

## **Toxoplasma-IgM AND IgG-AVIDITY IN SINGLE SAMPLES FROM AREAS WITH A HIGH INFECTION RATE CAN DETERMINE THE RISK OF MOTHER-TO-CHILD TRANSMISSION**

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### SUMMARY

Anti-*Toxoplasma* IgG-avidity was determined in 168 serum samples from IgG- and IgM-positive pregnant women at various times during pregnancy, in order to evaluate the predictive value for risk of mother-to-child transmission in a single sample, taking the limitations of conventional serology into account. The neonatal IgM was considered the serologic marker of transmission. Fluorometric tests for IgG, IgM (immunocapture) and IgG-avidity were performed. Fifty-one of the 128 pregnant women tested gave birth in the hospital and neonatal IgM was obtained. The results showed 32 (62.75%) pregnant women having high avidity, IgM indexes between 0.6 and 2.4, and no infected newborn. Nineteen (37.25%) had low or inconclusive avidity, IgM indexes between 0.6 and 11.9, and five infected newborns and one stillbirth. In two infected newborns and the stillbirth maternal IgM indexes were low and in one infected newborn the only maternal parameter that suggested fetal risk was IgG-avidity. In the present study, IgG-avidity performed in single samples from positive IgM pregnant women helped to determine the risk of transmission at any time during pregnancy, especially when the indexes of the two tests were analysed with respect to gestational age. This model may be less expensive in developing countries where there is a high prevalence of infection than the follow-up of susceptible mothers until childbirth with monthly serology, and it creates a new perspective for the diagnosis of congenital toxoplasmosis.

**KEYWORDS:** Congenital toxoplasmosis; Serology for toxoplasmosis; IgG-avidity.

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### INTRODUCTION

The risk of mother-to-child transmission of *Toxoplasma gondii* and the severity of sequelae in congenital infection have been correlated with gestational age in which maternal seroconversion occurs<sup>4</sup>. Significant reduction in the frequency and severity of disease at birth have been associated with *in utero* treatment of infected fetuses<sup>1,16</sup>, even though treatment does not prevent transmission of the parasite<sup>24</sup>. The high prevalence of *Toxoplasma* infection in pregnant women from Porto Alegre has led to the use of serological screening in the local health care system<sup>12</sup>.

IgM antibodies may appear earlier and decline more rapidly than IgG antibodies and are frequently the first class of antibodies detected after primary infection<sup>17</sup>. Despite the wide distribution of commercial kits to measure IgM antibodies, they lack standardization; therefore, quantitative results from different manufacturers cannot be compared. Several methods for IgM detection still have a high frequency of false-positives and in some patients, positive IgM is "residual", detected during the chronic phase of infection. A positive IgM result in an isolated sample can be interpreted as a true-positive in acute infection, a true-positive in chronic infection or as a false-positive<sup>17</sup>.

The IgM indexes measured in acute infection are significantly higher than those in residual ones<sup>25</sup>. With ELFA- enzyme-linked fluorescent assay, VIDAS system, increasing IgM indexes or over 3.5 were observed in acute infection, indexes between 3.0 and 3.5 in the transitional phase, and indexes between 0.65 and 3.0 in latent infection<sup>2</sup>.

An IgM test should be performed by a procedure with minimal nonspecific reaction, such as IgM-capture EIA<sup>2,5,25</sup>. The algorithm for serodiagnosis of toxoplasmosis in the USA includes an IgM measurement when time of infection needs to be defined. If IgM is positive at elevated indexes, infection has probably occurred in the prior three to six months. If it is positive at low indexes it may be a false-positive, be related to infection acquired in the prior two years or be related to reinfection. If it is negative, infection has occurred more than two years before. In individuals presenting with low IgM indexes, it was suggested by the authors that a second sample be collected, with both processed together to safely detect an increase of antibody levels<sup>25</sup>. In pregnant women with low IgM indexes, it is difficult to determine the time of infection and exclude the possibility of transmission.

In 1989, HEDMAN *et al.*<sup>9</sup> introduced the IgG-avidity test to help

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diagnose acute infection based on the binding strength of specific IgG to a multivalent antigen of the parasite, which is produced at low concentrations after primary infection and increases over time. High avidity at the beginning of pregnancy has been used as a parameter to exclude post-conception infection<sup>9,10</sup>.

