UNIVERSIDADE DE UBERABA

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PRESENÇA DE *Streptococcus mutans* E INTERLEUCINAS 6 E 10 EM AMOSTRAS DE LÍQUIDO AMNIÓTICO

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Lorrayne Borges Moreira

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Dissertação apresentada para a banca examinadora da Defesa de Mestrado em Odontologia - Área de Clínica Odontologia da Universidade de Uberaba.

Orientadora: Profa. Dra. Ruchele Dias Nogueira Geraldo Martins

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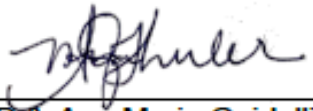

Profª. Drª. Ana Maria Guidelli Thuler
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Aos meus pais **Eder Moreira** e **Edilúcia Borges,**
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RESUMO

O período gestacional representa um momento importante para a mãe e filho, pois a exposição materna à antígenos microbianos pode determinar a colonização e desenvolvimento imunológico fetal. A cavidade bucal representa uma porta de entrada de inúmeras espécies bacterianas. Estas por sua vez, podem ocasionar doenças orais importantes, como a cárie dentária, sendo *Streptococcus mutans* (SM) o principal micro-organismo envolvido na sua etiologia. Estudos prévios demonstraram que neonatos apresentam anticorpos contra SM na saliva na ausência de níveis detectáveis deste micro-organismo, daí a necessidade de investigar a exposição antigênica na vida intrauterina. O objetivo do presente estudo foi avaliar a presença de SM e IL-6 e IL-10 em amostras de Líquido Amniótico (LA) e associar com dados clínicos. O estudo envolveu a participação de 26 mulheres com partos a termo e sem complicações gestacionais. Dados de saúde geral foram colhidos através de um questionário aplicado às participantes. As voluntárias foram examinadas oralmente, o CPOD foi calculado e salivas (SA) não estimuladas foram coletadas. A obtenção de LA foi realizada no Centro Obstétrico do MPHU durante as cesáreas estocadas em gelo. As amostras coletadas (SA e LA) foram encaminhadas ao Laboratório de Biologia Molecular da UNIUBE para análise laboratorial. A análise da presença de DNA de SM foi realizada por ensaios de PCR em tempo real com primers específicos. Os níveis de IL-6 e IL-10 foram realizados através de ELISA com kits específicos para estas interleucinas nas amostras de LA. Os resultados mostraram que 69,2% das amostras de LA apresentavam SM detectável, sendo que 65% das mulheres tinham a bactéria no LA e SA ao mesmo tempo. Houve uma associação positiva entre a presença de SM no LA e relato de não ir ao dentista frequentemente ($p<0.05$). Não houve diferença estatisticamente significante entre a frequência de LA com detecção ou não de SM e cáries ativas ($p>0.05$). No entanto, a os escores de CPOD das mulheres com LA positivo para SM foram superiores ao das mulheres sem SM detectável ($p<0.05$). Os níveis de IL-6 foram superiores ao de IL-10 no LA ($p<0.05$). Não houve diferenças significantes nos níveis de interleucinas entre amostras com SM detectável ou não no LA e nem mesmo com dados clínicos analisados ($p>0.05$). Os dados coletados permitiram concluir que o LA possui SM em níveis quantificáveis, provavelmente associados com a maior experiência de cárie, visto pelo maior índice de CPOD. Os níveis de interleucinas não estiveram relacionados com a presença de SM. A presença de SM no LA pode explicar a estimulação imune mucosa de crianças recém nascidas pela detecção prévia de anticorpos salivares contra SM.

Palavras chave: *Streptococcus mutans*, liquido amniótico, caries

ABSTRACT

The gestational period represents an important moment for the mother and child, since maternal exposure to microbial antigens can determine colonization and fetal immune development. The oral cavity represents a gateway for numerous bacterial species. Those bacteria can cause important oral diseases, such as dental caries, in which *Streptococcus mutans* (MS) being the main microorganism involved in its etiology. Previous studies have shown that neonates have antibodies to MS in their saliva in the absence of detectable levels of this microorganism, hence the need to investigate antigenic exposure in intrauterine life. The aim of the present study was to evaluate the presence of MS and IL-6 and IL-10 in samples of Amniotic Fluid (AF) and to associate with clinical data. The study involved the participation of 26 women with term deliveries and without gestational complications. General health data were collected through a questionnaire applied to the participants. The volunteers were examined orally, the DMFT was calculated and unstimulated saliva (SA) was collected. The acquisition of LA was performed at the Obstetric Center of MPHU during cesarean sections and stocked on ice. The collected samples (SA and AF) were sent to the Molecular Biology Laboratory of UNIUBE for laboratory analysis. The analysis of the presence of SM-DNA was performed by real-time PCR assays with specific primers. The levels of IL-6 and IL-10 were performed by ELISA with specific kits for these interleukins in the AF samples. The results showed that 69.2% of the AF samples had detectable MS, and 65% of the women had the bacteria in the LA and SA at the same time. There was a positive association between the presence of MS in AF and a report of not going to the dentist frequently ($p < 0.05$). There was no statistically significant difference between the frequency of AF with or without detection of MS and active caries ($p > 0.05$). However, the DMFT scores of women with positive AF for MS were higher than that of women without detectable MS ($p<0.05$). IL-6 levels were higher than IL-10 in AF ($p<0.05$). There were no significant differences in the levels of interleukins between samples with detectable MS or not in AF and not even with clinical data analyzed ($p>0.05$). The data collected allowed us to conclude that the AF has SM in quantifiable levels, probably associated with the greatest caries experience, and seen by the highest DMFT index. Interleukin levels were not related to the presence of MS. The presence of MS in AF may explain the mucosal immune stimulation of newborn children by the previous detection of salivary antibodies against MS.

Keywords: *Streptococcus mutans*, amniotic fluid, caries

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1. INTRODUÇÃO GERAL

O líquido amniótico (LA) é um complexo e dinâmico líquido biológico que proporciona proteção mecânica, nutrientes e substâncias necessárias para o crescimento fetal e seu bem-estar, além de possuir efeito antimicrobiano (Underwood et al., 2005). É um biofluido que se encontra em contato permanente com o feto e tem sua composição variada de acordo com a evolução gestacional (Underwood et al., 2005).

No primeiro trimestre, o LA é composto por várias moléculas que passam facilmente pela membrana provenientes do plasma materno (Hasimoto et al., 1993). Ao final desta fase, a sua composição começa a mudar, passando a se assemelhar ao plasma fetal; equilíbrio esse conseguido através da pele fetal e de outras vias de troca, como a placenta e o cordão umbilical (Hasimoto et al., 1993). A produção de urina, a deglutição fetal e o exsudato alveolar passam a ser as vias fundamentais para a mudança na composição do LA a partir do segundo trimestre (Hasimoto et al., 1993). Há inclusive entrada preferencial pela placenta de produtos teciduais, como prostaglandinas e a prolactina, que passam direto para o LA, sem circular pelo sangue materno (Hasimoto et al., 1993).

Tem sido amplamente refutada, em virtude das novas técnicas de sequenciamento metagenômico, a hipótese de que os recém nascidos são inicialmente estéreis e adquirem micro-organismos maternos vaginais e fecais somente quando iniciam o trânsito pelo canal vaginal, durante o trabalho de parto (Mackie et al., 1999). A investigação da presença de micro-organismos nestes ambientes, só é realizada quando há sintomas ou em algumas situações que podem levar a infecção como, por exemplo, a ruptura prematura de membranas ou dilatação cervical (Lee et al., 2007). Existem evidências clínicas de que há micro-organismos na placenta, sangue do cordão umbilical, líquido amniótico e mecônio em gestações a termo sem infecções evidentes (Bearfield et al., 2002; Steel et al., 2005; Jiménez et al., 2005, 2008; Stout et al., 2013; Aagaard et al., 2014).

Espécies virais como citomegalovírus (Endo et al., 2009), papilomavírus (Rombaldi et al., 2009) e zika vírus (Calvet et al., 2016) puderam ser detectadas no sangue do cordão umbilical de bebês recém nascidos. Estudo de Jimenez e colaboradores (2005), em amostras de sangue de cordão umbilical, detectaram a presença de várias espécies bacterianas tais como: Enterococcus faecium, Propionibacterium acnes, Staphylococcus epidermidis e Streptococcus sanguinis. Curiosamente, estas espécies são naturalmente presentes em crianças desde os primeiros dias de vida (Favier et al., 2002; Martin et al., 2003) e em crianças predispostas, pode haver o desenvolvimento de infecções oportunistas por tais espécies

(Jimenez et al., 2005). A análise de mecônio de neonatos saudáveis apresentou níveis detectáveis de *Enterococcus faecalis, Staphylococcus epidermides e Escherichia coli*, sugerindo que ao contrário do que se pensava, esse material biológico pode não ser estéril (Favier et al., 2002) e refletem a exposição microbiana do ambiente intrauterino.

Estas evidências de influxo microbiano entre mãe e filho, suscitam diversas discussões a respeito da transferência de micro-organismos comensais detectáveis na mãe para o feto, como por exemplo, os albergados na cavidade bucal. A cavidade oral possui mais de 700 tipos diferentes de bactérias, que de forma dinâmica, constituem a microbiota oral composta por micro-organismos transitórios e residentes. Estudos em modelos animais, de infecção ativa oral com micro-organismos comensais, permitiram a detecção de vários destes patógenos no feto e placenta (Lin et al., 2003; Boggess et al., 2005; Fardini et al., 2010). O sequenciamento de amostras de placentas mostrou que o microbioma placentário não se assemelha aos microbiomas vaginais ou intestinais, como se pensava anteriormente, pois é mais semelhante à microbiota oral normal, especialmente da língua e amígdala (Aagaard et al., 2014).

Patógenos orais, incluindo *Streptococcus mutans* e *Lactobacillus casei*, responsáveis pela doença cárie, podem estar presentes na cavidade amniótica (Morency et al., 2006; Gendrin et al., 2015), mas pouca informação e estudos longitudinais coesos abordam estes micro-organismos. Diferentemente dos micro-organismos periodontais, uma maior colonização por estes micro-organismos denominados cariogênicos resulta em uma menor incidência de partos prematuros (Dasanayake et al., 2005; Durand et al., 2009; Merglova et al., 2014).

A grande maioria dos estudos com gestantes e micro-organismos orais cariogênicos, especialmente *Streptococcus mutans*, envolve a transmissão destes micro-organismos da mãe para o filho, pois esta representa a principal fonte primária de infecção (Lapirattanakul et al., 2008; Binks e Duane, 2015; Da Silva Bastos et al., 2015). Alguns estudos têm demonstrado que mães com elevadas concentrações de *S. mutans* salivares, tendem a ter filhos altamente infectados, sendo que as mães pouco colonizadas, têm filhos com baixos níveis detectáveis deste micro-organismo (Berkowitz e Jordan, 1975; Rogers, 1977; Kohler et al., 1983; Li e Caufield, 1995; Li et al., 2000; Redmo Emanuelsson e Thornqvist, 2001; Tanzer et al., 2001; Azevedo et al., 2014). As primeiras informações a respeito da possível transferência de bactérias envolvidas no biofilme dentário foram relatadas por Ivanyi e Lehner (1978) que constataram uma correlação significativa antígeno-específica existente entre as respostas proliferativas de linfócitos maternos e neonatais para uma variedade de antígenos bacterianos

do biofilme dental. Os autores sugeriram que os linfócitos fetais estavam sendo sensibilizados por um fator materno solúvel que atravessa a placenta. Posteriormente, *Streptococcus mutans* e *Lactobacillus casei* foram detectados na cavidade amniótica (Bearfield et al., 2002; Dasanayake et al., 2005; Morency et al., 2006).

