Automated Method for 1) Assessing the Phases of Oestrus Cycle and 2) Quantifying Ovarian Follicles in Rat

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Introduction

Oestrus staging: Various drugs and chemicals cause endocrine disruption and interfere with the female reproductive system. Accurate and consistent determination of rat oestrus cycle phases helps understand these effects in nonclinical studies. This task needs to be performed by skilled pathologists. We propose a deep learning-based algorithm to identify the 4 phases of the oestrus cycle using combined morphology of rat's vagina and uterus.

Follicle counting: The counting of early stages of ovarian follicles to study the possible effects on fertility is recommended/required in multigenerational reproductive studies performed on rat. Manual counting of the different ovarian follicles is a tedious, error-prone and time-consuming task that must be done by well-trained pathologists. We propose an automated solution for the identification and quantification of Primordial type 1, Primordial type 2 and Growing follicles in rat ovary.

Materials and Methods

Oestrus staging: A training data set consisting of whole slide images of H&E-stained sections of uterus and vagina from 50 control Wistar rats was created by extracting 512x512 tiles corresponding to annotations made by pathologists on the same slide images. Customized U-Net with Efficient-Net B4 model was trained to segment various parameters from uterus and vagina sections. Further, features of these parameters were extracted using image processing techniques. Table 1 provides the list of parameters segmented and the features extracted. The extracted features were then passed through a random forest classifier for finding out the phase of the cycle in rodents.

Organ	Vagina	Vagina	Uterus	Uterus	Uterus	Uterus
Parameter	Layers segmentation – Stratum Corneum, Germinativum and Mucification	Leucocyte	Epithelial Height	Apoptosis present in Superficial Epithelium and Glands	Mitotic figures present in Superficial Epithelium and Glands	Luminal Dilation
Quantification Method	Percentage Area	Percentage Area	Median Height	Percentage Area	Percentage Area	Percentage Area

Table 1. List of parameters and quantification methods used for oestrus staging

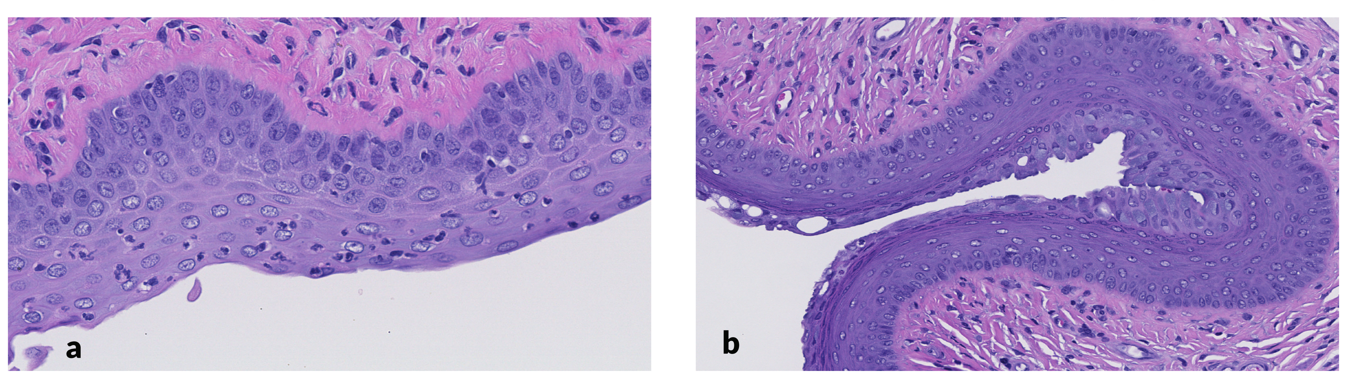

Fig. 1. Shows the parameters of vagina used for staging. (a) Infiltration of leucocytes. (b) Type and thickness of epithelial layers