The risk of acute infection, parasitemia and mother-to-child transmission has been associated with high levels of IgG<sup>14</sup>, presence of IgM<sup>14</sup> or IgM at high levels<sup>2,14,25</sup> and low IgG-avidity<sup>5,9,11,13,18,22,23</sup>. Serological responses, however, follow diverse, individual rhythms. Low avidity, as well as the presence of IgM can be detected long after primary infection in some individuals. High and even very high IgG is not always observed in acute infection, especially during the first weeks<sup>11</sup>.

At Hospital Materno-Infantil Presidente Vargas in Porto Alegre, serology for toxoplasmosis is requested at the first prenatal visit, but the follow-up of susceptible pregnant women is poorly conducted or is not done at all. Samples from positive IgG and IgM pregnant women, who start prenatal care late or are serologically tested only at the time of labor, are commonly seen. It would be of value if the patients' immune status could be obtained of these single samples, bearing in mind that ultrasonography, amniocentesis and PCR (polymerase chain reaction) are requested based on it.

The aim of this study was, therefore, to evaluate IgG-avidity in samples from IgG- and IgM-positive pregnant women in relation to its capacity to identify the risk of mother-to-child transmission of *Toxoplasma* from single samples, requested at any time during pregnancy, considering the limitations of conventional serology. Specific IgG levels and specific IgM and IgG-avidity indexes were analyzed, and neonatal IgM was used as the serologic marker of mother-to-child transmission.

## PATIENTS AND METHODS

Anti-*T. gondii* IgG-avidity was prospectively determined in all positive IgG and IgM samples obtained from pregnant women at their first prenatal visit to Hospital Materno-Infantil Presidente Vargas in Porto Alegre, Rio Grande do Sul, Brazil, between 2000 and 2004, regardless of gestational age. Fifty-one mothers gave birth at the hospital. A newborn blood sample was drawn to process specific IgG and IgM.

IgG anti- *T. gondii* was performed using the MEIA, AxSYM<sup>®</sup> SYSTEM Toxo IgG commercial kit (Abbott Laboratories, Chicago, IL, USA). Results were expressed as International Units per milliliter (IU/mL), negative: < 2.0, positive: > 3.0, inconclusive: between 2.0 and 3.0.

IgM anti- *T. gondii* was performed using the ELFA, VIDAS TOXO IgM, TXM commercial kit (bioMérieux SA, Lyon, France). The results were expressed as indexes, negative: < 0.55, positive: > 0.65, inconclusive: between 0.55 and 0.65.

VIDAS AVIDITY was also performed using the ELFA commercial kit and the results were expressed as indexes, low: < 0.2, high: > 0.3, inconclusive: between 0.2 and 0.3.

Newborn specific IgG and IgM were processed with the same techniques.

Fisher's Exact test was used to analyze (a) the association between categorized IgM and transmission, (b) categorized IgM and categorized avidity and (c) categorized avidity and transmission. The Student *t* test was used to analyze the association between avidity index and transmission. The Pearson correlation was used to analyze the correlation between IgM and avidity indexes.

The project was approved by the Ethics Committee of the Hospital Materno-Infantil Presidente Vargas.

## RESULTS

IgG-avidity was performed on 168 samples from positive IgG and IgM pregnant women (about 2.5% of the total number of serologies performed during this period) and was high in 128 (76%) and low or inconclusive in 40 (24%). The gestational age was known in 105 women (62.5%) of which 33 (31.4%) were in the first, 36 (34.3%) in the second and 36 (34.3%) in the third trimester of gestation. Fifty-one mothers (30%) gave birth at the hospital, thus making it possible to obtain neonatal IgM.

Among the 128 pregnant women with high avidity, 32 gave birth at the hospital. IgG levels ranged from 58 to 8,832 IU/mL and IgM indexes ranged between 0.65 and 2.4. Eighteen mothers had low IgM indexes (between 1.0 and 3.0) and 14 only very low amounts of IgM (between 0.65 and 0.9). Eleven mothers were in the first, seven in the second and 14 the third trimester of gestation. Neonatal IgM tests were negative in all newborns.

Among the 40 pregnant women with low or inconclusive avidity, 19 gave birth at the institution (Table 1). IgG levels ranged from 106 to 3,356 IU/mL and IgM indexes from 0.65 to 11.9. Six mothers had IgM indexes higher than 3.5 and avidity very low (M1 to M6), 11 had low IgM indexes (between 1.0 and 3.0) and two only traces of IgM (between 0.65 and 0.9). Two mothers were in the first, six were in the second and 11 were in the third trimester of gestation.