A disseminação de bactérias orais pela corrente circulatória é comum em pacientes submetidos a procedimentos dentais como exodontias, tratamento endodôntico e cirurgia periodontal (Li et al., 2000). Em gestantes a entrada de micro-organismos para a circulação seria facilitada pelos quadros de gengivites (Nierderman et al., 2013) devido as alterações hormonais decorrentes da gravidez (Offenbacker et al., 2006). Somados a isto, *S. mutans* pode estar associado à etiologia das endocardites bacterianas, devido a sua capacidade de causar uma bacteremia seguida pela adesão às células endoteliais (Kilian, 1982; Moreillon, 2004; Nakano et al., 2006, Nemoto et al., 2008). Ensaios longitudinais estabeleceram que escovação regular dos dentes com pasta fluoretadas esta associado com menor colonização por *S. mutans* e menores chances de cárie de infância (Plonka et al., 2013).

Neste sentido, as evidências literárias fortalecem a hipótese de que possa haver influxo de espécies bacterianas orais ou parte destas, por via hematogênica durante a gestação. Este estímulo bacteriano pode desempenhar um papel fundamental na estimulação ativa do sistema imune de mucosa de recém-nascidos e lactentes, tendo uma função biológica importante por apresentar uma coleção de antígenos que contribuem para a tolerância antigênica (Zaura et al., 2014) e também pode ser uma importante ferramenta para o entendimento da presença de anticorpos salivares específicos contra estreptococos orais encontrados, em recém nascidos, logo no primeiro dia de vida (Borges et al., 2015). Os resultados de uma pesquisa recente com gestantes do presente estudo (Mendes et al., 2018) permitiram observar a presença de *S. mutans* em amostras de sangue do cordão umbilical mostrando que este micro-organismo pode ser detectado no ambiente intrauterino e ainda, que hábitos de higiene oral em mais de 3 vezes ao dia podem colocar *S. mutans* na circulação pela permeabilidade da gengiva materna. Por outro lado, a maioria das amostras de colostro destas mesmas gestantes não apresentaram *S. mutans*, apesar de serem altamente colonizadas pela bactéria (Silva et al., 2019).

A produção de IgA específicos a determinados antígenos derivados de fontes alimentares e ambientais inicia-se antes do nascimento (Shah e Bapat, 2006). Foram encontrados IgA em salivas e mecônio de neonatos no primeiro dia de vida contra antígenos de *E. coli* (Mellander et al., 1986). Uma vez que o mecônio é formado a partir da 16ª semana de gestação e representa uma coleção de restos de células e secreções, bem como da ingestão de líquido amniótico, estes anticorpos são próprios do recém-nascido. Estudos prévios

revelaram que crianças recém nascidas apresentam anticorpos IgA salivares contra *S. mutans* e seus antígenos de virulência, independentemente da presença da bactéria na saliva da criança (Borges et al., 2015), o que sugere a presença de *S. mutans* no líquido amniótico, daí a importância da presente investigação.

As citocinas são peptídeos secretados pelas células da resposta imunológica inata e adaptativa e são as mediadoras de muitas funções dessas células (Kummar & Shafi, 2004) podendo ser produzidas durante a fase de ativação e fase efetora da imunidade para mediar e regular a resposta inflamatória e imunitária (Gallin et al., 1999). Estes peptídeos são liberados em resposta a micro-organismos e outros antígenos agressores, e atuam nas respostas envolvidas na inflamação e na imunidade do indivíduo. As diferentes citocinas podem ser enquadradas em diversas categorias, os interferons, interleucinas, fator estimulador de colônias (CSF), fator de necrose tumoral (TNF-α e TNF-β) e fator de crescimento (TGF-β). Podem ser pró e anti-inflamatórias, sendo que o desfecho final de um processo inflamatório dependerá do balanço entre elas. As citocinas pró-inflamatórias estimulam a inflamação, diretamente ou pela produção de outras citocinas, estando presentes em níveis elevados no sangue e soro durante a inflamação, sendo elas, a IL-1, IL-2, IL-6, IL-8, IL-12, TNF-α e IFN-γ (Meirovitz et al., 2010). As anti-inflamatórias: IL-4, IL-10 e IL-13 atenuam a inflamação pela restrição da produção de citocinas inflamatórias e aumentam a expressão dos receptores solúveis (Sarrouh et al., 2007).

A produção da IL-6 pode ser induzida facilmente por diversos estímulos como infecção viral, antígenos bacterianos, entre outros (Van damme et al.,1987; Fong et al., 1989; Ueno et al., 1989). Estudos em monócitos de neonatos mostraram que estímulos microbianos desencadeiam a produção de IL-6 por estas células em níveis similares aos produzidos por indivíduos adultos (Matsuzaki et al., 1990; Saito et al., 1990; Yachie et al., 1990). Estudos recentes mostram altos níveis de IL-6 no sangue do cordão umbilical em recém-nascidos com sepse neonatal precoce, cujas mães tiveram corioamnionite (Yoon et al., 2000; Naccasha et al., 2001, Chiesa et al., 2003). Sendo assim, a IL-6 é um mediador inflamatório neonatal importante. Além disto, um estudo de Redwine e colaboradores (2000) demonstraram que crianças de 8 a 9 anos, que sofreram algum tipo de estresse apresentam aumento dos níveis de IL-6 na saliva se comparadas com aquelas não submetidas a estresse. Perda de sono parece estar associada com a diminuição nos níveis noturnos de IL-6, o que poderia resultar em alterações do sistema imunológico (Redwine et al., 2000).

A IL-10 é produzida pelos linfócitos B e T “helper” tipo 2 (Th2) e pelos monócitos e possui uma função inibitória da proliferação dos linfócitos T “helper” tipo 1 (Th1) e da

produção de IFN-γ e IL-2, IL-1β, IL-6, IL-8, IL-12 e TNF-α. A função dos macrófagos como células apresentadoras de antígenos também é inibida pela IL-10 por meio da diminuição da expressão dos antígenos do complexo de histocompatibilidade de classe II, da molécula de adesão intercelular tipo 1 e da expressão do CD23. O aumento nos níveis de IL-10 encontrados no plasma e sangue do cordão umbilical de recém-nascidos prematuros avaliados para sepse foi associado à mortalidade, sendo considerado como um dos primeiros indicadores de prognóstico de sepse (Romagnoli et al., 2001; Cancelier et al., 2009).

Um estudo prévio mostrou que neonatos apresentam níveis de interleucinas tituláveis em salivas sem evidências de detecção de micro-organismos que pudessem estimular uma resposta imune própria (Sesso et al., 2014). Os níveis médios e a frequência de detecção de citocinas IL-6 e IL-10 em amostras salivares de recém nascidos foram significativamente maiores ao nascer do que após 3 meses de vida, especialmente em prematuros (Sesso et al., 2014), o que pode sugerir então que sejam derivadas no líquido amniótico.

Os dados encontrados até o momento mostram que o recém nascido possui anticorpos contra *S. mutans* circulantes derivados do sangue do cordão umbilical (Sesso et al., 2017) e salivares (Borges et al., 2015), que representam uma estimulação local. No entanto, há necessidade de se investigar o influxo de *S. mutans* através do líquido amniótico, que por sua vez, podem desempenhar um papel fundamental na estimulação ativa do sistema imune de mucosa do descendente.

A investigação da presença de *S. mutans* e citocinas no LA poderia ser uma importante ferramenta para o entendimento da presença destes componentes salivares encontrados em recém nascidos logo no primeiro dia de vida. Somados a isto, a abordagem sobre a investigação precoce sobre os agentes etiológicos associados a cárie é de interesse mundial, já que esta doença continua sendo um grande ônus para a saúde pública de bebês, pré-escolares e crianças que frequentam escolas primárias nos países desenvolvidos e menos desenvolvidos, sendo o maior ônus suportado por crianças das comunidades desfavorecidas (Kemoli et al., 2013). A cárie na primeira infância é o termo usado para descrever a cárie dentária em crianças com 71 meses ou menos (Zafar et al., 2009). É considerada uma doença com alta incidência entre as condições crônicas da infância e é cinco vezes mais frequente que a asma (Bagramian et al., 2009). Diante do exposto, a hipótese do presente estudo é a de que puérperas com alta experiência de cárie e níveis detectáveis de SM salivares possam apresentar mais frequentemente a bactéria no LA e níveis mais elevados de IL-6.

2. OBJETIVOS

O objetivo geral do presente estudo foi o de investigar a associação entre a detecção de SM e de interleucinas (IL-6 e IL-10) no LA de mulheres que tiveram gestações sem intercorrências e a presença de SM na saliva e dados clínicos. Os objetivos específicos incluíram:

- Avaliar a presença de SM no LA e na SA
- Associar a presença de SM nas SA e LA com informações coletadas em questionários;
- Correlacionar a presença SM nas SA e LA com a experiência de cárie materna;
- Comparar os níveis de DNA do SM detectado no LA e SA;
- Quantificar os níveis de IL-6 e IL-10 e comparar entre as gestantes com SM detectável no LA e SA.

3. CAPITULO 1 - ARTIGO CIENTÍFICO

Segundo as Normas da *The Journal of Maternal-Fetal & Neonatal Medicine* (ANEXO 1)

Presence of Streptococcus mutans and interleukin-6 and 10 in amniotic fluid

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Running Title: *S. mutans and cytokines in amniotic fluid*

ABSTRACT

The gestational period represents an important moment for the mother and child, since maternal exposure to microbial antigens can determine colonization and fetal immune development. The oral cavity represents a gateway for numerous bacterial species. Those bacteria can cause important oral diseases, such as dental caries, in which *Streptococcus mutans* (MS) being the main microorganism involved in its etiology. Previous studies have shown that neonates have antibodies to MS in their saliva in the absence of detectable levels of this microorganism, hence the need to investigate antigenic exposure in intrauterine life. The aim of the present study was to evaluate the presence of MS and IL-6 and IL-10 in samples of Amniotic Fluid (AF) and to associate with clinical data. The study involved the participation of 26 women with term deliveries and without gestational complications. General health data were collected through a questionnaire applied to the participants. The volunteers were examined orally, the DMFT was calculated and unstimulated saliva (SA) was collected. The acquisition of LA was performed at the Obstetric Center of MPHU during cesarean sections and stocked on ice. The collected samples (SA and AF) were sent to the Molecular Biology Laboratory of UNIUBE for laboratory analysis. The analysis of the presence of SM-DNA was performed by real-time PCR assays with specific primers. Levels of IL-6 and IL-10 were performed by ELISA with specific kits for these interleukins in the AF samples. The results showed that 69.2% of the AF samples had detectable MS, and 65% of the women had the bacteria in the LA and SA at the same time. There was a positive association between the presence of MS in AF and a report of not going to the dentist frequently ($p < 0.05$). There was no statistically significant difference between the frequency of AF with or without detection of MS and active caries ($p > 0.05$). However, the DMFT scores of women with positive AF for MS were higher than that of women without detectable MS ($p<0.05$). IL-6 levels were higher than IL-10 in AF ($p<0.05$). There were no significant differences in the levels of interleukins between samples with detectable MS or not in AF and not even with clinical data analyzed ($p>0.05$). The data collected allowed us to conclude that the AF has SM in quantifiable levels, probably associated with the greatest caries experience, and seen by the highest DMFT index. Interleukin levels were not related to the presence of MS. The presence of MS in AF may explain the mucosal immune stimulation of newborn children by the previous detection of salivary antibodies against MS.

