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BRIEF COMMUNICATION

EXPERIMENTAL PARACOCCIDIOIDOMYCOSIS IN PREGNANT RATS

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SUMMARY

Paracoccidioidomycosis (PCM), caused by the dimorphic fungus *Paracoccidioides brasiliensis* (Pb), is the most prevalent systemic mycosis in Latin America. There are few reports in the literature about the disease damages during pregnancy and the consequences to the fetuses and breeding. This study evaluated the implications of PCM during pregnancy on offspring and mothers in Wistar rats. Groups of rats were submitted to systemic Pb infection, by intraperitoneal infusion, and mated 30 days after the infection date. Immediately after birth, rats and neonates were sacrificed to obtain organs for standard histological examination, morphometric analysis, fungi recovery by plating (CFU) and dosing of anti-Pb antibodies by ELISA. There were no stillbirths or miscarriages, however, the fetuses from infected pregnant rats had lower body and organ weight but the fertility rate was 100%. The largest number of CFU was recovered from the organ of pregnant rats, the pathological examination revealed more severe infection in the same group, further on the largest number of granulomas and fungal field. It can be concluded that the PCM was more severe in the group of pregnant rats, with implications to the weight of offspring.

KEYWORDS: Paracoccidioidomycosis; Pregnant; *Paracoccidioides brasiliensis.*

Paracoccidioidomycosis (PCM), caused by the dimorphic fungus *Paracoccidioides brasiliensis* (Pb), is the most prevalent systemic mycosis in Latin America. PCM affects more than 10 million people; the highest mortality rate due to PCM occurs in south and southeast Brazil. The largest series of Pb infection was recorded in Brazil, with more than 80% of cases[2,8].

Infection occurs primarily through accidental inhalation of the pathogen by the host, which starts off in the lungs and then may spread throughout the body, causing damage to internal organs and the development of mucocutaneous lesions[3].

PCM is highly relevant to public health. The clinical manifestations of PCM result in irreversible physical disabilities that incapacitate the patient, usually in their most productive years[6], since approximately 85% of PCM cases occur between 30 and 59 years of age[5,15]. Indeed, PCM has the potential to be highly disabling[12].

It is believed that, in women, estrogen inhibits the fungus yeast-mycelium transition, thus limiting the infection[14]. However, the gestational period is reported to offer risk of death, both to mother and fetus[11]. Therefore the aim of this study was to evaluate the consequences of PCM during pregnancy to mothers and their offspring.

After being approved by the Ethics Committee for Animal Experiments and Practices (CEEAAP), the current study was conducted in the Experimental Laboratory of Physiotherapy and the Clinical Laboratory for Teaching, Research, and Extension, both at the State University of West Parana in Cascavel, Parana State.

Forty-five-day-old female Wistar rats were provided with a standard diet, housed in cages, and exposed to a light and dark cycle of 12 hours. The rats were distributed into four groups: i) Group Gp consisted of pregnant, Pb-infected rats; ii) Group GPb-free consisted of uninfected pregnant rats; iii) Group GPb consisted of ten non-pregnant rats, Pb-infected rats and, iv) Group GCA consisted of non-pregnant, uninfected rats.

The rats in the Gp and GPb groups were inoculated intraperitoneally with a suspension of 10^5 Pb yeats (Pb18 strain).

On Day 30 of infection, rats in the Gp and GPbfree groups simultaneously mated during a one-week period. Immediately after birth, the pups were weighed. Subsequently, all animals, including the pups, were sacrificed under anesthesia and the hearts, livers, lungs and spleens were removed for histological analysis of colony-forming units (CFU).

The intensity of PCM in the organs was classified as absent (without histopathological lesions), mild (presence of subtle histopathological lesions), moderate (diffuse histopathological lesions), or severe (histopathological lesions with diffuse necrotic cells). The number

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Table 1
Mean and standard deviation of the body and organ weights (in grams) of neonates in the control (Gp) and experimental groups (GPbfree)

Variables	Gp ($\bar{x}$/SD)	GPbfree ($\bar{x}$/SD)	% difference	*p*-Value
Body weight	6.205 ± 0.719	6.505 ± 0.885	4.83	0.02*
Heart	0.044 ± 0.005	0.045 ± 0.008	2.27	0.15
Liver	0.324 ± 0.044	0.373 ± 0.066	15.12	0.04*
Spleen	0.027 ± 0.004	0.035 ± 0.004	29.63	0.006**

* Indicates a statistically significant difference using the Wilcoxon test.

of giant cells, granulomas, and Pb were counted using the software ImageTool® (UTHSCSA Dental Diagnostic Science, San Antonio, Texas, USA). The Wilcoxon test was used for comparison of quantitative data, with the significance level set at 5%.

