Congenital transmission in a murine model with a Mexican *Trypanosoma cruzi* strain

Oscar A. Reboreda-Hernández[1] , Benjamín Nogueda-Torres[2] , Rocío Ortiz-Butron[3] , and Nayeli Gonzalez-Rodriguez[1]*

[1]Department of Morphology, National School of Biological Sciences; [2]Department of Parasitology, National School of Biological Sciences; [3]Department of Physiology, National School of Biological Sciences. IPN, Mexico City, Mexico

Abstract

Background: *Chagas´ disease, parasitosis, caused by the protozoan Trypanosoma cruzi, currently affects 7 million people worldwide. Although is primarily transmitted by vectors, it could also be adquired through blood transfusions, organ transplants, orally or congenitally.* **Objective:** *To conduct more research of global relevance that addresses congenital transmission, which has recently become more important due to population movements.* **Method:** *A T. cruzi Mexican parasite strain (NINOA, that belongs to DTU 1) was inoculated in female mice, infection was monitored parasitological, and serological though pregnancy and, afterbirth in the mother and in the pup until they reach adulthood; a histopathological analysis was performed.* **Results:** *Pregnancy was difficult to get, parasitemia lowed during pregnancy. Newborn mice not present parasitemia or histopathological changes but they had T. cruzi-antibodies.* **Conclusion:** *This study gives evidence that congenital transmission could happened in females with low parasitemia, and interestingly, this infection could be a factor to considerate in females that have complications to get pregnant. On the other hand, we provide data that support that congenital transmission implies mother-to-child antibody exchange even without patent parasitemia. Thus, we suggest that screening for T. cruzi infection should be mandatory in pregnant women, but it could also be important to perform it in women who have difficulties becoming pregnant.*

Keywords: *Congenital. Pregnancy. Chagas' disease.*

Transmisión congénita en un modelo murino con una cepa mexicana de *Trypanosoma cruzi*

Resumen

Antecedentes: *La enfermedad de Chagas, una parasitosis causada por el protozoario Trypanosoma cruzi, afecta actualmente a siete millones de personas en todo el mundo. Aunque se transmite principalmente por vectores, también puede transmitirse mediante transfusiones sanguíneas, trasplantes de órganos, oralmente y de forma congénita.* **Objetivo:** *Realizar más investigaciones de relevancia global que traten sobre la transmisión congénita, que ha ido adquiriendo mayor importancia últimamente debido a los movimientos poblacionales.* **Método:** *Se inoculó una cepa de parásitos T. cruzi mexicana (NINOA, perteneciente a la DTU 1) en ratones hembra, se monitoreó la infección parasitológica y serológica durante el embarazo y el parto en la madre y en las crías hasta que alcanzaron la adultez; se realizó un análisis histopatológico.* **Resultados:** *El embarazo fue difícil de lograr, la parasitemia disminuyó durante el embarazo. Las crías recién nacidas no presentaron*

parasitemia ni cambios histopatológicos, pero tenían anticuerpos contra T. cruzi. **Conclusión:** *Este estudio proporciona evidencia de que la transmisión congénita podría ocurrir en hembras con baja parasitemia y, curiosamente, esta infección podría ser un factor para considerar en mujeres que tienen complicaciones para quedar embarazadas. Por otro lado, proporcionamos datos que respaldan que la transmisión congénita implica el intercambio de anticuerpos de madre a hijo incluso sin parasitemia patente. Por lo tanto, sugerimos que la detección de la infección por T. cruzi debería ser obligatoria en mujeres embarazadas, pero también podría ser importante realizarla en mujeres que tienen dificultades para quedar embarazadas.*

Palabras clave: Congénito. Embarazo. Enfermedad de Chagas.

Introduction

Chagas´ disease is a protozoan infection caused by *Trypanosoma cruzi* that currently affects 7 million people worldwide mainly in the Americas (its endemic area) up to 30% if patients suffer cardiac complication, and up to 10% digestive manifestations that could lead to patients' death. Parasitosis can be treated in the acute phase with benznidazole and nifurtimox, in chronic cases, has no cure, but is useful to offered treatment to prevent congenital transmission[1].