Keywords: *Streptococcus mutans*, amniotic fluid, caries

Introduction

Childhood caries is a common infectious disease that remains one of the most common morbidities worldwide and may lead to several problems of public health for children due to lost of tooth and malocclusion, malnutrition, phonetic issues, low self-esteem and possible systemic interactions. The severity of this disease impacts the oral health-related quality of life of preschool children (Firmino et al., 2016). Brazil has a population highly exposed to *S. mutans*, in which children, with 5 to 11 months of age, have high levels of *S. mutans* in saliva (Alves et al., 2009). So, early prevention procedure should be used immediately after birth, especially against colonization by *S. mutans*, which is considered the primary etiological agent of dental caries (Loesche et al., 1975) due to its ability to adhere and to accumulate on tooth surface, forming the dental biofilms.

The mucosal immune response is the important source of oral protection; there are evidence that salivary IgA against antigens of *S. mutans*: Glucan binding protein B, glicosyltransferase C and Antigen I/II is associated to modulation of *S. mutans* infection, because high level of IgA reactive to those antigens were found in children with *S. mutans* undetectable in the oral cavity at 5-11 months of age (Nogueira et al., 2005). Detectable levels of salivary IgA antibodies reactive to *S. mutans* were detected in children within the first hours after birth (Nogueira et al., 2012), before the first breastfeeding. Furthermore, the salivary IgA antibody specificities to *S. mutans* virulence antigens appear to be influenced by the newborn gestational age at birth (Borges et al., 2015), which might reflect the level of immunological maturity of the mucosal immune system.

In order to clarify the role and development of mucosal immune response in neonates, these findings supported further study about the investigation of those mucosal antibodies and the microbial sources of stimulation from mother and intra uterine environment. The analysis of perinatal and intrauterine exposition to *S. mutans* needs to be investigated. Recently, few samples of colostrum presented *S. mutans* (Silva et al., 2019), excluding the immediate immune stimulation by the breastfeeding. The analysis of umbilical blood showed that the majority of

samples analyzed had *S. mutans* detectable, showing that it can be transferred during intrauterine life by the blood (Mendes et al., 2018). Several other microorganisms had been detected in the placenta, umbilical cord blood, amniotic fluid and meconium in term birth, without any signal of infection (Aasparg et al., 2014). These findings have fundamentally changed the understanding of gut microbiota acquisition and its role in human development and the traditional view of human microbiome acquisition of "sterile womb paradigm" and supports studies about the "in utero colonization hypothesis" (Perez-Munhoz et al., 2018). Particularly in relation to bacteria colonizing the placenta, evidence has been presented suggesting hematogenous spread of organisms from the mother to the amniotic cavity (Aasparg et al., 2014; Han et al., 2006; Fardini et al., 2010). Recent study with sequencing of humans' placentas showed that the microbiome of those samples were more similar to normal oral microbiota, especially the tongue and tonsil, than the vaginal or intestinal microorganisms, as previously described (Aasparg et al., 2014).

Cytokines are peptides secreted by cells of immune response to regulate the activation and effector phases of inflammatory response (Clapp, 2006). Proinflammatory cytokines, such as: IL-6, IL-8, IL-12, IFN-γ, stimulates the inflammation (Meirovitz et al., 2010), while the anti-inflammatory, IL-10, attenuates the inflammation by restricting the production of inflammatory cytokines and increase expression of soluble receptors (Sarrouh et al., 2007). These peptides can be released in response to microorganisms (Rang et al., 2001) and are important in controlling the response to antigenic challenges. Studies focused the analysis of cytokines in samples of amniotic liquid (Holst et al., 2005; Jacobsson et al., 2005), showing a positive correlation between the increases of proinflammatory cytokines, particularly IL-6 with prenatal intrauterine infection (Holst et al., 2005) and preterm birth (Holst et al., 2005; Jacobsson et al., 2005). A previous study showed that neonates have detectable interleukin levels in saliva without evidence of detection of microorganisms (such as *S. mutans* and *S. mitis*) that could stimulate their own immune response (Sesso et al., 2014), suggesting that those cytokines were remain of amniotic fluid.

Since amniotic fluid is a biofluid composed of several molecules that pass through the fetal membrane from the maternal plasma, it is swallowed during intrauterine life and the placental microbiome resembles the oral (Aaspard et al., 2014) so there is a need to investigate the immunological components and microbiological effects of this biofluid that are associated with the oral development of the neonate. Here, we investigated the presence of *S. mutans* and IL-6 and IL-10 in the amniotic fluid from voluntaries with term gestation and associated with clinical oral data.

Material and Methods

Thirty-nine healthy women, who were admitted at term to the Department of Obstetrics at the Mario Palmeiro University Hospital after a clinically normal pregnancy, were enrolled in the study. Clinical records were collected and examined in order to verify that the criteria were fulfilled. Inclusion criteria were: healthy mothers without alcohol consumption and smoking, no complications during pregnancy and delivery, full term deliveries (above 39 weeks of gestation), without using medications, such as: corticosteroids, antibiotics and anti-inflammatories. Informed consent was obtained from all the patients according to the protocol approved by the Committee for Ethical Issues in Human Research at the University of Uberaba (ANEXO 2 - CAAE 62821616.1.0000.5145). Informed written consent was sought for their participation in the study. General demographic details were collected from the women through a previously developed administered questionnaire. A total of 26 voluntaries were eligible to participate in the study. Thirteen did not participate of study because gave up continuing (n=3), started using antibiotics or anti-inflammatories (n=5), were edentulous (n=2), smokers (n=3).

Questionnaires collected information during the gestation period including age, level of education (primary, secondary, high school and university), general health (yes or no), dental visit frequent (yes or no), brushing frequency (one to two or more than 3 times in one day), dental treatment

during the gestation (yes or no), smoking (yes or no), alcohol consumption (yes or no), treatment/medication (yes or no).

Samples collections

On the day before caesarean section unestimulated saliva samples and Amniotic Fluid (AF) was taken from women attending for elective caesarean section. Amniotic fluid samples were obtained after spontaneous rupture of the membranes or by transvaginal amniotomy. The fluid was transported in ice in a sterile polystyrene tube to the laboratory where the fluid was divided into aliquots. A portion of the amniotic fluid was sent to the research laboratory for microbiologic evaluation. The remaining fluid was immediately centrifuged at 500g to 800g for 10 min with the supernatant stored at - 70 ° C for future evaluation of cytokine concentrations.

Dental Caries Assessment

The results of the clinical dental examination were recorded employing the decayed, missing, and filled teeth (DMFT) index, where “D” (decayed) denotes the present number of teeth with untreated and active caries, “M” (missing) expresses how many teeth are missing and have been extracted due to caries, and “F” (filled).

Detection of *S. mutans* in the samples

DNA was extracted with the PowerLyzer PowerSoil DNA Isolation Kit (MO-BIO, Carlsbad, CA, USA) according to the manufacturer’s instructions. Firstly, the samples were incubated with 20 mg ml^{-1} lysozyme (Sigma, Tokyo, Japan) solution; containing 50 mM Tris-HCL (Sigma, pH 8.0) and 20 mM EDTA (Sigma), and incubated at 37^{o}C for 30 min. Samples were immediately transferred to eppendorf tube containing bead Solution. After were vortexed for 2 min to ensure the release of bacteria to the suspension, which was then transferred to a PowerLyzer Glass Bead Tube (MOBIO). The concentration of the purified PCR product was measured with a NanoDrop 2000 spectrophotometer (Thermo Scientific). Sm479F/R primer pair (Sm479F: 5′-

TCGCGAAAAAGATAAACAAACA-3′ and Sm479R: 5′-GCCCCTTCACAGTTGGTTAG-3′) that is highly specific and sensitive for identification of *S. mutans* in mixed DNA samples was purchased from Invitrogen (Tokyo, Japan). The StepOne™ Real-Time PCR System (Thermo Fisher Scientific) performed quantitative-PCR. Each reaction tube contained reaction mixture, including 6.5 µL SYBR Green Master Mix (Roche, Ilhois, USA), 1µL of each primer, 4.5 µL de ultrapure water e 2µL of DNA extracted from samples. The cycling conditions were 45 cycles of 15 s at 94°C for denaturation, 30 s at 56°C for annealing, and 30 s at 72°C for extension followed by a melting curve analysis of the qPCR product. Two qPCR reactions were performed for all samples. To positive control was used DNA from *Streptococcus mutans* (UA159).

Quantification of Cytokines Using Elisa

Amniotic Fluid concentration of IL-6 and IL-10 were determined by ELISA commercial kits (Sigma) in according to the manufacturer's instructions. All tests were performed in triplicate with samples diluted 1:200. The ranges of the sensitivity standard curve of the ELISA kits were 4.7–150 pg/mL for IL-10 and 1.4-1340 pg/mL for IL-6.

Statistical Analysis

Analyses were performed using a computer statistics program (SPSS 13.0, SPSS Inc. Chicago, IL, USA). Detection frequencies were compared using Chi-square analysis or Fisher Exact test. Differences in the fluorescence intensity, levels of interleukin and DMTF were calculated by ANOVA with *post hoc* Bonferroni's analysis. The correlation between SM DNA detection and data of interview and caries experience were tested by Pearson analysis. A P value <0.05 was considered statistically significant.

Results

In total, 26 women with singleton pregnancy were admitted in the study. The ethnicity mix comprised 14 Caucasian, 4 Asian and 8 Afro women with a mean age of 27 years. The mean gestation period was 39.3 weeks and the mean weight at delivery was 3656 grams. All were singleton births with 13 males and 13 females. None women were primigravidae, smoking, alcohol consumption, treatment/medication during the gestation. No evidence of infective morbidity was found among the newborns.

Detection of SM in AF and salivas

Firstly, bacterial DNA was detected by 16S rDNA directed real time PCR, with either one or both sets of universal primers used, in 21/26 of the amniotic fluid samples. SM was detected in the AF of 18/26 of patients (Table 1). Twelve of 26 of patients demonstrated infection with SM DNA in both AF and saliva, but the difference was not significant (Figure 1, $p>0,05$).

No association was found between the frequencies of women with detection of SM DNA in AF as regards age, level of education, general health, frequent dental visits (Table 1, $p>0.05$). On the other hand, women that related to no visit frequently the dentist have more samples of amniotic fluid with microbial DNA detectable (Table 1, Fisher Exact test, $p=0.007$).

Levels of DNA SM in AF and salivas

The mean intensity of fluorescence emitted in CT (in unity of fluorescence) of SM DNA detected in the AF and in salivas were 205481 (SD:142751) and 182193 (SD:47154) respectively ($p>0.05$). Comparison between groups of SM DNA detected or not in AF showed that there were a statistically difference in the levels of fluorescence emitted in samples of salivas, because these levels were higher in women with *S. mutans* detected in the amniotic fluid (Figure 2, $p<0.05$).

Caries Experience and SM DNA detection in saliva and AF

The presence of active caries were found in 38.5% (10/26) and *S. mutans* detectable in saliva in 57.7% (15/26) of women. The prevalence of the presence of both caries and *S. mutans* in saliva, caries alone, and *S. mutans* alone was 23.1% (6/26), 15.4% (4/26), and 34.6% (9/26), respectively. In addition, 26.9% (7/26) of women did not have both caries and *S. mutans* in saliva. There were no difference in the detection of active caries between women with SM DNA detection or nor in the AF (Figure 3 $p>0.05$).

The experience of caries represented by DMFT scores was from 3 to 20. Figure 4 presents the DMFT scores of each woman by detection of SM DNA in the amniotic fluid. The mean of DMFT of women with SM DNA detected in AF was the double of values found in women with SM no detected in AF (10.5 ± 4.8 vs 5.1± 2.3, $p=0.0073$). There were positive correlation between DMFT and Intensity of fluorescence of SM DNA detection in AF and saliva ($p<0.05$ and $r>0.39$).

