shown in the morphology of its epithelial cells. The canine female in her reproductive life goes through different phases of activity and hormonal rest that are repeated cyclically. This is called the estrous cycle and consists of 4 stages: proestrus, estrus, diestrus and anestrus. The interpretation of vaginal cytology's, is a process to which a considerable amount of time is dedicated by its observation in the microscope and the same interpretation by the doctor which can become subjective and poorly performed, causing economic losses for the owners. Therefore, this work proposes an automatic system that will identify six types of cells and the quantity of them in the glass slide, based on a Faster R-CNN to determine in which stage of the estrous cycle the dog is. Our results show an accuracy of 91.6%. The proposed system will improve the efficiency and speed of cytology's to decreased from 1 h approximately to just a few seconds.

Keywords: Vaginal cytology · Estrous cycle · Faster R-CNN · Deep learning · Cells

1 Introduction

Dogs have always been a fundamental pillar in the different facets of people's lives. Hence the importance of dogs in society, not only to go for a walk with them but for other beneficial activities, such as: search and rescue, medical pets, emotional support, hunting, grazing and protection. The goal of every living being is the perpetuation of its species. To achieve this, they must fulfill the function of reproducing, which consists in the ability of every living being to produce another similar individual. This function

becomes a transcendent biological fact of great importance for the survival of the species, since it perpetuates life beyond the individual [1].

The canine female in her reproductive life goes through different phases of activity and hormonal rest that are repeated cyclically. This is called the estrous cycle which consists of 4 stages: proestrus, estrus, diestrus and anestrus [2]. The first estrus appears in female dogs between the first 6 and 10 months of their life and then they experience a new ovarian cycle approximately every 6 months. Canine infertility can derive from multiple etiologies, but statistically the rejection of the mating or the bad synchronization ovulation-mount, represent approximately 80% of the failures of reproduction in this species. Although artificial insemination resolves the rejections of the mating, it will not be successful, if it is not done at the right time, being currently the vaginal cytology as the best way to determine that moment [3].

Vaginal cytology is the most applied procedure to verify the status of the female reproductive system. Despite this method being old, it is preferred due to its remarkable scope, it is practical, fast and economical, and it can be done by any specialized veterinary professional. The study consists of introducing a clean swab - preferably sterilized - in the vagina of the female. Subsequently, epithelial cells should be analyzed under a microscope to find out the status of the female dog's reproductive system. However, this analysis can be very time-consuming and tedious, since the doctor will count and classify the cells seen in the microscope manually, consuming approximately 1 h for each study performed.

Under these conditions, we have been interested in detecting the dog's estrous cycle using an automatic system based on convolutional neural networks, mainly in the Faster Region-based CNN (R-CNN) [4]. The objective of this work is to detect, classify and count the 6 different types of cells that will be seen in the microscope in a faster and more efficient way, and thus get to know the state of the estrous cycle where the female dog is. Unlike another estrous cycle identification proposal [5], in which the system classifies the estrous cycle directly from the input image, our proposed system provides the number of different types of cells, which indicates the female dog's estrous cycle. In this sense, our proposal provides reasons about the final classification decision to expert.

This paper is organized as follows: Sect. 2 a brief review of deep learning in veterinary medicine. Section 3 shows the methodology of the proposed system and the creation of the database. In Sect. 4, we provide some experimental results. Finally, Sect. 5 concludes this paper.

2 Deep Learning in Veterinary Medicine

In some aspects, veterinary medicine can be compared with the human medicine. Many basic laboratory tests are still too expensive for pet owners or simply not feasible due to the lack of automated methods and the large number of different species.

An example would be the white blood cell count (WBC). In human medicine, this is one of the most basic blood tests and is performed almost every time blood is drawn. On the other hand, in birds and reptiles, this laboratory test must be done manually (unlike humans, the red blood cells in birds and reptiles have a nucleus that interferes with most automated counting methods). However, due to the large number of different species and

the morphological differences in their white blood cells, this test can only be performed by specialists in this field and, therefore, is rarely used in practice [6].

