UNIVERSIDADE ESTADUAL DE MONTES CLAROS
Victor Bruno da Silva

Transtorno do Espectro do Autismo, eventos adversos no parto e aleitamento materno: um estudo de caso controle

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RESUMO

O Transtorno do Espectro do Autismo (TEA) é um transtorno do neurodesenvolvimento, cujos sinais e sintomas manifestam-se de maneira precoce nos indivíduos afetados. O número de casos de TEA está em ascensão e as famílias afetadas estão sujeitas a grande impacto socioeconômico. A identificação de fatores associados ao TEA, principalmente dos que são passíveis de modificação, é importante pois viabiliza a criação de políticas públicas de saúde com o intuito de prevenção do TEA. O presente estudo objetivou avaliar a associação entre o TEA e a ocorrência de eventos adversos no parto e o aleitamento materno em crianças/adolescentes do norte de Minas Gerais, Brasil. Foi desenvolvido um estudo caso-controle constituído por 248 binômios mães/indivíduos com diagnóstico do TEA (grupo caso) e 886 crianças/adolescentes com desenvolvimento neurotípico (grupo controle). Um questionário semiestruturado foi utilizado como instrumento de coleta de dados. Para identificar os fatores associados ao TEA, foi utilizado o teste qui-quadrado na análise bivariada e modelo de regressão logística para realização da análise múltipla. Odds ratio (OR) bruta e ajustada foram utilizadas para estimar a magnitude das associações. Foram produzidos dois artigos: Artigo 1 - *Association between autism spectrum disorder and childbirth events: a case-control study*, que teve como objetivo avaliar a associação entre eventos adversos do parto e o TEA em crianças e adolescentes; Artigo 2 - *Breastfeeding and autism spectrum disorder: case-control study*, que buscou avaliar a associação entre aleitamento materno e o TEA. Entre as variáveis relacionadas aos eventos adversos do parto, as seguintes apresentaram associação com o TEA, ao nível de 0,05, na análise ajustada: parto cesárea de urgência (OR=2,38; IC95%=1,61-3,51) e presença de mecônio no líquido amniótico (OR=1,67; IC95%=1,06-2,65). Constatou-se também que crianças e adolescentes com TEA foram mais propensos a terem sido expostos a dois ou mais eventos adversos no parto (OR=1,59; IC95%=1,01-2,51). Quando avaliada a associação do TEA com o aleitamento materno, nos três modelos ajustados a ausência de aleitamento materno apresentou associação positiva e significativa com o transtorno (Modelo 1: OR=2,0, IC95%=1,1-3,8; Modelo 2: OR=2,1, IC95%=1,1-4,2; Modelo 3: OR=2,1, IC95%=1,1-4,1). Dada a importância crescente do TEA, a identificação precoce dos fatores de risco potencialmente modificáveis, tais como os eventos do parto e a presença do aleitamento materno, é importante ferramenta clínica e de saúde pública, podendo ser uteis na criação de medidas para intervenção e promoção de saúde.

Palavras-chave: Transtorno Autístico. Parto. Aleitamento Materno. Cesárea. Mecônio.

ABSTRACT

Autism Spectrum Disorder (ASD) is a neurodevelopmental disorder whose signs and symptoms manifest early in affected individuals. The number of ASD cases is on the rise and affected families are subject to a great socioeconomic impact. The identification of factors associated with ASD, especially those that are susceptible to modification, is important because it makes it possible to create public health policies aimed at preventing ASD. This study aimed to evaluate the association between ASD and the occurrence of adverse events at birth and breastfeeding in children/adolescents in northern Minas Gerais, Brazil. A case-control study consisting of 248 binomials mothers/individuals with diagnosis of ASD (case group) and 886 children/adolescents with neurotypical development (control group) was developed. A semi-structured questionnaire was used as a data collection tool. To identify the factors associated with TEA, the chi-square test was used in the bivariate analysis and logistic regression model for performing multiple analysis. Odds ratio (OR) gross and adjusted were used to estimate the magnitude of associations. Two articles were produced: *Article 1 - Association between autism spectrum disorder and childbirth events: the case-control study*, which aimed to evaluate the association between adverse events of childbirth and the TEA in children and adolescents; *Article 2 - Breastfeeding and autism spectrum disorder: case-control study*, which aimed to evaluate the association between breastfeeding and the TEA. Among the variables related to the adverse events of childbirth, the following presented an association with the ASD, at a level of 0.05, in the adjusted analysis: emergency cesarean section (OR=2.38; 95%CI=1.61-3.51) and presence of meconium in the amniotic fluid (OR=1.67; 95%CI=1.06-2.65). It was also found that children and adolescents with ASD were more likely to have been exposed to two or more adverse events at delivery (OR=1.59; 95%CI=1.01-2.51). When the association of ASD with breastfeeding was evaluated, in the three adjusted models the absence of breastfeeding showed a positive and significant association with the disorder (Model 1: OR=2.0, 95%CI=1.1-3.8; Model 2: OR=2.1, 95%CI=1.1-4.2; Model 3: OR=2.1, 95%CI=1.1-4.1). Given the growing importance of ASD, early identification of potentially modifiable risk factors, such as delivery events and the presence of breastfeeding, is an important clinical and public health tool and can be useful in creating measures for intervention and health promotion.

Key-words: Autistic Disorder. Parturition. Breast Feeding. Cesarean Section. Meconium.

LISTA DE ABREVIATURAS E SIGLAS

ANDA	Associação Norte-Mineira de Apoio ao Autista
CCEB	Critério de Classificação Econômica Brasil
CDC	*Centers for Disease Control and Prevention*
CEP	Comitê de Ética em Pesquisa
DP	Desvio Padrão
EUA	Estados Unidos da América
Fapemig	Fundação de Amparo à Pesquisa do Estado de Minas Gerais
IC	Intervalo de Confiança
M-CHAT	*Modified Checklist for Autism in Toddlers*
MG	Minas Gerais
OR	*Odds Ratio*
OR_a	*Odds Ratio* ajustada
OR_b	*Odds Ratio* bruta
PPGCS	Programa de Pós-Graduação em Ciências da Saúde
RPMO	Rotura Prematura de Membranas Ovulares
RUC	Revista Unimontes Científica
SPSS	*Statistical Package for the Social Sciences*
TCLE	Termo de Consentimento Livre e Esclarecido
TEA	Transtorno do Espectro do Autismo
Unimontes	Universidade Estadual de Montes Claros

APRESENTAÇÃO

Meu interesse pelo Transtorno do Espectro do Autismo - TEA despertou em 2013, quando, ainda aluno do segundo período do curso médico da Universidade Estadual de Montes Claros – Unimontes, fui convidado, por minha professora Fernanda Alves Maia, a participar do projeto de pesquisa do seu doutorado intitulado "Prevalência do Transtorno do Espectro do Autismo em crianças matriculadas na educação infantil em escolas da rede pública e privada da cidade de Montes Claros/Minas Gerais. À época, recém-chegado do ensino médio, não tinha muitos conhecimentos sobre o TEA, mas sabendo pessoalmente como o ambiente escolar pode ser difícil para crianças e adolescentes, principalmente com aqueles que apresentam características especiais, logo me interessei pelo projeto e pela oportunidade em trabalhar ativamente pela inclusão social.

No início participei como voluntário e busquei aprofundar meus conhecimentos sobre o TEA. Primeiramente, foi realizada uma revisão de literatura sobre o tema. Logo em seguida, ao identificar que existiam muitas dúvidas sobre a etiologia do TEA e, sem abandonar a motivação inicial, a inclusão, o projeto inicial foi desmembrado em dois: "Transtorno do Espectro do Autismo em crianças e adolescentes: um estudo de caso-controle na cidade de Montes Claros - MG" e "Transtorno do Espectro do Autismo: efeito de uma intervenção em pais e em profissionais da educação infantil".

Enquanto aprofundava meus conhecimentos sobre o tema, esse projeto alcançou financiamento em dois editais da Fundação de Amparo à Pesquisa do Estado de Minas Gerais – Fapemig. Nos anos subsequentes fui bolsista pela Fapemig e, desde então, participei ativamente de todas as etapas e processos para desenvolver este trabalho de extrema importância para nossa região e, principalmente, para as famílias com crianças/adolescentes com o diagnóstico do TEA.

Juntamente a outros estudantes de iniciação científica, auxiliei na elaboração de um instrumento para coleta de dados para o desenvolvimento do projeto. Foi realizada uma busca em grandes bases de dados (*SciELO, Lilacs, Medline, PubMed, Web of Science*) a fim de identificar as principais variáveis envolvidas no desenvolvimento do TEA, dando ênfase aos fatores pré-natais, perinatais e pós-natais. Dessa busca, foram produzidas duas revisões de

literatura, já publicadas, das quais também sou coautor (“Fatores Pós-Natais Relacionados ao Transtorno do Espectro do Autismo: Revisão Integrativa da Literatura” publicado na Revista Unimontes Científica - RUC, e “Fatores perinatais associados ao Transtorno do Espectro do Autismo: Revisão integrativa da literatura”, publicado na Revista Norte Mineira de Enfermagem - Renome).

Após a criação do instrumento, em 2016, participei das entrevistas às mães das crianças/adolescentes com e sem TEA, utilizando o instrumento de coleta de dados citado anteriormente, e o *Modified Checklist for Autism in Toddlers* (M-CHAT) para o grupo de crianças/adolescentes consideradas neurotípicas, com o objetivo de identificar àqueles que possuíam sinais do TEA. No total foram identificadas 120 crianças e adolescentes com rastreamento positivo pelo M-CHAT, as quais foram encaminhadas para avaliação multiprofissional mais detalhada. Foram entrevistadas 278 mães do grupo caso e 1006 do grupo controle. Após as entrevistas, participei ativamente do processamento e análise dos dados. Do resultado dessas coletas de dados, colaborei para a elaboração de três artigos científicos, sendo dois já publicados e outro em processo de submissão [“Transtorno do espectro do autismo e idade dos genitores: estudo de caso-controle no Brasil” publicado na revista Cadernos de Saúde Pública; “Transtorno do espectro do autismo e fatores pós-natais: um estudo de caso-controle no Brasil” publicado na Revista Paulista de Pediatria; “Estudos Psicométricos da versão Brasileira do *Modified Checklist for Autism in Toddlers* (M-CHAT)” em processo de submissão na revista Psicologia: Ciência e Profissão].

Em 2017, participei da equipe de acolhimento do estudo de intervenção oferecido a todos os professores e supervisores da educação infantil pública da cidade de Montes Claros, do qual 674 professores e supervisores participaram do evento. Ainda em 2017, por meio do Programa de Pós-Graduação em Ciências da Saúde – PPGCS, foi institucionalizado o Programa de Ensino, Pesquisa e Extensão sobre o Transtorno do Espectro do Autismo (SAMTEA), que conta com 18 subprojetos e abrange várias áreas do desenvolvimento da criança e objetiva amparar, instruir e assistir familiares e pessoas com TEA.

Após tantas conquistas, aprendizado e desenvolvimento pessoal ao participar desse projeto, prossegui nas minhas carreiras acadêmica e profissional com ingresso no programa de mestrado, objetivando seguir na busca ativa de conhecimentos a respeito do TEA.

SUMÁRIO

1 INTRODUÇÃO

1.1 Transtorno do Espectro do Autismo

1.1.1 Conceito e histórico

O Transtorno do Espectro do Autismo (TEA) é um complexo transtorno do desenvolvimento neurológico, no qual interação social e linguagem estão comprometidas, o que promove inúmeras manifestações motoras, sensoriais, emocionais e cognitivas, entre elas comportamentos e/ou interesses repetitivos ou restritos[1]. Plouller, em 1906, foi quem introduziu o termo "autismo" na psiquiatria, como forma de descrever o sinal clínico de isolamento frequente observado em algumas pessoas. Kanner, em 1943 nos Estados Unidos, descrevendo uma síndrome com o mesmo sinal clínico de isolamento extremo em artigo denominado "*Autistic Disturbances of Affective Contact*", alterou o termo para "distúrbio autístico do contato afetivo"[2]. Além do isolamento ele descrevia, também, outras características dos indivíduos do estudo (entre dois anos e quatro meses a onze anos, como: obsessividade, estereotipias e ecolalia)[2,3]. No ano subsequente um médico austríaco sem contato algum com Kanner, chamado Hans Asperger, descreveu na Áustria os mesmos sintomas que Kanner havia descrito[4].

Em 1961, após uma entrevista exibida no Reino Unido de uma mãe que falou ao público sobre as características de seu filho com autismo, houve um grande impacto, já que vários pais identificaram em seus filhos as mesmas características que foram descritas na entrevista. Isso gerou conscientização dos mesmos sobre um problema em comum e, logo após uma reunião dos mesmos em 1962, foi fundada a "*National Autistic Society"*, primeira associação de pais de crianças com autismo do mundo, tendo como símbolo uma peça de um quebra-cabeças, que segundo os fundadores era a melhor tradução do autismo para a sociedade[4].

O conceito do autismo passou por algumas modificações desde sua descrição inicial. No *Diagnostic and statistical manual of mental disorders* (DSM-III), em 1980, o mesmo foi definido como "autismo infantil", sendo apontado como um tipo dos vários transtornos

globais do desenvolvimento (TGD)[1]. No DSM-IV em 1994, outros transtornos foram adicionados à categoria: transtorno de Rett, transtorno desintegrativo da infância, transtorno de Asperger e transtorno global do desenvolvimento sem outra especificação[1]. Já em 2013, no DSM-V mais recente, todos esses termos foram substituídos por Transtorno do Espectro do Autismo (TEA). Dessa forma, entende-se que o diagnóstico é conceituado como um "espectro" que inclui todos os transtornos previamente definidos no DSM-IV, com exceção do transtorno de Rett[1].

O uso do termo espectro deve-se à variedade do grau de manifestação dos sintomas e a uma ampla gama de níveis de desenvolvimento e funcionamento. O espectro inclui as seguintes condições, antes tratadas como transtornos diferentes: autismo infantil precoce, autismo infantil, autismo de Kanner, autismo de alto funcionamento, autismo atípico, transtorno global do desenvolvimento sem outra especificação, transtorno desintegrativo da infância e transtorno de Asperger[1].

1.1.2 Características clínicas do TEA

O diagnóstico do TEA é clínico e segundo o DSM-V para confirmá-lo é necessário que o indivíduo apresente *déficits* significativos e que persistem nos campos da comunicação e da interação social, além de padrões restritos e repetitivos de comportamento e interesse. Essas características devem estar presentes no início da infância, entretanto não precisam se manifestar por completo desde o início, apresentando-se de maneira plena quando as demandas sociais excedem o limite da capacidade do indivíduo[1]. Normalmente as manifestações clínicas do TEA ocorrem antes dos 36 meses de idade e são mais evidentes quando as demandas sociais iniciam[1] (Quadro 1). Para os pais, normalmente a desconfiança de que há algo anormal com o filho é observada com o atraso da comunicação verbal e a ausência de resposta do indivíduo quando o chamam pelo nome, por volta dos 24 meses de vida[5-7]. No Quadro 1 estão apresentados os sinais sugestivos de TEA no primeiro e segundo anos de vida.

Quadro 1: Sinais sugestivos do Transtorno do Espectro do Autismo no primeiro e segundo ano de vida.

Sinais sugestivos de TEA no primeiro ano de vida		
Perda de habilidades já adquiridas, como balbucio ou gesto dêitico de alcançar, contato ocular ou sorriso social	Baixa frequência de sorriso e reciprocidade social, bem como restrito engajamento social (pouca iniciativa e baixa disponibilidade de resposta)	Irritabilidade no colo e pouca responsividade no momento da amamentação
Baixo contato ocular e deficiência no olhar sustentado	Baixa atenção à face humana (preferência por objetos)	Demonstração de maior interesse por objetos do que por pessoas
Não segue objetos e pessoas próximos em movimento	Apresentação de pouca ou nenhuma vocalização	Não aceita o toque
Não responde ao nome	Imitação pobre	Não se volta para sons, ruídos e vozes no ambiente
Interesses não usuais, como fixação em estímulos sensório-viso-motores	Incômodo incomum com sons altos	Distúrbio de sono moderado ou grave
Sinais sugestivos de TEA no segundo ano de vida		
Os comportamentos repetitivos tanto com o corpo como com objetos	Não compartilhamento de objetos	Não participação em brincadeiras coletivas
Poucas atitudes comunicativas	Baixo contato visual	Aumento da irritabilidade
Dificuldade maior que o habitual em regular as emoções negativas		

Fonte: Sociedade Brasileira de Pediatria, 2019.

Indivíduos com TEA podem apresentar grau variável de manifestações clínicas segundo a idade cronológica, o nível de desenvolvimento e de acordo com a própria gravidade do TEA, por isso utiliza-se o termo “espectro” para definir essa condição clínica. Além disso, um indivíduo classificado em determinado espectro e gravidade pode transitar entre os demais de

acordo com a demanda social e o grau de suporte oferecido[1,5]. Comprometimento intelectual pode ou não estar presente e os *déficits* de linguagem são frequentes. As alterações na fala variam de ausência total, atrasos e compreensão reduzida da fala, ecolalia (imitação da fala de outra pessoa com repetição da última palavra ou da frase ouvida) até linguagem explicita e literal[1,5,8] (Figura 1).

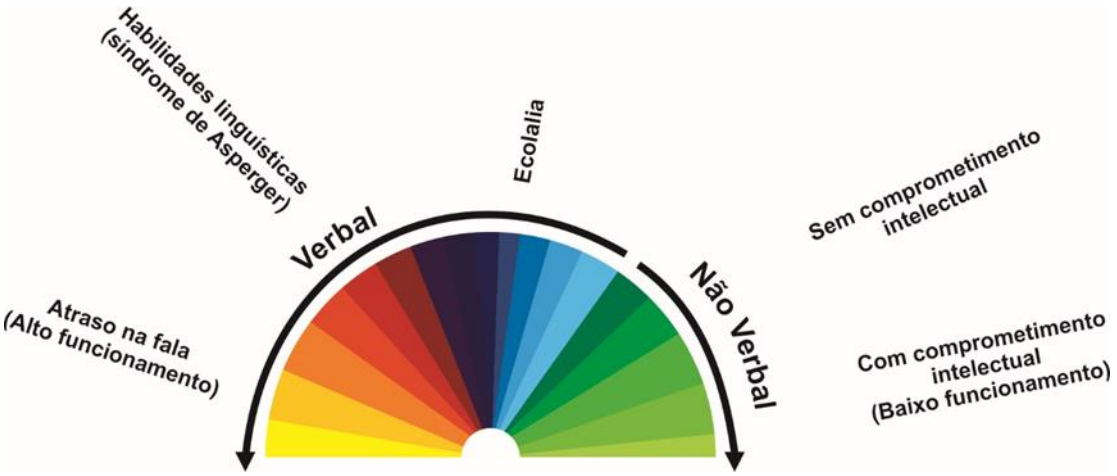

Figura 1. *Deficit* de linguagem no Transtorno do Espectro do Autismo.

Fonte: Feita com base em revisão de literatura.

Além das manifestações clínicas inerentes ao TEA, também se observa variadas manifestações associadas e comorbidades[8,9]. Outros transtornos neuropsiquiátricos são comuns nesses indivíduos como, por exemplo, os transtornos de ansiedade (fobias, transtorno obsessivo compulsivo, tiques motores, comportamentos autodestrutivos); transtorno do *déficit* de atenção e hiperatividade; e alterações sensoriais que variam da hiposensibilidade à hipersensibilidade aos diversos tipos de estímulos[8,10-12]. Doenças genéticas também podem estar associadas como a Síndrome do X Frágil e a Síndrome de Williams[8,13]. Manifestações comuns são os transtornos do trato gastrointestinal e os transtornos alimentares, apresentadas como constipação ou diarreia frequentes, hiperseletividade e intolerância alimentar[8,13-16]. Alterações motoras como dispraxia e alterações da marcha e alterações motoras finas também podem ocorrer[8].

1.1.3 Etiologia

Apesar de ser um transtorno de origem multifatorial e de ter implicado, entre seus fatores etiológicos, uma base genética envolvendo inúmeros genes e sua interação com o ambiente, os conhecimentos acerca desse tema ainda estão pouco fundamentados[17-23]. As interações entre múltiplos genes dentro do genoma do indivíduo bem como as diferentes combinações genéticas entre os indivíduos e a interação gene *versus* ambiente manifestam-se pela ampla variabilidade fenotípica do TEA[21]. Tem sido evidenciado que vários genes, cujas variantes, sejam elas herdadas ou de novo, participam de múltiplos modelos de herança genética[8].

Estudos realizados entre gêmeos monozigóticos têm evidenciado taxa de herdabilidade do TEA superior a 90%[24,25], apesar de outros sugerirem que há proporção similar de contribuição entre fatores genéticos e ambientais[18,21,26]. Estudo de revisão sobre o papel da interação genética e ambiental no TEA concluiu, ao comparar estudos com gêmeos monozigóticos e dizigóticos, que as taxas de concordância variam segundo o espectro autista, de forma que a concordância do TEA em gêmeos monozigóticos é incompleta[21]. Assim, torna-se evidente a contribuição dos fatores ambientais para a etiologia do TEA[21].

Entre os fatores ambientais já estudados e que apresentaram associação significativa com o TEA destacam-se: alterações do líquido amniótico, como variações no volume e presença de mecônio[27-30], rotura prematura de membranas[31], trabalho de parto induzido[30], tempo de trabalho de parto[30,32,33], parto cesárea[33-40], uso de anestesia[36], apresentação fetal[30], parto prematuro[31-33,41], peso ao nascer[32,42-44], icterícia neonatal[31,40], tabagismo materno[29,31,40], transtornos psiquiátricos e/ou doenças neurológicas e estado emocional da mãe durante a gestação[40,42,45-47], idades materna e paterna[31,32,36,40,42,48], e aleitamento materno[49-51]. A epigenética, a qual explica o efeito ambiental sobre a genética, tem sido implicada com papel de destaque na etiologia do TEA[13,20,21,23]. Modificações de proteínas histonas e dos micro RNAs, bem como metilação do DNA podem ser induzidas por fatores ambientais tais quais os descritos anteriormente[21,23,52]. Essas alterações são hereditárias e podem ser mantidas de maneira estável ao longo do tempo segundo as exposições ambientais, demonstrando sua importância para o desenvolvimento neurocognitivo, especialmente no período intrauterino, fase crítica do desenvolvimento[17,20-23].

1.1.4 Epidemiologia

O TEA tem se tornado uma condição cada vez mais prevalente, sendo um importante problema de saúde pública com grande impacto financeiro, social e familiar[53-55]. Segundo estimativas de prevalência realizadas pelo instituto americano *Centers for Disease Control and Prevention* (CDC), entre crianças americanas com oito anos de idade, o número de casos de TEA está em ascensão[53,56]. A prevalência estimada sofreu um aumento de uma criança com TEA para 111 crianças neurotípicas em 2006, para uma criança com TEA para 54 crianças neurotípicas, ou 1,85%, em 2016[53,56,57]. Além disso, o diagnóstico mais precoce (aos 36 meses de idade) vem se tornando cada vez mais frequente nos Estados Unidos[56]. Observa-se, ainda, taxa de recorrência dez vezes maior, em relação à população em geral, entre as crianças com um irmão mais velho com o transtorno[58].

As mudanças recentes nos critérios diagnósticos e a utilização de instrumentos diagnósticos e de rastreio com propriedades psicométricas adequadas podem justificar o aumento da prevalência do TEA[8]. Além disso, o aumento no número de avaliações e a realização das mesmas de maneira cada vez mais precoce também são justificativas possíveis[56]. Em 2014 nos Estados Unidos, 74% das crianças com quatro anos receberam avaliação aos 36 meses de idade, enquanto que em 2016 esse número subiu para 84%[56]. Entretanto, dois estudos independentes concluíram que esse aumento não pode ser explicado em sua totalidade por esses fatores[59,60].

Segundo o CDC, indivíduos do sexo masculino são mais acometidos que os do sexo feminino, com uma prevalência estimada de quatro meninos para cada menina com TEA[56]. No último levantamento do CDC em 2016, pela primeira vez não foi encontrada diferença global no número de crianças negras que apresentavam diagnóstico de TEA em comparação às crianças brancas. Entretanto, o número de crianças hispânicas com TEA é menor quando comparadas às brancas e negras[56]. Aproximadamente um terço das crianças com TEA apresentam deficiência intelectual[56]. A maioria dos estudos que avalia a prevalência do TEA é proveniente da Europa e dos Estados Unidos, de forma que a mesma não está bem documentada em muitos países. O Brasil e a América Latina, da mesma forma, ainda não possuem uma estimativa de prevalência conclusiva[61-64].

Dado o aumento do número de diagnósticos de TEA ao longo dos anos, observa-se também o aumento dos gastos familiares[53-55]. A maioria dos trabalhos disponíveis quanto aos gastos com o TEA são provenientes dos Estados Unidos - EUA e do Reino Unido – RU[65]. Estudos que buscaram avaliar os gastos financeiros com indivíduos com TEA nos EUA e no RU demonstraram gastos semelhantes – cerca de 2,4 milhões de dólares nos EUA e 2,2 milhões no RU para indivíduos com TEA e deficiência intelectual associada e de 1,4 milhão de dólares para indivíduos sem deficiência intelectual associada ao TEA em ambos os países[65]. Apesar dos gastos totais terem sido semelhantes, os gastos em categorias específicas foram diferentes. Nos EUA 79% dos custos foram com serviços, 12% com produtividade e 9% com o cuidador. No RU 56% dos custos foram com serviços, 42% devido à perda de emprego e 2% com cuidador[65]. Nos Estados Unidos é estimado que o orçamento familiar devido aos custos com uma criança com TEA aumenta em 17.000 dólares[66]. No Brasil os dados com os custos financeiros são escassos, sendo que em um estudo desenvolvido em Minas Gerais estimou um gasto médio *per capita* de 39 milhões de reais com indivíduos com TEA de baixo funcionamento (alto nível de comprometimento)[6].

