J Forensic Sci, September 2006, Vol. 51, No. 5
doi:10.1111/j.1556-4029.2006.00234.x
Available online at: www.blackwell-synergy.com

TECHNICAL NOTE

Sarah K. Paterson,[1,2,3] M.Sc.; Cynthia G. Jensen,[2] Ph.D.; Susan K. Vintiner,[3] B.Sc.(Hons); and Susan R. McGlashan,[2] Ph.D.

Immunohistochemical Staining as a Potential Method for the Identification of Vaginal Epithelial Cells in Forensic Casework*

ABSTRACT: There is currently no accurate method to identify vaginal epithelial cells uniquely. This study aimed to use a cell extraction procedure compatible with routine forensic sampling methods, and to investigate the expression of cytokeratin (CK), estrogen receptor-α (ERα), and phosphodiesterase 5 (PDE5) in order to distinguish between skin, buccal, vaginal, and external penile epithelial cells. Seminal fluid samples were also examined. Epithelial cell samples were fixed in formalin, embedded in agarose, and processed using histological methods. Antigen–antibody reactions were detected using the DAKO Envision+™ detection system. CK was present in all cells from all five sources confirming the origin of cells as epithelial. Both ERα and PDE5 positively labeled vaginal, buccal, and skin epithelial cells. Although an antigen unique to vaginal epithelial cells was not identified, we have described a cell extraction procedure for use in the immunohistochemical detection of a wide range of antigens, an approach compatible with forensic diagnostics.

KEYWORDS: forensic science, immunohistochemistry, epithelial cells, vagina, buccal, skin, cytokeratins, estrogen receptor-α, phosphodiesterase 5

It is currently not possible to distinguish specifically between skin, buccal, vaginal, and penile epithelial cells using either cytological approaches or DNA profiling techniques. Epithelial cells from the skin can be presumptively identified due to their lack of nuclei (1). However, shed buccal and vaginal cells, obtained from a crime scene sample for example, are morphologically indistinguishable when examined using standard histological stains such as hematoxylin and eosin (H&E) (2). In fact, vaginal and buccal cells are so similar that vaginal epithelial cells have been used as a model of buccal epithelium in studies of epithelial transport (3,4).

There are a number of cases in which the forensic identification of vaginal epithelial cells could provide important probative evidence (5). For example, in a case of alleged sexual assault, the defense proposes that female DNA recovered from a bottle originated from the complainant's buccal epithelial cells and were deposited when she drank from the bottle. However, the prosecution argues that the DNA originated from vaginal epithelial cells transferred at the time of the alleged sexual violation. This type of scenario is encountered in forensic investigations, and demonstrates a requirement for a method to distinguish between epithelial cells from different areas of the body. Although several studies have reported methods for the identification of vaginal cells and/or secretions, for example, Lugol's Iodine (6–8) or protein and ion detection (9–18), none show specificity to vaginal epithelial cells and are therefore unsuitable to use as evidential support in a court of law.

Immunohistochemistry (IHC) is a routine diagnostic technique used in surgical pathology. This technique can be used to detect antigens in samples that contain very few cells and is compatible with DNA extraction techniques currently used in routine forensic casework. A recent forensic study used IHC to identify a monoclonal antibody for a sweat-specific protein (19). IHC was also applied to detect brain tissue on a defendant's shirt during a double homicide investigation (20,21).

Therefore, an immunohistochemical approach may be suitable for identifying differential antigen expression between epithelial cells from different body sites/sources. The study aimed to modify an existing forensic procedure for extracting cells from cotton swabs and then to use this protocol to investigate several candidate proteins that may identify a marker unique to vaginal epithelial cells. We believe this approach will provide forensic scientists with a simple and reliable method for the detection of a wide range of antigens and the identification of specific cell types and body fluids.

Methods

Collection of Cell Samples

Cotton tip swabs (Biolab, Buckland, New Zealand) were used for the collection of cell samples. During all procedures, volunteers were asked to wash their hands before sampling. Researchers

[1]Department of Chemistry, University of Auckland, Auckland, New Zealand.
[2]Department of Anatomy with Radiology, University of Auckland, Auckland, New Zealand.
[3]Forensic Biology Group, Institute of Environmental Science & Research Limited (ESR), Auckland, New Zealand.