This emerging technology could provide a solution to this and other similar problems in veterinary medicine. In recent years, deep learning applications have been used to solve several problems in medical imaging, resulting in an improvement over previous generation results [7]. So far, to name just a few, this technique has been used in tumor detection, blood flow quantification, brain image analysis and pathology images [8]. In some cases, these deep learning techniques even surpassed humans in these tasks [6].

Unfortunately, despite its successful use in human medicine, deep learning applications are still lacking in veterinary imaging. It is argued that this should change, because not only deep learning can allow more accurate, faster and less expensive diagnoses in veterinary medicine and it can even be used easily by any veterinarian [8].

Currently there are studies conducted by the Faculty of Veterinary Medicine of Zagreb in Croatia, which decided to implement deep learning in a real-world task in veterinary medicine that is usually done manually, in this scope they counted the percentage of reticulocytes in cats [6] and also in [5] authors classify the estrus cycle in rats by using features of whole image and transfer learning.

3 Methodology

3.1 Dataset

In this work we create a dataset consisting of vaginal cytology samples in female dogs collected by the reproduction management laboratory of the Universidad Autónoma Metropolitana - Xochimilco. The vaginal samples are smeared into a glass slide and stained with pap smears [9]. The images were taken with an inverted microscope (see Fig. 1a) with an integrated camera with 40X magnification objective lens, without zoom, with the shutter open at 2.6. We obtained a total of 250 images of size 1600×1200 pixels in an RGB color space with jpg format. (see Fig. 1b). The next step was to manually label the cell types (see Fig. 2) in each of the images in the database, which was performed by an expert in the reproduction laboratory. Finally, the dataset was divided into two parts: 70% for training and 30% for test.

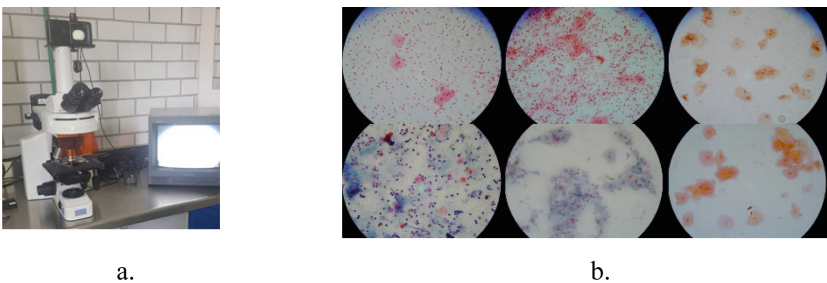


Fig. 1. a) Inverted microscope with integrated camera and b) Example of images obtained

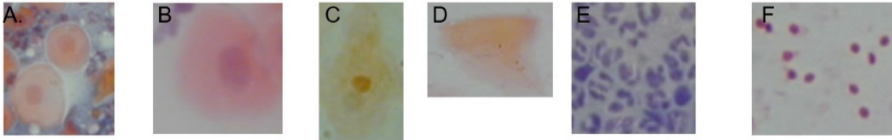


Fig. 2. Epithelial cell types to recognize during a vaginal cytology; A. Parabasal cells, B. Superficial cells with a pyknotic nucleus, C. Intermediate cells, D. Anucleate (squamous) cells, E. Neutrophils, F. Red blood cells.

3.2 Faster R-CNN Method for Identification of Cells

The Faster R-CNN is a method that allows the classification and localization of objects in an image, this is an improvement of its predecessors R-CNN [10] and Fast R-CNN [11] where execution time is decreased. The architecture of a Fast R-CNN (see Fig. 3) is composed by a Region Proposal Network (RPN) with a backbone CNN that extracts the features from the image. The RPN works on top of the feature map of the CNN and creates its own region proposals inside the network, then a Region Of Interest (ROI) Pooling layer warps the region proposal and takes the feature maps to fed them into fully connected layers that aim to predict the class probability and the bounding boxes. Finally, a regression is used to correct the offset of the proposed bounding boxes. Because this is a complete network that combines the extraction of features and the proposal of regions, its computational time is low.

