J Pediatr Rev. 2016 July; 4(2):e6174. doi: 10.17795/jpr-6174.

Published online 2016 July 23. **Review Article**

Impact of Maternal Folate Deficiencies on Early Neurological Development: A Narrative Review

Joshua T Emmerson,[1] and Nafisa M Jadavji[1,*]

[1]Department of Neuroscience, Carleton University, Ottawa, Canada

[*]*Corresponding author*: Nafisa M|Jadavji, Department of Neuroscience, Carleton University, Ottawa, Canada. Tel: +1-6135202600, E-mail: nafisa.jadavji@mail.mcgill.ca

Received 2016 March 30; **Revised** 2016 June 28; **Accepted** 2016 June 29.

Abstract

Context: Folates are B-vitamins that cannot be generated de novo and are therefore obtained from the diet. In the brain, these vitamins are involved in nucleotide synthesis, DNA repair, lipid metabolism, methylation and neurotransmitter synthesis. It is well established that adequate levels of maternal folates are required for closure of the neural tube within the first month of pregnancy, however, it is not clear whether maternal folates are needed throughout pregnancy for brain development and whether they influence offspring neurological function after birth. The aim of this review is to outline current literature from epidemiological and animal model studies that shows maternal supplementation of folates throughout pregnancy does indeed affect offspring neurological function after birth.

Evidence Acquisition: A Medline search was performed using the following mesh terms, maternal-fetal exchange, folic acid, offspring neurologic manifestations, methylenetetrahydrofolate reductase (MTHFR), embryology, and behavior.

Results: The studies described in the present review have reported that maternal deficiencies in folates during pregnancy result in changes in behavior as well as in blood and brain tissue in offspring, including altered methylation, including reduced levels of the global methyl donor S-adenosylmethionine (SAM), and increased levels of oxidative stress.

Conclusions: The data summarized here outlines the importance of adequate levels of folates throughout pregnancy to facilitate appropriate neurological development of offspring after birth.

Keywords: Behaviour, Embryology, Folic Acid, Maternal Fetal Exchange, Methylenetetrahydrofolate Reductase (MTHFR), Narrative Review, Neuologic Manifestations, Offspring Neurological Model

1. Context

The importance of folate, a B-vitamin, is well known for its' role during closure of the neural tube, the future brain and spinal cord, during in utero development (1), but what happens after birth? Do maternal contributions of folates during in utero development affect neurological function of offspring after birth?

Folates are B-vitamins that are mainly obtained from the diet, since they cannot be synthesized de novo. In the brain they are involved in nucleotide synthesis, DNA repair, lipid metabolism, methylation and neurotransmitter synthesis. Folates are a naturally occurring form of the vitamin that predominantly come from diet however they are also produced by gut bacteria, whereas folic acid is the form found in dietary supplements. Methylenetetrahydrofolate reductase (MTHFR) is an important enzyme involved in folate metabolism because it can reduce levels of homocysteine, a cytotoxic amino acid. Specifically, MTHFR reduces folates to 5-methyltetrahydrofolate (5-methylTHF), the main circulating form of folate and the primary methyl donor to homocysteine, ultimately reducing homocysteine levels and recycling it to methionine. Elevated levels of homocysteine are associated with increased risk for cardiovascular disease (2) as well as negative health consequences in the developing fetus (3). Therefore, homocysteine removal via proper folate metabolism may play a significant role in early neurological development. Folates influence methylation through homocysteine recycling to methionine and generation of S-adenosylmethionine (SAM), a global methyl donor. In the brain, SAM is involved in neurotransmitter synthesis and lipid metabolism. Interestingly, there is a polymorphism in *Mthfr* that has been reported in approximately 20% of the North American and European populations (4). The polymorphism occurs at base pair 677 (C $\rightarrow$ T) and results in reduced enzyme function as well as increased levels of homocysteine (5). A maternal polymorphism in MTHFR has been linked to increased neural tube defects (NTDs) in offspring (6).

