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Experimental Candidosis and Recovery of *Candida albicans* from the Oral Cavity of Ovariectomized Rats

Juliana Campos Junqueira*[,1], Carlos Eduardo Dias Colombo[1], Joyce da Silva Martins[1], Cristiane Yumi Koga Ito[1], Yasmin Rodarte Carvalho[1], and Antonio Olavo Cardoso Jorge[1,2]

[1]*Department of Biosciences and Oral Diagnosis, School of Dentistry of São José dos Campos, São Paulo State University, Francisco José Longo 777, São Dimas, São José dos Campos, CEP 12245–000, SP, Brazil, and* [2]*Department of Dentistry, University of Taubaté, Rua dos Operários 09, Centro, Taubaté, CEP 12020–270, SP, Brazil*

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***Abstract*: The aim of this study was to analyze the development of candidosis and the recovery of *C. albicans* from the oral cavity of ovariectomized and sham-ovariectomized rats. One hundred and twenty-four rats originally negative for *Candida* spp. in the oral cavity were divided into two groups: ovariectomized and sham-ovariectomized. Fifty-eight ovariectomized and the same quantity of sham-ovariectomized rats were inoculated with *C. albicans* for the study of candidosis development and recovery of yeast. Four animals from each group were not inoculated with yeast suspension and were submitted to tongue dorsum morphologic analysis by optical and scanning electron microscopy. The development of candidosis in the tongue dorsum was observed by optical and scanning electron microscopy in the periods of 6 hr, 24 hr, 7 days and 15 days after the last inoculation. Recovery of *C. albicans* was performed by oral samples plating on Sabouraud agar after 1, 2, 5 and 7 days and progressively at each 15-day interval until negative cultures for yeasts were obtained. The results were analyzed by Mann-Whitney and Student's *t* tests. The tongue dorsum of sham-ovariectomized and ovariectomized rats, not infected by *Candida*, presented normal aspect. Among the infected rats, the ovariectomized group showed less occurrence of candidosis lesions and lower recovery of *C. albicans* from the oral cavity in relation to the sham-ovariectomized group. It could be concluded that candidosis was less frequent from the oral cavities of ovariectomized rats in relation to sham-ovariectomized.**

***Key words*: Oral candidiasis, *Candida albicans*, Rat, Ovariectomy**

Candida gender fungi are commonly present in the oral cavity of healthy individuals, and *C. albicans* is the most prevalent species (27, 36). Changes in the oral microbiota or in the immunological system of the host can lead to candidosis development (2, 36).

Candidosis has become a human disease of increasing importance in the last decades due to the increasing number of patients with immunological involvement associated with the infection by the human immunodeficiency virus (HIV) and use of immunosuppressive agents after organ transplantation or antineoplasic therapy (27).

**Address correspondence to* Dr. Juliana Campos Junqueira, Department of Bioscience and Oral Diagnosis, School of Dentistry of São José dos Campos, São Paulo State University, Francisco José Longo 777, São Dimas, São José dos Campos, CEP 12245–000, SP, Brazil. Fax: +55–12–39479010. E-mail: julianacjunqueira@hotmail.com

Many researchers, in an attempt to understand the mechanisms related to the pathogenesis of oral candidosis, developed several experimental models in rats. These studies related that the oral cavity of these animals is easily colonized by *Candida* and develops similar lesions in relation to those observed among human beings (2, 27).

Many predisposing factors for oral candidosis were studied in experimental models, such as: broad-spectrum antibiotics therapy (4, 11), the use of acrylic prosthesis (29), diabetes mellitus (35), topical use of corticosteroids (8), xerostomia (14, 15) and immunosuppressive therapy (2, 32).

In addition to these factors, infection by *Candida*

Abbreviations: cfu, colony forming units; EBP, estrogen binding protein; HE, hematoxylin-eosin; HIV, human immunodeficiency virus; PAS, periodic acid-Schiff.

seems to be influenced by ovarian hormone levels. Previous studies related that ovariectomized rats are resistant to vaginal colonization by *Candida*. However, they developed long-term infections when treated with high doses of estrogen (10, 31). The mechanisms by which these hormones act in the vulvovaginal candidosis are not completely known, but many factors have been suggested such as: a) accumulation of glycogen in the epithelial tissue (7), b) increased adherence of *C. albicans* to epithelial cells (28), c) decreased immunological cell response to yeast (18) and d) induction of germination and growth of *C. albicans* (37, 40).

The influence of estrogen and progesterone levels in the vulvovaginal candidosis has been extensively investigated. However, similar studies of the oral mucosa are rarely described. Therefore, the purpose of this work was to verify the effects of ovariectomy on the development of candidosis and on the recovery of *C. albicans* from the oral cavity of female rats.

