

MORPHOLOGICAL ASPECTS OF THE VAGINAL EPITHELIUM OF ATHYMIC NUDE RATS

Aspectos morfológicos do epitélio vaginal de ratas nude atímicas

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Foi analisada à microscopia de luz a morfologia do epitélio vaginal de ratas congenitamente atímicas (rnu/rnu), homozigotas para o gene recessivo nude e de suas "littermates" eutímicas (rnu/+), heterozigotas para o mesmo gene, da linhagem Rowett, nas fases de proestro e de metaestro do ciclo estral.

Através destes estudos pudemos concluir que a morfologia do epitélio vaginal de ratas atímicas, nas duas fases estudadas, é semelhante à das eutímicas e compatível com a morfologia vaginal de ratas de outras linhagens descritas na literatura.

Unitermos: Epitélio vaginal - rato nude - morfologia.

Keywords: Epithelium vaginal - nude rats - morphology.

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Introduction

The recessive mutation leading to congenital athymia and lack of hair in rats was first observed in 1953 in a colony maintained at the Rowett Research Institute, in Aberdeen, Scotland and the symbol rnu was assigned to the mutant recessive nude gene (5).

Up to now, a few studies regarding this strain are found in the literature, although the immunological characteristics and the morphology of the lymphoid and endocrine organs have been well detailed (18,19). Morphologic similarities between ovaries of rnu/rnu and +/rnu rats have been observed (18). However, studies on the vaginal morphology have not been found.

The objective of the present investigation was to observe on light microscopy, the morphology of the vaginal epithelium of the rnu/rnu and +/rnu rats, in the proestrus and metestrus phases of the estrous cycle in order to detect possible alterations in rnu/rnu animals since they seem to present alterations in their hormonal status (15).

Material and method

The animals used in the present study were obtained from a colony of nude rats of the Pathological Anatomy Laboratory at the Butantan Institute, São Paulo, Brasil, maintained in semiconventional conditions of feeding, hygiene, controlled temperature (20°C) and mating rnu/rnu males with +/-rnu females whose matrixes were provided by the CNRS - Centre National de la Recherche Scientifique (France).

Ten adults rnu/rnu and ten +/-rnu rats, with ages ranging from three to four months and weighing approximately 250g were used. Both types of rats were randomly divided into two groups of five animals each, one group being sacrificed in proestrus and the other in metestrus. To determine these phases all animals were submitted to daily colpocytologic examinations and only after confirmation of at least two normal and consecutive estrous cycles they were used. After ether anesthesia, the vagina was withdrawn and fixed in Bouin liquid and embedded in paraffin. Five mm sections were obtained with a Minot type microtome and stained with hematoxylin-eosin.

Results

The morphology of the vaginal epithelium was similar in both rnu/rnu and +/-rnu rats.

In proestrus, the vagina was covered by a stratified keratinized pavement epithelium containing approximately 11 cell layers. Its basal portion consisted of one or two layers of cells containing an elongated nucleus and one or two nucleoli. Right above the basal portion an intermediary one was seen, consisting of almost six layers of cells, with spherical nuclei, one or more nucleoli and that show to be more and more flattened as they move towards the surface. The more superficial portion of the epithelium is distinguished by consisting of approximately three cell layers, with a pavement form containing or not a flattened, pyknotic nucleus, acidophilic cytoplasm and being keratinized at the luminal portion. Around the nucleus a bright halo separating it from the remainder of the cytoplasm was observed in some cells of the intermediary layer. Some mitotic figures were seen in the basal layer (figs. 1A/1B).

In metestrus, a stratified vaginal pavement epithelium consisting of four or six cell layers was observed. The basal layer consisted of cells containing an elongated nucleus with two or more nucleoles. In this layer mitotic figures were also observed. Above this layer, the presence of other two or three layers whose cells were elongated and with distinct cell limits, roundish nucleus with two or more nucleoli were observed. These cells were similar to those of basal layer but with the

longer axis parallel to the epithelial surface and a greater cytoplasmic volume. The portion which covers the vaginal epithelium consisted of one or two cell layers, with a fusiform aspect, characterized by elliptic nuclei sometimes pyknotic and deeply stained. Some epithelial surface areas were thinner and consisted of cells containing a pyknotic nucleus, greater part of this surface being covered with a thin acidophilic layer. In the whole epithelium, leukocytes infiltration was observed (figs. 2A/2B).

