NEONATAL SEPSIS: EVALUATION OF RISK FACTORS AND HISTOPATHOLOGICAL EXAMINATION OF PLACENTAS AFTER DELIVERY

SEPSE NEONATAL: AVALIAÇÃO DOS FATORES DE RISCO E ANÁLISE HISTOPATOLÓGICA DE PLACENTAS APÓS O PARTO

Pâmella Oliveira DUARTE[1]; Gláucia Elisete Barbosa MARCON[3]; Kenia Oenning AKAGI[1]; Zoraida Fernandez GRILLO[3]; Baldomero Antonio Kato da SILVA[4]; Doroty Mesquita DOURADO[1, 2]

1. Programa de Pós-Graduação em Doenças Infecciosas e Parasitárias, Universidade Federal de Mato Grosso do Sul, Campo Grande - MS, Brasil. pamella.duarte@hotmail.com.br; 2. Laboratório de Toxinologia e Plantas Medicinais, Universidade Anhanguera- Uniderp, Campo Grande - MS, Brasil; 3. Fundação Oswaldo Cruz de Mato Grosso do Sul, Campo Grande - MS, Brasil; 4. Programa de Pós-Graduação em Ciências Biomédicas, Universidade Federal do Piauí, Parnaíba, Piauí, Brasil.

ABSTRACT: Neonatal sepsis is a clinical syndrome defined by systemic signs of infection in newborns accompanied by bacteremia. Can be responsible for serious consequences for the newborn child, characterized at the birth as early sepsis or late onset sepsis, with high rate of neonatal morbidity and mortality. Pathological agents such as *Escherichia coli* (*E. coli*), *Streptococcus agalactiae* (*S. agalactiae*), *Ureaplasma urealyticum* and *Mycoplasma hominis* are most often responsible for intrauterine infections. The objective of this study is to evaluate the factors of neonatal sepsis predisposition in pregnant women through histopathological examination and the apoptotic index of placental tissues and detect DNA of *E. coli* and *S. agalactiae* using the Polymerase Chain Reaction (PCR). Histopathological analyses were made and the apoptotic index was determined to verify the levels of possible inflammatory infiltrates and cell death. Placenta samples were collected from November 2013 to May 2014. After DNA extraction, a PCR was performed amplifying the target fragment from the conserved regions of the rpoB (beta-RNA polymerase) polymorphism of *E. coli* and the factor 1 of *S. agalactiae.* The apoptosis index was tested with Acridine Orange and the histological procedure with Hematoxylin-Eosin staining. Among 100 samples of placental tissues analyzed by PCR, 48 represented the control group and did not present a risk factor associated with neonatal sepsis, and 52 samples representing the study group had at least one risk factor. Among these 52 samples, 7 (13.4%) had a PCR positive for *E. coli*. No placenta samples showed a positive PCR for *S. agalactiae*. The quantification of the apoptotic index did not show statistical significances between the groups and no inflammatory infiltrates were observed. However, histological sections showed fibrinoid necrosis, infarct areas and areas of calcification in all samples. Therefore, the results allow to conclude that the seven patients of experimental group with positive PCR for *E. coli* had eminent risk factors of neonatal sepsis, and the infection of the urinary tract (UTI) is the main aggravating circumstance. The histopathological examination demonstrated that the risk factors caused significant alterations, producing fibrinoid necrosis and infarcted areas in the placenta, contrary to apoptotic index that didn't differ from the group with unprecedented risk.

KEYWORDS: *Escherichia coli*. *Streptococcus agalactiae*. Histopathology. Neonatal sepsis.

INTRODUCTION

Neonatal sepsis is a clinical syndrome characterized by systemic signs of infection accompanied by bacteremia in the first month of life, and may or not present a positive blood culture. The neonatal sepsis are classified into early sepsis and late sepsis depending on the period of symptom onset and blood culture result. Blood culture is the most used test, considered gold standard (CANTEY; BAIRD, 2017), but the sensitivity does not exceed 80% (FHEMIG, 2013). In Brazil, the Ministry of Health classifies early sepsis as the one that presents diagnostic evidence during the first 48 hours of life with a maternal risk factor for infection. Late sepsis presents diagnostic evidence after 48 hours of life and may be caused by maternal genital tract microorganisms of hospital origin and other environmental risk factors (ANVISA, 2017).

Pathological agents such as *Escherichia coli*, *Streptococcus agalactiae*, *Ureaplasma urealyticum* and *Mycoplasma hominis* are most often responsible for intrauterine infections (HILLIER et al., 1991). *Escherichia coli* is the most common inhabitant of the gastrointestinal tract of humans. It belongs to *Enterobacteriacea* family, and

Received: 19/04/18
Accepted: 10/10/18

in women, it lives in the vaginal microbiota. In addition, it is responsible for 50% to 60% of cases of urinary tract infection and it is the most common pathogen in neonatal meningitis (da SILVA; CAMPOS, 2008), being the most common pathogen in newborns (STOLL et al., 2011; DONG; SPEER, 2014).

Group B *Streptococcus* (GBS), or *Streptococcus agalactiae*, is a Gram-positive bacterium that causes an invasive disease primarily in infants, pregnant or postpartum women. Is the main infectious cause of morbidity and mortality among infants in the United States (VERANI; MCGEE; SCHRAG, 2010).

Maternal infections caused by these agents are related to neonatal sepsis evidenced by the following maternal risk factors: premature labor, rupture of membranes over 18 hours before delivery, maternal colonization, maternal fever (≥ 38ºC) during or after delivery, newborns with a low birth weight (<2,500 grams), and previous baby with neonatal infection (XIAO et al., 2017; GOULART et al., 2006). The infection is premature in 75% of cases; it manifests usually before the 72 hours, but may be delayed, appearing between the 1st and 4th week of life (XIAO et al., 2017; JOLIVET, 2002).

The hemoculture is considered the gold standard test for infections and sepsis diagnostic (BLEVINS; BRONZE, 2010) and is the most used diagnostic test in newborn samples, in addition to non-specific tests that present positive predictive values and jointly guide the medical conduct. Blood culture is poorly sensitive and takes about 72 hours for the results to be released (RIEDEL; CARROLL, 2016; VIEIRA, 2004).