Cytokines concentrations

The distributions of cytokine levels in AF samples of mothers with SM DNA detection or not in are shown in Figure 5. All samples of AF presented IL-6 and IL-10. The mean levels of IL-6 showed were significantly higher than IL-10 in the AF ($p<0.05$; mean: 1795,54 ± 1918,23 vs 55,04 ± 41,67). Comparison of cytokine levels in the groups of women with SM DNA detected in the AF or not had no significant difference for both cytokines (Figure 5, $p>0,05$). Pearson correlation reveals no correlations between IL-6 and IL-10 and DMFT and clinical data ($p>0.05$, $r<0,01$).

Discussion

This study investigated the presence of genetic material of SM in amniotic fluid from mothers at delivery and compared with clinical data and levels of interleukin 6 and 10 and caries experience.

The results showed that SM DNA can be detected in the majority of AF samples (n=18, 69.2%), but there were not a positive correlation in the detection between detection of SM in saliva and active caries. On the other hand, the presence of SM in the AF was related to frequent visit to the dentist and caries experience.

In this study, 80% of the AF samples were found to be positive for microorganisms by using 16S rDNA PCR, which contributes to the hypothesis that the uterine cavity has a normal flora. The presence of SM in AF corroborates the findings of recent studies, in which several microorganisms could be isolated in samples of umbilical cord blood, amniotic fluid, and in the placenta with no clinical or histological evidence of infection or inflammation in the mother-child pairs (Stout *et al.*, 2013; Aagaard *et al.*, 2014) and refute the idea that the intrauterine environment is free of microorganisms and that fetuses acquire microorganisms only when they started transit through the vaginal canal and subsequently through contact with maternal skin (Mackie *et al.*, 1999). Metagenomic analysis of placental specimens has revealed the existence of a microbiome in the placenta with taxonomic profiles similar to those described in the oral environment, where periodontopathogenic bacteria are relatively abundant in placentas of healthy pregnancies (Aagaard *et al.*, 2014; Prince *et al.*, 2014).

The results in this study indicate that the majority of samples (69,2%) have *S. mutans* found in the AF as like were detected previously (Bearfield et al., 2002). Although in the case of *S. mutans*, absence from the saliva, 65% of voluntaries had the bacteria in both samples, showing the bacteria in AF may have an oral origin. Several routes of bacterial access to the placenta, including ascension from the lower genital tract, entry through the mother's bloodstream, or active transport of microbes by immune cells from the gut or oral cavity (Perez-Munhoz et al., 2018). Although that the placenta is considered a barrier to protect the fetus from microbial pathogens that invade the blood stream of the mother (Robbins & Bakardjiev., 2012).

Admittedly, SM is a common microorganism in the oral microbiota and is associated with the formation of dental caries. The earliest colonization frequently observed in the Brazilian babies (Alves et al., 2009) and the presence of salivary IgA against SM from infants at birth (Borges et al., 2015) can be due to exposition and swallowing of AF during intrauterine life.

Data collected from oral health and hygiene habits in the interview showed that number of brushings and dental treatment during the gestation did not associate to the presence of SM in the LA. On the other hand, women that frequented regularly the dentist office had less to have bacteria in the AF; i.e, 83% of women with SM detected in the AF did not frequent the dentist regularly.

The fluorescence obtained in CT represents the levels of DNA found in the samples. The results show that salivas have higher levels of SM than AF, which is expected, because the oral cavity is the environment of SM. Besides this women with detectable SM in AF had higher levels of bacteria in saliva. Comparison of clinical exams, especially the detection of actives caries and calculate of DMFT, with the detection of SM in the AF showed that the presence of active caries was not related to the detection of SM in the AF, but the experience of caries, by the DMFT score was correlated positively with detection of bacteria in the AF.

Although IL-6 can be induced by various stimuli, such as bacterial antigens, viral infections, and many others (Van Damme et al., 1987; Fong et al., 1989; Ueno et al., 1989), the results did not allow to relate the presence of IL-6 and IL-10 with the detection of SM in the AF, since there was no statistically significant difference ($p > 0.05$) between the groups of detectable SM or not, suggesting that this bacterium is not capable of inducing a significant inflammatory response in AF.

It is well established that intra-amniotic infection or intra-amniotic inflammation is associated with adverse pregnancy and neonatal outcomes (Romero et al., 2014; Lee et al., 2007; Combs et al., 2014). Amniotic fluid IL-6 has been traditionally considered as the markers for the identification

of intra-amniotic inflammation (Lee et al., 2007; Romero et al., 1993; Jun et al., 2000). However, patients with intra-amniotic inflammation without detectable microorganisms have similar outcomes to those with intra-amniotic inflammation associated with the presence of microorganisms (Romero et al., 2014; Lee et al., 2007; Combs et al., 2014; Chaemsaithong et al., 2016). Therefore, a key issue in determining outcome is the presence or absence of inflammation. Interestingly the interleukin levels found are consistent with those presented by Sesso et al (2014), who demonstrated a concentration of IL-6 higher than IL-10 in saliva from babies at birth.

In conclusion, the present results reinforces the theory that there are bacteria or part of them circulating in the intra uterine life, without causing an early interruption of gestation. This study opens a range of discussions on the transfer mechanism of oral microorganisms detectable between mother and fetus, which could contribute to installation of these bacteria in the newborn or even stimulate a mucosal immune response against its installation. SM was detected in a several samples of AF especially in women with high caries experience.

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Conflict of interest

The authors declare no conflict of interest.

FIGURES

Figure 1. Number of samples with detected SM DNA or not in salivas of women with microbial DNA detected or not in the amniotic fluid

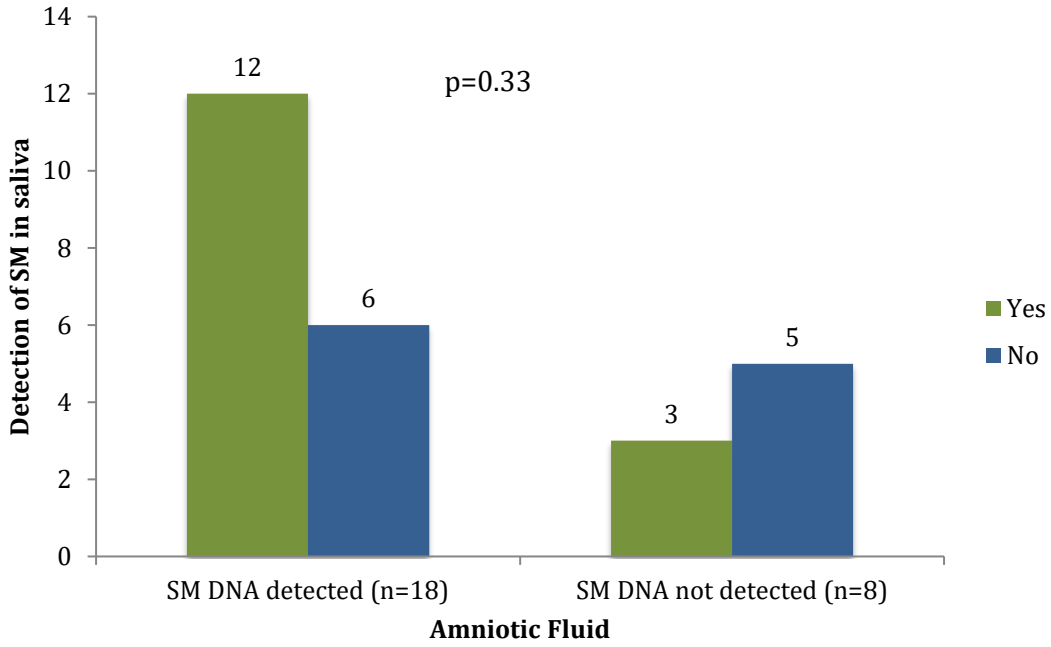


Figure 2. Intensity of fluorescence obtained in salivas samples of mothers with Microbial DNA detection in the AF (amniotic Fluid) or not.

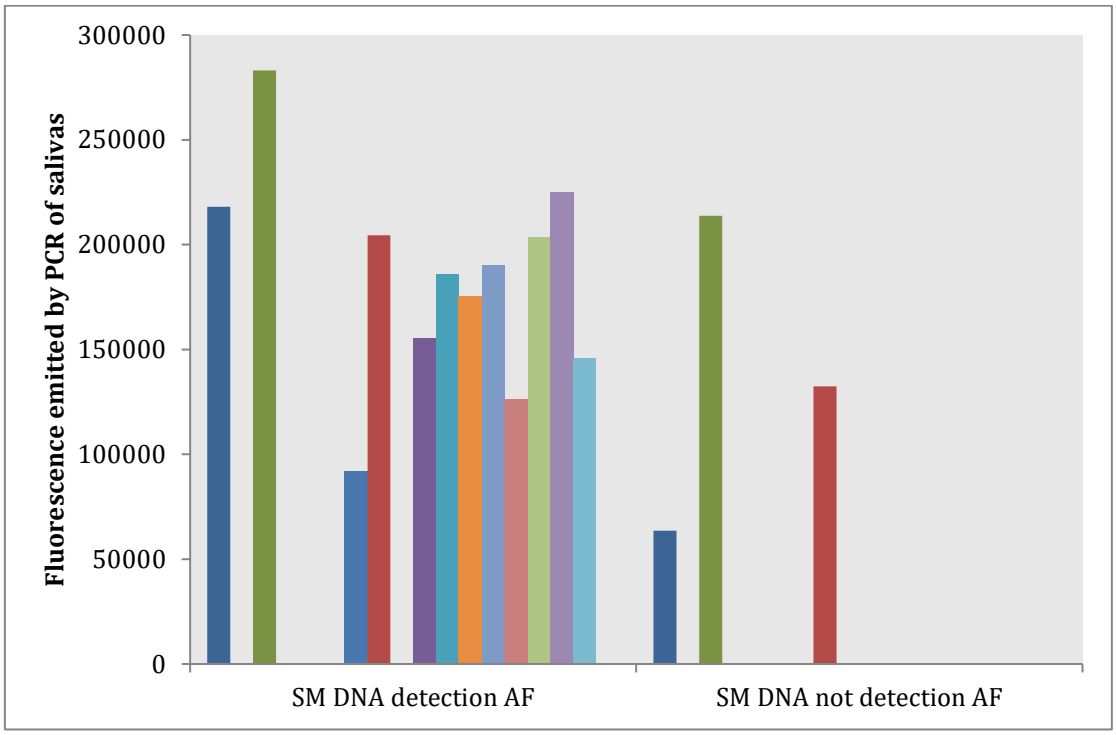


Figure 3 Number of samples with detected actives caries or not of women with microbial DNA detected or not in the amniotic fluid

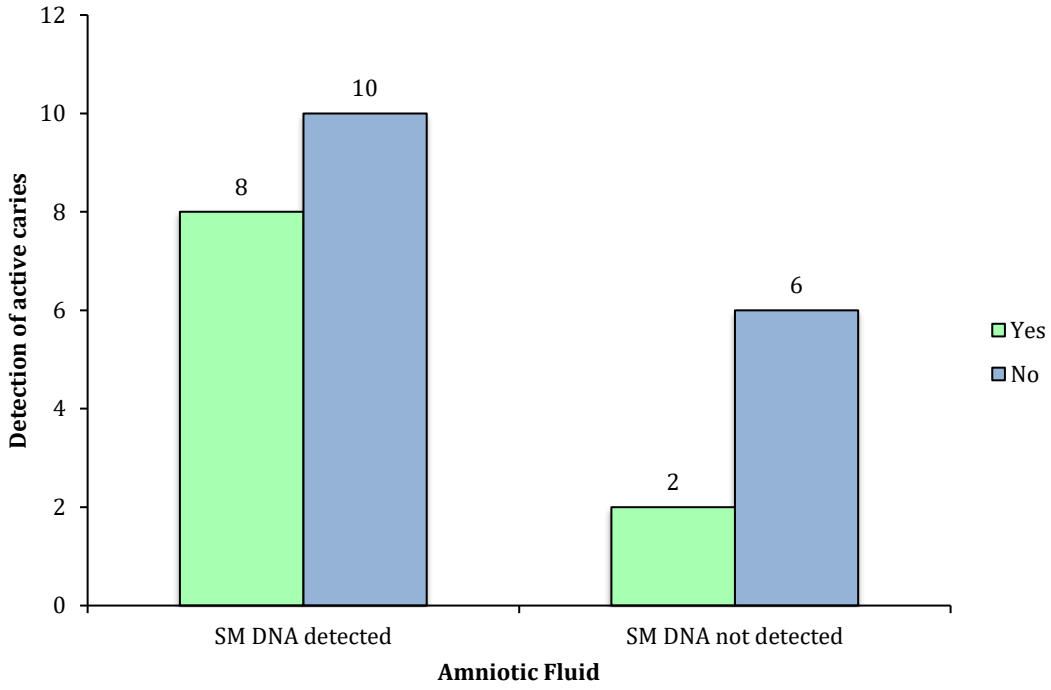


Figure 4 . Distribution of decayed, missing, and filled teeth (DMFT) by detection of SM in the samples of amniotic fluid.

