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# Language deficit in boys with autism spectrum disorder in relation to maternal reproductive health, endocrine disruptors, and delivery method

## Rečové schopnosti u chlapcov s poruchou autistického spektra v súvislosti s reprodukčným zdravím matky, endokrinnými disruptormi a typom pôrodu

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**Summary:** **Objectives:** The presented research aimed to identify prenatal factors involved in abnormal neurodevelopment and postnatal manifestation of an autistic phenotype in 107 boys (average age 4.31 ± 2.24 years). **Materials and methods:** Their biological mothers were asked to fill out a comprehensive questionnaire about their reproductive health, infections during pregnancy, oral contraceptive intake before conception, and potential substance abuse before and during pregnancy as well as delivery and newborn information. The boys were subsequently diagnosed with autism spectrum disorder (ASD) using the combination of Autism Diagnostic Observation Schedule (ADOS-2) and Autism Diagnostic Interview – Revised (ADI-R). Based on the ADOS-2 module chosen during diagnosis, boys diagnosed with Module 1 can be classified as nonverbal or minimally verbal (N = 68), while those diagnosed using Module 3 are fully verbal (N = 39). **Results:** According to our results, reproductive health related to the length of the menstrual cycle before pregnancy with the autistic child seems to play a role with regards to the severity of the disorder (P = 0.017) as well as the number of previous pregnancies (P = 0.026). Mothers of nonverbal children reported to have had a much shorter menstrual cycle (27.35 ± 6.60 days) than those with verbal children (30.14 ± 4.44 days) and reported more previous pregnancies (0.93 ± 1.07 vs. 0.51 ± 0.91), while not reporting the number of live births before they had the autistic child. Children who were later diagnosed as non-verbal had a longer delivery time (from 2 to 48 hours; on average 11.13 hours, SD = 9.49) than verbal ones (between 1 and 27 hours, which was on average 7.09 hours, SD = 8.91), P = 0.0182. Delivery method didn't play a role in this context, and neither did the type of conception (natural, insemination, etc.). **Conclusion:** Studying the involvement of prenatal factors in the etiology of autism based on the speech of the child seems to be a promising approach.

**Key words:** teratogens – neurodevelopmental disorder – prenatal development – etiology – autism spectrum disorder

## Introduction

Autism spectrum disorder (ASD) is a pervasive neurodevelopmental disorder characterized by deficits in the ability to initiate and sustain reciprocal social interaction and social communication, and by a range of restricted, repetitive, and inflexible patterns of behavior, interests, or activities [1]. Individuals on the autism spectrum exhibit a full range of intellectual functioning and language abilities, hence identification of their potential deficits is crucial for appropriate individualized support and selection of interventions.

For individuals with ASD, the average age of using first words is approximately 36 months, in contrast to the

**Súhrn:** **Ciele:** Predkladaný výskum sa zaoberá identifikáciou prenatálnych faktorov vplývajúcich na abnormálny neurovývin a postnatálnu manifestáciu autistického fenotypu v súbore 107 chlapcov (priemerný vek 4,31 ± 2,24). **Súbor a metódy:** Biologické matky autistických chlapcov poskytli údaje týkajúce sa ich reprodukčného zdravia, infekcií počas gravidity, užívaní orálnej antikoncepcie pred počatím, prípadne užívaním návykových látok pred a počas gravidity a tiež informácie týkajúce sa novorodenca. Následne boli chlapci diagnostikovaný na poruchy autistického spektra (PAS), pomocou diagnostických nástrojov ADOS-2 (Autism Diagnostic Observation Schedule) a ADI-R (Autism Diagnostic Interview – Revised). V ADOSE-2 bol zvolený diagnostický modul podľa rečových schopností dieťaťa, buď Modul 1 – neverbálny alebo minimálne verbálny chlapci (n = 68) a verbálni chlapci (n = 39). **Výsledky:** Na základe našich výsledkov, má reprodukčné zdravie matky súvisiace s dĺžkou menštruačného cyklu pred graviditou s autistickým dieťaťom, súvis s mierou rečového postihnutia (p = 0,017), taktiež počet predošlých tehotenstiev (p = 0,026). Matky neverbálnych detí uvádzali kratší cyklus (27,35 dní ± 6,60) ako matky verbálnych detí (30,14 days ± 4,44) a mali viac predošlých tehotenstiev (0,93 ± 1,07 vs. 0,51 ± 0,91). Neuviedli však počet živo narodených detí pred tehotenstvom s autistickým dieťaťom. Deti, ktoré boli neskôr diagnostikované ako neverbálne, mali dlhší pôrod (od 2 do 48 hod; v priemere 11,13 hod, SD = 9,49), ako verbálne (od 1 do 27 hod, čo bolo v priemere 7,09 hod, SD = 8,91), p = 0,0182. Spôsob pôrodu nezohrával úlohu, ani spôsob počatia (prirodzené vs. umelé). **Záver:** Skúmanie prenatálnych faktorov v etiológii autizmu z hľadiska rečového vývinu sa javí ako dobrý prístup.