The beginning of an empiric antibiotic therapy intra childbirth to prevent infections and inadequate volumes of samples are limiting factors in the hemoculture that can produce false-negative results. In neonates, mainly premature with low birth weight, the collection of blood is restricted to a single sample with minimum volume (1 mL), maiking the diagnosis very difficult when the bacteremia level is low (SINHA et al., 2018; MERMEL; MAKI, 1993). When there are risk factors and evidence of late sepsis, the newborn is monitored, and if symptoms such as fever, apathy, moaning etc. are found, the empirical use of antibiotics such as ampicillin and gentamicin is recommended (FHEMIG, 2013).

As a complementary method to blood culture, blood parameters and urine exams of the newborn, PCR directed to the placenta, targeting the main pathogens related to sepsis (*Escherichia coli*, *Streptococcus agalactiae*, *Ureaplasma urealyticum*, *Mycoplasma hominis* and GBS) may present an indication of maternal infection when associated with risk factors. Because it is a well-established molecular test with a high sensitivity and specificity, it can complement and guide the clinical management of the newborn's treatment.

The objective of this study was to evaluate the factors of neonatal sepsis predisposition in pregnant womens through histopathological examination and the apoptotic index of placental tissues and detect DNA of *E. coli* and *S. agalactiae* using the Polymerase Chain Reaction (PCR).

MATERIAL AND METHODS

Study Population

The study was approved by the Ethics Committee for Research Involving Human Beings of the Federal University of Mato Grosso do Sul (UFMS) on 26 November 2013, document number 467.571.

100 samples of placentas of pregnant women were included in the study (70% had vaginal delivery). All of them gave birth at the Cândido Mariano Maternity located in the city of Campo Grande, Mato Grosso do Sul state (Brazil), from November 2013 to May 2014. They had a mean age of 24.9 years and a mean gestational age of 38.4 weeks.

Among the 100 samples analyzed, 48% (48/100) represented the control group, of them 75% had vaginal delivery (36/48) and 25% had cesarean delivery (12/48), with no risk factor associated to neonatal sepsis, single pregnancy, normal evolution until the time of the study, in initial labor and with admission in the same sector of maternity. Approximately 52% (52/100) of the samples tested presented at least one risk factor, such as a history of urinary tract infection, twinning, fever at any time during pregnancy, rupture of membranes over 18 hours, abortion in previous pregnancy, adolescents under the age of 18 and with suspected infections. In order to identify the probable infection, risk factors were evaluated by using infectious identification forms targeted to pregnant women. The group composed by pregnant women with risk factors, 65.3% had vaginal delivery (34/52) and 34.6% had cesarean delivery (18/52).

In this research we excluded pregnant women with positive serological tests for infectious diseases (human immunodeficiency virus (HIV), hepatitis, syphilis, toxoplasmosis and rubella), preeclampsia, insufficient material for analysis and incorrectly filled form of infection identification.

Samples

Immediately after delivery**,** segments with 1 to 2 grams were sectioned using a sterile surgical scalpel in the neighboring region between the umbilical cord and the placenta, from the fetal face (or chorionic) to the maternal side. The segments were immediately packed in individual, sterile vials and sent for molecular and histological analysis. For molecular analysis, the tissues were stored in a freezer at -20°C for further extraction of the DNA in order to avoid environmental contamination.

Molecular Analysis

DNA isolation

Approximately 25 milligrams of the placental tissue were sectioned using disposable and sterile lamina, and submitted to DNA extraction using the Illustra Tissue & Cells Genomic *Prep Mini Spin*® kit (GE Healthcare, USA). Conventional PCR (SAIKI et al., 1985) was used to verify the feasibility of DNA with some modifications for human β–globin, as described by Bauer et al., (1991). The viability of samples and quality of DNA were evaluated with PCR of human β-globin gene using PC04: 5′ CAACTTCATCCACGTTCACC 3′ and GH20: 5′ GAAGAGCCAAGGACAGGTAC 3′ primers under the following PCR conditions: initial denaturation at 94°C for 5 min, 39 cycles with the cycling profile of 94°C for 1 min, 55°C for 1 min, 72°C for 1 min, and final extension for 5 min at 72°C.

PCR for *E. coli* and *S. agalactiae*

For the detection of *E. coli* and *S. agalactiae*, a PCR was performed using the primers E-coli-F/ 5'- CTG GTC GAC GAC AAG ATG CAC- 3'; E-coli-R/ 5'- GTC TTC CAG TTC GAT GTT GAT ACC- 3', generating a fragment of 323 bp (genes rpoB); and Sagalact-F/ 5'-GCA GCC AGT TGA AGA TCG TTA TG- 3'; Sagalact-R/ 5'-CCA GCA GCA CCA CGA ATT TC- 3', generating a fragment of 350 bp (factor 1). The primers were designed at Center for Molecular Biology and Genetic Engineering (Campinas University-UNICAMP). Were selected conserved regions of genes rpoB and used the Gene Runner program. The sequences were analyzed by BLAST program (Basic Local Alignment Search Tool) at the NCBI site. The PCR reaction was prepared with a final volume of 50 µL using 1 µL of DNA template, 50 mM of potassium chloride, 10 mM of Tris-HCl (pH 8.4), 1.5 mM $MgCl_2$, 2.0 mM dNTP mix (Ludwig Biotec), 3.0 units of Taq DNA Polymerase recombinant (Invitrogen) and 20 pmol of each primer. The amplification of the template DNA was processed in automatic thermocycler (MJ Research Minicycler). For *E. coli*, the reaction conditions were 95°C for 5 min followed by 40 cycles of denaturation at 94°C for 1 min, annealing at 60°C for 1 min, extension at 72°C for 1 min, with a final extension at 72°C for 5 min. For *S. agalactiae*, 95ºC for 5 min followed by 40 cycles of denaturation at 94ºC for 1 min, annealing at 57ºC for 1 min, extension at 72ºC for 1 min, with a final extension at 72ºC for 5 min. The amplified fragment of 323 bp and 350 pb was separated by electrophoresis on a 1.5% agarose gel stained with ethidium bromide and visualized by ultraviolet transilluminator.