*This study was presented as a poster at the 17th International Symposium of The Australian and New Zealand Forensic Science Society, April 2004, Wellington, New Zealand.

Received 8 Jan. 2006; and in revised form 7 May 2006; accepted 20 May 2006; published 15 Sept. 2006.

involved in the study wore disposable latex gloves during all subsequent handling of samples. Five female volunteers each provided two swabbings from the internal wall of the vagina, the buccal mucosa of the cheek, and skin from the underside of the forearm. To obtain cells of male origin, which are likely to be found in forensic samples containing vaginal epithelial cells, five male volunteers each provided two swabbings from the external penile shaft. Samples of seminal fluid were obtained from six male volunteers (Fertility Associates Laboratory, Auckland, New Zealand). All samples were collected according to guidelines approved by the University of Auckland Human Subjects Ethics Committee (Reference #: 2003/081).

Epithelial Cell Extraction and Identification

To ensure optimal morphology and antigenicity, epithelial cells were extracted from one set of swabs within 1–4 h of collection. In order to mimic processing times within a crime scene, a second set of swabs was left at room temperature for 48 h before extraction. For all samples, the swab head was subsequently removed using a sterile scalpel blade and placed in a 1.5 mL centrifuge tube containing 1 mL distilled water and allowed to sit for 15 min. The swab head was removed from the tube and the remaining contents were centrifuged at 13,800 × g for 15 min. The supernatant was removed and the pellets containing cells were fixed in 10% neutral-buffered formalin (NBF) for 1 h. Seminal fluid samples were fixed immediately after collection in 10% NBF. All fixed epithelial cells and seminal fluid samples were centrifuged for 5 min at 525 × g to form a pellet, which was mixed with molten 4% (w/v) agarose (Type IX-A: ultra-low gelling temperature, Sigma-Aldrich, Auckland, New Zealand) and subsequently centrifuged for 2 min at 82 × g. The cell-agarose mix was allowed to set for 15 min at 4°C to form a cell-agarose block termed a cellblock. The cellblocks were removed from the centrifuge tube and placed in a lidded embedding histology cassette (Techno-Plas, Dandenong South, Australia). The cassette was transferred to 10% NBF and processed according to a histological procedure involving dehydration in an alcohol series, wax impregnation, clearing in xylene, and embedding in paraffin wax. The details of this procedure are presented in Table 1. Four-micrometer-thick sections were used for all experimental conditions (22).

Sections from epithelial cellblocks were stained with routine H&E, and sections from seminal fluid cellblocks were stained with the Christmas tree stain (23). If cells were absent from the set of sections, the specific cellblock was removed from subsequent IHC procedures.

IHC

Four-micrometer-thick sections were cut and transferred onto poly-L-lysine-coated slides. Slides were immersed in preheated antigen retrieval solution (see Table 2) and heated either in a microwave at power level 10 for 2 × 5 min or placed in a pressure cooker at 15 psi for 1 min. The slides were allowed to cool and washed in running tap water for 5 min. Sections were incubated with 0.03% H_2O_2 (EnVision+ System, DakoCytomation Botany, NSW, Australia) for 5 min to block unspecific peroxidase activity. Sections were then washed with phosphate-buffered saline (0.1 M; PBS) for 3 × 5 min and incubated with primary antibody (see Table 2) for 30–60 min at room temperature. Each antibody was tested in duplicate. Primary antibodies were detected using the EnVision+System (the commercially available kit utilizes chain polymer-conjugated technology that reduces the amount of primary antibody and incubation times required). Sections were washed with PBS for 3 × 5 min and subsequently incubated for 30 min with labeled polymer. Sections were washed with PBS for 3 × 5 min and incubated with the 3,3′-diaminobenzidine (DAB) substrate-chromagen solution (EnVision+ System, DakoCytomation) for 5–10 min and rinsed in distilled water. Sections were counterstained with 0.4% hematoxylin to provide contrast and mounted in DEPEX (Ajax Finechem, Auckland, New Zealand). Positive control tissue used in this study was obtained from Novacastra (Newcastle-Upon-Tyne, U.K.) and is detailed in Table 1. For internal negative controls, the primary antibody was substituted with PBS. Swabs that had been fixed following storage at room temperature for 48 h were processed identically. The expression of cytokeratin (CK) (AE1/AE3) antibody was tested on these delayed fixation samples only.