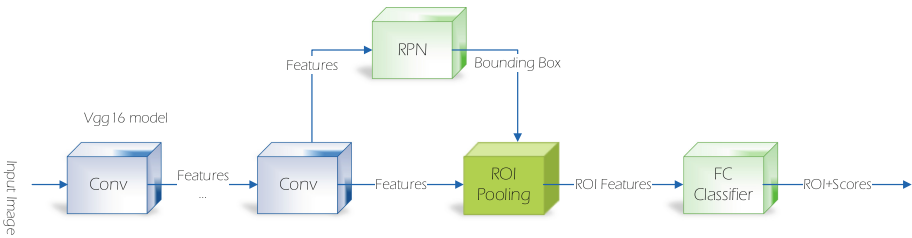


Fig. 3. Faster R-CNN architecture

3.3 Estrus Identification Automatic System on Female Dogs

There are several architectures for feature extraction widely used for classification tasks such as Resnet-101, Resnet-50, Inception V2, Vgg16, Vgg19 and so on. The aim of this scope is to achieve a high performance by using low computational resources in the shortest amount of time, therefore the Vgg16 architecture is chosen as the backbone for the Faster R-CNN. This feature extractor consists in 13 convolutional layers which have proven to be enough. For the training, 70% of the images were fed into the network along with their bounding boxes previously segmented by the experts.

The method for determining the estrous cycle consists in taking 6 photographs from the glass slide to acquire the most information from it. Then, from each photograph the different cells are counted and grouped among all images. According to the specialist,

to indicate the stage of the estrous cycle the presence of different cells is close to the percentages in the following Table 1, the percentages may vary but the relation between cells is close, the \pm sign indicates that the cells may appear but they are not relevant to the cycle, the + sign indicates that the cells will be present but the number of them is not relevant, and finally the - sign indicates the absence of the cell, although it is irrelevant to the cycle. This tells us that if we can find cells and count them, we can know in which stage of the estrous cycle the canine is.

Table 1. Approximate cell percentages for each stage of the canine estrous cycle [12].

Cells	Proestrus %	Estrus %	Diestrus %	Anestrus %
Anucleated	10	90	30	10
Superficial	30	8	20	10
Intermediate	50	2	20	20
Parabasal	10	0	30	60
Neutrophil	\pm	+	+	+
Erythrocytes	+	-	-	-

The proposed method (see Fig. 4) consists in use of the Faster R-CNN to detect the cells in the acquired images (6 images). Then, just by counting the number of bounding boxes and polling them given their label, we can compute the percentages of cells and determine the stage of the estrous cycle. This can be done easily and much faster than manual counting.

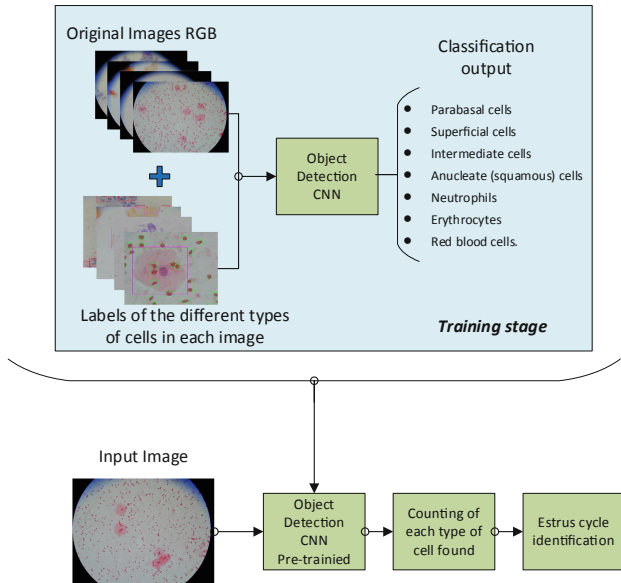


Fig. 4. System for estrus cycle identification

4 Results

For the detection the Faster R-CNN was used with the Vgg16 feature extractor as previously discussed, for the training parameters we used the stochastic gradient descent with momentum optimizer with a L2 regularization of 0.0005 and a momentum of 0.900, the model was trained in two stages for a total of 300 epochs, with an initial learning rate of 0.001 during 200 epochs and then with a learning rate of $5e-8$ for 100 epochs, the mini-batch size was set to 1. These parameters depend on the feature extractor chosen, for our scope these showed the best performance.