**Kľúčové slová:** teratogény – neurovývinové poruchy – prenatálny vývin – etiológia – poruchy autistického spektra

usual onset at around 18 months in neurotypical children, while expressive language delay is the most common reason parents seek diagnostics and interventions [2]. Only a small percentage of children develop functional language if it did not occur before the age of 5. It is estimated that around 25% to 35% of children with ASD remain nonverbal or minimally verbal [3].

The importance of assessing intellectual functioning and language abilities as a necessary part of the diagnostic process is highlighted in the new definition of ASD in the currently valid classification ICD-11. Many Slovak clinicians still use the diagnoses specified in the older International Classification of Disorders, 10$^{th}$ version (ICD-10), which is valid in Slovakia since 1992, and it puts autism under the group of pervasive developmental disorders dividing it into separate diagnoses such us childhood autism, atypical autism, or Asperger syndrome. In contrast, ICD-11 (World Health Organization, 2019), which should already be implemented, only operates with the unified term autism spectrum disorder, separated into 6 groups based on intellectual functioning and language abilities: ASD with/without disorder of intellectual development and with mild or no impairment of functional language/with impaired functional language (i.e., not able to use more than single words or simple phrases)/with complete or almost complete absence of functional language.

In recent years, a global increase in the prevalence of ASD has been observed. In 2000, according to the Center for Disease Control and Prevention (CDC) in the USA, one in 100 children was diagnosed with ASD. In 2014, it was one in 59 children with ASD, and in 2016, it was one in 54 children under the age of 8 [4]. Between 2014 and 2019, the prevalence in the US was 1.70% in children aged 4 years and 1.85% in children aged 8 years; in European children and adolescents under 18 years of age it ranged between 0.38 and 1.55% [5]. The increasing prevalence of PAS is probably caused by a combination of several factors. From a psychological point of view, it is mainly a shift in the understanding of the diagnosis, the refinement and expansion of diagnostic criteria, as well as the improvement of the availability and quality of screening and diagnostic tools used to detect the disorder. Secondly, it is attributed to more and more prevalent environmental risk factors, e. g. older age of parents, a too short time interval between pregnancies, increasingly frequent autoimmune diseases or overcoming a serious infection during pregnancy, use of certain medications, consumption of alcohol or smoking, but also exposure to various harmful substances caused by air pollution, and presence of heavy metals and endocrine disruptors, e. g. in cosmetics and cleaning agents or pesticides in food [6].

Autism spectrum disorder is primarily the result of disruption of function and interaction of certain brain structures responsible for the integration and synthesis of information coming from various sensory channels, as well as brain areas responsible for organizing behavior in general and social behavior [7,8].

ASD is a disorder with a multifactorial pathogenesis, involving the interaction of genetic susceptibility and environmental factors. Genetic influence is confirmed, for example, by studies involving twins, which began in 1977 and continue to this day [9–14]. They show that monozygotic twins have a significantly higher concordance for autism than dizygotic twins. Overall, the evidence suggests that hereditary factors can be identified in more than a half of all autism diagnoses [15]. Also, thanks to Whole Exome/Genome Sequencing, numerous candidate genes associated with ASD that carry *de novo* mutations have also been discovered, such as CHD8, UBE3A, SHANK, NLGN3, TCF4, and many others [16–19]. These genes are involved in synapse formation and

**Tab. 1. Mothers' age at the time of conception of the child with ASD and at the time of filling out the questionnaire.**
Tab. 1. Vek matiek v čase počatia dieťaťa s PAS a v čase vyplnenia dotazníka.

