 doi:10.20944/preprints201906.0030.v1

Is Excessive Prenatal Ultrasonography a Risk Factor for Autism? A Literature Review

Short Title: Excessive Prenatal Ultrasonography: Autism Risk Factor?

Hissa Moammar[a], MD FAAP and Rabi Sulayman[b], MD FAAP

[a] King Salman Center for Disability Research, Riyadh, Saudi Arabia; and [b] Chicago Medical School, Chicago, Illinois.

Address correspondence to:

Hissa Moammar, POB 76763, Rakah Post Office, Khobar 31952, Saudi Arabia; Tel: +966505805963; Fax +966138578685; Email: hissamoammar@yahoo.com

Rabi Sulayman, Professor, and Chair, Chicago Medical School, 4220 West 95th Street Suite 210, Oak Lawn, Chicago, Illinois 60453; Tel: +1708.684.5580; Fax +1708.684.4068; Email: rabi.sulaymanmd@gmail.com

Table of Contents Summary

The historic mutual rise of prenatal ultrasonography and autism spectrum disorder and their similarities and controversies in the literature are discussed in this review.

Scientific Abstract

For the past several decades, abdominal prenatal ultrasonography has been the most significant technology in obstetrics as a long-established practice. However, the frequency, exposure time, thermal and cavitation exposure indices, and increased acoustic output of the ultrasonic waves may be harmful to the embryo/fetus and might increase susceptibility to Autism Spectrum Disorder (ASD). The increase in the prevalence of ASD is associated with an affluent ethnicity, high socioeconomic status, and high parental education where prenatal ultrasonography is readily available and affordable. Enhanced biophysical adverse effects may link the analogous increase in prenatal ultrasonography and autism, and prenatal ultrasonography may emerge as a risk factor for autism. Radiography usage provides historical evidence for this fact: the predominant past opinion was that exposure to X-rays during pregnancy caused no significant risk to a fetus. However, the association between X-ray exposure and childhood leukemia was only established 40 years after X-ray use began. This review focuses on excessive PUS usage and ASD development.

Public Abstract

Advancements in medical technology over the past several decades have made prenatal ultrasound more frequently accessible to expecting mothers during their pregnancy, especially for the affluent. A parallel development is the increase in autism diagnoses (Autism Spectrum Disorder, or ASD) in children of affluent families. There is a general lack of studies of the impact of prenatal ultrasound on fetuses, especially around varying attributes such as frequency, duration of exposure, and thermal and cavitation indices. There is also a historical precedent set, where

 doi:10.20944/preprints201906.0030.v1

exposing fetuses to X-rays was not found to be harmful until it was linked to the development of childhood leukemia decades later. This paper seeks to establish a need to further study these attributes of prenatal ultrasound overuse and their possible impact on a developing fetus, with a special focus on the occurrence of Autism.

Keywords: autism; autistic spectrum disorder; children; behavior; ultrasonography; prenatal; pregnancy

Introduction:

The prevalence of autism spectrum disorder (ASD) has been rising markedly since the Centers for Disease Control and Prevention (CDC; Atlanta, GA, USA) first began recording its prevalence in 1988.[1] There is no known cause for ASD; regardless, the coinciding rise in abdominal prenatal ultrasonography (PUS) use and prevalence of ASD is challenging to disregard. However, any possible correlation between the two has not been well-explored. This review focuses on excessive PUS usage and ASD development.

History of PUS

Ultrasonography was first used in the field of obstetrics/gynecology in 1958[2] .Bang and Holm[3] in 1968, reported identifying a fetal heartbeat at ten weeks of gestation. In the early 1980s, an "ultrasonic boom" [4] ensued because of the introduction of a small, portable, and affordable ultrasonic real-time device that facilitated PUS examinations in the doctors' clinics worldwide.[5]

Ultrasonography involves pressure waves that are greater than 20 kilohertz in frequency to make ultra-oscillating sound waves that penetrate tissue as mechanical energy.[6,7] The contact between the ultrasonic wave and the scanned tissue produces the following biophysical effects: thermal effects, cavitation, and acoustic pressure flow in body fluids. The intensity of an ultrasonic wave (i.e., the acoustic power per unit of area) is measured in milliwatt per square centimeter (mW/cm^2). The highest exposure intensity within the beam (i.e., spatial peak) is averaged over the time of exposure (i.e., temporal average) and is named the spatial peak temporal average.[6-10]

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 4 June 2019 doi:10.20944/preprints201906.0030.v1

After ultrasound manufacturers developed output display standard (ODS) biosafety measures by displaying their levels on the ultrasonic screens, the Food and Drug Agency (FDA) in 1992 deregulated the acoustic output levels of clinical ultrasound systems, which produced better images and therefore improved diagnosis for patients. Ultrasonic acoustic output levels, emitted by the transducer, for fetal, neonatal, and pediatric imaging were augmented from 94 mW/cm^2 in 1986[11] to 720 mW/cm^2 in 1992,[12] including the peak exposure through the mechanical index, which enhanced potential biophysical effects exerted on the embryo and fetus.[4,6,10,13]

The ODS is composed of two indices to alert the end-user of temperature rise and mechanical impact. The soft tissue thermal index depends on three factors: transducer opening i.e. beam width, beam direction, and scanning mode. The ultrasonic beam can produce heat[6,7,10] and cause hyperthermia[6,9-11] to an embryo/fetus. The mechanical or cavitation index occurs because of an intense beam forming bubbles in soft tissue; if severe, mechanical injury causes cavitation in tissues.[14] The acoustic output leads to radiation forces flowing in fluids causing strain on tissues.[15]

The responsibility of the ultrasonographer is to keep these indices as low as possible and to maintain the acoustic pressure as low as reasonably achievable (ALARA). These safety regulations are clinician- and end-user-dependent.[11,13] Hence, the FDA recommended that ultrasound scanning be limited for valid medical indications and conducted by the professionally trained end-users. [11,13,15]

In the 1980s, the first diagnostic imaging devices evolved into a digital two-dimensional (2D) arrays. This advancement was followed, in the 1990s, by real-time processable three-dimensional (3-D) and four-dimensional (4-D) arrays, which is 3-D visualization with motion.[15,16]

History of ASD

In 1943, child psychiatrist Kanner[17] was the first to characterize autism as extreme social isolation and intolerance for change; he used the term “autos,” meaning “self” in Greek, to describe “early infantile autism.” Kanner also emphasized the rarity of infantile autism with

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 4 June 2019 doi:10.20944/preprints201906.0030.v1

150 cases per 20,000 "troubled children" observed over a 30-year career.

In 1944, Asperger[18] described autistic-like behavior in young boys with decreased social and communicative interactions combined with ordinary intelligence and language attainment. In 1966, an epidemiological survey in the County of Middlesex by Lotter[19] revealed that the prevalence of "autistic" cases was 4.5 per 10,000 population and was more common in boys.

In 1970, Treffert[20] of the state of Wisconsin in the United States published a 5-year study (conducted from 1962 to 1967) that investigated the prevalence of infantile autism, which was rare at a prevalence of 0.7 cases per 10,000 population. In the Treffert study, the ordinal position of the autistic child was not the firstborn male. The total prevalence of infantile autism in addition to childhood psychosis and psychotic disorders with brain damage was 4.8 cases per 10,000 population, which was similar to Lotter's figures of prevalence rates.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 4 June 2019 doi:10.20944/preprints201906.0030.v1

Prevalence of ASD

ASD has undergone various diagnostic modifications;[21-26] however, the prevalence of ASD grew exponentially and globally within the last 50 years,[27-49] as presented in the following Table:

Table: Global Prevalence of Autism from 1966 to 2016

Country	Diagnostic Criteria	Year(s)	Prevalence per 10,000 Population
Bahrain [28,42]	DSM-IV	2013	4.3
Canada [27,29,30,31]	DSM-IV-DSM V	1988, 2010, 2015	10.1, 79.1, 106
China [32]	DSM-V	2012	39
France [33]	ICD 10	1997–2003	36.5
India [34]	ADOS	2017	23
Israel [35]	DSM IV	2008–2013	38–43
Japan [27,36,37]	Kanner-DSM-IV	1982–2008	2.33–181.1
Korea [38]	DSM IV	2014	220
Oman [27,39,42]	DSM-IV	2010	1.4
Saudi Arabia [40,41,42]	DSM IV-CARS	2009	1.4–29
Sweden [27,43,44]	Rutter-Gillberg	1983–2006	5.6–35.3
United Arab Emirates [27,42,45]	DSM-IV	2007	29
United Kingdom [19,27,46]	Rating Scale-ICD10	1966–2006	4.1–80.4
United States of America [20,27,47,48,49]	Kanner-DSM-IV DSM V	1970, 2009, 2014	0.7, 110, 168

Note:
ADOS, Autism Diagnostic Observation Schedule; CARS, Childhood Autism Rating Scale;
DSM-IV and DSM-V, Diagnostic and Statistical Manual of Mental Disorders (edition IV and V, respectively);
ICD10, International Classification of Disease, 10th edition

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 4 June 2019 doi:10.20944/preprints201906.0030.v1

Over the course of the past decades, the proposed ASD prevalence increase has been controversial; some investigators[50] conclude that the observed increase in ASD may have been related to the "diagnostic shifting", improved public education and awareness of autism, whereas other investigators state that the increase in ASD is because of high referrals and earlier diagnosis of ASD.[51]

More developed countries[52] and higher socioeconomic populations[53] have a greater prevalence of ASD. This finding may be because of the higher usage of ultrasonic examinations in more affluent populations with higher health insurance coverage.[53] A study from California reported that ASD is associated with higher parental education.[54] From 2002 to 2010, the CDC annual surveillance revealed unaltered racial and ethnic variances in ASD prevalence; white children maintained the highest prevalence whereas Hispanic children remained the lowest.ASD consists of several diverse conditions; such that some autistic individuals are first identified in adulthood.[56] In 2010, Baxter et al.[57] estimated the prevalence of autism in the Global Burden Disease Study as 52 million. In 2016, Vos et al.[58] revised the estimate to 62 million ASD cases, which was an increase of 10 million ASD cases worldwide in 6 years.

