Reproductive efficiency of asymptomatic *Theileria equi* carriers mares submitted to an embryo transfer program

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ABSTRACT.- Bezerra LL, Jacob J.C.F., Santos H.A., Massard C.L., Silva P.C.A., Gaudêncio F.N. & Sá M.A.F. 2015. **Reproductive efficiency of asymptomatic *Theileria equi* carriers mares submitted to an embryo transfer program.** Pesquisa Veterinária Brasileira 35(3): 265-269. Departamento de Reprodução e Avaliação Animal, Departamento de Parasitologia Animal, Universidade Federal Rural do Rio de Janeiro, Seropédica, RJ 23890-000, Brazil. E-mail: luluzootec@uol.com.br

This study aimed to assess and evaluate the effects of *Theileria equi* infection on embryonic recovery, gestation and early embryonic loss. Thirteen Mangalarga Marchador *Theileria equi* positive donors (diagnosed through nested-PCR) and 40 embryos receptors were used. Donors were submitted to two embryo collections in two consecutive estrous cycles (GId); after, the same mares were treated with imidocarb dipropionate (1.2mg/kg IM) in order to collect more embryos in two more estrous cycles (GIId). Receptors were divided into two groups (control and with treated) with 20 animals each, where one group was the control (Glr) and the other one (Glr) treated with 1.2mg/kg IM of imidocarb dipropionate assessing the gestation rate at 15, 30, 45 and 60 days. After 52 embryo collections, the embryonic recovery rates were 53.84% (14/26) and 65.38% (17/26) (p> 0.05) for GId and GIId, respectively. The gestation rate was 70% (14/20) (p>0.05) at 15, 30, 45 and 60 days in group Glr and for Glr was 85% (17/20) (p>0.05) at 15 days, 80% (16/20) (p>0.05) at 30, 45 and 60 days. The treatment with imidocarb dipropionate did not cause significant improvement in the reproductive efficiency at an ET program.

INDEX TERMS: *Theileria equi*, babesiosis, biotechnology, nPCR, mares.

INTRODUCTION

The success of embryo transfer programs (ET) in equines can be affected by several known factors such as animal
body score, uterine status, day of embryo collection, quality and handling of the embryo, besides requirement of highly specialized workers. However, there are also some unknown factors that could interfere in the ET. Animals free of diseases, ectoparasites and endoparasites are necessary for an ideal embryo transfer program; however, due to Brazilian tropical climate and the lack of sanitary control, it is difficult obtaining animals free of some parasitosis, such as babesiosis.

The demand for assisted reproduction techniques development in equiculture has considerably increased. Brazil is one of the leading countries in the use of Embryo transfer (ET), along with the USA and Argentina. Although ET use has increased over the last decades, its numbers have fluctuated along with the financial health of the equine industry. Due to the high cost of the technique it has only been used in genetically superior animals as donors (Squires et al. 1999).

The babesiosis has been described as the main equine parasitosis, due to direct damages such as reduced performance and mortality, besides the indirect damages as commercialization restraint and specially exports (Friedhoff 1990). The mortality in infections by Theileria equi is low; in general the animals recover from the disease acute phase and of remaining parasite asymptomatic carriers. During the infection chronic phase, unspecified clinical signs, as impatience, weight loss and reduced physical and reproductive performance are common (Schein 1988).

Cases of congenital babesiosis have been observed, suggesting transplacental transmission (Santos et al. 2008), at the first trimester of gestation (Allsopp et al. 2007), besides the occurrence of aborted fetuses with jaundice and anemia, premature or born sick foals, showing symptoms as anemia, prostration, jaundice and reluctance to feed (Du Plessis & Basson 1966). Thus, considering the small number of embryos obtained per mare a year, a better pregnancy rate and lower embryonic loss are required. Accordingly, it is important assessing the influence of T. equi infection on embryonic recovery rates, gestation and early embryonic loss in a Commercial Program of Equine Embryo Transfer.

**MATERIALS AND METHODS**

The present study took place in a Commercial Center for Embryo Transfer, located in the city of Itaguaí, and on two stud farms in the city of Seropédica/RJ, Brazil. Thirteen donors and 40 receptors of embryos from the “Mangalarga Marchador” breed were used. The animals were between 3 and 10 years old, presented body score between four and five (NRC 2007) and were previously examined for congenital parasitosis, anemia, prostration, jaundice and reluctance to feed (Du Plessis & Basson 1966). Thus, considering the small number of embryos obtained per mare a year, a better pregnancy rate and lower embryonic loss are required. Accordingly, it is important assessing the influence of T. equi infection on embryonic recovery rates, gestation and early embryonic loss in a Commercial Program of Equine Embryo Transfer.