| | Age at the time of conception of the child with ASD | | Age at the time of completing the questionnaire | |
|---|---|---|---|---|
| | Module 1 | Module 3 | Module 1 | Module 3 |
| Min. | 19 | 19 | 22 | 34 |
| Max. | 41 | 39 | 48 | 51 |
| Average | 30.12 | 29.36 | 34 | 40 |
| SD | 5.19 | 4.75 | 5.32 | 4.14 |

ASD/PAS – autism spectrum disorder/poruchy autistického spektra, Min. – minimum age/minimálny vek, Max. – maximum age/maximálny vek, SD – standard deviation/smerodajná odchýlka

maintenance, function of neurotransmitters that affect the excitability of neurons, and are also correlated with the development of autistic traits [20]. In total, around 1,000 genes are believed to be implicated in autism [21].

In addition to genetic factors, the development of ASD is also influenced by various environmental factors in the prenatal, perinatal, and even postnatal period. A cross-sectional study conducted in the southwest of Iran demonstrated that children born to older parents are at a higher risk for having ASD [22]. The results of many studies collected in a meta-analysis also established a link between maternal infectious diseases during pregnancy and an increased risk for autism in the offspring [23]. An analysis combining data from six longitudinal studies indicated that prenatal alcohol exposure (PAE) can delay both receptive and expressive communication in children up to the age of 36 months [24]. Diabetic mothers have been reported to have more children with speech delay than relatively healthy mothers [25]. Another often mentioned risk factor for ASD is problematic childbirth, including premature birth, cesarean section, and other birth complications [26–28]. Moreover, numerous studies have explored the impact of socioeconomic status and family knowledge about autism concerning the age at which a child is diagnosed with autism [29].

Despite the considerable amount of scientific research devoted to ASD, it is remarkable that to our knowledge, there are few previous research studies focused on the genetic and epigenetic differences in verbal vs nonverbal children with ASD. These two groups have very different prognosis for future life and require very different support and interventions.

The goal of this research study was to explore the relationship between maternal health factors and degree of functional language impairment in boys later diagnosed with ASD. This effort not only advances our understanding of ASD etiology in general, but helps identify children with ASD which could benefit from early speech-focused intervention by identifying possible epigenetic risk factors responsible for future language impairment.

## Materials and methods

This epidemiological study included data from 107 biological mothers (mean age 36 years, SD = 5.67), who answered questions regarding their sexual and reproductive health, hormonal background (endocrine system), prenatal and perinatal period in relation to exposure to toxins, substance abuse, and infectious diseases. Delivery records and records on the health status of the newborn, who was later diagnosed with autism spectrum disorder (ASD) were required as part of the diagnostic process and were provided upon informed consent signed by the biological mother.

We chose a questionnaire as a research tool featuring a series of questions used to collect useful information from respondents. The questionnaire was anonymous and suitable for sensitive topics. This epidemiological questionnaire (EDO-20) was created with the aim of defining risk factors in addition to genetic ones, which play a role in the etiology of autism spectrum disorder. It can be found on the following link:
https://docs.google.com/forms/d/e/1FAIpQLSfcTw5lYwtRTapqTFy62kis5tQ7FLDZ62Mc9wJZ7DV_xi6PLg/viewform?vc=0&c=0&w=1

The questionnaire contained close-ended (restricted-choice) questions, offering a fixed set of choices to select from and also open-ended (long-form) questions, which allows respondents to provide answers in their own words. With close-ended questions, we collected categorical and also quantitative variables.

About 107 boys were diagnosed with ASD by properly trained clinicians at the Academic Research Center for Autism (ARCA) using the combination of the Autism Diagnostic Observation Schedule, second edition (ADOS-2), and the Autism Diagnostic Interview – Revised (ADI-R 2), which are considered to be the gold standard of ASD diagnostics. During diagnostics, each individual was administered one of five ADOS-2 modules, and selected based on their chronological age and level of expressive language proficiency. In the diagnosed sample, 68 individuals (63.55%) belonged to Module 1 (boys aged 3.53 years, SD = 1.05), representing children who are non-verbal or minimally verbal.