In comparing the two studies of Kogan et al. the estimated prevalence by parental report of currently diagnosed ASD in the 3–17 years age group was 1 in 91 in 2007.[59] This prevalence increased to 1 in 40 in 2016.[60]

Prenatal Ultrasonography and ASD

Although an "ultrasound boom"[61] coincided with an "autistic epidemic,"[44,62] correlation and causation between the two has yet to be investigated empirically.

In 2010, in a retrospective American study of children born from 1995 to 1999, Grether et al.[63] found that ultrasonic exposure in the second trimester of pregnancy was not a risk factor for ASD.

In 2012, Stoch et al.[64] from Australia examined an existing controlled study to evaluate the correlation between childhood ASD diagnosis and randomized prenatal ultrasonic

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 4 June 2019 doi:10.20944/preprints201906.0030.v1

exposure. Of 2,834 randomly selected single pregnancies, 1,415 pregnancies underwent one ultrasonic scan at 18 weeks of gestation whereas 1,419 pregnancies underwent several second- and third-trimester scans at 18, 24, 34, and 38 weeks. ASD rate did not show a significant variation between a single second-trimester scan versus several ultrasound scans in the second and third trimesters.

In 2016, Carlsson et al.[65] from Sweden analyzed the frequency of ASD in 30,000 children born from 1999 - 2003. In the study, 14,726 single pregnancies were randomly exposed to one ultrasonic scan at 12 weeks of gestation, and 14,596 pregnancies were exposed to several ultrasonic scans at 18, 24, 34, and 38 weeks of gestation. There was no significant difference in the ASD occurrence between early and late PUS exposure.

In 2013, World Federation for Ultrasound in Medicine and Biology, International Society of Ultrasound in Obstetrics and Gynecology, Asian Federation Societies of Ultrasound in Medicine, American Institute of Ultrasound in Medicine (AIUM), British Medical Ultrasound Society, and European Federation Societies for Ultrasound in Medicine and Biology[66] agreed on the safety and prudent use of PUS by applying the following conditions:

1. Limit fetal exposure time;
2. Restrict pulsed Doppler ultrasound use to clinically required indications;
3. Maintain the thermal index to less than 1;
4. Use as short as possible exposure time (no longer than 5-10 min), not exceeding 60 minutes.[12,66]

The acoustic output threshold was defined; therefore, the AIUM and other societies incorporated the “as low as reasonably achievable” (i.e., “ALARA”) principle in its guidelines to monitor and maintain the acoustic output.[67-70]

The Triple Hit Hypothesis

ASD varies from mild to severe disease. Casanova explains this spectrum in the “triple hit” hypothesis[71,72] wherein the development of autism is determined by the different interaction of

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 4 June 2019 doi:10.20944/preprints201906.0030.v1

three factors: the vulnerable stage of brain development, genetic susceptibility, and environmental impact such as ultrasonic exposure primarily during the first trimester. Casanova hypothesized that the severity of ASD depends on the timing, duration, and intensity of PUS scanning to the embryo/fetus.

In July 2016, Webb et al.[73] retrospectively analyzed a modification of Casanova's hypothesis by analyzing a possible association between ASD severity and PUS exposure within the first trimester of pregnancy with fetuses genetically predisposed to ASD. Genetic predisposition was determined, based on the presence or absence of ASD-associated copy number variations (CNVs) with structural duplications or deletions of deoxyribonucleic acid (DNA) base pairs in the genome sequence.[74]

The results of the Webb et al.[73] study supported the hypothesis that male ASD children with CNVs, who were exposed to first-trimester ultrasound, had a considerably reduced non-verbal intelligence quotient and more repetitive behaviors than male ASD children with CNVs without ultrasound exposure. It also demonstrated that first-trimester PUS exposure influences the outcome diversity in ASD children, whether CNVs were present or not. This variation was not associated with social affective impairment but increased parental reports of repetitive behaviors. The study suggested that the diversity in ASD symptoms may partially result from ultrasonic exposure during early prenatal development in children with particular genetic susceptibilities.

The AIUM Bioeffects Committee[75] responded to Webb's[73] study, by emphasizing that the study results did not determine a causal relationship between ultrasound use and autism. It advocated that PUS can be safely performed by qualified sonographers and clinicians for valid medical causes, and PUS exposure can be reduced when the ALARA principle is applied.

Safety of PUS

Biosafety studies of PUS on the embryo/fetus have been controversial, and the studies can be categorized into advocates,[67,69,76-78] neutralists,[6,79-80] and questioners.[81-84] Abramowitz[10,76,77] highlights the positive safety record reports of PUS on the human fetus and indicates that no

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 4 June 2019 doi:10.20944/preprints201906.0030.v1

scientific studies to date have shown any resulting fetal impairment. Also, the AIUM and ACOG have issued several reports on the prospective biological adverse effects of PUS that have assured its safety.[67-70]

Brightness modulation (B-mode) scanners, used in the mid-1990s, are the source of the prevalent safety verification of PUS.[10,65,79,81] The new devices such as pulsed Doppler, color flow, or scanners produce 10- to 15-fold higher acoustic outputs than those of the earlier 1990s scanners; however, there is a shortage of epidemiological biosafety studies on the new devices' usage.[79,81 82]

Left-handedness, speech delays, dyslexia are PUS-associated neurological symptoms.[80-82] The hypothesis is that PUS generated heat may raise the maternal core temperature. Thus, thermal heat cannot dissipate due to the lack or poorly developed blood circulation in the embryo/fetus respectively, inactivating fetal core enzymes.[82]

A survey of the FDA acoustic output data was grouped into three episodes: 1984-1989,1992-1997 and 2005-2010. The survey revealed a chronological increase in ultrasonic energy power and in Doppler mode utilization in fetal ultrasonic scanning. Doppler modes produce a significant rise in bone thermal index compared to the B-mode. Two ultrasonic factors affect tissue heating mainly the ultrasonic energy output and the higher mean frequency of the transducer which penetrates and is absorbed by the tissue resulting in augmented heating.[83]

A case-controlled study conducted by Rosman et al.[84,85] on three groups of patients; mainly ASD, developmentally delayed and neurotypical, showed a higher mean ultrasonic penetration rate in the ASD group in the first and second trimester, with no statistical difference of other ultrasonic variables. The depth of tissue penetrance can be recorded and counted on the PUS image however how the depth of tissue penetration was calculated continues to be controversial. Ultrasonic devices have calibers that are regularly used to improve image resolution at the expense of tissue penetration. Rosman et al. showed that in the first trimester an increase of 0.9 cm in the mean depth of ultrasonic penetration was observed in the ASD group compared to both the neurotypical and developmentally delayed groups. The higher mean depth of penetration denotes that greater than expected heat dissipation occurred at 3.5-megahertz

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 4 June 2019 doi:10.20944/preprints201906.0030.v1

transducer frequency and has affected fetal neural tissue development in ASD patients.[71,102,103,132]

Ten years after the implementation of ODS, a Swedish assessment[86] revealed that 33% of daily PUS end-users could comprehend the mechanical and thermal indices, 28% were aware of the location of safety indices on their screen, and merely 22% could modify the energy output on their machine. The inadequate knowledge of end-users of the biosafety indices or their screen appearance is widespread among medical professionals in many countries.[86-89]
The current clinical practice is to scan all pregnant women who have low- or high-risk pregnancies at every prenatal clinic visit, which may amount to 10 scans per pregnancy.[10,76,77]

Souvenir or keepsake fetal ultrasounds has expanded into a major industry and are conducted by nonmedical personnel.[90-93] The FDA[94] and the European Committee for Medical Ultrasound Safety,[95] strongly deter this nonmedical practice.

