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ORIGINAL ARTICLE

Olfactory sensory evaluation in newborn children of women infected with COVID-19 during pregnancy

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Abstract

Introduction: In adults, olfactory loss is one of the earliest and most frequent acute clinical manifestations of SARS-CoV-2 infection. The number of children infected with SARS-CoV-2 is relatively small, perhaps due to the lower expression of Angiotensin Converting Enzyme 2 (ACE2) in children compared to adults. Little is known about foetal impairment in mothers infected with SARS-CoV-2. This paper describes an ongoing scientific project on smell perception in infants.

Objective: The goal of the study is to develop and validate a behavioural evaluative scale of olfactory perception in healthy newborns and to apply this scale to newborn children of women infected with COVID-19 during pregnancy comparing to newborn children of women without COVID-19 infection history, in order to compare these groups.

Methods: This is a retrospective comparative analytical cohort study of 300 newborns exposed and unexposed to COVID-19 during pregnancy. The data collection will follow the experimental procedure in a previous study that explored odours of the maternal breastmilk, vanilla (sweet) and distilled water (neutral). A coffee smell was implemented as an addition to this previous study in order to include acid/bitterness category to the categories of stimuli.

Discussion: It is feasible to argue the hypothesis of the involvement of the foetus' olfactory bulb as one of the indelible pathophysiological manifestations to the clinical diagnosis of COVID-19 with neurosensory olfactory deficit in foetuses and newborns affected by intrauterine infection. This study aims to investigate if newborn children of women infected with COVID-19 during pregnancy have olfactory sensory changes. The clinical trial was registered in the Brazilian Registry of Clinical Trials (ReBEC- RBR-65qxs2).

Keywords: newborn, perception, odors, COVID-19, SARS-CoV-2

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Authors summary

Why was this study done?
Olfactory loss in adults is one of the initial and most frequent acute clinical manifestations of SARS-CoV-2 infection. The few studies that have evaluated this alteration in the paediatric range have shown that children have less olfactory sensory loss than adults. One possible explanation would be the lesser expression of ACE2 in the nasal mucosa compared to adults with consequently less binding of SARS-CoV2. The authors propose to evaluate the action of SARS-CoV-2 at the intrauterine level regarding the foetus' olfactory sensory impairment.

What did the researchers do and find?
This study aims to assess the olfactory sensory perception of newborns of women who previously tested positive for COVID-19 during pregnancy compared to newborns of women who did not test positive for COVID-19 during pregnancy. It also aims to develop and validate a behavioural evaluation scale of olfactory sensory-perceptual perception in newborns. This work is based on the scientific literature on the sensory-perceptual development of smell in foetuses and newborns. Newborns will be exposed to four odours: breast milk, vanilla (sweet), coffee (bitter) and distilled water (neutral). The exposure order will be the same in all experiments.

What do these findings mean?
There is a lack of data in the literature regarding foetal impairment by SARS-CoV-2. Younger children have a lower incidence of COVID-19 symptoms compared to adults including low frequency of sensory impairment, especially smell. The results of this study will clarify whether intrauterine SARS-CoV-2 can affect the foetal sensory system or not.

INTRODUCTION

The natural history of COVID-19, caused by the SARThe natural history of COVID-19, caused by the SARS-CoV-2 virus, is still being explored. Little is known about its effects on pregnancy, the puerperium and the health of the mother and foetus, as well as the impact on the long-term development of the children of infected mothers during pregnancy. Based on the positive data to date, the clinical picture of COVID-19 during pregnancy does not seem to differ when observed outside this period. The disease can be asymptomatic or present with clinical manifestations that vary in severity and symptomology, with the most common symptoms being fever (67%) and cough (66%), accompanied less frequently by malaise, dyspnoea, and diarrhoea[1].

Vertical transmission of SARS-CoV-2 was previously thoroughly assessed in six pregnant women affected by the disease in the third trimester[2]. This study tested the amniotic fluid, umbilical cord blood, breast milk and nasopharyngeal swab of newborns, which were all negative for the virus. Subsequently, two systematic review and two studies describing cases of pregnancy infection in hospitals of Wuhan, China and New York City, United States, found no evidence of vertical transmission[1,3–5]. Inconclusive reports of vertical transmission include that of newborns with specific anti-SARS-CoV-2 IgM, but with negative RT-PCR[5], as well as that of a 16h newborn with positive RT-PCR with 16h of life, but specific IgG and IgM remained negative until the 5th day[6]. The interpretation of these results requires prospective studies, which are known for the accuracy of molecular tests and the kinetics of anti-SARS-CoV-2.

Maternal SARS-CoV-2 infection does not expose the foetus and newborn only to the effects caused directly by the virus, but also to a variety of indirect effects. The consequences of the maternal and foetal inflammatory response, with the production of potentially cytotoxic cytokines, as well as the effect of the use of antiviral medications, have not been studied to date. Another important aspect concerns the risk of contamination of the newborn during or shortly after birth. Practices such as delayed cord clamping and skin-to-skin contact between mothers and newborns are not universally recommended and evidence on the risk of contagion during breastfeeding is still limited[7,8].

There are studies reporting infected adults who developed neurological diseases, such as mental confusion, stroke, seizure, or loss of smell, due to a direct or indirect effect on the central nervous system (CNS). Likewise, a picture of acute haemorrhagic encephalopathy diagnosed through magnetic resonance imaging was reported in a woman in the sixth decade of life affected by COVID-19[9]. The prevalence of neurological impairment by the disease is still being observed, but a study of 214 patients in Japan showed that 36% had some clinical finding related to the nervous system[10]. Though, not enough data is provided about the risk of fetuses or newborn infants being vulnerable to neurological sequels due to the COVID-19 infection.