The blood samples were collected from a puncture in the jugular vein, with 40x12 needles, placed on flasks with ethylenediaminetetraacetic acid 11% (EDTA) anticoagulant and sent in isothermal boxes to the Laboratory of Experimental Chemotherapy in Veterinary Therapy of the Federal Rural University of Rio de Janeiro. To access the hematimetry and leucoimetry a Poch 100 IV Roche electronic equipment was used. The differential leukocytes count was performed by stained blood smears and the total solids determination was accessed through refractometry.

For the reproductive test, the mares were every two days examined by transrectal palpation and ultrasonography until they presented about 30mm diameter follicle, when they began to be daily examined. When the largest follicle reached ≥ 35 mm diameter and uterine echogenicity suitable with estrus, ovulation was induced with 1000 UI of intravenous human chorionic gonadotropin (hCG). The Artificial Insemination (AI) was performed with fresh semen of assured quality 24 hours after the hCG administration. Whether there was no ovulation 48 hours after the AI, mares were submitted to the same procedure. The dose of 500x106 progressively motile spermatozoa was used to inseminate the mares. Seven and nine days post-ovulation (D7 and D9), embryo recovery attempt was performed in the donor mares (Fleury et al. 2001) at two consecutive estrous cycles (Gld). After, these same animals were treated with imidocarb dipropionate (1.2mg/kg IM monthly) for two more embryos in two estrous cycles (Gld). Embryo receptors were divided into two groups of 20 animals each, where one was the control (Glr) and, the other one treated with (Gld) imidocarb dipropionate. The receptors were then inovulated with a synchronicity degree from -1 to +5 (ovulation one day before, up to five days after the donors), and then the gestation rates were evaluated at 15, 30, 45 and 60 days.

The hematological parameters were transformed (natural log) and submitted to the t test (p>0.05) and the embryonic recovery rates, gestation and animals embryo quality were assessed by chi-square test with 5% significance level and 95% interval according to Sampaio, 2002.

**RESULTS**

In the present study, as the 13 embryo donors as the 40 receptors presented positive result for T. equi based on the nested-PCR diagnostic method at the beginning of the procedures. At the end of the experiments, after imidocarb dipropionate treatment, 69% (9/13) of embryo donors and 50% (10/20) of embryo receptors presented negative results for T. equi in the nPCR (Table 1 and 2). The receptors untreated group of receptors presented 20% (4/20) of negative animals, using the same diagnostic method (Table 2).

Donor and receptor mares presented normal erythrocytes count before and after treatment with imidocarb dipropionate. Regarding the leukogram, the embryo donors presented a decrease in leucocytes and total neutrophil, and a slight increase of lymphocytes and monocytes after treatment. The embryo recipients presented a blood count within normal parameters.

Before donor mares have been treated with imidocarb dipropionate, the embryonic recovery rate was 53.8% (14/26). After the treatment, the recovery rate increased to 65.9% (17/26). Even with this numerical superiority observed after treatment, the chi-square test did not show significative difference (p>0.05).

The gestation rate at 15, 30, 45 and 60 days in the un-
treated receptor group was 70% (14/20) with no embryonic loss. In the treated group the gestation rate at 15 days was 85% (17/20) and at 30, 45 and 60 days it was 80% (16/20), considering there was embryonic loss at 30 days of gestation in one of the treated mares. No significative difference (P>0.05) was observed among the gestation rates.

The treatment with imidocarb dipropionate caused improvement on the quality of the recovered blastocysts. Before treatment, it was obtained 71.43% of embryos (10/14) classified as grade I. After treatment the number of grade I embryos was 94.12% (16/17). An increase of 22.69% in the quality of grade I embryos was observed. The percentage of embryos classified as grade II before treatment was 28.57% (4/14) and after treatment was 5.88% (1/17). No significative difference was observed (p>0.05) among the results after the chi-square test.