Risk Factors of ASD

Advanced parental age and a higher age difference between parents have been linked as ASD risk factors.[96] ASD risk is also increased in the offspring of mothers with systemic lupus erythematosus,[97] diabetes mellitus,[98,99] pre-eclampsia,[100] and influenza virus infection or vaccination during pregnancy.[101]

Antepartum, intrapartum and perinatal complications were identified as ASD risk factors; including birth asphyxia with Apgar scores of <7 at 5 minutes after birth. [102,103] The association between prenatal fever and ASD, as demonstrated in the studies by Brucato[104] and Hornig,[105] can be an indirect warning of the temperature rise in the embryo/fetus when exposed to an undetected high thermal index during ultrasonography.

Genetics and ASD

The association between ASD and genetics is vastly studied. The genome-wide screening for ASD susceptibility loci,[106-108] has linked more than 100 genomic loci with ASD.[109]

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 4 June 2019 doi:10.20944/preprints201906.0030.v1

ASD comprises certain genetic syndromes, yet gene penetrance varies within a population. For example, the autistic features of fetal valproate syndrome affect approximately 10% of individuals, whereas those of Angelman's syndrome affect 80% of individuals. However, gene mutations in autism are rare and occur in <1% of the population.[110] Twin studies revealed that ASD has moderate genetic heritability and is significantly influenced by environmental factors.[111] Only 5%–15% of ASD cases are identified, based on genetic variations.[112] Genetic variations are involved in ASD; however, geneticists agree that a significant environmental effect is also associated with it.[106,112] They commend investigating other nongenetic causes of ASD.[106]

Neurodevelopment in ASD

Normal neurodevelopment extends from embryogenesis to late adolescence; yet, its critical embryonic/fetal neurodevelopmental growth is susceptible to environmental injuries.[113] The neurotypical brain attains 70% and 80% of its adult mass by one and two years of age, respectively.[114]

Neurodevelopmentally ASD demonstrates two variations: (1) the course of the autistic brain development deviates from the neurotypical one,[71,115-117] and (2) the diversity in ASD neuroanatomy depends on the severity of the autistic disorder.[115,117]

Magnetic resonance imaging (MRI), both functional and structural, is a noninvasive modality for research in children.[118,119] MRI research studies are not applicable before the diagnostic confirmation of ASD at 2–4 years of age. Head circumference measurements correspond accurately with the brain volume, based on structural MRI imaging in 1.7-year-old to 6-year-old normal children but not in the older age group.[121]

Three stages of ASD brain development exist. The first stage is verified by means of head circumference measurements.[116,121] The last two stages are confirmed by using longitudinal serial structural MRI.[117,118]

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 4 June 2019 doi:10.20944/preprints201906.0030.v1

Stage I

At birth, ASD newborns may have a normal or less than the 25th percentile head circumference. The growth of the head circumference/brain size subsequently accelerates to the 84th percentile in the first two years of life.[116,121-123] These changes are predominantly in the dorsolateral prefrontal cortex with an abnormal increase in the frontal and temporal lobe and amygdala.[124] In ASD, the overgrowth of the head circumference/brain size (i.e., macrocephaly) is one of the most frequent traits of the severe form of ASD.[122-125]

Stage II

At 6–10 years of age, the ASD brain's growth rate gradually decelerates to a plateau with the ultimate cessation of growth, which reduces the cortical thickness and brain volume.[126] These early changes affect the growth of brain architecture, and neural connectivity.[126,12]

Stage III

At 10–15 years of age, the brain growth rate in ASD intersects that of the growth percentile of neurotypical adolescents.[128] A prompt decline subsequently ensues in ASD brain volume and continues rapidly throughout late adolescence and adulthood with diminution of cortical thickness and ultimately cortical atrophy.[127-130]

Neuropathology of ASD

Embryonic/fetal neural development depends on vital sequential and genetic programming of the brain.[131] Individuals with the neurodevelopmental HOXA1 gene, which is associated with autism,[132] have a large head circumference. This finding confirms the significance of macrocephaly as a clinical sign of ASD.[124]

To determine whether the overgrowth of the young autistic brain is because of poor control of neuron production (i.e., dysregulation) or lack of cell death (i.e., apoptosis), Courchesne et al.[133] performed neuron cell counts in the postmortem prefrontal cortices. The study showed a 67% increase in neurons in seven autistics versus six normal males. In the dorsolateral and mesial segments of the ASD prefrontal cortex, the increase was 79% and 29%, respectively.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 4 June 2019 doi:10.20944/preprints201906.0030.v1

Prenatally, cortical neurons develop; therefore, this large number of neurons produced in the frontal cortex can only occur because of an abnormal prenatal event that may result in a gene defect or a neural deformity and may delineate the primary biologic etiology of ASD.
Postnatally, neurological development is incapable of producing such excessive frontal lobe neurons.[123-125,133,134,138]

In the ASD frontal lobe of the cerebral cortex, diffusion tensor imaging outlines reduced connections in the white matter association tract as well as horizontal commissural tracts connecting the two hemispheres, and vertical project tracts connecting the brainstem and the spinal cord, based on high values for the apparent diffusion coefficient in the whole frontal lobe.[135-137]

Ribonucleic acid (RNA) in situ hybridization markers reveal abnormal architecture and cortical disorganization in the prefrontal and temporal cortex in autistic children. A prenatal developmental dysfunction may cause this disorganization in neuronal layer formation and differentiation.[138] Neuropathologically, cortical gray matter was embedded in the white matter with multiple developmental defects in 92% of the brains of autistic patients.[139]

The amygdala is a cluster of nuclei in the temporal lobe [140] that regulates emotions such as fear, gratification, anger, and hostility. Dysfunctional amygdala causes lack of social interaction and absence of facial and emotional reaction in ASD.[141] Amygdalar volume growth in ASD is increased in early toddlerhood; however, it eventually slows down such that the neuronal cell number and size are reduced in adulthood.[117,142] Amygdalar enlargement is associated with intense apprehension, and deterioration of communication and social abilities.[142,143]

The cerebellum contributes to higher cognitive functions, such as language, cognitive, and emotional adaptation,[144,145] in addition to balance and motor function.[117]
In ASD children, diffusion tensor imaging[146,147] depicts abnormal connectivity between the cerebellar pathways and its rostral protrusion, where the cerebellar-cerebral disconnection is augmented by the reduced volume,[148]number and density[149] of the Purkingie cells which results in the behavioral and cognitive features of ASD.[150,151]

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 4 June 2019 doi:10.20944/preprints201906.0030.v1

Recent MRI cerebellar studies[152-154] show that autistic children have reduced cerebellar vermal volumes of lobes VI and VII. These findings are particular to nonsyndromic ASD. Vargas et al. [155] reported a pathological inflammation in the cerebral cortices, white matter, and cerebella of ASD patients, supported by proinflammatory cytokines in the ASD cerebrospinal fluid. The ASD postmortem reports of age-specific changes in the weight and volume of brain are probably due to an impediment in its development that causes a continuous inflammatory process.

Discussion

In 1966, before the advent of the portable sonogram, the incidence of ASD was 3 to 4 patients per 10,000 population.[19] By 2004, although various diagnostic criteria were implemented, the incidence had increased to 30–40 patients per 10,000 population.[156,157]

A literature review indicates a surge in the prevalence of ASD, which has affected an estimated 1% of all age groups among high-income countries.[44,56] In addition to the increased prevalence in ASD, the use of PUS has significantly increased.[61,158] The association of radiographic imaging during pregnancy and childhood leukemia was only established by MacMahon in 1962– forty years since the beginning of radiographic imaging.[159]

In 2013, the evaluation of randomized data of PUS usage from insurance sources revealed an average of 4 –5 prenatal scans per pregnancy with a 30%–50% increase in the utilization rate.[158]

In 2014, the lifespan cost of a low functioning ASD individual was $2.4 million in the United States versus $2.2 million in the United Kingdom whereas the lifespan cost of a high functioning ASD individual was $1.4 million in the United States and the United Kingdom.[160]

In 2015, the economic burden of ASD in the United States was forecasted to be $268 billion; 1.45% of the gross domestic product. By 2025, the economic burden is forecasted to increase to $461 billion; which will be 2.29% of the gross domestic product.[161]

The global disease burden of ASD was estimated at 9 million years lived with disabilities by 2016; 121 disability-adjusted life years per 100,000 population.[162]

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 4 June 2019 doi:10.20944/preprints201906.0030.v1

Studies have demonstrated excessive neuron production throughout the first two years of life in ASD patients. It is hypothesized that this is due to an aberrant prenatal event in the uterine environment.[83,115,116,122,126,133,134] Maternal infection during pregnancy, through maternal immune activation (MIA) dysregulation, may alter brain development programming during embryonic/fetal life.[134]

The tendency to conduct a PUS scan for every prenatal visit, which mostly involve low-risk pregnancies, is not medically indicated and is contrary to the American College of Obstetrics and Gynecology (ACOG) and AIUM safety guidelines.[69,70]

A PUS viability scan at 8–10 weeks of gestation should take a very brief dwell time. A second scan to evaluate morphology at 22–24 weeks of gestation ought to be as short as possible.

The medical history for all prenatal visits necessitates the acquisition of information regarding previous PUS scans, and patients must be cautioned to avoid commercial ultrasounds. The maternal temperature should be recorded prior to and during if ultrasonic scanning time is prolonged.[163] The mother must provide informed signed consent before undergoing PUS scanning. When ultrasonically scanning the patient, the ultrasonographer is required to monitor and to document the number of scans, the thermal and mechanical indices, the acoustic output, the depth of penetration and the dwell time.