**Tab. 2. Residence of the mothers of boys with ASD.**
Tab. 2. Bydlisko matiek chlapcov s PAS.

| Region | Module 1 | Module 3 |
|---|---|---|
| Bratislava Region | 45 | 22 |
| Nitra Region | 4 | 2 |
| Košice Region | 1 | 0 |
| Trnava Region | 6 | 3 |
| Banská Bystrica Region | 7 | 1 |
| Žilina Region | 2 | 0 |
| Trenčín Region | 1 | 0 |
| Prešov Region | 2 | 0 |

ASD/PAS – autism spectrum disorder/poruchy autistického spektra

Thirty-nine (36.45%) fully verbal children, able to talk using complex sentences and use past and future tense, were diagnosed using Module 3 (boys aged 6.18 years, SD = 3.12). These two groups were selected as they represent the polar opposites of the autism spectrum when considering a degree of functional language impairment. The average age of mothers of boys enrolled in the first group (nonverbal boys in module one) was 34 years (SD = 4.75), and in the second group (verbal boys in module three), it was 40 years (SD = 4.14) at the time of completing the questionnaire (Tab. 1).

Most of the mothers were residents of Bratislava (the capital of Slovakia) and the surrounding area (M1: 42.06%; M3: 20.56%). All of the country's regions were represented by at least one respondent/mother (Tab. 2).

The answers provided by the biological mothers were compared between the non-verbal and verbal group of boys using the statistical program GraphPad PRISM statistical packages, tested with the T-test, Chi-squared test, Mann-Whitney U-test, and Fisher's exact test, dependent upon the normality of data distribution.

## Results

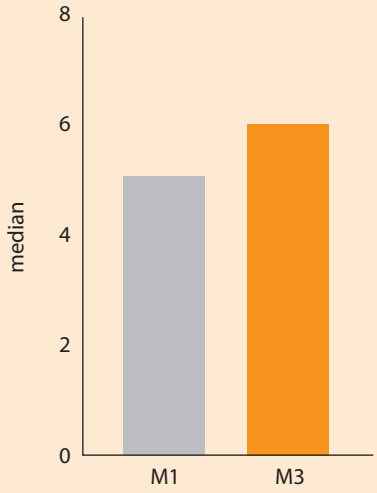
An epidemiological questionnaire was filled out by 107 biological mothers of 107 autistic boys. The questions reflect on the potential causes of autism including maternal factors (such as age, menstrual cycle, reproductive and sexual health) as well as substance abuse before and during pregnancy. The answers were compared between mothers of nonverbal (N = 68, aged 3.53 ± 1.05 years) and verbal children (N = 39, aged 6.18 ± 3.12 years), aimed at detecting those risk factors which might influence the degree of functional language impairment.

However, the most commonly quoted risk factors for autism spectrum disorder were not among those which seemed to be related to the degree of functional language impairment in the analyzed

**Tab. 3. Observed maternal and epigenetic factors in association with speech development in autistic children.**
Tab. 3. Sledované maternálne a epigenetické faktory v súvislosti s rečovým vývinom u artistických detí.

| | Questions answered by the biological mothers of verbal and nonverbal autistic boys | P-value |
|---|---|---|
| Vaginal infections before and during pregnancy | vaginal candidiasis | 0.40 |
| | bacterial vaginosis | 0.94 |
| | HPV infection (genital herpes) | 0.21 |
| | gonorrhea | N/A |
| | syphilis | N/A |
| | chlamydia | 0.99 |
| | genital warts (human papilloma virus) | 0.31 |
| Substance use and abuse | nicotine (tobacco products) | 0.07 |
| | sedatives | N/A |
| | antidepressants | 0.31 |
| | alcohol | 0.67 |
| | marijuana | 0.99 |
| | other substances causing dependency | 0.99 |
| Hormonal factors | polycystic ovaries | 0.52 |
| | obesity | 0.44 |
| | underweight | 0.71 |
| | hormonal therapy two years before conception or during pregnancy | 0.53 |
| | hypothyroidism | 0.27 |
| | hyperthyroidism | 0.69 |
| | duration of HC use | 0.67 |
| | duration of HC withdrawal before conception | 0.39 |
| Age factors | menarche | 0.59 |
| | age at first pregnancy | 0.5 |
| | age at pregnancy with autistic child | 0.74 |
| Menstrual cycle | length of menstrual cycle before the pregnancy with the autistic child | **0.017** |
| | days of menstrual bleeding before the pregnancy with the autistic child | 0.32 |
| | abnormalities of the menstrual cycle (amenorrhea, dysmenorrhea, very short cycle, very long cycle, irregular cycle) | 0.87 |