Problem

Among the symptoms provided by carriers of the new coronavirus, is an olfactory sensory alteration. Olfactory loss in adults is one of the earliest and most frequent acute clinical manifestations of SARS-CoV-2 infection. It is feasible to hypothesize the involvement of the foetus' olfactory bulb during intrauterine life as one of the indelible pathophysiological manifestations to the clinical diagnosis of COVID-19 with neurosensory olfactory deficit in foetuses and newborns affected by intrauterine infection. Based on these evidences, the following research question was raised: do newborn children of women infected with SARS-CoV-2 during pregnancy have olfactory sensory changes? Hence, this work is based on the scientific literature on the sensory-perceptual development of smell in foetuses and newborn babies[11-18] and aims to replicate methodological procedures of studies such as Bartocci *et al.*[12], adapting this to the hospital context and expanding it as categories of olfactory stimulus for the implementation of a measure of discrimination sensitivity.

The main goal of the study is to assess the olfactory sensory perception of newborn children of women infected with SARS-CoV-2 during pregnancy. The specific objectives are to develop a behavioural evaluation scale of olfactory perception in infants; to validate this scale in healthy newborns; and to compare the responses with that of newborn children of women infected with COVID-19 during pregnancy.

METHODS

Study Design

The present study is a retrospective comparative analytical cohort research of 300 newborns as part of a major project aiming to follow-up the development of children born from mothers that tested positively for COVID-19 during pregnancy entitled "Clinical outcomes of children of mothers exposed to SARS-CoV-2 infection during pregnancy".

Sample size calculation

No precise figure is available about the prevalence of SARS-CoV-2 infection among pregnant women in Brazil, but international reports estimate up to 15.3% of all pregnancies being exposed[1-4]. Recent data accounts for a birth rate of 44,195 newborns per year in the Federal District area, where Brasilia the capitol of Brazil, is located. Thus, considering an "infinite" population (>20.000 pregnant women), and assuming a 15% prevalence of SARS-CoV-2 exposed pregnancies, a confidence level of 95% and margin of error of 5%, the minimum size for a random sample of exposed women would be 195, yielding an expected similar figure for the exposed children. Taking into consideration an expected drop-out rate of up to 20% in the BORN sub-study, the required number of exposed mothers (to give birth to the BORN participants) would raise to 234.

Nevertheless, our sampling approach is based on convenience, not random chance, as eligible subjects present to the recruitment centres. The aforementioned calculations served primarily as a reference, so as not to overestimate the inclusion of participants. Given the limited available knowledge regarding the effects of SARS-CoV-2 on pregnancy and child development, thus conferring upon the study an eminently exploratory character, we adopted an approach of "as much as feasible, but no more than reasonable" for the sample size definition.

Everything considered, we set an a priori number of 300 exposed women in the PREGNANT phase, which would result in expected 300 exposed children in the BORN phase. We adopted a 1:1 allocation rate between exposed and controls, thus implying another 300 mothers and 300 children who did not test positive for COVID-19 to constitute the non-exposed control group. Hence, the overall sample size of the PROUDEST study was finally set at 1200 participants (600 mother-child dyads: 300 exposed, 300 control).

Study Location and Period

This study would take place at the outpatient clinic for newborns born to pregnant women with COVID -19 (and Regular Growth and Development Outpatient clinic with newborns born to pregnant women without Covid-19). This outpatient clinic was developed at the University Hospital of Brasília (HUB) aiming at accompanying these newborns up to five years of age. It is composed of a multidisciplinary team, such as paediatric doctors, nurses, occupational therapists, speech therapists and neuropsychologists. This study will investigate infants born between July 2020 and March 2021.

Study Population and Eligibility Criteria

The target group will be composed of newborns up to 14 days old, whose mothers tested positive on a test that detects genetic material of the virus using a laboratory technique called polymerase chain reaction (PCR) for SARS-CoV-2 during pregnancy. The control group will be composed of newborns up to 14 days old, whose mothers did not test positive for SARS-CoV-2 during pregnancy. The inclusion criteria for exposed mother-baby dyads are a maternal age of ≥18 years and laboratory evidence of SARS-CoV-2 infection during pregnancy through RT-PCR or serology (searching for IgG and IgM antibodies) markers. The results of the rapid test accompanied by information about the clinical symptoms or characteristic tomography were collected as supplementary data. For the control group of unexposed mother-baby dyads, the inclusion criteria are a maternal age of ≥18 years and serology for SARS-CoV-2 (IgG and IgM antibodies) at admission for delivery assistance. Exposed newborns will be included evidence of maternal SARS-CoV-2 infection during pregnancy.

Exclusion criteria for exposed women and unexposed women are: pre-existing chronic diseases requiring continuous use of medications, except diabetes and hypertension; smoking and / or alcohol consumption; suspected or confirmed other congenital infections such as toxoplasmosis, syphilis, rubella, herpes, Chagas and ZIKA; and impossibility of sequential follow-up until delivery. Exposed and unexposed newborns will be excluded if there are indications or confirmation of genetic disorder; suspected or confirmed other congenital infections, such as toxoplasmosis, syphilis, rubella, herpes Chagas and ZIKA; and impossibility of sequential follow-up until the age of five.

Data Collection

The maternal epidemiological data will be obtained at the first meeting with the pregnant woman or her legal representative and are those contained in the "Epidemiological data spreadsheet". Clinical data will be obtained during prenatal care, which will occur in consultations with the following maximum interval: monthly between 0 and 34 weeks, twice a week between 34 and 36 weeks and weekly between 36 weeks and delivery. The data referring to childbirth and puerperium assistance will be obtained during hospitalization in the maternity hospital from medical reports. The monitoring of child growth and development will follow the intervals determined by the Ministry of Health of Brazil and will take place in an outpatient clinic specifically created for this purpose, composed of a multidisciplinary team of paediatrics, psychologists, occupational therapists, speech therapists, physiotherapists and nurses. The assessment of child neurodevelopment up to 42 months of life will include cognitive, motor, socioemotional aspects, and aspects related to language and adaptive behaviour and will be carried out with the validated version of the Bayley III Child Development Scale[19]. As of two and a half years of age, aspects related to intellectual performance will also be assessed through the Wechsler Pre-School and Primary Intelligence Scale[20], with a half-yearly interval.