Regulatory reforms must be implemented to improve professional end-users' knowledge and modification of ultrasonic biosafety parameters. Medical ultrasonographers must acquire a license, which is to be renewed periodically, through strict training with a practical examination. [85-89]

The popular practice of commercial fetal video scanning of pregnant women,[90-93] should be prohibited for maternal and fetal safety.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 4 June 2019 doi:10.20944/preprints201906.0030.v1

Maternal blood biomarkers such as cell-free DNA [164] are available to test for aneuploidy and to replace ultrasonic nuchal translucency examinations which may take up to 45 minutes to uphold the fetus in the military position.

The study by Rosman et al.[84] regarding the association of prenatal ultrasonography and ASD is an essential step in the research on the impact of PUS usage on the fetus; however, case-controlled studies have limitations.

Although ASD research has focused on genetics, medical, and environmental causes, and delineated multiple risk factors and associations; a definite etiology of ASD has yet to be identified.

Prenatal ultrasonography, an important obstetrical tool, is not a commercial commodity and its usage needs to be medically indicated and restricted to safeguard the lives of our future children.

Conclusion

The long-term PUS biophysical effects[6,7] on the embryo/fetus featuring the acoustic output of new ultrasonic devices,[15,16,85] its overuse in clinical practice[10,77] and the commercial fetal video souvenirs [91-94] remain unknown.

A joint obstetric/pediatric, peer-reviewed, statistically significant study to determine whether a correlation prevails between excessive in utero ultrasonic exposure and the development of childhood ASD is long overdue.

 doi:10.20944/preprints201906.0030.v1

Financial Disclosure: The authors have no financial relationships relevant to this article to disclose.

Funding Source: No funding was secured for this study

Conflict of Interest: The authors have no conflicts of interest relevant to this article to disclose.

Abbreviations:

AIUM: American Institute of Ultrasound in Medicine ALARA: as low as reasonably achievable
ADOS: Autism Diagnostic Observation Schedule ACOG: American College of Obstetrics and Gynecology ASD: autism spectrum disorder
CARS: Childhood Autism Rating Scale
CDC: Centers for Disease Control and Prevention CNV: copy number variation
DNA: deoxyribonucleic Acid
DSM: Diagnostic and Statistical Manual of Mental Disorders FDA: Food and Drug Agency
ICD10: International Classification of Disease 10th Edition PUS: abdominal prenatal ultrasonography
MRI: magnetic resonance imaging ODS: output display standard

Contributors' Statement

Dr. Hissa Moammar[a] is a pediatric consultant, affiliated with King Salman Center for Disability Research, (Riyadh, Saudi Arabia). She conducted the literature search using the Cochrane Database of Systematic Reviews and Ovid Medline from 1966 to 2018. She manually retrieved articles written before 1966, based on the reference list. She reviewed the published medical literature on infantile autism, autism, pervasive developmental disorder, autistic spectrum disorder, and ultrasonography and prenatal ultrasonography.

Dr. Rabi Sulayman[b] is affiliated with Chicago Medical School (Chicago, Illinois). He has coauthored, edited, and reviewed the manuscript.

All authors have approved the final manuscript as submitted and agree to be accountable for all aspects of the work.

Acknowledgments

The authors thank Dr. Muhammad Mansur for his advice and recommendation of this literature review. He is a practicing Obstetrician and Gynecologist in Beirut, Lebanon with extensive training in Maternal-Fetal Medicine and Obstetrical Ultrasonography at McGill University, Montreal- Canada.

The authors acknowledge that the opinions expressed in this article are those of the authors and not necessarily those of King Salman Center for Disability Research or Chicago Medical School.

References

1. Bertrand J, Mars A, Boyle C, Bove F, Yeargin-Allsopp M, Decoufle P. Prevalence of autism in a United States population: The Brick Township, New Jersey, investigation. *Pediatrics* 2001; 108: e1155–e1161. https://www.ncbi.nlm.nih.gov/pubmed/11694696
2. Donald I, McVicar J, Brown T. The investigation of abdominal masses by pulsed ultrasound. *Lancet.* 1958; 271: e1188-e1195. https://www.ncbi.nlm.nih.gov/pubmed/13550965
3. Bang J, Holm HH. Ultrasonics in the demonstration of fetal heart movements. *Am J Obstet Gynecol*. 1968; 102: e956-e960. https://www.ncbi.nlm.nih.gov/pubmed/3299185
4. Campbell S. A short history of sonography in obstetrics and gynaecology. *Facts Views Vis Obgyn*. 2013; 5: e213-e229. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3987368/
5. Sippel S, Muruganandan K, Levine A, Shah S. Review Article: use of ultrasound in the developing world. *Int J Emerg Med.* 2011; 4: e72-e83. doi: 10.1186/1865-1380-4-72
6. Joy J, Cooke I, Love, M. Is ultrasound safe? *Obstet Gynaecol*. 2006; 8: e222-e227. doi: 10.156/toag.8.4.222.27271.
7. Chen Z. Assessment of ultrasound field properties and the potential effects on cells [Master's Thesis]. Stockholm, Sweden: Royal Institute of Technology; 2013. https://www.diva-portal.org/smash/get/diva2:637933/FULLTEXT02.pdf
8. American Institute of Ultrasound in Medicine: Standard for real-time display of thermal and mechanical indices on diagnostic ultrasound equipment., Laurel, MD, 1992 and National Electrical Manufacturers Association, Rosslyn, VA. https://www.nema.org/Standards/Pages/Standard-for-Real-Time-Display-of-Thermal-andMechanical-Acoustic-Output-Indices-on-Diagnostic-Ultrasound-Equipment.aspx
9. Safety Group of the British Medical Ultrasound Society. Guidelines for the safe use of diagnostic ultrasound equipment. Ultrasound. 2010; 18: e52-e59. https://www.bmus.org/static/uploads/resources/BMUS-Safety-Guidelines-2009-revisionFINAL-Nov-2009.pdf
10. Abramowicz, JS. First-Trimester Ultrasound: A Comprehensive Guide; Detroit MI, Springer, 2016. doi: 10.1007/978-3-319-20203-7
11. Food and Drug Administration (FDA). 510 (k) *Guide for Measuring and Reporting Acoustic Output of Diagnostic Ultrasound Medical Devices*. Rockville, MD: FDA Center for Devices and Radiological Health;1985.https://www.fda.gov/ucm/groups/fdagov-public/@fdagovmeddev-gen/documents/document/ucm578118.pdf
12. Food and Drug Administration (FDA). *Revised 510(k) Diagnostic Ultrasound Guidance for 1993*. Rockville, MD: FDA Center for Devices and Radiological Health; 1993. https://www.fda.gov/downloads/UCM070911.pdf
13. Stratmeyer ME, Greenleaf JF, Dalecki D, Salvesen KA. Fetal ultrasound: mechanical effects. *J Ultrasound Med*. 2008; 27: e597-e605. https://www.ncbi.nlm.nih.gov/pubmed/18359910
14. Henderson J, Wilson K, Jago JR, et al. A survey of the acoustic outputs of diagnostic ultrasound equipment in current clinical use. *Ultrasound Med Biol.* 1995;21: e699-e7051. https://www.ncbi.nlm.nih.gov/pubmed/8525560
15. Sheiner E, Hackmon R, Shoham-Vardi I, et al. A comparison between acoustic output indices in 2D and 3D/4D ultrasound in obstetrics. *Ultrasound Obstet Gynecol*.2007;29(3) e326–e328. doi:10.1002/uog.3933. 2007;29(3): e326-e328. doi:10.1002/uog.3933.