Five positive IgM newborns (M1, M2, M5, M11 and M13) and one stillbirth (M12) resulted from these mothers. In three infected newborns (M1, M2 and M5), the maternal IgM indexes were extremely high and in two infected newborns (M11 and M13) and the stillbirth (M12) these indexes were low. Maternal serologies of M2 and M5 were very similar: low levels of IgG and high indexes of IgM.

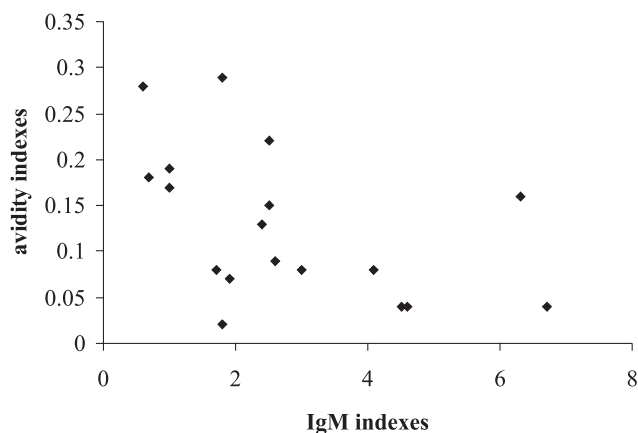
According to the Fisher's Exact test, there is a statistically significant association between categorized IgM of mothers (index higher than 3.0) and transmission ( $p < 0.028$ ), neonatal IgM being the serologic marker of transmission. High IgM is predicted to result in a higher number of infected children than low or inconclusive IgM. There is also a statistically significant association between categorized IgM and categorized avidity ( $p < 0.001$ ), high IgM being associated with low avidity and vice-versa. There is also an association between categorized avidity and transmission ( $p < 0.001$ ), low avidity associated with transmission and high avidity associated with lack of transmission using the manufacturer threshold of 0.2. Reducing the threshold to

**Table 1**  
Mothers with positive or inconclusive IgM and low or inconclusive avidity, according to gestational age and neonatal IgM

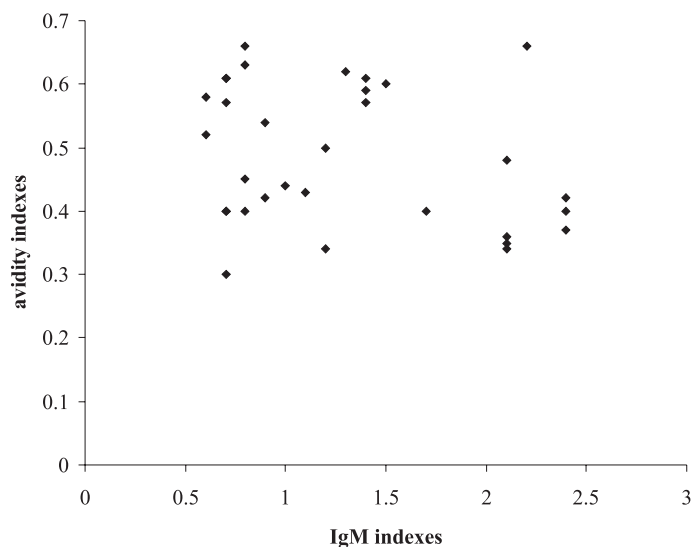
Mother	Gestational age-trimester	IgG IU/mL		IgM indexes	Avidity indexes	Neonate IgM indexes
		ELFA	MEIA			
M1	3rd	3356	> 300	11.9	0.05	positive (7.9)
M2	3rd	112	173	6.7	0.04	positive (6.5)
M3	1st	2656	> 300	6.3	0.16	negative
M4	1st	1060	> 300	4.6	0.04	negative
M5	3rd	155	> 300	4.5	0.04	positive (9.3)
M6	3rd	748	> 300	4.1	0.08	negative
M7	3rd	560	> 300	3.0	0.08	negative
M8	2nd	176	100	2.6	0.09	negative
M9	3rd	352	> 300	2.5	0.15	negative
M10	3rd	360	> 300	2.5	0.22	negative
M11	2nd	244	> 300	2.4	0.13	positive (1.4)
M12	3rd	2000	> 300	1.9	0.07	stillbirth
M13	3rd	212	165	1.8	0.02	positive (2.9)
M14	2nd	148	79	1.8	0.29	negative
M15	2nd	280	277	1.7	0.08	negative
M16	2nd	820	> 300	1.0	0.17	negative
M17	2nd	460	> 300	1.0	0.19	negative
M18	3rd	1416	> 300	0.7	0.18	negative
M19	3rd	106	28	0.6	0.28	negative

0.15 increases the association between low avidity and transmission. According to the Student *t* test, there is an association between the avidity index and transmission ( $p < 0.001$ ), with a lower avidity index associated with a higher chance of transmission.