**DISCUSSION**

Aiming to characterize the occurrence of *Theileria equi*, several researches have been performed in Brazil. Serological tests have been in epidemiological studies, showing the presence of antibodies and indicating, therefore, that these animals were in contact with the parasite in some moment of their lives. The greatest obstacle of these serological techniques lies in the fact that the antibodies can remain circulating for months or even for the entire life of the animals, characterizing positivity in serological tests. For asymptomatic carriers, the molecular tests are more indicated, since they are able to detect the presence of blood circulating protozoa at low rates. The nPCR has been cited as the best technique due to its amplification reactions, which ensures greater sensitivity to the technique (Baldani 2004). The highest percentage of negative animals in the treated donors and receptors groups is probably due to the prophylactic treatment with imidocarb dipropionate. It is believed that, once animals did not present any symptomatic, the parasitemia was already low, even when still detectable; and after treatment the parasitemia level became lower and probably undetectable by the method. The nPCR method based on the gene sequence *ema-1* of *T. equi*, is able to detect parasitemias up to 0.000006%, the equivalent of 5 infected cells from a total of 10^9 erythrocytes (Nicolaiwsky et al. 2001). In another evaluation of this PCR technique for *T. equi* routine detection (Rampersad et al. 2003) through blood samples analyses from healthy and clinically sick equines, the nPCR method was able to detect the parasite 3.6 times over than stained blood smears and 2.2 times over than the detection at the first PCR amplification, showing higher sensitivity when compared to the conventional parasitological methods. The negative results obtained with no treatment receptors at the end of the experiment have possibly been associated to the reduction in the parasitemia levels due to a better handling of these animals during mating season, reducing the contact with the vector tick and possible reinfections.

The treatment with imidocarb dipropionate in infected equines has demonstrated that this compound causes parasite eradication from blood; however, according to Bruning (1996) the horses remained always infected. Clinically infected animals receiving imidocarb dipropionate (5mg/kg) treatment presented parasitemia presented decrease after two days, from 20% to 10% of infected erythrocytes and untreated ponies presented an increase from 20 to 60% in three days (Simpson & Neal 1980).

During the chronic phase there is no significative alteration between uninfected equines hematocrit *T. equi carries* (Hailat et al. 1997). According to that author, the infected equines and those ones previously treated with imidocarb dipropionate did not present any clinical sign of the disease. The same authors still reported equines highly infected presenting a reduction in their hematocrit from around 20 to 30% (Hailat et al. 1997).

According to Schein (1988), equines infected by *T. equi* develop a solid immunity protecting them against clinical diseases in cases of re-exposure to the parasite. This protection has been attributed to a continuous stimulation of the immune system by parasites persisting in the organism during the chronic phase of the disease, even with low parasitemia rates. According to Bruning (1996) imidocarb dipropionate treatment to *T. equi* has proven removing the parasite from blood circulation, although, the infected equines have remained carriers through out their lives , being responsible for the propagation and maintenance of infection.

According to the study performed by Squires et al. (2003) the embryonic recovery rate was between 50 and 75%. Some studies have shown variations in the embryonic recovery rate of: 50.9 and 59.5% (Fleury et al. 2001), 63.4% in 658 collections (Jacob et al. 2002), and 60% (Gusmão et al. 2010). The stress caused in *T. equi* carries animals with disease clinical signs and treated with imidocarb dipropionate at therapeutic dose (4mg/kg, q72h, in four applications) has caused great reduction in the embryonic recovery rate. Besides, this parasiticide is related to episodes of cramps, salivation, irritability, gastrointestinal hyper-motility, renal and liver failure, etc. However, using prophylactic doses (1.2mg/kg) in chronic carrier mares, no undesirable side effects were observed, as well as, there was no decrease in the embryonic recovery rate and ges-
However, there were important increases of 11.54% in the embryonic recovery rate, 15% in the gestation rate at 15 days and 10% in the gestation rate at 30, 45 and 60 days in the group treated with imidocarb dipropionate in relation to the untreated group.

The treatment of *Theileria equi* infection with imidocarb dipropionate raised the quantity of collected grade I embryos.

**CONCLUSIONS**

It can be concluded that treatment with imidocarb dipropionate did not cause a significant improvement (p>0.05) in the embryonic recovery rates and gestation rates at 15, 30, 45 and 60 days.