16. Sheiner E, Shoham-Vardi I, Pombar X, Hussey MJ, Strassner HT, Abramowicz JS. An increased thermal index can be achieved when performing Doppler studies in obstetric sonography. *J Ultrasound Med.* 2007;26 (1):e71-e76.doi: 10.1371/journal.pone.0135717
17. Kanner L. Autistic disturbances of affective contact. *Nervous Child*. 1943; 2:217-250. https://psycnet.apa.org/record/1943-03624-001
18. Asperger H. Die autistisehen psychopathen in kindesalter. *Arch Psych Nervenkrankh.* 1944; 117: e76-e136.https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4725185/. In German.
19. Lotter V. Epidemiology of autistic conditions in young children. *Soc Psychiatry*. 1966; 1: 124-135. https://link.springer.com/article/10.1007/BF00584048
20. Treffert D. Epidemiology of infantile autism. *Arch Gen Psychiatry*. 1970; 22: e431-e438. doi:10.1001/archpsyc.1970.01740290047006
21. American Psychiatric Association. (APA). Diagnostic and Statistical Manual of Mental Disorders, third edition. Washington, DC: APA. 1980. http://displus.sk/DSM/subory/dsm3.pdf
22. American Psychiatric Association. (APA). Diagnostic and Statistical Manual of Mental Disorders, fourth edition. Washington, DC: APA. 1994. http://displus.sk/DSM/subory/dsm4.pdf
23. American Psychiatric Association. (APA). Diagnostic and Statistical Manual of Mental Disorders, fourth edition, text rev. Washington, DC: APA 2000. https://www.psychiatry.org/psychiatrists/practice/dsm
24. American Psychiatric Association. (APA). Diagnostic and Statistical Manual of Mental Disorders, fifth edition. Washington, DC: APA 2013. https://www.psychiatry.org/psychiatrists/practice/dsm
25. World Health Organization. *International Classification of Disease (ICD-10) Classification of Mental and Behavioural Disorders: "Blue Book" (Clinical Descriptions and Diagnostic Guidelines*) . https://www.who.int/classifications/icd/en/bluebook.pdf?ua=1
26. Reed GM. Toward ICD-11: improving the clinical utility of WHO's International Classification of mental disorders. *Prof Psychol Res Pract*. 2010;41: e457-e464. doi: 10.1037/a0021701
27. Elsabbagh M, Divan G, Koh YJ, et al. Global prevalence of autism and other pervasive developmental disorders. *Autism Res*. 2012, 5: e160–e179. doi: 10.1002/aur.23
28. Al-Ansari AM, Ahmed MM. Epidemiology of autistic disorder in Bahrain: Prevalence and obstetric and familial characteristics. *Mediterr Health J.* 2013; 19: e769- e774. https://www.ncbi.nlm.nih.gov/pubmed/24313037
29. Bryson SE, Clark BS, Smith IM. First report of a Canadian epidemiological study of autistic syndromes. *J Child Psychol Psychiatry.* 1988;29(4): e433-e445. http://dx.doi.org/10.1111/j.1469-7610.1988.tb00735.x
30. Lazoff TL, Piperni T, Fombonne E. Prevalence of pervasive developmental disorders among children at the English Montreal School Board. *Can J Psychiatry*. 2010; 55: e715e720. doi: 10.1002/aur.239
31. Public Health Agency of Canada. *2018 Report of the National Autism Spectrum Disorder (ASD) Surveillance System. Autism Prevalence among Children and Youth in Canada.2018*. https://www.canada.ca/en/public-health/services/publications/diseasesconditions/infographic-autism-spectrum-disorder-children-youth-canada-2018.html

32. Wang F, Lu L, Wang SB, Zhang L, et al. The prevalence of autism spectrum disorders in China: a comprehensive meta-analysis. *Int J Biol Sci.* 2018 May 12;14(7): e717-e725. doi: 10.7150/ijbs.24063.
33. Van Bakel MM, Delobel-Ayoub M, Cans C, et al. Low but increasing prevalence of autism spectrum disorders in a French area from register-based data. *J Autism Dev Disord.* 2015 Oct;45(10): e3255-e3261. doi: 10.1007/s10803-015-2486-6.
34. Rudra A, Belmonte MK, Soni PK, Banerjee S, Mukerji S, Chakrabarti B. Prevalence of autism spectrum disorder and autistic symptoms in a school-based cohort of children in Kolkata, India. *Autism Res*. 2017; 10(10): e1597-e1605. doi: 10.1002/aur.1812
35. Mahajnah M, Sharkia R, Shalabe H, *et al.* Clinical characteristics of autism spectrum disorder in Israel: impact of ethnic and social diversities. *Biomed Res Int.* 2015; 2015: e962093. doi: 10.1155/2015/962093**.**
36. Hoshino Y, Kumashiro H, Yashima Y, Tachibana R, Watanabe M. The epidemiological study of autism in Fukushima-ken. *Folia Psychiatr Neurol Jpn.* 1982;36: e115-e124. https://www.ncbi.nlm.nih.gov/pubmed/7129258
37. Kawamura Y, Takahashi O, Ishii T. Reevaluating the incidence of pervasive developmental disorders: impact of elevated rates of detection through implementation of an integrated system of screening in Toyota, Japan. *Psychiatry Clin Neurosci.* 2008; 62: e152-e159. doi: 10.1111/j.1440-1819.2008. 01748.x
38. Kim YS, Fombonne E, Koh YJ, Kim SJ, Cheon KA, Leventhal BL. A comparison of DSM-IV pervasive developmental disorder and DSM-5 autism spectrum disorder prevalence in an epidemiologic sample. *J Am Acad Child Adolesc Psychiatry.* 2014;53(5): e500-e508.doi: 10.1016/j.jaac.2013.12.021.
39. Al-Farsi YM, Al-Sharbati MM, Al-Farsi OA, Al-Shafaee MS, Brooks DR, Waly MI. Brief report: prevalence of autistic spectrum disorders in the Sultanate of Oman. *J Autism Dev Disord.* 2011;41: e821-e825. doi: 10.1007/s10803-010-1094-8
40. Al-Salehi SM, Al-Hifthy EH, Ghaziuddin M. Autism in Saudi Arabia: presentation, clinical correlates, and comorbidity. *Transcult Psychiatry*.2009;46: e340–e347. doi: 10.1177/1363461509105823
41. Faheem M, Mathew S, Al Qahtani M. et al. Autism spectrum disorder: knowledge, attitude, and awareness in Jeddah, Kingdom of Saudi Arabia. *BMC Genomics*.2016; 17(6): e62. doi: http://dx.doi.org/10.18203/2349-3291.ijcp20171683
42. Salhia HO, Al-Nasser LA, Taher LS, Al-Khathaami AM, El-Metwally AA. Systematic review of the epidemiology of autism in Arab Gulf countries. *Neurosciences*.2014;19: e291e296. https://www.ncbi.nlm.nih.gov/pubmed/25274588
43. Steffenburg S, Gillberg C. Autism and autistic-like conditions in Swedish rural and urban areas: a population study. *Br J Psychiatry.*1986; 149(1): e81-e87. https://www.ncbi.nlm.nih.gov/pubmed/3779317
44. Gillberg C, Cederlund M, Lamberg K, Zeijlon L. Brief report: "the autism epidemic". The registered prevalence of autism in a Swedish urban area. *J Autism Dev Disord.*2006;36: e429-e435. https://www.ncbi.nlm.nih.gov/pubmed/16568356
45. Eapen V, Mabrouk AA, Zoubeidi T, Yunis F. Prevalence of pervasive developmental disorders in preschool children in the UAE. *J Trop Pediatr*.2007;53: e202-e205 https://www.ncbi.nlm.nih.gov/pubmed/17244665.
46. Baird G, Simonoff E, Pickles A, et al. Prevalence of disorders of the autism spectrum in a population cohort of children in South Thames: the special needs and autism project (SNAP). *Lancet*. 2006; 368: e210-e215. https://www.ncbi.nlm.nih.gov/pubmed/16844490

47. Centers for Disease Control and Prevention (CDC). Prevalence of autism spectrum disorders—Autism and Developmental Disabilities Monitoring Network, United States, 2006. *MMWR Surveill Summ.*2009;58(SS10): e1-e20. https://www.cdc.gov/mmwr/preview/mmwrhtml/ss5810a1.htm.
48. Christensen DL, Braun KVN, Baio J, et al. Prevalence and characteristics of autism spectrum disorder among children aged 8 years—Autism and Developmental Disabilities Monitoring Network, 11 Sites, United States, 2012. *MMWR Surveill Summ*. 2018;65(13): e1-e23. doi: 10.15585/mmwr.ss6503a1
49. Baio J, Wiggins L, Christensen DL, et al. Prevalence of autism spectrum disorder among children aged 8 years — Autism and Developmental Disabilities Monitoring Network, 11 Sites, United States, 2014. *MMWR Surveill Summ* 2018;67(No. SS-6): e1–e23. http://dx.doi.org/10.15585/mmwr.ss6706a1
50. Barbaresi WJ, Colligan RC, Weaver AL, Katusic SK. The incidence of clinically diagnosed versus research-identified autism in Olmsted County, Minnesota, 1976-1997: results from a retrospective, population-based study. *J Autism Dev Disord*. 2009; 39: e464-e470. doi:10.1007/s10803-008-0645-8
51. Latif AH, Williams WR. Diagnostic trends in autistic spectrum disorders in the South Wales valleys. Autism. 2007; 11: e479-e487. http://aut.sagepub.com/cgi/reprint/11/6/479.
52. Thomas P, Zahorodny W, Peng B, et al. The association of autism diagnosis with socioeconomic status. *Autism*. 2012;16: e201-e213.doi:10.1177/1362361311413397
53. Durkin MS, Maenner MJ, Meaney FJ, et al. Socioeconomic inequality in the prevalence of autism spectrum disorder: evidence from a U.S. cross-sectional study. *PloS One*. 2010;5: e11551. https://doi.org/10.1371/journal.pone.0011551
54. Van Meter KC, Christiansen LE, Delwiche LD, Azari R, Carpenter TE, Hertz-Picciotto I. Geographic distribution of autism in California: a retrospective birth cohort. *Autism Res*. 2010; 3: e19-e29. doi: 10.1002/aur.110
55. Autism and Developmental Disabilities Monitoring Network Surveillance Year 2002 Principal Investigators; Centers for Disease Control and Prevention. Prevalence of autism spectrum disorders—autism and developmental disabilities monitoring network, 14 sites, United States, 2002. *MMWR Surveill Summ*. 2007;56: e12-e28. http://www.cdc.gov/mmwr/preview/mmwrhtml/ss5601a2.htm
56. Lai MC, Baron-Cohen S. Identifying the lost generation of adults with autism spectrum conditions. *Lancet Psychiatry*. 2015; 2: e1013-e1027. doi: 10.1016/S2215-0366(15)00277-1
57. Baxter AJ, Brugha TS, Erskine HE, Scheurer RW, Vos T, Scott JG. The epidemiology and global burden of autism spectrum disorders. *Psychol Med*. 2015; 45(3): e601-e613. doi: 10.1017/S003329171400172X
58. Vos T, Abajobir AA, Abate KH, et al. Global, regional, and national incidence, prevalence, and years lived with disability for 328 diseases and injuries for 195 countries, 1990–2016: a systematic analysis for the Global Burden of Disease Study 2016. *Lancet*; 2017;390: e1211-e1259. doi: 10.1016/S0140-6736(17)32154-2
59. Kogan MD, Blumberg SJ, Schieve LA, et al. Prevalence of parent-reported diagnosis of autism spectrum disorder among children in the US, 2007. *Pediatrics.*2009;124: e1395-e1403. doi: 10.1542/peds.2009-1522
60. Kogan, MD, Vladutiu CJ, Schieve LA et al. The Prevalence of parent-reported autism spectrum disorder among US children. *Pediatrics*. 2018; 142(6): pii: e20174161.