1.1.5 Eventos adversos do parto e o TEA

Fatores relacionados ao parto têm sido implicados na gênese do TEA desde 1956, com o primeiro estudo realizado por Pasmanick *et al.*, que demonstraram, por meio de um estudo caso-controle, uma ligação entre complicações na gestação e distúrbios comportamentais em crianças[67]. Os eventos que ocorrem durante o parto e, também, durante o período intra-uterino têm sido associados ao correto desenvolvimento cerebral[30,68]. Alterações ocorridas nessas fases podem estar envolvidas no aparecimento das características patofisiológicas do TEA[30,68]. Uma metanálise de mais de quarenta estudos identificou vários fatores associados ao maior risco do TEA, entre eles distócia de cordão umbilical, apresentação fetal não cefálica, gravidez gemelar, baixo peso ao nascer e aspiração de mecônio[30].

O que tem sido abordado é que não é o evento em si que promove dano neural, mas um estressor comum aos outros eventos do parto associados que promovem hipóxia e influenciam diretamente o neurodesenvolvimento[28,30,42]. A maioria dos estudos que abordam eventos ocorridos no parto e suas associações positivas ou negativas com o TEA estão relacionadas a

situações de hipóxia[30,69-72]. A hipóxia age sobre as células causando danos que podem levar à alterações no neurodesenvolvimento, além disso, também interfere na atividade dopaminérgica, a qual tem sido implicada nas bases fisiopatológicas do TEA[28,31,73].

O mecônio liberado no líquido amniótico é uma das situações que indicam hipóxia fetal. A liberação de mecônio após o nascimento é normal[28], entretanto, sua liberação no líquido amniótico é um indicador de hipóxia fetal[30,69]. Dessa forma, a exposição ao mecônio tem sido associada a uma maior probabilidade de TEA[28-30].

Tipo de parto, se cesárea ou eletiva, também tem sido bastante explorado na literatura, porém os resultados dos estudos são divergentes. Alguns estudos demonstram associação positiva do parto cesárea com o TEA[36-38], enquanto outros não identificaram tal associação[31,32,35,40]. Dentro dos estudos que evidenciam associação do parto cesárea com o TEA, há ainda os que dividem em parto cesárea de urgência/emergência e o eletivo, também com resultados conflitantes[39,40].

O tempo do trabalho de parto também é um fator de interesse que tem sido discutido na literatura. Mulheres sem trabalho de parto ou com trabalho de parto prolongado (tempo mais de 15 horas) têm apresentado maior probabilidade de terem filhos com TEA comparadas às demais com tempo normal[32,33,45]. Trabalho de parto induzido, principalmente se ocorrido em parto de indivíduo do sexo masculino, demonstrou aumentar a probabilidade de TEA em alguns estudos[29]. Outros eventos adversos do parto também têm sido discutidos: alterações no volume do líquido amniótico[27-30], rotura prematura de membranas[31], uso de anestesia no parto[36], apresentação fetal[30] e parto prematuro[31-33,41].

1.1.6 Aleitamento materno e o TEA

O aleitamento materno é uma ação que estabelece inúmeros benefícios ao binômio mãe-filho, os quais incluem, além da nutrição da criança, a formação de um profundo vínculo afetivo e social entre o binômio[74,75]. Segundo o Ministério da Saúde do Brasil e a Organização Mundial da Saúde o aleitamento materno deve ser exclusivo até os seis meses de vida e complementado com outros tipos de alimentos até os dois anos de idade[74,75]. O leite materno

provê todos os nutrientes necessários ao bom desenvolvimento do lactente até os seis meses de vida, incluindo as quantidades de água, micronutrientes (vitaminas e sais minerais) e macronutrientes (carboidratos, proteínas e lipídeos), e ajuda na nutrição do lactente até os dois anos de idade. Além disso, a partir dele também são dispensados, ao lactente, fatores protetores contra infecções e outros tipos de doenças, como os anticorpos, células de defesa, como os glóbulos brancos, e outros fatores imunológicos maternos[74-76].

Inúmeros são os benefícios do aleitamento materno que são concedidos não apenas ao lactente, mas sim ao binômio mãe-filho[74-80]. Para a mãe observa-se aumento do intervalo interpartal, redução dos custos financeiros devido à ausência de gastos com fórmulas infantis e redução de determinados tipos de câncer maternos[74,76,78]. Para o lactente, o leite materno tem participação ímpar na redução da morbimortalidade infantil e também no tempo de recuperação de diversas doenças, reduzindo a ocorrência de doenças como pneumonia, diarreia, otite média aguda, doenças alérgicas como asma, obesidade e doença celíaca[74-80]. Além disso, também contribui para o bom crescimento e desenvolvimento das estruturas orofaciais (relação intermaxilar sagital e vertical corretas, adequada posição dos dentes incisivos, ausência de protrusão dos lábios), limitando o aparecimento de hábitos orais inadequados[81].

Dado os diversos beneficios do aleitamento materno, inclusive melhora no desempenho escolar, aumento na produtividade e melhora no desenvolvimento intelectual e social, também tem sido explorada, recentemente, sua relação com o TEA[40,74,82-88]. A maioria dos estudos evidencia um papel protetor do aleitamento materno para o desenvolvimento do TEA, apesar dos resultados ainda serem inconsistentes[49,50,82-87].

Extensa metanálise publicada recentemente demonstrou uma redução de 58% na chance de TEA em crianças amamentadas e de 76% naquelas que foram submetidas ao aleitamento materno exclusivo, destacando a amamentação como importante fator na redução do risco de TEA[40]. Uma coorte dinamarquesa também demonstrou tendência de término precoce do aleitamento materno em mães que mais tarde teriam seus filhos diagnosticados com TEA e risco reduzido de TEA, caso a amamentação se prolongasse por mais de seis meses[50]. Tseng *et al.* (2017) em seu estudo de metanálise que reuniu 1463 indivíduos com TEA e 1180 sem TEA demonstrou que o grupo TEA apresentou menor chance de amamentação quando comparado ao grupo controle (OR = 0,61, IC95%= 0,45–0,83)[82]. Outro estudo demonstrou

chance duas vezes e meia maior de TEA em indivíduos que não receberam aleitamento materno se comparado aos que receberam[51]. Em contramão, um estudo americano não evidenciou associação estatística significativa após ajustes entre início da amamentação e TEA, existindo associação apenas na análise não ajustada[83].

Entre os estudos que apontam o leite materno como fator protetor as justificativas para esse fato dizem respeito ao papel da ocitocina e do eixo cérebro-intestino-microbiota agindo positivamente[87-94]. O ocitocina é um hormônio que tem sua produção estimulada pela sucção mamária que age estimulando a lactação[95]. Tem sido demonstrado que o leite materno possui níveis desse hormônio que são transmitidos ao lactente durante a amamentação, o qual junto ao processo de amamentar favorece o desenvolvimento do reconhecimento social, do vínculo social e do neurodesenvolvimento no bebê [91-93]. Além disso, a produção de ocitocina também afeta a mãe, reduzindo os níveis de estresse e ansiedade[93,95,96].

Quanto ao eixo cérebro-intestino-microbiota, o leite materno atua na formação de uma microbiota saudável que age no funcionamento intestinal e no desenvolvimento do sistema imunológico, os quais atuam no cérebro interferindo nas áreas cerebrais que ditam o desenvolvimento e o comportamento humano[87,89,94]. A microbiota intestinal é constituída por uma grande diversidade de microrganismos e já tem sua formação iniciada nos primeiros dias após o nascimento, daí a importância do leite materno (primeiro alimento que o ser humano tem contato) na sua formação e, consequentemente, no estabelecimento do eixo cérebro-intestino-microbiota[89].

Apesar de ainda não estar bem discriminado os mecanismos pelos quais a amamentação influi no TEA, a maioria dos estudos demonstra que a ausência de amamentação ou níveis abaixo do recomendado está associada ao maior risco de TEA, além de outros transtornos cognitivos e comportamentais[77,85,86]. Dessa forma, destaca-se que os profissionais da saúde devem chamar a atenção das famílias para os benefícios e para a importância do aleitamento materno, incentivando sua ocorrência.

Diante do exposto, tendo em vista que a prevalência do TEA está aumentando globalmente e que esse transtorno pode limitar significativamente a capacidade do indivíduo participar da sociedade, a identificação dos fatores associados ao TEA, principalmente os modificáveis, é fundamental para a sua prevenção. O conhecimento desses fatores pode contribuir com as

políticas públicas para que medidas de prevenção sejam direcionadas de forma mais assertiva para esse problema. Soma-se à relevância deste estudo, o ineditismo da proposta na América do Sul, visto que não foram identificados outros estudos que discorram sobre os fatores aqui relacionados, sendo de grande valia os resultados aventados para orientação dos profissionais de saúde, promoção de saúde e comparação entre estudos realizados com populações de outras regiões do mundo.

2 OBJETIVOS

2.1 Objetivo geral

- Investigar a associação entre Transtorno do Espectro do Autismo e associação de eventos adversos no parto e o aleitamento materno.

2.2 Objetivos específicos

- Verificar se existe associação entre tipo de parto e o TEA.
- Analisar a possível associação entre eventos adversos no parto e o TEA.
- Analisar a possível associação entre o aleitamento materno e suas variantes (exclusivo ou não exclusivo) com o TEA.

3 METODOLOGIA

3.1 Desenho do estudo e amostragem

Trata-se de um recorte do estudo caso-controle desenvolvido em Montes Claros, cidade localizada ao norte do estado de Minas Gerais, Brasil, intitulado “Transtorno do Espectro do Autismo em Montes Claros: um estudo de caso-controle”, que buscou avaliar associações entre TEA e fatores pré-natais, perinatais e pós-natais.

Para o cálculo do tamanho amostral do estudo de caso-controle independente[97], foi estimado um *odds ratio* (OR) de 1,9[98,99] dada a probabilidade 0,18 de exposição entre os indivíduos do grupo controle. Em função da análise de vários fatores de exposição, o fator idade materna no parto ≥ 35 anos foi considerado como parâmetro por ter proporcionado o maior tamanho amostral entre as demais variáveis testadas. O poder do estudo foi definido em 80% com nível de significância de 0,05, sendo quatro indivíduos do grupo controle para cada indivíduo do grupo caso. Foi realizada correção para efeito do desenho adotando *deff* = 1,5 devido amostragem por conglomerado e, com o objetivo de corrigir possíveis perdas, foram acrescentados 10% ao cálculo inicial. O tamanho amostral necessário foi definido em 213 indivíduos do grupo caso e 852 do grupo controle.

3.2 Seleção de casos e controles

Para a seleção do grupo caso foram analisados os cadastros da Secretaria Municipal de Saúde e dos planos de saúde a fim de identificar clínicas especializadas ao atendimento de crianças/adolescentes com TEA. Foram identificadas oito clínicas especializadas, entre elas seis com atendimento privado (particular e convênio médico) e duas com atendimento público. Além das clínicas, também foi identificada uma associação que acolhe especificamente crianças com TEA de toda a macrorregião norte de Minas Gerais, a Associação Norte-mineira de Apoio ao Autista (ANDA). Após identificação, foram realizadas visitas às clínicas e à ANDA, com o objetivo de esclarecer quanto a importância social e

acadêmica do trabalho. Todas consentiram a participação e viabilizaram uma lista com o contato de 398 mães de crianças/adolescentes diagnosticados com TEA.

Para serem incluídos no grupo caso, os indivíduos deveriam dispor de laudo médico constando o diagnóstico do TEA confirmado pelos profissionais que os assistiam nas instituições identificadas. Além disso, deveriam ter respondido positivamente à pergunta "Seu filho tem diagnóstico do TEA?", do instrumento de coleta de dados desenvolvido pelos autores (Apêndice A). Foram realizadas três tentativas de contato telefônico com as 398 mães apontadas, dessas, 332 atenderam a ligação e 304 concordaram com um agendamento para maiores esclarecimentos acerca do trabalho. Do total, 278 mães aceitaram participar, mas destas, 25 responderam negativamente à pergunta referente ao diagnóstico do TEA em seus filhos, sendo excluídas do estudo. Dessa forma, o grupo caso compreendeu 253 mães de crianças/adolescentes com idades entre dois e quinze anos que residiam na cidade de Montes Claros e em outras cidades da macrorregião norte do estado de Minas Gerais, tais como Bocaiúva, Pirapora, Janaúba, Januária, Salinas, entre outras.

Para o grupo controle, foram selecionadas crianças/adolescentes neurotípicos matriculados de maneira regular em 63 escolas da rede pública, filantrópica e privada de Montes Claros, nas quais indivíduos do grupo caso também estudavam. Todavia, catorze indivíduos do grupo caso de até quatro anos ainda não frequentavam a escola, sendo identificadas para o grupo controle 66 crianças, da mesma faixa etária, na atenção primária de Montes Claros, que também não frequentavam a escola e que não possuíam sinais de TEA. Foi almejada proporção semelhante para faixa etária (2 a 5; 6 a 10; e 11 a 15 anos) entre os indivíduos do grupo caso e controle, seguindo a razão de quatro controles para cada caso. A variável sexo não foi considerada, propositalmente, para seleção dos controles, com o objetivo de esclarecer a associação entre sexo e TEA na população estudada.

Todas as escolas identificadas foram visitadas e seus administradores esclarecidos quanto à importância e aos benefícios que o trabalho poderia oferecer. Logo em seguida, os gestores indicaram crianças/adolescentes para participação no trabalho, sendo excluídos àqueles que possuíam diagnóstico prévio de TEA ou suspeita de algum transtorno psiquiátrico.

Foi realizado contato com as mães das crianças/adolescentes indicados por meio de carta-convite, a qual continha informações quanto aos objetivos e relevância do trabalho, ou de

maneira presencial nas reuniões regulares de pais nas escolas. As que devolveram a carta-convite assinada foram contatadas via telefônica para agendamento presencial de mais esclarecimentos sobre o trabalho. Um total de 1006 mães de crianças/adolescentes neurotípicos concordou em participar do trabalho e responder o instrumento de coleta de dados.

Visando identificar crianças/adolescentes do grupo controle com sinais/sintomas de TEA, foi utilizado o instrumento de rastreio do TEA traduzido para o português: *Modified Checklist for Autism in Toddlers* (M-CHAT) (Anexo A). O M-CHAT é utilizado para rastreio do TEA em crianças de 18 a 24 meses de idade, compondo-se por 23 perguntas com duas opções de resposta (sim ou não), sendo seis perguntas específicas para o TEA[100]. Para as crianças/adolescentes que ultrapassavam a faixa etária de aplicação do M-CHAT, as mães foram orientadas a responder de acordo com os comportamentos da faixa etária referida. As mães que tiveram filhos com o rastreamento positivo (responderam positivamente duas das seis questões críticas) foram encaminhadas para melhor investigação diagnóstica e excluídas do estudo. Assim, o grupo controle foi composto por 886 crianças/adolescentes neurotípicos.

A fim de reduzir possíveis fatores de confusão, todas as crianças/adolescentes dos grupos caso e controle que possuíam comorbidades, geralmente associadas ao TEA, como síndrome de Down, síndrome de Rett, síndrome do X frágil, foram excluídas do estudo.

3.3 Instrumento de coleta de dados

Para construção do instrumento de coleta de dados foi realizada uma revisão da literatura em grandes bases de dados (*SciELO, Lilacs, Medline* e *PubMed*) de publicações dos anos 2000 a 2014, com o propósito de identificar os principais fatores pré-natais, perinatais e pós-natais associados ao TEA. Os seguintes descritores principais foram utilizados: "*pregnancy*", "*prenatal*", "*perinatal*", "*postnatal*" e "*neonatal*", associados a "*austism*" e "*asd*". Após análise dos estudos, foi produzido um questionário semiestruturado que incluía 213 questões subdivididas em oito grupos: caracterização do sujeito, características demográficas e socioeconômicas dos pais, fatores pré-natais, eventos ocorridos no parto, fatores neonatais, fatores pós-natais e fatores familiares (Apêndice A).

Após a construção inicial do instrumento, o mesmo foi revisado por uma equipe multiprofissional de especialistas no atendimento e acompanhamento de indivíduos com TEA, sendo três fonoaudiólogas, uma psicopedagoga, uma neuropediatra, uma pediatra, uma homeopata, uma psicóloga, uma bióloga e uma farmacêutica. Em seguida, foi realizado um estudo piloto que contou com a participação de dez mães de crianças com TEA e cem mães de crianças neurotípicas, a fim de averiguar a aplicabilidade do mesmo e sanar quaisquer erros eventualmente identificados durante a aplicação. Os questionários utilizados nesse pré-teste não foram considerados no universo amostral do presente estudo.

Após as devidas correções necessárias do instrumento, deu-se início ao procedimento de coleta de dados. Os indivíduos que constituíram o grupo caso foram entrevistados entre agosto do ano de 2015 e janeiro de 2016, enquanto que os do grupo controle entre fevereiro e setembro do ano de 2016. Para a coleta de dados, foi agendado encontro presencial e individual com as mães das crianças/adolescentes, em horário e local previamente definidos segundo a disponibilidade das mesmas.

A coleta de dados foi realizada por uma equipe composta por estudantes de iniciação científica dos cursos de medicina, de enfermagem e de engenharia. A equipe de coleta de dados foi previamente treinada e orientada, com o intuito de padronizar e uniformizar o procedimento de aplicação.

3.4 Variáveis do estudo

Na presente investigação foram realizados dois estudos nos quais foram considerados dois grupos de variáveis de exposição: (1) eventos do parto e (2) amamentação. O grupo eventos do parto foi constituído pelas seguintes variáveis: alteração no líquido amniótico/LA (presença ou não de oligodrâmnio), rotura prematura de membranas ovulares/RPMO (antes do início do trabalho de parto e após vinte semanas de gestação), parto induzido (aquele em que a mulher iniciou as contrações uterinas efetivas após uso de indutores e/ou que realizaram cesárea), tempo de trabalho de parto (utilizou-se como ponto de corte 12 horas, e aquelas que, mesmo após tentativa de indução, não obtiveram contrações efetivas, considerou-se como não

entrou em trabalho de parto), uso de ocitocina pré-parto (uso ou não uso para condução do parto), tipo de parto (vaginal, cesárea eletiva ou cesárea de urgência), uso de anestesia pré-parto (sim ou não), apresentação fetal (cefálico ou não), distócia de cordão umbilical (presença ou ausência) e mecônio (presença ou ausência no líquido amniótico no momento do parto).

O grupo de variáveis relacionadas com a amamentação foi composto por: presença ou ausência de aleitamento materno, tempo de aleitamento materno (seja exclusivo ou não) e tempo de aleitamento materno exclusivo. Essas duas últimas variáveis foram categorizadas em aleitamento até os seis meses de vida, mais de seis meses, menos de seis meses e não amamentou. Esse ponto de corte foi utilizado, pois, segundo a Organização Mundial de Saúde, o aleitamento materno deve ser exclusivo até os seis meses de idade, enquanto que o complementado deve ocorrer até os dois anos[101].

Além das variáveis de exposição, as seguintes variáveis foram consideradas nas análises ajustadas dos estudos: sexo da criança (masculino ou feminino), idade da mãe no parto (< 25 anos, entre 25 e 34 anos, ≥ 35 anos), cor da pele da mãe (autodeclarada e categorizada em branca e não branca), classe socioeconômica segundo critérios da Associação Brasileira de Empresas de Pesquisa – ABEP[102] (classes A/B, C ou D/E), gestação gemelar (presença ou ausência), TEA na família (presença ou ausência), prematuridade (idade gestacional ≥ 37 semanas ou < 37 semanas), choro ao nascer (presença ou ausência). No estudo 1 acrescenta-se a variável paridade (≤ 2 crianças e ≥ 3 crianças) e no estudo 2: mecônio no líquido amniótico (presença ou ausência) e admissão em unidade de terapia intensiva neonatal (presença ou ausência).

3.5 Análise dos dados

Em ambos os estudos procedeu-se a realização de análises descritivas das variáveis investigadas por meio da distribuição de frequência simples e relativa, por grupo (caso e controle). Foi utilizado o teste Qui-Quadrado (χ^2) com o objetivo de verificar a associação do TEA com as variáveis de exposição analisadas. As variáveis com nível descritivo (valor-p) inferior a 0,20 foram selecionadas para análise de regressão múltipla, na qual se adotou o

modelo de regressão logística, com procedimento passo a passo (*stepwise backward*). A magnitude da associação entre a variável desfecho e as independentes de exposição foi estimada pela *odds ratio* (OR) com seus respectivos intervalos de confiança de 95% (IC95%). Adotou-se nível de significância de $\alpha = 0,05$.

O teste de *Hosmer & Lemeshow* e a estatística pseudo R^2 *Nagelkerke* foram utilizados para verificar a qualidade do ajuste dos modelos. Foi verificada ausência de multicolinearidade entre as variáveis independentes. Todas as análises dos dados obtidos foram conduzidas utilizando o *software* estatístico *Statistical Package for the Social Sciences* - SPSS versão 23.0 (IBM - Chicago, EUA).

3.6 Aspectos éticos

O presente trabalho atendeu os preceitos éticos definidos pelo Conselho Nacional de Saúde para pesquisas com seres humanos estabelecidos pela resolução 466/2012. O Comitê de Ética em Pesquisa (CEP) da Universidade Estadual de Montes Claros aprovou o desenvolvimento deste trabalho sob o parecer número 534.000/14 (Anexo B). Todos os responsáveis pelas crianças/adolescentes que participaram do estudo assinaram o Termo de Consentimento Livre e Esclarecido – TCLE (Apêndice B).

4 PRODUTOS CIENTÍFICOS

Conforme as recomendações do Programa de Pós-Graduação em Ciências da Saúde, os resultados do presente estudo estão apresentados em forma de dois artigos científicos.

Artigo 01: "*Association between autism spectrum disorder and childbirth events: a case-control study*" está formatado de acordo com as normas para publicação do periódico *The Journal of Pediatrics*, já submetido.

Artigo 02: "*Breastfeeding and Autism Spectrum Disorder: case-control study"* segue o formato exigido pelo periódico *Journal of Autism and Developmental Disorders*, já submetido.

4.1 Produto 1

ASSOCIATION BETWEEN AUTISM SPECTRUM DISORDER AND CHILDBIRTH EVENTS: A CASE-CONTROL STUDY

"Autistic disorder and childbirth events"

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KEY-WORDS: Delivery, Obstetric; Parturition; Neurodevelopmental Disorders.

ABSTRACT

Objective: to assess the association between childbirth events and Autism Spectrum Disorder (ASD) development in children and adolescents is the purpose of this study. **Study design:** the current research is a case-control study developed in Northern Minas Gerais State, Brazil comprised by 248 children/adolescents diagnosed with ASD (case) and 886 children/adolescents without ASD diagnosis (control). A semi-structured questionnaire was applied mothers of children/adolescents, and the multiple logistic regression model was adopted for data analysis. Gross and adjusted odds ratios (OR) were used to estimate magnitude of the associations. **Results:** ASD was associated with presence of meconium in amniotic fluid (ORa: 1.67; 95% CI: 1.06-2.65) and cesarean delivery type (ORa 1.65; 95% CI: 1.17-2.32). Emeegency C-Section incraesedASD development likelihood (ORa: 2.38; 95% CI: 1.61-3.51). Children and adolescents with ASD were more likely to have been exposed to two or more, unfavorable childbirth events (ORa: 1.59; 95% CI: 1.01 - 2.51). **Conclusion:** the present study suggest that the presence of meconium in the amniotic fluid, emergency cesarean delivery, and experiencing more than one unfavorable childbirth event are important factors that should be taken into consideration in studies about ASD etiology. Given that unfavorable childbirth events are changeable and preventable, our results provide an approachable intervention for ASD prevention and, therefore, being an important clinical and public health tool.

INTRODUCTION

Autism Spectrum Disorder (ASD) has become an increasingly prevalent condition and a major public health issue with significant financial, social and family impacts[1,2,3]. ASD is among the top ten causes of disability for 5-to-9-year-old children worldwide[4]. This neurodevelopmental disorder is characterized by impaired social interaction and communication, in addition to stereotyped and restrictive movement patterns[5].

Although it is a multifactorial disorder that involves, among its etiological factors, a genetic basis comprising numerous genes and their interaction with the environment, the knowledge about this subject remains unclear[6,7]. Moreover, incomplete concordance between monozygotic twins and epigenetic mechanisms that explain the effects of environmental factors on gene expression[6,8] reinforces the contribution of non-genetic factors to the etiology of this disorder[6,9].

Childbirth events stand out among factors involved in the pathophysiology of ASD. Pasamanick , Rogers & Lilienfeld (1956) have conducted the first investigation about this subject and showed, through a case-control study, the link between pregnancy complications and children behavioral disorders. Since then, several factors associated with childbirth events have been investigated and correlated to ASD development, particularly changes in amniotic fluid, such as changes in meconium volume and presence[11,12,13,14], premature rupture of membranes[15], induced labor[14], labor duration[14,16,17], C-section[17,18,19,20,21,22,23,24],anesthesia use[20] and fetal presentation[14].

Given the scarcity of studies conducted in Latin America and divergences between previous studies, the aim of the present research was to analyze the association between childbirth events and Autism Spectrum Disorder development in children/adolescents.

METHODS

The current research is part of a larger study entitled "Autism Spectrum Disorder in Montes Claros: a case-control study", developed Montes Claros, Minas Gerais State, Brazil. The aim of the aforementioned study was to investigate possible associations between ASD and several factors such as childbirth events. The same population examined in the aforementioned study was investigated in recently published studies, in which methodological information is described in detail[25,26].

