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ORIGINAL ARTICLE

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The forensic value of the immunohistochemical detection of oestrogen receptors in vaginal epithelium

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Abstract This study investigated whether oestrogen receptors can be immunohistochemically detected in paraffin-embedded vaginal mucosa samples using monoclonal antibodies and whether the method would be suitable for the identification of vaginal cells in cytological smears. Samples of vaginal mucosa were obtained from living females and female corpses, as vaginal smears. For comparison purposes, resected prepuce samples and samples of postmortem male urethral mucosa were also investigated. Nuclear oestrogen receptors could be regularly detected in the basal, parabasal and deeper intermediate epithelium layers of freshly fixed vaginal mucosa but proved to be highly sensitive to autolytic changes. In the cytological smears obtained from living subjects, no oestrogen receptors were detectable. Oestrogen receptors were also detected in the basal epidermal cells of the male prepuce.

Key words Oestrogen receptor · Vaginal epithelium · Immunohistochemistry · Forensic value

Introduction

The morphological detection of vaginal cells in stains or penile swabs continues to be problematical. Systematic investigations have shown that the Lugol's staining method can no longer be used as proof of the presence of vaginal cells on penile swabs from a suspected sexual offender, as glycogen-containing squamous epithelial cells can also be found in the male urethral mucosa [9–11]. A reliable method for the specific morphological identification of vaginal epithelial cells is not yet available. In recent years reports have been published demonstrating that oestrogen receptors are found not only in breast cancer tissue and in tissue obtained from the upper female genital tract, but also in the vaginal mucosa [8, 15, 18, 25, 26]. Therefore we investigated whether oestrogen receptors can be immunohistochemically detected in paraffin-embedded vaginal epithelium and whether the method would be suitable for the cytological detection of vaginal cells in cytological smears with regard to forensic investigations.

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Material and methods

In an initial study we examined 20 mucosal biopsies obtained from gynaecology patients between 17 and 71 years old. Indications for biopsy were suspicious inflammatory lesions of the cervix uteri and/or vagina. Only such smears without any pathological changes were included in this study. The phase of the menstrual cycle and the use of exogenous hormones were recorded. Immediately after sampling the biopsy specimens, measuring up to about 0.3 cm in diameter, were fixed for 24 h in 4% buffered formalin and embedded in paraffin. For the immunohistochemical detection of oestrogen receptors using the streptavidin/biotin-peroxidase method, we employed specific monoclonal IgG 1 mouse antibodies (Immunotech, # 1344) and detection with the Universal Peroxidase detection kit (Immunotech, # 059). After prior examination, samples were incubated with primary antibody for 12 h at room temperature. The remaining detection parameters and the internal controls, complied with the recommended protocols. Freshly fixed breast cancer tissue was employed as the external positive control material. In a second study ten postmortem samples of vaginal mucosa and endometrium (uterine corpus) were investigated. The age of these subjects was between 16 and 56 years, the postmortem interval varied between 6 and 48 h, and the mean fixation duration was 32 h (4% buffered formalin). A third series comprised 160 cytological vaginal swabs from healthy women aged between 24 and 73 years (screening investigations, n = 40) obtained using cotton-tipped swabs or cyto-brushes. In each case, one swab was air-dried, and three others were fixed in isopropanol (98%), isopropanol formalin solution (1:1) or in acetone (at 4° C). The preparations were incubated for 90 min with undiluted primary antibody (Immunotech, # 1344) at room temperature, and further processed according to the recommended protocols.

Freshly fixed resected prepuce samples (n = 5) with a mean fixation duration similar to the female epithelium and serial sections taken from the fossa navicularis and pars cavernosa of formalin-fixed postmortem male urethral mucosa (n = 2) served as control material.

