HISTOLOGICAL AND IMMUNOHISTOCHEMICAL STUDIES ON THE VULVA OF SHE-CAMEL

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ABSTRACT

Ten adult she-camels were used to study histological structure of the vulva and the immunohitochemical localization of estrogen receptor alpha (ERα) and progesterone receptor (PR). Histologically, the vulvar lip consists of mucosal surface, cutaneous surface and connective tissue core. The mucous surface is lined with keratinized stratified squamous epithelium while the cutaneous surface is covered with skin and its appendages except sweat gland. Immunohistochemically, ERα is identified in the epithelia of both surfaces of the vulva but PR is not identified in the skin of vulva of camel. Both ERα and PR are localized in the nuclei of the basal and parabasal cell layers in the vulvar epithelia indicating their role in growth and differentiation.

Keywords: Histology, estrogen receptor alpha, progesterone receptor, vulva, camel.

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1. INTRODUCTION

Camels were, and still are, valued as riding, baggage and work animals, as well as providers of meat and milk (Ibrahim, 2008). Although the whole female genital system of camels received appropriate works from histological studies, yet the vulva of she-camels were histologically studied in a narrow scale (El-Tayeb, 1981 and El-Hariri et al., 1988). Growth, differentiation and function of the female genital system are regulated by estrogen and progesterone via their estrogen receptors (ERα) and progesterone receptors (PR) respectively. These receptors are members of the steroid receptor superfamily (Carson-Jurica et al., 1990). Therefore the present study aims to investigate the histological structure of the vulva in adult she-camels with special reference to the immunohistochemical localization of both ERα and PR in their tissues.

2. MATERIALS AND METHODS

2.1. Animal and tissue processing

Small specimens were taken from different parts of the vulvae of 10 non pregnant, apparently normal, she-camels with ages ranged 5-10 years were collected from El-Warrak abattoir in Giza Governorate, Egypt. The age of these, animals were determined according to Williamson and Payne (1978). The specimens were immediately fixed in formalin, dehydrated in ascending grades of ethyl alcohol, cleared in xylene and embedded in paraffin wax.

2.2. Histology and Histochemistry

Paraffin sections of 5 micrometer thickness from vulva of she-camels were cut and stained with the following techniques for

Harris's alum haematoxylin and aqueous, Masson's trichrome stain, Gomeri's reticulin stain, Verhoeff's stain and Bielschowsky's silver stain. The fixative and staining methods were used as outlined by Bancroft et al. (1994).

2.3. Immunohistochemistry

Paraffin sections of 5 micrometer from vulva of she-camels were collected on positive charged microscope slides. Sections were deparaffinized in xylene, cleared in absolute ethanol, rehydrated sequentially in 95% ethanol, 70% ethanol, distilled water, and rinsed in phosphate buffered saline (PBS). Sections were incubated with the preheated antigen retrieval solution in steamer for 40 minutes at 99°C. Then, sections were incubated in 3% hydrogen peroxide in absolute methanol for 20 minutes to reduce the endogenous peroxidase activity. For blocking of the non-specific reactions, sections were incubated in a humidified chamber for 60 minutes with PBS containing 10% normal goat serum (Santa Cruz Biotechnology Inc., CA, USA). Sections were incubated overnight at 4°C in a humidified chamber with rabbit polyclonal antibody against estrogen receptor (ERα) and progesterone receptor (PR) diluted by 1:300 and 1:200 respectively. All the antibodies were purchased from (Santa Cruz Biotechnology Inc., CA, USA). Sections were incubated for 30 minutes at RT with anti-rabbit IgG, diluted 1:400 in PBS, as a secondary antibody (Vector Laboratories, Inc., Burlingame, CA, USA). The visualization was performed using the ready to use Vectastain ® Elite ABC Reagent (Vector Laboratories, Inc., Burlingame, CA, USA). Sections were treated with a liquid diaminobenzidine (DAB) substrate chromagen system (Dako Cytomation, CA, USA), counterstained with haematoxylin, dehyderated, cleared in xylene, mounted by xylene based mounting and covered with a coverslip. For negative controls, the primary antibodies were omitted and exchanged with normal rabbit IgG.