An odds ratio (OR) equal to 1.9 was estimated[27,28] with 0.18 likelihood of exposure among control subjects[29] in order to calculate the sample size. The power of the study was set at 80%, at 0.05 significance level, with four controls for each case. The sample size was increased by 10% to mitigate possible losses and *deff* = 1.5 was adopted to correct the design effect. Thus, the sample size included 213 case and 852 control individuals.

Case group-inclusion criteria included individuals' whose medical record reported ASD diagnosis, had this diagnosis further confirmed by Northern Minas Autistic Support Association and specialized clinics medical professionals and their mothers had to answer positively to the "Was your child diagnosed with ASD?" question in the data collection instrument. Thus, the case group included 253 mothers of children/adolescents 2-15 years-old.

Control group-inclusion criteria included individuals belonging to the same age group and enrolled in the same schools as the case group ones. Individuals showing signs of ASD, based on the Modified Checklist for Autism in Toddlers[30], and/or suspected of having other psychiatric disorders, as well as any malfunction or syndrome associated with ASD, were excluded from the study. After exclusion criteria were applied, control group included 886 mothers.

Data collection was individually conducted in person, at a pre-scheduled time and place,

according to mothers' availability. A previously trained research team individual made the appointments and conducted the interviews. A semi-structured instrument was elaborated based on a literature review and reviewed by a multiprofessional team. A pilot study was conducted prior to data collection.

The childbirth event variables analyzed in the current study were: change in amniotic fluid/AF (presence or absence of oligohydramnios), premature rupture of ovular membranes/PROM (before labor onset and after 20 pregnancy weeks), induced labor (effective uterine contractions after the use of inducers and/or cesarean section), labor duration (12 hours was used as cut-off point, whereas women who did not show effective contractions, even after attempted induction, were classified as not going into labor), use of pre-delivery oxytocin (use, or non-use, for labor induction), delivery type (vaginal delivery, elective cesarean section and emergency cesarean section), use of pre-delivery anesthesia (use or non-use), fetal presentation (cephalic or not), umbilical cord dystocia (presence or absence) and meconium (presence or not in amniotic fluid at delivery time). Initial analyses have categorized delivery types as vaginal and cesarean section.

All assessed variables were descriptively analyzed based on their frequency distributions in both groups. The Chi-square test (χ^2) was used to assess the association between ASD and other variables; variables presenting significance level lower than 0.20 (p-value < 0.20) were subjected to multiple analysis. The stepwise backward logistic regression model was adopted in the multiple analyses; association magnitude between outcome and independent variables was estimated through odds ratio (OR), at 95% confidence intervals (95% CI). The number of unfavorable birth events associated with ASD was also evaluated and divided in three groups: hypoxia (presence of meconium in amniotic fluid and umbilical cord dystocia), changes in amniotic fluid (PROM and oligohydramnios) and in labor and delivery type (fetal presentation, use of pre-delivery oxytocin, induced delivery and delivery type).

The analyzed adjustment variables were: child sex (boy or girl), parity (≤ 2 children and ≥ 3 children), mother's age at childbirth (< 25 years old, from 25 to 34 years old, ≥ 35 years old), mother's skin color (self-reported and categorized as white and non-white), socioeconomic class (classes A/B, C or D/E)[31], twin pregnancy (presence or absence), family history of ASD (presence or absence), prematurity (gestational age ≥ 37 weeks or < 37 weeks) and crying at birth (presence or absence). Hosmer & Lemeshow test and pseudo R^2 Nagelkerke statistics were used to assess the quality of the adjustment. A correlation matrix between dependent variables was performed and results did not show multicollinearity between them. All data analyses were conducted in the Statistical Package for Social Sciences - SPSS statistical software version 23.0 (IBM - Chicago, USA).

The present study followed the ethical precepts defined by the National Health Council for research conducted with human beings, according to resolution 466/2012. State University of Montes Claros Research Ethics Committee (REC) has approved current research development under opinion number 534.000/14. The legal guardians of all children/adolescents included in the study signed the Informed Consent Form (ICF).

RESULTS

Final sample included 248 children/adolescents diagnosed with ASD, since 5 cases with comorbidities associated with ASD were excluded, and 886 children/adolescents without signs of this disorder. Similarities in mean age ($p = 0.398$), age groups ($p = 0.305$), type of attended school ($p = 0.561$) and social class ($p = 0.320$) were observed between groups. ASD group consisted of 4 four time more boys than girls when compared to the control group ($p < 0.001$).

Based on bivariate analysis, childbirth event variables showing association with ASD were preterm birth, presence of oligohydramnios, presence of meconium in amniotic fluid, non-cephalic fetal presentation, induced labor, prolonged labor and/or not going into labor, anesthesia use and cesarean delivery type (elective or emergency). All these variables were subjected to multiple analyses (Table 1).

It is noteworthy that 34.9% of women who used anesthesia at childbirth did not know which anesthesia was used in them. Among those who remembered it, similar proportions were observed between the analyzed categories, which recorded approximately 50% of epidural and/or spinal anesthesia use in both groups.

Based on the multiple analyses, the presence of meconium in amniotic fluid and emergency cesarean delivery were associated with ASD (Table 2). Based on analyses in which delivery type was only categorized into vaginal and cesarean delivery, cesarean delivery also remained significant after adjustments (p-value: 0.004; ORa 1.65; 95% CI: 1.17-2.32). However, the cesarean delivery category was split into elective and emergency delivery. It was possible seeing that the magnitude of the association was higher in the group presenting two, or more, unfavorable childbirth events than in the group presenting only one (Table 3).

Based on the categorization of the number of unfavorable childbirth events associated with ASD into three groups, only the group ‘changes in amniotic fluid’ did not show statistically significant association with ASD. Groups ‘hypoxia’ and ‘changes in labor and delivery type’ showed statistically significant association with the disorder (Figure 1).

DISCUSSION

Non-genetic factors associated with ASD development have been increasingly investigated; childbirth events, mainly the ones causing fetal hypoxia, have shown association with this disorder. The presence of meconium in amniotic fluid and emergency cesarean section have shown association with ASD in the population investigated in the current study, even after adjustments in genetic (family members with ASD) and non-genetic factors (child's sex, mother's parity, age and skin color, socioeconomic class, twin pregnancy, family history of ASD, prematurity, crying at birth).

In accordance to previous studies, children/adolescents with ASD were approximately twice as likely to have been exposed to meconium in the amniotic fluid as the ones in the control group[11,12,13]. According to Miller et al. (2017), children exposed to meconium were more likely to be diagnosed with ASD than non-exposed children[12]. Increased risk of ASD development was also identified in the adjusted analyses[12,13]. Such association between ASD and meconium aspiration by babies was not evidenced in the meta-analysis performed by Gardener et al. (2011) and in the cohort study conducted by Miller et al. (2017)[12,14]. It is worth noting that exposure to meconium does not mean aspiration[12,14]. It was not possible to investigate whether children/adolescents assessed in the present study presented meconium aspiration syndrome.

Meconium (feces resulting from undigested waste) is often eliminated soon after childbirth[12]. However, meconium released during the intrauterine period may be associated to incidence of fetal stressors such as hypoxia[14, 32]. Thus, it is likely that meconium exposure itself is not the one causing neural damage, but a common stressor that influences both meconium release and neurodevelopment[12]. Hypoxia can cause cellular trauma and trigger meconium release; however, the absence of respiratory distress may hinder neonatal hypoxia

detection and treatment, a fact that may lead to some neurological impairment level, as seen in ASD[12]. Thus, events leading to fetal hypoxia have been identified as a common mechanism for several ASD risk factors[14,33,34,35].

Burstyn et al. (2011) have evidenced that male children who suffered fetal hypoxia presented higher risk of developing ASD[32]. In addition, individuals diagnosed with ASD tend to have complications during pregnancy, which often involve situations that lead to fetal hypoxia[35]. Fetal hypoxia is associated with increased dopaminergic activity, which was already associated with ASD[15, 36].

In addition to the presence of meconium in the amniotic fluid, other factors indicating hypoxia indicate preterm birth, birth weight, fetal distress, absence of crying at birth, premature rupture of ovular membranes and umbilical cord dystocia - they are oxygen deprivation factors that can cause brain damage[37]. Premature rupture of ovular membranes and umbilical cord dystocia were not associated with ASD, based on the gross analysis conducted in the present study while absence of crying at birth has shown association with ASD, after confounding factor adjustment, in a previously published study conducted with this very same population[26].

Delivery type is another factor that has been associated with increased likelihood of ASD development. The children/adolescents with ASD investigated in the current study presented higher likelihood of being born by cesarean section than children/adolescents without ASD. However, based on the multiple analysis conducted after cesarean section was categorized into elective and emergency cesarean delivery, only emergency cesarean section maintained significant association with ASD. This outcome has evidenced that children/adolescents with ASD were twice as likely to have been born by emergency cesarean section.

Previous studies focused on analyzing only cesarean section delivery, without specifying the cesarean section type, have also found association between C-section and ASD[20, 21,22]. However, other studies did not find such association[15,16,19,24]. According to Yip et al. (2017), cesarean section delivery, whether of elective or emergency type, increases the risk of ASD development when it is performed between 36 and 42 pregnancy weeks in comparison to vaginal delivery[23]. Similar results were found in the meta-analysis conducted by Zhang et al. (2019), who suggested that cesarean delivery, regardless of its type, was associated with increased risk of ASD development[24]. However, the meta-analysis conducted by Gardner et al. (2011) did not find association between ASD and cesarean delivery[14].

Elective and emergency caesarean deliveries are characterized by different factors; therefore, they may be associated with neurodevelopment in a different way[24]. Emergency cesarean sections are often performed due to some complication[16,35]. Thus, ASD may not be associated with the surgical procedure itself, but with factors that led to it[19,24].

However, despite being a life-saving procedure in the case of possible complications, there is no evidence that cesarean delivery performed without indication is beneficial to the child[24]. On the contrary, cesarean section is associated with a number of short- and long-term health issues such as neurodevelopmental impairment[24]. Several hypotheses have attempted to explain the association between cesarean delivery and ASD, such as oxytocin dysregulation, the microbiota-gut-brain axis and nervous system toxicity caused by anesthesia application during cesarean sections[22]. Thus, the fact that the number of cesarean deliveries has increased considerably in recent years has raised significant concern. According to estimates, cesarean deliveries account for approximately 20% of all childbirths worldwide; they range from 7% in Africa to 41% in Latin America and in the Caribbean[38]. This representation is even higher in Brazil, where cesarean sections account for almost 50% of all childbirths; this index is approximately three times higher than that recommended by the World Health

Organization[39,40]. This trend is likely due to medical professionals and pregnant women's preferences rather than to adverse clinical conditions[41].

Other childbirth events such as the incidence of oligohydramnios, induced labor, labor duration, anesthesia use and fetal presentation are also associated with delivery type; these events remained associated with ASD in the present study - based on the bivariate analysis - and lost significance after adjustments. This outcome draws attention to other factors, whether they are genetic or not, which may be behind this association.

With respect to the number of unfavorable events at childbirth, the group presenting two, or more, unfavorable events presented positive association with ASD. These results are consistent with previous studies, which showed that children with ASD were more likely to have had at least one unfavorable event during pregnancy and/or childbirth than their neurotypical siblings[41], as well as that the incidence of any type of complication was higher in the group of children with ASD than in the group of children without ASD[24]. These data reinforce the importance of monitoring individuals who have had at least one unfavorable event during childbirth in order to help identifying signs of ASD and to facilitate its early diagnosis.

The current study had some limitations: data source was based on mothers' reports, which may have been subject to memory bias; ASD diagnosis was performed by different teams; the impossibility to determine the indications for emergency cesarean sections and to confirm whether or not they were elective based on medical records analysis of; and the impossibility to confirm whether the individuals included in the study had meconium aspiration syndrome. It is worth noting that an adaptation of the use of the M-CHAT screening instrument was carried out beyond the expected age range (mothers oriented to answer about the children's characteristics during the expected age range).

However, this is, to our knowledge, the first study to address ASD and childbirth events in Latin America, based on a sample size of this magnitude (248 cases and 886 controls). In addition, analyses were adjusted for several factors known to be associated with ASD and that may influence childbirth events, and the diagnosis of individuals belonging to the case group was not only based on reports but further confirmed by qualified professionals.

Childbirth events that emerged as significant risk factors for ASD in the present study, such as the incidence of meconium in the amniotic fluid and emergency cesarean delivery, have suggested that fetal hypoxia is likely to be an important factor for ASD development. These findings have shown the complexity of factors associated with childbirth events in ASD etiology. Results in the current study have also indicated that children/adolescents with ASD were more likely to have been exposed to two, or more, unfavorable childbirth events.

It is worth emphasizing that unfavorable childbirth events are preventable and changeable, as well as that understanding these factors is important to help preventing ASD and to support the development of public policies focused on actions aimed at favoring its diagnosis and immediate intervention and, consequently, at improving the prognosis of individuals with ASD, at supporting their family members, as well as at reducing public spending on this disorder. Longitudinal studies may better clarify the causal relationship between these factors and ASD.

LIST OF ABBREVIATIONS AND ACRONYMS

ASD	Autistic Spectrum Disorder

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TABLES

Table 1: Features of the case (n = 248) and control (n = 886) groups, based on childbirth events. Crude odds ratio and respective confidence intervals. Montes Claros County - MG, Brazil, 2016.

Childbirth events	Case	Control	Total*	OR_c	p-
	n (%)	n (%)	n (%)	(CI95%)	value**
Presence of oligohydramnios					
Yes	21 (8.7)	27 (3.6)	48 (4.9)	2.53 (1.40-4.57)	**0.001**
No	220 (91.3)	716 (96.4)	936 (95.1)	1.00	
Premature rupture of ovular membranes (PROM)					
Yes	22 (9.1)	99 (11.8)	121 (11.2)	0.74 (046-1.21)	0.227
No	221 (90.9)	738 (88.2)	959 (88.8)	1.00	
Induced labor					
Yes	125 (50.6)	336 (38.3)	461 (41.0)	1.65 (1.24-2.20)	**<0.001**
No	122 (49.4)	542 (61.7)	664 (59.0)	1.00	
Labor duration					
> 12 hours	34 (15.0)	70 (8.6)	104 (10.0)	1.71 (1.04-2.68)	**0.018**
Did not go into labor	37 (16.4)	194 (24.0)	231 (22.3)	0.67 (0.45-0.99)	**0.048**
≤12 hours	155 (68.6)	546 (67.4)	701 (67.7)	1.00	
Prepartum oxytocin use					
Yes	57 (24.5)	219 (26.9)	276 (26.3)	0.88 (0.63-1.23)	0.462
No	176 (75.5)	598 (73.1)	772 (73.7)	1.00	
Delivery type					
Elective cesarean section	74 (30.1)	244 (27.8)	318 (28.3)	1.64 (1.16-2.33)	**0.005**
Emergency cesarean section	88 (35.8)	178 (20.3)	266 (23.7)	2.68 (1.90-3.78)	**<0.001**

Vaginal	84 (34.1)	455 (51.9)	539 (48.0)	1.00	
Anesthesia use					
Yes	217 (88.6)	692 (79.3)	909 (81.3)	2.03 (1.32-3.10)	**0.001**
No	28 (11.4)	181 (20.7)	209 (18.7)	1.00	
Fetal presentation					
Non-cephalic	39 (15.7)	89 (10.0)	128 (11.3)	1.67 (1.11-2.51)	**0.012**
Cephalic	209 (84.3)	797 (90.0)	1006 (88.7)	1.00	
Umbilical cord dystocia					
Yes	16 (6.6)	58 (6.6)	74 (6.6)	1.00 (0.60-1.80)	0.974
No	226 (93.4)	827 (93.4)	1053 (93.4)	1.00	
Presence of meconium in the amniotic fluid					
Yes	47 (19.0)	89 (10.0)	136 (12.0)	2.09 (1.42-3.08)	**<0.001**
No	201 (81.0)	797 (90.0)	998 (88.0)	1.00	

ORc= Crude Odds Ratio; CI: Confidence Interval.* Variable associated with *missings.*** Chi-square test.

Table 2: Multiple logistic regression analysis of factors associated with Autism Spectrum Disorder. Crude and adjusted odds ratio (n = 1123) and respective confidence intervals. Montes Claros County - MG, Brazil, 2016.

Variables	ORc (95% CI)	ORa (95% CI)	p-value
Presence of meconium in the amniotic fluid			
Yes	2.09 (1.42-3.08)	1.67 (1.06-2.65)	**0.027**
No	1.00	1.00	
Delivery type			
Elective cesarean section	1.64 (1.16-2.33)	1.24 (0.83-1.87)	0.299
Emergency cesarean section	2.68 (1.90-3.78)	2.38 (1.61-3.51)	**<0.001**
Vaginal	1.00	1.00	

ORg= Crude Odds Ratio; OR_a= Adjusted Odds Ratio; CI: Confidence Interval.Model adjusted to child gender, mother's parity, age and skin color, socioeconomic class, twin pregnancy, family history of ASD, prematurity, crying at birth.$X^2_{HL} = 0.333$; Pseudo $R^2_N = 0.249$; -2 $\log_{L} = 938.00$. HL= Hosmer-Lemeshow test; N= Nagelkerke; L= likelihood.

Table 3: Multiple regression model concerning the number of childbirth complications associated with Autism Spectrum Disorder: Adjusted Odds Ratio and respective 95% confidence intervals. Montes Claros County, MG, Brazil, 2016.

Number of complications	**Case**	**Control**	**OR_a(95% CI)***	**p-value**
	n (%)	**n (%)**		
1	42 (16.9)	246 (27.8)	0.87 (0.51 -1.48)	0.596
≥ 2	173 (69.8)	451 (50.9)	1.59 (1.01 – 2.51)	**0.045**
None	33 (13.3)	189 (21.3)	1.00	

OR_a= Adjusted Odds Ratio; CI: Confidence Interval.* Model adjusted to child gender, mother's parity, age and skin color, socioeconomic class, twin pregnancy, family history of ASD, prematurity, crying at birth.

Figure 1: Crude analysis of number of adverse childbirth events associated with Autism Spectrum Disorder, categorized into three groups of unfavorable events. Montes Claros County, MG, Brazil, 2016.

Figure 1A: Factors related to hypoxia: presence of meconium in amniotic fluid and umbilical cord dystocia.

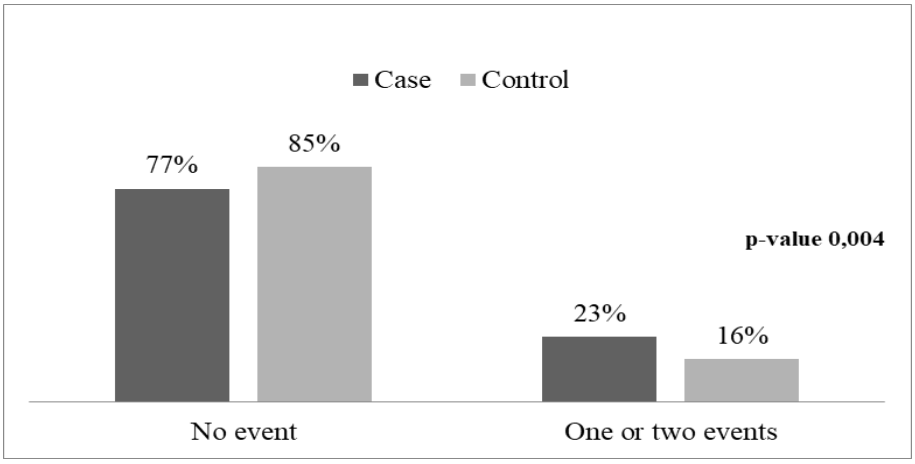


Figure 2B: Changes in amniotic fluid: PROM and oligohydramnios.

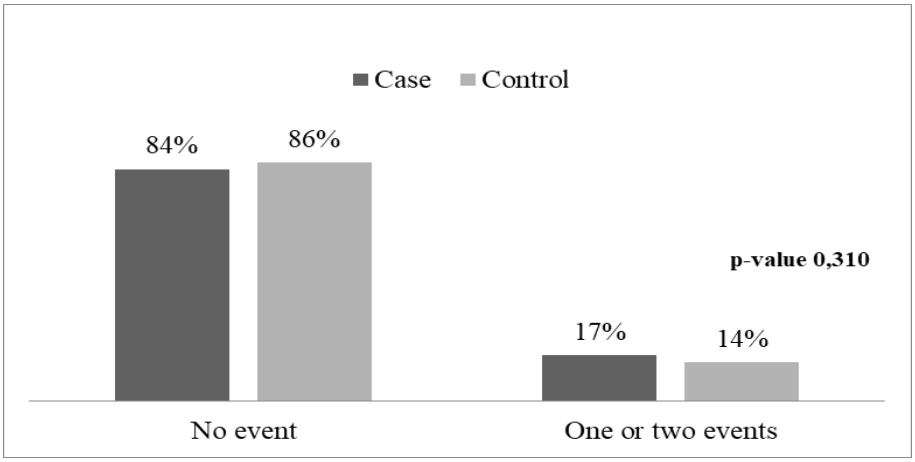
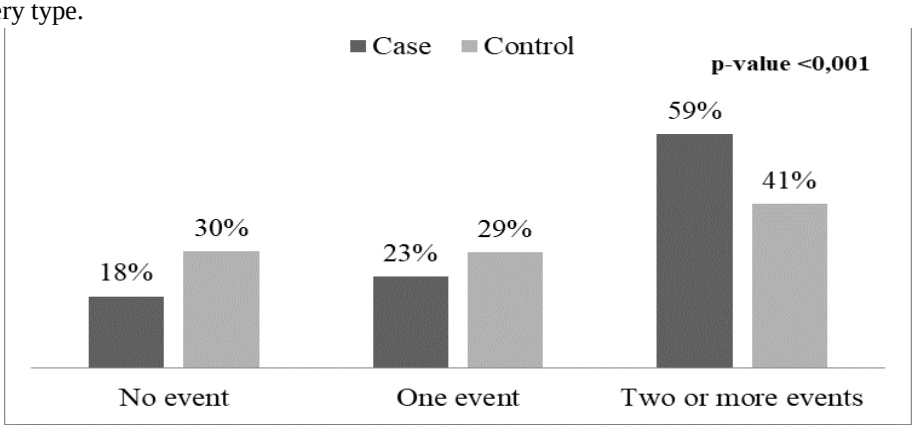


Figure 3C: Changes in labor and delivery type: fetal presentation, use pre-delivery oxytocin, induced delivery and delivery type.



4.2 PRODUTO 2

BREASTFEEDING AND AUTISM SPECTRUM DISORDER: CASE-CONTROL STUDY

“Breastfeeding and Autism”

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ABSTRACT

Autism Spectrum Disorder is a neurodevelopmental disorder, which symptoms manifest early in affected individuals. This is a case-control study carried out in the north of the state of Minas Gerais, Brazil, which included 248 children and adolescents diagnosed with Autism (case group) and 886 children and adolescents without diagnosed ASD (control group). For data analysis, a multiple logistic regression model was adopted. The magnitude of the associations wasestimated by the Odds ratio (OR). Three multiple models were adjusted: Model 1 - presence or absence of breastfeeding; Model 2 - duration of breastfeeding; Model 3 - duration of exclusive breastfeeding. Autism was associated with the absence of breastfeeding in the three adjusted models. The duration of breastfeeding did not show a statistically significant association with ASD. The results of the present study indicated that children with ASD were more likely to have not received breastfeeding.

KEYWORDS: Feeding, Breast. Disorder, Autistic. Study, Case-Control. Disorder, Neurodevelopmental.

INTRODUCTION

Autism Spectrum Disorder (ASD) is increasingly prevalent, causing great socioeconomic impact among families that have an individual with this disorder (Baio, Wiggins, Christensen 2018; Crafa and Warfa 2015; Gal, Abiri, Reichenberg 2012). According to the latest survey conducted by the American Institute for Centers for Disease Control and Prevention (CDC), the prevalence of ASD among American children aged eight years was from one child with ASD to every 54 neurotypic children in 2016 (Maenner, Shaw, Baio 2016) .

The phenotype of individuals with ASD is heterogeneous and genetic and environmental factors are implicated in its genesis (American Psychiatric Association 2013; Newschaffer 2007; Crawford 2015) Among the environmental factors, the age of the parents, congenital malformation, neonatal jaundice, absence of crying at birth, seizure in childhood and breastfeeding can be highlighted (Maia, Oliveira, Almeida, Alves, Saeger, Silva, et al. 2019; Maia, Oliveira, Alves, Bandeira, Silva, Nunes, et al. 2018; Tseng, Chen, Stubbs 2017).

Individuals with ASD have restricted and repetitive behavioral patterns, in addition to persistent deficiencies in communication and social interaction, which greatly impairs socialization (American Psychiatric Association 2013). Breastfeeding is one of the first experiences of social interaction in humans, which is why its absence has been implicated in neurodevelopmental disorders (Raju 2011). It is characterized by being a unique, dynamic and bidirectional social process in which there is much more than the simple transfer of nutrients necessary for the child's development, and the child's first social bond is also established (Raju 2011). The transfer of oxytocin through breast milk positively interferes in some areas that are normally compromised in individuals with ASD, contributing to social recognition and the establishment of a social bond, in addition to contributing, also, to neurodevelopment process (American Psychiatric Association 2013; Lim, Bielsky and Young 2005; Krol, Rajhans, Missana, Grossmann 2015; Dolen 2015; Soke, Maenner, Windham 2019).