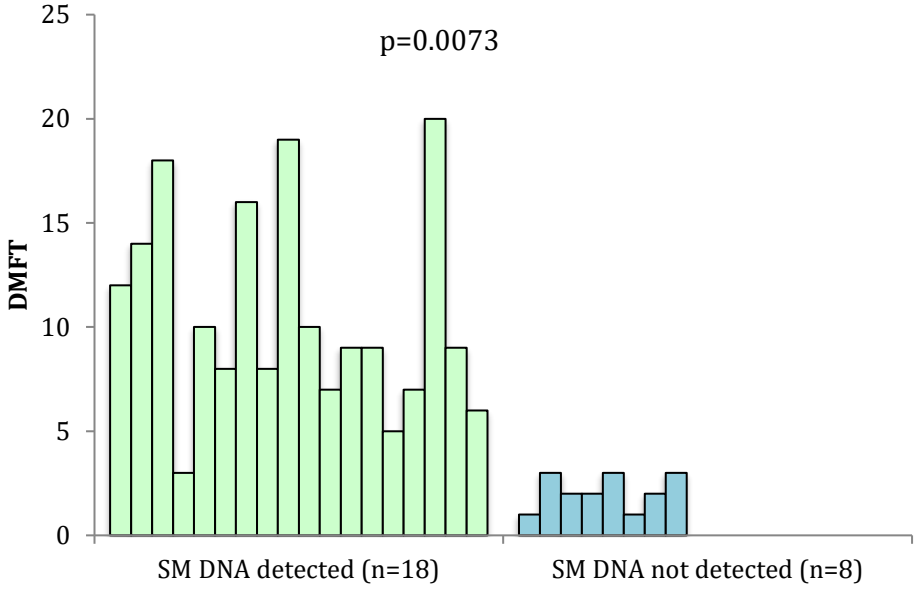
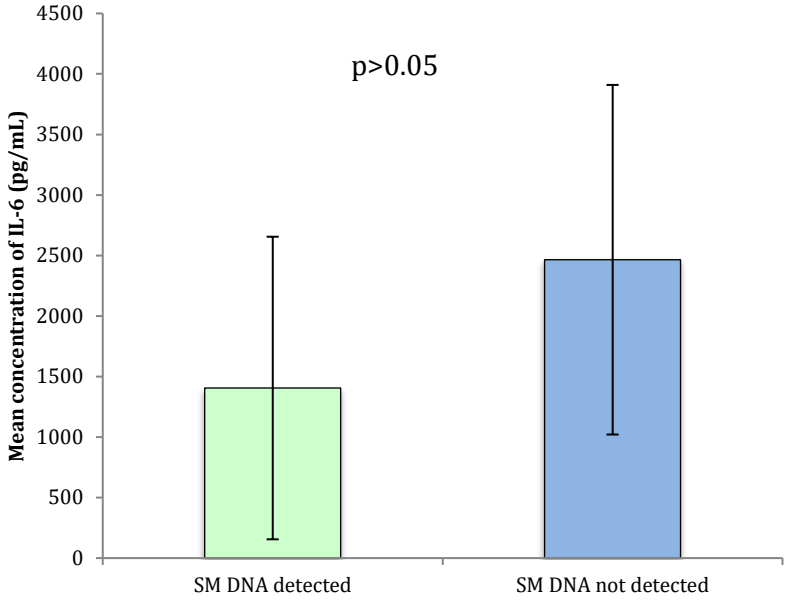
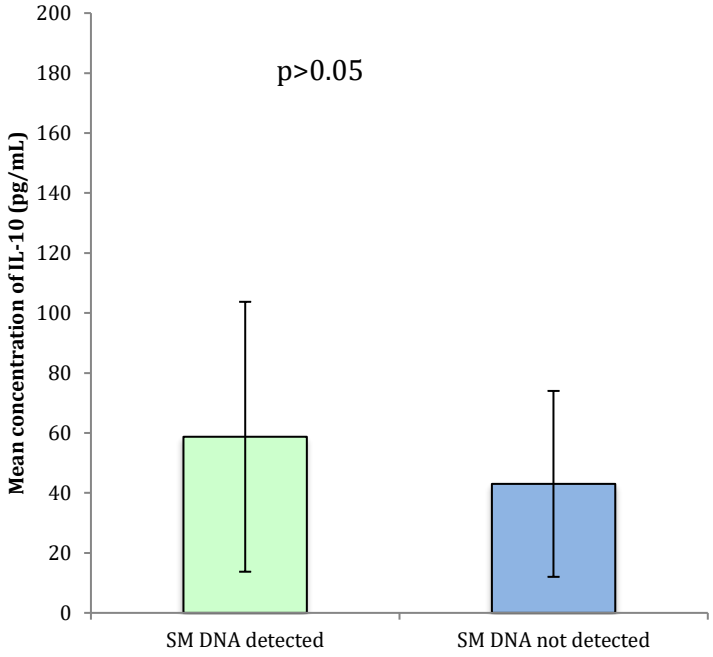


Figure 5. Levels of Interleukin 6 and 10 in samples of AF with SM DNA detected or not in the amniotic fluid





TABLE

Table 1. Demographic and clinical characteristics of the women with respect to the presence and the absence of Microbial DNA detected in the amniotic fluid

	Amniotic Fluid		***p-value***
Parameter Recorded	SM DNA detection (n = 18)	SM DNA not detection (n=8)	
Race/Ethnicity Cohort			
no Caucasian, n (%)	8 (45)	4 (50)	0.86
Caucasian, n (%)	10 (55)	4 (50)	
***Mean Maternal Age** (SD)*	28.1 (6.7)	22.5 (4.5)	0.06
Levels of education			
Primary /Secondary, n (%)	5 (28)	2 (25)	
High School, n (%)	10 (56)	5 (63)	0.93
University, n (%)	3 (16)	1 (12)	
Dental visit frequent			
Yes, n (%)	3 (17)	6 (75)	0.007
No, n (%)	15 (83)	2 (25)	
Dental Treatment during the gestation			
Yes, n (%)	2 (11)	0 (0)	0.55
No, n (%)	16 (89)	8 (100)	
Number of brushing times in one day			
1 to 2 (n) %	10 (56)	5 (63)	1.00
over 3 (n) %	8 (44)	3(37)	

References

1. AAGAARD KM, 2014 Author Response To Comment On "The Placenta Harbors A Unique Microbiome". Sci Transl Med 254: 17-16.

2. ALVES AC, Nogueira Rd, Stipp Rn, Pampolini F, Moraes Ab, Gonçalves Rb, Höfling Jf, Li Y, Mattos-Graner Ro, 2009. Prospective Study Of Potential Sources Of Streptococcus Mutans Transmission In Nursery School Children. J Med Microbiol 58: 476-481.

3. BEARFIELD C, Davenport Es, Sivapathasundaram V, Allaker Rp, 2002. Possible Association Between Amniotic Fluid Micro-Organism Infection And Microflora In The Mouth. Bjog 109: 527-533.

4. BORGES MC, Sesso Ml, Roberti Lr, De Menezes Oliveira Ma, Nogueira Rd, Geraldo-Martins Vr, Ferriani Vp, 2015. Salivary Antibody Response To Streptococci In Preterm And Fullterm Children: A Prospective Study. Arch Oral Biol 60: 116-125.

5. CHAEMSAITHONG, Piya Et Al. A Point Of Care Test For Interleukin-6 In Amniotic Fluid In Preterm Prelabor Rupture Of Membranes: A Step Toward The Early Treatment Of Acute Intra-Amniotic Inflammation/Infection. The Journal Of Maternal-Fetal & Neonatal Medicine, V. 29, N. 3, P. 360-367, 2016.

6. CLAPP, D.W., 2006. Developmental Regulation Of The Immune System. Semin. Perinatol. 30, 69-72.

7. COMBS CA, Gravett M, Garite Tj, Et Al. Amniotic Fluid Infection, Inflammation, And Colonization In Preterm Labor With Intact Membranes. Am J Obstet Gynecol 2014;210:125.E1–E15

8. FARDINI Y, Chung P, Dumm R, Joshi N, Han Yw, 2010. Transmission Of Diverse Oral Bacteria To Murine Placenta: Evidence For The Oral Microbiome As A Potential Source Of Intrauterine Infection. Infect Immun 78: 1789-1796.

9. FIRMINO RT, Gomes Mc, Clementino Ma, Martins Cc, Paiva Sm, Granville-Garcia Af 2016. Impact Of Oral Health Problems On The Quality Of Life Of Preschool Children: A Case-Control Study. Int J Paediatr Dent 26: 242-249.

10. FONG TC, Babbitt Jt, Bloom Et. Increase In Tac Mrna Expression In Natural Killer-Like Cell Line In Response To Interleukin-1 And Interleukin-2 Shown At The Population And Cellular Level By In Situ Hybridization. Nat Immun Cell Growth Regul. 1989;8(3):165-72.

11. HAN YW, Ikegami A, Bissada Nf, Herbst M, Redline Rw, Ashmead Gg. Transmission Of An Uncultivated *Bergeyella* Strain From The Oral Cavity To Amniotic Fluid In A Case Of Preterm Birth. *J Clin Microbiol*, 2006. **44**(4):1475–83. Doi:10.1128/Jcm.44.4.1475-1483.(2006).

12. HOLST, R.M., Mattsby-Baltzer, I., Wennerholm, U.B., Hagberg, H., Jacobsson, B., 2005. Interleukin-6 And Interleukin-8 In Cervical Fluid In A Population Of Swedish Women In Preterm Labor: Relationship To Microbial Invasion Of The Amniotic Fluid, Intra-Amniotic Inflammation, And Preterm Delivery. Acta Obstet. Gynecol. Scand. 84, 551-557.

13. JACOBSSON, B., Mattsby-Baltzer, I., Hagberg, H., 2005. Interleukin-6 And Interleukin-8 In Cervical And Amniotic Fluid: Relationship To Microbial Invasion Of The Chorioamniotic Membranes. Bjog. 112, 719-724.

14. JUN JK, Yoon Bh, Romero R, Kim M, Moon Jb, Ki Sh, Et Al. Interleukin 6 Determinations In Cervical Fluid Have Diagnostic And Prognostic Value In Preterm Premature Rupture Of Membranes. Am J Obstet Gynecol. 2000; 183(4):868–73. Https://Doi.Org/10.1067/Mob.2000.109034 Pmid: 11035328.

15. LEE SE, Romero R, Jung H, Et Al. The Intensity Of The Fetal Inflammatory Response In Intraamniotic Inflammation With And Without Microbial Invasion Of The Amniotic Cavity. Am J Obstet Gynecol 2007;197:294.E1–6

16. LOESCHE WJ, Rowan J, Straffon Lh, Loos Pj, 1975. Association Of Streptococcus Mutants With Human Dental Decay. Infect Immun 11: 1252-1260.