According to the Pearson correlation, there is a significant correlation between IgM and avidity indexes, moderately and inversely. Thus, a high avidity index corresponds to a low IgM index and vice-versa ( $r = -0.548$ ,  $p < 0.001$ ). The relationship between IgM and avidity indexes when avidity is low, is shown in Fig. 1 ( $r = -0.472$ ;  $p = 0.041$ ), and when it is high, in Fig. 2 ( $r = -0.275$ ;  $p = 0.128$ ).



**Fig. 1** - The moderate and inverse correlation between IgM and avidity indexes obtained in the samples with low avidity ( $r = -0.472$ ,  $p = 0.041$ ).



**Fig. 2** - The non-significant correlation between IgM and avidity indexes, obtained in samples with high avidity. ( $r = -0.275$ ,  $p = 0.128$ ).

## DISCUSSION

When routine testing for *Toxoplasma* antibodies is performed in pregnant women, the first serum sample is usually taken at the first antenatal health care visit confirming pregnancy, usually between eight and twelve weeks. At Hospital Materno-Infantil Presidente Vargas, the first serum sample from a pregnant woman is frequently obtained later, after 20 weeks of pregnancy, or even as a parturient. If the presence of

IgM antibodies in single samples is the parameter used to identify acute infection, many women will be falsely identified as probably or possibly infected, according to the classification system produced by the working group of the European Research Network on Congenital Toxoplasmosis<sup>14</sup>, and they will undergo unnecessary diagnostic amniocentesis and/or antiparasitic treatment.

For these reasons, positive IgG and IgM in single samples were analyzed with regard to three parameters, IgG in IU/mL by MEIA and ELFA and IgM and IgG-avidity indexes by ELFA. The parameters were compared to neonatal infection determined by neonatal IgM, in order to assess the risk of mother-to-child transmission. The absence of neonatal IgM in the 32 infants born to mothers with high avidity, low indexes of IgM and even very high levels of IgG reinforces the importance of high avidity in evaluating the risk of transmission in any gestational age.

The presence of neonatal IgM in five infants born to mothers with low avidity and one stillbirth reinforces the importance of low avidity concerning the risk of transmission when it is measured in IgM-positive pregnant women. High indexes of IgM (M1, M2 and M5) also support the importance of IgM levels concerning the risk of transmission. M3 and M4 were under 20 weeks and their neonates were IgM negative, probably because the risk of transmission is lower in the first half of pregnancy.

The VIDAS avidity test has been standardized and optimized to exclude recent seroconversion in pregnant women presenting with elevated anti-*Toxoplasma* IgM levels in the first trimester of pregnancy<sup>6</sup>. The association between categorized avidity and transmission ( $p < 0.001$ ) using the manufacturer recommended threshold of 0.2 is increased when reducing the threshold from 0.2 to 0.15, with six cases of fetal transmission resulting from 12 mothers.

Without measuring avidity, the infected infants born to M11 and M13 with low levels of IgG and low indexes of IgM would not have been detected during pregnancy. In one infected newborn (M13), the only maternal parameter that strongly suggested fetal risk was IgG-avidity. These are the results from conventional serologies, most difficult to correlate with the risk of transmission, as IgM can be residual in a descending curve.

The moderate and inverse correlation between IgM and avidity indexes obtained in the samples with low avidity (Fig. 1) in this series ( $r = -0.472$ ,  $p = 0.041$ ) is very similar to the correlation found by JENUM *et al.*<sup>11</sup> between avidity indexes and estimated the time of infection ( $r = 0.46$ ). The correlation between IgM and avidity indexes obtained in the samples with high avidity (Fig. 2) in this series ( $r = -0.275$ ,  $p = 0.128$ ) is non-significant, meaning that high avidity did not show any relationship with IgM. There was a better association of transmission with avidity ( $p < 0.001$ ) than with categorized IgM, index higher than 3.0 ( $p < 0.028$ ).

