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HISTOLOGICAL, HISTOCHEMICAL AND IMMUNOHISTOCHEMICAL LOCALIZATION OF SEX HORMONES RECEPTORS IN THE WATER BUFFALO'S VULVA

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ABSTRACT

This study was done on twenty buffalo-cows to study the histological, histochemical and immunohistochemical structure of the vulva and evaluates the hormonal receptors of estrogen, progesterone and androgen hormones. The vulva consisted of two surface, mucosal and cutaenous. It was lined by stratified squamous keratinized and pigmented epithelium. Fibroelastic CT represented the core of the vulva. Estrogen receptors alpha was localized in the nuclei of the epithelial cells of the mucosal and cutaenous surfaces and the secretory cells of sebaceous glands of the vulva. Progesterone receptors were localized in the nuclei of epithelial cells of the vulval mucosal surface and cells in the basal and parabasal layers. Androgen receptors were localized in the nuclei of the epithelial cells of the mucosal surface and the secretory cells of sebaceous glands of the vulva.

KEYWORDS: Water Buffalo-Immunohistochemical-Vulva-Sex Hormones-Histology

INTRODUCTION

The vulva is the external opening to the reproductive system. It is external vertical opening of genital tract just below anus. Diameter is larger than that of vagina. The vulva has three main functions: the passage of urine, the opening for mating and serves as part of the birth canal. Vulva walls supplied with glands which are active during excitement .The vulva lips are located at the sides of the opening and appear wrinkled and dry when the cow is not in estrus. As the animal approaches estrus, the vulva will usually begin to swell and develop a moist red appearance. (Kunbhar et al., 2003).

The vulva consists of two labia, covered by skin with sebaceous glands and fine hair. The dermis and hypodermis have a vascular plexus that becomes congested during estrus, especially in sows and bitches. While constrictor vulvae muscle is located in the hypodermis (Inomata et al., 1993) and (Eljarah et al.,2012) in female Arabian oryx. Most of available literature dealt with the histology of the external genitalia are few and were done by (Bareedy, 1977 and Badawy et al., 1978) in buffalo, (Friess, 1972; Blazquez et al., 1987b and Inomata et al., 1982) in cow, (Bulmer, 1964 and Restall, 1966) in sheep, (Steinbach and

Smidt, 1970 and Inomata et al., 1993) in pig, (Spurgeon and Reddy, 1986 and Wang et al., 2006) in dog, (Inomata et al., 2009 and Plavec and Pavlin 2012) in cat, (Murakami and Mizuno, 1984; Inomata et al., 1985 and Anderson and Clark, 1990) in rat and Suzuki et al. (2002) in mice and (Marshall, 1978 and Puerta-Fonolla, 1998) in human. Most of the previous studies revealed that the sex hormones receptors were demonstrated in the ovary, oviduct, uterus, cervix, vagina and mammary glands (Schams et al., 2003; Ulbrich et al., 2003; Boos et al., 2006; Juengel et al., 2006; Salvetti et al., 2007; Tienthai et al., 2008 and Kunkittia et al., 2011).

Materials and Methods

The vulva of twenty buffalo-cows at age range of 3-9 years was collected from El-Basatin slaughter house. It was fixed by buffered formalin solution and transported to laboratory for tissue confirmation and estrogen, progesterone and androgen receptors immunohistochemistry. All tissues were evaluated by general, special and histochemical stains (Wilson and Gamble*, 2002).* Immunostaining for estrogen, progesterone and androgen receptors were done on cryostat sections by peroxidase- antiperoxidase (PAP) method using the ERICA monoclonal kit (Abbot, North Chicago , IL) in brief 4um cryostat sections were mounted on glass slide ,coated with the tissue adhesive provided in the kit and placed in 3.7% formaldehyde in phosphate –buffered saline (PBS) for 10 minutes. Sections were treated with 0.3% hydrogen peroxide diluted in methanol to block endogenous peroxidase activity and incubated with normal goat serum. Then the slides incubated with antiestrptrophilin monoclonal antibody(H222) or control ratimmunoglobulimne (Ig) G for 30 minutes at room temperature, followed by treatment with goatanti-rat IgG antiserum(bridging antibody) and with PAP complex (Kiernan,2008). Finally, diaminobenzidine and 0.06% hydrogen peroxide diluted in PBS were applied .Counter staining was done with hematoxylin or methyl green. The specific staining was observed as brown colored granules, and the control slides as treated with control antibody yielded negative results.

