Artificial Intelligence based Spermatogenesis Staging to aid Reproductive Toxicology study in Wistar Rat

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OBJECTIVE

- To propose an automated solution for identification of the fourteen stages of spermatogenic cycle in digital images of Wistar rat testes using artificial intelligence based technologies
- To examine the efficiency of the proposed solution in terms of accuracy by comparison with the results of manual staging done by pathologists

INTRODUCTION

Histopathological examination of testicular tissue is considered to be the most sensitive tool to detect toxicological effects on male reproductive function. Regardless of the type of toxicity study, testes should always be examined with an awareness of the spermatogenic cycle to ensure identification of subtle changes. This examination involves classification of seminiferous tubules into different stages of spermatogenic cycle which is a painfully demanding task. We present an automated method to identify the fourteen stages of spermatogenesis in digital images of rat testes using artificial intelligence based technologies. The results of the method are in concordance with that of the manual staging done by pathologists.

MATERIALS AND METHODS

Materials

- Training dataset of 10 Periodic Acid Schiff (PAS) stained whole slide images of Wistar rat testes
- Test dataset of 20 PAS stained whole slide images of Wistar rat testes
- Leica SCN400 scanner for image acquisition

Methods

a) Training, Testing & Fine-Tuning

- Knowledge transfer from domain experts – Normal Spermatogenic Cycle
- Segmentation of seminiferous tubules by training a customized variant of *VGG Net* (deep learning network) on 1200 tiles of size 512x512 at 10x magnification taken from the training data set
- Mapping of segmented tubules from 10x to 40x for accurate detection of various germ cells relevant for characterizing the different stages of the tubules (as per Table 1)
- Germ cells used for staging are *Elongated Spermatids (ESp), Spermatocytes (Spc), Round Spermatids (RSp), Residual bodies (RB), Meiotic bodies (MB)*
- Detection of germ cells using a customized variant of ResNet (deep residual network)
- Tubules in stages 2/3 and 4/5 grouped together due to closely overlapping features
- Stage-frequency maps representing the comparative counts of the tubules into various stages were generated for the test data set

b) Validation

- Algorithm was tested on 20 PAS stained rat testes slide images and the results were verified by the pathologists
- The average stage-frequency map obtained from the above exercise was compared with Chapin et al[1] and Hess et al[2]

Table 1: Characteristic features for stages used in automated staging

Stages	Characteristic Features
1	*ESp heads:* limited bundling; *ESp location:* close to lumen with very few moving towards base; *Spc size:* smaller than stages 2/3
2, 3	*ESp heads:* frequent bundling; *ESp location:* majority in the mid-epithelial region; *Spc size:* smaller than stages 4/5 and 6
4, 5	*ESp heads:* prominent bundling; *ESp location:* majority within lower third of the epithelium; *Spc size:* larger than stages 2/3
6	*ESp heads:* prominent bundling; *ESp location:* majority towards lumen; *Spc size:* larger than stages 4/5
7	*RSp shape:* round; *ESp location:* aligned around the lumen; *RB size:* smaller than stage 8; *RB location:* random with respect to ESp heads
8	*RSp shape:* slightly eccentric; *RB size:* larger than stage 7; *RB position:* mostly below ESp heads
9	*RSp shape:* towards elliptical; *RB location:* may be present in lumen and within epithelium; *No ESp*
10	*RSp shape:* elliptical and starts to elongate; *RB location:* may be present in lower third and base of epithelium; *No ESp*
11	*ESp shape:* banana shape; *RB location:* may lie in the lower third and base of epithelium; *No RSp*
12	*ESp shape:* longer and thinner than stage 11; *Chromatin density of large pachytene* denser than stage 13; *No RSp*
13	*ESp shape:* similar or thinner than stage 12; *Chromatin density of large pachytene* less than stage 12; *No RSp*
14	*ESp shape:* similar to stage 13; *At least one MB; RSp* may also be present

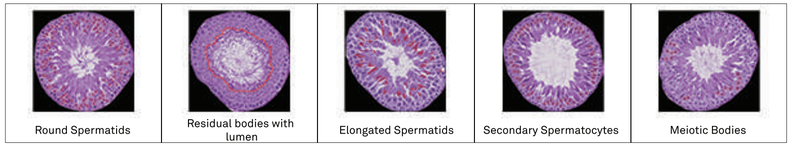

Fig 1: Segmentation Results for various germ cells

RESULTS

- The results of automated staging (a snap-shot depicted in Fig 2) are in concordance with that of the manual staging done by the pathologists
- The software generated average stage frequency map (shown in Fig 3) largely conforms to those of Chapin et al[1] and Hess et al[2].

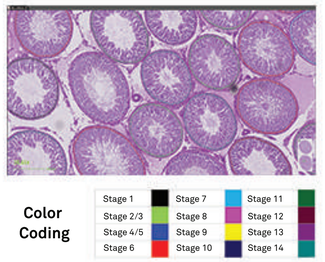

Fig 2: Classification of seminiferous tubules into respective stages using automated staging

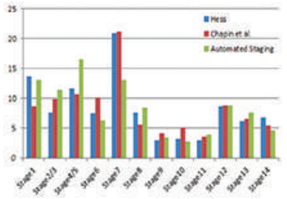

Fig 3: Comparison of stage frequency map with Chapin et al[1] and Hess et al[2]

CONCLUSION/FUTURE DIRECTION

- This automated solution helps in fast and accurate spermatogenesis staging by overcoming the cumbersome manual process.
- This solution can thus act as an effective tool to aid male reproductive toxicology studies in terms of spermatogenesis staging.
- Spermatogenesis staging using similar criteria is under development on H&E stained images.

References

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