The metabolic evaluation will be carried out by measuring blood glucose, insulin, lipid profile (total cholesterol and fractions, triglycerides), thyroid function (TSH, free T4) and basal cortisol at the end of the 12th and 24th months of life. Blood, CSF and placenta samples will be stored following the current rules for handling biological materials until their analysis.

Data Collection Procedure

The data collection will follow an experimental procedure from Bartocci *et al.*[12] that explores odours of the maternal breastmilk, vanilla (sweet), coffee (acid/bitter) and distilled water (neutral). The coffee smell is an addition to the previous study in order to include the acid/bitterness category to the categories of stimuli. Each test epoch consists of 30 s of baseline definition followed by 30 s of smell exposure with a two-minute interval for washout effect (figure 1).

The research assistants will wear surgical gloves for each handling of the solutions, and they will be prepared in an auxiliary room to avoid the spread of the smell of the solutions in the environment. Each solution will be kept in a hermetically sealed flask before and during all session to avoid smell impregnation. The room temperature will be maintained between 19-21ºC. The baby will be accommodated and cuddled on the lap of a familiar caregiver to prevent social stress and in a calm, relatively quiet (between 40 and 50dB(A)) environment reserved inside the Hospital Paediatric Follow-Up facility. The behavioral state[21] during the session will be monitored and registered for each stage of smell exposure ranging from state 1 (deep sleep) to sate 6 (crying). In case of the baby achieving state level six (cry – intense cry or high motor activity) or in need of a diaper change, the session will be paused and resumed as soon the baby returns to the state five or less. Mothers will be instructed to not use moisturizers or perfumes and to bring the babies already breastfed at least 15 min before the session.

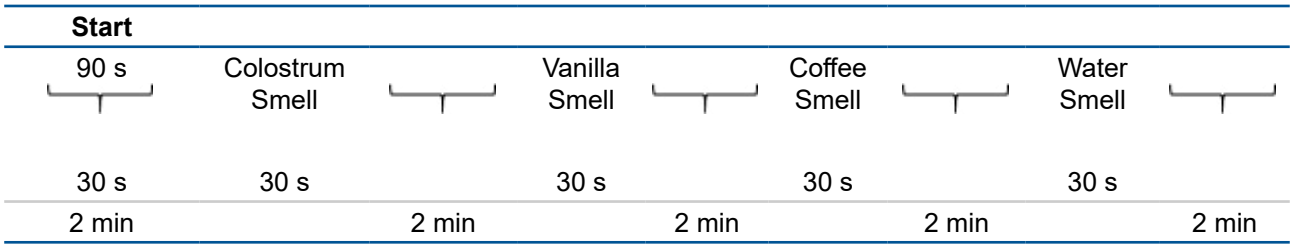

Figure 1: Timeline of odour exposure during one session adapted from Bartocci *et al.*[12]

Session phases

Step I

Collection of socio-demographic and clinical information through specific instruments and consultation of the electronic medical record.

Step II

1.Select the sample according to the inclusion criteria – participant invitation.
2.Explain to the mother how the research will be carried out and collect the consent form from the person responsible for the newborn.
3.Ask if the mother had followed the requirement to not use moisturizers or perfumes.
4.Ask the mother to remove 1 ml of breast milk and put it on a hermetic flask.
5.Turn on the video recorder.
6.Follow the following steps with the baby sitting on the companion's lap (not the mother):

A. Basal Phase: start filming the baby's face for at least 30 seconds before the first odour presentation and keep filming the entire session.
B. Stimulus Phase I: present the cotton swab soaked in breast milk for 30 seconds 2 cm from the baby's nose, maintaining the filming throughout this period, discard the cotton swab.
C. Wash out phase I: continue filming the baby for 2 minutes while the research assistant discards the cotton swab dipped in breast milk.
D. Stimulus Phase II: present the cotton swab soaked in vanilla solution for 30 seconds 2 cm from the baby's nose, maintaining the filming throughout this period, discard the cotton swab.
E. Wash out phase II: continue filming the baby for 2 minutes.
F. Stimulus Phase III: present the cotton swab soaked in coffee solution for 30 seconds 2 cm from the baby's nose, maintaining the filming throughout this period, discard the cotton swab.
G. Washout phase I: continue filming the baby for 2 minutes.
H. Control Phase: present the cotton swab soaked in water for 30 seconds 2 cm from the baby's nose, maintaining the filming throughout this period, discard the cotton swab.
J. Washout phase II: continue filming the baby for 2 minutes.

Step III - Image coding

1.Phase I coding: will be carried out for the development of the olfactory sensory evaluation scale. The videos will be analyzed frame by frame to survey categories of behavioural responses to the four different olfactory stimuli. The rating levels will consider two response categories "yes" or "no", for present and absent reaction to the smell. Factor Analysis will be performed with analyses of the main components for the construction of the instrument and analysis of the sample data.

2.Phase II coding: will be carried out for comparative evaluation of newborn children of pregnant women with and without a diagnosis for Covid-19.

Data Analysis

A mixed design will be run, with treatment (colostrum, vanilla, coffee, water) as within-subject variable and COVID-19 (covid vs. noncovid babies) as between-subject variable (group variable). The dependent variable will be the dichotomous responses. Continuous variables will be described as mean and standard deviation or median and interquartile range (IQR), as appropriate. Categorical variables will be described as frequencies and percentages. The analyses will be run to determine the perception thresholds, sensitivity parameters, and ROC curves (Receiver Operating Characteristic). In addition to fixed effects models, random effects models are estimated and also adjusted for possible confounders. Statistical significance will be considered when the p value is less than .05. The data will be stored in a SPSS-21 file, with an alphanumeric code that prevents the identification of the patient; curve fitting will be done with the MATLAB curve-fitting tool. To control for possible selection bias, propensity score matching (PSM) will be performed, followed by re-analyses as part of the robustness analyses.