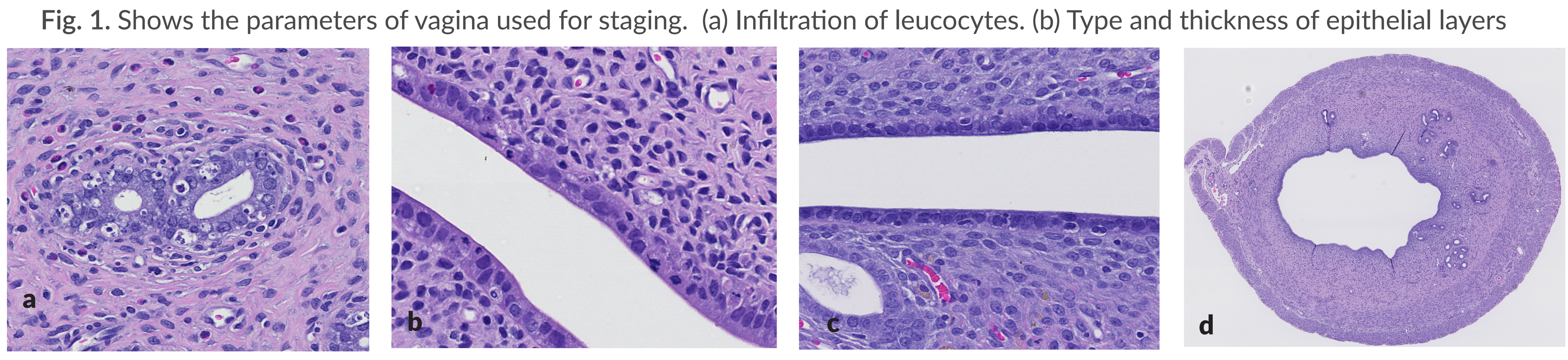

Fig. 2. Shows the parameters of the uterus used for staging.
(a) Quantification of apoptosis. (b) Quantification of mitotic figures. (c) Thickness of the epithelium. (d) Quantification of luminal dilation

Fig. 3. Outlines the entire algorithmic pipeline for oestrus staging

The algorithm created was validated with a validation data set of uterus and vagina sections from 110 control Wistar rats using ground truth labels provided by expert pathologists.

Follicle counting: A training data set consisting of whole slide images of H&E-stained sections of the ovaries from 60 control Wistar rats was created by extracting tiles corresponding to annotations made by pathologists on the same slide images. A customised U-Net model was trained on this data to segment out Primordial Type 1, Primordial Type 2 and Growing follicles. The segmented outputs were then refined using various image-processing techniques.

Parameters	Primordial Type 1 and Primordial Type 2	Growing follicles
Magnification	40x	20x

Table 2. List of parameters trained for follicle counting with their magnification

The algorithm created was validated with a validation data set of uterus and vagina sections from 110 control Wistar rats using ground truth labels provided by expert pathologists.

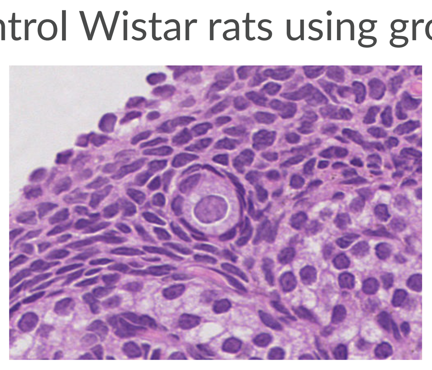

Fig. 4. Shows the Primordial Follicle Type 1, characterised by a visible oocyte surrounded by one layer of flat to cuboidal granulosa cells with flat to oval nuclei

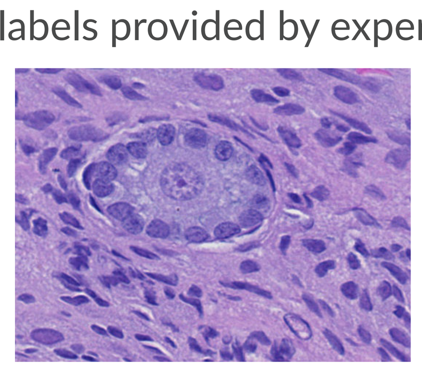

Fig. 5. Shows the Primordial Follicle Type 2, characterised by a visible oocyte surrounded by one layer of cuboidal to columnar granulosa cells with round nuclei