Materiais and Methods

Animals. This study was approved by the Research Ethics Committee of the São José dos Campos School of Dentistry/UNESP, under protocol number 028/2001. One hundred and twenty-four female rats (*Rattus norvegicus*, Albinus, Wistar), negative for the presence of *Candida* spp. in the oral cavity and weighing approximately 250 g, were included in the study and divided into two experimental groups: sham-ovariectomized (n=62) and ovariectomized (n=62).

Body weight. Twenty sham-ovariectomized animals and 20 ovariectomized were weighed on the day of the ovariectomy and 7, 14, 21 and 28 days after the surgery.

Dosage of estrogen and progesterone. Blood samples were obtained from eight sham-ovariectomized rats and eight ovariectomized at intervals of 31, 38 or 46 days after ovariectomy. Vaginal scrapings were previously obtained from the sham-ovariectomized rats to identify the estrous cycle stage and only the ones that were in the proestrus phase were used for the hormone dosage, according to Nequin et al. (24). Dosages of estradiol and progesterone were performed by radioimmunoassay (Diagnostic Product Corporation, Los Angeles, Calif., U.S.A.) and the results were expressed as pg estradiol/ml plasma and ng progesterone/ml plasma.

Morphological evaluation of the tongue dorsum. Twenty-eight days after the surgery of ovariectomy or sham-ovariectomy, eight rats not infected by *C. albicans* were sacrificed, four from each group. The tongues were removed and analyzed macroscopically and microscopically. From each group, two specimens underwent light microscopy analysis and two specimens underwent scanning electron microscopy analysis.

Inoculation of C. albicans *in the oral cavity of the rats*. Twenty-eight days after the surgery, 116 rats received oral inoculation of *C. albicans*, 96 rats were used for the experimental candidosis study and 20 for the recovery of *C. albicans*.

A suspension of *C. albicans*, containing 5×10^8 viable cells/ml was prepared according to Reed et al. (25). A standard strain isolated from a patient with denture stomatitis was used. For the inoculation of this suspension, the animals were sedated with solution of xylazine chloride (Bayer, SP, Brazil) and ketamine (Virbac, SP, Brazil), in the proportion of 1/0.5 ml, at the dose of 0.05 ml/100 g of body weight, intramuscularly. The suspension of *C. albicans* (0.2 ml) was dripped into the mouth of the animals with the aid of a 1 ml syringe and 30×8 mm blunt needle. Then the material was spread on the tongue dorsum with a swab previously soaked in the suspension. This procedure was repeated for 3 consecutive days.

Experimental candidosis. Out of the 96 rats included in the experimental candidosis study, 48 were sham-ovariectomized and 48 were ovariectomized. These animals were sacrificed 6 hr, 24 hr, 7 days and 15 days after the last inoculation of *C. albicans*. After the sacrifice, the tongues were removed and analyzed by stereomicroscopy. At each sacrifice period, 10 rats were used for analysis under optical microscopy and 2 for analysis under scanning electron microscopy.

For light microscopy analysis, the tongues were fixed in 10% formalin for 24 hr and hemisected in the sagittal plane. Then the cuts were mounted in paraffin. The cuts (7 μm) were stained with hematoxylin-eosin (HE) and periodic acid-Schiff (PAS).

A semiquantitative analysis was performed in order to assess the degree of colonization of the epithelium by *Candida*, based in the methodology proposed by Freire-Garabal et al. (12). Twenty-eight histological fields of the tongue dorsum of each cut, in anteroposterior direction, were analyzed using a 40× objective lens. A score ranging from 0 to 4 was given for each histological field: score 0—absence of colonization; score 1—1 to 5 hyphae; score 2—6 to 15 hyphae; score 3—16 to 50 hyphae; score 4—over 50 hyphae. Two histological sections, selected randomly, were analyzed from each animal in a total of 56 scores per animal. For statistical analysis, an average score was determined from the 56 scores.

For the scanning electron microscopy observation, the specimens were fixed in 2.5% glutaraldehyde in phosphate buffer (0.1 M and pH 7.3) for 24 hr at 4 C.

The samples were rinsed with phosphate buffer (0.2 M and pH 7.3) three times and post fixed in 1% osmium tetroxide in phosphate buffer (0.2 M and pH 7.3) for 1 hr. After two other rinses with this buffer, the specimens were dehydrated in acetone and dried to the critical point with CO_2 (Denton Vacuum DCP 1, N.J., U.S.A.). The samples were mounted on aluminum stubs, coated with gold (Denton Vacuum Desk II, Fla., U.S.A.) and examined by scanning electron microscopy (Jeol JSM5600, Tokyo).