The importance of maternal contributions of folic acid during the first month of brain in utero development are well established (7). Many countries around the world have implemented mandatory fortification to grains, in an at-

tempt to reduce the incidence of NTDs (8). Since 1999 in Costa Rica mandatory fortification has led to a 60% reduction in the number of NTDs and in Chile the reduction is closer to 55% (8), however it is not clear whether folic acid is required for the remaining 8 months of pregnancy. Recent data suggests that maternal contributions of folates throughout pregnancy have a significant impact on their offspring's development and growth after birth through an array of changes. The purpose of this review is to outline current evidence from epidemiological and animal model studies that shows maternal contributions of folates during early development does indeed affect neurological function after birth.

2. Evidence Acquisition

A Medline search was performed using the following mesh terms: maternal-fetal exchange, folic acid, offspring neurological model, neurologic manifestations, methylenetetrahydrofolate reductase (MTHFR), embryology, and behavior. Preference was given to sources that reported key findings were sought. Additional sources were found using the bibliographies of retrieved articles.

3. Results

3.1. Epidemiological Research

3.1.1. Maternal Folate Status Associations with Fetal Growth

Folates serve a central role in nucleic acid synthesis, thus a maternal reduction in availability will likely impair essential processes for development of a fetus such as cellular growth and replication (9). Only recently, the epigenetic profiles of newborns were investigated and mothers with increased plasma folate levels were associated with decreased global DNA methylation in offspring, specifically identifying 43 of the 365 CpG's involved in folate metabolism were altered (10).This data suggests that epigenetic factors play a role on fetal development and growth. This statement has also been recognized in the realm of neurodevelopmental disorders such as Autism Spectrum Disorder (11).

Due to ethical constraints, more research has been conducted on blood samples of mothers and their newborn children and as a result, there appears to be various relationships between the two. For example, blood homocysteine levels of pregnant women, measured at 30 - 34 weeks of gestation, were slightly correlated negatively with birthweight of their newborns however no correlation was found with Vitamin B12 and folate with birth weight, respectively (12, 13). Also, the Rotterdam predict study which found that of 186 women, there was a significant association between RBC folate status and offspring cerebellar growth given periconceptual (defined as 4 weeks before to 8 weeks after conception) folic acid supplementation (14). As reviewed by van Uitert et al. (2013), there were a handful of studies which investigated the association of RBC folate status and fetal growth parameters including birth weight and head circumference. Despite minor variations between these studies including the time of study, sample size and study type, the visible trend appears to be that birth weight and head circumference are inversely associated with increased maternal folate status (15). While some studies are finding significant associations between maternal folate status and fetal growth, others such as Sutton et al. (2012) in Dublin found no association between RBC folate, vitamin B12 nor total homocysteine and congenital malformations (besides NTD's) including cleft palate, musculoskeletal malformations and midline defects (16). Perhaps folate status is moreso related to specific aspects of fetal growth as opposed to others which is indicative of a more narrow and specific mechanism of action however this remains unclear. Adding to the complexity and elusiveness is the findings of one particular study consisting of 654 pregnant women of India reporting that plasma homocysteine levels, but not Vitamin B12 status in mothers of 30 weeks gestation was associated with insulin status in offspring (13).

3.1.2. Folate Status Associations with Methylenetetrahydrofolate Reductase (MTHFR) Polymorphism

Interestingly, another study in 2014 using two cohorts reported that an increase in maternal homocysteine, attributed to the MTHFR polymorphism (677C $\rightarrow$ T), is associated with decreased birthweight (17), suggesting implications of other components of folic acid metabolism playing key roles in proper fetal development and growth. It is apparent that mothers with the *Mthfr* polymorphism are more vulnerable to homocysteine accumulation and face an increased risk to defects in development and growth. In fact, one study assessed the relationship between MTHFR genotype and folate levels in both RBC's and plasma of both pregnant and non-pregnant females (18). The authors found that circulating folate levels were decreased in both pregnant and non-pregnant women with the *MTHFR*TT genotype. Similarly, plasma folate levels were lower in the TT compared to the CC women, however this was only observed in the pregnant female group. This data suggests that pregnant women with a polymorphism in MTHFR may require supplementation (18) with 5-methylTHF. From a larger perspective, the explanation for the benefits of increased maternal folate status for offspring development and growth may alternatively rely more on enzymes in-

volved in its metabolism.