Sections were examined using bright-field microscopy using either a × 40 or a × 60 lens (Nikon Eclipse E800 microscope, Nikon, Auckland, New Zealand), and images were captured using a Nikon digital camera DXM1200F (Nikon). Positive cells were characterized by orange/brown DAB labeling, whereas negative cells showed no labeling. Results were expressed as the percentage of samples containing positively labeled cells within each epithelial cell type.

TABLE 1—*Histological processing schedule used in the current study.*

Fluid	Time (min)
Formalin	Holding
70% ethanol	30
80% ethanol	30
95% ethanol	30
95% ethanol	30
Absolute ethanol	30
Absolute ethanol	30
Xylene	30
Xylene	30
Paraffin wax	15
Paraffin wax	15

TABLE 2—*Antibodies, corresponding control tissue, and the experimental conditions used in this study.*

Antibody	Antigen Retrieval	Dilution	Positive Control Tissue	Source of Antibody
Anti-human CK (clone AE1/AE3)	Microwave	1:100	Skin or Glandular epithelium	Dako Cytomation
Anti-human CK5 (clone XM26)	Pressure cooker	1:200	Skin or Glandular epithelium	Novocastra, U.K.
Anti-human ERα (clone 1D5)	Microwave	1:35	Breast	Dako Cytomation
Anti-human PDE5	Microwave	1:100	Lung	Dr. Mauro Giorgi, University of L'Aquila, L'Aquila, Italy

CK, cytokeratin; ERα, estrogen receptor-α; PDE5, phosphodiesterase 5.

Results

All epithelial samples and seminal fluid samples were examined for cells. One skin (1/5) and one external penile swab (1/5) did not contain cellular material and were removed from further testing. In all H&E-stained sections, both agarose and fibers from the cotton swab were identified (Fig. 1; asterix and arrowheads). Fibers were pale pink with dark blue refractile spots and ranged from