**REFERENCES**

Alkopp M.T.E.P., Lewis B.D. & Penzhorn B.L. 2007. Molecular evidence for transplacental transmission of *Theileria equi* from carrier mares to their apparently healthy foals. Vet. Parasitol. 140:130-136.

Baldani C.D. 2004. Estudo comparativo de técnicas diretas (esfregaço sanguíneo, cultivo in vitro e reação em cadeia polimerase) e indiretas (reação de imunofluorescência, ensaio imunoenzimático e fixação de complemento) no diagnóstico de *Babesia equi* em equinos naturalmente infectados. Tese de Doutorado, Faculdade de Ciências Agrárias e Veterinárias, Universidade Estadual Paulista, Jaboticabal, SP. 93p.

Bruning A. 1996. Equine piroplasmosis: an update on diagnosis, treatment and prevention. Brit. Vet. J. 152:139-151.

Du Plessis J.L. & Basson P.A. 1996. Babesiosis in aborted equine fetuses: a report on two cases in South Africa. J. Am. Vet. Med. Assoc. 179:267-269.

Fleury J.J., Pinto A.J., Marques A., Lima C.G. & Arruda R.P. 2001. Fatores que afetam a recuperação embrionária e os índices de prenhez após transferência transcervical em equinos da raça Mangalarga. Braz. J. Vet. Res. Anim. Sci. 38:29-33.

Friedhoff K.T., Tenter A.M. & Muller I. 1990. Haemoparasites of equines: impact on international trade of horses. Rev. Sci. Tech. Off. Int. Epiz. 9:1187-1194.

Gomes G.M., Jacob J.C.F. & Domingues L.B. 2004. Utilização de mares após parto para uso como embrião receptores em um programa de transferência transcervical em eqüinos da raça Mangalarga. Tese de Doutorado, Faculdade de Ciências Agrárias e Veterinárias, Universidade Estadual Paulista, Jaboticabal, SP. 93p.

Gusmão A.L., Feitosa T.A.L., Moura J.C.A., Resende M.V., Baptista L.E.P. & Tavares N.R. 2002. The impact of degree of synchrony between donors and recipients in a commercial equine embryo transfer program. Theriogenology 57:545.

Hailat N.Q., Lafi S.Q., Al-Darraji A.M. & Al-Ani F.K. 1997. Equine babesiosis associated with strenuous exercise: clinical and pathological studies in Jordan. Vet. Parasitol. 69:1-14.

Jacob J.C.F., Domingues L.B., Castel E.L., Castel M.O., Silva A.G., Mello C.M. & Gasparetto F. 2002. The impact of degree of synchrony between donors and recipients in a commercial equine embryo transfer program. Theriogenology 57:545.

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Rampersad J., Cesar E., Campbell M.D., Samlal M. & Ammons D. 2003. A field evaluation of PCR for the routine detection of *Babesia equi* in horses. Vet. Parasitol. 114:81-87.

Rocha A.N., Lopes E.P., Guimarães J.D., Siqueira J.B., Torres C.A.A., Pinho R.O. & Carvalho G.R. 2007. Pregnancy rates and early embryo loss in a commercial embryo transfer program in Mangalarga Marchador mares. Anais 17º Congresso Brasileiro de Reprodução Animal, Curitiba, PR, p.160. (Abstract)

Sampaio I.B.M. 2002. Estatística Aplicada à Experimentação Animal, 2ª ed. Fundação de Estudo e Pesquisa em Medicina Veterinária e Zootecnia, Belo Horizonte. 265p.

Santos T.M., Santos H.A. & Massard C.L. 2008. Diagnóstico molecular de babesia congênita em potros neonatos no estado do Rio de Janeiro, Brasil. Revta Bras. Parasitol. 17:348-350.

Schein E. 1988. Equine babesioses, p.197-208. In: Ristic M. (Ed.), Babesiosis of Domestic Animals and Man. CRS Press, Boca Raton.

Simpson C.F. & Neal F.C. 1980. Ultrastructure of *Babesia equi* in ponies treated with imidocarb. Am. J. Vet. Res. 41:267-271.

Squires E.L., McCue P.M. & Vanderwal D.K. 1999. The current status of equine embryo transfer. Theriogenology 51:91-104.

Squires E.L., Carnevale E.M., McCue P.M. & Bruemmer J.E. 2003. Embryo technologies in the horse. Theriogenology 59:151-170.