The dynamics of antibody formation must be remembered in interpreting the maternal results, especially in single samples. Considering the screening techniques used at Hospital Materno-Infantil Presidente Vargas, the sensitivity of MEIA-IgG<sup>3</sup> and the capture design of ELFA-IgM<sup>2</sup>, the risk of mother-to-child transmission is related to

the time of antibody appearance: (1<sup>st</sup>) IgM > 3.0/ IgG < 20 UI/mL, (2<sup>nd</sup>) IgM > 3.0/ IgG between 20 and 300 UI/mL, (3<sup>rd</sup>) IgM > 3.0/ IgG > 300 UI/mL, (4<sup>th</sup>) IgM < 3.0/ IgG positive at any level. The last group, therefore, benefit most from obtaining the result of avidity, with low avidity indicating a higher risk, high avidity indicating a lower risk of transmission.

In JENUM *et al.*<sup>11</sup> series, high IgG-avidity (above 20%), can be used to exclude recent acute infection in the first 20 weeks, which reinforces the findings by LAPPALAINEN *et al.*<sup>13</sup> who found a positive predictive value of 100% for high avidity in the five preceding months.

In FLORI *et al.* series<sup>6</sup>, the increase of avidity indexes was low; the average time to reach the threshold of 0.3 was 14.2 months. No women reach this threshold either at four months or at six months after seroconversion. According to the authors, it is justifiable to decrease the threshold for exclusion of a recent infection of less than four months to 0.2 and use the company threshold of 0.3 for exclusion of infections which have occurred more than six months ago. The kinetics of the avidity test in five groups of women based on the type and duration of treatment given has also been evaluated and there was no significant difference when comparisons were made between the groups.

The best approach to prevent and control congenital toxoplasmosis is not clear. In France, a country with a high prevalence of infection, prevention is performed through serological screening and follow-up of susceptible mothers until childbirth with monthly serology<sup>1,16,21</sup>. Seroconversion indicates maternal acute infection. Parasite detection in amniotic fluid can be performed by *in vitro* culture, mouse inoculation and PCR to confirm fetal infection.

PCR for detection of *T. gondii* has revolutionized prenatal diagnosis by enabling an early diagnosis to be made, thereby avoiding the use of more invasive procedures on the fetus, thanks to its sensitivity, specificity and rapidity<sup>7,17</sup>. However, the results of a European collaborative study highlighted the lack of homogeneity between PCR protocols and performance and underlined the need for an external quality assurance scheme<sup>19</sup>. There are, also, marked differences in PCR sensitivity depending on gestational age at the time of the amniocentesis. Furthermore, PCR on amniotic fluid is not recommended for HIV-infected women, because of the risk of transmitting the HIV virus to the fetus during the procedure<sup>17</sup>.

DUNN *et al.*<sup>4</sup> analyzed the status of congenital infection in 554 cases. The overall maternal-fetal transmission rate was 29%, which masked a sharp increase in risk from 6% at 13 weeks to 72% at 36 weeks. Women who seroconverted at 24-30 weeks carried the highest risk (10%) of having a congenitally infected child with early clinical signs and at risk of long-term complications.

Most infected newborn babies do not have overt signs of disease at birth but are at substantial risk of developing long-term sequelae: chorioretinal disease in up to 85% of infected children and neurological abnormalities. Marked reductions in frequency and severity of disease at birth have been associated with *in utero* treatment. Significant reductions in expected long-term complications have been associated with extended post-natal treatment regimens started in the newborn period or as a continuation of *in utero* treatment<sup>1,16,21</sup>.

Despite the use of advanced methods, some cases of congenital toxoplasmosis cannot be detected early, which underlines the importance of careful follow-up of newborns at risk<sup>21</sup>. In Denmark, a country with a low prevalence of infection, a neonatal screening programme based solely on detection of *Toxoplasma*-specific IgM antibodies evaluated from the PKU card could identify more than 75% of infected infants born to untreated mothers<sup>15</sup>. This strategy could be the most appropriate one for countries with a low prevalence of infection<sup>8,15</sup>. In the United States, an isolated serum sample, often obtained either late in the first trimester or during the second or third trimester, is the only source of information on whether the fetus is at risk<sup>26</sup>.