The relationship between breastfeeding and ASD has been explored by other studies, however the results are still inconsistent (Tseng, Chen, Stubbs 2017; Soke, Maenner, Windham 2019; Ghozy, Tran, Naveed 2019). In general, what has been observed is that children with ASD have a higher breastfeeding absence rate, or when it is present, the period is shorter compared to children considered neurotypic, thus suggesting a possible breastfeeding protective role (Tseng, Chen, Stubbs 2017; Soke, Maenner, Windham 2019; Ghozy, Tran, Naveed 2019; Cheng, Eskenazib, Widjaja 2019; Al-Farsi, Al-Sharbati, Waly, Al-Farsi, Al-Shafaee, Al-Khaduri, et al. 2012; Ravi, Chandrasekaran, Kattimani, Subramanian 2016; Manohar, Pravallika, Kandasamy, Chandrasekaran, Rajkumar 2018). Recently published meta-analysis showed a 58% ASD risk reduction among breastfed children and 76% in those who received exclusive breastfeeding (Ghozy, Tran, Naveed 2019).

Given the importance of breastfeeding for human growth and development and the inconsistency between studies must already regarding its relationship with ASD, the aim of this study was to evaluate an association between breastfeeding and children/adolescents ASD development from northern Minas Gerais, Brazil.

METHODS

The present study is an excerpt from the research “Autism Spectrum Disorder in Montes Claros: a case-control study”, carried out in Montes Claros, a city located in the state of Minas Gerais, Brazil. The original study aimed to find possible associations of prenatal, perinatal and postnatal factors with ASD, among which the relationship between breastfeeding and ASD stands out. Other information about the studied population and methodological details are available in recently published studies (Maia, Oliveira, Almeida, Alves, Saeger, Silva, et al. 2019; Maia, Oliveira, Alves, Bandeira, Silva, Nunes, et al. 2018).

Estimated odds ratio (OR) of 1.9 and exposure probability of 0.18 among individuals in the control group were adopted to calculate the sample size (Quinlan, McVeigh, Driver, Govind, Karpati 2015; Budi, Sitaresmi, Windiani 2015; Xavier, Jannotti, Silva, Martins 2013).The power of the study was defined at 0.80 significance level of 0.05 and four individuals in the control group for each one in the case group. Deff = 1.5 was adopted to correct the design effect and 10% was added to the sample size to reduce the impacts of possible losses. Thus, the estimated sample size was 213 individuals for the case group and 852 for the control group.

Individuals included in the case group had a medical report confirming the ASD diagnosis, later ratified by professionals who accompanied them at the Associação Norte Mineira de Apoio à Autista (ANDA) and at specialized clinics. In addition, when asked about the data collection instrument, all mothers answered positively to the question "Does your child have a diagnosis of ASD?". In all, the case group corresponded to 253 individuals aged between two and fifteen.

The control group included individuals in the same age group who studied at the same school as those who belonged to the case group. Children / adolescents who showed signs of ASD after screening with Modified Checklist for Autism in Toddlers (Losapio and Pondé 2008) and/or who had a suspicion of another associated psychiatric disorder, as well as any malformation or syndrome were excluded from the study. Thus, the control group included 886 individuals.

A semi-structured instrument for data collection was produced after reviewing the literature and reviewed by a multiprofessional team. For data collection, meetings were scheduled at a predefined time and

place, in person and individually, according to the mothers' availability. Schedules and interviews were carried out by a previously trained team. Data collection was preceded by a pilot study to identify possible flaws in the data collection instrument and correct them.

The exposure variables analyzed were presence or absence of breastfeeding, time of breastfeeding (whether exclusive or not) and time of exclusive breastfeeding. These last two variables were categorized as breastfeeding until the age of six months, more than six months, less than six months and did not breastfeed. This cutoff point was used, as according to the World Health Organization, breastfeeding should be exclusive until six months of age (World Health Organization 2007).

Descriptive analyzes were carried out through the distribution of frequencies and, in order to verify the association of ASD with the analyzed variables, the Chi-Square test ($\chi 2$) was used. Variables with a significance level below 0.20 were selected for multiple regression analysis.

The logistic regression model was adopted with a stepwise backward procedure, so that the magnitude of the association between the outcome variable and the independent variables was estimated by the odds ratio (OR) with their respective 95% confidence intervals (95% CI %). Significance level of $\alpha = 0.05$ was adopted. Three multiple models were adjusted: Model 1 - included the variable presence or absence of breastfeeding; Model 2 - included the variable duration of breastfeeding; Model 3 - included duration of exclusive breastfeeding. All three models were adjusted for the following variables: gender of the child (male or female), mother's age at birth (<25 years, between 25 and 34 years, ≥ 35 years), mother's skin color (self-reported and categorized into white and non-white), socioeconomic class according to the Brazilian Economic Classification Criterion (classes A/B, C or D/E) (Associação Brasileira de Empresas de Pesquisa 2016), twin pregnancy (presence or absence), ASD in the family (presence or absence), prematurity (gestational age ≥ 37 weeks or <37 weeks), crying at birth (presence or absence), meconium in the amniotic fluid (presence or absence) and admission to the neonatal intensive care unit (yes or no).

Hosmer & Lemeshow test and the pseudo R^2 Nagelkerke statistic were used to verify the quality of the fit. There was an absence of multicollinearity between the dependent variables after performing a correlation matrix. The statistical software Statistical Package for the Social Sciences - SPSS version 23.0 (IBM - Chicago, USA) was used to conduct the data analysis.

This study was approved by the Ethics Research Committee (CEP) of the State University of Montes Claros under opinion number 534.000/14. All individuals who participated in the work had the Free and Informed Consent Term - TCLE clarified and signed by their respective legal guardians.

RESULTS

The total sample consisted of 1134 children and adolescents. Of these, 248 were children/adolescents with ASD and 886 without signs of this disorder. The mean age in both groups was similar (p-value = 0.398), being 6.4 years (± 3.5) in the case group and 6.6 (± 3.4) in the control group. In the case group, there were about four boys for each girl with ASD, while in the control group, one boy for each girl (p <0.001). The other characteristics of the case and control group are shown in Table 1.

In bivariate analyzes, breastfeeding was associated with ASD, so that a greater number of children in the case group did not receive breastfeeding or had shorter duration of breastfeeding. This association persisted even when the time and type of breastfeeding were discriminated (whether exclusive or non-exclusive) (Figure 1).

In the multiple analysis, in the three adjusted models, the absence of breastfeeding showed an important statistical association with ASD (Table 2). It was observed that children with ASD were more likely to have been exposed to shorter breastfeeding time in models two and three, despite not having a statistically significant association.

DISCUSSION

The benefits of breastfeeding for newborns and infants are undoubted. In the present study, children and adolescents in the case group were less likely to have received breastfeeding compared to those in the control group. The absence of breastfeeding showed a positive and significant association with ASD in all models analyzed, considering exclusive or non-exclusive breastfeeding.

Problems with social interaction and communication are some of the pillars for the diagnosis of individuals with ASD5, hence the importance of exploring the factors that influence the development of these characteristics. It is already known the positive role played by breastfeeding in reducing the occurrence of various medical conditions in the short and long term, as well as in the future child intellectual development, promoting an increase in the intelligence quotient indexes (Horta, Souza, Mola 2018; Quigley, Hockley, Carson 2012; Bar, Milanaik, Adesman 2016; American Academy of Pediatrics 2020).

In addition to the aforementioned benefits, other advantages of breastfeeding are being explored, which are important, especially for individuals with ASD (Aguiar e Silva 2011; World Health Organization, Unicef 2003; Mello, Ho, Dias, Andrade 2013). Among these advantages are fewer family expenses since TEA

incurs a high financial cost for families, which can be reduced by not using costly breast milk substitutes. In addition, it has been shown that breast milk works by improving communication and adaptability scores, areas affected by these individuals (Aguiar e Silva 2011; World Health Organization, Unicef 2003; Oddy, Li, Whitehouse, Zubrick, Malacova 2011). An Australian cohort showed that infants who received breastfeeding for four months or more had better mean scores for adaptability and communication at one year of age, while those who were breastfed for less than four months had a 1.82 risk of late adaptability and 1.66 of late communication when compared to the others (Oddy, Li, Whitehouse, Zubrick, Malacova 2011).

One of the justifications for the possible protective factor of breast milk in the development of ASD is related to the action that it plays in the development of the immune and neural systems through, for example, the intestinal microbiota (Manohar, Pravallika, Kandasamy, Chandrasekaran, Rajkumar 2018 ; Diaz Heijtz 2016). The intestinal microbiota is formed in the first years after birth and includes a variety of microorganisms (Diaz Heijtz 2016; O'Sullivan, Farver, Smilowitz 2015). It interacts directly with the central nervous system through the enteric plexus, constituting the brain-intestine-microbiota axis, which directly influences child development and behavior (Diaz Heijtz 2016). The intestinal microbiota produces substances that are essential for immune maturation and neural plasticity, so that changes in its composition have been decisive for the development of neuropsychiatric disorders, including ASD (Borre, O'Keeffe, Clarke, Stanton, Dinan, Cryan 2014; Bravo, Forsythe, Chew, Escaravage, Savignac, Dinan, et al. 2011; Ribeiro, Nicoli, Santos, Lima-Santos 2019). Breastfeeding action over brain-intestine-microbiota axis occurs through healthy microbiota formation and subsequent neural development (Diaz Heijtz 2016; O'Sullivan, Farver, Smilowitz 2015). Changes in intestinal microbiota have also been associated with clinical manifestations and the ASD severity spectrum, for example, gastrointestinal disorders present in children with ASD, be it constipation or diarrhea, which can be aggravated due to mircobiota alterations (Ribeiro, Nicoli, Santos, Lima-Santos 2019; Brown, Lozupone, Kang 2015).

Another justification for the protective effect of breastfeeding is due to the transfer of oxytocin through breast milk during the breastfeeding process (American Psychiatric Association 2013; Lim, Bielsky and Young 2005; Krol, Rajhans, Missana, Grossmann 2015; Dolen 2015; Green and Hollander 2010; Yamasue and Domes 2017). This neuropeptide consists of nine amino acids and is produced by magnocellular neurons in the paraventricular nucleus and in the supraoptic nucleus of the hypothalamus and is involved human social and cognitive behavior modulation (American Psychiatric Association 2013; Lim, Bielsky and Young 2005; Krol, Rajhans, Missana, Grossmann 2015; Dolen 2015; Green and Hollander 2010; Yamasue and Domes 2017).

Studies have shown that oxytocin increases group confidence and improves emotional recognition (Van IJzendoorn and Bakermans-Kranenburg 2012; Bakermans-Kranenburg and Van IJzendoorn 2013). As social and cognitive deficits are ASD hallmarks, oxytocin has been implicated in the pathophysiology of this disorder (Green and Hollander 2010).

In the present study, individuals with ASD were less likely to be exposed to breastfeeding. Other studies also corroborate the results presented here (Tseng, Chen, Stubbs 2017; Ghozy, Tran, Naveed 2019; Schultz, Klonoff-Cohen, Wingard, Akshoomoff, Macera, Ji, et al. 2006). A recently published meta-analysis that brought together 1463 individuals with ASD and 1180 without ASD demonstrated that the first group was less likely to have received breastfeeding (OR = 0.61, 95% CI = 0.45–0.83 , P = 0.002), and the data persisted when counting those who received artificial breastfeeding (Tseng, Chen, Stubbs 2017). A case-control study conducted in 2005 demonstrated two and a half times greater chances of ASD among children who did not breastfeed compared to those who breastfed for more than six hours (Schultz, Klonoff-Cohen, Wingard, Akshoomoff, Macera, Ji, et al. 2006).

Another result of the present study shows that individuals with ASD, when they were breastfed, had shorter duration of breastfeeding compared to those in the case group. Some studies show that the increase in breastfeeding time is related to a reduction in diagnoses of ASD (Al-Farsi, Al-Sharbati, Waly, Al-Farsi, Al-Shafaee, Al-Khaduri, et al. 2012; Boucher, Julvez, Guxens 2017; Shafai , Mustafa, Hild , Mulari, Curtis 2014; Bittker and Bell 2018). A multicenter cohort developed in Spain showed that breastfeeding for a longer period improves cognitive development and reduces the manifestation of ASD traits even after adjustments for confounding factors (Boucher, Julvez, Guxens 2017). Children with breastfeeding for a longer time tend to have greater neural sensitivity to body expressions that indicate happiness, so that breastfeeding interferes with neural tendencies to manifest fear and happiness (Dolen 2015).

Shorter breastfeeding duration in ASD individuals observed in the present study may have occurred because breastfeeding was herder for mothers in the case group (Manohar, Pravallika, Kandasamy, Chandrasekaran, Rajkumar 2018; Lawrence 2014; Lemcke, Parner, Bjerrum., Thomsen, Lauritsen 2018). Even before diagnosis, children with ASD already presented some of the associated characteristics such as reduced social interaction and cooperation group (Manohar, Pravallika, Kandasamy, Chandrasekaran, Rajkumar 2018; Lawrence 2014; Lemcke, Parner, Bjerrum., Thomsen, Lauritsen 2018).

Another interesting data obtained in this work concerns the continuation of exclusive breastfeeding after six months of age. This finding contradicts the recommendations of the main international bodies, such as the

World Health Organization and the Ministry of Health of Brazil, in addition to going against other studies that demonstrate early initiation of complementary feeding (Dallazen, Silva, Gonçalves 2018; Schincaglia, Oliveira, Sousa, Martins 2015). This can be explained by the fact that Brazil is a developing country and the region in which the study was developed is very poor and some families are unable to feed their children. However, breast milk is unable to meet all the child's nutritional needs after the sixth month of life, so that the prolongation of exclusive breastfeeding after the sixth month, as well as the early introduction of food, is also unfavorable, causing nutritional deficiencies (Brasil, 2005).

Some limitations must be considered in the present study. The main one concerns the mothers' self-reporting of information, in which memory bias may have occurred, which prevented the exact delimitation of the days when the child received breastfeeding. Despite this, it is important to emphasize the importance of the study, with the large sample size (248 cases and 886 controls) and the adjustment of the models for the variables that demonstrated statistical association with the ASD in previous studies.

CONCLUSION

In the present study, it was found that children and adolescents with ASD were more likely to have not received breastfeeding, which shows a further benefit of it in a Latin American population. The high financial and social cost that TEA demands from families that have an individual with this disorder could be avoided with the simple act of breastfeeding. It is up to health professionals, from the preconception period, throughout pregnancy and postnatal to clarify about the numerous benefits of breastfeeding, and also about the problems that may arise in their absence, such as the greater chance of having children with TEA. The realization of a longitudinal study may clarify this relationship in a more appropriate way and reinforce, once again, the importance of breastfeeding for good growth and child development.

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Table 1: Distribution of case and control groups according to socioeconomic and demographic characteristics of parents and children and childbirth characteristics. Montes Claros, Minas Gerais, Brazil, 2016.

Variables	Case (n=248)	Cotrol (n=886)	Total (n=1134)	Value-p*
	n (%)	n (%)	n (%)	
Sex of the Children/Adolescent				<0.001
Male	47 (19.0)	437 (49.3)	484 (42.7)	
Female	201 (81.0)	449 (50.7)	650 (57.3)	
Prematurity				0.054
≥ 37 weeks	194 (81.5)	731 (86.8)	925 (86.1)	
< 37 weeks	44 (18.5)	111 (13.2)	155 (13.9)	
Twin Pregnancy				0.013
No	235 (94.8)	866 (97.7)	1101 (97.1)	
Yes	13 (5.2)	20 (2.4)	33 (2.9)	
Delivery type				
Vaginal	84 (34.1)	455 (51.9)	539 (48.0)	
Elective cesarean section	74 (30.1)	244 (27.8)	318 (28.3)	0.005
Emergency cesarean section	88 (35.8)	178 (20.3)	266 (23.7)	<0.001
Presence of crying at birth				<0.001
Yes	205 (82.7)	823 (92.9)	1028 (90.7)	
No	43 (17.3)	63 (7.1)	106 (9.3)	
Meconium Presence				<0.001
Yes	201 (81.0)	797 (90.0)	998 (88.0)	
No	47 (19.0)	89 (10.0)	136 (12.0)	
Neonatal ICU admission				<0.001
Yes	206 (83.1)	824 (93.0)	1030 (90.8)	
No	42(16.9)	62 (7.0)	104 (9.2)	
ASD Family History				<0.001
No	197 (79.4)	830 (93.7)	1027 (90.6)	
Yes	51 (20.6)	56 (6.3)	107 (9.4)	

Mother's age (at birth)				<0.001
< 25 years	46 (18.5)	293 (33.1)	339 (29.9)	
25 to 34 years	148 (59.7)	437 (49.3)	585 (51.6)	
≥ 35 years	54 (21.8)	156 (17.6)	210 (18.5)	
Socioeconomic class				0.320
A/B	149 (60.1)	493 (56.2)	642 (57.1)	
C	87 (35.1)	351 (40.0)	438 (38.9)	
D/E	12 (4.8)	33 (3.8)	45 (4.0)	
Skin Color (maternal)				0.001
Non-White	180 (73.2)	716 (82.8)	916 (81.0)	
White	66 (26.8)	149 (17.2)	215 (19.0)	

Table 2: Multiple regression models of the association between breastfeeding and Autism Spectrum Disorder: adjusted Odds Ratio ($OR_{a)}$ with respective 95% confidence intervals (95%CI). Montes Claros, Minas Gerais, Brazil, 2016.

Variables	Model 1*		Model 2**		Model 3***	
	ORa (95%CI)	p-value	ORa (95%CI)	p-value	ORa (95%CI)	p-value
Breastfeeding						
Yes	Reference					
No	2.0 (1.1-3.8)	**0.041**				
Breastfeeding duration						
6 months			Reference			
> 6 months			0.8 (0.5-1.3)	0.308		
< 6 months			1.2 (0.8-1.8)	0.288		
Didn't Breastfeed			2.1 (1.1-4.2)	**0.038**		
Exclusive breastfeeding duration						
6 months					Reference	
> 6 months					0.8 (0.4-1.4)	0.371
< 6 months					1.2 (0.9-1.8)	0.253
Didn't Breastfeed					2.1 (1.1-4.1)	**0.031**

The analyzed adjustment variables were: child gender. mother's age and skin color. socioeconomic class. twin pregnancy. family history of ASD. prematurity. delivery type. crying at birth. presence of meconium in the amniotic fluid. admission to the Neonatal Intensive Care Unit.

*X^2_{HL} = 0.503; Pseudo R^2_N =0.254; **X^2_{HL} = 0.943; Pseudo R^2_N =0.262; ***X^2_{HL} = 0.634; Pseudo R^2_N =0.260.

HL= Hosmer-Lemeshow test; N= Nagelkerke.

Figure 1: Distributions of the case and control groups according to the characteristics of the breastfeeding. Montes Claros County, Minas Gerais, Brazil, 2016.

Figure 1A – Breastfeeding.

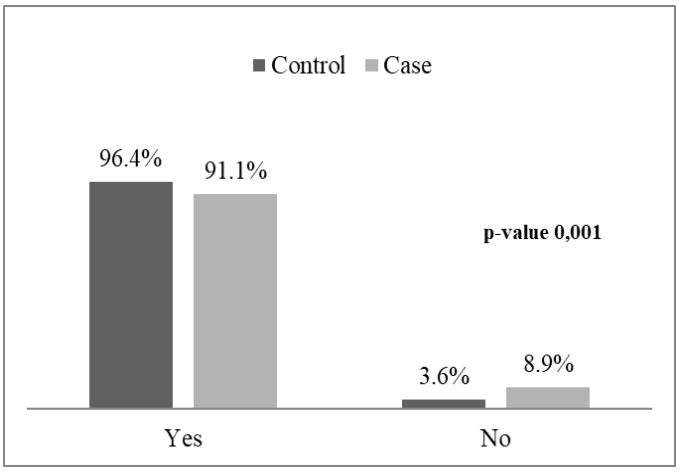


Figure 1B – Breastfeeding duration.

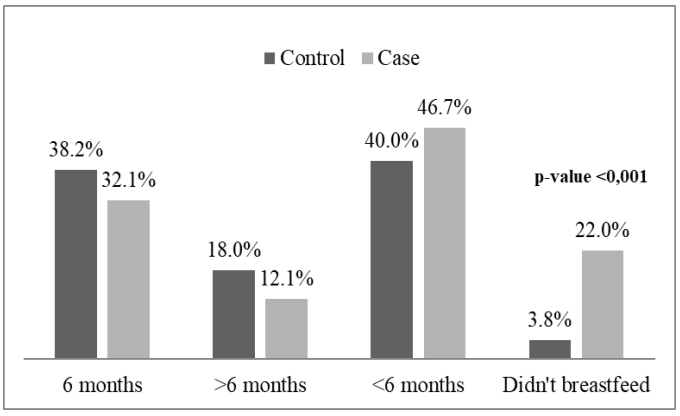
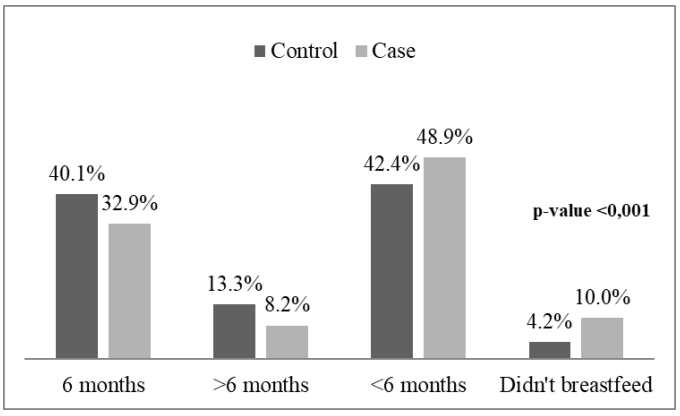


Figure 1C – Exclusive breastfeeding duration.



5 CONSIDERAÇÕES FINAIS

O presente estudo sugere que a exposição ao parto cesárea de urgência esteve associado ao TEA. Verificou-se também que a presença de mecônio no líquido amniótico foi mais comum entre os indivíduos do grupo caso, sugerindo associação com o TEA. Ambos os fatores podem ser evitados com uma assistência adequada durante o período pré-natal e o que antecede de imediato o parto, podendo interferir diretamente na vida de toda uma família. Vale destacar que estudos longitudinais poderão esclarecer melhor a relação de causalidade entre esses fatores e o TEA.

Este estudo destaca a importância do aleitamento materno para o desenvolvimento infantil ao identificar que os indivíduos com TEA tiveram maiores chances de exposição à ausência de aleitamento materno. O aleitamento materno, de acessibilidade universal no período pós-parto, sempre deve ser estimulado no período pré-concepcional, durante a gestação e imediatamente após o parto.

Outro ponto relevante observado durante a realização deste estudo foi o elevado número de crianças identificadas com sinais do TEA no grupo controle quando foi aplicado o instrumento de ratreio M-CHAT. Nos últimos anos ocorreram avanços no que diz respeito à identificação e ao diagnóstico precoce do TEA no Brasil, entretanto o número de indivíduos sem diagnóstico ou com diagnóstico inadequado ainda é expressivo. Nesse sentido, o Programa de Ensino, Pesquisa e Extensão sobre o Transtorno do Espectro do Autismo (SAMTEA) viabilizou o acesso ao diagnóstico do TEA e de outros transtornos mentais ao encaminhar as crianças do grupo controle identificadas com sinais de TEA para atendimento especializado.

A partir de todos os estudos realizados no âmbito do projeto de pesquisa sobre os fatores pré, peri e pós natais associados ao TEA, está em processo de elaboração uma síntese dos seus principais resultados com vistas à produção de um material didático. Esse material será destinado aos profissionais que integram a assistência à população na atenção primária e secundária à saúde no Brasil, contribuindo, dessa forma, com a disseminação do conteúdo científico em prol da saúde pública.

Os achados desse estudo têm uma importante contribuição para a epidemiologia do TEA, na perspectiva de disponibilizar, aos estudiosos desse transtorno, o conhecimento acerca dos fatores associados ao TEA. O que poderá contribuir na redução da sua prevalência na população infantil. A identificação desses fatores, especialmente daqueles que são modificáveis, poderá nortear políticas públicas de saúde, que oriente os profissionais e a própria população quanto aos fatores que devem ser evitados. Visto que, os fatores implicados na gênese do TEA, como os genéticos e os mecanismos epigenéticos, são de difícil controle ou não disponíveis.

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APÊNDICES

APÊNDICE A - Instrumento de coleta de dados

UNIVERSIDADE ESTADUAL DE MONTES CLAROS
PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIAS DA SAÚDE

TEA - COMVIVER

QUESTIONÁRIO DE FATORES PRÉ-NATAL, PERINATAL E PÓSNATAL ASSOCIADOS AO RISCO DO TRANSTONO DO ESPECTRO DO AUTISMO/TEA

Data: ___/___/_____

Início:_____:_____ Fim:_____:_____ Tempo:_____h_____min.