The PCR samples positive for *E. coli* were purified using the NucleoSap® enzyme – mix of Exonuclease and Alkaline Phosphatase (Molecular Biotecnologia, Brazil), following the recommendations of the manufacturer, and sent for sequencing at Centro de Pesquisas sobre o Genoma Humano e Células- Tronco, Instituto de Biociências – Universidade de São Paulo.

***E. coli* and *S. agalactiae* Positive Control**

E. coli ATCC 35218 (Newprov) and *S. agalactiae* ATCC 27591 (conceded by Dr. Carlos Emílio Levy/Laboratory of Microbiology of the Division of Clinical Pathology, Campinas University-UNICAMP) was used as the positive control. The strain was reactivated and inoculated according to ANVISA (2013). The bacterial DNA was extracted using the Illustra Blood Genomic *Prep Mini Spin*® kit (GE Healthcare, USA).

Histopathological Analysis

Fragments of placental samples weighing 1 to 2 grams collected during delivery were immediately fixed in 10% buffered formalin for 24 hours, processed (Alcohol-Xylene), embedded in paraffin, cut to a final 5 µm thickness and placed on the slide for Hematoxylin-Eosin histological staining for visualization in 400X magnification of the placental morphology. The images were captured using a video camera Samsung®, attached to a Bioval L2000C microscope, using ImageLab program version 2.4 for quantification of fibrinoid necrosis, infarct areas and areas of calcification.

Apoptosis Identification

Fragments of placental samples weighing approximately 200 milligrams were homogenized and processed for staining with Acridine orange, following the protocol described by Rovozzo and Burke (1973) with modifications by Mauro et al., (2011). Staining makes it possible to observe cells

using a fluorescence Zeiss Axiolab microscope (HBO 50). Three slides (triplicate) of each sample were prepared and a thousand cells were counted in each slide. For each triplicate, we calculated the arithmetic mean and the mean value of apoptotic cells. Cytological modifications were used to detect apoptotic cells. To avoid statistical errors, all slides were analyzed by only one observer. The formula used for index apoptotic calculation was: IA = (Number of apoptotic cells/ total number of cells) x 100.

Statistical Analysis

The values of numerical variables were expressed as mean ± standard deviation. Comparisons between the apoptotic indexes were performed using the Mann-Whitney test. The level of significance was $p \leq 0.05$.

The tabulation of results was made in the Microsoft Office Excel 2013 software, and the water calculation was performed using Bioestat 5.0. Comparison of the data and preparation of graphs were performed using the GraphPad Prism 5.0 software.

RESULTS

Histopathological and molecular analysis were performed in 100 placenta samples. The pregnant women had a mean age of 24.9±5.9 years, gestational age of 38.4±1.7 weeks. The study showed that 13.4% (7/52) of pregnant women with risk factors were PCR positive for *E. coli*. They had risk factors associated with neonatal sepsis during pregnancy, two of them had urinary tract infection (P60 and P66), one of them had a preterm birth and UTI (P69), one of them had fever and UTI (62), one of them was an adolescent and had UTI (P61), urinary tract infection in a previous pregnancy (P68), and one woman had an abortion in a previous pregnancy (P65) (Table 1).

Table 1. Distribution of PCR positive samples for *Escherichia coli*, co-related with risk factor associated to neonatal sepsis, Campo Grande- MS.

Positive PCR	
Samples	**Risk Factor**
P60	UTI (Urinary Tract Infection)
P61	gestation in adolescence and UTI
P62	fever and UTI
P65	abortion in a previous pregnancy
P66	UTI
P68	UTI in a previous pregnancy
P69	UTI and preterm birth

There were 52% had risk factors for infections such as teenage pregnancy (7.6%), urinary tract infection (71.1%), twin pregnancy (1.9%), fever during and after delivery (5.7%), abortion in previous pregnancy (13.4%), gestational age between 36 and 37 weeks (23%) and low-weight newborns (7.6%) (Table 2).

Molecular Analysis

The one hundred placental tissues were subjected DNA extraction and to PCR test for the *E. coli* and *S. agalactie*. Among the 52 samples of pregnant women presenting risk factors, seven (13.4%) were positive for *E. coli* (Figure 1) and none of them were positive for *S. agalactiae*. Among the 48 samples included in the control group without any risk factor, there was no detection of *E. coli* and *S. agalactiae* by PCR.

Among the seven PCR positive samples for *E. coli*, six were sequenced and showed a similarity of 87-97% with *E. coli* sequences isolated from urine and fecal samples deposited in the GenBank.

Table 2. Distribution in percentage of pregnant women with risk factors associated to neonatal sepsis, Campo Grande – MS, 2015 (n = 52).

Groups	Risk Factors	
	Yes	No
	n %	n %
Teenage Pregnancy	4 (7,6%)	48 (92,4%)
Urinary Tract Infection	37 (71,1%)	15 (28,9%)
Twin Pregnancy	1 (1,9%)	51 (98,1%)
Fever	3 (5,7%)	49 (94,3%)
Abortion in previous pregnancy	7 (13,4%)	45 (86,6%)
Gestational age between 36 and 37 weeks	12 (23%)	40 (77%)
Low birth weigth	4 (7,6%)	48 (92,4%)

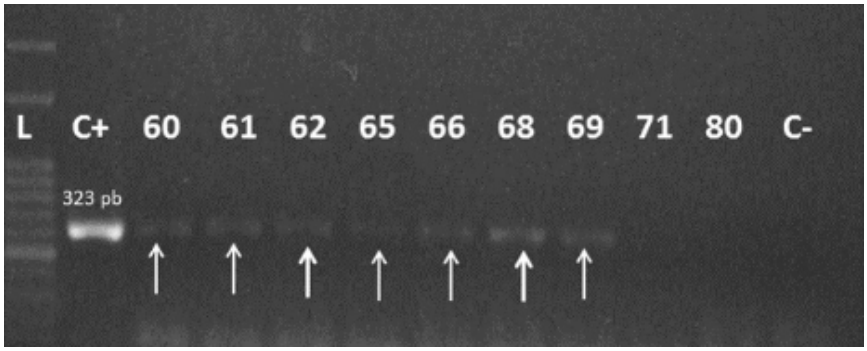

Figure 1. Molecular analysis for *Escherichia coli*; 100 bp DNA Ladder (L); positive control (C+); samples (60-80); negative control (C-); white arrows: positive samples (60, 61, 62, 65, 66, 68, 69).