Results

The vulva of the buffalo-cow appeared large in size and dark pigmented color. It had two surface, mucosal (inner) and cutaenous (outer) surface. The mucosal surface (inner surface) was covered by stratified squamous keratinized epithelium (Fig.1). Numerous long thin and thick papillae extend from the epithelium and extruded into the propria-submucosa. The

cutaenous surface was covered by pigmented stratified squamous epithelium containing hair follicle and sebaceous glands (Fig.2). The connective tissues around sebaceous glands show alcianophilic reaction (fig.3), while PAS positive reactions were demonstrated in hair root sheath and papillary muscles (Fig.4).

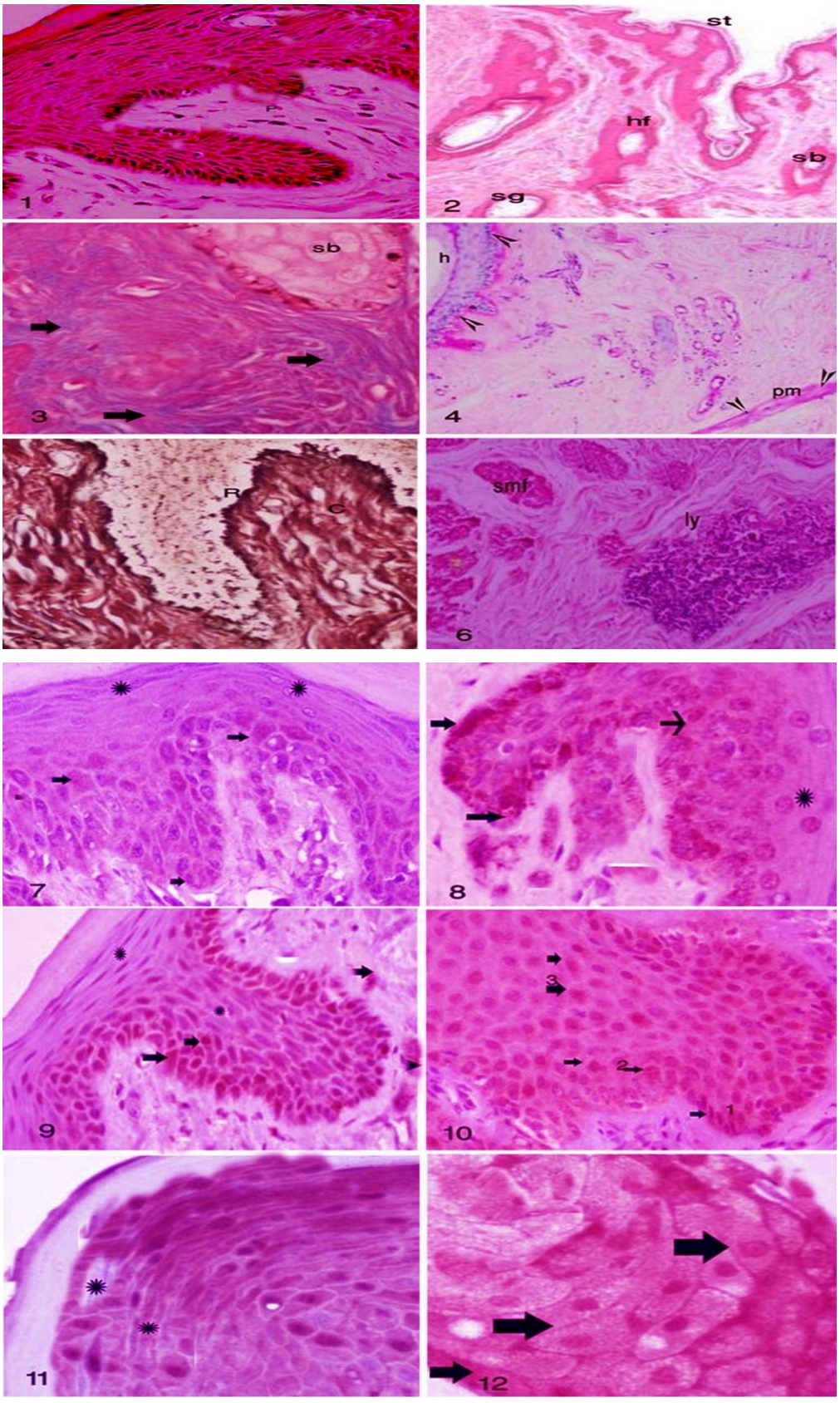
The vulvar core fills the area between the inner and outer surface. It mainly consisted of fibroelastic CT that identified within propria-submucosa of mucosal surface and reticular layer of cutaenous surface (Fig.5). It is consisted mainly of dense collagenous connective tissue with presence of few smooth muscle cells. Numerous lymphocytes were located in the propria-submucosa of the mucosal surface (Fig.6)