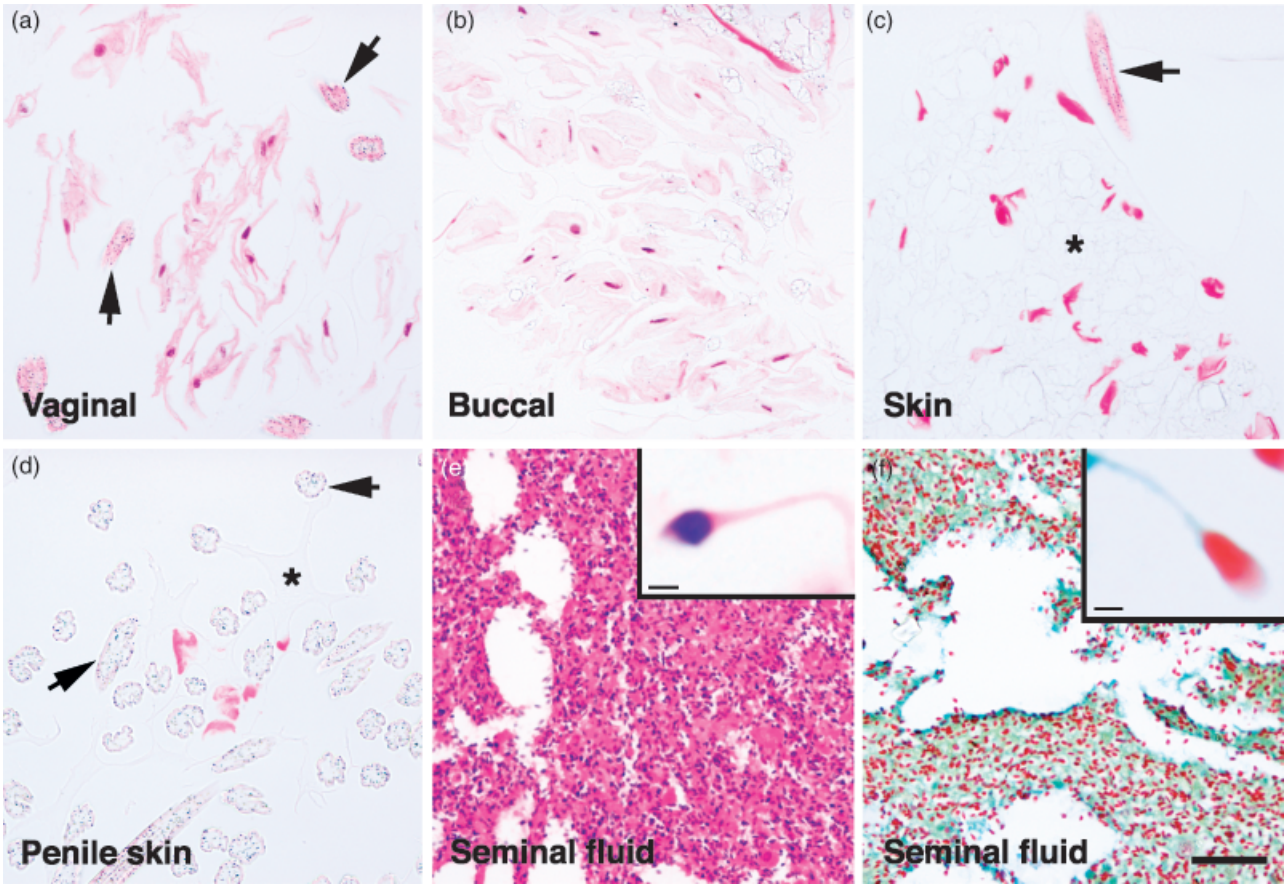

FIG 1—*Hematoxylin and eosin-stained sections from the (A) vagina, (B) buccal, (C) skin, (D) penile skin, and (E) seminal fluid. Samples also contain fibers from cotton-tipped swabs (arrows). Agarose within each sample can also be identified (*). (F) Spermatozoa stained using the Christmas tree method. All images were obtained at the same magnification. Scale bar = 40 μm. (E inset) Spermatazoan heads stain blue/purple and tails pink. (F inset) Spermatazoan heads stain red and tails blue-green. Scale bars = 5 μm.*

20 to 50 μm in length. In both vaginal and buccal samples, cells stained with a pale pink cytoplasm and purple/blue nuclei (Fig. 1*A* and *B*), whereas skin epithelial cells stained intense pink and there was no evidence of nuclei (Fig. 1*C*). Similarly, the cytoplasm of penile skin cells stained intense pink and no nuclei were detected (Fig. 1*D*). Seminal fluid samples contained spermatozoa. Sperm heads stained with H&E were purple/blue, and the tails stained pale pink (Fig. 1*E* and inset). With the Christmas tree stain, sperm heads were red and the tails blue-green (Fig. 1*F* and inset).

A broad-spectrum CK antibody, AE1/AE3, and an antibody specific to CK5 were used to classify cells as being of epithelial origin. All vaginal, buccal, and skin epithelial cell samples comprised cells positive for AE1/AE3 (Fig. 2*A*–*C*) and CK5 (data not shown), and the pattern of staining for the two antibodies was identical. AE1/AE3 (Fig. 2*D*)- and CK5 (data not shown)-positive cells were also present in 55% and 22% of the external penile epithelial cell samples, respectively (see Table 2). Seventeen percent of seminal fluid samples contained "squamous" cells that were AE1/AE3 (Fig. 2*E*) and CK5 positive. In positive control tissue, epithelial cells present in skin labeled positively. All internal negative controls showed no brown labeling.

Estrogen receptor-α (ERα) was not detected in any of the epithelial cell samples including "squamous" cells from seminal fluid samples (images not presented; see Table 2). However, ERα was expressed in the tails of spermatozoa present in seminal fluid samples. Labeling was present in the corresponding skin-positive control tissue (data not shown). In contrast, phosphodiesterase 5 (PDE5) was expressed in the cytoplasm of all female and male epithelial cell samples and the "squamous" cells in seminal fluid samples. The pattern of staining was the same as that for AE1/AE3 and therefore the images are not presented. However, PDE5 was also present in sperm tails. A summary of the proportion of positive samples using each antibody is presented in Table 3.

The antigenicity of the cell samples using AE1/AE3 was not altered following a 48 h delay in fixation. All cells labeled positively and there was no difference between the intensity of staining of the cells fixed within 1–4 h compared with cells that were fixed 48 h later.