17. MACKIE RI, Sghir A, Gaskins Hr, 1999. Developmental Microbial Ecology Of The Neonatal Gastrointestinal Tract. Am J Clin Nutr 69: 1035s-1045s.

18. MENDES, M. M. Et Al. Streptococcus Mutans In Umbilical Cord Blood, Peripheral Blood, And Saliva From Healthy Mothers. **Curr Microbiol**, V. 75, N. 10, P. 1372-1377, Oct 2018.

19. MEIROVITZ, A., Kuten, M., Billan, S., Abdah-Bortnyak, R., Sharon, A., Peretz, T., Sela, M., Schaffer, M., Barak, V., 2010. Cytokines Levels, Severity Of Acute Mucositis And The Need Of Peg Tube Installation During Chemo-Radiation For Head And Neck Cancer: A Prospective Pilot Study. Radiat. Oncol. 25, 5-16.

20. NOGUEIRA RD, Alves Ac, Napimoga Mh, Smith Dj, Mattos-Graner Ro.Characterization Of Salivary Immunoglobulin A Responses In Children Heavil Yexposed To The Oral Bacterium Streptococcus Mutans: Influence Of Specific Antigen Recognition In Infection. Infect Immun. 2005 Sep;73(9):5675-84.

21. NOGUEIRA, R.D., Sesso, M.L., Borges, M.C., Mattos-Graner, R.O., Smith, D.J., Ferriani, V.P., 2012. Salivary Iga Antibody Responses To *Streptococcus Mitis* And *Streptococcus Mutans* In Preterm And Fullterm Newborn Children. Arch. Oral. Biol. 57, 647-653.

22. PEREZ-MUÑOZ ME, Arrieta Mc, Ramer-Tait Ae, Walter Ja 2017. Critical Assessment Of The "Sterile Womb" And "In Utero Colonization" Hypotheses: Implications For Research On The Pioneer Infant Microbiome. Microbiome 28; 5(1):48.

23. PRINCE AL, Antony Km, Chu Dm, Aagaard Km, 2014. The Microbiome, Parturition, And Timing Of Birth: More Questions Than Answers. J Reprod Immunol 104: 12-19.

24. STOUT MJ, Conlon B, Landeau M, 2013. Identification Of Intracellular Bacteria In The Basal Plate Of The Human Placenta In Term And Preterm Gestations. Am J Obstet Gynecol 208: 226-227.

25. ROBBINS JR, Bakardjiev AI. Pathogens and the placental fortress. Curr Opin Microbiol. 2012;15:36–43.

26. ROMERO R, Yoon Bh, Mazor M, Gomez R, Gonzalez R, Diamond Mp, Et Al. A Comparative Study Of The Diagnostic Performance Of Amniotic Fluid Glucose, White Blood Cell Count, Interleukin-6, And Gram Stain In The Detection Of Microbial Invasion In Patients With Preterm Premature Rupture Of Membranes. Am J Obstet Gynecol. 1993; 169(4):839–51. Pmid: 7694463. 35.

27. ROMERO R, Miranda J, Chaiworapongsa T, Et Al. Prevalence And Clinical Significance Of Sterile Intra-Amniotic Inflammation In Patients With Preterm Labor And Intact Membranes. Am J Reprod Immunol 2014;72:458–74

28. SILVA CBD, Mendes Mm, Rodrigues Br, Pereira Tl, Rodrigues Dbr, Rodrigues Junior V, Ferriani Vpl, Geraldo-Martins Vr, Nogueira Rd. Streptococcus Mutans Detection In Saliva And Colostrum Samples. Einstein (Sao Paulo). 2019 Jan

29. SESSO Ml, Borges Mc, Ferriani Vp, Geraldo-Martins Vr, Rodrigues Db, Nogueira Rd. Prospective Evaluation Of Cytokine In Saliva Of Preterm And Fullterm Neonates. Immunobiology. 2014 Nov;219(11):830-5.

30. UENO Y, Hays Ef, Hultin L, Uittenbogaart Ch. Human Thymocytes Do Not Respond To Interleukin-2 After Removal Of Mature "Bright" Cd5 Positive Cells. Cell Immunol. 1989 Dec;124(2):239-51.

31. VAN DAMME J, Cayphas S, Van Snick J, Conings R, Put W, Lenaerts Jp, Simpson Rj, Billiau A. Purification And Characterization Of Human Fibroblast-Derived Hybridoma

Growth Factor Identical To T-Cell-Derived B-Cell Stimulatory Factor-2 (Interleukin-6). Eur J Biochem. 1987 Nov 2;168(3):543-50.

4. CONSIDERAÇÕES FINAIS e CONCLUSÕES

Os resultados do presentes estudo reforçam a teoria de que as bactérias orais ou parte delas circulam na vida intrauterina, sem causar interrupção precoce da gestação. Este estudo abre uma série de discussões sobre o mecanismo de transferência de microrganismos orais detectáveis entre mãe e feto, o que poderia contribuir para a instalação dessas bactérias no recém-nascido e estimular uma resposta imune da mucosa contra sua instalação, o que justifica a presença de IgA salivar contra SM logo após o nascimento.

A presença de SM foi detectada em várias amostras de Líquido amniótico independentemente da detecção de cárie ativa materna, mas esteve associada a uma maior experiência de cárie e menor frequência de controle da doença, devido a não visita periódica ao dentista.

Os níveis de citocinas não estiveram relacionados com a infecção por SM e nem mesmo com dados de saúde geral, hábitos ou exame clínico.

5. REFERÊNCIAS BIBLIOGRÁFICAS

1. AAGAARD KM, 2014 Author Response To Comment On "The Placenta Harbors A Unique Microbiome". **Sci Transl Med** 254: 17-16.
2. ALVES AC, Nogueira Rd, Stipp Rn, Pampolini F, Moraes Ab, Gonçalves Rb, Höfling Jf, Li Y, Mattos-Graner Ro, 2009. Prospective Study Of Potential Sources Of Streptococcus Mutans Transmission In Nursery School Children. **J Med Microbiol** 58: 476-481.
3. AZEVEDO MR, Menezes AM, Assunção MC, Gonçalves H, Arumi I, Horta BL, Hallal PC. Tracking of physical activity during adolescence: the 1993 Pelotas Birth Cohort, Brazil. **Rev Saude Publica**. 2014 Dec;48(6):925-30. doi: 10.1590/S0034-8910.2014048005313.
4. BAGRAMIAN RA, Garcia-Godoy F, Volpe AR. The global increase in dental caries. A pending public health crisis. **Am J Dent.** 2009 Feb;22(1):3-8.
5. BEARFIELD C, Davenport Es, Sivapathasundaram V, Allaker Rp, 2002. Possible Association Between Amniotic Fluid Micro-Organism Infection And Microflora In The Mouth. Bjog 109: 527-533.
6. BERKOWITZ RJ, Jordan HV, White G. The early establishment of Streptococcus mutans in the mouths of infants. **Arch Oral Biol**. 1975 Mar;20(3):171-4.
7. BINKS C, Duane B. Mother-to-child transmission of Streptococcus mutans. **Evid Based Dent**. 2015 Jun;16(2):39-40. doi: 10.1038/sj.ebd.6401089.
8. BOGGESS KA, Bailit JL, Singer ME, Parisi VM, Mercer BM. Projected benefits of universal or scheduled antepartum corticosteroids to prevent neonatal morbidity:a decision analysis. **Am J Obstet Gynecol**. 2005 Oct;193(4):1415-23.
9. BORGES MC, Sesso Ml, Roberti Lr, De Menezes Oliveira Ma, Nogueira Rd, Geraldo-Martins Vr, Ferriani Vp, 2015. Salivary Antibody Response To Streptococci In Preterm And Fullterm Children: A Prospective Study. **Arch Oral Biol** 60: 116-125.
10. CANCELIER, A.C., Petronilho, F., Reinke, A., Constantino, L., Machado, R., 2009. Inflammatory and oxidative parameters in cord blood as diagnostic of early-onset neonatal sepsis: a case-control study. Pediatr. Crit. **Care Med**. 10, 467–471.
11. CALVET G, Aguiar Rs, Melo As, Sampaio Sa, De Filippis I, Fabri A, Araujo Es, De Sequeira Pc, De Mendonça Mc, De Oliveira L, Tschoeke Da, Schrago Cg, Thompson Fl, Brasil P, Dos Santos Fb, Nogueira Rm, Tanuri A, De Filippis Am (2016) Detection and sequencing of Zika virus from amniotic fluid of fetuses with microcephaly in Brazil: a case study. **Lancet Infect Dis** 16:653-660.
12. CHAEMSAITHONG, Piya Et Al. A Point Of Care Test For Interleukin-6 In Amniotic Fluid In Preterm Prelabor Rupture Of Membranes: A Step Toward The Early Treatment Of Acute Intra-Amniotic Inflammation/Infection. **The Journal Of Maternal-Fetal & Neonatal Medicine**, V. 29, N. 3, P. 360-367, 2016.
13. CHIESA, C., Pellegrini, G., Panero, A., Osborn, J.F., Signore, F., Assumma, M., 2003. C-Reactive protein, interleukin-6, and procalcitonin in the immediate postnatal period: influence of illness severity, risk status, antenatal and perinatal complications, and infection. **Clin. Chem**. 49, 60-68.
14. CLAPP, D.W., 2006. Developmental Regulation Of The Immune System. Semin. **Perinatol**. 30, 69-72.

15. COMBS CA, Gravett M, Garite Tj, Et Al. Amniotic Fluid Infection, Inflammation, And Colonization In Preterm Labor With Intact Membranes. **Am J Obstet Gynecol** 2014;210:125.E1–E15

16. DASANAYAKE AP, Li Y, Wiener H, Ruby JD, Lee MJ. Salivary Actinomyces naeslundii genospecies 2 and Lactobacillus casei levels predict pregnancy outcomes. **J Periodontol**. 2005 Feb;76(2):171-7.

17. DA SILVA BASTOS R, Olympio KP, Bijella VT, Buzalaf MA, de Magalhães Bastos JR. Trends in dental caries prevalence in 12-year-old schoolchildren between 1976 and 2001 in Bauru, Brazil. **Public Health**. 2005 Apr;119(4):269-75

18. DURAND R, Gunselman EL, Hodges JS, Diangelis AJ, Michalowicz BS. A pilot study of the association between cariogenic oral bacteria and preterm birth. **Oral Dis**. 2009 Sep;15(6):400-6.

19. ENDO T, GOTO K, ITO K, SUGIURA T, TERABE K, CHO S, NISHIYAMA M, SUGIYAMA K, TOGARI H (2009) Detection of congenital cytomegalovirus infection using umbilical cord blood samples in a screening survey. **J Med Virol** 81:1773-1776.

20. FARDINI Y, Chung P, Dumm R, Joshi N, Han Yw, 2010. Transmission Of Diverse Oral Bacteria To Murine Placenta: Evidence For The Oral Microbiome As A Potential Source Of Intrauterine Infection. **Infect Immun** 78: 1789-1796.

21. FAVIER CF, Vaughan EE, De Vos WM, Akkermans AD. Molecular monitoring of succession of bacterial communities in human neonates. **Appl Environ Microbiol.**2002 Jan;68(1):219-26.

22. FIRMINO RT, Gomes Mc, Clementino Ma, Martins Cc, Paiva Sm, Granville-Garcia Af 2016. Impact Of Oral Health Problems On The Quality Of Life Of Preschool Children: A Case-Control Study. **Int J Paediatr Dent** 26: 242-249.