Recovery of C. albicans. Out of the 20 rats used for the recovery of *C. albicans*, 10 were sham-ovariectomized and 10 ovariectomized. After the last inoculation of *C. albicans*, samples were collected from the oral cavity of the animals by using a swab after 1, 2, 5 and 7 days and after that every 15 days until two negative cultures of yeast were obtained for each animal, based on the methodology proposed by Freire-Garabal et al. (12), Jorge et al. (14), Takakura et al. (32) and Totti et al. (34).

The swab was dropped into Sabouraud dextrose agar (Difco) with chloramphenicol (Carlo Erba, Rio de Janeiro, Brazil; 0.1 mg/ml) in duplicate and incubated for 48 hr at 37 C. Cream-colored, pasty and smooth colonies on Sabouraud agar plates were throughout the experiment considered to be yeast.

Statistical analysis. Data of body weight (%), dosages of estradiol (pg/ml) and progesterone (ng/ml), in sham-ovariectomized and ovariectomized groups were compared by the Student's *t* test. For comparison between the scores of the sham-ovariectomized and ovariectomized groups, obtained in the semiquantitative analysis of the epithelium colonization by *Candida*, Mann-Whitney test was applied. The level of significance adopted was of 5% ($P<0.05$).

Results

Body Weight

Mean and standard deviation values of the percentage of increased body weight after 28 days of the ovariectomy was 11.59±3.86% for the sham-ovariectomized rats and 20.36±3.02% for the ovariectomized rats. The difference between the groups was statistically significant (Student's *t* test; $\alpha=5\%$, $P=0.000$).

Dosage of Estrogen and Progesterone

The mean of the plasmatic levels of estradiol and progesterone was higher in the sham-ovariectomized group in relation to the ovariectomized one (Table 1).

Morphological Evaluation of the Tongue Dorsum

In the macroscopic analysis of the eight rats not infected by *C. albicans*, by light microscopy and scanning electron microscopy, it was observed that the tongues of the ovariectomized group presented normal aspect similar to the sham-ovariectomized group.

Experimental Candidosis

Macroscopic analysis: Out of the 96 rats infected by *C. albicans* in the experimental candidosis study, the presence of clinical lesions on the animal tongues was verified after 7 and 15 days of the inoculation. After 7 days, clinical lesions were observed in 66.66% of the sham-ovariectomized animals and 41.60% of the ovariectomized. After 15 days, the clinical lesions were found in 75.00% of the sham-ovariectomized rats and 33.00% of the ovariectomized rats. These lesions were characterized by areas of papillary atrophy, situated in the region of simple conic papillae, near the giant papillae (Fig. 1).

Light microscopy and scanning electron microscopy analyses: The sham-ovariectomized and ovariectomized rats presented candidal lesions with similar characteristics in all sacrifice periods.

6 hr—Light microscopy examination indicated the presence of candidosis in all rats both sham-ovariectomized and ovariectomized. Candidal lesions presented variable quantities of yeasts and hyphae in the keratin (Fig. 2). The other layers of the epithelium and the lamina propria were normal. However, when there was a great quantity of *Candida*, many polymorphonuclear leucocytes in the prickle and basal cell layers were observed, also forming intraepithelial microabscesses (Fig. 3).

In the scanning electron microscopy analysis, it was possible to observe the presence of bacteria and yeasts adhered to the filiform papillae. In regions with a large quantity of yeasts, the filiform papillae area was covered by biofilm (Fig. 4). In this period of observation, no hyphae were found on the tongue dorsum.

Table 1. Means and standard deviations of the plasmatic concentrations of estradiol and progesterone for the sham-ovariectomized ($n=8$) and ovariectomized ($n=8$) groups

	Sham-ovariectomized	Ovariectomized
Estradiol (pg/ml)	57.14±16.00*	18.88±1.53*
Progesterone (ng/ml)	15.78±4.64**	3.96±0.81**

*Statistically significant difference (*t*-Student test; $\alpha=5\%$, $P=0.0003$). **Statistically significant difference (*t*-Student test; $\alpha=5\%$, $P=0.0002$).

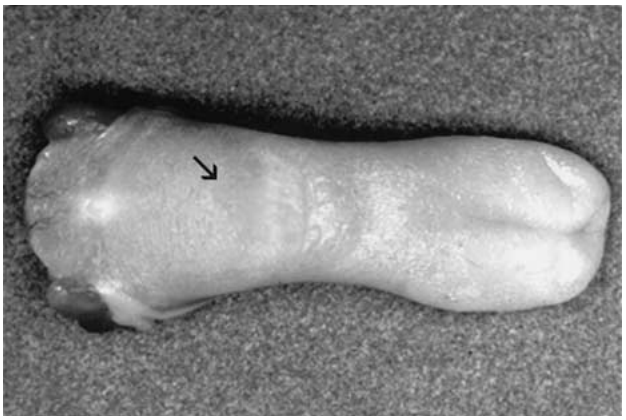

Fig. 1. Macroscopic aspect of the tongue dorsum of rat from the sham-ovariectomized group 15 days after inoculation by *Candida albicans*. Areas of papillar atrophy can be verified (→). Original increase: 6.5×.