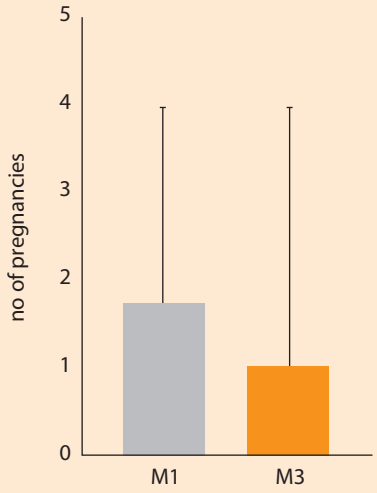
HPV – human papillomavirus/ľudský papilomavírus, N/A – not applicable (no report on this condition)/neuplatňuje sa




ASD/PAS – autism spectrum disorder/ /poruchy autistického spektra, M1 – Module 1/Modul 1, M3 – Module 3/ /Modul 3

**Graph 1. Number of menstrual cycle days before pregnancy with an autistic child.**

Graf 1. Počet dní cyklu pred tehotenstvom s autistickým dieťaťom.




ASD/PAS – autism spectrum disorder/ /poruchy autistického spektra, M1 – Module 1/Modul 1, M3 – Module 3/ /Modul 3, no – number/počet

**Graph 2. Number of pregnancies before pregnancy with an autistic child.**

Graf 2. Počet tehotenstiev pred tehotenstvom s autistickým dieťaťom.

group of ASD boys. Sexually transmitted diseases (STDs) and genital infections were not related to the severity of ASD, and neither were hormonal factors, age factors, nor the consumption of substances causing dependency before pregnancy (Tab. 3). Regarding STDs, there was no case of gonorrhea, syphilis, or chlamydia (NA) in the mother (NA) (Tab. 3). However, mothers reported infections such as vaginal candidiasis (16 cases in M1 mothers, 5 cases in M3 mothers), bacterial vaginosis (7 cases in M1 mothers, 4 cases in M3 mothers), genital herpes (only 1 reported case of an M3 mother), and genital warts (only 1 case in the M3 mothers' group) during pregnancy.

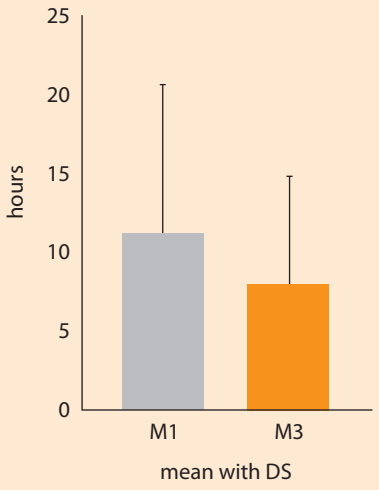
Regarding hormonal factors, 11 mothers reported to suffer from polycystic ovary syndrome (6 in the nonverbal children group) and 5 in the verbal children group. Nine mothers of nonverbal children reported to have undergone hormonal therapy (in the verbal children's group it was 3); a total of 11 mothers reported to suffer from hypothyroidism (8 in the non-verbal group and 3 in the verbal group) and 2 from hyperthyroidism (1 in each group). It is not clear which of the mothers were administered thyroidal hormones. Twenty-two (32.35%) M1 mothers and 12 (31.59%) M3 mothers used hormonal contraceptives before pregnancy with the autistic child. Most of them stopped taking the hormonal contraceptive 12 months prior conceiving the child later diagnosed with ASD (24 in the M1 group and 12 in the M3 group). The average time years of taking HC in the non-verbal mothers' group was 5.67 (SD = 3.23), while in the verbal mothers' group it was 6.58 (SD = 3.15). Seven M1 mothers and 4 M3 mothers reported to have been obese during childhood or adulthood and only one mother from the non-verbal group was underweight (Tab. 3).

Age factors did not play a role. Mothers of nonverbal children started menstruating at an average age of 12.91 years (SD = 1.46), while mothers of verbal children started at 13.05 years (DS = 1.45).