Entrevistador(a):_____ Local:_____

Num Questionário:

IDENTIFICAÇÃO

1. Data de **NASCIMENTO** da criança**:**	2.DNCRI___I___I___I___I___I___I___I___I
2. **IDADE** atual da criança em **ANOS**:	3.IDADECRI___I___I
3. Nome da **ESCOLA** onde estuda a **CRIANÇA**:	
4. A **ESCOLA** é: 0. Pública 1. Privada	5.ESCOLAI___I
5. Qual **ANO/SÉRIE**:	6.SERIEI___I___I

DADOS PESSOAIS DOS PAIS

6. **ENDEREÇO** atual da **MÃE**:	
7. **TELEFONE** de contato da **MÃE**:	
8. Idade **ATUAL** da **MÃE** (em anos):	10.IDAMAEAT I___I___I
9. Idade da **MÃE** na data do **PARTO** da criança (em anos):	11.IDAMAEPARTO I___I___I
10. **TELEFONE** de contato do **PAI**:	
11. Idade **ATUAL** do **PAI** (em anos):	14.IDAPAIAT I___I___I
12. Idade do **PAI** na data do **PARTO** da criança (em anos):	15.IDAPAIPARTO

	I___I___I

DADOS DA MÃE

13. Tipo **SANGUÍNEO** da **MÃE**: 0. A 1. B 2. AB 3. O 4. Não sei/Não lembro	16.TIPOSANMAE I___I
14. Fator **RH**: 0. Positivo 1. Negativo 2. Não sei/Não lembro	17.FATORRHMAE I___I
15. Qual era o seu **ESTADO CIVIL** durante a gestação: 1. Casada 3. União consensual ou estável 2. Separada/Divorciada/Desquitada/Ex-união consensual 3. Solteira 4. Viúva	18.ESTCVMAEGES I___I
16. Qual é a melhor opção que define sua **COR DE PELE/ETNIA**: 1. Parda/Morena 3. Branca/caucasiana 2. Preta/ ascendência negra 4. Vermelha/ ascendência indígena 3. Amarela/ ascendência oriental 5. Não quis responder	19.CORPELEMAE I___I
17. Qual é o seu grau de **ESCOLARIDADE**? 0. Analfabeto 1. 4ª série incompleta (antigo primário incompleto) 2. 4ª série completa (antigo primário completo) 3. 8ª série incompleta (fundamental incompleto) 4. 8ª série completa (fundamental completo) 5. Ensino médio incompleto (não terminou o 3°científico) 6. Ensino médio completo (terminou o 3°científico) 7. Superior incompleto 8. Superior completo 9. Pós-graduação	20.GRAUESCMAE I___I
18. QUAL é a sua **PRINCIPAL OCUPAÇÃO** atualmente? 0. Empregado de empresa privada 4. Funcionário público 1. Empresário/empregador 5. Profissional liberal/Autônomo 2. Aposentado/encostado 6. Desempregado 3. Estudante 7 . Dona de casa/do lar 8.Outros, qual (is)	21.PROFMAEATUALI___I
19. Você **TRABALHOU** com **PRODUTO TÓXICO** antes ou durante a gestação? 0. Sim 1. Não (se **NÃO** vá para a **QUESTÃO 24**) 2. Não sei/não lembro	22.PRODTOXI___I
20. Com **QUAL** produto?	23.QUALPRODTOXI___I___I
21. Você **TRABALHOU** durante a gestação? 0. Sim 1. Não	24.MAETRAGESI___I
22. Qual é aproximadamente a **RENDA TOTAL** mensal da sua **FAMÍLIA** (das pessoas que moram com você)? R$788,00 (salário mínimo vigente) 0. Menor que 1 salário mínimo (menor que R$788,00) 1. De 1 a 2 salários mínimos (de R$788,00 a R$1.576,00) 2. De 2 a 4 salários mínimos (de R$1.576,00 a R$3.152,00) 3. De 4 a 6 salários mínimos (de R$3.152,00 a R$ 4.728,00) 4. De 6 a 8 salários mínimos (de R$ 4.728,00 a R$ 6.304,00) 5. Maior que 8 salários mínimos (maior que R$ 6.304,00) 6. Não quero responder	25.RENDAFAMI___I

23. **QUANTIDADE** de membros na família (considere as pessoas que moram juntas na mesma residência, exceto os empregados domésticos):_____pessoas — 26.QUANMEMFAMI__I

24. Tipo de **RESIDÊNCIA**:
0. Alugada 1. Casa própria 2. Cedida — 27.TIPORESI___I

25. Quais e quantos dos itens abaixo **HÁ** em sua **CASA** (lembre-se que devem ser itens **FUNCIONANTES**)? — 28. CRITBRASIL I___I___I

BENS QUE POSSUI	NÚMERO DE ÍTENS				
Televisão em cores	0	1	2	3	4 ou mais
Rádio (não vale de carro)	0	1	2	3	4 ou mais
Banheiro (tenha vaso sanitário)	0	1	2	3	4 ou mais
Automóvel (só carros. Não vale veículos que usados somente para trabalho e se for usado para tal não contar)	0	1	2	3	4 ou mais
Empregada mensalista	0	1	2	3	4 ou mais
Aspirador de pó	0	1	2	3	4 ou mais
Máquina de lavar	0	1	2	3	4 ou mais
Vídeo cassete/ou DVD	0	1	2	3	4 ou mais
Geladeira	0	1	2	3	4 ou mais
Freezer (aparelho independente ou parte da geladeira duplex	0	1	2	3	4 ou mais

DADOS DO PAI

26. Tipo **SANGUÍNEO** do **PAI**:
0. A 1. B 2. AB 3. O 4. Não sei/Não lembro — 29.TIPOSANPAI I___I

27. Fator **RH** do **PAI**:
0. Positivo 1. Negativo 2. Não sei/Não lembro — 30.FATORRHPAI I___I

28. Qual é o grau de **ESCOLARIDADE** do **PAI**? — 31. ESCOLPAII___I
0. Analfabeto
1. 4ª série incompleta (antigo primário incompleto)
2. 4ª série completa (antigo primário completo)
3. 8ª série incompleta (fundamental incompleto)
4. 8ª série completa (fundamental completo)
5. Ensino médio incompleto (não terminou o 3°científico)
6. Ensino médio completo (terminou o 3°científico)
7. Superior incompleto
8. Superior completo
9. Pós-graduação
10. Não sei

29. QUAL é a **PRINCIPAL OCUPAÇÃO** do **PAI** atualmente? — 32.PROFPAIATI___I____I
0. Empregado de empresa privada 4. Funcionário público
1. Empresário/empregador 5. Profissional liberal/Autônomo

2. Aposentado/encostado 6. Desempregado 3. Estudante 7. Não sei 4. Outros, qual (is)?	
30. O PAI **TRABALHOU** com **PRODUTO TÓXICO** antes da gestação? 0. Sim 1. Não (se **NÃO** vá para a **QUESTÃO 35**) 2. Não sei/não lembro	33.PRODTOXPAII ___I
31. Com **QUAL** produto?	34.QUALPRODTO XIPAI___I___I

FATORES PRÉ-NATAIS DA CRIANÇA

32. Tipo de **GRAVIDEZ**: 0. Única (em caso de gravidez ÚNICA vá para a **QUESTÃO 39)** 1-Múltipla (Gêmeos)	35.TIPOG RAV I___I
33. **NÚMERO** de gêmeos: 0. Dois 1. Três 2. Quatro ou mais	36.NUMGEMI ___I
34. Os gêmeos são **IDÊNTICOS** (monozigóticos): 0. Sim 1. Não 2. Não sei	37.MONOZIGI___ I
35. Sexos **DIFERENTES** entre os gêmeos: 0. Sim 1. Não	38.SEXDI FGI___I
36. A **CONCEPÇÃO** foi realizada: 0. Naturalmente/tradicionalmente 3. Fertilização *in vitro* 1. Estimuladores da ovulação 4. Outros, qual (is)? 2. Inseminação intra uterina	39.CONCEPÇ AO I___I
37. Realizou o **PRÉ-NATAL** em que local? 0. Público (inclui o SUS) 3. Não realizou pré-natal 1. Privado (inclui plano de saúde/convênio) 2. Público/privado (ambos)	40.LOCALPR EI___I
38. **NÚMERO** de consultas pré-natais:	41.NCONSPRENA T I ___I___I
39. **PARIDADE** (nº de filhos nascidos vivos):	42.NFILH O I___I___I
40. Qual a **ORDEM** de nascimento do filho **em questão**? 0. Primeiro 1. Segundo 2.Terceiro 3. Quarto 4.Outro. Qual?	43.ORDNASC RI___I___I
41. Teve **ABORTOS** anteriores ao filho **em questão**? 0. Sim 1. Não (se **NÃO**, vá para a **QUESTÃO 46**)	44.ABORANTI___ I
42. **QUANTOS** abortos? 0. Um 1. Dois 2. Três 3. Quatro 4. Cinco ou mais	45.QUANTAB ORTI___I
43. Qual é o **INTERVALO** (anos) entre os partos do filho em questão e do filho anterior a ele?	46.INTPARTOI___ I___I
44. Gravidez foi **PLANEJADA**? 0. Sim 1. Não	47.GRAPLAN I___I
45. Gravidez foi bem **ACEITA**? 0. Sim 1. Não	48.GRAA CEII___I

46. Teve algum tipo de **ESTRESSE** durante gestação? 0. Sim 1. Não	49.STRES GRA I___I
47. Teve **DEPRESSÃO** e/ou **TRISTEZA** e/ou **ANSIEDADE** durante a gestação? 0. Sim 1. Não	50.DEPT RISANSI ___I
48. Quantas **HORAS** diárias trabalhadas (fora de casa) durante a gestação?	51.HORASTR MAE I___I___I
49. Teve **AJUDA** de alguém nos trabalhos **DOMÉSTICOS** durante a gestação? 0. Sim 1. Não	52.ATRD OMGESI ___I
50. Como você classificaria seu estado de saúde **ANTES** da **GESTAÇÃO**? 0. Muito bom 2. Bom 4. Regular 6. Ruim 1. Muito ruim 3. Não sabe 5. Não quis responder	53.SAUD EANTGE SI___I
51. Apresentou **DIABETES GESTACIONAL?** 0. Sim 1. Não 2. Não sei/não lembro	54.DIAB ETEGESI ___I
52. Apresentou **PRÉ-ECLAMPSIA/ ECLAMPSIA**? 0. Sim 1. Não 2. Não sei/não lembro	55.ECLA GESI___I
53. Apresentou **NÁUSEAS/VÔMITOS** durante a gestação? 0. Sim 1. Não (se **NÃO** vá para a **QUESTÃO 58**)	56.NAUS/ VOM I___I
54. As náuseas/vômitos durante a gestação **PREJUDICARAM** a alimentação/saúde? 0. Sim 1. Não 2. Não sei/Não lembro	57.NAUS PREJ I___I
55. Usou algum **MEDICAMENTO** nos anos que **ANTECEDERAM** a gravidez por mais de um mês seguido? 0. Sim 1. Não 2. Não sei/Não lembro	58.USOREMA NTGESI___I
56. Usou **MEDICAMENTOS** durante a gestação? 0. Sim 1. Não (se **NÃO** vá para a **QUESTÃO 63**)	59.MEDD URGES I___I
57. Em que **PERÍODO** da gestação? 0. Primeiro trimestre 1. Segundo trimestre 2. Terceiro trimestre	60.PERU SOMEDI ___I
58. **QUAL** classe de medicamento **DURANTE** a gestação? 0. Antitérmicos/ Analgésicos 4. Anti-inflamatórios 1. Antibióticos 5. Hormônios 2. Anticonvulsivantes 6. Antieméticos (enjoo, náusea, vômitos) 3. Antidepressivo 7. Corticoides 8. Outros, qual (is)?	61.QUALMEDGE S I___I
59. Com qual **FREQUÊNCIA**? 0. Esporadicamente 1. Continuamente	62.FREQMEDGES I___I
60. Você **PINTAVA** ou **ALISAVA** o cabelo nos 10 anos que **ANTECEDERAM** a gravidez?	63.PINTAALISAA NTI___I

0. Sim 1. Não 2. Não sei/não lembro	
61. Você **PINTOU** ou **ALISOU** o cabelo **DURANTE** da gestação? 1. Sim 1. Não 2. Não sei/não lembro	64.PINTOUALISODURI___I
62. Você recebeu **VACINA** durante a gestação? 0. Sim 1. Não (se **NÃO** vá para a **QUESTÃO 67**) 2. Não sei/Não lembro	65.VACINAGESI___I
63. **QUANTAS** doses? 0. Uma 1. Duas 2. Três 3. Quatro 4. Mais de quatro 5. Não sei/não lembro	66.QUANDOSEVACII___I
64. Realizou tratamento **DENTÁRIO** durante a gestação? 0. Sim 1. Não 2. Não sei/Não lembro	67.TRADENTEGESI___I
65. Teve uso de **ANESTESIA** durante o tratamento **DENTÁRIO**? 0. Sim 1. Não 2. Não sei/Não lembro	68.ANESTESIADENTEI___I
66. Realizou **RX** durante a gestação? 0. Sim 1. Não 2. Não sei/Não lembro	69.RXGEST I___I
67. Você teve **INTERNAÇÕES** durante a gestação? 0. Sim 1. Não (se **NÃO**, vá para a **QUESTÃO 73**) 2. Não sei/não lembro	70.INTERMAE I___I
68. **QUANTAS** internações durante a gestação?	71.NUMINTERGES I___I___I
69. Qual o **MOTIVO**? 0. Sangramento 2. Hipertensão 1. Infecção 3. Outros, qual (is)?	72.MOTINTERI___I
70. Você teve **SANGRAMENTO** durante gestação? 0. Sim 1. Não (se **NÃO**, vá para a **QUESTÃO 75**) 2. Não sei/não lembro	73.SANGGESTI___I
71. Em **QUAL** período? 0. Primeiro trimestre 1. Segundo trimestre 2. Terceiro trimestre 3. Não sei/não lembro	74.PERSANGESI___I
72. Teve **INFECÇÕES** durante gestação? 0. Sim 1. Não (se **NÃO**, vá para a **QUESTÃO 77)**	75.INFECGESI___I
73. **QUAL**? 0. Urinária 3. Garganta 5. Sinusite 7. Bronquite 1. Ouvido 4. Intestinal 6. Vaginal 8. Asma 2. Influenza A 9.Outro, qual (is)?	76.QUALINFECI___I
74. Você teve **FEBRE** durante a gestação? 0. Sim 1. Não 2. Não sei/ Não lembro	77.FEBREGESI___I
75. Quantos **QUILOS** você **GANHOU** na gestação?	78.KGGESI___I___I
76. Você é ou já foi **FUMANTE**, ou seja, já fumou, ao longo de sua vida, pelo menos 100	79.MAETABG

cigarros (cinco maços de cigarros)? 0. Sim 1. Não (se **NÃO** vá para a **QUESTÃO 87**)	I___I
77. Hábito de **FUMAR** durante a **GESTAÇÃO**: 0. Sim (se **SIM**, vá para a **QUESTÃO 83**) 1. Não	80.FUMOGES I___I
78. Parou de fumar **ANTES** da gestação? 0. Sim 1. Não (se **NÃO**, vá para a **QUESTÃO 84**)	81.FUMANTGE I___I
79. Quanto **TEMPO ANTES** da gestação (meses)?	82.PAROUANTGESI___I___I
80. **QUANTOS** cigarros por dia **DURANTE** a gestação?	83.QTCIGDIAGES I___I___I
81. Fumou por quanto **TEMPO** (meses) durante a gestação?	84.TEMPFUGES I___I
82. **PAROU** de fumar durante a gestação? 0. Sim 1. Não (Se **NÃO** vá para a **QUESTÃO 87)**	85.PAROUDURGES I___I
83. Em qual **MÊS** de gestação parou?	86.MESPARFU I___I
84. Você faz uso de bebida alcoólica **ATUALMENTE?** 0. Sim 1. Não	87.USOALCATMAEI___I
85. Você **FEZ USO** de **BEBIDA** alcoólica durante a gestação? 0. Sim 1. Não (Se **NÃO**, vá para a **QUESTÃO 93)**	88.ALCOLGEST I___I
86. **QUAL** bebida você consumiu durante a gestação? 0. Cerveja 3. Pinga 1. Whisky 4. Vinho 2. Vodca 5. Outros, qual (is)?	89.QUALBEBDAMAE I___I
87. Quantos **EPISÓDIOS** (vezes) por semana (durante a gestação)?	90.EPISBEBMAEI___II___I
88. Número de **DOSES** por episódio**:_____** Considere uma dose: **meia garrafa ou 1 lata de cerveja, um cálice de vinho ou 1 dose de bebidas destiladas (aguardente, *whisky*, etc.)**	91.NDOSEMAEI___I___I
89. Por quanto **TEMPO** (anos) faz ou fez uso de bebida alcoólica?_____	92.TEMPALMAEI___I___I
90. Você já **FEZ/FAZ** uso de **DROGAS ILÍCITAS**? 0. Sim 1.Não (se **NÃO**, vá para a **QUESTÃO 96)**	93.DROGAMAEGES I___I
91. **QUAL**? 0. Maconha 3. Cocaína 1. Crack 4. Não sei/não quis responder 2. Heroína 5. Outros, qual (is)?	94.QUALDROGMÃE I___I

92. Por quanto **TEMPO (**anos**)** foi usuário (antes da gestação)?	95.TEMPUSDROMAE I___I___I
93. O **PAI** é ou já foi **FUMANTE**, ou seja, já fumou, ao longo de sua vida, pelo menos 100 cigarros (cinco maços de cigarros)? 0. Sim 1. Não	96.PAIFUMAI___I
94. O **PAI** fazia uso de **BEBIDA ALCOÓLICA** antes da gestação? 0. Sim 1. Não 2. Não sei/não lembro	97.PAIALCOOGESI___I
95. O **PAI** fez uso de **DROGAS ILÍCITAS** antes da gestação? 0. Sim 1. Não 2. Não sei/não lembro	98.PAIDROGESI___I
96. O **PAI RESIDIU/CONVIVEU** com você durante a **GESTAÇÃO**? 0. Sim 1. Não (Se **NÃO**, vá para a **QUESTÃO 112**)	99.PAIRESMAEGESI___I
97. O **PAI** tinha hábito de **FUMAR** durante a gestação? 0. Sim (Se **SIM**, vá para a **QUESTÃO 103**) 1. Não 2. Não sei/não lembro	100.PAIFUMOGESI___I
98. Se **NÃO**, pai parou de fumar **ANTES** da gestação? 0. Sim 1. Não (Se **NÃO** vá para a **QUESTÃO 104**)	101.PAIPAROFUMANTES I___I
99. Quanto **TEMPO** antes da gestação (meses)?	102.QTTEMFUMOPAII___I
100. **QUANTOS** cigarros por dia durante a gestação?	103.CIGARROPAIDIA I___I___I
101. **PAI** fez uso de **BEBIDA** alcoólica durante a **GESTAÇÃO**? 0. Sim 1. Não (se **NÃO**, vá para a **QUESTÃO 109**)	104.ALCOLPAIGES I___I
102. **QUAL** bebida? 0. Cerveja 3. Pinga 1. Whisky 4. Vinho 2. Vodca 5. Outros, qual (is)?	105.BEBIDAPAI I___I
103. **QUANTOS** episódios (vezes) por semana?	106.QTOEPIPAII___I
104. Número de **DOSES** por episódio**:** Considere uma dose : **meia garrafa ou 1 lata de cerveja, um cálice de vinho ou 1 dose de bebidas destiladas (aguardente, *whisky*, etc.)**	107.DOSEPAII___I
105. Por quanto **TEMPO** (anos) o pai faz ou fez uso de bebida alcoólica?	108.TMPALCOOLPAII___I
106. **PAI** fez uso de **DROGAS** ilícitas durante a **GESTAÇÃO**: 0. Sim 1. Não (se **NÃO**, vá para a **QUESTÃO 112**)	109.DROGAPAIGES I___I
107. **QUAL**? 0. Maconha 3. Cocaína 1. Crack 4. Não sei/não quis responder 2. Heroína 5. Outros, qual (is)?	110.QUALDROGPAI I___I
108. Quanto **TEMPO (**anos**)** foi usuário antes da gestação?	111.TEMUSDROP

	AI I___I___I
109. Qual a quantidade de **LÍQUIDO AMNIÓTICO** durante a gestação? 0. Normal 1. Pouco (oligoidrâmnio) 3. Muito (polidrâmnio) 4. Não sei/não lembro	112.LIQAMNIOTICOI___I
110. Fez uso de **SULFATO FERROSO**? 0. Sim 1. Não (se **NÃO**, vá para a **QUESTÃO 115**) 2. Não sei/não lembro	113.SULFATOFERRO I___I
111. Em **QUAL** período? 0. Alguns meses antes da gestação 1. Durante gestação 2. Antes e durante gestação 3. Após o parto 4. Não sei/não lembro	114.PERIODOSULFATOI___I
112. Fez uso de **ÁCIDO FÓLICO?** 0. Sim 1. Não (se **NÃO**, vá para a **QUESTÃO 117**) 2. Não sei/não lembro	115.USOACFOLICO I___I
113. Em **QUAL** período? 0. Alguns meses antes da gestação 1. Durante gestação 2. Antes e durante gestação 3. Não sei/não lembro	116.PERIODOACFOLICOI___I
114. Você teve **DEPRESSÃO PÓS-PARTO**? 0. Sim 1. Não 2. Não sei/não lembro	117.DEPRESAOPOSPA RTOI___I

EVENTOS OCORRIDOS NO PARTO

115. Entrou em **TRABALHO** de **PARTO** naturalmente? 0. Sim 1. Não 2. Não sei/não lembro	118.TRABPARTO I___I
116. Quanto tempo durou o **TRABALHO** de **PARTO**? 0. Menos de 8 horas 1. Entre 8 e 12 horas 2. Mais de 12 horas 3.Não sei/não lembro 4. Não entrei em trabalho de parto	119.TRABPARTODEMI___I
117. Houve indução por **OCITOCINA** (medicamento para aumentar as contrações)? 0. Sim 1. Não 2. Não sei/não lembro	120.OCITOCINAPARTOI___I
118. Qual foi o tipo de **PARTO**? 0. Cesárea 1. Normal (se **normal** vá para a **QUESTÃO 123**) 2. Outro, qual(is)?	121.TIPO PARTO I___I
119. Se **CESÁREO**: 0. Planejado 1. Forçado induzido/Urgência	122.PARTOCESAR I___I
120. Foi aplicada **ANESTESIA** no parto? 0. Sim 1. Não (se **NÃO**, vá para a **QUESTÃO 125**) 2. Não sei/não lembro	123.ANESTPARTO I___I
121. **QUAL**? 0. Peridural 3. Raquidiana 6. Local 1. Geral 4. Combinada (peridural + Raquidiana) 2. Não sei/não lembro 5. Outros, qual (is)?	124.QUALANESTESIAI___I

122. **ROMPEU** precocemente a bolsa (após 20 semanas de gestação e antes do início das contrações que indicam o trabalho de parto)? 0. Sim 1. Não 2. Não sei/não lembro	125.RUPTURAMEM I___I

FATORES PERINATAIS DA CRIANÇA

123. A criança **NASCEU** com quantas **SEMANAS**?	126.IGRN I___I___I
124. **SEXO**: 0. Masculino 1. Feminino	127.SEXORN I___I
125. **PESO** ao nascer em gramas:	128.PESONASC I___I___I___I___I
126. **ESTATURA**: _____ cm.	129.ESTATRN I___I___I
127. **APGAR** 1. 1º min _____ 2. 5º min _____	130.APGAR1 I___I___I 130.1APGAR5 I___I___I
128. Circunferência da **CABEÇA**: Ao nascer:_____cm 1 ano:_____cm 5 anos_____cm	131.CIRCUNCABNI___I___I 131.1.CIRCUNCABUMI___I___I 131.2CIRCUNCABDOI___I___I
129. Apresentação **FETAL** normal (cabeça estava encaixada)? 0. Sim 1. Não 2. Não sei/Não lembro	132.APRESFETAL I___I
130. **PRESENÇA** de **CHORO** ao nascer? 0. Sim 1. Não 2. Não sei/Não lembro	133.PRESCHORO I___I
131. O recém-nascido teve **SOFRIMENTO FETAL**, lesão ou trauma no nascimento? 0. Sim 1. Não 2. Não sei/Não lembro	134.SOFRIFETALI___I
132. O recém-nascido teve **HIPÓXIA** (faltou oxigênio) fetal? 0. Sim 1. Não 2. Não sei/Não lembro	135.HIPOXIAFETAL I___I
133. O recém-nascido teve dificuldade de iniciar **RESPIRAÇÃO** ao nascer? 0. Sim 1. Não 2. Não sei/Não lembro	136.DIFRESPIRAR I___I
134. O recém-nascido recebeu **TRATAMENTO** com **OXIGÊNIO**? 0. Sim 1. Não 2. Não sei/Não lembro	137.TRAOXIG I___I
135. O recém-nascido teve complicações com o **CORDÃO UMBILICAL**? 0. Sim 1. Não 2. Não sei/Não lembro	138.COMCORDUM I___I
136. O recém-nascido teve **ICTERÍCIA** (nasceu amarelinho)? 0. Sim 1. Não 2. Não sei/Não lembro	139.RNICTERÍCIA I___I

137. Presença de **MECÔNIO** (presença de fezes no líquido amniótico)? 0. Sim 1. Não 2. Não sei/Não lembro	140.MECONIO I___I
138. O recém-nascido nasceu com **ANEMIA**? 0. Sim 1. Não 2. Não sei/Não lembro	141.ANEMIANEO I___I
139. O recém-nascido teve alguma **INFECÇÃO**? 0. Sim 1. Não (se **NÃO**, vá para a **QUESTÃO 144**) 2. Não sei/Não lembro	142.INFECÇAONEO I___I
140. **QUAL**? 0. Conjuntivite 1. Pneumonia 2. Meningite 3. Sepse (infecção generalizada) 4. Outros, qual (is)?	143.QUALINFECNEO I___I
141. O recém-nascido teve **FEBRE**? 0. Sim 1. Não 2. Não sei/Não lembro	144.FEBRERNI___I

FATORES PÓS-NATAIS (do nascimento até a data atual)