Histopathological Analysis and Apoptotic Index

Inflammatory infiltrates were not observed in the 52 samples with risk factors and in the 48 samples of the control group, although there was fibrinoid necrosis, areas of placental infarction and calcification in all samples. There was a statistically significant result in the quantification of fibrinoid necrosis and infarcted areas, causing a small decrease in these variables in placentas that showed risk factors associated with neonatal sepsis when compared to those who did not have it (Table 3). The placental morphology is shown in Figure 2.

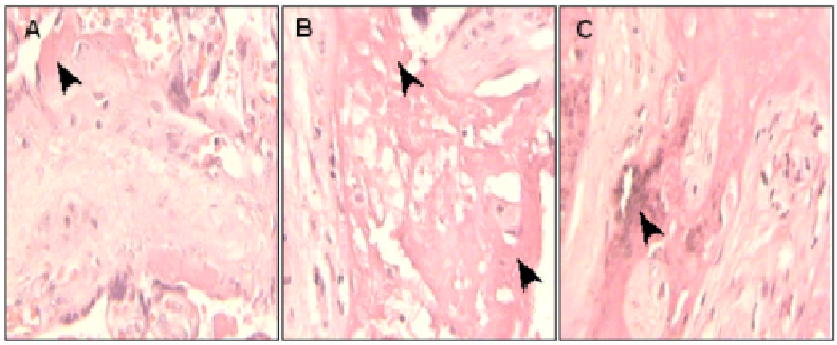

Figure 2. Photomicrograph of human placenta stained with Hematoxylin-Eosin (HE) showing (A) Fibrinoid Necrosis, (B) infarcted areas, (C) calcification (400X).

Apoptotic cells were quantified and the apoptotic index was calculated. However, the results were not statistically significant after comparing the group with risk factors for neonatal infection in relation to the group without such factors (Table 3).

Table 3. Mean values ± interquartile range (minimum - maximum) of the measured values of the variables apoptotic index, fibrinoid necrosis, infarcted areas and calcification in relation to absence or presence of risk factor, n = 100.

	Without risk factors	**With risk factor**	***P* value**
Index apoptotic	9,13±1,01 (8,03-11,63)	9,12±1,13 (7,57-11,50)	0,4752
Fibrinoid necrosis	1,250,09 (1,10-1,55)	1,400,06 (1,35-1,65)	0,0417
Infarcted areas	0,40±0,01 (0,35-0,50)	0,45±0,05 (0,40-0,50)	0,0427
Calcification	0,05±0,05 (0,00-0,10)	0,05±0,03 (0,00-0,10)	0,5785

DISCUSSION

It is important to emphasize the presence of at least one risk factor in each of the seven pregnant women who presented a positive PCR for *E. coli*, demonstrating the direct relation between the pathogen and the presence of these factors. In cases where women have a risk factor, the clinical management is the empirical administration of antibiotics to newborns to treat or prevent neonatal sepsis.

Thus, it is interesting to evaluate the risks and benefits considering that antibiotics select multi-resistant flora and may contribute to fungal infections and late neonatal sepsis. Often, doubts resulting from laboratory tests lead to unnecessary treatment of newborns (SINHA et al., 2018; VIEIRA, 2004). *Escherichia coli* is considered one of the most common pathogens related with the precocious sepsis in full-term newborns and with low weight birth (CORTESE et al., 2016), and the increasing resistance degree to antibiotics it has been decisive in the increment of pathogen virulence (BEHMADI et al., 2016; STOLL et al., 2002). Mohsen et al., (2017) found larger resistance of Gram-negative bacteria to ampicillin, cephalosporins and piperacillin-tazobactam, and smaller resistance was evidenced to aminoglycosides, carbapenems and quinolones. It was also detected multiresistance mainly in isolated of Gram-negative.

The higher sensibility of the PCR technique, comparing with blood culture, can detect probable cases of sepsis because it is less influenced by the previous use of antibiotics. In addition, the technique identifies bacterial DNA, bacteria not viable and intracellular bacteria, which often are not detectable by blood culture, therefore van den BRAND et al., (2014) proposed an adapted multiplex Real-time PCR which allows the direct detection of the main bacterial pathogens of late neonatal sepsis. Although blood culture represents the gold standard to evidence the agents that cause sepsis, it is considered a laborious and time-consuming technique. PCR may be a complementary alternative because the results can be released within 24 hours.

Blood culture may take up to 72 hours to obtain results, and it presents a low sensitivity, with both false negative results and false positives (VIEIRA, 2004), which may directly interfere with the clinical management in relation to early sepsis. In this research the placenta samples were collected in the wire region, which is more likely to be colonized by pathogens, immediately after delivery using disposable and sterile slides. In addition, they were immediately frozen at -20°C in sterile flasks and subjected to DNA extraction. Such care minimizes the contamination of samples, generating reliability of results.