Immunohistochemistry

Estrogen receptors alpha was localized in the nuclei of the epithelial cells (mucosal and cutaenous surfaces) and the secretory cells of sebaceous glands of the vulva .In epithelium of the mucosal surface, cells of the superficial layer show negative immunoreactivity for estrogen receptors alpha (Fig.7). Cells in the basal, parabasal and intermediate layers show positive immunoreactivity for estrogen receptors alpha , but the cells of upper layers show negative immunoreactivity for estrogen receptors alpha . In epithelium of the cutaenous surface, cells of stratum basale and spinosum show positive immunoreactivity for estrogen receptors alpha (Fig.8), but cells of startum granulosum show negative immunoreactivity for estrogen receptors alpha.

Progesterone receptors was localized in the nuclei of epithelial cells of the vulvar mucosal surface. Basal and parabasal cell layers show positive immunoreactivity while cells of the intermediate and superficial layer show negative immunoreactivity for progesterone receptors (Fig.9).

Androgen receptors was localized in the nuclei of the epithelial cells of mucosal surface and the secretory cells of sebaceous glands of the vulva. Cells in the basal, parabasal and intermediate layers show positive immunoreactivity for androgen receptors (Fig.10), but cells of the superficial layer show negative immunoreactivity for androgen receptors (Fig.11). The cells in the older buffalo-cows showed intense immunostaining for androgen receptors . The secretory cells of the sebaceous glands show weak immunostaining for androgen receptors (Fig.12).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Discussion

Vulva of adult buffalo-cows was bounded by vulvar lips which had mucosal (inner) and cutaenous (outer) surfaces that was similar to the findings of Bareedy (1977) and Badawy et al. (1978) in buffalo's vulva. Mucosal surface of the buffalo-cows vulva was covered with mucous membrane of keratinized stratified squamous epithelium that supported by the results of Bareedy (1977) and Badawy et al. (1978) in buffalo and Jones (1983); Yang et al. (2005) and Farage and Maibach (2006) in women, however Raghavan and Kachroo (1964) in cow and Trautmann and Fiebiger (1957) 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). It was characterized by presence of numerous epithelial papillae extending into the underlying propria-submucosa which was consisted of highly vascularized dense collagenous connective tissue with spiral blood vessels. In addition, vulvar propria-submucosa of buffalo-cow had many lymphatic infiltrations that agreed with Trautmann and Fiebiger (1957) and Miller et al. (1964) in mare. Nodules of lymphocytic aggregations were seen deep to the vulvar core indicating increased immune response.