142. O Bebê recebeu **ALEITAMENTO MATERENO**? 0. Sim 1. Não (Se **NÃO**, Vá para a **QUESTÃO 147**)	145.LEITEMATERNI___I
143. Quanto tempo (em meses) durou o **ALEITAMENTO** materno **EXCLUSIVO**?	146.ALEITMATEXCLUI___I
144. A partir de qual mês foi introduzido o **LEITE** de **VACA** ou outro tipo de leite?	147.LEITEVACAMESI___I__I
145. Qual é o **TIPO SANGUÍNEO** da **CRIANÇA**? 0. A 1. B 2. AB 3. O 4. Não sei/Não lembro	148.TIPOSANFILHO I___I
146. Qual é fator **RH** da criança: 0. Positivo 1. Negativo 2. Não sei/Não lembro	149.FATORHFILHO I___I
147. A criança ficou **INTERNADA** no CTI e/ou UTI? 0. Sim 1. Não (se **NÃO**, vá para a **QUESTÃO 152**) 2. Não sei/não lembro	150.INTERÇAOFILHO I___I
148. Quanto **TEMPO** em dias?	151.TEMPO I___I___I___I
149. A criança fez **CIRURGIA**? 0. Sim 1. Não (se **NÃO**, vá para a **QUESTÃO 154**)	152.CIRURGIAFILHOI___I
150. **QUAL**? 0. Coração 1. Hérnia inguinal 2. Hérnia umbinical 3. Fimose 3. Intestino 4. Adenoide/amidala 5. Outros, qual (is)?	153.QUALCIRFILHO I___I
151. A criança tem **EPILEPSIA**? 0. Sim 1. Não 2. Não sei/Não lembro	154.EPILEPSIAFILHO I___I
152. A criança tem ou teve **CONVULSÕES**?	155.CONVUL

0. Sim 1. Não (se **NÃO**, vá para a **QUESTÃO 158**) 2. Não sei/Não lembro	FILHO I___I
153. Qual o **TOTAL** de eventos convulsivos ao longo da vida?	156.TOTALCONVULSAOI___I
154. O **PRIMEIRO** evento convulsivo ocorreu com **QUANTOS** anos?	157.PRIMEIRACONVUI___I
155. A criança teve **TRAUMATISMO CRANIANO ENCEFÁLICO** (hemorragia/hematoma na cabeça)? 0. Sim 1. Não 2. Não sei/Não lembro	158.TRAUMACEI___I
156. A criança teve **INFLAMAÇÃO** do **SISTEMA NERVOSO** (meningite, encefalite)? 0. Sim 1. Não 2. Não sei/Não lembro	159.INFLAMAÇAOSNC I___I
157. A criança teve **DIAGNÓSTICO** de **Transtorno do Espectro do Autismo/TEA**? 0. Sim 1. Não (se **NÃO** vá para a **QUESTÃO 162)**	160.FILHOTEAI___I
158. Quantos **ANOS** a criança tinha quando recebeu o **DIAGNÓSTICO**?	161.IDADEDIAGTEAII___I___I
159. A **CRIANÇA** nasceu com alguma **MALFORMAÇÃO** e/ou **DOENÇA GENÉTICA**? 0. Sim 1. Não (se **NÃO** vá para a **QUESTÃO 164**)	162.NASCMALFILHO I___I
160. **QUAL**? 0. Síndrome de Down 3. Síndrome de Rett 5. Fenilcetonúria 1. X frágil 4. Outros, qual (is)? 2. Não identificada	163.QUALMALFILHO I___I
161. Você teve algum **OUTRO FILHO** com alguma **MALFORMAÇÃO**? 0. Sim 1. Não (se **NÃO**, vá para a **QUESTÃO 166**)	164.OUTFILHOMALFOR I___I
162. **QUAL** malformação**?** 0. Síndrome de Down 3. Síndrome de Rett 1. X frágil 4. Outros, qual (is)? 2. Não identificada	165.QUALMALOUTFIL I___I
163. A criança tomou a **VACINA TRÍPLICE VIRAL** (contra sarampo, caxumba e rubéola) dada aos bebês aos 12 meses? 0. Sim 1. Não (se **NÃO**, vá para a **QUESTÃO 170**) 2. Não sei/Não lembro	167.VACTRI I___I
164. A criança teve **FEBRE** após a vacina? 0. Sim 1. Não 2. Não sei/Não lembro	167.FEBREPOSVACII___I
165. Notou algum **COMPORTAMENTO** diferente na criança após tomar a vacina tríplice viral? 0. Sim 1. Não (se **NÃO**, vá para a **QUESTÃO 170**) 2**.** Não sei/Não lembro	168.COMPOSVACI___I
166. **QUAL**? 0. Agressividade 3. Mais calmo 1. Sonolência 4. Outro, qual (is)? 2. Parou de falar	169.QUACOMVAC I___I
167. A criança é **ALÉRGICA**? 0. Sim 1. Não (se **NÃO**, vá para a **QUESTÃO 172**) 2. Não sei/Não lembro	170.ALERGIAFILHO I___I

168. A **QUE**? 0. Leite 4. Glúten 7. Poeira 1. Lactose 5. Cheiro 2. Pêlos de animais 6. Corante 3. Outros. Qual(is)?	171.ALERGAQUEI___I
169. A criança possui histórico frequente de **CONSTIPAÇÃO** ou **DIARREIA** até 1 ano de idade? 0. Sim 1. Não 2. Não sei/Não lembro	172.DIARREIABEBEI___I
170. A criança fez/faz alguma **RESTRIÇÃO ALIMENTAR**? 0. Sim 1. Não (se **NÃO**, vá para a **QUESTÃO 176**)	173.RESTALIMI___I
171. **QUAL**? 0. Caseína 1. Glúten 2. Lactose 3.Outros carboidrato 4. Outro, qual (is)?	174.QUALRESALIM I___I
172. A criança **MELHOROU** as queixas intestinais **APÓS** a restrição alimentar? 0. Sim 1. Não 2. Não sei	175.MELHORAAPOSRAI___I
173. A criança faz ou fez uso de **VITAMINAS** e/ou **MINERAIS**? 0. Sim 1. Não (se **NÃO**, vá para a **QUESTÃO 181**)	176.USOVITMII___I
174. **QUAL**? 0. Vitaminas do complexo B. QUAL?____ 2.Vitamina D 1. Ferro 3.Multivitamínicos 4. Outro, qual (is)?	177.QUALVITMII___I
175. Por quanto **TEMPO** (meses)?	178.TEMPOVITMII___I___I
176. Você achou que a criança **MUDOU** o **COMPORTAMENTO** após o uso de vitaminas/restrição alimentar? 0. Sim 1. Não (se NÃO, vá para a **QUESTÃO 181**) 2. Não sei/não lembro	179.MUDOBSPOSVITRA I___I
177. **QUAL** mudança observada? 0. Mais concentrado 4. Mais calmo 1. Mais flexível 5. Mais sonolento 2. Mais agressivo 6. Intolerante 3. Nervoso 7. Outros, qual (is)?	180.QUALMUTNMED I___I
178. A criança faz ou fez uso de algum **HORMÔNIO**? 0. Sim 1. Não (se **NÃO**, vá para a **QUESTÃO 186**)	181.USOHORMI___I
179. **QUAL**? 0. Melatonina 3. Hormônio do crescimento (GH) 1. Tiroxina 4. Outro, qual (is)? 2. Ocitocina	182. QUALHORMI___I
180. Por quanto **TEMPO** (meses)?	183. HORMTEMPOI__

	_I___I
181. Você achou que seu filho **MUDOU** o comportamento após o uso de **HORMÔNIO**? 0. Sim 1. Não (se **NÃO**, vá para a **QUESTÃO 188**) 2.Não sei/não lembro	184.MUDCOMHORI___I
182. **QUAL** mudança observada? 0. Mais concentrado 4. Mais calmo 1. Mais flexível 5. Mais sonolento 2. Mais agressivo 6. Intolerante 3. Nervoso 7. Outros, qual (is)?	185.QUALMUDHORI___I

FATORES FAMILIARES

183. Há casos de **Transtorno do Espectro do Autismo/TEA** na **FAMÍLIA**? 0. Sim 1. Não (se **NÃO**, vá para a **QUESTÃO 188**) 2. Não sei/Não lembro	186.FAMTEAI___I
184. **QUEM (em relação à criança)**? 0. Mãe 4. Pai 8. Outros, qual (is)? 1. Irmã/irmão 5.Tio (a) Paterno 2. Tio (a) Materna 6. Avó/avô Paterno 3. Avó/avô Materno 7. Primo (a) de primeiro grau	187.QUEMTEAFAMI___I
185. Há casos de **HIPERATIVIDADE** diagnosticados na família? 0. Sim 1. Não (se **NÃO**, vá para a **QUESTÃO 190**) 2. Não sei/Não lembro	188.FAMHIPERI___I
186. **QUEM (em relação à criança)**? 0. Mãe 4. Pai 8. Outros, qual (is)? 1. Irmã/irmão 5.Tio (a) Paterno 2. Tio (a) Materna 6. Avó/avô Paterno 3. Avó/avô Materno 7. Primo (a) de primeiro grau	189. QUEMHIPERI___I
187. Há casos de **RETARDO MENTAL** diagnosticados na família? 0. Sim 1. Não (se **NÃO**, vá para a **QUESTÃO 192**) 2. Não sei/Não lembro	190.RMENTFAMI___I
188. **QUEM (em relação à criança)**? 0. Mãe 4. Pai 8. Outros, qual (is)? 1. Irmã/irmão 5.Tio (a) Paterno 2. Tio (a) Materna 6. Avó/avô Paterno 3. Avó/avô Materno 7. Primo (a) de primeiro grau	191.QUEMRMFAM I___I
189. Há casos de **MALFORMAÇÃO** e/ou **DOENÇA GENÉTICA** diagnosticado na família? 0. Sim 2. Não (se **NÃO**, vá para a **QUESTÃO 195**) 2. Não sei/Não lembro	192.MALFAM I___I
190. **QUAL**? 0. Síndrome de Down 3. Síndrome de Rett 1. X frágil 4. Outros, qual (is)? 2. Não identificada	193.QUALMALFAMI___I
191. **QUEM (em relação à criança)**?	194.QUEMMALFA

0. Mãe 4. Pai 8. Outros, qual (is)? 1. Irmã/irmão 5.Tio (a) Paterno 2. Tio (a) Materna 6. Avó/avô Paterno 3. Avó/avô Materno 7. Primo (a) de primeiro grau	M I___I
192. Há casos de **EPILEPSIA** diagnosticados na família? 0. Sim 1. Não (se **NÃO**, vá para a **QUESTÃO 197**) 2. Não sei/Não lembro	195.EPLEPSIFAM I___I
193. **QUEM (em relação à criança)**? 0. Mãe 4. Pai 8. Outros, qual (is)? 1. Irmã/irmão 5.Tio (a) Paterno 2. Tio (a) Materna 6. Avó/avô Paterno 3. Avó/avô Materno 7. Primo (a) de primeiro grau	196.QUEMEPIL FAM I___I
194. Há casos de **DIABETES** diagnosticados na família? 0. Sim 1. Não (se **NÃO**, vá para a **QUESTÃO 199**) 2. Não sei/Não lembro	197.DIABETEFAM I___I
195. **QUEM (em relação à criança)**? 0. Mãe 4. Pai 8. Outros, qual (is)? 1. Irmã/irmão 5.Tio (a) Paterno 2. Tio (a) Materna 6. Avó/avô Paterno 3. Avó/avô Materno 7. Primo (a) de primeiro grau	198.QUEMDIA BFAM I___I
196. Há casos de **HIPERTENSÃO** diagnosticados na família? 0. Sim 1. Não (se **NÃO**, vá para a **QUESTÃO 201**) 2. Não sei/Não lembro	199.HIPERTEN SAOFAMI___I
197. **QUEM (em relação à criança)**? 0. Mãe 4. Pai 8. Outros, qual (is)? 1. Irmã/irmão 5.Tio (a) Paterno 2. Tio (a) Materna 6. Avó/avô Paterno 3. Avó/avô Materno 7. Primo (a) de primeiro grau	200.QUEMHIPE RTFAMI___I
198. Há casos de **CÂNCER** diagnosticados na família? 0. Sim 1. Não (se **NÃO**, vá para a **QUESTÃO 203**) 2. Não sei/Não lembro	201.CANCERFAM I___I
199. **QUEM (em relação à criança)**? 0. Mãe 4. Pai 8. Outros, qual (is)? 1. Irmã/irmão 5.Tio (a) Paterno 2. Tio (a) Materna 6. Avó/avô Paterno 3. Avó/avô Materno 7. Primo (a) de primeiro grau	202.QUEMCAN CERFAM I___I
200. Há casos de doença **AUTOIMUNE** diagnosticados na família? 0. Sim 1. Não (se **NÃO**, vá para a **QUESTÃO 205**) 2. Não sei/Não lembro	203.AUTOIMUNEF AMI___I
201. **QUEM (em relação à criança)**? 0. Mãe 4. Pai 8. Outros, qual (is)? 1. Irmã/irmão 5.Tio (a) Paterno 2. Tio (a) Materna 6. Avó/avô Paterno 3. Avó/avô Materno 7. Primo (a) de primeiro grau	204.QUEMAUTOIM UNEFAMI___I
202. Há história de **MORTE** de crianças na família por **MALFORMAÇÕES**? 0. Sim 1. Não (se **NÃO**, vá para a **QUESTÃO 207**) 2. Não sei/Não lembro	205.OBMA LFAM I___I
203. **QUEM (em relação à criança)**? 0. Mãe 4. Pai 8. Outros, qual (is)? 1. Irmã/irmão 5.Tio (a) Paterno 2. Tio (a) Materna 6. Avó/avô Paterno 3. Avó/avô Materno 7. Primo (a) de primeiro grau	206.QUEMOBMAL FAMI___I
204. As avós ou algum parente próximo tiveram **ABORTOS**? 0. Aborto único 1. Aborto repetidos 2. Não	207.ABORTOF

	AM I___I
205. Você e o pai da criança são **PARENTES**? 0. Sim 1. Não	208.CONSANGUEP AIS I___I
206. Há **CONSANGUINIDADE** (filhos entre parentes) na família? 0. Sim 1. Não 2. Não sei/Não lembro	209.CONSANGUEF AM I___I
207. **QUEM (refere ao parente da criança)**? 0. Mãe 4. Pai 8. Outros, qual (is)? 1. Irmã/irmão 5.Tio (a) Paterno 2. Tio (a) Materna 6. Avó/avô Paterno 3. Avó/avô Materno 7. Primo (a) de primeiro grau	210.QUEMCON SANGUE I___I

DADOS SÓCIO-ECONÔMICOS

208. A **ÁGUA** utilizada no seu domicilio é **PROVENIENTE** de? 0. Rede geral de distribuição 1. Poço ou nascente 2. Outro meio	
209. Considerando o trecho da rua do seu domicílio, você diria que a **RUA** é: 0. Asfaltada/Pavimentada 1. Terra/Cascalho	
210. Qual é o **GRAU** de **INSTRUÇÃO** do **CHEFE** da família? Considere como chefe da família a pessoa que contribui com a maior parte da renda do domicílio. 0. Analfabeto / Fundamental I incompleto 1. Fundamental I completo / fundamental II incompleto 2. Fundamental completo/médio incompleto 3. Médio completo / superior incompleto 4. Superior completo	

APOIO:

FAPEMIG
Fundação de Amparo à Pesquisa do Estado de Minas Gerais

Unimontes
Universidade Estadual de Montes Claros

anda
ASSOCIAÇÃO NORTE MINEIRA DE APOIO AO AUTISTA
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NÚCLEO DE PESQUISA, REABILITAÇÃO E INCLUSÃO

APÊNDICE B - Termo de Consentimento Livre e Esclarecido

TERMO DE CONSENTIMENTO LIVRE E ESCLARECIDO PARA PARTICIPAÇÃO EM PESQUISA

Título da pesquisa: Transtorno do Espectro do Autismo em crianças e adolescentes: um estudo de caso-controle na cidade de Montes Claros-MG

Instituição promotora: Universidade Estadual de Montes Claros

Patrocinador:FAPEMIG

Coordenador: Marise Fagundes Silveira

Atenção: Antes de aceitar participar desta pesquisa, é importante que você leia e compreenda a seguinte explicação sobre os procedimentos propostos. Esta declaração descreve o objetivo, metodologia/procedimentos, benefícios, riscos, desconfortos e precauções do estudo. Também descreve os procedimentos alternativos que estão disponíveis a você e o seu direito de sair do estudo a qualquer momento. Nenhuma garantia ou promessa pode ser feita sobre os resultados do estudo.

1-Objetivo: Investigar os fatores associados ao Transtorno do Espectro do Autismo/TEA em crianças e adolescentes da cidade de Montes Claros com diagnóstico desse transtorno.

2-Metodologia/procedimentos: Será realizado um estudo de caso-controle para investigar a associação entre o TEA e as variáveis sócio-econômicas, demográficas e etiológicas. A população-alvo do presente estudo será constituída pelas mães de crianças e adolescentes com diagnóstico do TEA. Para cada mãe de criança/adolescente com o TEA (grupo caso), serão selecionadas duas mães de crianças e adolescentes que não apresentam características do TEA (grupo controle). As crianças e adolescentes que compuserem o grupo controle serão da mesma idade e classe sócio-econômicas. Será aplicado às mães do grupo controle o mesmo questionário aplicado no grupo caso.

3-Justificativa: A prevalência do TEA aumentou ao longo dos últimos anos, o que justifica uma necessidade crescente em determinar a contribuição dos fatores de risco associados com o TEA. Identificar os fatores sócio-econômicas e demográficos, bem como os fatores pré, peri e neonatais pode melhorar a prevenção da doença, o diagnóstico precoce e antecipar o início do tratamento. Embora não haja nenhuma cura conhecida, o diagnóstico precoce e a intervenção imediata contribui para reduzir a probabilidade de cronificação do TEA, aumentam as possibilidades de tratamento e minimizam vários sintomas.

4-Benefícios: Este projeto propõe contribuir, de forma significativa, para a melhoria das políticas públicas de saúde: no estabelecimento de programas, na disponibilização dos serviços, na redução dos custos com esses serviços e no preparo dos profissionais para identificar e acolher famílias com maior probabilidade de ter filhos com o TEA. Além disso, trata-se de um estudo inédito no Brasil e acredita-se que os dados encontrados poderão contribuir com a ciência na busca de novas descobertas, incentivar o desenvolvimento de novos projetos e pesquisas e favorecer o crescente benefício da saúde e da qualidade de vida dessa população.

5- Desconfortos e riscos: Este estudo não apresenta desconforto nem risco para os envolvidos.

6- Danos: Este estudo não trará nenhum dano aos participantes.

7- Metodologia/procedimentos alternativos disponíveis: não se aplica.

8- Confidencialidade das informações: O pesquisador garante o sigilo e a confidencialidade dos dados coletados**.**

9- Compensação/indenização: Não se aplicam

10- Outras informações pertinentes

11- Consentimento:

Li e entendi as informações precedentes. Tive oportunidade de fazer perguntas e todas as minhas dúvidas foram respondidas a contento. Este formulário está sendo assinado voluntariamente por mim, indicando meu consentimento para participar nesta pesquisa, até que eu decida o contrário. Receberei uma cópia assinada deste consentimento.

Nome do participante	Assinatura do participante	Data
Nome da testemunha	Assinatura da testemunha	Data
Marise Fagundes Silveira		
Nome do Coordenador	Assinatura do Coordenador	Data

ENDEREÇO DO PESQUISADOR: Rua Três, 259 – Barcelona Parque - **TELEFONE:** 38 91813030

ANEXOS

ANEXO A - *Modified Checklist for Autism in Toddlers* (M-CHAT)

***Modified Checklist for Autism in Toddlers* (M-chat)**

1 Seu filho gosta de se balançar, de pular no seu joelho, etc?	Sim	Não
2. Seu filho tem interesse por outras crianças?	Sim	Não
3. Seu filho gosta de subir em coisas, como escadas ou móveis?	Sim	Não
4. Seu filho gosta de brincar de esconder e mostrar o rosto ou de esconde-esconde?	Sim	Não
5. Seu filho já brincou de faz-de-conta, como, por exemplo, fazer de conta que está falando no telefone ou que está cuidando da boneca, ou qualquer outra brincadeira de faz-de-conta?	Sim	Não
6. Seu filho já usou o dedo indicador dele para apontar, para pedir alguma coisa?	Sim	Não
7. Seu filho já usou o dedo indicador dele para apontar, para indicar interesse em algo?	Sim	Não
8. Seu filho consegue brincar de forma correta com brinquedos pequenos (ex. carros ou blocos), sem apenas colocar na boca, remexer no brinquedo ou deixar o brinquedo cair?	Sim	Não
9. O seu filho alguma vez trouxe objetos para você (pais) para lhe mostrar este objeto?	Sim	Não
10. O seu filho olha para você no olho por mais de um segundo ou dois?	Sim	Não
11. O seu filho já pareceu muito sensível ao barulho (ex. tapando os ouvidos)?	Sim	Não
12. O seu filho sorri em resposta ao seu rosto ou ao seu sorriso?	Sim	Não
13. O seu filho imita você? (ex. você faz expressões/caretas e seu filho imita?)	Sim	Não
14. O seu filho responde quando você chama ele pelo nome?	Sim	Não
15. Se você aponta um brinquedo do outro lado do cômodo, o seu filho	Sim	Não

olha para ele?		
16. Seu filho já sabe andar?	Sim	Não
17. O seu filho olha para coisas que você está olhando?	Sim	Não
18. O seu filho faz movimentos estranhos com os dedos perto do rosto dele?	Sim	Não
19. O seu filho tenta atrair a sua atenção para a atividade dele?	Sim	Não
20. Você alguma vez já se perguntou se seu filho é surdo?	Sim	Não
21. O seu filho entende os que as pessoas dizem?	Sim	Não
22. O seu filho às vezes fica aéreo, "olhando para o nada" ou caminhando sem direção definida?	Sim	Não
23. O seu filho olha para o seu rosto para conferir a sua reação quando vê algo estranho?	Sim	Não

Tradução Milena Pereira Pondé e Mirella Fiuza Losapio

ANEXO B - Parecer Consubstanciado do Comitê de Ética e Pesquisa

UNIVERSIDADE ESTADUAL DE MONTES CLAROS - UNIMONTES

PARECER CONSUBSTANCIADO DO CEP

DADOS DO PROJETO DE PESQUISA

Título da Pesquisa: Prevalência do Transtorno do Espectro do Autismo em Crianças Matriculadas na Educação Infantil de Escolas da Rede Pública e Privada da Mesorregião Norte de Minas.

Pesquisador: Fernanda Alves Maia

Área Temática:

Versão: 2

CAAE: 24933614.5.0000.5146

Instituição Proponente:

Patrocinador Principal: Financiamento Próprio

DADOS DO PARECER

Número do Parecer: 534.000

Data da Relatoria: 21/02/2014

Apresentação do Projeto:

A prevalência de casos do Transtorno do Espectro do Autismo- TEA tem aumentado de forma significativa durante as últimas décadas.

Objetivo da Pesquisa:

Investigar o Transtorno do Espectro do Autismo em crianças matriculada na educação infantil e associar os fatores socioeconômicos e etiológicos com o transtorno.

Avaliação dos Riscos e Benefícios:

Há risco de identificação de resultados falso-positivos por se tratar de diagnóstico com características subjetivas. Contudo os resultados poderão servir para planejamento de uma política de apoio aos portadores de TEA.

Comentários e Considerações sobre a Pesquisa:

Acredita-se que um estudo sobre a prevalência do TEA em crianças matriculadas nas
escolas de educação infantil possa contribuir de forma significativa para a melhoria nas políticas da educação e da saúde, no estabelecimento de programas, na disponibilização dos serviços, na redução dos custos com esses serviços e no preparo dos profissionais para identificar e diagnosticar crianças com TEA.

Endereço: Av.Dr Rui Braga s/n-Camp Univers Profº Darcy Rib
Bairro: Vila Mauricéia **CEP:** 39.401-089
UF: MG **Município:** MONTES CLAROS
Telefone: (38)3229-8180 **Fax:** (38)3229-8103 **E-mail:** maisa.leite@unimontes.br

UNIVERSIDADE ESTADUAL DE MONTES CLAROS - UNIMONTES

Considerações sobre os Termos de apresentação obrigatória:

Adequados.

Recomendações:

Apresentação de relatório final por meio da plataforma Brasil, em "enviar notificação".

Conclusões ou Pendências e Lista de Inadequações:

Aprovado.

Situação do Parecer:

Aprovado

Necessita Apreciação da CONEP:

Não

Considerações Finais a critério do CEP:

O projeto respeita os preceitos éticos da pesquisa em seres humanos, sendo assim somos favoráveis à aprovação do mesmo.

MONTES CLAROS, 19 de Fevereiro de 2014

Assinador por:
SIMONE DE MELO COSTA
(Coordenador)

Endereço: Av.Dr Rui Braga s/n-Camp Univers Profª Darcy Rib
Bairro: Vila Mauricéia **CEP:** 39.401-089
UF: MG **Município:** MONTES CLAROS
Telefone: (38)3229-8180 **Fax:** (38)3229-8103 **E-mail:** maisa.leite@unimontes.br

ANEXO C – Normas da revista *The Journal of Pediatrics* para o produto científico 1

General Information

The Journal of Pediatrics has an open access mirror journal, *The Journal of Pediatrics: X. The Journal of Pediatrics* publishes the following peer-reviewed (single-blind) material: Original Research Articles, Brief Reports, reviews of Medical Progress in pediatrics and related fields, Grand Rounds (clinicopathologic conferences [CPC] or didactic discussions), Commentaries, Association of Medical School Pediatric Department Chairs, Inc. (AMSPDC) commentaries, clinical pictures or images accompanied by a brief clinical description (Rediscovering the Physical Exam and Insights and Images), Letters to the Editor, Workshop/Symposium Summaries and Supplements. There is no charge to submit or publish in *The Journal*, unless an article contains color figures in the print version (See Figures). Authors choosing to publish articles as open access in *The Journal of Pediatrics: X* will pay an article publishing charge (APC), have a choice of license options, and retain copyright. *The Journal* does not publish animal studies or basic science articles without direct clinical relevance.