The group of risk was composed by 52 pregnant women, of which 34 (65,4%) had normal delivery and 18 (34,6%) had cesarean delivery. Of the seven positive pregnant women for *E. coli*, five had normal delivery, being one of the most important risk factor for nenonatal sepsis. According to Mussi-Pinhata et al., (1999) the transmission of infection may also occur during labor by aspiration of contaminated amniotic fluid or by contact of skin, mucous membranes of the

eyes and gastric tissues of newborn with blood and genital secretions or maternal feces. The main causes of morbidity and mortality are neonatal infections acquired in the uterus and during delivery (MIRANDA et al., 2012). The results suggest a possible fetal infection associated with the presence of *E. coli* in the placenta since the bacteria may go up, reaching the fetal membranes (LAJOS et al., 2008). A research developed by Jacociunas and Ulrich (2007) in Campo Bom (Rio Grande do Sul state, Brazil) showed a predominance of *E. coli* in urinary samples of pregnant women, representing 76% of the cases. Bolton et al., (2012) studied experimental models and demonstrated that non-disseminated UTI is associated with adverse perinatal outcome, such as the reduction of fetal weight and the evident inflammatory response in utero-placental tissues.

A comparative study in 1977-1987 and 1988-1998 showed that the etiology of microorganisms that cause neonatal infection has changed over the years. By the introduction of antibiotics, there was a predominance of Gram-negative microorganisms, being *E. coli* was detected in both decades (CECCON et al., 1999). Chen et al., (2013) carried out a comparison of incidence of *S. agalactiae* from 1990 to 2010 and they noted a significant decrease mainly in cases of premature sepsis, falling from 1.7 to 0.4 in each 1,000 newborns. This study complemented the findings in the literature because *S. agalactiae* DNA was not detected in none of the tested samples.

By staining using Hematoxylin-Eosin (H&E), was observed the presence of fibrinoid necrosis areas and calcification. There was a statistically significant result in the quantification of fibrinoid necrosis and infarcted areas, causing a small decrease in these variables in placentas that showed risk factors associated with neonatal sepsis when compared to those who did not have it. Placental fibrinoids are observed in normal placentas; however, in case of gestational hypertension, chronic hypertension and preeclampsia, an increase in perivillous space can be observed as a result of trophoblast cells secretion or maternal blood coagulation. For this reason, it is not considered restricted to pathological processes (SOUZA et al., 2011).

Areas of fibrinoid necrosis represent products of immunological reactions involving coagulation, anticoagulants and fibrinolysis. It is not the result only of diffusion of plasmatic proteins within a possible placental damage (LABARRERE; FAULK, 1991). Based on these data, it is suggested that the decrease in placental fibrinoids is due to placental physiological changes influenced by risk factors associated with neonatal sepsis; pathologies occurring during pregnancy usually cause changes in placental morpho-functional structure. The literature that addresses the issue is still scarce and more research is needed to prove the influence of risk factors on placental fibrin deposition.

Regarding the apoptotic index analysis, there had been no statistically significant difference between the groups. The placenta is a fetus-maternal organ that has its own stages of growth, development and senescence (GHEORGHE et al., 2010). The placental apoptosis is a physiological process during normal gestation, appearing at smaller quantities during the first quarter and increasing from the third quarter of pregnancy. A higher increase of apoptosis is usually observed in pathologies such as maternal diabetes, preeclampsia, choriocarcinoma and restricted intrauterine growth (SHARP et al., 2010). Therefore, apoptosis occurs naturally during pregnancy until term, and constitutes part of placental senescence, favoring the release of fetus. The investigation of the apoptotic index was important because, in the literature, Abrahams et al., (2004) demonstrated through apoptosis analyses that the immune response to infections may contribute to complications during pregnancy, such as preterm birth and preeclampsia.

The research is particularly relevant in Brazil due to the high prevalence of *E. coli* in the country. Studies published by Freitas et al., (2012) and Pinheiro et al., (2009) mentioned neonatal infection rates between 28.5% and 30.6% for Gram-positive and Gram-negative bacteria. The DNA amplification of *E. coli* by PCR is a sensitive, specific and easily performed technique, and does not require cell culture. It is useful to complement the diagnosis, focusing on the treatment, and assisting in the support for newborns in preventing late sepsis.

The identification of *E. coli* through PCR in placental tissues of pregnant women with risk factors for neonatal sepsis enhances the inclusion of the test in the Brazilian National Health System (SUS), mainly for women included in the risk group of sepsis. The test possesses accuracy and speed (ANDRADE et al., 2017) compared to the tests accomplished at the hospitals in patients with clinical signs of sepsis (DELLINGER et al., 2013). Besides, it can avoid the empiric use of antibiotics, because reflects the most probable pathogens, contributing with the knowledge of the local epidemiology and the causes of the sepsis (ALVES, 2011) being possible to optimize the prophylactic

treatment in newborn, reducing the death risk and expenses.

The results allow to conclude that the seven patients diagnosed with *E. coli* by PCR, had eminent risk factors of neonatal sepsis, and the UTI was the main aggravating. The histopathological examination demonstrated that the risk factors caused significant alterations, producing fibrinoid necrosis and infarcted areas in the placenta, contrary to apoptotic index that didn't differ from the group with unprecedented risk.

ACKNOWLEDGMENTS

We thank the Center for Molecular Biology and Genetic Engineering - CBMEG; Laboratory of Microbiology of the Division of Clinical Pathology, Campinas University-UNICAMP; Laboratory for Toxicology and Medicinal Plants, University Anhanguera - Uniderp, Campo Grande-MS; the Graduate Program in Infectious and Parasitic Diseases, Federal University of Mato Grosso do Sul by contributions and Coordination for the Improvement of Higher Education Personnel (CAPES) for financial support.