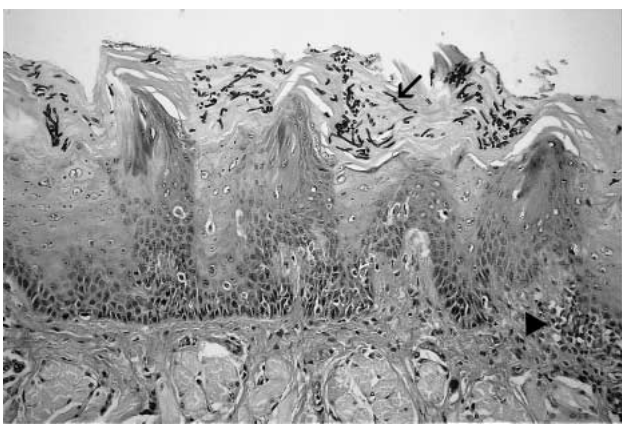

Fig. 2. Sagittal cut of the tongue dorsum of rat from the sham-ovariectomized group 6 hr after *C. albicans* inoculation. A great quantity of yeast and hyphae in the keratin layer can be observed (→). The epithelium shows areas of duplication of the basal layer and exocytosis (▶). PAS; original magnification 200×.

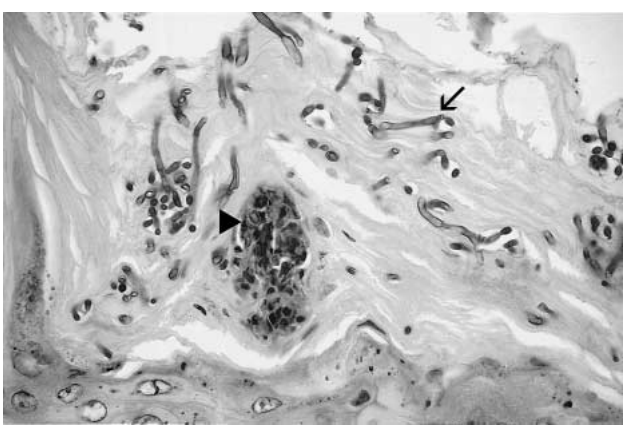

Fig. 3. Sagittal cut of the tongue dorsum of rat from the sham-ovariectomized group 6 hr after *C. albicans* inoculation. Hyphae (→) and intraepithelial microabscesses (▶) can be observed. PAS; original magnification 630×.

24 hr—The presence of candidosis in all animals of both groups, sham-ovariectomized and ovariectomized, was observed by light microscopy. Candidosis was characterized by a great number of hyphae in the keratin layer; the hyphae were much longer than in the 6-hr observation period. In the areas of candidosis, some polymorphonuclear leucocytes were observed on the basal and prickle cell layers of the epithelial tissue. Epithelium presented hydropic degeneration and spongiosis, especially in the basal and parabasal layers (Fig. 5). In some areas, there was keratin desquamation with agglomeration of hyphae and inflammatory cells.

The scanning electron microscopy analysis showed yeast and a large quantity of hyphae among the filiform

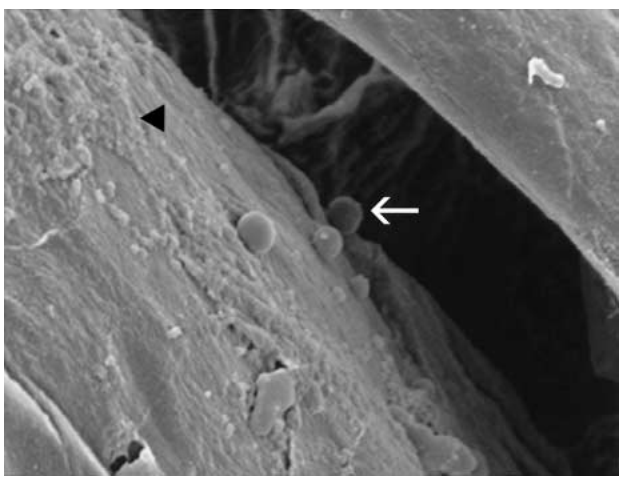

Fig. 4. Scanning electron microscopy of the tongue dorsum of rat from the ovariectomized group 6 hr after *C. albicans* inoculation. Yeast (→) and biofilm (▶) adhered to the anterior surface of the simple conic papillae. Original magnification: 1,900×.