Duplicate/Prior/Overlapping Publication or Submission

Manuscripts are accepted for review with the stipulation that they are submitted solely to *The Journal of Pediatrics.*Submission of an article implies that the work described has not been published previously (except in the form of an abstract or as part of a published lecture or academic thesis or as an electronic preprint, see https://www.elsevier.com/postingpolicy) that it is not under consideration for publication elsewhere, that its publication is approved by all authors and tacitly or explicitly by the responsible authorities where the work was carried out, and that, if accepted, it will not be published elsewhere including electronically in the same form, in English or in any other language, without the written consent of the copyright-holder. Authors should disclose prior publication as an abstract or an electronic preprint in the Cover Letter.

If any part of a manuscript by the same author(s) contains any information that was previously published, is in press, or is under consideration by another publication, a reprint of the previous article or a copy of the other manuscript must be submitted to the Editor at the point of submission, with a justification or explanation by the authors of any potential overlap or duplication. It is not necessary to disclose submissions that were rejected by another journal.

The Editors are disinclined to publish more than one paper arising from the study of the same patient population. Please combine papers from the same study whenever possible. If you are unable to combine the papers, a reprint of the other article(s) or a copy of the other manuscript(s) must be submitted to the Editor at the point of submission, with a justification or explanation by the authors as to why the papers could not be combined.

If the Editor is made aware of such overlapping or duplicate manuscripts that have not been disclosed by the authors, a written explanation will be requested. If, in the judgment of the Editor, the explanation is inadequate, the submission will be rejected. If there is no disclosure, an appropriate official of the primary author's academic institution will be notified.

Conflict of Interest/Disclosure Policy

According to the World Association of Medical Editors (WAME):

"Conflict of interest (COI) exists when there is a divergence between an individual's private interests (competing interests) and his or her responsibilities to scientific and publishing activities such that a reasonable observer might wonder if the individual's behavior or judgment was motivated by considerations of his or her competing interests. COI in medical publishing affects everyone with a stake in research integrity including journals, research/academic institutions, funding agencies, the popular media, and the public. Journals are interested in COI as it relates to a specific manuscript.

"Everyone has COIs of some sort. Having a competing interest does not, in itself, imply wrongdoing. However, it constitutes a problem when competing interests could unduly influence (or be reasonably seen to do so) one's responsibilities in the publication process. If COI is not managed effectively, it can cause authors, reviewers, and editors to make decisions that, consciously or unconsciously, tend to serve their competing interests at the expense of their responsibilities in the publication process, thereby distorting the scientific enterprise. This consequence of COI is especially dangerous when it is not immediately apparent to others. In addition, the appearance of COI, even where none actually exists, can also erode trust in a journal by damaging its reputation and credibility."

Authors are required to disclose on the title page of the initial manuscript any potential, perceived, or real conflict of interest. Authors must describe the role of the study sponsor(s), if any, in 1) study design; 2) the collection, analysis, and interpretation of data; 3) the writing of the report; and 4) the decision to submit the manuscript for publication. Authors should include statements even when the sponsor had no involvement in the above matters.

Authors should also state who wrote the first draft of the manuscript and whether an honorarium, grant, or other form of payment was given to anyone to produce the manuscript. If the manuscript is accepted for publication, the disclosure statements will be published.

Editors who make decisions about manuscripts have no COI with the authors or their institutions, study group, research funders, overlapping (similar or competing) research, etc. A list of COI for all Editors and Editorial Board members is available at http://www.jpeds.com/content/ed_board_bios. If Editors or Editorial Board members have a COI for particular manuscripts, they must recuse themselves as the handling Editor, in which case the manuscript will be assigned to a new Editor. Editorial Board members will serve as Guest Editors when appropriate (e.g., the author is an Editor of *The Journal of Pediatrics*, the authors of a manuscript are at the Editor's institution, the Editor has recused him/herself for whatever reason). Editors and Editorial Board members are blinded to any submissions for which they are authors.

Reviewers are required to disclose any real or potential conflicts of interest, as outlined in the Guidelines for Reviewers.

Additional information regarding conflicts of interest can be found at http://www.wame.org/conflict-of-interest-editorial#ref1, "Conflict of Interest in Peer-Reviewed Medical Journals: The World Association of Medical Editors (WAME) Position on a Challenging Problem." (This Editorial may appear in other medical and biomedical journals whose editors are members of WAME.)

Formatting of Funding Sources

List funding sources on the title page of the manuscript in a standard way to facilitate compliance to funder's requirements. For example, Supported by the National Institutes of Health (<grant number xxx> [to <author's initials>]); the Bill & Melinda Gates Foundation, Seattle, WA (<grant number yyy>]); and the Centers for Disease Control and Prevention (<grant number zzz> [to <author's initials>]).

It is not necessary to include detailed descriptions on the program or type of grants and awards. When funding is from a block grant or other resources available to a university, college, or other research institution, submit the name of the institute or organization that provided the funding. If no funding has been provided for the research, please indicate on the title page that no funding was received.

Authorship Criteria

Authors are expected to consider carefully the list and order of authors before submitting their manuscript and provide the definitive list of authors at the time of the original submission. Each author's contributions must be detailed in the Authorship Agreement and Contribution form uploaded at initial submission. If there are questions or concerns about whether each person in the author list fulfills the criteria for authorship according to the International Committee of Medical Journal Editors' (ICMJE) "Recommendations for the Conduct, Reporting, Editing, and Publication of Scholarly Work in Medical Journals," we will request further information from the corresponding author and, if necessary, request additional details for each person's work. All individuals who fulfill ICMJE's conditions for authorship should be included in the author list. Individuals who have contributed to the study, but do not meet the requirements for authorship, should be included in the Acknowledgments section (e.g., Department Chair, "honorary author," anyone who provided technical or writing assistance). All authors of a submitted manuscript must sign the Authorship Agreement and Contribution form declaring that they meet ICMJE's Recommendations for authorship and agreeing to the publication of the article and must be included at the time of submission.

Although *The Journal* does not allow for "co-first" authorship per se, authors may indicate a maximum of two authors in the byline who contributed equally ("*" next to their names and "* contributed equally" at the end of the affiliations section). Please note, however, that this will not change how the authors appear in future citations to the article.

If the byline includes the name of a study group, a list of all members of the study group and their affiliations must be provided and would be published as an online Appendix.

Addition, Deletion, or Rearrangement of Author Names

The authorship list and author order should be determined **before** submitting to *The Journal of Pediatrics* and authorship contributions should be detailed on the Authorship Agreement and Contribution form uploaded at initial submission.

Before the accepted manuscript is published in an online issue: In accordance with the policies of the Committee on Publication Ethics (COPE), requests to add, remove, or rearrange author names must be e-mailed to the Editorial Office (journal.pediatrics@cchmc.org) from the corresponding author of the accepted manuscript and must include the reason the name should be added or removed, or the author names rearranged. Confirmation e-mails from each author that they agree with the addition, removal, or rearrangement is also required; in the case

of addition or removal of authors, this includes confirmation from the author being added or removed. Requests that are not sent by the corresponding author will be forwarded by the Editorial Office to the corresponding author, who must follow the procedure as described above. Note that the Journal Manager will inform the Editorial Office of any such requests, and online publication of the accepted manuscript will be suspended until authorship has been finalized.

After the accepted manuscript is published in an online issue: Any requests to add, delete, or rearrange author names in an article published in an online issue will follow the same policies as noted above and may result in an erratum.

Acknowledgments Section

The names, degrees, and affiliations, along with any conflicts of interest, funding sources, and industry-relation, of persons who have contributed substantially to a study but do not fulfill the criteria for authorship as outlined by the International Committee of Medical Journal Editors (ICMJE) are to be listed in the Acknowledgments section, which will be published in the print and/or online version of *The Journal of Pediatrics*. This section should include individuals who provided any writing, editorial, and/or statistical assistance, as well as Department Chairs, "honorary authors," etc. Authors should inform all individuals in the Acknowledgments section that they are being listed on the submission.

Open Researcher and Contributor ID (ORCID)

"ORCID is an open, non-profit, community-driven effort to create and maintain a registry of unique researcher identifiers and a transparent method of linking research activities and outputs to these identifiers." Authors are encouraged to create an ORCID account, which provides a unique identification number that can be linked to manuscripts and publications for which they serve as authors. This can be helpful in distinguishing authors with common names. ORCIDs can be linked to EES user accounts, and also may be helpful when compiling a list of authored publications. Additional information about ORCID is available at http://orcid.org/content/initiative.

Ethical Approval of Studies, Informed Consent, and Identifying Details

Studies on patients or volunteers require ethics committee and/or independent review board (IRB) approval, which should be documented in the Methods section of the paper. If this study was not approved by the appropriate ethics committee or IRB, include a statement as to why it was exempt.

Manuscripts describing research involving human subjects should indicate that written informed consent was obtained from the parents or guardians of the children who served as subjects of the investigation and, when appropriate, assent from the subjects themselves. In the event that either the Editors or the reviewers question the propriety of the human investigation with respect to the risk to the subjects or to the means by which informed consent was obtained, The *Journal of Pediatrics* may request more detailed information about the safeguards employed and the procedures used to obtain informed consent. Copies of the minutes of the committees that reviewed and approved the research also may be requested. Authors should verify compliance with the Health Insurance Portability & Accountability Act of 1996(HIPAA) prior to submission.

Additionally, manuscripts describing research involving human subjects should ensure that the work described has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans (http://www.wma.net/en/30publications/10policies/b3/index.html); Uniform Requirements for manuscripts submitted to biomedical journals (http://www.icmje.org).

Patients have a right to privacy. Therefore identifying information, including patients' images, names, initials, or hospital numbers, should not be included in videos, recordings, written descriptions, photographs, and pedigrees unless the information is essential for scientific purposes and you have obtained written informed consent for publication in print and electronic form from the patient (or parent, guardian, or next of kin where applicable). If such consent is made subject to any conditions, Elsevier must be made aware of all such conditions. Written consents must be provided to Elsevier on request.

Even where consent has been given, identifying details should be omitted if they are not essential. If identifying characteristics are altered to protect anonymity, such as in genetic pedigrees, authors should provide assurance that alterations do not distort scientific meaning and editors should so note. If such consent has not been obtained, personal details of patients included in any part of the paper and in any supplementary materials (including all illustrations and videos) must be removed before submission.

Reporting Guidelines

The Journal of Pediatrics endorses the use of an appropriate reporting guideline when writing any health research manuscript. You must submit a completed checklist and flow diagram for all randomized trials (CONSORT guidelines) and for all meta-analyses (PRISMA guidelines) at the time of initial submission.

We strongly urge authors to submit completed checklists for all other relevant guidelines (and flow diagram if applicable), such as STROBE (observational studies), CHERRIES (on-line surveys), SAMPL (statistical reporting), etc. Editable checklists for reporting guidelines can be found on the EQUATOR Network site, which

also provides general information on how to choose the correct guideline and why guidelines are important. Using a checklist helps to ensure you have used a guideline correctly.

At minimum, your article should report the content addressed by each item of the identified checklist or state that the item was not considered in the study and, if relevant, the reason why not (for example, if you did not use blinding, your article should explain this). Meeting these basic reporting requirements will greatly improve the value of your manuscript, may facilitate/enhance the peer review process, and may enhance its chances for eventual publication.

Checklists are not simply an administrative hurdle. We ask you to complete a checklist because this helps to ensure that you have included all of the important information in your article, and because it helps our editors and reviewers to complete the same check. If the checklist indicates an item that you have not addressed in your manuscript, please either explain in the manuscript text why this information is not relevant to your study or add the relevant information.

Table. Common types of studies and corresponding reporting guidelines. Some reporting guidelines are required at submission.

Study type	**Reporting guideline**	***The Journal of Pediatrics*' Policy**
Randomized trials	CONSORT	Required
Meta-analyses	PRISMA	Required
Observational studies	STROBE	Encouraged
Web-based surveys	CHERRIES	Encouraged
Case reports	CARE	Encouraged
Qualitative research	SRQR	Encouraged
Diagnostic/prognostic studies	STARD	Encouraged
Quality improvement studies	SQUIRE	Encouraged
Economic evaluations	CHEERS	Encouraged
Study protocols	SPIRIT	Encouraged
Statistical reporting	SAMPL	Encouraged

Clinical Trials Registration

The Journal of Pediatrics follows recommendations from the World Health Organization and the ICMJE pertaining to clinical trial registration and reporting. All manuscripts reporting results from clinical trials must be registered in an approved clinical trial registry **prior** to the enrollment of the first participant.

According to the World Health Organization:

"For the purposes of registration, *a clinical trial is any research study that prospectively assigns human participants or groups of humans to one or more health-related interventions to evaluate the effects on health outcomes.* Interventions include but are not restricted to drugs, cells and other biological products, surgical procedures, radiological procedures, devices, behavioral treatments, process-of-care changes, preventive care, etc."

According to the ICMJE:

"The purpose of clinical trial registration is to prevent selective publication and selective reporting of research outcomes, to prevent unnecessary duplication of research effort, to help patients and the public know what trials are planned or ongoing into which they might want to enroll, and to help give ethics review boards considering approval of new studies a view of similar work and data relevant to the research they are considering."

A list of International Committee of Medical Journal Editors (ICMJE)-approved clinical trial registries and additional guidelines for registering RCTs are available at: http://www.icmje.org/recommendations/browse/publishing-and-editorial-issues/clinical-trial-registration.html. If a trial was not registered prior to the enrollment of the first participant and/or it was registered in an unapproved registry, you must provide an explanation in the initial letter of submission, which will be assessed by the Editors on a case-by-case basis.

A completed CONSORT checklist and CONSORT flow diagram are required for clinical trials submitted to *The Journal of Pediatrics*. Please refer to the Reporting Guidelines section for additional information. You must include the site of the registry and the trial registration number at the end of the abstract, as well as the first time the trial

name is used in the manuscript (usually the Methods section). Finally, the dates of patients' enrollment must be included in the Results section. This information will be collected at the time of submission.

Negative Studies

The Journal of Pediatrics agrees with the International Committee of Medical Journal Editors (ICMJE) statement regarding the obligation to publish negative studies: "Editors should consider seriously for publication any carefully done study of an important question, relevant to their readers, whether the results for the primary or any additional outcome are statistically significant. Failure to submit or publish findings because of lack of statistical significance is an important cause of publication bias" (http://www.icmje.org/). *The Journal* seeks original work which then undergoes peer-reviewed scrutiny with editorial oversight. Over the years *The Journal* has accepted articles that clearly documented a lack of efficacy of therapeutic agents or procedures. *The Journal* believes that evidence-based medicine must be based on the best evidence, which may include negative studies.

Animal Studies

The Journal of Pediatrics does not publish animal studies without direct clinical relevance. If you believe that an animal study has direct clinical relevance, it must be carried out in accordance with the U.K. Animals (Scientific Procedures) Act, 1986 and associated guidelines, the EU Directive 2010/63/EU for animal experiments http://ec.europa.eu/environment/chemicals/lab_animals/legislation_en.htm; or the National Institutes of Health guide for the care and use of Laboratory animals (NIH Publications No. 8023, revised 1978) and it must indicate clearly in the manuscript that such guidelines have been followed. All animal studies need to ensure they comply with the ARRIVE guidelines http://www.nc3rs.org.uk/page.asp?id=1357. **Online Resources for Authors**
A list of online resources, including reporting guidelines and resources for publication ethics, which may be beneficial to English speaking and non-native English speaking authors, is available at http://www.jpeds.com/content/resourcesforauthors.

Preparation of Manuscripts

General Information

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Manuscripts must adhere to the American Medical Association's (AMA) Manual of Style, as well as additional layout and length guidelines, outlined below, using the default settings in Word (or other word processing software) for font size and margins (e.g., 12 point font, 1" margins). All text should conform to standard American English style and usage. Authors for whom English is not their native language are strongly encouraged to seek the aid of a professional English language medical editing service. Although *The Journal of Pediatrics* does not endorse any particular English language editing services, many are available online to edit your manuscript for a fee.

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It is the policy of *The Journal of Pediatrics* to publish new and original work. Text copied from copyrighted works from third parties, even in an introduction or methods section, should never be used without clearly identifying the other source (either by quotations or indentations). Every paper should present some novelty and new results in the form of a unique paper written in an author's own words. *The Journal of Pediatrics* uses CrossCheck powered by iThenticate software to screen for originality on all submitted manuscripts.

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The cover letter should include the following:

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- A statement of any potential conflict of interest, real or perceived; this includes a description of the role of the study sponsor(s), if any, in: (1) study design; (2) the collection, analysis, and interpretation of data; (3) the writing of the report; and (4) the decision to submit the paper for publication. Include statements even when the sponsor had no involvement in the above matters. This information must also appear on the title page of the manuscript. Additional information is available at http://jpeds.com/authorinfo#conf.

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Title Page

The title page should include authors' full names and highest academic degrees; departmental and institutional affiliations of each author; sources of financial assistance (see Formatting of Funding Sources) or potential conflicts of interest, if any (see Conflicts of Interest/Disclosure Policy), and disclose prior presentation of study data as an abstract or poster. A data sharing statement may also be listed on the title page (see Data Statement). Listed authors should include only those individuals who have made a significant, creative contribution to the manuscript as defined by the International Committee of Medical Journal Editors (www.icmje.org). The authorship list and author order should be determined **before** submitting to *The Journal of Pediatrics* and authorship contributions should be detailed on the Authorship Agreement and Contribution form uploaded at initial submission. One author must be designated as the correspondent, with complete address, business telephone number, fax number, and e-mail address. The corresponding author is responsible for communicating with the Editorial Office and all other co-authors; the Editorial Office will not provide status updates or decision information to anyone other than the corresponding author. Proofs and order forms for reprints will be sent to the corresponding author if the manuscript is published. Include a list of key words not in the title, as well as a short title (8-word maximum). Trade names of drugs and other products must not appear in the article title.

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A list of abbreviations and acronyms that appear >3 times should be included in the manuscript, along with the expansion of each. All abbreviations and acronyms should be expanded, followed by the abbreviation or acronym in parentheses, upon first use in the abstract, as well as in the first use in the body of the manuscript. All subsequent uses, including tables and figures, should use the abbreviation or acronym. Because abbreviations and acronyms are designed to assist readers, they should be limited to those defined in the AMA Manual of Style, those that are commonly used by general pediatricians, and those that shorten the names of study groups.

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Use nonproprietary names of drugs, devices, and other products, unless the specific trade name is essential to the discussion. The trade name may appear once in the Abstract and once in the Introduction or Methods section, followed by the nonproprietary name, manufacturer, and manufacturer location in parentheses; all other mention of the product must use the generic name. Trade names of drugs and other products must not appear in the article title.

Laboratory Values

Laboratory values should be described in metric mass units. The International System of Units (SI units) should be provided in parentheses immediately after metric units. Conversion tables are available (see JAMA 1986; 255:2329-39 or Ann Intern Med 1987; 106:114-29).

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References

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Examples of references (if 6 or fewer authors or editors, list all; if 7 or more, list first 6 and add et al):

For journal articles

Kramarz P, DeStefano F, Gargiullo PM, Chen RT, Lieu TA, Davis RL, et al. Does influenza vaccination prevent asthma exacerbations in children? J Pediatr 2001; 138:306-10.

Cozzi F, Morini F. Possible mechanisms of pacifier protection against SIDS [letter]. J Pediatr 2001;138:783.

For Articles in Press (online)
Hellems MA, Gurka KK, Hayden GF. A review of *The Journal of Pediatrics*: The first 75 years. J Pediatr (2008). doi:10.1016/j.jpeds.2008.08.049.

For books
Rosenstein BJ, Fosarelli PD. Pediatric pearls: the handbook of practical pediatrics. 3rd ed. St Louis: Mosby; 1997.

Virginia Law Foundation. The medical and legal implications of AIDS. Charlottesville (VA): The Foundation; 1987.

For chapters in books
Neufeld EF, Muenzer J. The mucopolysaccharidoses. In: Scriver CR, Beaudet AL, Sly WS, et al, eds. The metabolic and molecular bases of inherited diseases. New York: McGraw-Hill; 2001. p. 3421-52.

For websites
American Medical Association [homepage on the Internet]. Chicago: The Association; c1995-2002 [updated 2001 Aug 23; cited 2002 Aug 12]. AMA Office of Group Practice Liaison; [about 2 screens]. Available from: http://www.ama-assn.org/ama/pub/category/1736.html

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Tables are to be uploaded into EES as separate documents, formatted in .doc or .xls. A concise title should be supplied for each. Tables should be self-explanatory and should supplement, not duplicate the text. If a table or any data therein have been previously published, a footnote must give full credit to the original source. (See Permissions).

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A concise legend for each Figure must be included in the manuscript file, not in the Figure files. If a Figure has been previously published or has been adapted from a prior publication, the legend must give full credit to the original source.(See Permissions).

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Original Articles

Full-length manuscripts for the Original Articles section of *The Journal of Pediatrics* must include a structured abstract of less than 250 words, to appear after the title page, with the following headings: Objective(s), Study design, Results, and Conclusion(s). The Objective(s) should put the study in context with the current literature (i.e., what is new, not textbook background information) and reflect the purpose of the study, that is, the hypothesis that is being tested or the question being asked (e.g., "To assess…," "To evaluate…"). The Study design should include the study methodology, the setting for the study, the subjects (number and type), the treatment or intervention, principal outcomes measured, and the type of statistical analysis. The Results section should include the outcome of the study and statistical significance, if appropriate. The Conclusion(s) states the significance of the results and limitations of the study.

Do not include line numbers. Failure to comply with length restrictions may result in a delay in the processing of your paper. The following length targets are recommended for Original Articles:

Structured Abstract: less than 250 words (Objective must contain a concise hypothesis of 1-2 sentences, beginning with "To test…," "To assess…," "To evaluate…," etc., which is free of background information that is more appropriate for the Introduction.)
Introduction: 1 page
Methods: 2-3 pages
Results: 2-3 pages
Discussion: 3-5 pages
Graphics: No more than 4 tables + figures total for print consideration. Additional tables or figures can be considered for online-only content.
Total page length: 18 manuscript pages, including title page, *not including references and online-only content (Online-only content includes appendices, tables, figures, videos, audio clips, and PowerPoint presentations. Unless extremely long and detailed, portions of the manuscript should not be separated into online appendices.)

Brief Reports

Brief Reports are either (1) brief, focused studies, with a single question or hypothesis, related to a topic of interest to the general academic pediatrician; (2) a small series of diagnostic or therapeutic interventions that provide a novel observation or conclusion; or (3) "case reports" that provide novel insight into pathophysiology, diagnosis, or treatment of an entity that does not represent a coincidental association. Please note that Brief Reports are not designed to present information that is generally available in textbooks, even if the reported entity is novel. Brief Reports are designed to provide readers with new information and stimulate new approaches to diagnosis, clinical management, or research. Do not include line numbers. Brief Reports should be approximately 9 double-spaced, numbered manuscript pages (including the title page), a brief, unstructured abstract of <50 words, and a combined total of no more than 2 tables + figures for print consideration. Additional tables or figures can be considered for online-only content. Length targets do not include references and online-only content. (Online-only content includes appendices, tables, figures, videos, audio clips, and PowerPoint presentations. Portions of the manuscript should not be separated into online appendices.)

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all submissions must have a title. Submissions will undergo review by the Editors, and their decision to accept or reject will be final.

Do not submit a Quiz with your Insights and Images manuscript. The Editor selects which accepted Insights and Images articles should be highlighted on jpeds.com with a Quiz.

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Letters to the Editor

Letters to the Editor should pertain to papers published in *The Journal of Pediatrics* within the past year or to related topics and should not exceed 300 words. Provide a unique title for the Letter on the title page with complete contact information for the author(s). Double-space the text of the Letter. References, including reference to the pertinent article(s) in *The Journal*, should conform to style for manuscripts (see References).

The Editors may decide to send Letters to the Editor to the authors of the article about which the Letter was written for review and/or Reply. If the Editors choose to publish the Reply, it will be published in the same volume as the Letter to the Editor. Replies are not sent to Letter authors prior to publication.

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Authors who wish to propose a review article for the Medical Progress section must e-mail a proposal letter and formal academic outline of the manuscript (i.e., introduction, thesis statement, supporting ideas, and conclusion), identifying the article type for the Editors to assess, and outline to journal.pediatrics@cchmc.org for approval *before* submitting the full manuscript. (Editors will not assess full manuscripts prior to submission.) Medical Progress articles are a focused summary on the latest evidence-based advancements in a rapidly changing field. Practical guidelines, diagnostic algorithms, comment on case management issues, and summation of results of outcomes research may be appropriate. Articles considered for this section should not be review articles, opinion, or advocacy pieces. One or all contributing authors should be recognized expert(s) in the subject matter, as illustrated by their record of impactful publications in peer reviewed journals. Do not include line numbers. Medical Progress manuscripts should be approximately 18 double-spaced, numbered pages, including the title page, tables, and figures (not including references and online-only content). (Online-only content includes appendices, tables, figures, videos, audio clips, and PowerPoint presentations. Portions of the manuscript should not be separated into online appendices.)

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evidence and experience and emphasizing reasoning as practiced by master clinicians, similar to Grand Rounds presented at a major academic center. One or all contributing authors should be recognized expert(s) in the subject matter, as illustrated by their record of impactful publications in peer reviewed journals. Do not include line numbers. Grand Rounds manuscripts should be approximately 16 double-spaced, numbered pages, including the title page, tables, and figures (not including references and online-only content). (Online-only content includes appendices, tables, figures, videos, audio clips, and PowerPoint presentations. Portions of the manuscript should not be separated into online appendices.)

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Do not include line numbers. Workshop/Symposium Summary manuscripts should be approximately 18 double-spaced, numbered pages, including the title page, tables, and figures (not including references). If the manuscript significantly exceeds the suggested length target, it should be proposed as a sponsored Supplement to *The Journal* (see Supplement). An abstract should not be provided, and online only appendices, tables, and figures are not encouraged. However, authors are welcome to include videos, cartoons, audio clips, etc., as multi-media files (see Multi-Media).