RESUMO: A sepse neonatal é uma síndrome clínica definida por sinais sistêmicos de infecção em recém-nascidos acompanhados de bacteremia. Pode ser responsável por sérias consequências para o recém-nascido, caracterizadas ao nascimento como sepse precoce ou sepse tardia, com alta taxa de morbidade e mortalidade neonatal. Agentes patológicos como *Escherichia coli* (*E. coli*), *Streptococcus agalactiae* (*S. agalactiae*), *Ureaplasma urealyticum* e *Mycoplasma hominis* são mais frequentemente responsáveis por infecções intra-uterinas. O objetivo deste estudo é avaliar os fatores de predisposição da sepse neonatal em gestantes através do exame histopatológico e do índice apoptótico de tecidos placentários e detectar DNA de *E. coli* e *S. agalactiae* utilizando a reação em cadeia da polimerase (PCR). Análises histopatológicas foram realizadas e o índice apoptótico foi determinado para verificar os níveis de possíveis infiltrados inflamatórios e morte celular. Amostras de placenta foram coletadas de novembro de 2013 a maio de 2014. Após a extração de DNA, foi realizada uma PCR amplificando o fragmento alvo das regiões conservadas do polimorfismo rpoB (polimerase beta-RNA) de *E. coli* e o fator 1 de *S. agalactiae*. O índice de apoptose foi testado com alaranjado de acridina e o procedimento histológico com coloração de hematoxilina-eosina. Entre 100 amostras de tecidos placentários analisados por PCR, 48 representaram o grupo controle e não apresentaram fator de risco associado à sepse neonatal, e 52 amostras representativas do grupo de estudo apresentaram pelo menos um fator de risco. Entre essas 52 amostras, 7 (13,4%) apresentaram PCR positivo para *E. coli*. Nenhuma amostra de placenta foi positivo para *S. agalactiae* na PCR. A quantificação do índice apoptótico não mostrou significância estatística entre os grupos e não foram encontrados infiltrados inflamatórios. No entanto, cortes histológicos mostraram necrose fibrinóide, áreas de infarto e áreas de calcificação em todas as amostras. Portanto, os resultados permitem concluir que as sete pacientes do grupo experimental com PCR positivo para *E. coli* apresentavam fatores de risco eminentes de sepse neonatal, sendo a infecção do trato urinário (ITU), o principal agravante. O exame histopatológico demonstrou que os fatores de risco causaram alterações significativas, produzindo necrose fibrinóide e áreas infartadas na placenta, ao contrário o índice apoptótico que não diferiu do grupo sem precedentes de risco.

PALAVRAS-CHAVE: *Escherichia coli*. *Streptococcus agalactiae*. Histopatologia. Sepse neonatal.

REFERENCES

ABRAHAMS, V. M.; BOLE-ALDO, P.; KIM, Y. M.; STRASZEWSKI-CHAVEZ, S. L.; CHAIWORAPONGSA, T.; ROMERO, R.; MOR, G. Divergent Trophoblast Responses to Bacterial Products Mediated by TLRs. **J Immunol**, v. 173, n. 7, p. 4286-96, oct. 2004. https://doi.org/10.4049/jimmunol.173.7.4286

ALVES, R. I. F. **Tratamento das infecções neonatais bacterianas e fúngicas: fundamentos teóricos para uma aplicação prática**. 31 f. 2011. Dissertação (Mestrado Integrado em Medicina) – Instituto de Ciências Biomédicas Abel Salazar, Universidade do Porto, Porto, 2011.

ANDRADE, P. D.; RUSSO, J. S.; GOUVEIA, J. B.; COSTA, C. R. C.; OLIVEIRA, K. G.; GIANETTI, M.; ANJOS, E. B. V.; PAVAN, T. B. S.; MARTINS, M. F. S.; FRANCO, J.; COSTA, M. L.; NOMURA, M. L.; LEVY, C. E.; PASSINI JÚNIOR, R.; COSTA, S. C. B. Molecular Characterization of Group B Streptococcus Serotypes By Multiplex Polymerase Chain Reaction. **Medical Express**, v. 4, n. 4, p. 1-6, aug. 2017. https://doi.org/10.5935/MedicalExpress.2017.04.06

ANVISA. **Microbiologia Clínica para o Controle de Infecção Relacionada à Assistência à Saúde**. Brasília: Agência Nacional de Vigilância Sanitária, 2013.

ANVISA. **Critérios Diagnósticos de Infecção Associada a Assistência a Saúde Neonatologia**. Brasília: Agência Nacional de Vigilância Sanitária, 2017.

BAUER, H. M.; TING, Y.; GREER, C. E.; CHAMBERS, J. C.; TASHIRO, C. J.; CHIMERA, J.; REINGOLD, A.; MANOS, M. M. Genital human papillomavirus infection in female university students as determined by a PCR-based method. **JAMA**, v. 265, n. 4, p. 472-477, june. 1991.

BEHMADI, H.; BORJI, A.; TAGHAVI-RAD, A.; SOGHANDI, L.; BEHMADI, R. Prevalence and Antibiotic Resistance of Neonatal Sepsis Pathogens in Neyshabour, Iran. **Arch Pediatr Infect Dis**, v. 4, n. 2, p. e33818, apr. 2016. https://doi.org/10.5812/pedinfect.33818

BLEVINS, S. M.; BRONZE, M. S. Robert Koch and the "golden age" of bacteriology. **Int J Infect Dis**, v. 14, n. 9, p. e744–51, apr. 2010. https://doi.org/10.1016/j.ijid.2009.12.003

BOLTON, M.; HORVATH, D. J.; LI, B.; CORTADO, H.; NEWSOM, D.; WHITE, P.; PARTIDA-SANCHEZ, S.; JUSTICE, S. S. Intrauterine Growth Restriction Is a Direct Consequence of Localized Maternal Uropathogenic *Escherichia coli* Cystitis. **PloS One**, v. 7, n. 3, p. 1-9, mar. 2012. https://doi.org/10.1371/journal.pone.0033897

CANTEY, J. B.; BAIRD, S. D. Ending the culture of culture-negative sepsis in the neonatal ICU. **Pediatrics**, v. 140, n. 4, p. e20170044, oct. 2017. https://doi.org/10.1542/peds.2017-0044

CECCON, M. E. J.; FEFERBAUM, R.; GIOLO, C. R.; VIEIRA, R. A.; DINIZ, E. M. A.; KREBS, V. J.; ARAUJO, M. C. K.; GALVANI, A. L. S.; VAZ, F. A. C. Sepse neonatal - análise comparativa entre duas décadas (1977-1987 e 1988-1998) em relação à incidência dos agentes etiológicos e da morbimortalidade. **Pediatria**. v. 21, n. 4, p. 287-297, mar. 1999.