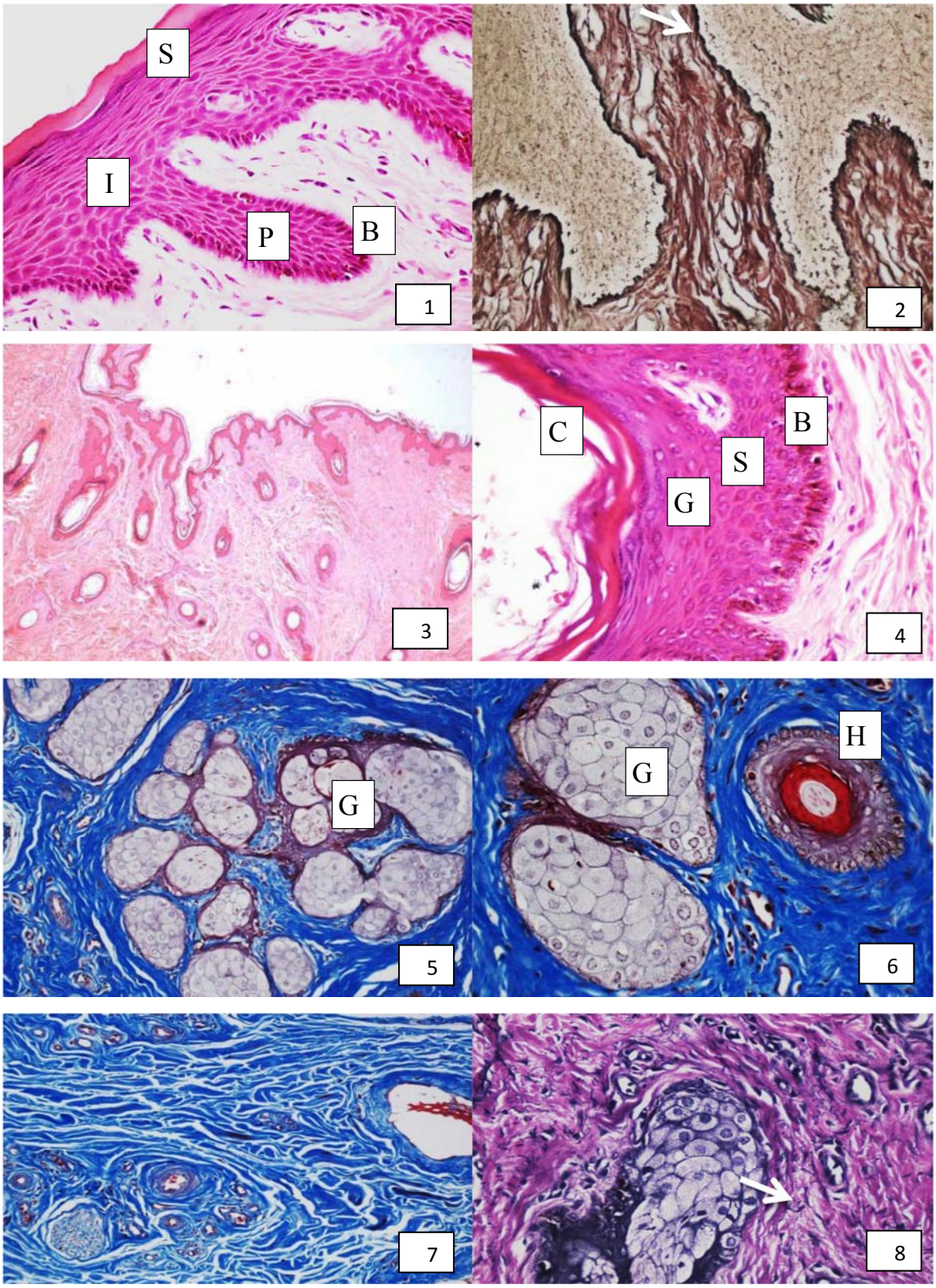
3. RESULTS

Histological sections of the vulva of adult she-camels revealed that each vulvar lip has mucosal surface, cutaneous surface and core.

The mucosal surface is the inner surface of the vulvar lips, which is covered with cutaneous mucous membrane of keratinized stratified squamous epithelium. Which consist of pigmented basal, parabasal, intermediate and superficial cell layers (Fig.1) that is rested on basement membrane with clear reticular lamina (Fig.2).