Ethical and Legal Aspects of the Research

The study was approved by the Research Ethics Committee from the University of Brasilia School of Medicine (http://www.fm.unb.br/cep-fm - CAAE 32359620.0.0000.5558) and was registered in the Brazilian Registry of Clinical Trials (ReBec) under number RBR-65qxs2. The complete protocol can be accessed at ReBec "Effects of COVID-19 on pregnancy, childbirth, puerperium, neonatal period and child development" [cited 2020 Sep 17]. Available from: http:// ensaiosclinicos.gov.br/rg/ RBR-65qxs2/).

DISCUSSION

Pregnant women can be infected with SARS-CoV-2, with a possible consequent infection of their foetuses and newborns. SARS-COV-2 infection can cause an immune overreaction that is manifested by the excessive activation of immune cells and the production of a large amount of interferon and cytokines that can affect foetal development and increase the risk of neurological diseases in the neonatal period[5].

Like SARS-CoV, SARS-CoV-2 uses angiotensin converting enzyme 2 (ACE2) as a functional receptor to infect human cells. Studies have shown that ACE2 is expressed mainly in the respiratory, cardiovascular and digestive systems, which makes these organs more susceptible to this new virus. ACE2 is less mature in the youngest children and thus may not function as an appropriate receptor for SARS-CoV-2. The number of children infected with SARS-CoV-2 is relatively small. Thus, one hypothesis for this low infection rate is the low expression of ACE2 in children. Bunyavanich[22] conducted a retrospective study that examined the nasal epithelium of 305 individuals aged 4 to 60 years and observed that the gene expression of ACE2 in the nasal epithelium was age dependent. ACE2 gene expression was lower in younger children and increased with age.

A neurological symptom that has been frequently described in patients who tested positive for COVID-19 is a temporary impairment of taste and smell, which has been reported in 49-70% of infected people[23]. Somekh *et al*.[24] evaluated sensory function in patients (children and adults) who had COVID-19 documented by laboratory tests. Of the 73 patients, 37 (51%) reported having had a change in taste or smell, 25.8% of which were children. Children aged 5 to 10 years did not report sensory impairment[23]. However, there are still no reports about infants, especially newborn infants on the risk of being infected during pregnancy or delivery time.

One of the proposed mechanisms for altering smell and taste related to COVID-19 is the ability of SARS-CoV-2 to bind to ACE2 in the nasal and oral mucosa. Among people who tested positive for COVID-19, olfactory sensory sensation was significantly less impaired in children than in adults ($p = .00014$). The significant difference in olfactory sensory impairment between children and adults, and particularly between younger children and middle-aged adults, is in line with the finding that the expression of ACE2 in the nasal epithelium and in the oral cavity is more intense among adults, corroborating the possibility that the distribution and expression of ACE2 in the oral cavity and nasal epithelium may contribute to differences in sensory impairment[22]. Little is known about foetal impairment in mothers infected with SARS-CoV-2. By evaluating the olfactory sensory perception of newborns of women infected with COVID-19 during pregnancy, we can quantify such involvement.

Author Contributions

RMT, GMF KSFC and LRM devised the original study protocol, which was amended by TL, JS, JALJ and KNC. RMT, KNC, KSFC wrote the first draft of the manuscript that was then critically reviewed and revised by the other co-authors. All authors approved the final version of the manuscript for submission. RMT is the guarantor, and affirms that the manuscript is an honest, accurate and transparent account of the study being reported; and that any discrepancies from the study as planned have been explained.

Conflicts of Interest

The authors have no conflict of interest to declare.

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Resumo

Introdução: Em adultos, a perda olfativa é uma das manifestações clínicas agudas mais precoces e frequentes da infecção por SARS-CoV-2. O número de crianças infectadas com SARS-CoV-2 é pequeno, talvez devido à menor expressão da Enzima Conversora da Angiotensina 2 (ACE2) em crianças em comparação com adultos. Pouco se sabe sobre o comprometimento fetal em mães infectadas com SARS-CoV-2. Este artigo descreve um projeto em andamento sobre a percepção olfativa em bebês recém-nascidos.

Objetivo: o objetivo do presente estudo é desenvolver e validar uma escala de avaliação comportamental da percepção olfativa em recém-nascidos saudáveis e aplicá-la a recém-nascidos de mulheres infectadas com COVID-19 durante a gravidez e comparar a recém-nascidos de mulheres sem histórico de infecção por COVID-19.

Método: Este é um estudo de coorte analítico comparativo retrospectivo de 300 recém-nascidos expostos e não expostos ao COVID-19 durante a gravidez. A coleta de dados seguirá o procedimento experimental de estudo anterior que explorou odores do leite materno, baunilha (doce) e água destilada (neutro). Um cheiro de café foi implementado como um complemento a este estudo anterior, a fim de incluir a categoria ácido / amargo nas categorias de estímulos.

Discussão: É possível argumentar a hipótese do envolvimento do bulbo olfatório do feto durante a vida intrauterina como uma das manifestações fisiopatológicas indeléveis para o diagnóstico clínico de COVID-19 com déficit olfatório neurossensorial em fetos e recém-nascidos afetados por infecção intrauterina. Este estudo tem como objetivo investigar se filhos recém-nascidos de mulheres infectadas com COVID-19 durante a gravidez apresentam alterações sensoriais olfativas. O ensaio clínico foi registrado no Registro Brasileiro de Ensaios Clínicos (ReBEC-RBR-65qxs2).

Palavras-chave: recém-nascido, percepção, odores, COVID-19, SARS-CoV-2

摘要

嗅觉丧失是成人感染新型冠状病毒(SARS-CoV-2)最早和最常见的急性临床表现之一. 与成人相比，儿童血管紧张素转换酶2（ACE2）的表达水平较低，推测儿童发病人数较成人少

与此有关. 尽管如此，但对于在感染新型冠状病毒(SARS-CoV-2)孕妇子宫内胎儿的嗅觉损伤的情况还不清楚.