The system is capable of recognizing parabasal, superficial, intermediate, neutrophil, erythrocytes and anucleate cells, plus it is also capable of recognizing red blood cells which provide some extra information; however, it does not belong to the estrous cycle. The system was tested on the 30% remaining information and its performance was evaluated by using the Average Precision (AP) metric given by (1).

$$AP = \int_0^1 p(r)dr \quad (1)$$

The definition of AP represents the area under the precision-recall curve, the precision (2) tells us how accurate the predictions are and recall (3) tells how good it can find all the correct regions.

$$Precision = \frac{TP}{TP + FP} \quad (2)$$

$$Recall = \frac{TP}{TP + FN} \quad (3)$$

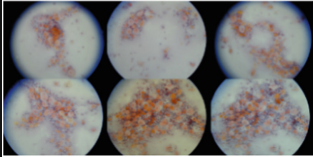
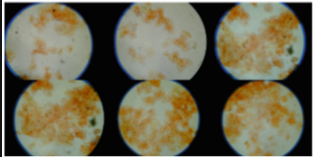
Where TP is true positive, FP is false positive, and FN is false negative, respectively. A TP is defined when the ratio of the intersection of the two areas (predicted and ground truth) over the union of the two areas is greater than a certain threshold in this case 0.75. Table 2 shows the results for the classification of every cell, showing a lower performance for red blood cells which does not represent an inconvenience for the estrus cycle.

Table 2. Performance of the detection of cells

Type of cell	Average precision
Anucleated	0.91
Superficial	0.91
Intermediate	0.97
Parabasal	0.94
Neutrophil	0.86
Erythrocytes	0.88
Red blood cells	0.23

Then the counting of the cells is done by counting the bounding boxes with a higher score of 90%, an example is show in Table 3 where the Anestrus and Estrus cycles are identified.

Table 3. Demonstration of cell count for a previously diagnosed cytology.

Images belonging to a smear	Diagnosis of the proposed system	Diagnosis by manual cell count (veterinarian)
	<p>The total cells detected in the image are:</p> <p>Anucleated = 3 ->>>3.49% Superficial = 6 ->>>6.98% Intermediate = 74 ->>>86.05% Parabasal = 3 ->>>3.49% Neutrophil = 3 Erythrocytes = 9 Red blood cells = 0</p> <p>This cytology belongs to Proestrus</p>	Proestrus
	<p>The total cells detected in the image are:</p> <p>Anucleated = 26 ->>>92.86% Superficial = 2 ->>>7.14% Intermediate = 0 ->>>0.00% Parabasal = 0 ->>>0.00% Neutrophil = 32 Erythrocytes = 0 Red blood cells = 0</p> <p>This cytology belongs to Estrus</p>	Estrus
Diagnostic time	5 sec	1 hour

Finally, we evaluated our proposed system with 12 new tests, which had already been classified by an expert (see Table 4), of which we were right at 11, having a percentage of identification of the estrous cycle of 91.6%.

Table 4. Number of new samples for evaluation by an expert and our proposed system

Estrus cycle	Veterinarian	System proposed	Detection rate %
Anestrus	3	3	100
Proestrus	3	3	100
Estrus	3	3	100
Diestrus	3	2	66.6
Total	12	11	91.6

5 Conclusion

In this paper a new system to identify the estrus cycle of a female dog is proposed, it is based on a Faster R-CNN detection model with the Vgg16 as feature extractor. In general, it achieved an accurate prediction of the different types of cells, except in red blood cells, which although it is not necessary to decide the classification of the estrus cycle, it might be of great importance to detect any anomaly different from our objective. On the other hand, in the test of the complete system the experts are still better to classify, the difference with our system lies in the time to classify the estrus cycle, being 5 s, which is much faster compared to approximately 1 h that the expert takes. The results have shown that our proposed approach in this classification task is effective and accurate. However, we still need to improve the robustness of our method by increasing the size of the database. It is worth noting that our proposed system provides the number of different types of cells to determine the estrus cycle, which is the same procedure performed by experts, and then the proposed system can be expanded easily for any other purposes, such as determine some specific diseases of dogs.

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