23. FONG TC, Babbitt Jt, Bloom Et. Increase In Tac Mrna Expression In Natural Killer-Like Cell Line In Response To Interleukin-1 And Interleukin-2 Shown At The Population And Cellular Level By In Situ Hybridization. **Nat Immun Cell Growth Regul.** 1989;8(3):165-72.

24. GENDRIN C, Vornhagen J, Ngo L, Whidbey C, Boldenow E, Santana-Ufret V, Clauson M, Burnside K, Galloway DP, Adams Waldorf KM, Piliponsky AM, Rajagopal L. Mast cell degranulation by a hemolytic lipid toxin decreases GBS colonization and infection. **Sci Adv**. 2015 Jul 17;1(6):e1400225.

25. HAN YW, Ikegami A, Bissada Nf, Herbst M, Redline Rw, Ashmead Gg. Transmission Of An Uncultivated *Bergeyella* Strain From The Oral Cavity To Amniotic Fluid In A Case Of Preterm Birth. **J Clin Microbiol**, 2006. 44(4):1475–83. Doi:10.1128/Jcm.44.4.1475-1483.(2006).

26. HASHIMOTO BE, Kramer DJ, Brennan L. Amniotic fluid volume: fluid dynamics and measurement technique. **Semin Ultrasound CT MR**. 1993 Feb;14(1):40-55.

27. HOLST, R.M., Mattsby-Baltzer, I., Wennerholm, U.B., Hagberg, H., Jacobsson, B., 2005. Interleukin-6 And Interleukin-8 In Cervical Fluid In A Population Of Swedish Women In Preterm Labor: Relationship To Microbial Invasion Of The Amniotic Fluid, Intra-Amniotic Inflammation, And Preterm Delivery. Acta Obstet. **Gynecol. Scand**. 84, 551-557.

28. IVANYI L, Lehner T. The relationship between caries index and stimulation of lymphocytes by Streptococcus mutans in mothers and their neonates. **Arch Oral Biol**. 1978;23(10):851-6.

29. JACOBSSON, B., Mattsby-Baltzer, I., Hagberg, H., 2005. Interleukin-6 And Interleukin-8 In Cervical And Amniotic Fluid: Relationship To Microbial Invasion Of The Chorioamniotic Membranes. **Bjog.** 112, 719-724.

30. JIMENEZ, E. et al. Isolation of commensal bacteria from umbilical cord blood of healthy neonates born by cesarean section. **Curr Microbiol**, v. 51, n. 4, p. 270-4, Oct 2005.

31. JUN JK, Yoon Bh, Romero R, Kim M, Moon Jb, Ki Sh, Et Al. Interleukin 6 Determinations In Cervical Fluid Have Diagnostic And Prognostic Value In Preterm Premature Rupture Of Membranes. **Am J Obstet Gynecol**. 2000; 183(4):868–73. Https://Doi.Org/10.1067/Mob.2000.109034 Pmid: 11035328.

32. KEMOLI AM. Global Disparity In Childhood Dental Caries: Is There A Remedy? **East Afr Med J**. 2013 Apr;90(4):130-6. Review.

33. KILIAN J. [Assessment of the reliability of the criteria for age determination by teeth in persons over 15 years of age]. **Cesk Stomatol**. 1982 May;82(3):228-34.

34. KÖHLER C. A morphological analysis of vasoactive intestinal polypeptide (VIP)-like immunoreactive neurons in the area dentata of the rat brain. **J Comp Neurol.** 1983 Dec 10;221(3):247-62.

35. KUMMAR S, Shafi NQ. False elevations in prostate-specific antigen levels affecting patient management. **Clin Adv Hematol Oncol**. 2004 Sep;2(9):599-601; discussion 602.

36. LAPIRATTANAKUL J, Nakano K, Nomura R, Hamada S, Nakagawa I, Ooshima T. Demonstration of mother-to-child transmission of Streptococcus mutans using multilocus sequence typing. **Caries Res**. 2008;42(6):466-74.

37. LEE SE, Romero R, Jung H, Et Al. The Intensity Of The Fetal Inflammatory Response In Intraamniotic Inflammation With And Without Microbial Invasion Of The Amniotic Cavity. **Am J Obstet Gynecol** 2007;197:294.E1–6

38. LIN D, SMITH MA, Champagne C, Elter J, Beck J, Offenbacher S (2003) Porphyromonas gingivalis infection during pregnancy increases maternal tumor necrosis factor alpha, suppresses maternal interleukin-10, and enhances fetal growth restriction and resorption in mice. **Infect Immun** 71: 5156-5162

39. LI Y, Caufield PW. The fidelity of initial acquisition of mutans streptococc by infants from their mothers. **J Dent Res**. 1995 Feb;74(2):681-5.

40. Li X, Kolltveit KM, Tronstad L, Olsen I. Systemic diseases caused by oral infection. **Clin Microbiol Rev**. 2000 Oct;13(4):547-58. Review.

41. LOESCHE WJ, Rowan J, Straffon Lh, Loos Pj, 1975. Association Of Streptococcus Mutants With Human Dental Decay. **Infect Immun** 11: 1252-1260.

42. MACKIE RI, Sghir A, Gaskins Hr, 1999. Developmental Microbial Ecology Of The Neonatal Gastrointestinal Tract. **Am J Clin Nutr** 69: 1035s-1045s.

43. MARTIN A, Gaillard M, Miot S, Riethmuller D, Schaal JP. [Lactate measurements and acid-base balance in cord blood]. **J Gynecol Obstet Biol Reprod** (Paris). 2003 Dec;32(8 Pt 1):713-9. French.

44. MATSUZAKI N, Saji F, Kameda T, Yoshizaki K, Okada T, Sawai K, Tanizawa O. In vitro and in vivo production of interleukin-6 by fetal mononuclear cells. **Clin Immunol Immunopathol.** 1990 May;55(2):305-14.

45. MELLANDER L, Björkander J, Carlsson B, Hanson LA. Secretory antibodies inIgA-deficient and immunosuppressed individuals. **J Clin Immunol**. 1986 Jul;6(4):284-91.

46. MEIROVITZ, A., Kuten, M., Billan, S., Abdah-Bortnyak, R., Sharon, A., Peretz, T., Sela, M., Schaffer, M., Barak, V., 2010. Cytokines Levels, Severity Of Acute Mucositis And The Need Of Peg Tube Installation During Chemo-Radiation For Head And Neck Cancer: A Prospective Pilot Study**. Radiat. Oncol**. 25, 5-16.

47. MENDES, M. M. Et Al. Streptococcus Mutans In Umbilical Cord Blood, Peripheral Blood, And Saliva From Healthy Mothers. **Curr Microbiol**, V. 75, N. 10, P. 1372-1377, Oct 2018.

48. MERGLOVA V, Koberova-Ivancakova R, Broukal Z, Dort J. The presence of cariogenic and periodontal pathogens in the oral cavity of one-year-old infants delivered pre-term with very low birthweights: a case control study**. BMC Oral Health.** 2014 Sep 1;14:109.

49. MORENCY AM, Bujold E. Comment on "Pregnancy outcome after early detection of bacterial vaginosis" [Eur. J. Obstet. Gynecol. Reprod. Biol. 128 (2006) 40-45]. **Eur J Obstet Gynecol Reprod Biol.** 2007 May;132(1):129; author reply 130.

50. MOREILLON P, Que YA. Infective endocarditis. **Lancet.** 2004 Jan 10;363(9403):139-49.

51. NACCASHA N, Hinson R, Montag A, Ismail M, Bentz L, Mittendorf R. Association between funisitis and elevated interleukin-6 in cord blood. **Obstet Gynecol.** 2001 Feb;97(2):220-4.

52. NAKANO K, Inaba H, Nomura R, Nemoto H, Takeda M, Yoshioka H, Matsue H, Takahashi T, Taniguchi K, Amano A, Ooshima T. Detection of cariogenic Streptococcus mutans in extirpated heart valve and atheromatous plaque specimens**. J Clin Microbiol**. 2006 Sep;44(9):3313-7.

53. NEMOTO H, Nakano K, Nomura R, Ooshima T. Molecular characterization ofStreptococcus mutans strains isolated from the heart valve of an infectiveendocarditis patient. **J Med Microbiol.** 2008 Jul;57(Pt 7):891-5.

54. NIEDERMAN R. Pregnancy gingivitis and causal inference. **Evid Based Dent**. 2013Dec;14(4):107-8.

55. NOGUEIRA RD, Alves Ac, Napimoga Mh, Smith Dj, Mattos-Graner Ro.Characterization Of Salivary Immunoglobulin A Responses In Children Heavil Yexposed To The Oral Bacterium Streptococcus Mutans: Influence Of Specific Antigen Recognition In Infection. **Infect Immun**. 2005 Sep;73(9):5675-84.

56. NOGUEIRA, R.D., Sesso, M.L., Borges, M.C., Mattos-Graner, R.O., Smith, D.J., Ferriani, V.P., 2012. Salivary Iga Antibody Responses To *Streptococcus Mitis* And *Streptococcus Mutans* In Preterm And Fullterm Newborn Children. Arch. **Oral. Biol**. 57, 647-653.

57. OFFENBACHER S, Boggess Ka, Murtha Ap, Jared Hl, Lieff S, Mckaig Rg, Mauriello Sm, Moss Kl, Beck Jd (2006) Progressive periodontal disease and risk of very preterm delivery. **Obstet Gynecol** 107: 29-36.

58. PEREZ-MUÑOZ ME, Arrieta Mc, Ramer-Tait Ae, Walter Ja 2017. Critical Assessment Of The "Sterile Womb" And "In Utero Colonization" Hypotheses: Implications For Research On The Pioneer Infant Microbiome. **Microbiome** 28; 5(1):48.

59. PRINCE AL, Antony Km, Chu Dm, Aagaard Km, 2014. The Microbiome, Parturition, And Timing Of Birth: More Questions Than Answers. **J Reprod Immunol** 104: 12-19.

60. PLONKA KA, Pukallus ML, Holcombe TF, Barnett AG, Walsh LJ, Seow WK. Randomized controlled trial: a randomized controlled clinical trial comparing aremineralizing paste with an antibacterial gel to prevent early childhood caries. **Pediatr Dent**. 2013 Jan-Feb;35(1):8-12

61. STOUT MJ, Conlon B, Landeau M, 2013. Identification Of Intracellular Bacteria In The Basal Plate Of The Human Placenta In Term And Preterm Gestations. **Am J Obstet Gynecol** 208: 226-227.

62. REDMO EMANUELSSON IM, Thornqvist E. Distribution of mutans streptococci in families: a longitudinal study. **Acta Odontol Scand**. 2001 Apr;59(2):93-8.

63. REDWINE, L., Hauger, R.L., Gillin, J.C., Irwin, M., 2000. Effects of sleep and sleep deprivation on interleukin-6, growth hormone, cortisol, and melatonin levels in humans. **J. Clin. Endocrinol. Metab**. 85, 3597-3603.

64. ROBBINS JR, Bakardjiev AI. Pathogens and the placental fortress. **Curr Opin Microbiol**. 2012;15:36–43.

65. ROGERS LF, Goldstein HM. Roentgen manifestations of radiation injury to the gastrointestinal tract. **Gastrointest Radiol.** 1977 Dec 20;2(3):281-91.

66. ROMAGNOLI, C., Frezza, S., Cingolani, A., De Luca, A., Puopolo, M., et al., 2001. Plasma levels of interleukin-6 and interleukin-10 in preterm neonates evaluated for sepsis. Eur. **J. Pediatr**. 160, 345–350.

67. ROMBALDI Ri, Serafini, Ep, Mandelli J, Zimmermann E, Losquiavo Kp, 2009. Perinatal Transmission Of Human Papillomavirus DNA. **Virol J** 6: 83.