3.1.3. Other Folate Status Associations

Studies have examined the relationship between mothers and newborns in terms of folate, vitamin B12 and homocysteine levels (19). They found that maternal blood folate and vitamin B12 were both positively correlated with umbilical cord levels in newborns. Interestingly, serum levels of folate and vitamin B12 in mothers were both negatively correlated with homocysteine levels in both mothers and newborns, the same was observed in plasma (10).Also, increased total homocysteine measured both in the mothers blood and umbilical cord was found in mothers diagnosed with pre-eclampsia (3). This data further provides evidence that a dietary maternal deficiency in folates or elevated levels of homocysteine influences levels in offspring (19) which could potentially explain, in part, how offspring are being born with deficits in neurological development. More physiological studies using animal models are at least required to investigate a causal relationship. Interestingly, a different study examined the effects of homocysteine and folate on delivery term and found that mothers with lower blood folate levels were associated with pre-term deliveries (< 37 week gestational period, excluding twins from the study). Even though delivery term may not be considered a key aspect of neurological development, it may be an indicator that reflects or contributes to developmental delays. These data suggest that a mothers' folate levels do indeed have an impact on offspring development.

3.1.4. Maternal Folate Status Associations with Early Childhood Behavior

Identifying relationships between maternal folic acid deficiencies on behavior of young children has also been investigated. One study explored the mental and psychomotor development of 5 year old children of mothers with varying blood folate levels during 19, 26 and 37 weeks of gestation, revealing that after a battery of psychological testing, there were no differences between the folate deficient and adequate folate nutritional status groups (20). These data suggest that adequate folate during later pregnancy (after the first trimester) may not be sufficient to influence childhood mental and psychomotor development. The authors suggested that perhaps a maternal folate deficiency has to occur at a critical window during fetal development such as early pregnancy, in order for a significant impact to occur on the development of the fetus (20). One such study by Schlotz et al (2010), provides supporting evidence of this idea. They investigated the potential effects of a maternal folate deficiency on behavioral deficits in their offspring and found that both maternal total folate and red blood cell folate levels measured at early stages of gestation (14 weeks) was negatively correlated with offspring hyperactivity and peer problems, suggesting that, while remaining consistent with the previous idea, an early folate maternal deficiency may lead to impairments in offspring development and behavior (21). In addition, a study was performed using 3209 mothers and their 3 year old children to assess the effects of maternal plasma folic acid deficiency, as a result of MTHFR genotype, on child emotional and behavioral impairments (22). The authors found that there was an association between decreased maternal plasma folates and increased emotional problems, such as social withdrawal and emotional reactivity, but not in behaviors, such attention and aggression. An interesting finding in this study is that mothers who began supplementation during early pregnancy showed reduced risk for child emotional problems compared to late pregnancy supplementation (22). Evidently there are behavioral and emotional impairments in offspring associated with a maternal folic acid deficiency during early pregnancy. Therefore, an early pregnancy based folic acid deficiency appears to be a factor worth taking into consideration for future studies and practices. Aside from emotional and social problems, a recent population-based study using a cohort of Dutch children between 6 - 8 years old reported that kids with prenatal maternal folate deficiencies, high homocysteine and low vitamin B12 levels, were associated with smaller brain volumes (via MRI), language performance and visuo-spatial deficits (23). So in terms of defined behavioral subsets, there does not appear to be a specific set of behavioral deficits in children of these reports compared to the more narrow findings previously mentioned in fetal growth. Other studies have investigated associations between maternal folate status and neurodevelopmental disorders such as Autism Spectrum Disorder (ASD) (11). For example, the CHARGE case-control study found that children aged 2 - 5 years of typical development were significantly associated with greater maternal folic acid intake (> 600 μg/day) for 3 months before to the end of the first month of pregnancy and this association was enhanced when both the mother and offspring possess the *Mthfr*CT variant genotypes (24). A Norwegian cohort study examining maternal folic acid status of 85,176 children aged 3 to 10 years only found a 0.1% reduction in ASD diagnoses in folic acid supplemented mothers however supplementation of 400 ug/day folic acid occurred from 1 month before to 2 months after conception (25). Aside from ASD, Mohanty et al. (2014) identified a significant association between low maternal serum folate levels with children diagnosed with Down's Syndrome but there was no significant prevalence of the *Mthfr* polymorphism when compared to controls. Compared to fetal growth and

development, it is difficult to infer that a broad physiological problem, such as folate deficiency, could be a cause of or provide an increased risk to neurodevelopmental disorders such as ASD or Down's Syndrome behavioral diagnoses. Nonetheless, there appears to be an array of associations between maternal folate status and early childhood behaviors that remains to be investigated more thoroughly.