Congenital transmission of the infection can occur during pregnancy, childbirth, and even during breastfeeding, even in asymptomatic pregnant women. Neonates may be asymptomatic or present with edema, fever, low birth weight, cardiomegaly, myocarditis, pneumonitis, hepatosplenomegaly, and even be born prematurely[2].

In endemic areas, there is congenital transmission happening even in areas where the control of the vector and blood bank transfusion is certificated by the local govern[3]. It is estimated that there are 40,000 infected pregnant women and 2,000 infected newborns in North America[4].

There are no current data of the women childbearing infected with *T. cruzi* in Latin America, but as is the Chagas´ endemic area, numbers could be higher than in USA because in the past years was reported that 10-40% of the newborn children have the infection and showed meningoencephalitis, hepatosplenomegaly, or hearth failure, rising the neonatal death risk[5].

Due the lack of recognition of the importance of congenital Chagas disease infection in several non-endemic countries, this disease has become global due to population movements from endemic countries. Recently, there are reports of Chagas´ disease congenital transmission in non-endemic areas such as Japan[6], Ireland[7], and Spain[8]. In a meta-analysis, the risk of congenital transmission was calculated in approximately 3.5%[9].

At present, congenital transmission represents the main route if the transmission in vector-free regions, moreover, this neglected tropical disease was approved at the World Health Organization global plan to interrupt four transmission routes by 2030 (vector, blood transfusion/organ transplant/congenital)[4].

One interesting data regarding this issue is that in blood banks routine, *T. cruzi* infection testing is mandatory[10] not so in pregnant women or in newborns[11]. It has been described that one of the factors that heavily influence the risk of vertical transmission is the parasite strain[12]. Thus, the goal of this study is to analyze the vertical transmission of a Mexican strain regards of its ability to be vertically transferred to the products in terms of patent parasitemia, antibodies, and tissular changes.

Materials and methods

Parasite

NINOA *T. cruzi* strain (TcI) (metacyclic tripomastigote).

Harem formation

BALB/c healthy mice 20 20 ± 2 g. (around 6 weeks old) was provided the physiology facilities (Animal House, Department of Physiology, Biological Sciences National School, National Polytechnic Institute). All animals were maintained and treated strictly according to the NOM-062-ZOO-1999[13], and all the procedures and experimental protocols were performed according to the Biological Sciences National School Bioethics Committee. Two harems, comprised six females and one male each (control and experimental groups), were established. From the moment of formation, vaginal introitus of the females was observed at 8-h intervals to ascertain the presence of the mating plug, thereby confirming successful copulation.

Mice and experimental groups

BALB/c healthy mice 20 20 ± 2 g. (around 6 weeks old) was provided the physiology facilities (Animal House, Department of Physiology, Biological Sciences National School, National Polytechnic Institute). All animals were maintained and treated strictly according to

the NOM-062-ZOO-1999[13], and all the procedures and experimental protocols were performed according to the Biological Sciences National School Bioethics Committee. Two six female mice group were used, one was remains without pregnancy but intraperitoneally infected with 1×103 metacyclic trypomastigotes as control, and the second group with pregnancy (coming from the harem) was intraperitoneally infected with 1×103 metacyclic trypomastigotes. Parasitemia levels were determined in counting the parasite number in 5 μL of caudal vein mice blood using a Neubauer's chamber Brand™ (Thermo Fisher Scientific, Waltham, Massachusetts, USA)[14].

Blood samples were taken, and then, pups were sacrificed at birth, at days 7, 14, 21, 28 (acute phase), and 90 (chronic phase) to obtained skeletal hind leg muscle and heart tissue, as these tissues were reported as the target tissues of this strain[15].