The number of yeasts, granulomas, and giant cells per field was determined by histology, and the number of viable fungi recovered by plating was compared between the groups (mothers and fetuses). Anti-Pb antibody titers were determined by an enzyme-linked immunosorbent assay (ELISA), as described by RAMOS *et al.*[9].

There were no stillbirths or abortions in the four groups and the fertility rate of the females was 100%. The average body weight of the newborns and the average weight of the organs of the newborns in the control group were higher than those of the newborns in the experimental group (Table 1).

Based on a qualitative analysis of the histological examination of the lungs and liver, both groups of infected females exhibited classic features of PCM, as follows: granuloma formation with giant cells, which were usually organized and delimited by a lymphocyte ring; Pb with and without budding; inflammatory cell infiltrates; and proliferation of collagen fibers.

The Gp group had severe lung and liver infection in all animals. The rats in the GPb group developed moderate infection in the lungs (50% of mothers) and liver (75% of the group), mild degree was observed in 50% and 25% of the organs, respectively (Fig. 1). The pups from both groups had normal histologic findings.

In the Gp group, a larger number of granulomas, giant cells, and Pb were noted compared to the GPb group (Fig. 2).

Based on colony formed units (CFU), Pb was recovered from the organs of infected females in the Gp and GPb groups. The average was 90 and 59 CFU/g of macerated lung of the rats in the Gp and GPb groups, respectively ($p = 0.001$). The average was 32 and 21 CFU/g of macerated liver in the Gp and GPb groups, respectively ($p = 0.003$). The other groups had no colony growth by plating.

The ELISA testing showed higher titers of anti-Pb antibodies, but there was no statistical significance between the Gp and GPb groups (1.33 and 1.55, respectively).

In the present study, there were no miscarriages or deaths of the pups among the different groups. The study also showed a statistically significant difference in mean body weights, and spleen and liver weights of pups from the Gp group and the other three groups. Similar findings were described by FREIRE DE CARVALHO & MONTENEGRO,

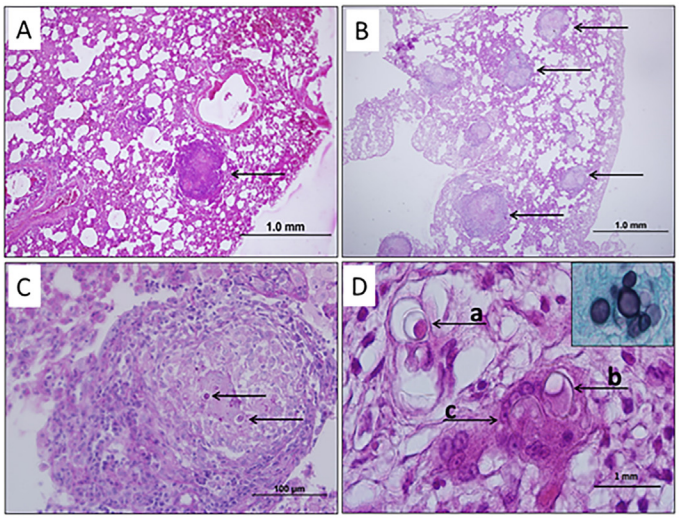

Fig. 1 - Photomicrographs of the lung of Wistar rats infected by *Paracoccidioides brasiliensis*, hematoxylin-eosin staining. A - arrow pointing to a single lung granuloma in a field of an animal sample from the GPb group. B - arrows indicate multiple pulmonary granulomas of an animal sample from the Gp group. C - mature lung granuloma as seen in B at a higher magnification; the arrows indicate the presence of *P. brasiliens*is within the granuloma. D - the same lung granuloma shown in C: arrow "a" shows *P. brasiliensis* with a birefringent membrane, arrow "b" shows a fungal cell inside a giant cell, and arrow "c" shows a giant cell containing fungi.