doi: 10.1542/peds.2017-4161.
61. McKinlay A. The ultrasonic boom—focus on health and safety. *Prog Biophys Mol Biol.* 2007; 93: e1-e2. doi:10.1016/j.pbiomolbio.2006.07.008
62. Hertz-Picciotto I, Delwiche L. The rise in autism and the role of age at diagnosis. *Epidemiology*. 2009;20(1): e84-e90. doi: 10.1097/EDE.0b013e3181902d15.
63. Grether JK, Li SX, Yoshida CK., Croen LA. Antenatal ultrasound and risk of autism spectrum disorders. *J Autism Dev Disord.* 2010; 40:238-245. doi: 10.1007/s10803-0090859-4
64. Stoch YK, Williams CJ, Granich J, et al. Are prenatal ultrasound scans associated with the autism phenotype? Follow-up of a randomised controlled trial. *J Autism Dev Disord.* 2012; 42:2693-2701. doi: 10.1007/s10803-012-1526-8.
65. Carlsson LH, Saltvedt S, Anderlid BM, et al. Prenatal ultrasound and childhood autism: long-term follow-up after a randomized controlled trial of first- vs second-trimester ultrasound. *Ultrasound Obstet Gynecol.* 2016; 48: e285-e288. doi: 10.1002/uog.15962
66. World Federation for Ultrasound in Medicine and Biology/International Society of Ultrasound in Obstetrics and Gynecology (WFUMB/ISUOG). WFUMB/ISUOG statement on the safe use of Doppler ultrasound during 11–14-week scans (or earlier in pregnancy). *Ultrasound Med Biol* 2013;39(3): e373. doi: 10.1016/j.ultrasmedbio.2012.11.025.
67. American Institute of Ultrasound in Medicine. As low as reasonably achievable (ALARA) principle; 2014. http://www.auim.org/publications/viewStatement.aspx?id=39.
68. ACOG Practice Bulletin No. 175: Ultrasound in pregnancy. *Obstet Gynecol*. 2016;128(6): e241-e256. doi:10.1097/AOG.0000000000001815
69. ACOG Committee on Obstetric Practice. ACOG Committee Opinion No. 723, Guidelines for diagnostic imaging during pregnancy and lactation. *Obstet Gynecol* 2017;130(4): e210e216.https://www.acog.org/Clinical-Guidance-and-Publications/Committee-Opinions/Committee-on-Obstetric-Practice/Guidelines-for-Diagnostic-Imaging-DuringPregnancy-and-Lactation?IsMobileSet=false
70. American Institute of Ultrasound in Medicine. AIUM-ACR-ACOG-SMFM-SRU Practice parameter for the performance of standard diagnostic obstetric ultrasound examinations. *J Ultrasound Med*.2018;37(11): e13-e24. doi: 10.1002/jum.14831.
71. Casanova, MF. The neuropathology of autism. *Brain Pathol*. 2007; 17: e422-e433. doi: 10.1111/j.1750-3639.2007.00100.x
72. Williams EL, Casanova MF. Potential teratogenic effects of ultrasound on corticogenesis: implications for autism. *Med Hypotheses*. 2010; 75: e53-e58. doi: 10.1016/j.mehy.2010.01.027
73. Webb SJ, Garrison MM, Bernier R, McClintic AM, King BH, Mourad PD. Severity of ASD symptoms and their correlation with the presence of copy number variations and exposure to first trimester ultrasound. *Autism Res*. 2017; 10: e472e484.doi:10.1002/aur.1690
74. Sharp AJ, Locke DP, McGrath SD, et al. Segmental duplications and copy-number variation in the Human Genome. *Am J Hum Genet.* 2005; 77: e78-e88. doi:10.1086/431652
75. AIUM Bioeffects Committee. AIUM responds to autism study. http://www.aium.org/soundWaves/article.aspx?aId=965&iId=20160907

76. Abramowicz JS. Ultrasound and autism: association, link, or coincidence? *J Ultrasound Med.* 2012; 31(8): e1261-e1269. https://www.ncbi.nlm.nih.gov/pubmed/22837291
77. Abramowicz JS. Benefits and risks of ultrasound in pregnancy. *Semin Perinatol*. 2013;37(5): e295-e300. doi: 10.1053/j.semperi.2013.06.004
78. Miller DL. Safety assurance in obstetrical ultrasound. *Semin Ultrasound CT MR*. 2008;29(2):e156-e164 doi: 10.1053/j.sult.2007.12.003
79. Salvesen KÅ, Lees C. Ultrasound is not unsound, but safety is an issue. *Ultrasound Obstet Gynecol*. 2009; 33:502-505. doi: 10.1002/uog.6381
80. Kieler H, Cnattingius S, Haglund B, Palmgren J, Axelsson O. Sinistrality--a side-effect of prenatal sonography: a comparative study of young men. *Epidemiology*. 2001;12:618-623. https://www.ncbi.nlm.nih.gov/pubmed/11679787
81. Marinac-Dabic D, Krulewitch, CJ. Moore, RJ. The safety of prenatal ultrasound exposure in human studies. *Epidemiology*: 2002;13 (3): eS19-eS22 https://www.ncbi.nlm.nih.gov/pubmed/12071478
82. Rodgers C. Questions about prenatal ultrasound and the alarming increase in autism. *Midwifery Today Int Midwife*. 2006; 80:66-67. https://www.ncbi.nlm.nih.gov/pubmed/17265825
83. Cibull SL. Harris GR, Nell DM. Trends in diagnostic ultrasound acoustic output from data reported to the US Food and Drug Administration for device indications that include fetal applications. *J Ultrasound Med* 2013; 32:e1921– e1932. doi:10.7863/ultra.32.11.1921
84. Rosman NP, Vassar R, Doros G, et al. Association of prenatal ultrasonography and autism spectrum disorder. *JAMA* Pediatr. *2018*; *172* (4): e*336*-e *344*. https://www.ncbi.nlm.nih.gov/pubmed/29435580
85. SzaboTL, Lewin PA. Ultrasound Transducer Selection in Clinical Imaging Practice. *J Ultrasound Med.* 2013; 32 (4); e573-e582. https://doi.org/10.7863/jum.2013.32.4.573
86. Marsál K. The output display standard: has it missed its target. *Ultrasound Obstet Gynecol*. 2005; 25: e211-e214. https://doi.org/10.1002/uog.1864
87. Sheiner E, Shoham-Vardi I, Abramowicz JS. What do clinical users know regarding safety of ultrasound during pregnancy? *J Ultrasound Med*. 2007;26: e319-e325. https://www.ncbi.nlm.nih.gov/pubmed/17324981
88. Piscaglia F, Tewelde AG, Righini R, Gianstefani A, Calliada F, Bolondi L. Knowledge of the bio-effects of ultrasound among physicians performing clinical ultrasonography: results of a survey conducted by the Italian Society for Ultrasound in Medicine and Biology (SIUMB**)**. *J Ultrasound*. 2009;12(1): e6-e11. doi: 10.1016/j.jus.2008.12.001
89. Akhtar W, Arain MA, Ali A, et al. Ultrasound biosafety during pregnancy: What do operators know in developing world? National survey findings from Pakistan. *J Ultrasound Med*. 2011;30: e981-e985. https://www.ncbi.nlm.nih.gov/pubmed/21705731
90. Wax JR, Cartin A, Pinette MG, Blackstone J. Nonmedical fetal ultrasound: knowledge and opinions of Maine obstetricians and radiologists. *J Ultrasound Med*. 2006; 25:e331e335.https://onlinelibrary.wiley.com/doi/pdf/10.7863/jum.2006.25.3.331
91. Dowdy D. Keepsake ultrasound: taking another look. *J Radiol Nurs*. 2016;35(2): e119e132. doi: 10.1016/j.jradnu.2016.02.006
92. Barnett SB, Abramowicz JS, Ziskin MC, et al. WFUMB symposium on safety of nonmedical use of ultrasound. *Ultrasound Med Biol*. 2010;36 (8): e1209-e1212. doi: 10.1016/j.ultrasmedbio.2010.04.005