The cutaneous surface is the outer surface of the vulvar lips which is covered by pigmented skin with its appendages involving hair follicles and sebaceous glands, but no sweat glands can be identified (Fig.3). The skin epidermis consists of stratum basale, spinosum, granulosum and corneum (Fig.4). Some of the sebaceous glands are not associated with hair follicles and present in the form of groups (Fig.5), but others are associated to hair follicles (Fig.6). The core of the vulvar lips is consisted mainly of vascularized dense collagenous connective tissue with presence of few smooth muscle cells (Fig.7). Elastic fibers are identified within core of the vulva and are concentrated around hair follicles and sebaceous glands (Fig.8). The vulvar lip core is highly innervated (Fig.9). Lymphocytic aggregations are present deep in the vulvar lip cores close to muscles (Fig.10). There is a sheet of striated muscle of constrictor vulvae (Fig.11).

Immunohistochemically, ERα is localized in the nuclei of the epithelial cells (mucosal and cutaneous surfaces) of the vulva. There is no immunostaining for ERα in the negative control sections. In epithelium of the mucosal surface, cells of the superficial layer show negative immunoreactivity for ERα. Cells in the basal, parabasal and intermediate layers show positive immunoreactivity for ERα in the breeding season (Fig.12). In epithelium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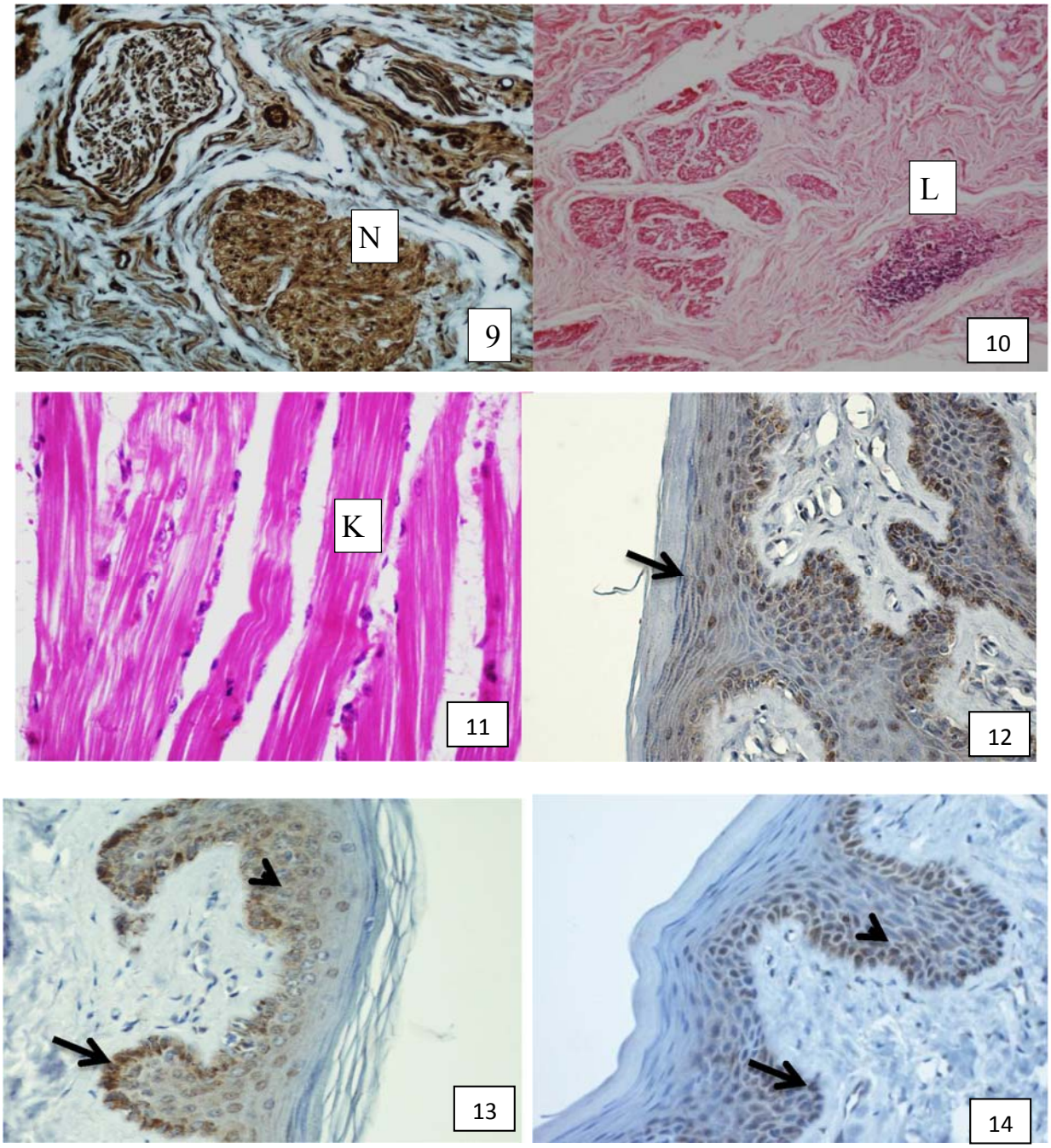