Discussion

The major aim of this study was to modify an existing forensic cell extraction procedure that was compatible with collection methods used at crime scenes and within a forensic laboratory, and for use within a valid immunohistochemical protocol. A second aim was to use this procedure to identify an antigen unique to vaginal epithelial cells compared with buccal and skin. Samples were collected with cotton tip swabs because larger amounts of cellular material can be retrieved using this collection method compared with other methods such as cell smearing and scraping (24,25). Cotton swabs are routinely used for evidential collection by forensic scientists and by doctors when examining sexual assault victims and suspects. Although alternative processing methods such as direct smears have been reported for use in

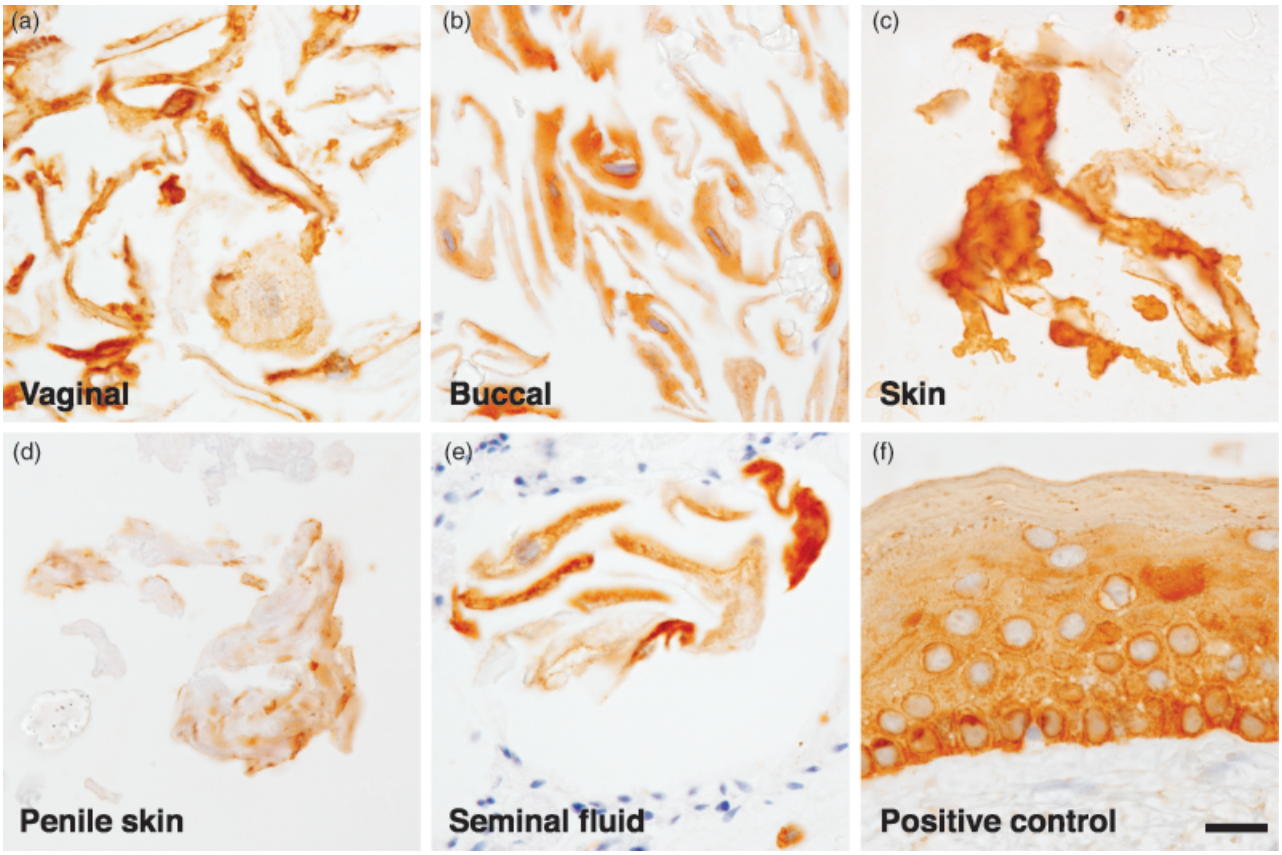

FIG 2—*Cytokeratin expression (orange/brown labeling) in the cytoplasm of (A) vaginal, (B) buccal, (C) skin, (D) penile skin, (E) seminal fluid, and (F) positive control epithelial cells of skin using anti-human broad-spectrum cytokeratin (clone AE1/AE3). (E) Although no cytokeratin expression is present in spermatozoa, squamous-epithelial-like cells show positive labeling in seminal fluid samples. All images were obtained at the same magnification. Scale bar = 10 μm.*