93. Raucher MS. What they mean by "good science": the medical community's response to boutique fetal ultrasounds. *J Med Philos.* 2009;34(6): e528-e544. doi:10.1093/jmp/jhp039
94. Food and Drug Administration. Avoid fetal "keepsake" images, heartbeat monitors. 2016.https://www.fda.gov/ForConsumers/ConsumerUpdates/ucm095508.htm
95. European Committee for Medical Ultrasound Safety (ECMUS). Souvenir scanning statement 2006. http://www.efsumb.org/ecmus
96. Sandin S, Schendel D, Magnusson P, et al. Autism risk associated with parental age and with increasing difference in age between the parents. *Mol Psychiatry*. 2016;21: e693-e700. doi: 10.1038/mp.2015.70
97. Vinet É, Pineau CA, Clarke AE, et al. Increased risk of autism spectrum disorders in children born to women with systemic lupus erythematosus: Results from a large population-based cohort. *Arthritis Rheumatol*. 2015;67: e3201-e3208. doi:10.1002/art.39320
98. Xiang AH, Wang X, Martinez MP, et al. Association of maternal diabetes with autism in offspring. *JAMA.* 2015;313(14): e1425-e1434. doi: 10.1001/jama.2015.2707
99. Wan H, Zhang C, Li H, Luan S, Liu C. Association of maternal diabetes with autism spectrum disorders in offspring: A systemic review and meta-analysis. *Medicine (Baltimore).* 2018;97(2): e9438. doi: 10.1097/MD.0000000000009438
100. Curran EA, O'Keeffe GW, Looney AM, et al. Exposure to hypertensive disorders of pregnancy increases the risk of autism spectrum disorder in affected offspring. *Mol Neurobiol.* 2017: e1-e8. doi: 10.1007/s12035-017-0794-x
101. Zerbo O, Qian Y, Yoshida C, Fireman BH, Klein NP, Croen LA. Association between influenza infection and vaccination during pregnancy and risk of autism spectrum disorder. *JAMA Pediatr*. 2017;171: e163609. doi: 10.1001/jamapediatrics.2016.3609
102. Hadjkacem I, Ayadi H, Turki M, et al. Prenatal, perinatal, and postnatal factors associated with autism spectrum disorder. *J Pediatr*. 2016; 92: e595-e601. doi: 10.1016/j.jped.2016.01.012
103. Getahun D, Fassett MJ, Peltier MR, et al. Association of perinatal risk factors with autism spectrum disorder. *Am J Perinatol.* 2017;34: e295-e304. doi: 10.1055/s-0036-1597624
104. Brucato M, Ladd-Acosta C, Li M, et al. Prenatal exposure to fever is associated with autism spectrum disorder in the Boston Birth Cohort. *Autism Res.* 2017;10: e1878-e1890. doi: 10.1002/aur.1841
105. Hornig M, Bresnahan MA, Che X, et al. Prenatal fever and autism risk. *Mol Psychiatry.* 2018; 23:759. doi: 10.1038/mp.2017.119
106. Frye RE, Slattery J, Kahler SG. Editorial: beyond genetics in autism. *Autism Research Review International.* 2015;29(3). https://www.autism.com/editorial_genes_ak.
107. Liu J, Nyholt DR, Magnussen P, et al. A genomewide screen for autism susceptibility loci. *Am J Hum Genet*. 2001;69: e327-e340. https://www.ncbi.nlm.nih.gov/pubmed/11452361
108. Yonan AL, Alarcon M, Cheng R, et al. A genome-wide screen of 345 families for autism susceptibility loci. *Am J Hum Genet*. 2003;73: e886-e897. https://www.ncbi.nlm.nih.gov/pubmed/13680528

109. Betancur C. Etiological heterogeneity in autism spectrum disorders: more than 100 genetic and genomic disorders and still counting. *Brain Res.* 2011;1380: e42-e77. doi: 10.1016/j.brainres.2010.11.078
110. Casanova M, Casanova E. Editorial: gene penetrance in autism. *Autism Research Review International.* 2015;29 (3) https://www.autism.com/editorial_genes_casanova
111. Hallmayer J, Cleveland S, Torres A, et al. Genetic heritability, and shared environmental factors among twin pairs with autism. *Arch Gen Psychiatry*. 2011;68: e1095-e1102. doi: 10.1001/archgenpsychiatry.2011.76
112. Edelson S. Editorial: Genetics, the environment, and autism**.** *Autism Research Review International*. 2015;29(3). https://www.autism.com/editorial_genes_edelson
113. Rice D, Barone S. Critical periods of vulnerability for the developing nervous system: evidence from humans and animal models. *Environ Health Perspect*. 2000;108: e511e533. https://www.ncbi.nlm.nih.gov/pubmed/10852851
114. Knickmeyer RC, Gouttard S, Kang C, et al. A structural MRI study of human brain development from birth to 2 years. *J Neurosci.* 2008;28: e12176-e12182. doi: 10.1523/JNEUROSCI.3479-08.2008
115. Ecker C. The neuroanatomy of autism spectrum disorder: an overview of structural neuroimaging findings and their translatability to the clinical setting. *Autism*. 2017;21(1): e18-e28. doi: 10.1177/1362361315627136.
116. Courchesne E. Abnormal early brain development in autism. *Mol Psychiatry*. 2002;7 (Suppl. 2): eS21-eS23. https://www.ncbi.nlm.nih.gov/pubmed/12142938
117. Courchesne E, Carper R, Akshoomoff N. Evidence of brain overgrowth in the first year of life in autism. *JAMA.* 2003; 290: e337-e344 https://www.ncbi.nlm.nih.gov/pubmed/12865374
118. Amaral DG, Schumann CM, Nordahl CW. Neuroanatomy of autism. *Trends Neurosci*.2008;31: e137-e145. doi: 10.1016/j.tins.2007.12.005
119. Wilke M, Holland SK, Myseros JS, Schmithorst VJ, Ball WS. Functional magnetic resonance imaging in pediatrics. *Neuropediatrics*. 2003;34(5):225-233. doi: 10.1055/s-2003-43260.
120. Belmonte MK, Mazziotta JC, Minshew NJ, et al. Offering to share how to put heads together in autism neuroimaging. *J Autism Dev Disord.* 2008;38(1): e2-e13. doi: 10.1007/s10803-006-0352-2
121. Bartholomeusz HH, Courchesne E, Karns CM. Relationship between head circumference and brain volume in healthy normal toddlers, children, and adults. *Neuropediatrics.* 2002;33(5): e239-e241. https://www.ncbi.nlm.nih.gov/pubmed/12536365
122. Hazlett HC, Poe M, Gerig G. Magnetic resonance imaging and head circumference study of brain size in autism: birth through age 2 years. *Arch Gen Psychiatry.* 2005;62(12): e1366e1376. https://www.ncbi.nlm.nih.gov/pubmed/16330725
123. Courchesne E, Karns C, Davis HR, et al. Unusual brain growth patterns in early life in patients with autistic disorder: an MRI study. *Neurology.* 2001; 57: e245-e254. https://www.ncbi.nlm.nih.gov/pubmed/11468308
124. McCaffery P, Deutsch CK. Macrocephaly and the control of brain growth in autistic disorders. *Prog Neurobiol.* 2005;77(1-2): e38-e56. doi: 10.1016/j.pneurobio.2005.10.005.