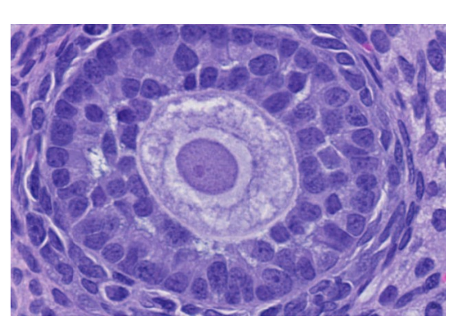

Fig. 6. Shows the Growing Follicle, characterised by a visible oocyte surrounded by cuboidal to columnar granulosa cells in two to five cell-layers with round nuclei and without a crescent-shaped antrum

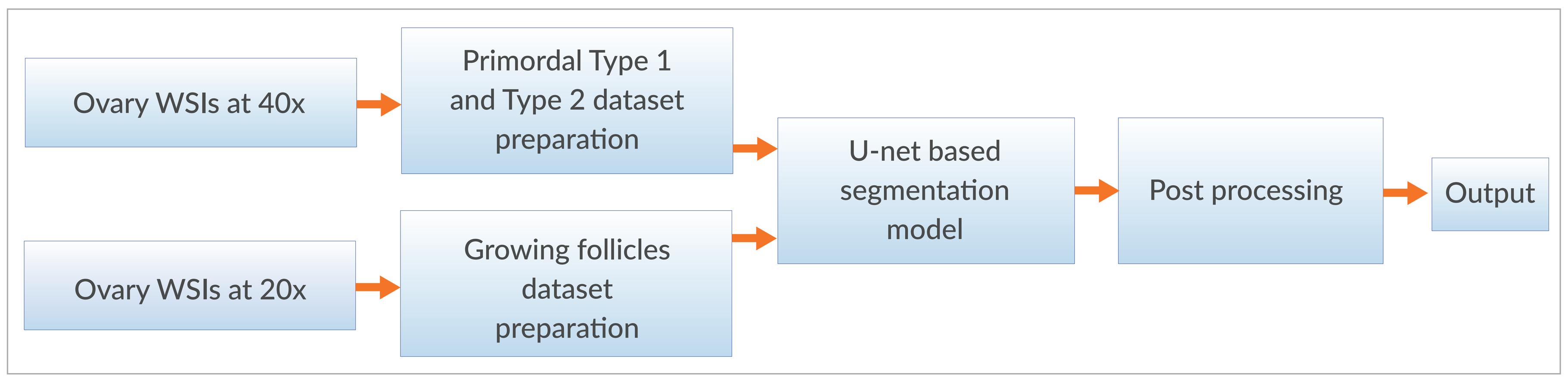

Fig. 7. Outlines the entire algorithmic pipeline for follicle counting

Results

The results of both algorithms correlate very well with the ground truth labels provided by pathologists, with both high sensitivity and specificity.

Oestrus Staging

Stage	Proestrus	Estrus	Metestrus	Diestrus
Sensitivity (%)	92.6	88.5	87.1	100
Specificity (%)	100	97.6	96.2	95.2

Table 3. Performance metrics of our model's prediction with respect to ground truth for oestrus staging