immunohistochemical studies, there are many reports of variable or poor preservation of cell morphology and antigenicity (26–29). The methods of cell collection and processing into cellblocks used in the current study provided samples, in the majority of cases, that contained many cells and showed good cell morphology. In particular, nuclei were clearly identified in buccal and vaginal sections and spermatozoa were structurally intact (see Fig. 1). Fibers and agarose also stained using H&E (see Fig. 1) but were easily identified and subsequently excluded from further examination. The use of cellblocks also allowed multiple immunohistochemical labeling with several different antibodies. Cellblocks can also be stored for many years, which is suitable for retrospective forensic investigations. An adapted histological processing schedule was developed with increased incubation times in paraffin wax compared with standard procedures, to allow adequate impregnation.

CKs are one of the three types of intermediate filaments that constitute the cytoskeleton of epithelial cells. As different epithelia express different CK isoforms (30), all epithelia can be classified based upon CK expression (31). Anti-human broad-spectrum CK antibody (clone AE1/AE3) identifies the majority of human CKs (31,32) and therefore was used in this study to identify all epithelia. Cells can be further classified as *stratified* epithelia with the use of anti-CK5, which was positive in all epithelial cells examined in the present study. However, CK5 expression has been reported in other cell types (31) and therefore may not be suitable in forensic classification where the health, age, and environmental exposure of cells are unknown. Interestingly, both antibodies to CK labeled the squamous cells in seminal fluid samples, suggesting that these cells may also be of epithelial origin and are likely to have originated from the lining of the urethral tract.

Antibodies to ERα and PDE5 were selected as potential markers for differentiating vaginal epithelial cells from buccal and skin epithelial cells. The selection of these proteins was based on previous reports of their expression in different sites of the body. ERs are ligand-dependent proteins and their primary function is to mediate the transcriptional response of estrogen in target cells

TABLE 3—*Summary of the percentage (%) of epithelial cell samples that comprised positively labeled cells using four different antibodies.*

Antigen	Vaginal	Buccal	Skin	Penile Skin	Spermatozoa in Seminal Fluid	Squamous Cells in Seminal Fluid
Cytokeratin (AE1/AE3)	100	100	100	55	0	17
CK 5	100	100	100	22	0	17
ERα	0	0	0	0	33	0
PDE5	100	100	100	60	66	0

CK, cytokeratin; ERα, estrogen receptor-α; PDE5, phosphodiesterase 5.

(33,34). ERα expression in the vagina has been reported as being restricted to the basal, parabasal, and intermediate cell layers and not present in the nuclei of superficial vaginal epithelial cells (35,36). It is, however, absent from the buccal mucosa (37) and epidermis (34), consistent with the finding of the current study. Similarly, ERα was not present in vaginal samples, suggesting that these cells were likely to have originated from the superficial layers only. ERα was, however, detected in spermatazoan tails although some seminal samples did not show any positive labeling likely due to a loss in viability resulting from a delay in fixation of samples obtained from the fertility clinic.

PDE5 belongs to the 3′, 5′ cyclic nucleotide phosphodiesterase family and is a regulator of cyclic guanosine monophosphate (cGMP) function (38–41). In the current study, PDE5 was present in vaginal cells, confirming reports that it is expressed in superficial vaginal cells (42). However, there are no reports on the expression of this enzyme in either buccal mucosa or epidermis. Although this study is the first to report PDE5 expression in buccal and skin epithelia, its expression in all epithelial samples tested therefore makes it unsuitable for differentiating one cell type from another.

Delayed fixation is an important consideration in forensic science due to the history and nature of case samples involved in these investigations. Case samples, which may contain vaginal epithelial cells, are often received in the forensic laboratory several days after the event under investigation. We show, for CK labeling at least, that the antigenicity of all epithelial samples is not affected by a 48 h delay to fixation. If further investigations identify a unique vaginal epithelial cell marker, delayed fixation studies would have to form part of the validation for use of that antibody in forensic studies.

In conclusion, we have developed a cell collection technique for IHC, which is compatible with current forensic science practice. We have also shown that a delay in fixation of samples does not alter antigenicity of epithelial cell samples. The study further examined the expression of several proteins that may be suitable for distinguishing between vaginal, buccal, and skin epithelial cells for forensic casework. However, antibodies to ERα and PDE5 were unable to uniquely identify vaginal cells. Further investigations using the cell extraction and IHC protocols developed in this study may lead to the discovery of an antigen unique to vaginal epithelial cells.