68. ROMERO R, Yoon Bh, Mazor M, Gomez R, Gonzalez R, Diamond Mp, Et Al. A Comparative Study Of The Diagnostic Performance Of Amniotic Fluid Glucose, White Blood Cell Count, Interleukin-6, And Gram Stain In The Detection Of Microbial Invasion In Patients With Preterm Premature Rupture Of Membranes. **Am J Obstet Gynecol**. 1993; 169(4):839–51. Pmid: 7694463. 35.

69. ROMERO R, Miranda J, Chaiworapongsa T, Et Al. Prevalence And Clinical Significance Of Sterile Intra-Amniotic Inflammation In Patients With Preterm Labor And Intact Membranes. **Am J Reprod Immunol** 2014;72:458–74

70. SAITO S, Saito M, Kato Y, Maruyama M, Moriyama I, Ichijo M. Production of IL-6 (BSF-2/IFN beta 2) by mononuclear cells in premature and term infants. **J Reprod Immunol.** 1990 Mar;17(1):17-26.

71. SARROUH, B.F., Rivaldi, D., Gambarato, B.C., Santos, D.T., Silva, S.S., 2007. Importância das citoquinas no sistema imunológico: propriedades e características funcionais. **Rev. Anal**. 32, 42-48.

72. SESSO Ml, Borges Mc, Ferriani Vp, Geraldo-Martins Vr, Rodrigues Db, Nogueira Rd. Prospective Evaluation Of Cytokine In Saliva Of Preterm And Fullterm Neonates. **Immunobiology**. 2014 Nov;219(11):830-5.

73. SHAH S, Bapat MM. Parental history of allergy, maternal serum IgE & cord serum IgE. **Indian J Med Sci**. 2006 Jan;60(1):13-8.

74. SILVA CBD, Mendes MM, Rodrigues BR, Pereira TL, Rodrigues DBR, Rodrigues Junior V, Ferriani VPL, Geraldo-Martins VR, Nogueira RD. Streptococcus mutans detection in saliva and colostrum samples. **Einstein** (Sao Paulo). 2019 Jan 31;17(1):eAO4515.

75. Steel JH, O'donoghue K, Kennea NL, Sullivan MH, Edwards AD. Maternal origin of inflammatory leukocytes in preterm fetal membranes, shown by fluorescence in situ hybridisation. **Placenta.** 2005 Sep-Oct;26(8-9):672-7. Epub 2004 Dec 8.

76. STOUT MJ, Conlon B, Landeau M, 2013. Identification Of Intracellular Bacteria In The Basal Plate Of The Human Placenta In Term And Preterm Gestations. **Am J Obstet Gynecol** 208: 226-227.

77. TANZER JM, Livingston J, Thompson AM. The microbiology of primary dental caries in humans. **J Dent Educ**. 2001 Oct;65(10):1028-37.

78. UENO Y, Hays Ef, Hultin L, Uittenbogaart Ch. Human Thymocytes Do Not Respond To Interleukin-2 After Removal Of Mature "Bright" Cd5 Positive Cells. **Cell Immunol**. 1989 Dec;124(2):239-51.

79. UNDERWOOD MA, Gilbert WM, Sherman MP. Amniotic fluid: not just fetal urine anymore. **J Perinatol**. 2005 May;25(5):341-8.

80. VAN DAMME J, Cayphas S, Van Snick J, Conings R, Put W, Lenaerts Jp, Simpson Rj, Billiau A. **Purification And Characterization Of Human Fibroblast-Derived**

81. YACHIE A, Takano N, Yokoi T, Kato K, Kasahara Y, Miyawaki T, Taniguchi N. Thecapability of neonatal leukocytes to produce IL-6 on stimulation assessed by whole blood culture. **Pediatr Res**. 1990 Mar;27(3):227-33.

82. YOON BH, Romero R, Park JS, Kim M, Oh SY, Kim CJ, Jun JK. The relationship among inflammatory lesions of the umbilical cord (funisitis), umbilical cord plasma interleukin 6 concentration, amniotic fluid infection, and neonatal sepsis. **Am J Obstet Gynecol**. 2000 Nov;183(5):1124-9.

83. ZAFAR S, Hafeez A, Qureshi F, Arshad N, Southall D. Structured training in the management of emergencies in mothers, babies and children in a poorly resourced health system: logbooks to document skill use. **Resuscitation**. 2009 Apr;80(4):449-52.

84. ZAURA E, Nicu Ea, Krom Bp, Keijser Bj (2014) Acquiring and maintaining a normal oral microbiome: current perspective. **Front Cell Infect Microbiol** 26: 85.

6. ANEXOS

ANEXO 1 - NORMAS DA REVISTA

Instructions for authors

Thank you for choosing to submit your paper to us. These instructions will ensure we have everything required so your paper can move through peer review, production and publication smoothly. Please take the time to read and follow them as closely as possible, as doing so will ensure your paper matches the journal's requirements.

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ANEXO 2 - APROVAÇÃO DO COMITE DE ÉTICA HUMANO

UNIVERSIDADE DE UBERABA - UNIUBE

PARECER CONSUBSTANCIADO DO CEP

DADOS DO PROJETO DE PESQUISA

Título da Pesquisa: Investigação dos níveis de imunoglobulinas, citocinas e presença de Streptococcus mutans em amostras de líquido amniótico e salivas

Pesquisador: Ruchele Dias Nogueira

Área Temática:

Versão: 2

CAAE: 62821616.1.0000.5145

Instituição Proponente: SOCIEDADE EDUCACIONAL UBERABENSE

Patrocinador Principal: Financiamento Próprio

DADOS DO PARECER

Número do Parecer: 1.991.600

Apresentação do Projeto:

Trata-se da segunda apresentação da proposta "Investigação dos níveis de imunoglobulinas, citocinas e presença de Streptococcus mutans em amostras de líquido amniótico e salivas", coordenada pela Dra Ruchele Dias Nogueira e colocada "em pendência" na reunião de com o seguinte parecer: "Observa-se apenas a falta da explicitação do risco de perda de confidencialidade, bem como as medidas que serão tomadas para minimizá-lo."

Segundo a proponente "trata-se de um estudo de investigação cuja originalidade é primária pois pouco se sabe sobre a presença de bactérias orais e componentes imunológicos no líquido amniótico. É um estudo analítico observacional pois as amostras coletadas serão analisadas para análise da presença ou não de material genético microbiano e também de citocinas de gestações normais sem evidências de doença ou intercorrências durante a gravidez. O período de segmento é transversal pois será realizada coletas em um único momento. Será analisada a prevalência da presença da bactéria e das citocinas em um estudo autocontrolado, aleatorizado e duplo cego."Segundo a pesquisadora, o projeto se justifica tendo em vista que "A investigação da transmissão de Streptococcus mutans (SM) representa a maior chave para o entendimento da cárie precoce na população brasileira. Estudos recentes demonstram que no sangue do cordão umbilical e na saliva de neonatos há a

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presença de SM e de anticorpos contra esta bactéria que parecem modular a infecção. Uma vez que o feto deglute o líquido amniótico, há necessidade de se investigar a presença de SM e de componentes imunológicos neste biofluido

Objetivo da Pesquisa:

Retira-se da proposta o que se segue:

analisar a presença de SM e componentes imunológicos no LA e comparar com amostras salivares de neonatos.

As principais metas do projeto são:

- Verificar a presença de S. mutans em amostras de LA e associar com a detecção nas salivas;
- Avaliar do níveis de IgA na saliva e LA;
- Comparar a complexidade da resposta de IgA contra antígenos de virulência de SM entre as amostras;
- Quantificar os níveis de citocinas em LA e saliva.
- Divulgar e publicar os resultados encontrados em revistas de bom impacto;
- Formar alunos de mestrado e doutorado em várias áreas de conhecimento;
- Divulgar a importância da higiene oral materno e do neonato.

Avaliação dos Riscos e Benefícios:

A participação no projeto não implica em risco desmedido para as gestantes, uma vez que a metodologia não prevê procedimentos invasivos ou que tragam algum tipo de risco. Retira-se da proposta: "Este projeto envolverá a participação de 100 gestantes saudáveis que forem realizar o parto no Mario Palmério Hospital Universitário. Estas gestantes serão convidadas e apresentadas ao projeto. Após a aprovação de sua participação e do recém nascido, as voluntárias responderão a um questionário sócio econômico, de saúde geral e oral. Nestas gestantes serão coletadas assepticamente amostras de líquido amniótico e após o nascimento as salivas dos neonatos. No primeiro dia pós-parto será coletada amostra da saliva materna. "Ressalte-se que a coleta de líquido amniótico será realizada no momento do parto, sem intervenção específica para essa finalidade antes do mesmo.

A pesquisadora assegura que "O presente projeto não apresenta riscos aos participantes por se tratar de coletas seguras, não invasivas e indolores". O risco de perda de confidencialidade foi adequado pela pesquisadora, reforçando no TCLE que os dados pessoais e identificadores dos participantes não serão divulgados.

Como benefícios, a pesquisadora esclarece "Os benefícios aos participantes concentrarão nas instruções individuais sobre prevenção sobre importância da prevenção de doença oral materna

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durante a gestação e do desenvolvimento de cárie dentária precocemente na criança com demonstração, no próprio bebê, de como higienizar a boca da criança, após as mamadas com auxílio de uma gaze estéril. Além de instrução quanto a possível transmissão de microrganismos de mães para filhos."

Comentários e Considerações sobre a Pesquisa:

A pesquisa é pertinente e tem valor científico. A pesquisadora é especialista na área, tendo vários trabalhos publicados nessa linha de pesquisa, que envolve vários outros pesquisadores, além de alunos de graduação e pós-graduação.

Considerações sobre os Termos de apresentação obrigatória:

Toda a documentação pertinente é apresentada, como cartas de autorização, TCLE, folha de rosto, projeto original.

Recomendações:

Não há

Conclusões ou Pendências e Lista de Inadequações:

Não há pendências.

Considerações Finais a critério do CEP:

Em reunião do dia 30/03/2017, a plenária votou de acordo com o relator pela aprovação do projeto, com a recomendação de encaminhamento de relatórios parcial e final conforme recomenda a resolução 466/12, item XI.2 d

Este parecer foi elaborado baseado nos documentos abaixo relacionados:

Tipo Documento	Arquivo	Postagem	Autor	Situação
Informações Básicas do Projeto	PB_INFORMAÇÕES_BÁSICAS_DO_PROJETO_832007.pdf	03/02/2017 13:46:27		Aceito
Outros	cartarespostaCEP2017.pdf	03/02/2017 13:46:02	Ruchele Dias Nogueira	Aceito
TCLE / Termos de Assentimento / Justificativa de Ausência	TCLE2017amniotico.pdf	03/02/2017 13:45:04	Ruchele Dias Nogueira	Aceito

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Continuação do Parecer: 1.991.600

Projeto Detalhado / Brochura Investigador	ProjetoLiquidoamnioticocomitedeetica.pdf	05/12/2016 11:23:55	Ruchele Dias Nogueira	Aceito
Outros	CurriculoRuchele.pdf	05/12/2016 11:23:21	Ruchele Dias Nogueira	Aceito
Outros	2012_cartaencaminhamentoLiquidoamniotico.pdf	05/12/2016 11:19:30	Ruchele Dias Nogueira	Aceito
Declaração de Instituição e Infraestrutura	cartaGalvani.pdf	05/12/2016 11:18:41	Ruchele Dias Nogueira	Aceito
Folha de Rosto	FolharostoAssinadaamniotico2.pdf	05/12/2016 11:12:42	Ruchele Dias Nogueira	Aceito

Situação do Parecer:
Aprovado

Necessita Apreciação da CONEP:
Não

UBERABA, 30 de Março de 2017

Assinado por:
Sálua Cecílio
(Coordenador)

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