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Announcements and Upcoming Events

Announcements of scheduled meetings, symposia, or postgraduate courses of interest to the pediatric readership may be sent to the Editorial Office via e-mail for consideration at least 2 months in advance of the meeting date or deadline. News items of general interest to pediatricians and related specialists will also be considered. Approved Announcements will be published in the online version of *The Journal of Pediatrics*. *The Journal* requests a reciprocal posting back to www.jpeds.com; however, the organization's decision to link to *The Journal's* website will not be a barrier to *The Journal's* willingness to post this Announcement or Event.

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Authors will receive e-mail notification from the Editorial Office of *The Journal of Pediatrics* after a decision has been made. It is very rare that peer-reviewed manuscripts are accepted upon initial submission. Requesting a revised manuscript should be seen as a positive step towards potential publication (although requesting a revision does not guarantee acceptance). We request that all revised manuscripts are submitted 4 weeks from the date of the revise decision. If you are unable to submit your revision in 4 weeks, we require that you send an e-mail to journal.pediatrics@cchmc.org, explaining why you are unable to submit a revision within the allotted time, as well as when you anticipate submitting the revision. We will then determine the merit of the requested extension.

All accepted manuscripts are subject to editorial revision and shortening. Authors should avoid redundancy between sections of text and between illustrations and text. Due to page limitations, the Editors may decide that figures, appendices, tables, acknowledgments, and other material will be published in the online version of *The Journal* and referenced in the print edition. Elsevier will automatically deposit any manuscripts that received funding from the National Institutes of Health (NIH) directly to PubMed Central as a service to authors, provided that NIH fundering is declared on the title page of the manuscript and Elsevier's copyright form. Following the deposit by Elsevier, authors will receive further communications from the NIH with respect to the submission. Additional information is available at https://www.elsevier.com/about/publishing-guidelines/policies/open-access-policies/funding-body-agreements/elsevier-nih-policy-statement.

Content Innovations

Inquiries Regarding Decisions
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This journal encourages and enables you to share data that supports your research publication where appropriate, and enables you to interlink the data with your published articles. Research data refers to the results of observations or experimentation that validate research findings. To facilitate reproducibility and data reuse, this journal also encourages you to share your software, code, models, algorithms, protocols, methods and other useful materials related to the project.

Below are a number of ways in which you can associate data with your article or make a statement about the availability of your data when submitting your manuscript. If you are sharing data in one of these ways, you are encouraged to cite the data in your manuscript and reference list. Please refer to the "References" section for more information about data citation. For more information on depositing, sharing and using research data and other relevant research materials, visit the research data page.

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To foster transparency, we encourage you to state the availability of your data in your submission. This may be a requirement of your funding body or institution. Data sharing statements should be included on the title page.

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Please visit our Open Access page for more information.

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The Journal of Pediatrics is pleased to announce the implementation of CrossMark, beginning with the August 2014 issue. CrossMark is a multipublisher initiative to provide a standard way for readers to locate the current version of an article. By implementing CrossMark, *The Journal* and Elsevier are committing to maintaining the content it publishes and alerting readers to changes if and when they occur. Clicking on the CrossMark logo will indicate whether an article is current or updates have been published. Additional information about CrossMark can be found on CrossMark's website (http://www.crossref.org/crossmark/), as well as The Editors' Perspective published in the August 2014 issue of *The Journal* (http://www.jpeds.com/article/S0022-3476(14)00537-X/fulltext).

Retraction Guidelines from the Committee on Publication Ethics (COPE)
The retraction guidelines published by the Committee on Publication Ethics (COPE) can be found at http://publicationethics.org/files/u661/Retractions_COPE_gline_final_3_Sept_09__2_.pdf

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Checklist for Manuscripts

Review Guide for Authors and instructions for submitting manuscripts through Elsevier Editorial System (EES), the electronic submission website at https://ees.elsevier.com/jpeds.

Please click here to find a table describing article types that appear in *The Journal of Pediatrics*.

• Cover letter

o Disclosure of prior publications or submissions (excluding rejected submissions) with any overlapping information, including studies and patients; a copy of the work(s) must be uploaded. Although poster presentations and abstracts are not considered duplicate publication, they should be stated in the cover letter. If there are no prior publications or submissions with any overlapping information, provide the following statement: "There are no prior publications or submissions with any overlapping information, including studies and patients." Additional information is available at http://jpeds.com/authorinfo#dup; o A statement of any potential conflict of interest, real or perceived; this includes a description of the role of the study sponsor(s), if any, in: (1) study design; (2) the collection, analysis, and interpretation of data; (3) the writing of the report; and (4) the decision to submit the paper for publication. Include statements even when the sponsor had no involvement in the above matters. This information must also appear on the title page of the manuscript. Additional information is available at http://jpeds.com/authorinfo#conf.

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- List of key words not in the title;
- Source of funding and conflict of interest statement, if applicable;

• Abstract (double-spaced), structured (less than 250 words) for Original Article or unstructured (50 words) for Brief Reports
• Article proper (double-spaced), including
- List of abbreviations (double-spaced)
- References (double-spaced), on a separate page
- Figure legends (double-spaced), on a separate page

• Tables including title (double-spaced), each on a separate page, saved as a separate file
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Updated April 2019

ANEXO D – Normas da revista *Journal of Autism and Developmental Disorders* para o produto científico 2

Manuscript Submission

Manuscript Submission

Submission of a manuscript implies: that the work described has not been published before; that it is not under consideration for publication anywhere else; that its publication has been approved by all co-authors, if any, as well as by the responsible authorities – tacitly or explicitly – at the institute where the work has been carried out. The publisher will not be held legally responsible should there be any claims for compensation.

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Please follow the hyperlink "Submit online" on the right and upload all of your manuscript files following the instructions given on the screen.

Please ensure you provide all relevant editable source files. Failing to submit these source files might cause unnecessary delays in the review and production process.

Title page

The title page should include:

- The name(s) of the author(s)
- A concise and informative title
- The affiliation(s) and address(es) of the author(s)
- The e-mail address, telephone and fax numbers of the corresponding author

Abstract

Please provide an abstract of 120 words or less. The abstract should not contain any undefined abbreviations or unspecified references.

Keywords

Please provide 4 to 6 keywords which can be used for indexing purposes.

Text

Text Formatting

Manuscripts should be submitted in Word.

- Use a normal, plain font (e.g., 10-point Times Roman) for text.
- Use italics for emphasis.
- Use the automatic page numbering function to number the pages.
- Do not use field functions.
- Use tab stops or other commands for indents, not the space bar.
- Use the table function, not spreadsheets, to make tables.
- Use the equation editor or MathType for equations.
- Save your file in docx format (Word 2007 or higher) or doc format (older Word versions).

Headings

Please use no more than three levels of displayed headings.

Abbreviations

Abbreviations should be defined at first mention and used consistently thereafter.

Footnotes

Footnotes can be used to give additional information, which may include the citation of a reference included in the reference list. They should not consist solely of a reference citation, and they should never include the bibliographic details of a reference. They should also not contain any figures or tables.

Footnotes to the text are numbered consecutively; those to tables should be indicated by superscript lower-case letters (or asterisks for significance values and other statistical data). Footnotes to the title or the authors of the article are not given reference symbols.

Always use footnotes instead of endnotes.

Acknowledgments

Acknowledgments of people, grants, funds, etc. should be placed in a separate section on the title page. The names of funding organizations should be written in full.

Body

- The body of the manuscript should begin on a separate page. The manuscript page header (if used) and page number should appear in the upper right corner. Type the title of the paper centered at the top of the page, add a hard return, and then begin the text using the format noted above. The body should contain:
- Introduction (The introduction has no label.)
- Methods (Center the heading. Use un-centered subheadings such as: Participants, Materials, Procedure.)
- Results (Center the heading.)
- Discussion (Center the heading.)

Headings

Please use no more than three levels of displayed headings.

Level 1: Centered

Level 2: Centered Italicized

Level 3: Flush left, Italicized

Footnotes

Center the label “Footnotes” at the top of a separate page. Footnotes can be used to give additional information, which may include the citation of a reference included in the reference list. They should not consist solely of a reference citation, and they should never include the bibliographic details of a reference. They should also not contain any figures or tables.

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Always use footnotes instead of endnotes. Type all content footnotes and copyright permission footnotes together, double-spaced, and numbered consecutively in the order they appear in the article. Indent the first line of each footnote 5-7 spaces. The number of the footnote should correspond to the number in the text. Superscript arabic numerals are used to indicate the text material being footnoted.

Author Note

The first paragraph contains a separate phrase for each author’s name and the affiliations of the authors at the time of the study (include region and country).

The second paragraph identifies any changes in the author affiliation subsequent to the time of the study and includes region and country (wording: “authors name is now at affiliation”.)

The third paragraph is Acknowledgments. It identifies grants or other financial support and the source, if appropriate. It is also the place to acknowledge colleagues who assisted in the study and to mention any special circumstances such as the presentation of a version of the paper at a meeting, or its preparation from a doctoral dissertation, or the fact that it is based on an earlier study.

The fourth paragraph states, “Correspondence concerning this article should be addressed to…” and includes the full address, telephone number and email address of the corresponding author.

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Terminology

- Please always use internationally accepted signs and symbols for units (SI units).

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Scientific style

- Generic names of drugs and pesticides are preferred; if trade names are used, the generic name should be given at first mention.
- Please use the standard mathematical notation for formulae, symbols etc.:Italic for single letters that denote mathematical constants, variables, and unknown quantities Roman/upright for numerals, operators, and punctuation, and commonly defined functions or abbreviations, e.g., cos, det, e or exp, lim, log, max, min, sin, tan, d (for derivative) Bold for vectors, tensors, and matrices.

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References

Citation

Cite references in the text by name and year in parentheses. Some examples:

- Negotiation research spans many disciplines (Thompson 1990).
- This result was later contradicted by Becker and Seligman (1996).
- This effect has been widely studied (Abbott 1991; Barakat et al. 1995; Kelso and Smith 1998; Medvec et al. 1999).

Ideally, the names of six authors should be given before et al. (assuming there are six or more), but names will not be deleted if more than six have been provided.

Reference list

The list of references should only include works that are cited in the text and that have been published or accepted for publication. Personal communications and unpublished works should only be mentioned in the text. Do not use footnotes or endnotes as a substitute for a reference list.

Reference list entries should be alphabetized by the last names of the first author of each work.

Journal names and book titles should be *italicized*.

- Journal article Harris, M., Karper, E., Stacks, G., Hoffman, D., DeNiro, R., Cruz, P., et al. (2001). Writing labs and the Hollywood connection. *Journal of Film Writing, 44*(3), 213–245.
- Article by DOI Slifka, M. K., & Whitton, J. L. (2000) Clinical implications of dysregulated cytokine production. *Journal of Molecular Medicine*, https://doi.org/10.1007/s001090000086
- Book Calfee, R. C., & Valencia, R. R. (1991). *APA guide to preparing manuscripts for journal publication.* Washington, DC: American Psychological Association.

- Book chapter O'Neil, J. M., & Egan, J. (1992). Men's and women's gender role journeys: Metaphor for healing, transition, and transformation. In B. R. Wainrib (Ed.), *Gender issues across the life cycle* (pp. 107–123). New York: Springer.
- Online document Abou-Allaban, Y., Dell, M. L., Greenberg, W., Lomax, J., Peteet, J., Torres, M., & Cowell, V. (2006). Religious/spiritual commitments and psychiatric practice. Resource document. American Psychiatric Association. http://www.psych.org/edu/other_res/lib_archives/archives/200604.pdf. Accessed 25 June 2007.

For authors using EndNote, Springer provides an output style that supports the formatting of in-text citations and reference list.

Tables

- All tables are to be numbered using Arabic numerals.
- Tables should always be cited in text in consecutive numerical order.
- For each table, please supply a table caption (title) explaining the components of the table.
- Identify any previously published material by giving the original source in the form of a reference at the end of the table caption.
- Footnotes to tables should be indicated by superscript lower-case letters (or asterisks for significance values and other statistical data) and included beneath the table body.

Each table should be inserted on a separate page at the back of the manuscript in the order noted above. A call-out for the correct placement of each table should be included in brackets within the text immediately after the phrase in which it is first mentioned. Copyright permission footnotes for tables are typed as a table note.

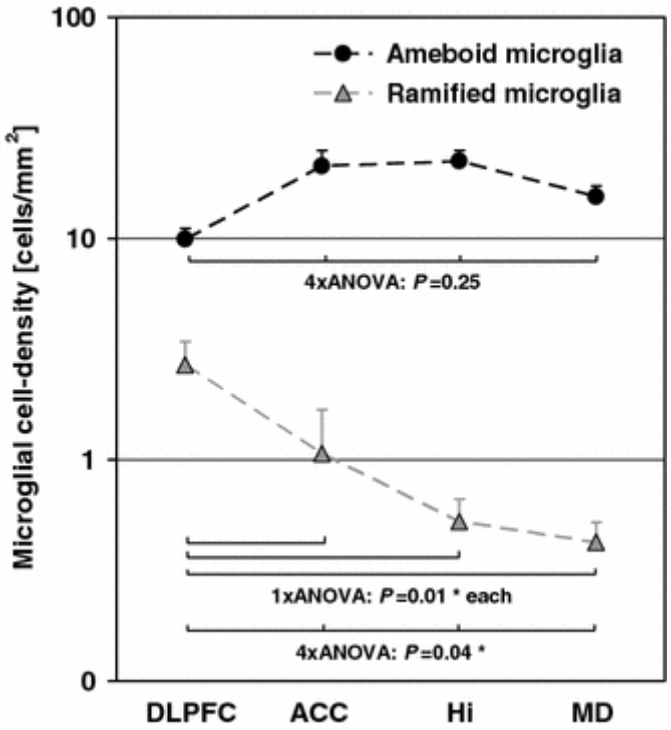
Artwork and Illustrations Guidelines

Electronic Figure Submission

- Supply all figures electronically.
- Indicate what graphics program was used to create the artwork.

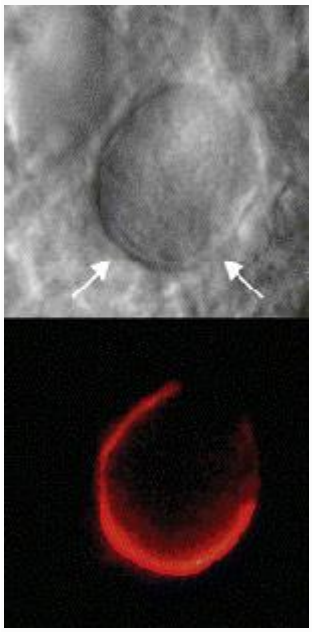
- For vector graphics, the preferred format is EPS; for halftones, please use TIFF format. MSOffice files are also acceptable.
- Vector graphics containing fonts must have the fonts embedded in the files.
- Name your figure files with "Fig" and the figure number, e.g., Fig1.eps.

Line Art



- Definition: Black and white graphic with no shading.
- Do not use faint lines and/or lettering and check that all lines and lettering within the figures are legible at final size.
- All lines should be at least 0.1 mm (0.3 pt) wide.
- Scanned line drawings and line drawings in bitmap format should have a minimum resolution of 1200 dpi.
- Vector graphics containing fonts must have the fonts embedded in the files.

Halftone Art



- Definition: Photographs, drawings, or paintings with fine shading, etc.
- If any magnification is used in the photographs, indicate this by using scale bars within the figures themselves.
- Halftones should have a minimum resolution of 300 dpi.

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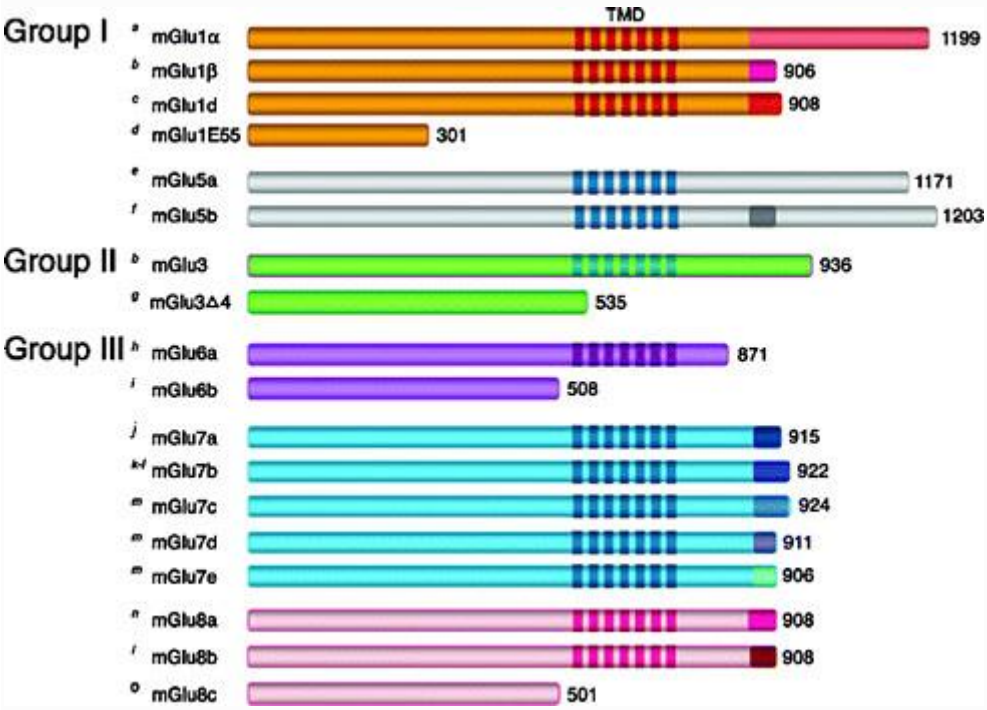

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- Combination artwork should have a minimum resolution of 600 dpi.

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- Color art is free of charge for online publication.
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- If the figures will be printed in black and white, do not refer to color in the captions.
- Color illustrations should be submitted as RGB (8 bits per channel).

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- To add lettering, it is best to use Helvetica or Arial (sans serif fonts).
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- Variance of type size within an illustration should be minimal, e.g., do not use 8-pt type on an axis and 20-pt type for the axis label.
- Avoid effects such as shading, outline letters, etc.
- Do not include titles or captions within your illustrations.

Figure Numbering

- All figures are to be numbered using Arabic numerals.
- Figures should always be cited in text in consecutive numerical order.
- Figure parts should be denoted by lowercase letters (a, b, c, etc.).
- If an appendix appears in your article and it contains one or more figures, continue the consecutive numbering of the main text. Do not number the appendix figures,"A1, A2, A3, etc." Figures in online appendices (Electronic Supplementary Material) should, however, be numbered separately.

Figure Captions

- Each figure should have a concise caption describing accurately what the figure depicts. Include the captions in the text file of the manuscript, not in the figure file.
- Figure captions begin with the term Fig. in bold type, followed by the figure number, also in bold type.
- No punctuation is to be included after the number, nor is any punctuation to be placed at the end of the caption.
- Identify all elements found in the figure in the figure caption; and use boxes, circles, etc., as coordinate points in graphs.
- Identify previously published material by giving the original source in the form of a reference citation at the end of the figure caption.

Figure Placement and Size

- Figures should be submitted separately from the text, if possible.
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- For large-sized journals the figures should be 84 mm (for double-column text areas), or 174 mm (for single-column text areas) wide and not higher than 234 mm.
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In order to give people of all abilities and disabilities access to the content of your figures, please make sure that

- All figures have descriptive captions (blind users could then use a text-to-speech software or a text-to-Braille hardware)
- Patterns are used instead of or in addition to colors for conveying information (colorblind users would then be able to distinguish the visual elements)
- Any figure lettering has a contrast ratio of at least 4.5:1

Figure caption sheet

The figure caption sheet contains a list of only the captions for all figures used. Center the label "Figure Captions" in uppercase and lowercase letters at the top of the page. Begin each caption entry flush left, and type the word "Figure", followed by the appropriate number and a period, all in italics. In the text of the caption (not italicized), capitalize only the first word and any proper nouns. If the caption is more than one line, double-space between the lines, and type the second and subsequent lines flush left. Table notes: Copyright permission footnotes for figures are typed as part of the figure caption.

- Each figure should appear on a separate page. The page where the figure is found should have the figure number and the word "top"[ie, Figure 1 top] typed above the figure. Figures or illustrations (photographs, drawings, diagrams, and charts) are to be numbered in one consecutive series of arabic numerals. Figures may be embedded in the text of a Word or Wordperfect document. Electronic artwork submitted on disk may be in the TIFF, EPS or Powerpoint format (best is 1200 dpi for line and 300 dpi for half-tones and gray-scale art). Color art should be in the CYMK color space. Assistance will be provided by the system administrator if you do not have electronic files for figures; originals of artwork may be sent to the system administrator to be uploaded. *** After first mention in the body of the manuscript, a call-out for the correct placement of each figure should be included in brackets on a separate line within the text.

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Electronic Supplementary Material

Springer accepts electronic multimedia files (animations, movies, audio, etc.) and other supplementary files to be published online along with an article or a book chapter. This feature can add dimension to the author's article, as certain information cannot be printed or is more convenient in electronic form.

Before submitting research datasets as electronic supplementary material, authors should read the journal's Research data policy. We encourage research data to be archived in data repositories wherever possible.

Submission

- Supply all supplementary material in standard file formats.
- Please include in each file the following information: article title, journal name, author names; affiliation and e-mail address of the corresponding author.
- To accommodate user downloads, please keep in mind that larger-sized files may require very long download times and that some users may experience other problems during downloading.

Audio, Video, and Animations

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- Supported file formats: avi, wmv, mp4, mov, m2p, mp2, mpg, mpeg, flv, mxf, mts, m4v, 3gp

Text and Presentations

- Submit your material in PDF format; .doc or .ppt files are not suitable for long-term viability.
- A collection of figures may also be combined in a PDF file.

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- Spreadsheets should be submitted as .csv or .xlsx files (MS Excel).

Specialized Formats

- Specialized format such as .pdb (chemical), .wrl (VRML), .nb (Mathematica notebook), and .tex can also be supplied.

Collecting Multiple Files

- It is possible to collect multiple files in a .zip or .gz file.

Numbering

- If supplying any supplementary material, the text must make specific mention of the material as a citation, similar to that of figures and tables.
- Refer to the supplementary files as “Online Resource”, e.g., "... as shown in the animation (Online Resource 3)", “... additional data are given in Online Resource 4”.
- Name the files consecutively, e.g. “ESM_3.mpg”, “ESM_4.pdf”.

Captions

- For each supplementary material, please supply a concise caption describing the content of the file.

Processing of supplementary files

- Electronic supplementary material will be published as received from the author without any conversion, editing, or reformatting.

Accessibility

In order to give people of all abilities and disabilities access to the content of your supplementary files, please make sure that

- The manuscript contains a descriptive caption for each supplementary material
- Video files do not contain anything that flashes more than three times per second (so that users prone to seizures caused by such effects are not put at risk)

ANEXO E - Carta de solicitação do uso do M-CHAT versão traduzida

UNIVERSIDADE ESTADUAL DE MONTES CLAROS
CENTRO DE CIÊNCIAS BIOLÓGICAS E DA SAÚDE
PROGRAMA DE PÓS GRADUAÇÃO EM CIÊNCIAS DA SAÚDE

Montes Claros, 05 de novembro de 2017.

Prezadas
Milena Pereira Pondé e Mirela Fiuza Losápio,

Espero que estejam bem e com saúde!
Sou Marise Fagundes Silveira, professora de bioestatística, pesquisadora e coordenadora do Programa de Pós-Graduação em Ciências da Saúde da Universidade Estadual de Montes Claros, MG (conceito 6-Capes). A Universidade Estadual de Montes Claros (UNIMONTES) está localizada na região Norte do estado de Minas Gerais.
Eu e meu grupo de pesquisa, investigamos sobre o Transtorno do Espectro do Autismo (TEA) e temos interesse em avaliar as propriedades psicométricas do *Modifi ed Checklist for Autism in Toddlers* (M-CHAT) quando aplicado em população no norte de Minas Gerais-Brasil. Temos interesse também em realizar um estudo de prevalência desse transtorno na nossa região e para tal pretendemos adotar o M-CHAT no rastreamento de crianças com sinais/sintomas do TEA. Esses dois estudos estão vinculados à instituição supracitada.
Nesta perspectiva, gostaria da sua autorização oficial para utilizar a versão traduzida, no Brasil, do *Modifi ed Checklist for Autism in Toddlers* (M-CHAT).

Estou à disposição para quaisquer esclarecimentos e antecipo agradecimentos,

Profa. Marise Fagundes Silveira
Coordenadora do Programa de Pós-Graduação e Ciências da Saúde-PPGCS
Universidade Estadual de Montes Claros - UNIMONTES

----- Mensagem encaminhada -----
De: Mirella Losapio <mfl_ssa@hotmail.com>
Para: Marise Fagundes <ciaestatistica@yahoo.com.br>
Enviado: terça-feira, 21 de novembro de 2017 09:19:43 BRST
Assunto: RE: Solicitação uso do M_CHAT versão traduzida para português

Bom dia Marise!
Desculpa a demora em responder.
Será uma satisfação contribuir de alguma forma com seu estudo, que é bastante interessante.
Se puder contribuir de mais alguma forma, estou à disposição.

Atenciosamente,

Mirella

De: Marise Fagundes <ciaestatistica@yahoo.com.br>
Enviado: domingo, 5 de novembro de 2017 14:29
Para: mfl_ssa@hotmail.com
Assunto: Solicitação uso do M_CHAT versão traduzida para português

Prezada Mirela,

Em anexo, solicitação de utilização da versão traduzida para o português do instrumento M-CHAT.

Att.,

Profª. Marise Fagundes
Universidade Estadual de Montes Claros
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