研究目的：本研究的目的是开发和验证健康新生儿的嗅觉行为评价量表，然后运用该量表对比评估妊娠期感染COVID-19的母亲分娩的新生儿和妊娠期未感染COVID-19的母亲分娩的新生儿的嗅觉感知.

研究方法：该研究是一个回顾性对比分析队列研究，研究对象为300个在妊娠期暴露和未暴露在新型冠状病毒（COVID-19）的新生儿. 数据采集将遵循一个早前对气味探究的实验程序. 本研究为了将将酸/苦也纳入刺激类别，在原先只有母乳、香草(甜)、和蒸馏水(中性)的气味的基础上又加入了咖啡气味.

讨论：一个可能的假说认为，胎儿的嗅球子宫内发育过程中受累是在临床诊断宫内感染SARS-CoV-2并伴神经感觉性嗅觉障碍的胎儿和新生儿中重要病理生理表现之一.

本研究旨在探讨妊娠期感染COVID-19的新生儿是否存在嗅觉改变. 该临床试验注册在巴西临床试验注册中心(ReBEC-RBR-65qxs2).

关键词：新生儿， 知觉，嗅觉，2019冠状病毒病，严重急性呼吸综合征冠状病毒2.

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ORIGINAL ARTICLE

标题：妊娠期感染COVID-19的母亲分娩的新生儿的嗅觉感官评估

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摘要

嗅觉丧失是成人感染新型冠状病毒(SARS-CoV-2)最早和最常见的急性临床表现之一. 与成人相比，儿童血管紧张素转换酶2（ACE2）的表达水平较低，推测儿童发病人数较成人少

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作者总结 - *Authors summary*

为什么做了这项研究？ - *Why was this study done?*
嗅觉丧失是成人感染新型冠状病毒(SARS-CoV-2)最早和最常见的急性临床表现之一. 少数在儿科范围内的相关研究表明儿童的嗅觉丧失几率低于成人. 对此一个可能的解释是，与成人相比，儿童血管紧张素转换酶2（ACE2）在鼻黏膜的表达较低，因此与SARS-CoV-2的结合较少. 本项研究中作者提议评估SARS-CoV-2对母亲子宫内的胎儿嗅觉的影响.

研究人员做了什么？有什么发现？ - *What did the researchers do and find?*
该研究旨在评估对比妊娠期感染COVID-19的母亲分娩的新生儿和妊娠期未感染COVID-19的母亲分娩的新生儿的嗅觉感知. 该研究以有关胎儿和新生儿嗅觉感官发展的科学文献为基础，旨在开发和验证健康新生儿的嗅觉行为评价量表。新生儿会按照相同的实验顺序暴露在四种气味下：母乳，香草（甜），咖啡（苦）和蒸馏水（中性）.

这些发现意味着什么? - *What do these findings mean?*
根据现有的文献，关于SARS-CoV-2对胎儿的损伤目前还尚不清楚. 和成人相比，低龄儿童感染后出现的症状更少也较轻，包括感觉障碍，尤其是出现嗅觉障碍出现的频率很低. 本研究结果将阐明宫内感染SARS-CoV-2是否会影响胎儿感觉系统.

1引言

由SARS-CoV-2引起的COVID-19的自然历史仍在不断探索中. 目前还尚不清楚其对妊娠、产褥期、母胎健康以及对在妊娠期受感染母亲的子女长期发育的影响. 根据目前为止的阳性数据，在孕期和此期外观测到的COVID-19的临床表现并没有明显的差异. COVID-19的临床表现有时是无症状的，最常见的症状是发热 (67 %) 和咳嗽 (66 %)，伴发身体不适、可能也会出现呼吸困难和腹泻[1]等具有不同严重程度症状的临床表现.

对于新冠病毒是否存在母婴垂直传播的问题，有研究对六例感染COVID-19并处于妊娠晚期的孕妇进行了相关的研究2. 该研究表明包括羊水、脐带血、母乳和新生儿鼻咽拭子在内的所有样本的检测结果均为阴性. 随后，中国武汉和美国纽约市两家医院对妊娠感染病例的系统回顾和描述病例研究也均报告未发现有垂直传播的证据1，3-5. 尽管如此，目前尚不能排除本研究中母婴垂直传播的可能性. 因为有研究发现，有些新生儿虽然RT-PCR呈阴性，但血清SARS-CoV-2 IgM偏高5， 以及出生16个小时RT-PCR呈阳性的新生儿患儿，血清新冠病毒抗体IgM一直到第五天仍是阴性6， 要解释这些结果还需要进行更准确的前瞻性研究.

母源性SARS-CoV-2感染不仅会对胎儿和新生儿有直接引影响，还会产生许多间接影响. 新生儿在分娩时或出生后不久可能会受感染是一个重要方面，因此并不建议延迟断脐和母婴之间有皮肤接触. 目前尚未证据显示，新冠病毒可以通过母乳传播. 其他方面包括母婴炎症反应的后果，潜在的细胞毒性细胞因子的产生和抗病毒药物的使用效果还尚未见研究.

不仅如此，有研究表明感染COVID-19成年人由于新冠病毒对中枢神经系统 (CNS) 直接或间接的影响而出现精神障碍、中风、癫痫或嗅觉丧失等神经系统疾病. 甚至出现更严重的症状，一名感染的六十岁妇女的脑部磁共振成像检查结果显示急性出血性脑炎9 由COVID-19引起的神经功能损害的患病率目前仍在观察中，但一项对214例日本患者的研究表明，36 % 的患者都出现了一些与神经系统有关的临床表现10. 此外，并没有足够的证据表明胎儿或新生儿因感染了COVID-19而容易遭受神经功能后遗症.