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Fig.1: Photomicrograph of she-camel's vulvar epithelium (mucosal surface) showing pigmented basal layer (B), parabasal (P), intermediate (I) and superficial (S). H&E X100. Fig.2: Photomicrograph of she-camel's vulvar epithelium (cutaneous surface) showing reticular lamina of its basement membrane (arrow). Gomori's reticulin X100. Fig.3: Photomicrograph of she-camel's vulva showing typical feature of its cutaneous surface which covered with skin and its appendages. H&E X20. Fig.4: Photomicrograph of she-camel's vulvar skin (cutaneous surface) showing its layers; stratum basale (B), spinosum (S), granulosum (G) and corneum (C). H&E X100. Fig.5: Photomicrograph of she-camel's vulva showing groups of sebaceous glands (G) which are not associated with hair follicles. Masson's trichrome X100. Fig.6: Photomicrograph of she-camel's vulva showing sebaceous glands (G) associated with hair follicles (H). Masson's trichrome X200. Fig.7: Photomicrograph of she-camel's vulva showing dense collagenous connective tissue core. Masson's trichrome X40. Fig.8: Photomicrograph of she-camel's vulva showing several elastic fibers (arrow) around sebaceous glands and hair follicle. Verhoeff's

method X200. Fig.9: Photomicrograph of she-camel's vulva showing typical feature of nerve bundles (N). Bielschowsky's silver X100. Fig.10: Photomicrograph of she-camel's vulva showing nodule of lymphocytic aggregation (L) close to the muscular wall. H&E X40. Fig.11: Photomicrograph of she-camel's vulva showing skeletal muscles of the constrictor vulvae muscles (K). H&E X100. Fig.12: Immunohistochemical staining for ERα in epithelium of she-camel's vulva (mucosal surface) showing immunostaining in all cell layers except superficial layer (arrow). Negative control for ERα and PR using normal rabbit IgG (inset). X100. Fig.13: Immunohistochemical staining for ERα in epithelium of she-camel's vulva (cutaneous surface) showing immunostaining in cells of stratum basale (arrowhead) and spinosum (arrow). Negative control for ERα and PR using normal rabbit IgG (inset). X100. Fig.14: Immunohistochemical staining for PR in epithelium of she-camel's vulva (mucosal surface) showing immunostaining in basal (arrow) and parabasal cells (arrowhead).X100.

of the cutaneous surface in both seasons, cells of stratum basale and spinosum show positive immunoreactivity for ERα, but cells of startum granulosum show negative immunoreactivity for ERα (Fig.13).

PR is localized in the nuclei of epithelial cells of the vulva (only mucosal surface) from both breeding and non-breeding seasons. There is no immunostaining for PR in the negative control sections. Cells in the basal and parabasal layers show positive immunoreactivity for PR, but cells of the intermediate and superficial layer show negative immunoreactivity for PR (Fig.14).