Histopathological analysis

Heart and skeletal muscle tissue sections were fixed in 10% buffered formalin for 24 h, dehydrated in absolute ethanol (Merck Millipore, Rahway, New Jersey, USA), cleared in xylene (Merck Millipore, Rahway, New Jersey, USA), and embedded in paraffin (Merck Millipore, Rahway, New Jersey, USA). Five-micron tissue sections were mounted on glass slides Isoslide™ (Merck Millipore, Rahway, New Jersey, USA), deparaffinized, and processed using hematoxylin and eosin staining technique. We analyzed each tissue section for randomly selected, 10-microscopic fields (×100 magnification), and examined three different tissue sections/organs/animals. The tissue sections were evaluated microscopically by two individuals in a blinded manner, using an optical microscope Nikon™ Eclipse 50i (Tokyo, Japan). The presence of inflammatory cells in H&E-stained sections was scored as 0 (absent), 1 (focal or mild, 0-1 foci), 2 (moderate, 2 foci), 3 (extensive inflammatory foci, minimal necrosis, and retention of tissue integrity), and 4 (diffused inflammation with severe tissue necrosis, interstitial edema, and loss of integrity).

Immunological analysis

Newborn sera were obtained 5 μL through a small cut of the caudal vein mice using a blood sampling device for newborn (Vygon, Écuen, France), and the microhematocrit capillary tubes (Kimble, Vineland, New Jersey, USA) were centrifuged at 3000 rpm/15 min. Sera were kept −4°C[16].

T. cruzi antigen

The antigenic extract was prepared with *T. cruzi* NINOA epimastigotes cultured in liver infusion tryptose, then the parasite was counted, using a Neubauer's chamber. Then, epimastigotes were washed by triplicate using PBS 0.1 M pH 7.2. The total volume was sonicated 3 times of 18 kHz for 1 min, employing a sonicator SFX250 (Branson Ultrasonics, Brookfield, Connecticut, USA). Later, the volume was centrifugated 12,000 g 1 h at 4°C, the supernatant protein content was estimated by Lowry's method. Finally, the solution was kept at −20°C until use[17].

Enzyme-linked immunosorbent assay (ELISA)

We performed a non-commercial indirect ELISA based on local antigens from *T. cruzi* NINOA strain that belongs to *T. cruzi* lineage I, predominating lineage in Mexico, because it has a slightly higher sensitivity compared to commercial serological test. Briefly, flat bottom microplates Costar™ (Corning, Corning, New York, USA) were coated with the *T. cruzi* antigen protein, at a concentration of 10 μg/mL in 0.1 M carbonate buffer pH 9.6. Then, unbound antigen was discarded with three washes of PBS 0.05% Tween, the plates were blocked to prevent false positive results with 200 μL of albumin (Hycel, Mexico), and incubated for 1 h at 37°C[17].

Later, plates were incubated with 100 μL mouse sera at a final dilution of 1:500 (in duplicate). After being incubated overnight at 4°C, they were washed 3 times with PBS-0.05% Tween. After the washes, 50 μL of a peroxidase-coupled goat anti-mouse IgG antibody (Sigma Aldrich, St. Louis, Missouri, USA) at a dilution of 1:7500 in PBS Tween was added to each well and incubated for 2 h at 37°C. After carrying out three washes, 100 μL of a solution of ortho-phenylenediamine (Sigma Aldrich, St. Louis, Missouri, USA), pH 7 carbonate buffer, distilled water, and hydrogen peroxide (Merck Millipore, Rahway, New Jersey, USA) were added after 3 min, the reaction was stopped with H_2SO_4 (Merck Millipore, Rahway, New Jersey, USA) and the plates were read at 490 nm in an ELISA HREADER1 reader (Hlab, Mexico).

International guidelines

The study was conducted rigorously following the ARRIVE 2.0 guidelines to ensure good practices in animal experimentation.