Acknowledgments

The authors would like to thank Dr. Mauro Giorgi for the generous gift of PDE5 antibody, and Jacqui Ross for her assistance with images and plates. We thank the Biomedical Imaging Research Unit, Department of Anatomy with Radiology, University of Auckland, for technical support and advice.

References

1. Burkitt HG, Young B, Heath JW, editors. Wheater's functional histology. A text and colour atlas. 3rd ed. London: Churchill Livingstone, 1994.
2. Ziskin D, Moulton R. A comparison of oral and vaginal epithelial smears. J Clin Endocrinol 1948;8:146–65.
3. van der Bijl P, Thompson IO, Squier CA. Comparative permeability of human vaginal and buccal mucosa to water. Eur J Oral Sci 1997; 105(6):571–5.
4. van der Bijl P, van Eyk AD, Thompson IO. Permeation of 17beta-estradiol through human vaginal and buccal mucosa. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 1998;85(4):393–8.
5. Juusola J, Ballantyne J. Messenger RNA profiling: a prototype method to supplant conventional methods for body fluid identification. Forensic Sci Int 2003;135(2):85–96.
6. Randall B, Riis RE. Penile glycogenated epithelial cells as an indicator of recent vaginal intercourse. Am J Clin Pathol 1985;84(4):524–9.
7. Randall B. Glycogenated squamous epithelial cells as a marker of foreign body penetration in sexual assault. J Forensic Sci 1988;33(2):511–4.
8. Rothwell TJ, Harvey KJ. The limitations of the Lugol's iodine staining technique for the identification of vaginal epithelial cells. J Forensic Sci Soc 1978;18(3–4):181–4.
9. Willott GM. L-tartrate inhibitable acid phosphatase in semen and vaginal secretions. J Forensic Sci Soc 1972;12(2):363–6.
10. Itoh Y. Analysis of human vaginal secretions using rabbit anti-human vaginal secretions: detection of non-serum proteins. Nippon Hoigaku Zasshi 1990;44(4):267–71.
11. Itoh Y, Furuhata A, Sato Y. Immunohistochemical studies on the localization of non-serum proteins detectable in human vaginal secretions. Nippon Hoigaku Zasshi 1991;45(1):26–9.
12. Itoh Y, Manaka M. Analysis of human vaginal secretions by SDS-polyacrylamide gel electrophoresis. Forensic Sci Int 1988;37(4):237–42.
13. Tsutsumi H, Kido A, Katsumata Y, Sato K, Oya M, Yada S. An immunological study of human nasal and vaginal secretions. Act Crim Japon 1982;48(3):121–6.
14. Blake ET, Cook CE Jr., Bashinski JS. Evidence that "vaginal peptidase" is a bacterial gene product. J Forensic Sci 1987;32(4):888–99.
15. Divall GB. Methods for the identification of menstrual blood. In: Lee HC, Gaensslen RE, editors. Advances in forensic science. Foster City: Biomedical Publications, 1985:1–14.
16. Divall GB. The application of electrophoretic techniques in the field of criminology. Electrophoresis 1985;6:249–58.
17. Adams EG, Wraxall BG. Phosphatases in body fluids: the differentiation of semen and vaginal secretion. Forensic Sci 1974;3(1):57–62.
18. Martin PD, Cheshire SK. A comparative study of the citrate and lactate concentrations in stains from semen, vaginal secretion and mixtures of the two using isotachophoresis. In: Brinkmann B, Henningsen K, editors. Advances in forensic haemogenetics 1. Berlin: Springer-Verlag, 1986: 299–301.
19. Sagawa K, Kimura A, Saito Y, Inoue H, Yasuda S, Nosaka M, et al. Production and characterisation of a monoclonal antibody for sweat-specific protein and its application for sweat identification. Int J Legal Med 2003;117:90–5.
20. Miller RT. Focus on immunohistochemistry—tissue protection immunohistochemistry. Dallas: ProPath Laboratory, 2002.
21. Miller RT, Grantham R, Lockett B, Temple-Camp C, Pang J. Immunohistochemical analysis of forensic evidence from a double homicide. Am J Forensic Med Pathol 2002;23(4):321–5.
22. Bancroft J, Stevens A, editors. Theory and practice of histological techniques. 4th ed. New York: Churchill Livingston, 1996.
23. Allery JP, Telmon N, Mieusset R, Blanc A, Rouge D. Cytological detection of spermatozoa: comparison of three staining methods. J Forensic Sci 2001;46(2):349–51.
24. Bright J. Forensic analysis of trace DNA [masters]. Auckland: University of Auckland, 2000.
25. Sweet D, Lorente M, Lorente JA, Valenzuela A, Villanueva E. An improved method to recover saliva from human skin: the double swab technique. J Forensic Sci 1997;42(2):320–2.
26. Dalquen P, Sauter G, Epper R, Kleiber B, Feichter G, Gudat F. Immunocytochemistry in diagnostic cytology. Recent Results Cancer Res 1993;133:47–80.
27. Nadji M, Ganjei P. Special report. Immunocytochemistry in diagnostic cytology: a 12 year perspective. Am J Clin Pathol 1990;94(4): 470–5.
28. Shield PW, Perkins G, Wright RG. Immunocytochemical staining of cytologic specimens. How helpful is it? Am J Clin Pathol 1996;105(2): 157–62.
29. Suthipintawong C, Leong AS, Vinyuvat S. Immunostaining of cell preparations: a comparative evaluation of common fixatives and protocols. Diagn Cytopathol 1996;15(2):167–74.
30. Reis-Filho JS, Simpson PT, Martins A, Preto A, Gartner F, Schmitt FC. Distribution of p63, cytokeratins 5/6 and cytokeratin 14 in 51 normal and 400 neoplastic human tissue samples using TARP-4 multi-tumor tissue microarray. Virchows Arch 2003;443(2):122–32.
31. Chu PG, Weiss LM. Keratin expression in human tissues and neoplasms. Histopathology 2002;40(5):403–39.
32. Merne M, Syrjanen S. The mesenchymal substrate influences the epithelial phenotype in a three-dimensional cell culture. Arch Dermatol Res 2003;295:190–8.
33. McKenna NJ, Lanz RB, O'Malley BW. Nuclear receptor coregulators: cellular and molecular biology. Endocr Rev 1999;20(3):321–44.