A recent review on the impact of vitamins in health reported that although supplementation of vitamins such as Vitamin B12 and B6 during pregnancy is recommended, an excess of vitamin intake may in fact put oneself at increased risk for other health problems. This topic remains under investigation, especially in terms of implications for long term effects (26). Despite many correlative studies, the underlying mechanisms to explain how maternal folic acid levels impact neural development and growth of offspring remain unknown.

3.2. Animal Models Research

3.2.1.Mechanisms Through Which Maternal Folate Contributions Affect Brain Development

3.2.1.1. Methylation

While human research has identified an unexplained impact of maternal folic acid deficiency on proper development of offspring, determining causal link(s) are of current interest. So far, many studies using rodent models have been used to further characterize and comprehend underlying mechanisms. For example, metabolism of the free form of folate, 5-methylTHF, is intimately involved with the synthesis of methionine, an amino acid critical in DNA methylation and repair. It has been reported that SAM, a global methyl donor, is reduced in the livers and brains of mouse pups of their mothers subjected to a dietary folic acid deficiency, and interestingly, this reduction was exacerbated after a shock stressor (27), suggesting that these pups have reduced DNA methylation due to a dietary folic acid deficiency. A similar study using rats found similar results in the Purkinje cells of the cerebellum (28), which may imply disrupted motor coordination. In addition, McKay et al (2011) examined the methylation profiles of candidate genes in folic acid deficient mouse dams (post-weaning of their pups) and found that there was altered methylation of insulin-like growth factor 2 (Igf2), an important growth factor involved with development and growth, in blood, liver and kidney tissue (29, 30). Alternatively, excessive maternal dietary supplementation of folic acid resulted in reduced global methylation in placental tissue of Wistar albino rats (31). Multiple sources have suggested that maternal folic acid inadequacy may predispose offspring to a secondary metabolic stressor and would thus lead to abnormal behaviors such as reduced locomotor activity and increased conditioned escape response (27, 29). These findings provide evidence that confirm findings from epidemiological data and further suggest that offspring of maternal folic acid deficiencies may lead to altered methylation profiles in offspring.

3.2.1.2. Growth Factors

To understand maternal folic acid deficiency from a molecular perspective, the primary goal is to understand what changes are being observed. For example, synapsins, which play a central role in neuroplasticity, synapse function and neurotransmitter release, were found to be expressed 2.2 fold less in the folic acid deficient offspring compared to control rats (28). In addition, mothers that were given an excess of folic acid but were deprived of vitamin B12 had offspring with reduced brain derived neurotrophic factor (BDNF) protein and mRNA levels as well as nerve growth factor (NGF) protein levels (32), both of which are important for neuronal growth and survival. Perhaps a maternal folic acid deficiency causes or at least contributes to a reduction in neurotrophic factors essential for proper offspring neuronal development which may explain the increased incidence of NTD's in offspring of folic acid deficient mothers.

3.2.1.3. Oxidative stress

One possible mechanism by which folic acid deficient dams affect proper neurological development of their offspring could be through an activation of oxidative stress pathways. Interestingly, one report demonstrated that rat pups of hyperhomocysteinemic moms had significantly increased global lipid peroxidation (mitochondrial most of all but also nuclear and cytosolic) as well as reduced but not significant glutathione (GSH) levels in brain tissue (33). In addition, malonialdehyde (MDA), a measure of lipid peroxidation, was increased in folic acid deficient dams (34). These findings suggest that oxidative stress in offspring may be a result of maternal deficiencies in folates. Authors from the studies linked hyperhomocysteinemic moms with reduced apoptotic regulation via B-cell-lymphoma (Bcl-2) and increased apoptotic signaling via p53, a tumor suppressor protein, suggesting a potential mechanism of cell death that has been described by many (33).