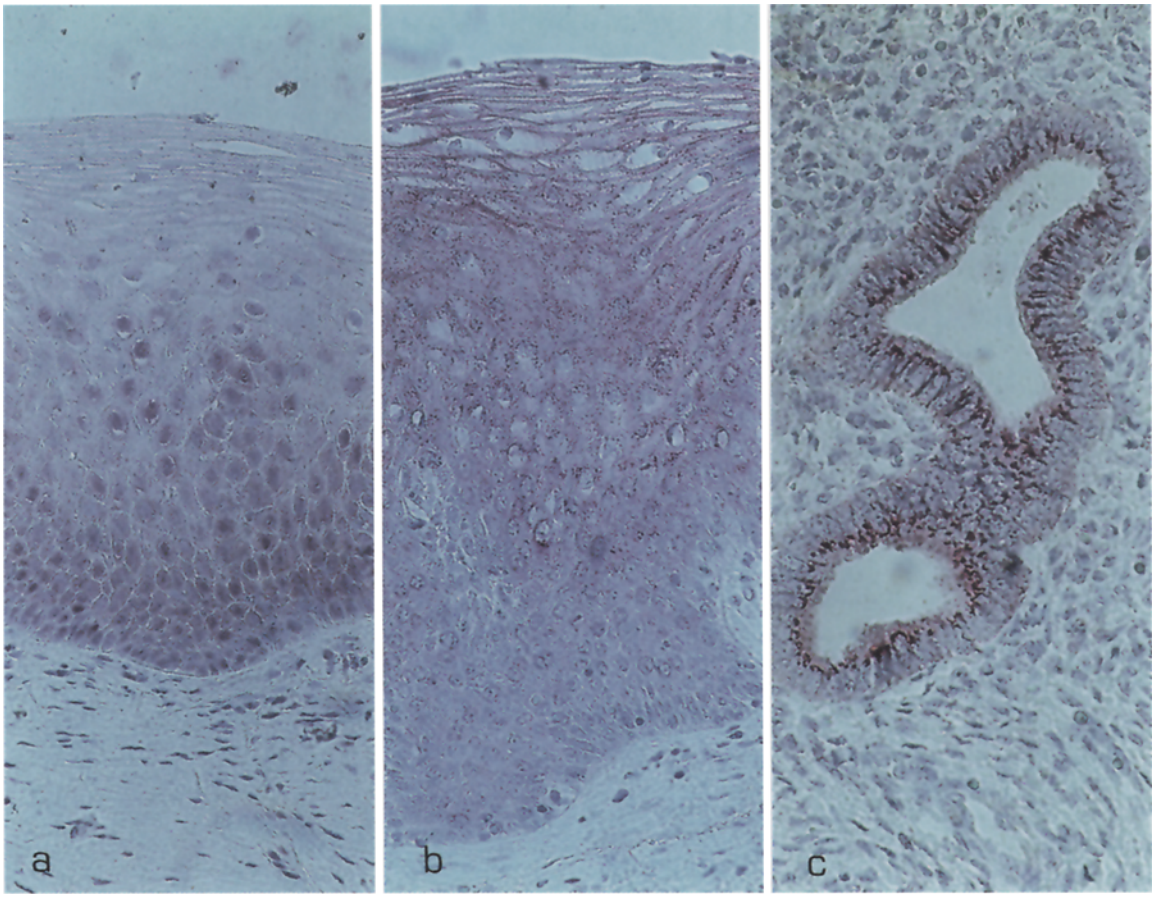

Fig. 1 Immunohistochemical staining of oestrogen receptors in paraffin-embedded female epithelium by use of monoclonal antibodies (streptavidin/biotin-peroxidase method, AEC-staining, approx. × 250) **a**) Freshly fixed vaginal mucosa sample obtained from a 25-year-old woman: intranuclear oestrogen receptors in the basal, parabasal, and deep intermediate epithelial layers. Receptor-free superficial cells. **b**) Postmortem vaginal tissue obtained from a 37-year-old woman (postmortem interval 8 h): diffuse, partly fine granular staining of the vaginal epithelium sharply demarcated from the unstained stroma. **c**) Postmortem endometrial sample of the same case: scattered nuclear staining for oestrogen receptor in glandular epithelium