Results

The observation of the mating plug determined copulation success, with both females *T. cruzi* infected and females *T. cruzi* uninfected exhibiting a 100% copulation success rate. However, despite achieving a 100% mating success in females, the percentage of births was < 30% in females infected with *T. cruzi* (Fig. 1) (p < 0.05). A parasitemia curve was conducted to compare the course of acute infection in the studied groups (Females *T. cruzi* infected, pregnant females *T. cruzi* infected). Over the observation days, three well-differentiated phenomena were evident. First, the onset of parasite presence in the bloodstream showed that in the non-pregnant group, parasitemia initiated earlier than in the pregnant female group (day 14 post-inoculation). In addition, a higher quantity of parasites in the blood (≈ 40,000) was observed in the non-pregnant group. The subsequent phenomenon was the peak of parasitemia, graphically evidenced by a higher number of parasites in the blood in non-pregnant females (day 23 post-inoculation), contrasting with pregnant females who exhibited a lower parasitic burden at this point. Pregnant females reached 100% mortality by day 32 post-inoculation, unlike non-pregnant females, who showed an end to parasitemia by day 27 post-inoculation (Fig. 2). The levels of serum antibodies against *T. cruzi* were assessed in newborn mice from infected mothers that gave birth and were compared with intraperitoneally inoculated newborns. The observed curve in neonates from infected mothers showed a high content of antibodies against *T. cruzi* from day 0, reaching a maximum value of 3.4 µg. In contrast, mice infected intraperitoneally did not exhibit serum concentration of these antibodies at the same time point. While neonates from infected mothers displayed a drastic decrease on day 7, mice infected intraperitoneally showed a gradual increase over the days, reaching their peak concentration on day 8; however, the entire group succumbed. Mice from infected mothers experienced another decrease in antibody concentration on day 20, remaining constant until the end of the assay with 100% survival (Fig. 3).

Discussion

T. cruzi involves a complex transmission, so it could serve as a unique model for the study of the adaptative parasite-host model[12] because it seems to have a wide range of variations according to several factors, including the strain. *T. cruzi* strains present different capabilities to
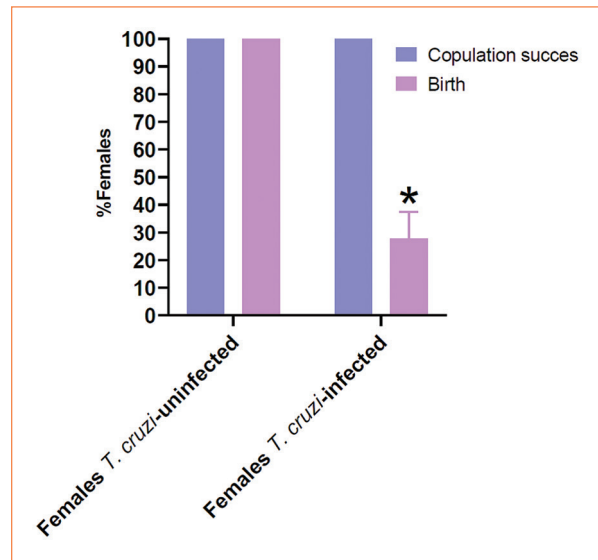

Figure 1. Copulation success. Mating plug (blue bar) was a 100% in all groups showing no statistical difference among groups, on the other hand, the birth (purple bar) was lower in *Trypanosoma cruzi*-infected females (p = 0.05).

cause congenital infection, for example, no congenital murine infection was observed with *T. cruzi* K98 clone isolated from a congenital case VC/TcVI, this isolate has a higher survival rate in the placenta than Tulahuén strain in placenta[4].

During pregnancy is necessary to have immune tolerance by the fact that the fetus express paternal antigens, that in other circumstances are recognized, and eliminated. Maternity immunity in the fetus-placental unity is deviated to an Th2 response to protect this process, explaining the presence of pregnancy-protecting cytokines as interleukin (IL)-10, transforming growth factor-b, and IL-4. These circumstances could alter the susceptibility of the host against protozoan infections, favoring congenital transmission[18].

T. cruzi-infected pregnant women who transmitted the infection to their fetus blood cells have a functional deficiency in the IL-2 production and an unbalance in the production of IL-10 and interferon-gamma (IFNγ); *T. cruzi*-infected pregnant women who did not transmit the infection to their fetus blood cells have normal secretion of IFNγ and IL-2[18].