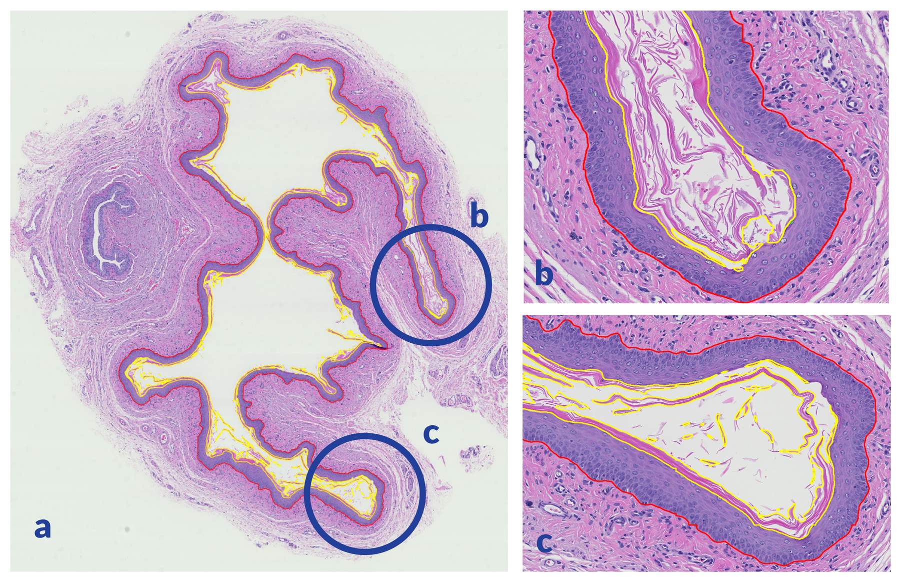

Fig. 8. Shows our final segmented output of vagina with its parameters: (a) Estrus, (b) and (c) represent higher magnification of the epithelial layers of (a)

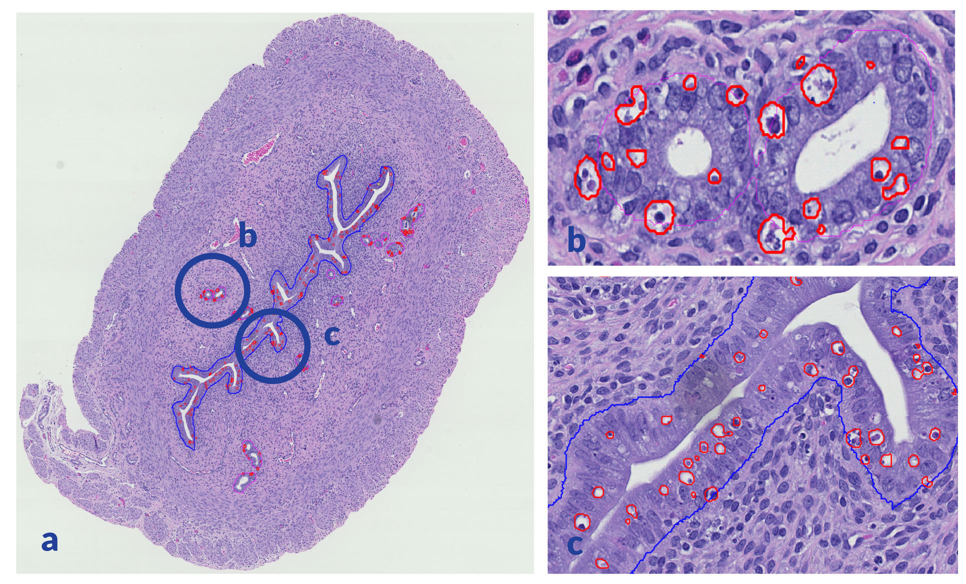

Fig. 9. Shows our final segmented output of uterus with its parameters: (a) Estrus, (b) and (c) represent higher magnification of glands and superficial epithelium of (a)

Follicle Counting

Follicle Type	Primordial follicles Type 1	Primordial follicles Type 2	Growing follicles
Sensitivity (%)	93.7	96.6	96.0
Specificity (%)	93.3	99.5	99.5

Table. 4. Performance metrics of our model's prediction with respect to ground truth for follicle counting