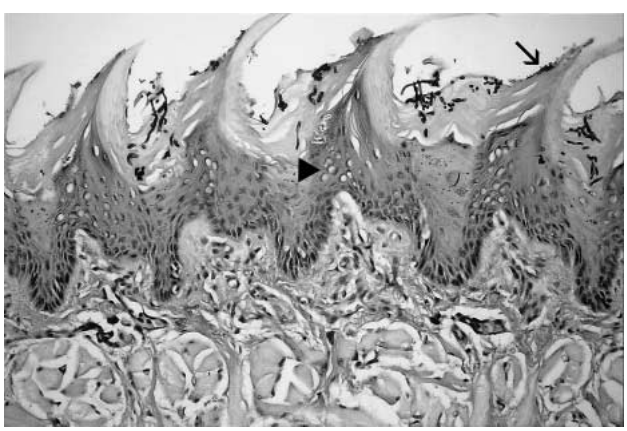

Fig. 5. Sagittal cut of the tongue dorsum of rat from the ovariectomized group 24 hr after *C. albicans* inoculation. The presence of bacteria (→) adhered to the anterior surface (convex) of the simple conic papillae, yeast and hyphae in the keratin and hydropic degeneration (▶) in epithelium are verified. PAS; original magnification 200×.

papillae. Moreover, there was tissue degradation and filiform papillae desquamation (Fig. 6).

7 days—Observed by optical microscopy was less colonization by hyphae and areas with intense tissue lesion, characterized by loss of filiform papillae, hyperparakeratosis, epithelial hyperplasia (Fig. 7), spongiosis, duplication and loss of the basal cell layer stratification, besides finding inflammatory cells in all layers sometimes forming microabscesses.

In the scanning electron microscopy examination, areas of atrophy and destruction of the filiform papillae and increased interpapillar surface were observed. At this period of observation, yeasts or hyphae were rarely observed in the tongue dorsum (Fig. 8).

15 days—By optical microscopy, the presence of hyphae in the keratin of only some animals was verified. The rats presented tissue lesions, similar to what was observed at 7 days. The lamina propria also exhibited mononuclear and polymorphonuclear inflammatory cell infiltrate.

In the scanning electron microscopy examination, yeasts and hyphae were no longer found. In the region between the simple conical and giant papillae, tissue lesion characterized by papillar atrophy and tissue flattening could still be observed.

In the semiquantitative analysis of the epithelial colonization by *Candida*, by optical microscopy, the ovariectomized group presented a lower mean score

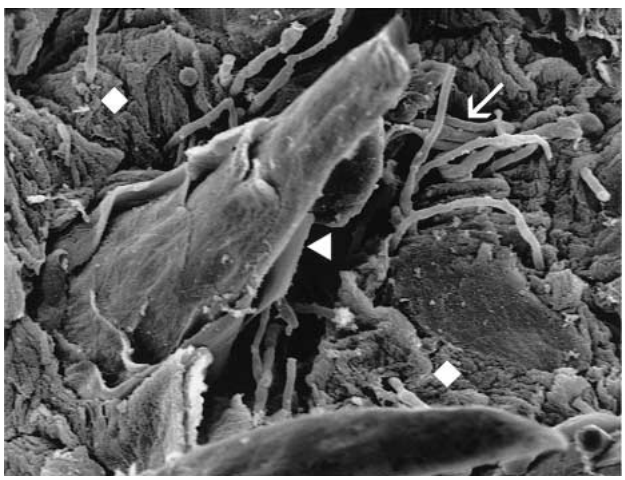

Fig. 6. Scanning electron microscopy of the tongue dorsum of rat from the ovariectomized group 24 hr after *C. albicans* inoculation. Hyphae (→), desquamated cells (▶) and tissue degradation (◆) are observed. Original magnification: 1,000×.

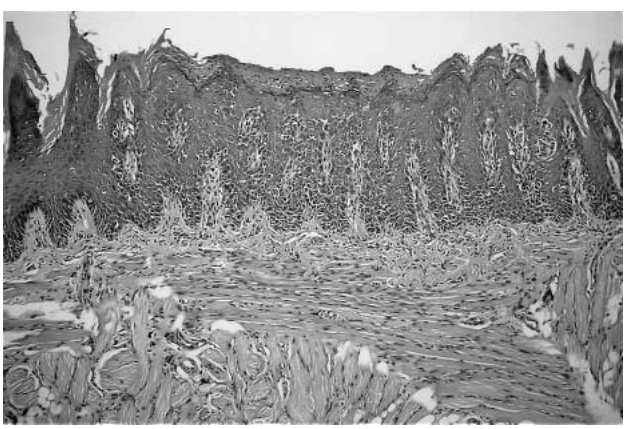

Fig. 7. Sagittal cut of the tongue dorsum of rat from the sham-ovariectomized group 7 days after *C. albicans* inoculation. Tissue lesion characterized by loss of filiform papillae, hyperparakeratosis and epithelium hyperplasia. HE; original magnification 100×.