It has been reported that vertical transmission is more likely to happen when the parasitemia is higher in the mother[19] related to the tissue infection, none of the pups has parasites in their heart or blood even when other TcI strains (AQ1-7) do have showed amastigotes in the tissues of the pups[20], possibly because
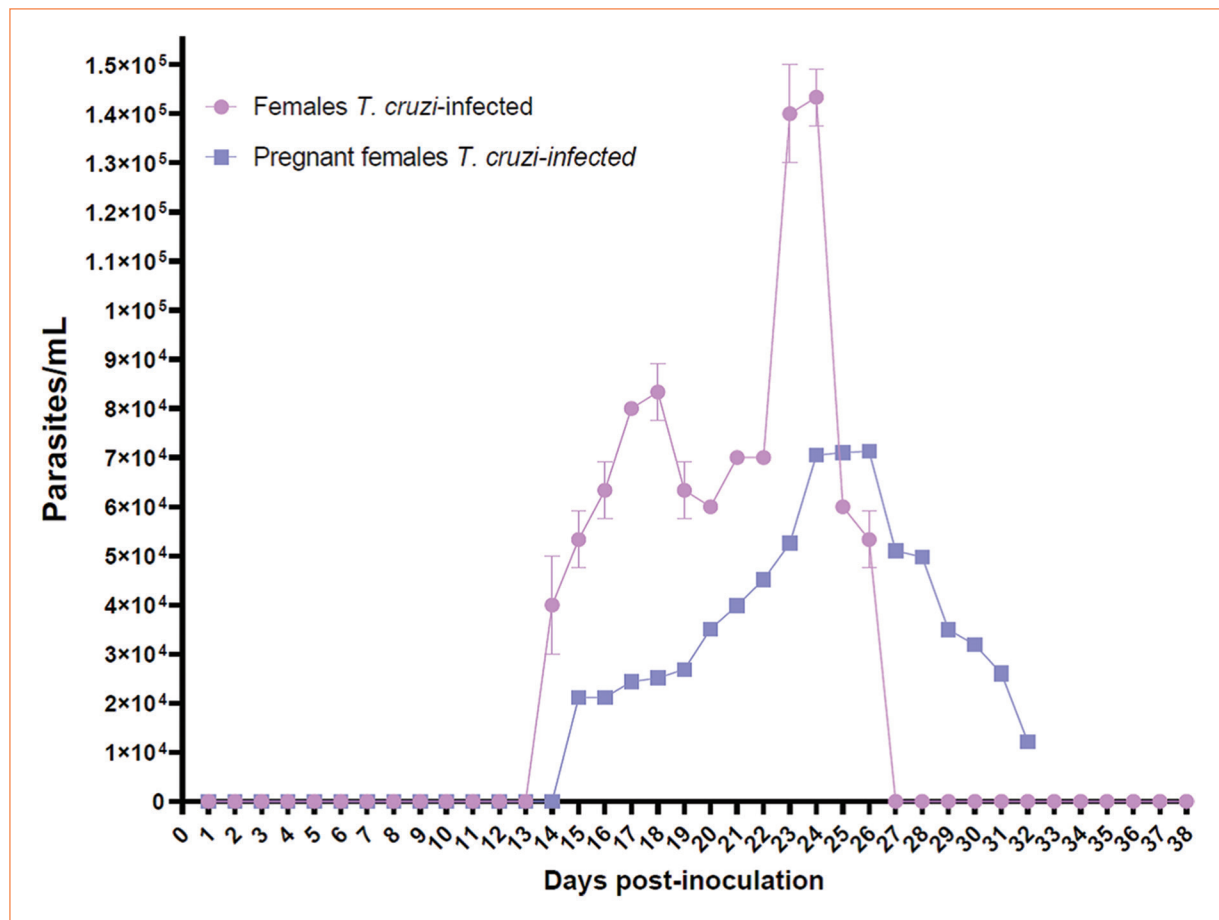
Figure 2. Parasitemia curve. Non-pregnant females exhibited an onset of parasitemia on day 14 post-inoculation, with subsequent peak of parasitemia occurring on day 25, and parasitemia terminating on day 27. In pregnant females, onset of parasitemia occurred on day 15 post-inoculation, with peak of parasitemia observed on day 24. However, the end of parasitemia was not recorded because all pregnant females succumbed by day 32. Notably, non-pregnant females displayed higher parasitic density (p = 0.05) compared to the pregnant counterparts.

low infection that the pregnant females presented, but it could be the consequence of the immune response that happens during the pregnancy at placental level; briefly, it has been proposed that during congenital transmission blood trypomastigotes cross placental barrier, infects syncytiotrophoblast, cytrotrophoblast, and villious stroma, there the parasites differentiate into amastigotes, proliferate, and differentiates into trypomastigotes, and invade fetal capillary, and the fetus but this could be interrupted by the immune fetus defense[21]. It is important to take in count that each strain infects differentially the placenta, resulting of the virulence of each strain[22], therefore is important to determine the strain that infects the pregnant female to help with an adequate management of each case.