Differences could be found in the length of the menstrual cycle (P = 0.017), indicating a shorter menstrual cycle before pregnancy with the ASD child in mothers of nonverbal children when compared to the verbal ones (Graph 1). The cycle of M1 mothers lasted on average 27.35 days (SD = 6.61 days), while in M3 mothers it was 30.14 days, (SD = 4.38). Surprisingly, they also reported more pregnancies prior to that with the autistic child (P = 0.026, Graph 2). Mothers of nonverbal children reported zero to four previous pregnancies (on average 0.93, SD = 1.06), while mothers of verbal children had zero to two pregnancies (on average 0.32, SD = 0.92). Unfortunately, it is not clear whether the previous pregnancies led to an abortion, stillbirth, or represented live births, since not all of the mothers were willing to report on this.

Most of the children diagnosed with autism were conceived naturally; an assisted reproduction method was performed only in 4 (5.88%) cases in the non-verbal group and 1 case (2.56%) in the verbal group.

Regarding the perinatal period, differences could be found in the reported length of the delivery from first contractions to delivery of the baby (P = 0.018, Graph 3). Mothers of nonverbal children reported a delivery time from 2 to 48 hours (on average 11.13 hours, SD = 9.49), while mothers of verbal children had a much shorter delivery time, between 1 and 27 hours, which was on average 7.09 hours (SD = 8.91). Other perinatal factors such as vaginal delivery vs. caesarian section didn't seem statistically relevant (P = 0.28). Thirty-four (50%) of the non-verbal children were delivered by caesarian section; in the verbal children group, it was 19 (51.35% out of 37). Forceps or a vacuum extractor was used in 6 (8.82%) cases of nonverbal children, while in the group of verbal children it was 2 (5.12%). Thirty-




M1 – Module 1/Modul 1,
M3 – Module 3/Modul 3

**Graph 3. Length of childbirth. Difference of means and standard deviation.**
Graf 3. Dĺžka pôrodu. Rozdiel v priemeroch a priemerná odchýlka.

**Tab. 4. Perinatal factors observed in relation to speech development in autistic children.**
Tab. 4. Perinatálne faktory sledované v súvislosti s rečovým vývinom u autistických detí.

| | Questions answered by the biological mothers of verbal and nonverbal autistic boys | P-value |
|---|---|---|
| Delivery data and the newborn | natural conception vs. assisted | 0.65 |
| | number of pregnancies before the pregnancy with the autistic child | **0.026** |
| | delivery date (week of pregnancy) | 0.38 |
| | delivery (vaginal/section) | 0.28 |
| | forceps, vacuum extraction | 0.71 |
| | epidural | 0.31 |
| | labor time from first contractions to the delivery of the child | **0.018** |
| | baby length | 0.82 |
| | baby weight | 0.12 |
| | newborn jaundice | 0.43 |

-six (52.94%) mothers of the non-verbal group had epidural analgesia; in the verbal group, it was 16 (41.02%). Most of the babies were born on time after the 37th week; on average it was in the 38.55th week (SD = 2.75) in the non-verbal group and in the 38.09th week (SD = 2.95) in the verbal group. Only 7 babies (10.29%) from the non-verbal group and 4 babies (10.26%) from the verbal group were born prematurely (between the 27th and 35th week). None of these factors could be statistically related to the degree of functional language impairment of the diagnosed autistic boys (Tab. 4).

There were no statistically significant differences regarding the newborns' measures (Tab. 4). Babies from the non-verbal group were born with an average birth weight of 3,306.03 grams (SD = 720.92) and those from the verbal group weighed 3,105.08 grams (SD = 632.80). The average birth length in the non-verbal group was 49.86 cm (SD = 3.77), and in the verbal group it was 49.54 cm (SD = 3.64). More than one half of the newborns suffered from newborn jaundice; 42 (65.63% out of 64 answers) from the non-verbal group and 28 (73.68% out of 38 answers) from the verbal group. Approximately 37.5% of the babies with jaundice from the non-verbal group and 12 (31.58 %) from the verbal group required special treatment including phototherapy (Tab. 4).

## Discussion

Despite a number of different studies and a relatively wide range of different risk factors for the development of ASD, according to our knowledge, there is a lack of studies focused on the explanation of genetic and epigenetic factors acting on the speech development or the lack of it in children with ASD. Paying more attention to this topic is therefore crucial. In particular, the rate of speech impairment in children with ASD is very significant because verbal children with ASD have a much better life prognosis.

In our study, we investigated a fairly wide range of possible risk factors, including age factors, the use of certain medications, hormonal influences, consumption of alcohol or smoking, overcoming a serious infection during pregnancy, and problematic childbirth including premature birth, cesarean section, and other birth complications.