CHEN, V. L.; AVCI, F. Y.; KASPER, D. L. A maternal vaccine against Group B Streptococcus: Past, Present, and Future. **Vaccine**, v. 31, n. 4, p. 13-19, aug. 2013. https://doi.org/10.1016/j.vaccine.2012.12.080

CORTESE, F.; SCICCHITANO, P.; GESUALDO, M.; FILANINNO, A.; DE GIORGI, E.; SCHETTINI, F.; LAFORGIA, N.; CICCONE, M. M. Early and late infections in newborns: where do we stand? A review. **Pediatr Neonatol**, v. 57, n. 4, p. 265-73, aug. 2016. https://doi.org/10.1016/j.pedneo.2015.09.007

da SILVA, D. C.; CAMPOS, L. C. *Escherichia coli* que Causa Infecções Extra-Intestinais (ExPEC – Extraintestinal Pathogenic *E. coli*). In: TRABULSI, L. R.; ALTERTHUM, F. (Ed.). **Microbiologia**. São Paulo: Atheneu, 2008. p. 311-18.

DELLINGER, R. P.; LEVY, M. M.; RHODES A.; ANNANE, D.; GERLACH, H.; OPAL, S. M.; SEVRANSKY, J. E.; SPRUNG, C. L.; DOUGLAS, I. S.; JAESCHKE, R.; OSBORN, T. M.; NUNNALLY, M. E.; TOWNSEND, S. R.; REINHART, K.; KLEINPELL, R. M.; ANGUS, D. C.; DEUTSCHMAN, C. S.; MACHADO, F. R.; RUBENFELD, G. D.; WEBB, S. A.; BEALE, R. J.; VINCENT, J. L.; MORENO, R. Surviving Sepsis Campaign: international guidelines for management of severe sepsis and septic shock: 2012. **Crit Care Med**, v. 41, n. 2, p. 580-637, feb. 2013. https://doi.org/10.1097/CCM.0b013e31827e83af

DONG, Y.; SPEER, C. P. Late-onset neonatal sepsis: recent developments. **Arch Dis Child Fetal Neonatal Ed**, v. 100, n. 3, p. F257-F263, nov. 2014. https://doi.org/10.1136/archdischild-2014-306213

FHEMIG. **Sepse Neonatal**. Minas Gerais, aug. 2013.
Avaible from: <http://www.fhemig.mg.gov.br/index.php/docman/Protocolos_Clinicos-1/132-031-sepse-neonatal-07082014/file>.

FREITAS, B. A.; PELOSO, M.; MANELLA, L. D.; FRANCESCHINI, S. C.; LONGO, G. Z.; GOMES, A. P.; SIQUEIRA-BATISTA, R. Sepse tardia em pré-termos de uma unidade de terapia intensiva neonatal: análise de três anos. **Rev Bras Ter Intensiva**, v. 24, n. 1, p. 79-85, mar. 2012. https://doi.org/10.1590/S0103-507X2012000100012

GHEORGHE, C. P.; GOYAL, R.; MITTAL, A.; LONGO, L. D**.** Gene expression in the placenta: maternal stress and epigenetic responses. **Int J Dev Biol**, v. 54, n. 2-3, p. 507–523, jan. 2010. https://doi.org/10.1387/ijdb.082770cg

GOULART, A. P.; VALLE, C. F.; DAL-PIZZOL, F.; CANCELIER, A. C. Fatores de risco para o desenvolvimento de sepse neonatal precoce em hospital da rede pública do Brasil. **Rev Bras Ter Intensiva**, v. 18, n. 2, p. 148-153, apr/june. 2006.

HILLIER, S. L.; KROHN, M. A.; KIVIAT, N. B.; WATTS, D. H.; ESCHENBACH, D. A. Microbiologic causes and neonatal outcomes associated with chorioamnion infection. **Am J Obstet Gynecol**, v. 165, n. 4, p. 955-61, oct. 1991. https://doi.org/10.1016/0002-9378(91)90447-Y

JACOCIUNAS, L. V.; ULRICH, S. Avaliação de Infecção Urinaria em Gestantes no Primeiro Trimestre de Gravidez. **Rev Bras Anal Clin**, v. 39, n. 1, p. 55-57, dec. 2007.

JOLIVET, R. R. Early- onset neonatal group B streptococcal infection: 2002 guidelines for prevention. **J Midwifery Womens Health**, v. 47, n. 6, p. 435- 446, nov./dec. 2002. https://doi.org/10.1016/S1526-9523(02)00348-3

LABARRERE, C. A.; FAULK, W. P. Factor VIII procoagulant: a marker of fibrinoid necrosis in normal term human placentae**. J Reprod Immunol**, v. 19, n. 2, p. 167-177, feb. 1991. https://doi.org/10.1016/0165-0378(91)90015-I

LAJOS, G. J.; JUNIOR, R. P.; NOMURA, M. L.; AMARAL, E.; PEREIRA, B. G.; MILANEZ, H.; PARPINELLI, M. A. Colonização bacteriana do canal cervical em gestantes com trabalho de parto prematuro ou ruptura prematura de membranas. **Rev Bras Ginecol Obstet**, v. 30, n. 8, p. 393-9, aug. 2008. https://doi.org/10.1590/S0100-72032008000800004

MAURO, M. O.; SARTORI, D.; OLIVEIRA, R. J.; ISHII, P. L.; MANTOVANI, M. S.; RIBEIRO, L. R. Activity of selenium on cell proliferation, cytotoxicity, and apoptosis and on the expression of CASP9, BCL-XL and APC in intestinal adenocarcinoma cells. **Mutat Res**, v. 715, n. 1-2, p. 7-12, june. 2011.

MERMEL, L. A.; MAKI, D. G. Detection of bacteremia in adults: consequences of culturing an inadequate volume of blood. **Ann Intern Med**, v. 119, n. 4, p. 270–72, aug. 1993. https://doi.org/10.7326/0003-4819-119-4-199308150-00003

MIRANDA, M. M. S.; SOUZA, L. M. G.; AGUIAR, R. A. L. P.; CORRÊA JR, M. D.; MAIA, M. M. M.; BORGES, R. S.; MELO, V. H. Rastreamento das infecções perinatais na gravidez: realizar ou não?. **Femina**, v. 40, n. 1, p. 14-22, jan/feb. 2012.