125. Courchesne E, Pierce K, Schumann CM et al. Mapping early brain development in autism. *Neuron.* 2007;56(2): e399-e413. https://www.ncbi.nlm.nih.gov/pubmed/17964254
126. Chaste P, Klei L, Sanders SJ, et al. Adjusting head circumference for covariates in autism: clinical correlates of a highly heritable continuous trait. *Biol. Psychiatry.* 2013;74: e576e584. doi: 10.1016/j.biopsych.2013.04.018
127. Carper RA, Moses P, Tigue ZD, et al. Cerebral lobes in autism: early hyperplasia and abnormal age effects. *Neuroimage.* 2002;16(4): e1038-e1051. https://www.ncbi.nlm.nih.gov/pubmed/12202091
128. Lange N, Travers BG, Bigler ED, et al. Longitudinal volumetric brain changes in autism spectrum disorder ages 6–35 years. *Autism Res.* 2015;*8*(1): e82-e93. doi: 10.1002/aur.1427
129. Hua X, Thompson PM, Leow AD. et al. Brain growth rate abnormalities visualized in adolescents with autism. *Hum Brain Mapp*. 2013;34(2): e425- e436. doi: 10.1002/hbm.21441
130. Hadjikhani N, Joseph RM, Snyder J, et al. Anatomical differences in the mirror neuron system and social cognition network in autism. *Cereb Cortex*. 2006;16(9):e1276-e1282. https://www.ncbi.nlm.nih.gov/pubmed/16306324
131. Stiles J, Jernigan TL. The basics of brain development. *Neuropsychol Rev*. 2010;20(4): e327-e348. doi: 10.1007/s11065-010-9148-4
132. Conciatori M, Stodgell CJ, Hyman SL, et al. Association between the HOXA 1 A218G polymorphism and increased head circumference in patients with autism. *Biol Psychiatry*. 2004;55(4):e413-e439. https://www.ncbi.nlm.nih.gov/pubmed/14960295
133. Courchesne E, Mouton PR, Calhoun ME et al. Neuron number and size in prefrontal cortex of children with autism. *JAMA.* 2011;306(18):e2001-e2010. doi: 10.1001/jama.2011.1638
134. Lombardo MV, Moon HM, Su J, Palmer TD, Courchesne E, Pramparo T. Maternal immune activation dysregulation of the fetal brain transcriptome and relevance to the pathophysiology of autism spectrum disorder. *Mol Psychiatry*. 2017;23(4): e1001-e1013. https://www.ncbi.nlm.nih.gov/pubmed/28322282
135. Sundaram SK, Kumar A, Makki MI, Behen ME, Chugani HT, Chugani DC. Diffusion tensor imaging of frontal lobe in autism spectrum disorder. *Cereb Cortex*. 2008;18(11): e2659-e2665. doi: 10.1093/cercor/bhn031
136. Barnea-Goraly N, Lotspeich LJ, Reiss AL. Similar white matter aberrations in children with autism and their unaffected siblings: a diffusion tensor imaging study using tract based spatial statistics. *Arch Gen Psychiatry*. 2010;67(10): e1052-e1060. doi:10.1001/archgenpsychiatry.2010.123
137. Jou RJ, Mateljevic N, Kaiser MD, Sugrue DR, Volkmar FR, Pelphrey KA. Structural neural phenotype of autism: preliminary evidence from a diffusion tensor imaging study using tract-based spatial statistics. *AJNR Am J Neuroradiol*. 2011;32(9):e1607-e1613.
138. Stoner R, Chow ML, Boyle MP, et al. Patches of disorganization in the neocortex of children with autism. *N Engl J Med*. 2014;27;370(13):1209-1219. doi: 10.3174/ajnr.A2558
139. Weigel J, Kuchna I, Nowicki K, et al. The neuropathology of autism: defects of neurogenesis and neuronal migration and dysplastic changes. *Acta Neuropathol*. 2010;119: e755-e770. doi: 10.1007/s00401-010-0655-4

140. Sah P1, Faber ES, Lopez De Armentia M, Power J. The amygdaloid complex: anatomy and physiology. *Physiol Rev*. 2003;83;803-834
141. Davis M, Whalen PJ. The amygdala: vigilance and emotion. *Mol Psychiatry*. 2001;6:e13e34. https://www.ncbi.nlm.nih.gov/pubmed/11244481
142. Juranek, J. et al. Association between amygdala volume and anxiety level: magnetic resonance imaging (MRI) study in autistic children. *J. Child Neurol*.2006;21:e1051e1058. doi: 10.1177/7010.2006.00237
143. Munson, J. et al. Amygdalar volume and behavioral development in autism. *Arch. Gen. Psychiatry*.2006: 63:e686-e693.https://www.ncbi.nlm.nih.gov/pubmed/16754842
144. Schmahmann JD, Caplan D. Cognition, emotion, and the cerebellum. *Brain.* 2006;129: e290-e292. https://www.ncbi.nlm.nih.gov/pubmed/16434422
145. Strick PL, Dum RP, Fiez JA. Cerebellum and nonmotor function. *Annu Rev Neurosci*. 2009;32;e413-e434. doi: 10.1146/annurev.neuro.31.060407.125606
146. Sivaswamy L. Kumar A. Raman D, et al. A diffusion tensor imaging study of the cerebellar pathways in children with autism spectrum disorder. *J Child Neurol*. 2010;25:e1223-e1231. doi: 10.1177/0883073809358765
147. Takarae Y, Minshew NJ, Luna B, Sweeney JA. Atypical involvement of fronto-striatal systems during sensorimotor control in autism. *Psychiatry Res*. 2007;156:e117-e127. https://www.ncbi.nlm.nih.gov/pubmed/17913474
148. Wegiel J, Flory M, Kuchna I, et al. Brain-region-specific alterations of the trajectories of neuronal volume growth throughout the lifespan in autism. *Acta Neuropathol Commun*. 2014a;2:28. https://doi.org/10.1186/2051-5960-2-28
149. Wegiel J, Flory M, Kuchna I, et al. Stereological study of the neuronal number and volume of 38 brain subdivisions of subjects diagnosed with autism reveals significant alterations restricted to the striatum, amygdala, and cerebellum. *Acta Neuropathol Commun.* 2014b2:141. doi: 10.1186/s40478-014-0141-7
150. Rogers TD, McKimm E, Dickson PE, Goldowitz D, Blaha CD, Mittleman G. Is autism a disease of the cerebellum? An integration of clinical and pre-clinical research. *Front Syst Neurosci.* 2013;7:15. doi: 10.3389/fnsys.2013.00015
151. Becker EB, Stoodley CJ. Autism spectrum disorder and the cerebellum. *Int Rev Neurobiol*. 2013;113;e1-e34. . doi: 10.1016/B978-0-12-418700-9.00001-0
152. Stanfield AC, McIntosh AM, Spencer MD, Philip R, Gaur S, Lawrie SM. Towards a neuroanatomy of autism: a systematic review and meta-analysis of structural magnetic resonance imaging studies. *Eur Psychiatry.* 2008;23:e289-e299. https://www.ncbi.nlm.nih.gov/pubmed/17765485
153. Webb SJ, Sparks BF, Friedman SD, et al. Cerebellar vermal volumes and behavioral correlates in children with autism spectrum disorder. *Psychiatry Res*. 2009;172:e61-e67. doi: 10.1016/j.pscychresns.2008.06.001
154. Traut N, Beggiato A, Bourgeron T, et al. Cerebellar volume in autism: literature meta-analysis and analysis of the autism brain imaging data exchange cohort. *Biol Psychiatry*. 2018;83(7):e579-e588. doi: 10.1016/j.biopsych.2017.09.029.
155. Vargas DL, Nascimbene C, Krishnan C, Zimmerman AW, Pardo CA. Neuroglial activation and neuroinflammation in the brain of patients with autism. *Ann Neurol*. 2005;57:e67-e81. . https://www.ncbi.nlm.nih.gov/pubmed/15546155
156. Rutter M. Aetiology of autism: findings and questions. *Journal of Intellectual Disability Research*. 2005;49(4):e231-e238. doi: 10.1111/j.1365-2788.2005.00676.x.

157. Williams JG, Higgins JPT, Brayne CEG. Systematic review of prevalence studies of autism spectrum disorders. *Arch Dis Child*. 2006;91:e8-e15. doi: 10.1136/adc.2004.062083.
158. O'Keeffe DF, Abuhamad A. Obstetric ultrasound utilization in the United States: data from various health plans. *Semin Perinatol*. 2013;37(5):e292-e294. doi: 10.1053/j.semperi.2013.06.003.
159. MacMahon B. Prenatal X-ray exposure and childhood cancer. *J Natl Cancer Inst*. 1962;28:e1173-e1191. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2814780/
160. Buescher A, Cidav Z, Knapp M, et al. Costs of autism spectrum disorders in the United Kingdom and the United States. *JAMA Pediatr*. 2014;168(8):e721-e728. doi: 10.1001/jamapediatrics.2014.210.
161. Leigh JP, Du J. Brief report: forecasting the economic burden of autism in 2015 and 2025 in the United States. *J Autism Dev Disord*. 2015;45(12):4135-4139. doi: 10.1007/s10803-015-2521-7.
162. Hay SI, Abajobir AA, Abate KH, et al. Global, regional, and national disability-adjusted life-years (DALYs) for 333 diseases and injuries and healthy life expectancy (HALE) for 195 countries and territories, 1990-2016: a systematic analysis for the Global Burden of Disease Study 2016. *Lancet*. 2017;390:e1260-e1344. doi: 10.1016/S0140-6736(17)32130-X.
163. Ziskin MC, Morrissey J. Thermal thresholds for teratogenicity, reproduction, and development. *Int J Hyperthermia*. 2011;27(4):e374-e87. doi 10.3109/02656736.2011.553769
164. Fairbrother G, Burigo J, Sharon T, Song K. Prenatal screening for fetal aneuploidies with cell-free DNA in the general pregnancy population: a cost-effectiveness analysis. *J Matern Fetal Neonatal Med.* 2016;29 (7):e1160-e1164. https://www.ncbi.nlm.nih.gov/pubmed/26000626