问题

嗅觉的变化是感染新冠状病毒的症状之一.嗅觉丧失是成人感染新型冠状病毒 (SARS-CoV-2) 最早和最常见的急性临床表现之一.一个可能的假说认为，胎儿的嗅球子宫内发育过程中受累是在临床诊断宫内感染SARS-CoV-2并伴神经感觉性嗅觉障碍的胎儿和新生儿中重要病理生理表现之一. 在上述证据的基础上，研究者提出以下的问题：感染SARS-CoV-2的孕妇的新生儿是否存在嗅觉的改变?本研究以有关胎儿和新生儿嗅觉感官发展的科学文献为基础，在Bartocci等人研究方法为基础11-18，并进行调整使其适应于医院情境. 扩展其实验嗅觉刺激的类别，用于测量判别敏感度.

本研究的主要目标是评估妊娠期感染COVID-19的母亲分娩的新生儿的嗅觉感知. 具体目标是制定一个婴幼儿嗅觉感知行为测评量表. 在健康新生儿中检验该量表，然后与妊娠期感染COVID-19的母亲分娩的新生儿的嗅觉感知反应进行比较.

2研究方法

2.1研究设计

本研究是一项回顾性比较分析队列研究，该研究旨随访在COVID-19呈阳性的孕妇所分娩的新生儿的发育情况，题为“感染SARS-CoV-2孕妇所分娩的新生儿的临床结局”.

样本量计算

目前关于巴西孕妇感染SARS-CoV-2的情况尚无确切数字说明。但据国际报告估计，SARS-CoV-2在所有孕妇中的感染率高达15.3%1-4. 根据最新的数据，巴西首都巴西利亚所在的联邦区每年有44，195名新生儿出生. 因此，考虑到孕妇群体是个‘无限大’的群体（>20.000名孕妇），并假设孕期感染SARS-CoV-2的几率为15%，置信水平为95%，误差范围为5%，则暴露组妇女的随机样本量最少是195人，相应预计暴露组儿童为类似样本数量. 同时考虑到在BORN分项研究中可能大概会有20% 的被试中途退出研究，所需的暴露组（感染）妇女数量至少为234人.

现阶段关于SARS-CoV-2对妊娠和儿童发育有什么样的影响还尚不清楚，因此使得本研究具有显著探索性的特点. 本研究并不是随机招募被试，而是基于被试是否符合相应的研究条件，并采用了”尽可能可行，但必须合理”的样本量定义方法. 综上所述的预估样本数量可以作为参考，以避免高估被试的人数.

综合考虑，我们在PREGNANT阶段设定了300名暴露组妇女，相应的在BORN阶段预期有300名暴露组儿童. 暴露组和对照组按照1：1比率分配，即非暴露对照组包括300名COVID-19呈阴性的母亲和儿童. 因此，PROUDEST研究的总体样本量最终为1200

名被试(600名母子配偶：300名暴露组，300名对照组）.

2.2研究地点和时间

该研究在巴西利亚大学医院(HUB)儿科的新生儿门诊进行，该儿科门诊由儿科医师、护士、职业治疗师、言语治疗师、神经心理学家等多学科团队组成，主要接收五岁以内的儿童病患. 本研究的调查对象为2020年7月至2021年3月在该门诊出生的新生儿（母亲孕期感染COVID-19和母亲孕期未感染COVID-19）.

2.3研究人群与纳入标准

本研究的目标群体将由出生14天以下的新生儿组成，其母亲孕期核酸检测（PCR检测) 呈阳性. 对照组也由出生14天以下的的新生儿组成，但其母亲孕期PCR检测呈阴性. 暴露组母婴二元体的纳入标准是：孕妇年龄≥18岁，且RT-PCR或血清IgM抗体检测证明妊娠期间感染SARS-CoV-2. 快速测试的结果以及有关的临床症状或特征断层扫描的信息将作为补充数据收集. 对于未暴露母婴二元体的对照组，纳入标准根据孕妇年龄≥18 岁以及入院分娩辅助时的SARS-CoV-2（IgG和IgM抗体）血清学检测阴性.

暴露和未暴露母亲的排除标准为：患有需要持续用药的慢性疾病；患有糖尿病和高血压；吸烟和/或饮酒；疑似或证实有其他先天性感染疾病如弓形虫病、梅毒、风疹、疱疹、南美锥虫病和寨卡病，以及不能进行连续随访直至分娩. 暴露和未暴露新生儿的排除标准为：如有迹象表明或确认的遗传综合征，疑似或确认的其他先天性感染，弓形虫病、梅毒、风疹、疱疹、南美锥虫病和寨卡病等，且不能后续随访至5岁，若符合上述则排除在外.

2.4数据收集

产妇流行病学调查资料将在与孕妇或其法定代表人的第一次会面时获得，这些数据收录在”流行病学数据电子表格”. 临床资料将在产前保健期间的会诊中获得：妊娠0 ~ 34周间每月一次会诊，妊娠34 ~ 36间每周两次会诊，妊娠36周至分娩每周一次会诊. 有关分娩和产褥期预后的资料将从产妇住院期间的医学报告中获得. 对小儿生长发育的监测将按照由巴西卫生部确定的时间间隔，并将在专门为此目的设立的门诊进行. 此外，本研究将使用修订的贝利婴幼儿发展量表第三版（Bayley III Child Development Scale）对从出生到42个月的婴幼儿进行包括认知，运动，社会情感以及与语言和适应性行为的有关方面的神经发育的评估. 婴幼儿从两岁半开始，每隔半年通过韦氏学龄前儿童智力测验量表（Wechsler Pre-School and Primary Intelligence Scale）20 评估儿童智力表现有关的方面. 同时通过测定婴幼儿第12、24个月末的血糖、胰岛素、血脂指标(总胆固醇和分数、甘油三酯)、甲状腺功能 (TSH、游离T4) 和基础皮质醇等指标对婴幼儿的代谢进行评估. 实验中的血液、脑脊液和胎盘样本将按照现行的处理生物材料的规则保存，直至分析完毕.