MOHSEN, L.; RAMY, N.; SAIED, D.; AKMAL, D.; SALAMA, N.; HALEIM, M. M. A.; ALY, H. Emerging antimicrobial resistance in early and late-onset neonatal sepsis. **Antimicrob Resist Infect Control**, v. 6, n. 1, p. 63, june. 2017.

MUSSI-PINHATA, M. M, YAMAMOTO, A. Y. Infecções congênitas e perinatais. **J Pediatr**, v. 75, n. 1, p. 15-30. 1999.

PINHEIRO, M. S.; NICOLETTI, C.; BOSZCZOWSK, I.; PUCCINI, D. M.; RAMOS, S. R. Infecção hospitalar em Unidade de Terapia Intensiva Neonatal: há influência do local de nascimento?. **Rev Paul Pediatr**, v. 27, n. 1, p. 6-14, mar. 2009. https://doi.org/10.1590/S0103-05822009000100002

RIEDEL, S.; CARROLL, K. C. Early identification and treatment of pathogens in sepsis: molecular diagnostics and antibiotic choice. **Clin Chest Med**, v. 37, n. 2, p. 191-207, june. 2016.

ROVOZZO, G. C.; BURKE, C. N. A Manual of Basic Virological Techniques. **Prentice Hall**, p. 159, feb. 1973.

SAIKI, R. K.; SCHARF, S.; FALOONA, F.; MULLIS, K. B.; HORN, G. T.; ERLICH, H. A.; ARNHEIM, N. Enzymatic amplification of beta-globin genomic sequences and restriction site analysis for diagnosis of sickle cell anemia. **Science**, v. 230, n. 4732, p. 1350–1354, dec. 1985. https://doi.org/10.1126/science.2999980

SHARP, A. N.; HEAZELL, A. E.; CROCKER, I. P.; MOR, G. Placental apoptosis in health and disease. **Am J Reprod Immunol**, v. 64, n. 3, p. 159-169, mar. 2010. https://doi.org/10.1111/j.1600-0897.2010.00837.x

SINHA, M.; JUPE, J.; MACK, H.; COLEMAN, T. P.; LAWRENCE, S. M.; FRALEY, S. I. Emerging Technologies for Molecular Diagnosis of Sepsis. **Clin microbiol rev**, v. 31, n. 2, p. e00089-17, feb. 2018. https://doi.org/10.1128/CMR.00089-17

SOUZA, D. A.; BEZERRA, A. F.; WANDERLEY, D. C.; SOUTO, C. M. Aumento no material fibrinoide perivilositário nas placentas de gestações com pré-eclâmpsia. **J Bras Patol Med Lab**, v. 47, n. 1, p. 71-77, feb. 2011. https://doi.org/10.1590/S1676-24442011000100010

STOLL, B. J.; HANSEN, N. I.; SÁNCHEZ, P. J.; FAIX, R. G.; POINDEXTER, B. B.; MEURS, K. P. V.; BIZZARRO, M. J.; GOLDBERG, R. N.; FRANTZ III, I. D.; HALE, E. C.; SHANKARAN, S.; KENNEDY, K.; CARLO, W. A.; WATTERBERG, K. L.; BELL, E. F.; WALSH, M. C.; SCHIBLER, K.; LAPTOOK, A. R.; SHANE, A. L.; SCHRAG, S. J.; DAS, A.; HIGGINS, R. D.; SHRIVER, E. K. Early Onset Neonatal Sepsis: The Burden of Group B Streptococcal and *E. coli* Disease Continues. **Pediatrics**, v. 127, n. 5, p. 817- 826, may. 2011.

STOLL, B. J.; HANSEN, N.; FANAROFF, A. A.; WRIGHT, L. L.; CARLO, W. A.; EHRENKRANZ, R. A.; LEMONS, J. A.; DONOVAN, E. F.; STARK, A. R.; TYSON, J. E.; OH, W.; BAUER, C. R.; KORONES, S. B.; SHANKARAN, S.; LAPTOOK, A. R.; STEVENSON, D. K.; PAPILE, L. A.; POOLE, W. K.Changes in pathogens causing early-onset sepsis in very-low-birth-weight infants. **N Engl J Med**, v. 347, n. 4, p. 240-7, july. 2002.

van den BRAND, M.; PETERS, R. P.; CATSBURG, A.; RUBENJAN, A.; BROEKE, F. J.; van den DUNGEN, F. A.; van WEISSENBRUCH, M. M.; van FURTH, A. M.; KÕRESSAAR, T.; REMM. M.; SAVELKOUL, P. H. M.; BOS, M. P. Development of a multiplex real-time PCR assay for the rapid diagnosis of neonatal late onset sepsis. **J Microbiol Methods**, v. 106, p. 8-15, nov. 2014.

VERANI, J. R.; MCGEE, L.; SCHRAG, S. J. Prevention of Perinatal Group B Streptococcal Disease - Revised Guidelines from CDC. **MMWR Morb Mortal Wkly Rep**, v. 59, n. RR-10, p. 1-36, nov. 2010. https://doi.org/10.1016/j.mimet.2014.07.034

VIEIRA, A. A. Sepse no período neonatal. In: MOREIRA, M. E.; LOPES, J. M.; CARVALHO, M. **O recém-nascido de alto risco: teoria e prática do cuidar** [online]. Rio de Janeiro: Editora FIOCRUZ; 2004. p. 564. Available from: <http://books.scielo.org/id/wcgvd/pdf/moreira-9788575412374.pdfoc>.

XIAO, T.; CHEN, L. P.; LIU, H.; XIE, S.; LUO, Y.; WU, D. C. The analysis of etiology and risk factors for 192 cases of neonatal sepsis. **BioMed Res Int**, Fujian, v. 2017, n. 4, p. 1-6, july. 2017.