BRIEF COMMUNICATION

EXPERIMENTAL PARACOCCIDIIDOMYCOSIS IN PREGNANT RATS

Eduardo Alexandre LOTH(1), Vanessa CECATTO(1), Samia Khalil BIAZIM(1), José Henrique Fermino FERREIRA(1), Caroline DANIELLI(1), Rodrigo Daniel GENSKE(1), Rinaldo Ferreira GANDRA(2) & Marcello Fabiano de FRANCO(3)

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 the highest mortality rate due to PCM occurs in south and southeast Brazil. 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.

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.

Fourty-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 $10^7$ 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

(1) Universidade Estadual do Oeste do Parán, Departamento de Fisioterapia, Laboratório de Microbiologia Experimental, Cascavel, PR, Brasil.
(2) Universidade Estadual do Oeste do Parán, Departamento de Farmácia, Laboratório de Análises Clínicas, Ensino, Pesquisa e Extensão, Hospital Universitário do Oeste do Parán, Cascavel, PR, Brasil.
(3) Universidade Federal de São Paulo – UNIFESP, Departamento de Patologia, São Paulo, SP, Brasil.

Correspondence to: Eduardo Alexandre Loth. Universidade Estadual do Oeste do Parán – UNOESTE. Depto. de Fisioterapia, UNOESTE. Caixa Postal 701. Rua Universitária 1.619, Jardim Universitário 8519-110 Cascavel, PR, Brasil. Telephone: +55 45 3220-7315/ +55 45 9813-0342. E-mail: ealoth@hotmail.com
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,

### Table 1

| Variables           | Gp (x/SD) | GPbfree (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.
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.\textsuperscript{10} 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.\textsuperscript{3} 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\textsuperscript{3}.

In contrast, SLEVOGT et al.\textsuperscript{15} 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.\textsuperscript{15} 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.\textsuperscript{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 \textit{Paracoccidioides brasiliensis} (Pb) é a micose sistémica de maior prevalência na América Latina. Há poucos relatos na literatura sobre os danos da doença durante a gestação e as alterações para os conceitos e reproduçôes. 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 crianças 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 presas, o exame anatômico-patológico revelou infecção 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 presas, com implicações sobre o peso da prole.

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