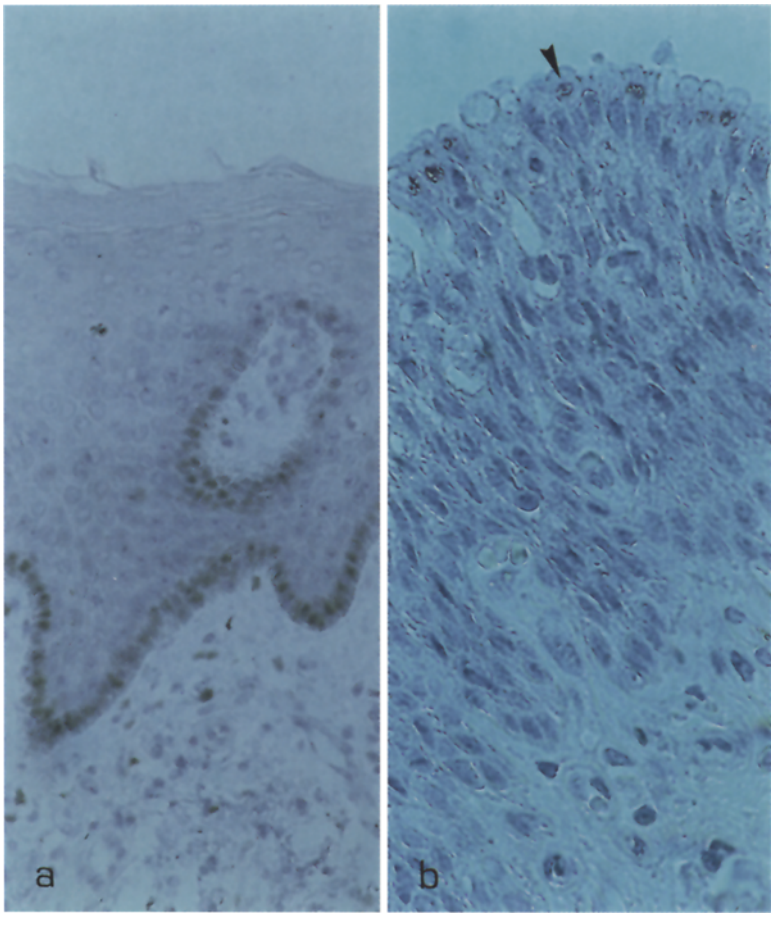

Fig. 2 Immunohistochemical detection of oestrogen receptors in paraffin-embedded male epithelium (streptavidin/biotin-peroxidase method, DAB staining) **a**) Surgically resected preputium obtained from a 60-year-old man: nuclear oestrogen receptors in the basal cell layer of the squamous epithelium (× 250) **b**) Postmortem male urethra (pars cavernosa) of a 45-year-old man: columnal epithelium with discrete fine granular staining in the cytoplasm (× 400)

Results

Intranuclear oestrogen receptors could be detected in all the gynaecological vaginal mucosal biopsy samples, irrespective of the age of the individual and the exogenous use of hormones. The greatest densities were found in the basal and parabasal cells. The number of receptors decreased towards the surface of the epithelium, and the superficial layer never showed oestrogen receptors (Fig. 1a). In the postmortem samples detection of hormone receptors was not possible even after a short postmortem interval of about 8 h. Only a diffuse, fine granular marking of the vaginal epithelium was found which was sharply demarcated from the non-staining subepithelial stroma (Fig. 1b). In contrast, individual intranuclear oestrogen receptors were still detectable in one postmortem endometrium section (Fig. 1c). Oestrogen receptors were never detectable in any of the cytological smears, irrespective of the nature and duration of fixation and the sampling technique but out of a total of 5 surgically resected prepuce samples 3 were found to contain intranuclear hormone receptors in the basal cells (Fig. 2a). In the postmortem samples from male urethra the results varied depending on the topographical region. No oestrogen receptors could be detected in the squamous epithelium of the fossa navicularis but the mucosa of the pars cavernosa showed a fine granular positive reaction in the cytoplasma of the columnal epithelial cells (Fig. 2b).

Discussion

Numerous biochemical, autoradiographical and immunohistochemical investigations have been performed [1, 2, 4, 12, 13, 19, 21, 23] on the presence of oestrogen receptors in human breast cancer tissue and their clinical significance for endocrine treatment and the prognosis. In addition, oestrogen receptors have been found in the tissues of the female genital tract [5, 7, 16, 20, 24], in some carcinomas of the colon [14], in the liver [6] and in the kidney [3]. Oestrogen receptors were first found in the vaginal mucosa in 1980 by quantitative radioimmunoassay [25]. Press et al. [18] reported the distribution of oestrogen receptors in fresh frozen vaginal epithelial sections and in other tissues of the female reproductive tract. The results reported so far with respect to the cycle dependence of the concentration of oestrogen receptors are varied [8, 15, 17, 22]. To our knowledge, this study is the first to report the immunohistochemical detection of oestrogen receptors in paraffin-embedded vaginal epithelium. In agreement with the results obtained in frozen sections [26], we found a high receptor density in the nuclei of the basal and parabasal cells of the vaginal mucosa in all freshly fixed biopsies. Only the superficial part of the cell layer was receptor-free. There was no evidence of any dependence of incidence and topographic receptor distribution on age, cycle phase or exogenous hormone use but reliable data would require further investigations. The diffuse antigen-antibody reaction in postmortem tissue samples even after short postmortem interval indicates a high level of sensitivity of the oestrogen receptors towards external influences. In addition to the general changes of autolysis, the acidic, bacteria contaminated vaginal environment in particular might impair the detection. In contrast to vaginal epithelium, scattered nuclear oestrogen receptors were detectable in the endometrium obtained after comparable postmortem intervals. It was shown that no oestrogen receptors were detectable in freshly fixed vaginal smears. Possible reasons for this are such external influences as drying or structural changes of the material by the fixation medium. In addition, despite the use of cytobrushes, the smears were composed almost exclusively of superficial and large intermediate cells from the upper epithelial layers where, according to the histological examinations, no oestrogen receptors are present.

On the basis of these results, the immunohistochemical determination of oestrogen receptors appears to be unsuitable alternative to the Lugol's staining method, which is still used but nevertheless unspecific, for the identification of vaginal cells.

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