Optimization for Hematoxylin and Eosin Staining for Cultured Ovarian Cortical Stripes of *Sus scrofa domesticus*

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Staining is an integral part of modern histopathology and without proper staining histology would be a mere concept. Haemotoxylin and Eosin (H&E) stain have been evolved during last several decades and considered as the golden standard for histological examinations for paraffin or resin embedded animal tissues which have been fixed, processed, embedded and sectioned. It has been widely used and has recognized as the best method to highlight the fine structure of cells without compromising the morphology of the cell. Among many synthetic aniline dyes Haemotoxylin is the only natural dye which is an extract of the Logwood (*Haematoxylum campechianum*). After many attempts of using dyes to stain animal tissues such as ovary from *Sus scorfa domesticus* which is inevitably rich with large fat globules, it has been discovered that traditional methods were not very effective. The objective of current study was to optimize existing H&E staining to achieve better results in high fat cultured tissues than low fat fresh tissues. Briefly, following procedures were adapted with strict time control methods. Deparaffinization of the sections on glass slides was performed. Two dips in xylene for 4 min each and followed by rehydration of the sections with a series of submerge in Ethanol (ETOH). Starting from 100% ETOH two dips followed by 90%, 70%, 50% alcohol and rehydration was completed with a final two dip of distilled water. All these steps were done for 2 min each. Subsequently sections were submerged in Haemotoxylin for 15 min followed by gentle washes with running tap water for another 10 min. Then all slides were subjected to another dip in Eosin for 30 sec. Slides were washed gently again for further 2 min with running tap water. Finally all the slides were dipped at 1-2 sec in 70%, 90%, 100% alcohol serial and xylene respectively. As soon as completion of the last xylene step, cover slips were placed on top of the permount mounting medium which are already placed on the tissue section and allowed the slides to dried-up for 24-36hrs before microscopic observation. In conclusion, it has been discovered double staining of high fat cultured tissue with Haemotoxylin and Eosin with this modified technique has evident best microscopic observations, provided example images showing results produced using modified protocol, as well as commentary on the strengths and limitations of the approach. This study was supported by Sabaragamuwa University Research Grant, SUSL/RG/2015/06.

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