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Role of Androgen in the Clitoris of Camel

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Absrtact: Ten adult female camels with ages ranging from 6 to 8 years were used to define the role of androgen in the clitoris via immunolocalization of the androgen receptors (AR) in the clitoral tissues during rutting and non-rutting seasons. AR was expressed in the cells of prepuce, pacinian-like corpuscles, stroma and chondrocytes of the clitoris from both rutting and non-rutting camels. Expression of AR in the clitoris was higher in non-rutting camels than rutting ones. This seasonal difference suggests that the expression of AR in the clitoris of camel may be hormonally controlled.

Key words: Androgen receptors · Immunohistochemistry · Clitoris · Camel

INTRODUCTION

Camel is an important livestock species in Arabic countries. Humans depend on camel not only for meat, milk and hide but also as mode of transportation especially in the desert "Ship of the desert" [1]. Androgen plays an essential role in follicular development, atresia and luteinization as autocrine or paracrine agents [2-6]. Androgens are the immediate precursor for the synthesis of estrogens in the female. Consequently, imbalance in androgen biosynthesis or metabolism may cause undesirable effects on female sexual reproductive functions [7]. Androgens act on its target tissues through their receptors which are members of steroid receptor superfamily [8]. To better define the role of androgen in the functions of the clitoris of camel; we have proceeded to immunolocalize AR during rutting and non-rutting season.

MATERIAL AND METHODS

Animals: A total of 10 of adult female camels were collected from Toukh abattoir in Kalyobyia Governorate, Egypt. The ages of the animals were determined according to William and Payne [9].

Tissues Specimens and Processing: Immediately after slaughter, different parts from the clitoris were collected. Specimens were immediately fixed in formalin, then were dehydrated in ascending grades of ethyl alcohol, cleared in xylene and embedded in paraffin wax.

Immunohistochemistry: Paraffin sections of 5 micrometer from clitoris of the rutting and non-rutting camels were collected on positive charged microscope slides. Sections were deparaffinized in xylene, 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 (Dako Cytomation, CA, USA) in steamer for 40 minutes at 99°C. Then, sections were incubated in 3% hydrogen peroxide in methanol for 15 minutes. For blocking of the non-specific reactions, sections were incubated in a humidified chamber for 60 minutes with PBS containing 10% normal goat serum (NGS) (Santa Cruz Biotechnology Inc., CA, USA). Sections were incubated with the primary antibody overnight at 4°C in dilution 1:300. The primary antibody was rabbit polyclonal antibody (N-20:sc-816), against peptide mapping at the N-terminus of AR of human origin and cross react with mouse, rat, human, equine, canine, bovine and procine, (Santa Cruz Biotechnology Inc., CA, USA). Sections were incubated for 30 minutes at room temperature with anti-rabbit IgG, diluted 1:600 in PBS, as a secondary antibody (Vector Laboratories, Inc., Burlingame, CA, USA). The visualization was performed using Vectastain ® Elite ABC Reagent (Vector Laboratories, Inc., Burlingame, CA, USA). Sections were treated with diaminobenzidine substrate chromagen

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Table 1: Immunohistochemical grading scores

Intensity score (IS)		Proportional score (PS)	
Score 0	No staining	Score 0	No positive cells
Score 1	Weak staining	Score 1	<1% positive cells
Score 2	Moderate staining	Score 2	1-9% positive cells
Score 3	Strong staining	Score 3	10-32% positive cells
Score 4	Very strong staining	Score	4 33-65% positive cells
		Score	5 >65% positive cells

system (Dako Cytomation, CA, USA) and counterstained with haematoxylin. For negative controls, the primary antibodies were omitted and a normal rabbit IgG (Santa Cruz Biotechnology Inc., CA, USA) were used at a concentration equal to that of the primary antibodies.

Morphometry: AR immunostaining in the clitoris of camels were scored according to the immunohistochemical grading scores method of Vermeirsch *et al.* [10] and Vermeirsch *et al.* [11] as shown in (Table 1). The total score (TS) is obtained by the addition of a proportional score (PS) to an intensity score (IS). The total score of AR immunoreactivity is scored as: 1-3, weak grade; 4-6, moderate grade; and 7-9, strong grade.

RESULTS

AR is localized in the nuclei of the epithelial cells of the prepuce, pacinian-like corpuscles, stroma and chondrocytes of the clitoris from both rutting and non-rutting seasons. There is no immunostaining for AR in the negative control sections. In rutting season, few epithelial cells of the clitoral prepuce show AR immunoreactivity that is weak immunostining (Fig.1), while in non-rutting season, almost all of epithelial cells of the clitoral prepuce show stronger immunostaining for AR (Fig.2). Comparing the intensity and proportion of positive epithelial cells shows significant seasonal difference as it is higher during non-rutting season (p<0.05, Table 2). Both stromal cells and cells of pacinian-like corpuscles from both rutting and non-seasons show moderate AR immunostaining (Figs.1,2,5) with no significant seasonal difference (p<0.05, Table 2). Several chondrocytes show positive immunoreactivity for AR in the non-rutting season with strong immunostaining (Fig.4), but it is weaker and fewer in rutting season (Fig.3). A significant higher AR immunoreactivity in chondrocytes from the non-rutting season than in the rutting season is detected (p<0.05, Table 2).