Immunologically, fetus and neonates' congenital infection has interesting points: on one hand, their inflammatory cells could be activated by *T. cruzi*; on the other hand, the fact that they have maternal antibodies could help to contain and even eliminate the infection by opsonized the parasites, resulting in a brief congenital infection without the development of the disease[23]. This strong immune response could help to explain that 50% premature babies born of Chagas´ disease infected mothers do not survive[21]. Here, we do not detect parasites on pup's blood smear by direct observation; however, IgG antibodies were detected, the antibody presence coincides with previously reported data in guinea pigs[24], and supports the proposal that the pups do not develop the disease could be due the antibody-dependent enhancement mechanism, resulting into the activation of the phagocytic FcγR pathway of the host cells[25].

Given that there is no direct blood exchange between the fetus and the mother, high parasitemia would not
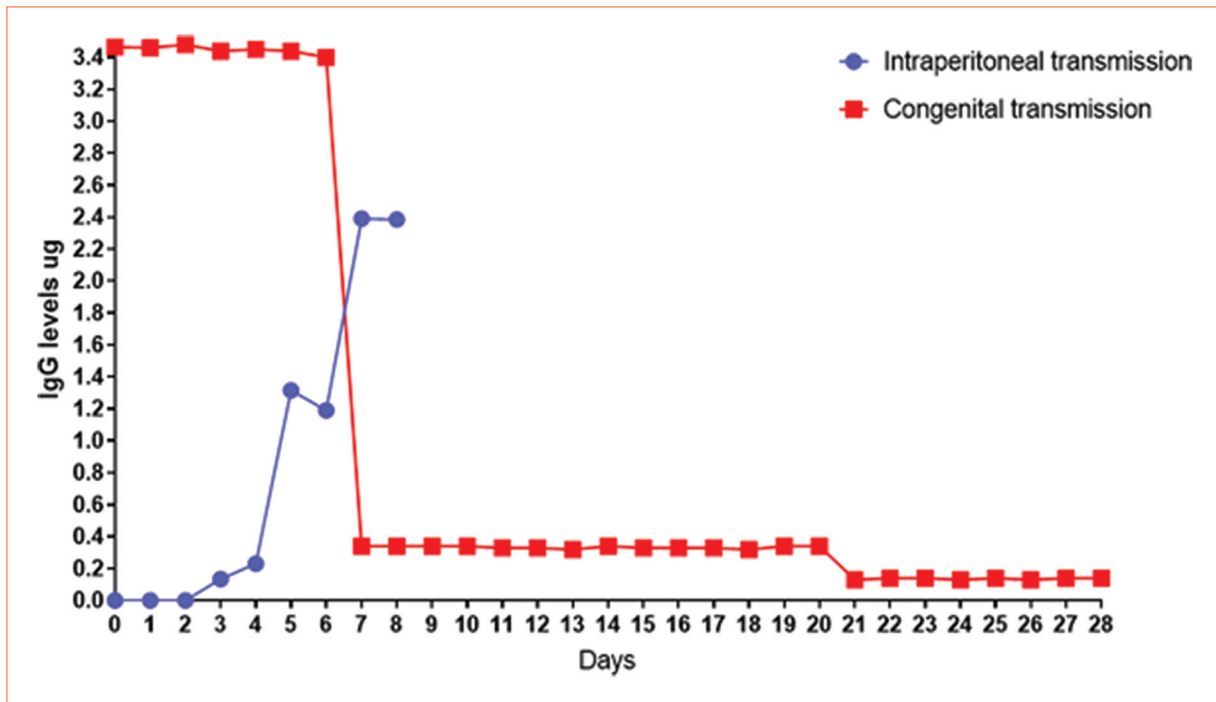
Figure 3. Immunoglobulinemia. The mouse pups infected intraperitoneally showed a gradual increase in antibodies against *Trypanosoma cruzi*. Reaching their maximum concentration on day 7 (2.4 µg), subsequently on day 8 these pups died due to the parasitic infection. Pups with congenital transmission showed the highest concentration of antibodies against *T. cruzi* from day 0 (3.4 µg), decreasing considerably on day 7 (0.4 µg), remaining constant for 13 days, a second decrease on day 21 (0.2 µg) remaining constant until day 28. Pups with congenital infection show a higher quantity of antibodies than those infected intraperitoneally (p = 0.05).