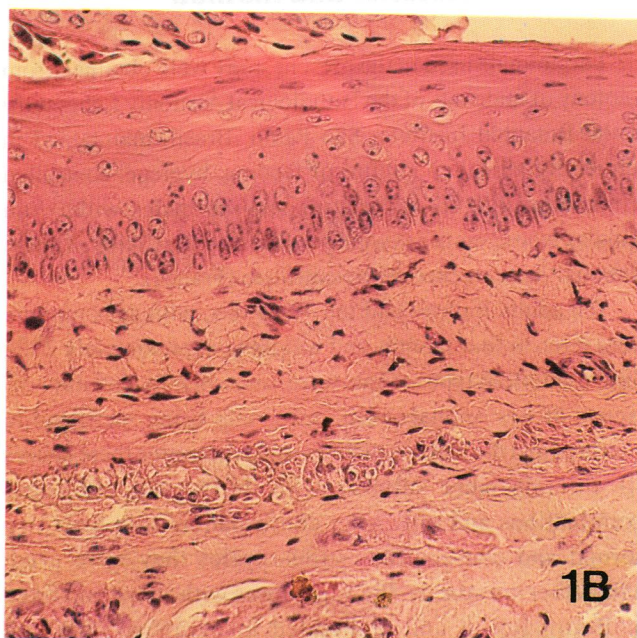
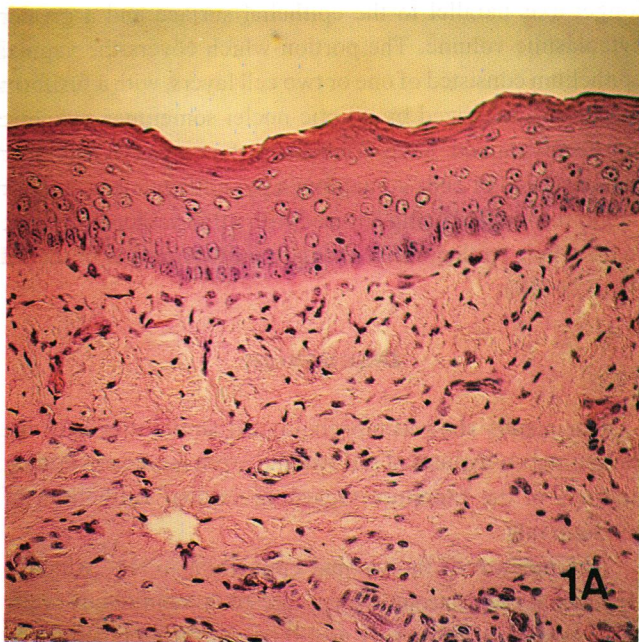
Discussion

Our results showed morphological similarities between the vaginal epithelium of rnu/rnu and +/-rnu rats corroborating findings in the literature regarding vaginal epithelium of common rats.

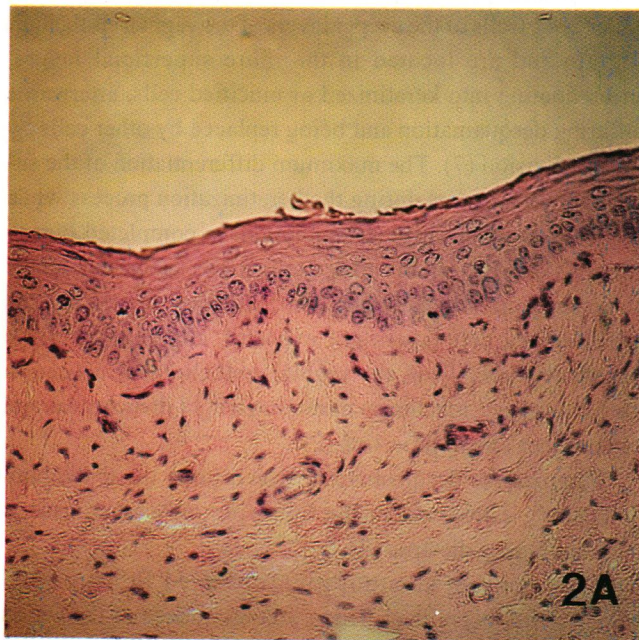
The renewal of the vaginal epithelium in rats, which is repeated at each estrous cycle is a result of continuous cyclic and dynamic processes of proliferation, differentiation and cell desquamation inducing morphological modifications which present patterns peculiar to each phase of the estrous cycle (2, 4). Cells of the deeper layers of the vaginal epithelium migrate and are located in the more superficial layers, differentiating into keratinized or mucified cells, afterwards suffering desquamation and being replaced by other cells by mitotic division (7). The maximum differentiation of the superficial cells occurs during the keratinization process when the renewal of the vaginal epithelium is completed during estrus phase (2,4). In this phase innumerable superficial layers of the keratinized cells suffer a desquamation process and the intermediary cell layers are exposed, characterizing the metestrus phase. Thus, the vaginal epithelium is reduced in the estrus phase, reaching a minimum height in metestrus and a maximum one in proestrus (2).