We managed to identify rarely described risk factors possibly influencing speech development in children with ASD – number of pregnancies before ASD pregnancy, days of the menstrual cycle before ASD pregnancy, and baby delivery time. On the contrary, many often described factors in the literature did not prove to be significantly different. However, this does not mean that they are not meaningful in general for the development of ASD. Based on our study, we can only determine that they probably do not affect whether or not a child with ASD will have language impairment. They may still contribute to the development of ASD, but not specifically affect speech development.

## Maternal early life factors associated with children's ASD

Our study explored a range of maternal factors, including reproductive health, substance abuse, hormonal influences, and obstetric history. Surprisingly, traditional risk factors commonly associated with autism spectrum disorder (ASD), such as sexually transmitted diseases (STDs), hormonal imbalances,

and substance abuse, did not appear to correlate with the degree of functional language impairment in the analyzed group. Instead, the study highlighted associations between certain maternal health conditions and the verbal abilities of autistic boys.

For instance, maternal infections during pregnancy, such as vaginal candidiasis and bacterial vaginosis, were reported more frequently among mothers of nonverbal children compared to those of verbal children. Vaginal candidiasis was reported by 16 mothers from the non-verbal group and only 5 from the verbal group. Bacterial vaginosis was reported by 7 mothers from the verbal group and 4 mothers from the non-verbal group. Genital herpes was reported in only one mother from the nonverbal group, as well as genital warts.

Additionally, hormonal factors, including polycystic ovary syndrome (PCOS) and hypothyroidism, were more prevalent in mothers of nonverbal children. These findings suggest a potential link between maternal health status and the degree of functional language impairment in autistic boys, although further research is needed to elucidate the underlying mechanisms.

Furthermore, differences in menstrual cycle length and previous pregnancies were observed between the two groups ($P = 0.017$), with mothers of nonverbal children reporting shorter menstrual cycles (on average 27.35 days) and a higher number of previous pregnancies (on average 0.93). These findings raise intriguing questions about the impact of maternal reproductive history on neurodevelopmental outcomes in autistic individuals.

The largest study of over 10,000 ASD cases drawn from Danish electronic health registers reported that maternal hospitalization for viral infection in the first trimester and any infection or bacterial infection in the 2nd trimester were associated with increased ASD risk [30]. Another study showed that use of ADs during the 2nd and/or 3rd trimester was associated with an 87% increased risk of ASD, even after taking into account potential confounders; no association was observed between use of ADs during the first trimester and the risk of ASD [31]. The main meta-analysis, with 22 observational studies comprising of 795,632 cases and 1,829,256 control participants, used a random-effects model to find no significant association between maternal smoking during pregnancy and ASD in offspring (pooled odds ratio (OR) = 1.16, 95% CI: 0.97–1.40) [32].

Many studies have shown that having a physical or mental disease or having any kind of infection during pregnancy (Rubella (German measles), Cytomegalovirus (CMV), Influenza (flu), Toxoplasmosis, and Herpes simplex virus (HSV)) can increase the possibility of having a child with autism [33,34].

In addition, the use of medication to address or control these conditions during pregnancy may heighten the likelihood of giving birth to a child diagnosed with autism [33,35].

For instance, research focusing on mothers who experienced a viral infection in the first trimester and a bacterial infection in the second trimester revealed a noteworthy correlation with the occurrence of autism in their children [36,37]. The monitored sample in the illustrative case-control study included 407 cases and 2075 frequency-matched controls.

Conversely, having a viral infection alone did not exhibit an association with the likelihood of having a child with autism [33]. Furthermore, a systematic review indicated that pregnant women diagnosed with various autoimmune diseases (such as rheumatoid arthritis, lupus, and multiple sclerosis) faced a 34% increased risk of having a child with autism [35].

## Number of previous pregnancies

Mothers of nonverbal children reported a higher number of previous pregnancies compared to mothers of verbal children (Graph 2). While the nature of these prior pregnancies (live births, abortions, and stillbirths) remains unclear, it warrants further investigation to understand if pregnancy history plays a role in the etiology of autism itself and the level of speech development in autistic children. However, the other study states that the offspring of women with a history of abortion were at higher risk of ASD [38]. Besides ASD as a neurodevelopmental disorder, previous studies demonstrated a positive association between abortion and other neurodevelopmental disorders such as attention-deficit/hyperactivity disorder [39,40], intellectual disability [41], and cerebral palsy [42]. Since abortion is a common adverse pregnancy outcome, valid and robust evidence of its effect on adverse outcomes in children is a research need that can be addressed using large-scale prospective studies.