DISCUSSION

Clitoris of the camel expressed AR in its preputial epithelium, pacinian-like corpuscles, stroma and chondrocytes during both rutting and non-rutting seasons. This work identified a significant seasonal difference in expression of AR in the clitoris of camel, while, Vermeirsch *et al.* [11] in the vagina and vulva of bitch and Cardenas and Pope [12] in the uterus of sow, did not found any cyclic changes in AR immunostaining in these tissues. In addition, Pessina *et al*. [13] found no effect of ovariectomy and estradiol replacement on AR expression in the vagina of rat. This work showed higher expression of AR in the camel's clitoris during the non-rutting season (progesterone dominant season) and lower expression of AR during the rutting season (estrogen dominant season) indicating that estrogen down-regulates expression of AR, while progesterone up-regulates expression of AR in the clitoris of camel which is completely differed from findings of West *et al.* [14], Slayden *et al.*[15] and Slayden and Brenner [16] as they stated that estrogen increases and progesterone decreases expression of AR in the endometrial tissues of monkey and woman. The localization of AR basically in the nuclei of basal and intermediate of epithelial cells of the clitoral prepuce of camel suggests that they may be

Table 2: Immnuostaining proportional (PS) scores, intensity (IS) scores and total (TS) scores ± standard deviation (SD) for AR in the camel's clitoris during both rutting and non-rutting seasons

	AR (Rutting season)			AR (Non-rutting season)		
	PS	IS	TS	PS	IS	TS
Preputial epithelium	1.8±1.0	0.8±0.5	2.5±1.5*	5.00	2.5±0.6	7.5±0.6*
Pacinian corpuscle	3.6±0.5	1.4±0.4	5.0±0.5	4.00	1.8±0.4	5.8±0.4
Stroma	2.8±0.5	1.8±0.4	4.6±0.7	2.8±0.5	1.6±0.5	4.4±0.5
Chondrocytes	2.8±0.5	2.4±0.4	5.2±0.5*	5.00	3.5±0.5	8.5±0.5*

* Within TS row, means a significant difference (P < 0.05) in comparison between rutting and non-rutting data.

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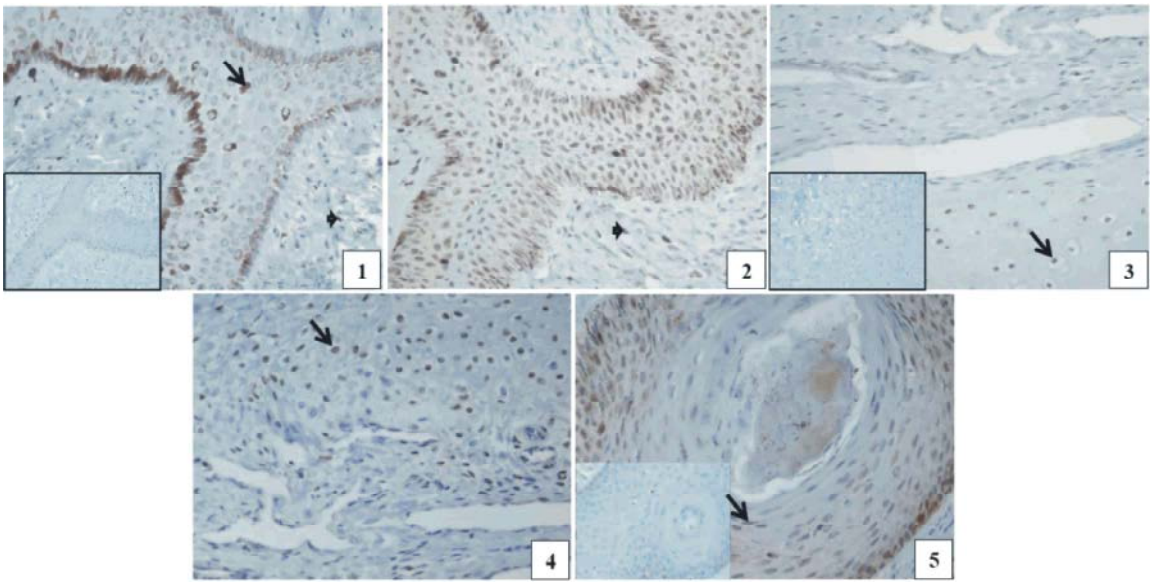

Fig. 1: Prepuce of clitoris of rutting camel showing few and weaker immunostaining for AR (arrow) compared to Fig.2. Stromal cells showing AR immunostining (small arrow). (Inset showing negative control). X40 Obj.

Fig. 2: Prepuce of clitoris of non-rutting camel showing more and stronger immunostaining for AR compared to Fig.1. Stromal cells showing AR immunostining (small arrow) X40 Obj.

Fig. 3: Cartilage of clitoris of rutting camel showing few and weaker immunostaining for AR (arrow) compared to Fig.4. (Inset showing negative control). X40 Obj.

Fig. 4: Cartilage of clitoris of non-rutting camel showing stronger immunostaining for AR (arrow) compared to Fig.3. X40 Obj.

Fig. 5: Pacinian-like corpuscle in prepuce of clitoris of rutting camel showing positive immunostaining for AR (arrow) in its cells. (Inset showing negative control). X60 Obj.

involved in the growth and differentiation of this epithelium that was supported by Buchanan *et al.* [17] and Hodgins *et al.* [18]. In addition, AR may be involved in the healing of the wounds in this epithelium that was supported by Hodgins *et al.* [18] and Krzysiek-Maczka [19]. Moreover, the presence of AR in the pacinian-like corpuscles suggests that it has role in sexual arousal and sensation.

CONCLUSION

This study revealed expression of AR in the clitoris of camel and showed significant difference between the rutting and non-rutting seasons. We suggest that androgen has role in the function of clitoris especially sexual arousal and sensation.

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