Epitope Retrieval from Thick Endometrial Sections Demonstrating Three Dimensional Relationships of Neuronal Networks in Endometriosis

Frank Manconi, Eleanor PW Kable, Robert Markham, Ian S Fraser

1Queen Elizabeth II Research Institute for Mothers and Infants, Dept of Obstetrics and Gynaecology. 2Australian Centre for Microscopy and Microanalysis, The University of Sydney, NSW, Australia, 2006.
E-mail: frank.manconi@sydney.edu.au

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INTRODUCTION
Detection of unique nerve fibres in endometrium of women with endometriosis\(^1\) has lead to increased interest in studying their relationship with infertility and pain. All studies so far involved in description of these neuronal structures have relied on conventional histological tissue sections, but have rarely studied the networks in 3-dimensions. Biological systems exist and operate in three-dimensional surroundings; therefore it makes good sense to gain access to three-dimensional quantitative information, based upon three-dimensional projections.

METHODOLOGY
We utilised thick (≥50µm) formalin-fixed endometrial sections, low temperature, heat-induced epitope retrieval, immunofluorescence and Laser Scanning Confocal Microscopy in order to visualize and quantitatively assess three-dimensional relationships of endometrial neuronal structures. Polyclonal antibodies [Rabbit anti-PGP9.5 and rabbit anti-goat tetramethylrhodamine (TRITC)] were used to target and visualize neuronal structures and monoclonal antibodies [mouse anti-human cytokeratin and mouse anti-goat fluorescein isothiocyanate (FITC)] were employed to label and visualize epithelial tissue. Digital images were acquired using a Nikon Eclipse E800 microscope equipped with laser attachment.

RESULTS
Computationally reassembled image stacks utilising ImarisBasic 4.1 visualisation suite provided a three-dimensional reconstruction of neuronal architecture and glandular epithelium of uterine endometrial sections. Additionally, demonstrated that utilisation of a lower temperature (60°C) combined with a longer time period (18-24 hours) has improved the retrieval method for two antibodies requiring heat-induced epitope retrieval, (cytokeratin and Protein gene product 9.5) without altering morphology.

DISCUSSION
This protocol continued previous three-dimensional reconstructive work illustrating the endometrial microvasculature\(^2\)\(^-\)\(^3\). Previous studies have indicated that blood vessels and nerve fibres course throughout the body in an orderly pattern, often alongside one another\(^7\) but may not do so in endometrium of women with endometriosis. Three dimensional neuronal relationships may be involved in pin generation and infertility mechanisms in endometriosis.

REFERENCES