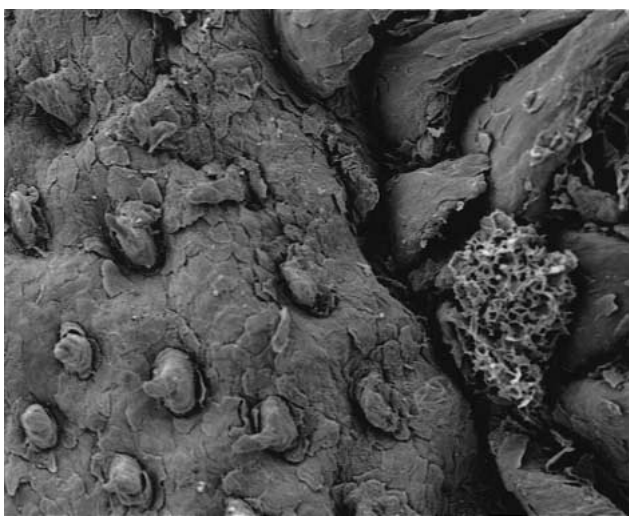

Fig. 8. Scanning electron microscopy of the tongue dorsum of rat from the sham-ovariectomized group 7 days after *C. albicans* inoculation. Atrophy of filiform papillae and increase of interpapillar surface are verified. Original magnification: 200×.

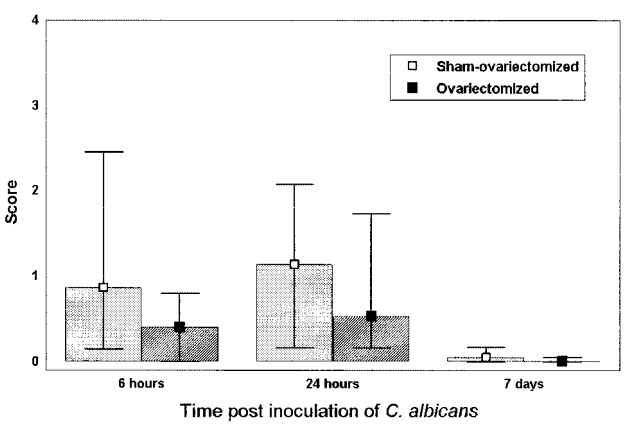

Fig. 9. Maximum, minimum and mean values obtained in the semiquantitative analysis of the epithelial colonization by *C. albicans*. No significant statistical difference (Mann-Whitney test) was detected between the sham-ovariectomized ($n=30$) and ovariectomized ($n=30$) rats for the periods of 6 hr ($P=0.12$), 24 hr ($P=0.19$) and 7 days ($P=0.34$).

Table 2. Number of rats positive to *Candida* in the oral cavity of sham-ovariectomized ($n=10$) and ovariectomized ($n=10$) groups in the different periods of recovery

Time post inoculation of *C. albicans* (days)	Sham-ovariectomized ($n=10$)	Ovariectomized ($n=10$)
1	10	10
2	10	10
5	10	10
7	10	10
22	10	5
37	8	0
52	4	0
67	1	—
82	0	—
97	0	—

than the sham-ovariectomized one in the periods of 6 hr, 24 hr and 7 days after the inoculation by *C. albicans*. However, no significant statistical difference was observed between the groups (Fig. 9). In the 15-day period, the epithelium rarely showed colonization by *Candida*.

Recovery of C. albicans *from the Oral Cavity of Rats*

In the sham-ovariectomized group, *C. albicans* was recovered up to 67 days after inoculation and in the ovariectomized group only up to 22 days (Table 2).

Discussion

Besides their being a good experimental model for the study of oral candidosis, rats have been largely used for understanding the mechanisms involved in menopause. The estrogen and other hormonal deficiency observed during menopause can be induced in experimental animals through ovariectomy surgery (38).

The success of this surgery in reducing the ovarian hormone levels is reflected in the changes of the animals' body weight. The decrease of estrogen levels results in weight gain because it induces increased food intake and consequent fat accumulation (1). The increased body weight 28 days after the ovariectomy was equivalent to 11.59% for the sham-ovariectomized rats and to 20.36% for the ovariectomized. This difference was statistically significant. This result was similar to the results of Rico et al. (26), who observed 13% body weight increase for the control animals and 24% for the ovariectomized rats 30 days after the surgery.