数据采集过程：

该研究数据采集将遵循Bartocci等人12 对气味探究的实验程序. 研究者为了将将酸/苦也纳入刺激类别在原先只有母乳、香草(甜)、和蒸馏水(中性)的气味的基础上又加入了咖啡气味. 每个试验周期由基线时30 s和30 s的气味暴露期组成，为了不受其他气味的干扰，上一个与下一个气味暴露期中间有一个2 分钟间隔 (图1).

研究助手在实验中每次处理溶液时，都要戴上外科手术手套并在辅助室内进行准备，以避免溶液中的气味散布到环境中. 此外，在每次实验之前和实验过程中，每种溶液都应保存在密封的烧瓶中，以免产生异味. 为防止婴儿产生社会压力，实验中婴儿被安置在熟悉的护理员的膝盖上. 实验环境应保持平静，相对安静（40至50dB（A）之间）且室温维持19-21ºC之间. 在实验期间监测婴幼儿在每一阶段的气味暴露下的行为状态21，并把状态按照从1级 (深睡眠)到6级(哭泣)的程度记录下来. 如果婴儿状态达到6级(哭泣-剧烈哭泣或运动活跃)或需要更换尿布，则实验将暂停，并在婴儿回到5级或以下时以重新开始实验. 母亲将被告知在实验期间不要使用保湿剂或香水，并在实验开始至少15分钟前已经将哺乳过的婴儿带到实验室.

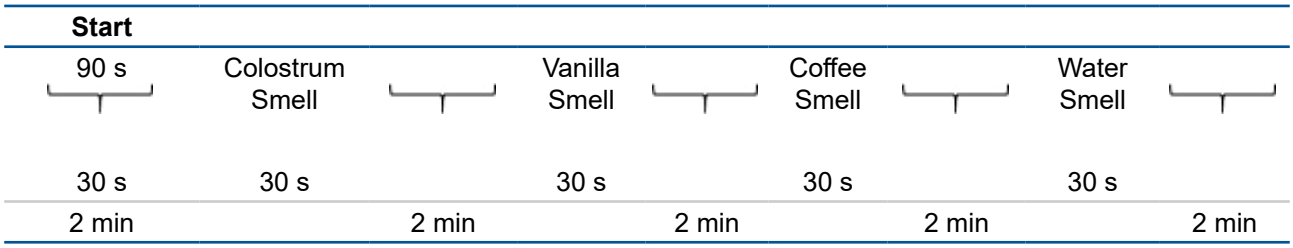

图1 .实验期间的气味暴露时间表—改编自Bartocci等人12

实验步骤：

第一步：

通过特定工具收集母亲的社会人口统计学和临床信息，并查阅电子病历.

第二步：

1. 根据被试纳入标准选择样本.
2. 向母亲解释该研究是如何进行，并向新生儿的监护人收集知情同意书.
3. 询问母亲是否遵循了不使用保湿剂或香水的要求.
4. 请求母亲取出1毫升母乳，置于密封瓶中.
5. 打开录像机.
6. 婴儿坐在陪同的护理员的大腿上（不是母亲），并按照以下步骤操作：

A. 基础阶段：在第一次出现气味之前，对婴儿的脸至少进行30秒的拍摄.

B. 刺激阶段I：将浸泡过母乳的棉签呈现在婴儿鼻子前2 cm处30秒，拍摄整个过程，然后丢弃棉签.

C. 冲洗阶段I：继续拍摄婴儿2分钟.

D. 刺激阶段II：将浸泡过香草溶液的棉签呈

现在婴儿鼻子前2 cm处30秒，拍摄整个过程，然后丢弃棉签.
E. 冲洗Ⅱ期：继续拍摄婴儿2分钟.
F. 刺激第三阶段：将浸泡过咖啡的棉签呈现在婴儿鼻子前2 cm处30秒，拍摄整个过程，然后丢弃棉签.
G. 冲洗Ⅲ期：继续拍摄婴儿2分钟.
H. 控制阶段：将浸泡过清水的棉签呈现在婴儿鼻子前2 cm处30秒，拍摄整个过程，然后丢弃棉签.
J. 冲洗IV期：继续拍摄婴儿2分钟.

第三步-图像编码：

1. 第一阶段编码：开发嗅觉感官评价量表. 研究者通过对视频的逐帧分析调查婴儿对四种不同嗅觉刺激的行为反应类别. 并将婴儿对气味出现或无反应的评定等级考虑为 ‘有’ 或 ‘无两个反应类别. 然后对样本数据进行因素分析.
2. 第二阶段编码：将比较评估感染和未感染SARS-CoV-2孕妇所分娩的新生儿.

2.5数据分析

该实验是一个混合设计，以气味(初乳、香草、咖啡、水)为被试内变量，Covid (COVID与非COVID婴儿) 为被试间变量 (组变量)，因变量为二分（有或无）反应. 采用SPSS-21软件对研究数据进行统计分析，用字母数字代码命名患者. 连续变量是酌情使用均值和标准差或中位数和四分位间距（IQR）值来描述，分类变量采用频率和百分比进行描述. 分析将用于确定感知阈值、灵敏度参数和ROC曲线 (受试者工作特征曲线). 除了固定效应模型外，为了对可能的干扰因素进行调整也估算出了随机效应模型. 在MATlab使用曲线拟合工具完成曲线拟合，此外为了控制可能的选择偏差，将进行倾向得分匹配 (PSM)，然后重新分析作为稳健性分析的一部分. 所有检验以双侧 $p<0.05$ 为差异有统计学意义.

2.6研究的伦理和法律方面

该研究经巴西利亚大学医学院研究伦理委员会批准 (http://www.fm.unb.br/cep-fm-CAAE32359620.0.0000.5558)，以编号RBR-65qxs2在巴西临床试验登记处 (ReBec) 注册. 可以在ReBec上通过 ‘COVID-19对妊娠、分娩、产褥期、新生儿期和儿童发育的影响’ （引用自2020年9月17日）中查阅完整的协议. 文稿可以从：http：//ensaiosclinos.gov.br/rg/RBR-5qxs2/)获得.