be directly responsible for infection[12]. Previously, it has been correlated vertical transmission with a positive hemoculture, suggesting that to *T. cruzi* could be vertically transmitted is mandatory that the mother had elevated parasitemia[26]. In the present work, the parasitemia of the mothers lower during pregnancy contribute with evidence that mothers displayed an inflammatory response to control the infection to prevent the transmission, supporting that it has been reported higher levels of INF-γ[27]. Even more, there is a hypothesis that proposes that borne vector toward vertical infection should impart mother-to-child some protection against a challenge with more virulent strains[28].

T. cruzi lineages discrete typing units (DTUS) seem to influence vertical transmission but there are not know the specific factors that contribute to it; moreover, it seems that every strain influences this transmission[27]. In one Brazilian and one St. Catherine's Island (USA) strain, was reported that the newborns died in approximately 2 weeks after birth, and the mothers died during the experiment[12]. In the present study, using NINOA stain, the newborns grow with no

problems as it happened in a previous work performed with H4 strain in guinea pigs, both DTU I strains[15,24]. Then, the present assay shown and supports the idea that the DTUS have a big influence in the acute infection leading or not to the death of the hosts infected with *T. cruzi*.

Finally, we want to point out that acute *T. cruzi*-infected females were hard to get pregnant, data that support the proposal that *T. cruzi* appears to induce low reproductive rates in copulating female mice[20]. May be due the strong physiological inflammation status, moreover, in the present study, even when female mice got pregnant some of them present miscarriages maybe due the strong defense promote by the infection that implies the induction of inflammasome signaling in placental trophoblasts, the hyperactivation of blood cells and the releasing of proinflammatory cytokines such as IFN-γ that activates monocytes/macrophages and stimulates, in synergy with TNF-α the generation of NO to kill parasites[23]. Thus, we could suggest that the females that continue the pregnancy process displayed an inflammation status less aggressive than the ones that

had miscarriages, supporting the previous data that in pregnant women, the predominant response is TH2[18].

In the present study, we performed our work employing NINOA strain and the parasitemia in the neonates remained subpatent parasitocopically, data that coincide with the information about other TcI *T. cruzi* strains (X10)[20,29]. However, further studies related to the factors that influence parasite´s multiplication during pregnancy is needed.

Conclusion

In one hand, *T. cruzi* strain and parasitemia load of the pregnancy female are two important factors to take in account to dilucidated the possibility of a vertical transmission; it seems that every strain behaves differently. On the other hand, the pregnancy itself acts as a protective factor against the newborn possible infection due the *T. cruzi*-antibody production.

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Conflicts of interest

The authors declare no conflicts of interest.

Ethical disclosures

Protection of human and animal subjects. This work was approved by the Ethics and Research Committee of the National School of Biological Sciences (CEI-ENCB) with folio number: ENCB/CIC-UAL/028/2023, protocol number ZOO-008-2023 and AUT- B-05219-052.

Confidentiality of data. The authors declare that no patient data appear in this article. Furthermore, they have acknowledged and followed the recommendations as per the SAGER guidelines depending on the type and nature of the study.

Right to privacy and informed consent. The authors declare that no patient data appear in this article.

Use of artificial intelligence for generating text. The authors declare that they have not used any type of generative artificial intelligence for the writing of this manuscript nor for the creation of images, graphics, tables, or their corresponding captions.

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