Cutaneous surface of the buffalo-cows vulva was covered by pigmented skin with its appendages that was similar to Bareedy (1977) and Badawy et al. (1978) in buffalo, Raghavan and Kachroo (1964); Getty (1975) and Blazquez et al. (1987c) in cow, Miller et al. (1964) and Getty (1975) in mare and sow, and Yang et al. (2005); Farage and Maibach (2004) and O'Connell et al. (2008) in women. Basement membranes of inner and outer root sheathes are PAS positives. Smooth muscles of arrector pilli muscles were present close to hair follicles.

Core of the buffalo-cows 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, but Miller et al. (1964) in mare stated that it is rich in smooth muscle cells and elastic fibers. Some of fat cells are present deeply that agreed with Trautmann and Fiebiger (1957) in sow. the connective tissue core was highly vascularized that agreed with Puppo (2011) in women. Superficially, near the epithelium, there were several blood capillaries and small blood vessels, while several medium sized blood vessels were seen deeply. Moreover, the vulvar core was highly innervated that agreed with Puppo (2011) in women. Deep to the vulvar core, there was thick sheet of smooth muscle cells which were in both circular and longitudinal arrangement. aggregations of lymphocytes were present at deep layer of the connective tissue

core and close to muscles. There was a sheet of striated muscle of constrictor vulvae which was considered as continuation of constrictor vestibuli muscle that simulated to Raghavan and Kachroo (1964) in cow and Getty (1975) in mare, bitch and cat queen.

Vulva of the buffalo-cow showed estrogen receptors alpha immunoreactivity in its epithelium that was similar to those obtained by Vermeirsch et al. (2002b) 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 estrogen receptors alpha immunoreactivity in vulva of woman.

This work detected estrogen receptors alpha in the basal and parabasal cells of mucosal and skin surfaces of the vulva that agreed with Martin-Alguaci et al. (2008) who detected estrogen receptors alpha staining in basal and suprabasal epidermal cells of the woman vulva, while Hodgins et al. (1998) observed estrogen receptors alpha in basal cells of epidermis of woman, and Taylor et al. (2008) did not detect estrogen receptors alpha immunoreactivity in the basal cells of woman's vulva. Estrogen receptors alpha immunostaining was obviously seen in the secretory cells of sebaceous glands that differed from the findings of Onnis et al. (1985) and Hodgins et al. (1998) as they denied presence of estrogen receptors alpha within the skin appendages of woman's vulva. Estrogenic receptors were demonstrated in epidermal keratinocytes and dermal fibroblasts of the vulva and perineum, hair and non-hair bearing (Maclean et al.,1990).

Vulva of the buffalo-cow showed progesterone receptors 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 progesterone receptors immunoractivity clearly in basal and parabasal layers of vulvar epithelium (mucosal surface), .This investigation showed no progesterone receptors immunoreactivity in the vulvar skin or skin appendages that agreed with Hodgins et al. (1998) in the skin appendages of woman's vulva and disagreed with Vermeirsch et al. (2002b) who detected immunolocalized progesterone receptors in the skin epithelium of the vulva of bitch.

Vulva of the buffalo-cows showed androgen receptors immunoreactivity in the epithelial cells of its mucosal surface and in the secretory cells of sebaceous glands. The current work detected androgen receptors in basal, parabasal and intermediate cells of the epithelium of mucosal surface of the vulva, however Hodgins et al., (1998) showed androgen receptors in the basal and parabasal cells of woman's vulvar epidermis, also Taylor et al. (2008) identified androgen receptors immunostaining in the parabasal cells of the normal vulvar epidermis and

was absent from the basal cells. On the other hand, Onnis et al. (1985) denied androgen receptors immunolocalization in the vulva of woman at all. The cells of sebaceous glands showed androgen receptors immunoreactivity that supports the findings of Hodgins et al. (1998) in vulvar skin of woman, but disagreed with Onnis et al. (1985) and Vermeirsch et al. (2002b) as they did not identify androgen receptors in the vulvar skin appendages of woman and bitch.

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