The massive desquamation, which turns the epithelium thin during estrus and in the following metestrus phase is minimal during diestrus (7).

The intensive keratinization observed in estrus and proestrus has been identified as a consequence of the action of high levels of plasmic estrogen (9). Administration of steroid hormones in castrated rats elucidates the eventual participation of these hormones in the mucification or keratinization process of the vaginal epithelium. Estrogen activates the keratinization process while progesterone seems to inhibit it in addition to intensifying the epithelial mucification (1). Bertalanffy & Lau (2) suggested that exogenous progesterone determines the interruption of the mitotic activity as well as maturation of the vaginal epithelium inhibiting its cyclic modification. The joint administration of estrogen and progesterone promotes the thickening and



Figs. 1A/1B - Photomicrograph of the vaginal portion of (A) *rnu/rnu* and (B) *+rnu* rats, showing the histological aspect of this organ in proestrus. Observe the stratified pavement epithelium keratinized. H.E. 330 X and 440 X respectively.



Figs. 2A/2B - Photomicrograph of the vaginal portion of (A) *rnu/rnu* and (B) *+rnu* rats, showing the histological aspect of this organ in metestrus. Observe the stratified pavement epithelium is less developed than in proestrus. H.E. 330 X and 520 X respectively.

micification of the superficial cell layers of the vaginal epithelium (6,13).

In the present study the morphological results of the vaginal epithelium obtained using light microscopy, both for *rnu/rnu* and *+rnu* rats presented a thick epithelium consisted of approximately 11 layers of cells in proestrus. In metestrus, the vaginal epithelium showed to be thin and consisted of approximately four layers of cells. Cells of the intermediary layers of the epithelium in both phases studied presented a

polygonal shape, acidophilic cytoplasm, nucleus with condensed chromatin and evident nucleolus. Cells of the most superficial layers had a polygonal shape, a pyknotic or absent nucleus and acidophilic cytoplasm. These findings are in agreement with those of Jaworski (7) and Bertalanffy & Lau (2).

The interdependence of functions of the endocrine and immunological systems has been suggested by many experiments performed in athymic animals or those submitted to thymectomy. Prepuberal rats (3) and neonatal mice (3, 10,

11, 12, 16) presented ovarian dysgenesis and delay puberty after thymectomy. In addition, thymus graft is able to restore fertility in nude mice (17). Kopf-Maier & Mboneko (8) also reported reduced levels of estrogen and progesterone in athymic mice.

Although rnu/rnu rats have normal ovarian (18), uterine (14) and vaginal epithelium morphologies, these females have

a difficult reproduction and their serum levels of estrogen and progesterone seem to be altered (15).

Detailed studies on hormonal-endocrine mechanisms inherent to these athymic organisms, such as detection of possible alterations in the amount of hormonal receptors, are needed in order to elucidate the sexual behaviour of these females of the Rowett strain.

Summary

The morphology, on light microscopy, of the vaginal epithelium of congenitally athymic (rnu/rnu) rats, of the Rowett strain, homozygous for the recessive nude gene and of their bearing-thymus littermates (+/rnu), heterozygous for the same gene were analyzed in proestrus and metestrus of the estrous cycle.

It could be concluded that, the morphology of the vaginal epithelium of the athymic rats, in both phases studied, is similar to that of bearing-thymus ones and compatible with the vaginal morphology of rats of other strains described in the literature.

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