4. DISCUSSION

Mucosal surface of the she-camel's vulva was covered with mucous membrane of keratinized stratified squamous epithelium that agreed with Bareedy (1977) and Badawy et al. (1978) in buffalo; however Raghavan and Kachroo (1964) in cow and Miller et al., (1964) in mare identified non-keratinized epithelium. The mucosal surface was considered as a continuation of vestibular mucous membrane that agreed with Raghavan and Kachroo (1964). Cutaneous surface of the she-camel's vulva was covered by pigmented skin with its appendages that was similar to Bareedy (1977) and Badawy et al. (1978) in buffalo, Blazquez et al. (1987) in cow, and Getty (1975) in mare and sow. In contrary to others, our results did not identify sweat glands, but hair follicles and sebaceous glands were identified. Some of the sebaceous glands were present associated to hair follicles, but others were not associated with hair follicles and present in form of groups that agreed with Bareedy (1977) in buffalo. Core of the she-camel's vulvar lip was consisted of dense collagenous connective tissue with presence of few smooth muscle cells that was similar to Yang et al. (2005) in women. The connective tissue core was highly vascularized that agreed with Puppo (2011) in women. Moreover, the vulvar core was highly innervated that agreed with Puppo (2011) in women. There was a sheet of striated muscle of constrictor vulvae, which was considered as continuation of constrictor vestibuli muscle that simulated to Getty (1975) in mare, bitch and cat queen. The presence of lymphocytic aggregations and nodule indicates the high immune response in the vulva which is considered as the external orifice of the female genital tract. Vulva of the she-camel showed ERα immunoreactivity in its epithelium that was similar to those obtained by Vermeirsch et al. (2002) in bitch and MacLean et al. (1990); Hodgins et al. (1998); Martin-Alguaci et al. (2008) and Taylor et al. (2008) in woman, while completely differed with Onnis et al. (1985) who did not demonstrate ER immunoreactivity in vulva of woman. This work detected ERα in the basal and parabasal cells of mucosal and skin surfaces of the vulva of she-camel that agreed with Martin-Alguaci et al. (2008) who detected ERα staining in basal and suprabasal epidermal cells of the woman vulva, while Hodgins et al. (1998) observed ER in basal cells of epidermis of woman, and Taylor et al. (2008) did not detect ERα

immunoreactivity in the basal cells of woman's vulva. Vulva of the she-camel showed PR immunoreactivity at low scale as it was only seen at its mucosal surface that was similar to those obtained by Hodgins et al. (1998) in the inner surface of labia minora of woman. This work detected PR immunoractivity clearly in basal and parabasal layers of vulvar epithelium (mucosal surface). PR immunoreactivity in the vulvar skin or skin appendages during both seasons that agreed with Hodgins et al. (1998) in the skin appendages of woman's vulva and disagreed with Vermeirsch et al. (2002) who detected immunolocalized PR in the skin epithelium of the vulva of bitch. It is worthy to note that both ER and PR are localized mainly in basal and parabasal cell layers in the epithelia of the vulva that indicates the role of both estrogen and progesterone in growth and differentiation of the epithelial cells of the vulva of she-camel that is supported by Buchanan et al. (1998) and Hodgins et al. (1998). In addition, they may be involved in the healing of the wounds in these epithelia that was supported by Onnis and Becagli (1986); Brincat et al. (2005) and Krzysiek-Maczka (2005).

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دراسات هستولوجية وهستوكيميائية مناعية على فرج الناقة

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الملخص العربي

أجريت هذه الدراسات على 10 نوق بالغة لمعرفة التركيب النسيجي للفرج وكذلك للتعبير الهستوكيميائى المناعي لمستقبلات الإستروجين ألفا والبروجيسيترون في أنسجة الفرج. الدراسة الهستولوجية أوضحت أن شفرة الفرج لها سطح مخاطي وأخر جلدي ولب من الأنسجة الضامة. أما السطح المخاطي فكان مغطى بطبقة ظهارية متعددة ومغطاة بالكيراتين وأما السطح الجلدي فكان مغطى بالجلد وملحقاته ما عدا الغدد العرقية. الدراسة الهستوكيميائية المناعية أوضحت تعبيرا لمستقبلات الإستروجين ألفا في الطبقة الظهارية لسطحي شفرة الفرج أما مستقبلات البروجيستيرون لم تظهر في السطح الجلدي لشفرة الفرج. وقد ظهرت التعبيرات لمستقبلات الإستروجين ألفا والبروجيستيرون في أنوية خلايا الطبقة السفلى والجار سفلى في الظهارة المغطاة لشفرة الفرج مما يدل على الدور البارز للإستروجين والبروجيستيرون في نمو وتطور ظهارة شفرتي الفرج.

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