34. Thornton MJ. The biological actions of estrogens on skin. Exp Dermatol 2002;11(6):487–502.
35. Wolf H, Wandt H, Jonat W. Immunohistochemical evidence of estrogen and progesterone receptors in the female lower urinary tract and comparison with the vagina. Gynecol Obstet Invest 1991;32(4):227–31.
36. Press MF, Nousek-Goebl NA, Bur M, Greene GL. Estrogen receptor localization in the female genital tract. Am J Pathol 1986;123(2):280–92.
37. Leimola-Virtanen R, Pennanen R, Syrjanen K, Syrjanen S. Estrogen response in buccal mucosa—a cytological and immunohistological assay. Maturitas 1997;27(1):41–5.
38. Corbin JD, Turko IV, Beasley A, Francis SH. Phosphorylation of phosphodiesterase-5 by cyclic nucleotide-dependent protein kinase alters its catalytic and allosteric cGMP-binding activities. Eur J Biochem 2000;267(9):2760–7.
39. Kotera J, Fujishige K, Omori K. Immunohistochemical localization of cGMP-binding cGMP-specific phosphodiesterase (PDE5) in rat tissues. J Histochem Cytochem 2000;48(5):685–93.
40. Lin CS, Lau A, Tu R, Lue TF. Expression of three isoforms of cGMP-binding cGMP-specific phosphodiesterase (PDE5) in human penile cavernosum. Biochem Biophys Res Commun 2000;268(2):628–35.
41. Soderling SH, Beavo JA. Regulation of cAMP and cGMP signaling: new phosphodiesterases and new functions. Curr Opin Cell Biol 2000;12(2):174–9.
42. D'Amati G, di Gioia CR, Bologna M, Giordana D, Giorgi M, Dolci S, et al. Type 5 phosphodiesterase expression in the human vagina. Urology 2002;60(1):191–5.

Additional information and reprint requests:
Susan R. McGlashan, Ph.D.
Department of Anatomy with Radiology
University of Auckland
Private Bag 92019
Auckland, New Zealand
E-mail: s.mcglashan@auckland.ac.nz