In rats with intact ovaries, estradiol and progesterone plasmatic concentrations vary according to the phase of the estrous cycle (1). According to Levine et al. (22), the plasmatic levels of estradiol range approximately between 30 and 50 pg/ml. In our work, for the hormone dosage, a standardization of the phase of the estrous cycle was necessary. Therefore, the sham-ovariectomized rats were killed in proestrus, the phase in which both the estradiol and progesterone levels are high (24).

The plasmatic estradiol level was about three times higher in the sham-ovariectomized group in relation to the ovariectomized one (57.14 and 18.88 pg/ml of plasma). This difference was even higher for the progesterone dosage, in which the sham-ovariectomized group presented five times higher hormonal level in relation to the ovariectomized group (15.78 and 3.96 ng/ml of plasma). These data indicate that the ovarian excision was successful and that the ovariectomized animal model was able to reflect the hormonal stage of women in menopause.

It was observed by the light microscopy analysis of the rat's tongue not infected by *C. albicans* that the ovariectomized animals presented normal aspect similar to the sham-ovariectomized. These results agree with the findings of Hertz et al. (13), who described normal morphological aspects of the papillae of the tongue dorsum and of the epithelium of the buccal mucosa in women after menopause. Leimola-Virtanen et al. (21) also observed similarity between the cell pattern of the exfoliative cytology in the buccal mucosa of young and postmenopausal women.

In the scanning electron microscopy analysis, the tongue dorsum of the ovariectomized rats presented similar aspect to the sham-ovariectomized rats. However, Yucel et al. (39) demonstrated that hormone alterations during pregnancy change the morphology of the lingual papillae, leading to an increase in gustatory sensibility. In scanning electron microscopy examination, these authors observed that the tongue of pregnant rats presented deeper sulcus around the circumvallate papilla, non-circular laminar configuration in the fungiform papillae, and disorganized direction of the filiform

papillae.

The animals that received inoculation of *C. albicans* in the oral cavity developed clinical and microscopic lesions of candidosis in the tongue dorsum even without presenting predisposing factors, such as use of antibiotics, immunosuppression, carbohydrate-rich diet or xerostomia. These data confirm that the experimental candidosis can be induced by a simple inoculation of a pathogenic strain of *C. albicans* (3).

Oral candidosis could be favored by the animal sedation during the inoculation. Takakura et al. (32) verified that the severity of the candidosis lesion increased with the time of sedation after inoculation. According to these authors, the animal sedation impairs the ingestion of the *C. albicans* suspension, avoiding the occurrence of systemic candidosis and increases the permanence of yeast in the oral cavity, favoring the formation of germ tubes and filamentation which are responsible for tissue invasion.

The experimental candidosis induced in this work was less frequent in the ovariectomized group in relation to sham-ovariectomized, but clinical and microscopic characteristics were similar for both groups.

Clinical lesions of candidosis were observed in the rats sacrificed after 7 and 15 days of inoculation. Similar results were found by Allen et al. (3), who sacrificed the animals in various subsequent periods of inoculation and verified macroscopic lesions after 7 days.

Six hours after *C. albicans* inoculation by light microscopy the presence of yeasts and hyphae agglomerates in the keratin layer, in different regions of the tongue dorsum was observed. Using the same methodology as the present work, Junqueira (16) verified that the formation of hyphae occurs 4 hr after the inoculation. These findings confirm that the production of hyphae by *C. albicans* is an important mechanism in the microorganism tissue invasion (36).

In scanning electron microscopy examination, a large quantity of yeast among the filiform papillae covered by biofilm was observed. These data indicate a possible interaction between *C. albicans* and other microorganisms present in the biofilm, which can have contributed to its colonization and proliferation in the oral cavity (36).

Also, in relation to the 6-hr period, the presence of hyphae was observed by optical microscopy but not by scanning electron microscopy. Allen et al. (3) reported that hyphae being observed by scanning electron microscopy are very rare since they are present in the interior of the keratin.

After 24 hr of *C. albicans* inoculation, the optical microscopy analysis showed a great quantity of hyphae and keratin desquamation. Shakir et al. (29) showed that *Candida* infection increased the number of mitotic figures per unit length of the basal layer of the epithelium and the quantity of desquamation. According to these authors, these changes are defense mechanisms against fungal invasion.

The tissue degradation and keratin desquamation with hyphae agglomeration, observed 24 hr after inoculation, can possibly explain the large quantity of hyphae found in scanning electron microscopy examination at this observation period.