RICCI *et al.*<sup>20</sup> compared the results of the two approaches in a population with a high infection rate in order to evaluate the effectiveness of these strategies in 5,288 susceptible pregnancies, 188 identified as infected, and 9,730 neonates. In this series, neonatal screening for toxoplasmosis seems to be less effective than pregnancy screening. In a study from Porto Alegre, neonatal screening identified cases of congenital toxoplasmosis acquired and transmitted in late pregnancy and undetected by prenatal screening<sup>12</sup>.

There is a high prevalence of congenital toxoplasmosis in Porto Alegre<sup>12</sup>. Prevention programs using single samples should be approached carefully. It is necessary to choose the best combination of assays<sup>11,22</sup>. Because the IgG-avidity test is largely employed as a 2<sup>nd</sup> level test, 117 cases of toxoplasmosis in pregnancy divided into the risk categories according to Lebech's criteria were re-examined including avidity that could define 77 out of 117 (65.8%) with only a single serum sample. The avidity test proved to be a useful method to classify the *Toxoplasma* infection in a single serum sample, especially during pregnancy<sup>23</sup>.

A large European multicenter study<sup>22</sup> explored the diagnostic performances of 20 antibody assays and their combinations in assessing the time of primary infection with *T. gondii*. The sample criteria permitted evaluation of test performance in terms of clinical sensitivity and specificity rather than analytical sensitivity and specificity. Excellent diagnostic performances, specificity near 99% maintaining sensitivities of 95% or higher, were reached by sequential use of highly sensitive IgM assays and methods that explored IgG quality, differential agglutination and avidity assays. The prerequisite for IgG seropositivity may have ruled out samples in the first two weeks of infection, when not all the antibody isotypes have yet been expressed.

For Brazilian public health institutions, the best serological model to prevent congenital toxoplasmosis using single samples would be analyzing for IgG and IgM antibodies using sensitive methods. Avidity should be done in all positive IgM serologies. High avidity helps to exclude acute infection in the last 20 weeks. Low avidity is associated with risk of transmission which increases during gestation; therefore, neonatal IgM should be performed in all low IgG-avidity maternal serologies. Negative neonates, even asymptomatics, should be carefully followed-up during the first year to exclude congenital toxoplasmosis.

## CONCLUSIONS

From these results it can be concluded that IgG-avidity performed

in single samples from positive anti-*Toxoplasma* IgM pregnant women can determine the risk of transmission at any time during pregnancy, especially if the indexes of the two tests are analysed compared to gestational age. This model may be less expensive in developing countries with a high infection rate than the follow-up of susceptible mothers until childbirth with monthly serology, and it creates a new perspective for the diagnosis of congenital toxoplasmosis.

## RESUMO

### Avidéz de IgM e IgG de *Toxoplasma* em amostras de áreas com alta taxa de infecção pode determinar risco de transmissão materno-fetal

A avidéz de IgG anti-*Toxoplasma* foi realizada em 168 amostras IgG e IgM positivas de gestantes, coletadas em qualquer período da gestação, para avaliar o valor preditivo do risco de transmissão materno-fetal em amostra única, considerando as limitações da sorologia convencional. A IgM neonatal foi considerada o marcador sorológico de transmissão. Testes fluorométricos foram realizados para IgG, IgM (imunocaptura) e avidéz de IgG. Cinquenta e uma das 128 gestantes testadas tiveram os partos realizados na instituição e a IgM neonatal foi obtida. Os resultados mostraram 32 (62.75%) gestantes com avidéz alta, índices de IgM entre 0,6 e 2,4 e nenhum recém-nascido infectado. Dezenove (37.25%) tiveram avidéz baixa ou inconclusiva, índices de IgM entre 0,6 e 11,9, cinco recém-nascidos infectados e um natimorto. Em dois recém-nascidos infectados e no natimorto, os índices maternos de IgM foram baixos e em um recém-nascido infectado, o único parâmetro materno que sugeriu risco para o feto foi a avidéz de IgG. No presente estudo, a avidéz de IgG realizada em amostras isoladas de gestantes IgM positivas auxiliou a determinar o risco de transmissão durante toda a gestação, especialmente quando os índices dos dois testes foram analisados em relação à idade gestacional. Este modelo pode ser menos oneroso para países em desenvolvimento com alta prevalência da infecção e cria nova perspectiva para o diagnóstico da toxoplasmose congênita.

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