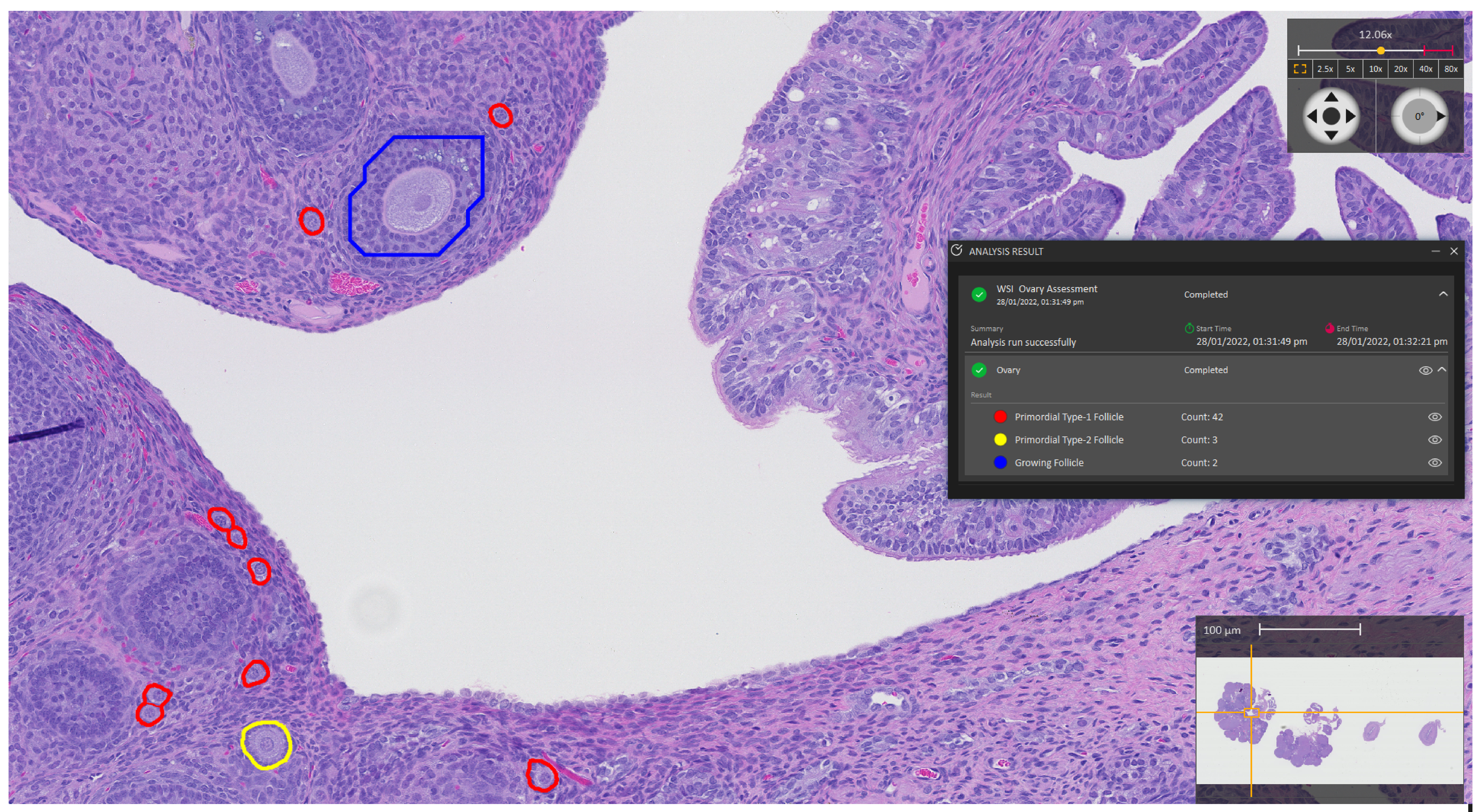

Fig. 10. Shows our final segmented output with the number and type of follicles in our inhouse viewer Airadesk

Conclusion

The deep learning method that we developed was consistent, efficient, and less labour-intensive than manual methods for classifying and quantifying the required parameters for oestrus staging and follicle counting. As a result, our solution helps in bringing workflow efficiency and accuracy for both oestrous staging and follicle counting.

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