Seven and 15 days after *C. albicans* inoculation, by optical microscopy, the presence of a small quantity of hyphae and of tissue lesion characterized by loss of filiform papillae, acanthosis and epithelium hyperplasia was observed. Samaranayake and Samaranayake (27) reported that, although the infection by *Candida* is restricted to the keratin layer in the epithelial surface, tissue changes could occur in the deepest layers of the epithelium and in the lamina propria. These effects can probably be attributed to extracellular enzyme production, such as proteinase and phospholipase.

In the semiquantitative analysis of the epithelial colonization by *C. albicans*, the ovariectomized group presented lower results in comparison to the sham-ovariectomized one for the periods of 6 hr, 24 hr and 7 days. These data agree with studies about vulvovaginal candidosis that showed influence of the ovarian hormone levels in the infection by *C. albicans*.

The mechanisms by which these hormones act on vulvovaginal candidosis are not yet completely understood, but many studies have shown that the estrogen presents indirect effects on the host tissue, favoring *C. albicans* invasion (7, 18, 31), and direct effects on the microorganism, shaping its pathogenicity mechanisms (37).

Sobel et al. (31) verified that ovariectomized rats are resistant to the development of experimental candidosis because their vaginal epithelium is composed by non-cornified columnar cells. However, these animals developed vaginal candidosis after treatment with estrogen. This hormone induces the thickening and cornification of the epithelium, favoring the adhesion of *C. albicans*. Since in this work the histological characteristics of the tongue dorsum of the sham-ovariectomized and ovariectomized rats were similar, possibly the lowest development of candidosis in the ovariectomized animals is related to the direct effect of the estrogen on *C. albicans*.

Kinsman and Collard (19) collected vaginal fluid of rats uninfected by *Candida* to study the *in vitro* effects of ovarian hormones on the germination of yeast. *C. albicans* strain was added to the vaginal washings after it had been centrifuged and filtered to eliminate epithe-

lial cells. After a 3-hr incubation period, the percentage of germ tubes formation was 11.5 for the sham-ovariectomized group; 8.9 for the ovariectomized group; 16.5 for the ovariectomized group treated with estrogen and 13.1 for the ovariectomized group treated with progesterone. The authors concluded that factors not related to the characteristics of the vaginal epithelium cells are important for the production of germ tubes.

Many *in vitro* studies showed that *C. albicans* undergoes changes in its morphology and growth rate in response to estrogen, increasing its pathogenicity (37). Buckman and Miller (6) and Madani et al. (23) suggested that *C. albicans* response to this hormone is mediated by the estrogen binding protein (EBP), present in the yeast cytoplasm.

In our work, *C. albicans* was recovered from the oral cavity of the animals in the sham-ovariectomized group up to 67 days after inoculation and from the ovariectomized group up to 22 days.

Sobel et al. (31) also studied the recovery of *C. albicans* from the vaginal fluid of rats after many inoculation periods (10^8 viable cells/ml) and verified that after 96 hr all ovariectomized animals presented negative cultures for *Candida*, which indicates that the presence of estrogen is essential to vaginal colonization by this microorganism.

The effects of ovarian hormones on candidosis of women have also been studied. Epidemiologic studies demonstrated that vulvovaginal candidosis is uncommon before menarche and after menopause, and during hypoestrogenic periods. On the other hand, the vaginal *Candida* infection increases during pregnancy and in women using high estrogen-containing oral contraceptives (9, 20, 30).

Some studies investigated the correlation between the level of adherence *in vitro* of *C. albicans* to human exfoliated vaginal epithelial cells and the hormonal status of the cell donors. Segal et al. (28) verified increase of *C. albicans* adherence during pregnancy and the first or fourth weeks of the menstrual cycle. However, Botta (5) found that adherence of *C. albicans* to vaginal epithelial cells was highest during the third week. Kalo and Segal (17) studied the effect of estradiol and progesterone on the binding of the yeasts to vaginal epithelial cells and verified that just progesterone was related to increase in the adherence.

Although the influence of the hormonal status on both vaginal candidosis and *C. albicans* adherence to vaginal epithelial cells *in vitro* has been shown to be significant, similar studies were not developed for oral candidosis. Theaker et al. (33) demonstrated that the adherence of *C. albicans* to women epithelial cells was significantly higher collected on day 5 of the menstrual cycle when compared with days 15, 22 and 28, indicating that hormonal influences should be considered when buccal cells are used *in vitro* to assess candidal adherence.

In this work, the experimental candidosis and the recovery of *C. albicans* from the oral cavity of rats was less frequent in the ovariectomized group in relation to the sham-ovariectomized one, suggesting that the ovarian hormones have a significant influence on oral candidosis.

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