3讨论

孕妇作为一个特殊的群体感染SARS-CoV-2后，其胎儿和新生儿可能也会随之感染. SARS-COV-2感染会引起免疫过度反应，主要表现为免疫细胞过度激活，产生大量干扰素和细胞因子，这些结果会增加新生儿患神经系统疾病的风险，并影响胎儿发育5. 与SARS-CoV一样， SARS-CoV-2 利用血管紧张素转换酶2 (angiotensin converting enzyme 2，ACE2) 作为功能受体感染人体细胞. 研究表明，ACE2主要在呼吸道、心血管和消化系统中表达，这使得这些器官更容易感染这种新的病毒. 儿童ACE2蛋白的表达不足或发育及功能不成熟，可能是儿童SARS-CoV-2感染率较低的原因. Bunyavanich22对305名4 ~ 60岁个体鼻上皮的回顾性分析研究发现随着年龄的增长，ACE2在鼻上皮中的表达变化呈显著的线性趋势. ACE2在低龄儿童中表达水平较低，且随年龄增长而升高.

据报道有49-70%的COVID-19感染者报告出现暂时性味觉和嗅觉障碍的神经系统症状. Somekh等人24 通过实验室检查评估了COVID-19的患者（儿童和成人）的感觉功能. 检查发现，在73例患者中，37例 (51%) 报告有味觉或嗅觉改变，其中25.8%为儿童. 此外，5 ~ 10岁间的儿童并未报告感觉障碍23. 但目前关于婴儿，尤其是在孕期或分娩时有感染风险的新生儿的相关报道却寥寥无几.

有人提出SARS-CoV-2与鼻腔和口腔粘膜中ACE2结合的能力是COVID-19改变嗅觉和味觉的机制之一. 在成人COVID-19检测阳性的人群对比，儿童嗅觉障碍程度明显较低 ($p = 0.0014$). 嗅觉障碍在儿童与成人之间，尤其是小儿与中年人之间存在显著差异，这一发现与成人鼻上皮和口腔中ACE2的高表达相一致，证实了ACE2在口腔和鼻上皮中的分布和表达可能导致了感觉障碍的差异. 母亲感染SARS-CoV-2对胎儿产生的影响目前尚未清楚，但通过评估孕期感染COVID-19的母亲分娩的新生儿的嗅觉感觉，我们可以量化这种影响.

作者贡献：

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Abstract

Introduction: In adults, olfactory loss is one of the earliest and most frequent acute clinical manifestations of SARS-CoV-2 infection. The number of children infected with SARS-CoV-2 is relatively small, perhaps due to the lower expression of Angiotensin Converting Enzyme 2 (ACE2) in children compared to adults. Little is known about foetal impairment in mothers infected with SARS-CoV-2. This paper describes an ongoing scientific project on smell perception in infants.

Objective: The goal of the study is to develop and validate a behavioural evaluative scale of olfactory perception in healthy newborns and to apply this scale to newborn children of women infected with COVID-19 during pregnancy comparing to newborn children of women without COVID-19 infection history, in order to compare these groups.

Methods: This is a retrospective comparative analytical cohort study of 300 newborns exposed and unexposed to COVID-19 during pregnancy. The data collection will follow the experimental procedure in a previous study that explored odours of the maternal breastmilk, vanilla (sweet) and distilled water (neutral). A coffee smell was implemented as an addition to this previous study in order to include acid/ bitterness category to the categories of stimuli.

Discussion: It is feasible to argue the hypothesis of the involvement of the foetus' olfactory bulb as one of the indelible pathophysiological manifestations to the clinical diagnosis of COVID-19 with neurosensory olfactory deficit in foetuses and newborns affected by intrauterine infection. This study aims to investigate if newborn children of women infected with COVID-19 during pregnancy have olfactory sensory changes. The clinical trial was registered in the Brazilian Registry of Clinical Trials (ReBEC- RBR-65qxs2).

Keywords: newborn, perception, odors, COVID-19, SARS-CoV-2

Resumo

Introdução: Em adultos, a perda olfativa é uma das manifestações clínicas agudas mais precoces e frequentes da infecção por SARS-CoV-2. O número de crianças infectadas com SARS-CoV-2 é pequeno, talvez devido à menor expressão da Enzima Conversora da Angiotensina 2 (ACE2) em crianças em comparação com adultos. Pouco se sabe sobre o comprometimento fetal em mães infectadas com SARS-CoV-2. Este artigo descreve um projeto em andamento sobre a percepção olfativa em bebês recém-nascidos

Objetivo: o objetivo do presente estudo é desenvolver e validar uma escala de avaliação comportamental da percepção olfativa em recém-nascidos saudáveis e aplicá-la a recém-nascidos de mulheres infectadas com COVID-19 durante a gravidez e comparar a recém-nascidos de mulheres sem histórico de infecção por COVID-19.

Método: Este é um estudo de coorte analítico comparativo retrospectivo de 300 recém-nascidos expostos e não expostos ao COVID-19 durante a gravidez. A coleta de dados seguirá o procedimento experimental de estudo anterior que explorou odores do leite materno, baunilha (doce) e água destilada (neutro). Um cheiro de café foi implementado como um complemento a este estudo anterior, a fim de incluir a categoria ácido / amargo nas categorias de estímulos.

Discussão: É possível argumentar a hipótese do envolvimento do bulbo olfatório do feto durante a vida intrauterina como uma das manifestações fisiopatológicas indeléveis para o diagnóstico clínico de COVID-19 com déficit olfatório neurossensorial em fetos e recém-nascidos afetados por infecção intrauterina. Este estudo tem como objetivo investigar se filhos recém-nascidos de mulheres infectadas com COVID-19 durante a gravidez apresentam alterações sensoriais olfativas. O ensaio clínico foi registrado no Registro Brasileiro de Ensaios Clínicos (ReBEC- RBR-65qxs2).

Palavras-chave: recém-nascido, percepção, odores, COVID-19, SARS-CoV-2