## Length of labor

Mothers of nonverbal children reported significantly longer labor times (on average four hours longer labor) compared to mothers of verbal children (Graph 3). This suggests a potential association between complications during delivery and speech outcomes in autism. We found no literature referencing this finding.

## Alcohol consumption and substance abuse

The obtained results unequivocally indicate the absence of a correlation between the use of substances causing addiction before pregnancy, such as alcohol, nicotine, and others, and the degree of functional language impairment of an autistic child. Compared with previous similar studies, our results are somewhat consistent, as previous researchers have found disagreements on this issue. According to a study which investigated the effects of prenatal alcohol exposure (PAE) at various levels on

child neurodevelopment at 2 years of age, initial findings suggested a potential positive correlation between low-level PAE and cognitive development; this was reduced after considering environmental and social factors [34]. Notably, early binge drinking followed by continued lower-level PAE was linked to increased sensation-avoiding behavior in children. However, no significant associations were found between PAE and overall neurodevelopment after adjusting for key confounders, emphasizing the need for further research to uncover subtle or delayed effects.

In addition, a meta-analysis evaluating the link between maternal alcohol consumption during pregnancy and ASD in the offspring and analyzing data from five studies, they did not find an independent association between maternal drinking and ASD risk in children, with consistent results across different levels of alcohol intake and pregnancy trimesters [36].

Also, there is conflicting evidence that challenges the presented results. For example, studies of children with fetal alcohol spectrum disorder (FASD) demonstrate that they face significant language difficulties compared to controls [37]. Specifically, they found pronounced deficits in both receptive and expressive language skills, with younger children struggling in relational vocabulary and sentence imitation, and older children showing delays in word ordering, grammatical comprehension, and malapropisms.

Another updated systematic review of the effects of PAE on language, speech, and communication development in children up to preschool age, as well as analyzing longitudinal studies from 1970 onwards, found that PAE was significantly associated with delayed receptive and expressive communication, highlighting the negative impact of alcohol on fetal development [43]. Even after accounting for environmental factors, the association persisted up to 36 months.

Finally, a study was conducted that examined the impact of PAE on brain white matter integrity in toddlers aged 2–3 years, comparing children with PAE to unexposed controls. It found that PAE was associated with alterations in white matter microstructural integrity, particularly in the brain stem, limbic, and association tracts [44]. White matter integrity of the uncinate fasciculus is associated with learning, memory, attention, and language skills in children [45].

Thus, our study's findings emphasize the complexity of understanding the impact of PAE on ASD, revealing no direct correlation with language deficits in autistic children. Despite some studies suggesting potential impacts of PAE on cognitive and language development, this work, alongside a meta-analysis, underscores a lack of significant association after adjusting for confounding factors. It is advised to conduct further research on the impact of alcohol consumption before and during pregnancy on the neurodevelopmental outcomes in children.

We suggest that our results will be interpreted in accordance with some limits of our research, namely the fact that mothers filled out the questionnaire retrospectively, shortly before their children were diagnosed in ARCA, so the reported information could be slightly affected by time. The reliability of mother reports could have also been affected by the fact that the questionnaire was not fully anonymized (later linked to the child due to the child's code assigned before diagnostics), so it is possible that the relatively low reporting of substance abuse was purposefully lower than in reality. Finally, the most serious deficit is the current absence of a control sample for a reliable comparison of the prevalence of these epigenetic risk factors in the general population of parents of neurotypical children in Slovakia. In the future, we would like to collect a sufficiently large sample of Slovak mothers with neurotypical children in order to do a comparison.

## Conclusion

In conclusion, as we still know relatively little about the development of speech in children diagnosed with ASD and possibly the causes of their often-observed impairments, our study contributed to this important debate. Since previous research studies focusing on analyzing the genes possibly responsible for speech development in ASD have not yet reached convincing conclusions, it is safe to assume it is necessary to investigate genetic as well as epigenetic factors or their combinations. Identifying early risk factors for delayed or impaired speech development in ASD is crucial, as it would allow quicker and targeted interventions focused on language and speech development for children at risk, potentially resulting in significantly improved future prognosis.

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