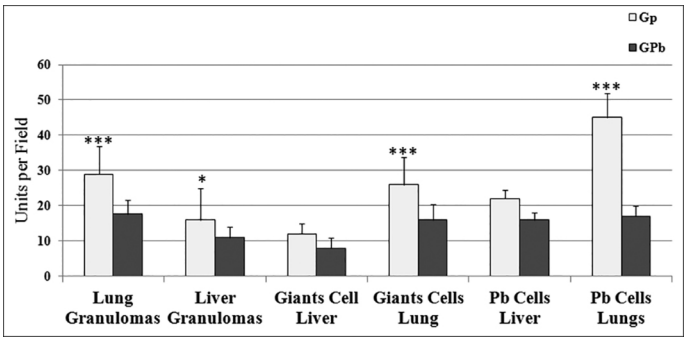

Fig. 2 - Morphometric analysis of a section of the liver and lungs of animals in groups of female rats infected with *P. brasiliensis*, pregnant and non-pregnant. Average count conducted at three fields in the organs of animals groups: Gp - pregnant, Pb-infected rats, and Group GPb, non-pregnant, Pb-infected rats. *** - indicates statistically significant difference between the infected groups ($p = 0.001$); * - indicates statistically significant difference between the infected groups ($p = 0.05$), Wilcoxon test.

who reported that hamster pups from Pb-infected females weighed significantly less than the ones from uninfected females. Moreover, no changes in fertility rates between females in the control and infected groups were noted; however, a number of miscarriages occurring among the group of infected females were reported. Conversely, in the current study, we observed no complications, such as miscarriages and stillbirths. It is noteworthy that in the present model the symptoms of chronic disease were reproduced, while the work by FREIRE DE CARVALHO & MONTENEGRO mimicked the acute form of the disease using an intracardiac route of infection.

In the present study, we detected a significant difference in the number of granulomas and giant cells in the lungs and livers of rats in the Gp group compared to the GPb group, and the number of CFU recovered by plating was also significantly higher in the Gp group.

FREIRE DE CARVALHO & MONTENEGRO conducted the only experimental work on the subject that has been published in the literature. The authors found significant differences in the number of CFUs between their experimental and control groups and suggested that the increased levels of estrogen did not inhibit Pb growth factor or modified the host-parasite relationship resulting in more severe infection.

SHIKANAI-YASUDA *et al.*[11] reported that the specific immunological changes which occur during pregnancy may aggravate the natural course of systemic mycoses. Thus, the number of miscarriages and stillbirths in pregnant women with PCM may increase. In addition, ALSHARIF *et al.*[1] reported that the low estrogen level that occurs after birth can favor PCM. In fact, the growth of Pb can be affected by high levels of estrogen and progesterone[7].

In contrast, SLEVOGT *et al.*[13] reported an interesting case of a pregnant woman at 16 weeks of gestation with PCM who presented cervical and axillary lymphadenopathy. After treatment with rifampicin, isoniazid and ethambutol, the lymphadenopathy regressed completely; however, four months postpartum the lymphadenopathy recurred. SLEVOGT *et al.*[13] suggested that the growth of Pb may have been affected by an increased level of estrogen in late pregnancy in combination with the known effect of rifampicin on the fungus. Therefore, the hormonal decline after childbirth may have contributed to reactivation of infection. RESTREPO *et al.*[10] reported that estrogen inhibits transformation of the fungus yeast-to-mycelium, but the growth of the fungus and budding are not affected by estrogen.

In this context, anti-Pb titers have not been reported in the literature. Very few articles have been published in the scientific literature on PCM during pregnancy (humans and experimental models), which has prevented further discussion on the topic. Certainly, additional studies are needed to expand our knowledge and understanding of Pb infection during pregnancy. Nevertheless, we conclude that PCM was more severe in the pregnant rats group, where the pups presented lower body weights at birth.

RESUMO

Paracoccidioidomicose experimental em ratas grávidas

Paracoccidioidomicose (PCM), causada pelo fungo dimórfico *Paracoccidioides brasiliensis* (Pb) é a micose sistêmica de maior prevalência na América Latina. Há poucos relatos na literatura sobre os danos da doença durante a gestação e as alterações para os conceptos e reprodutoras. O estudo avaliou as implicações da PCM durante o período gestacional sobre a prole e genitora em ratas Wistar. Grupos de ratas foram submetidos à infecção sistêmica por Pb, por meio de infusão intraperitoneal e acasaladas, 30 dias após a data da infecção. Imediatamente após o nascimento, as ratas e neonatos foram sacrificados para obtenção dos órgãos para exames histológicos padrão, análise de morfometria, recuperação de fungos por plaqueamento (UFC) e dosagem de anticorpos anti-Pb por ELISA. Não houve natimortos ou abortos, porém, os conceptos advindos de prenhas infectadas apresentaram menor peso corporal e dos órgãos, entre os grupos e a taxa de fecundidade foi de 100%. O maior número de UFC foi recuperado dos órgãos das ratas prenhas, o exame anátomo-patológico revelou infeção mais grave, no mesmo grupo, além do maior número de granulomas e fungos por campo. Pode-se concluir que a PCM ocorreu de modo mais grave no grupo de ratas prenhas, com implicações sobre o peso da prole.

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