3.2.1.4. Animal Behavior

Not many studies in the context of maternal folic acid deficiency have included measures of animal behavior, so it remains unknown whether the changes in brain tissue of offspring do indeed translate to functional changes. One

group of folic acid deficient mothers experienced a significantly prolonged gestational period, and its offspring demonstrated increased anxiety behaviors (35). Other behavioral deficits have been documented as early as 1951, where offspring of folic acid deficient dams exhibited reduced maze learning ability (36). Also, in 1976, a study using 35 day old mice demonstrated that folic acid deficient dams bore offspring that scored significantly lower on locomotor activity and conditioned escape behavior (27). Most recently, visual and spatial short term memory impairments (37) as a result of maternal MTHFR or folic acid deficiencies have been reported in wild-type offspring, possibly as result of increased maternal homocysteine levels. Abnormalities in negative geotaxis and linear walking scores (28) in wild-type folic acid deficient offspring have also been observed. Given the small amount of offspring behavior studies of folic acid deficient dams, there appears to be a general trend that maternal folic acid deficiencies result in offspring behavioral abnormalities.

4. Conclusions

A survey of the current literature strongly suggests that maternal supplementation of folates during critical areas (at least within the first month) of development are required for normal neurological function after birth. Epidemiological research has suggested, while still unclear, some links and trends between maternal folate contributions on early childhood development. Investigations of animal models has moved this research area forward and provided much needed evidence to help understand the likely underlying molecular machinery. Having understood that adequate maternal folate are required for neural tube closure, future studies should be directed towards dissecting out the critical time window and dosage for maternal folate supplementation are required as well a more extensive understanding of whether impairments in neurological function continue into adulthood and increase susceptibility to other diseases.

Acknowledgments

The authors would like to thank Patrice D. Smith for comments on manuscript text.

Footnote

Funding/Support: Joshua T Emmerson was a recipient of graduate award for Ontario students for research in dementia studentship. Nafisa M Jadavji was funded by council of Ontario universities postdoctoral women's health scholars and then national science engineering research council (NSERC) Fellowship.

References

1. Mayanil CS, Ichi S, Farnell BM, Boshnjaku V, Tomita T, McLone DG. Maternal intake of folic acid and neural crest stem cells. 1 ed. Netherlands: Elsevier Inc.; 2011.
2. Castro R, Rivera I, Blom HJ, Jakobs C, Tavares de Almeida I. Homocysteine metabolism, hyperhomocysteinaemia and vascular disease: an overview. *J Inherit Metab Dis.* 2006;**29**(1):3–20. doi: 10.1007/s10545-006-0106-5. [PubMed: 16601863].
3. Kim MW, Hong SC, Choi JS, Han JY, Oh MJ, Kim HJ, et al. Homocysteine, folate and pregnancy outcomes. *J Obstet Gynaecol.* 2012;**32**(6):520–4. doi: 10.3109/01443615.2012.693984. [PubMed: 22779952].
4. Schneider JA, Rees DC, Liu YT, Clegg JB. Worldwide distribution of a common methylenetetrahydrofolate reductase mutation. *Am J Hum Genet.* 1998;**62**(5):1258–60. doi: 10.1086/301836. [PubMed: 9545406].
5. Frosst P, Blom HJ, Milos R, Goyette P, Sheppard CA, Matthews RG, et al. A candidate genetic risk factor for vascular disease: a common mutation in methylenetetrahydrofolate reductase. *Nat Genet.* 1995;**10**(1):111–3. doi: 10.1038/ng0595-111. [PubMed: 7647779].
6. Botto LD, Yang Q. 5,10-Methylenetetrahydrofolate reductase gene variants and congenital anomalies: a HuGE review. *Am J Epidemiol.* 2000;**151**(9):862–77. [PubMed: 10791559].
7. Daly LE, Kirke PN, Molloy A, Weir DG, Scott JM. Folate levels and neural tube defects. Implications for prevention. *JAMA.* 1995;**274**(21):1698–702. [PubMed: 7474275].
8. Castillo-Lancellotti C, Tur JA, Uauy R. Impact of folic acid fortification of flour on neural tube defects: a systematic review. *Public Health Nutr.* 2013;**16**(5):901–11. doi: 10.1017/S1368980012003576. [PubMed: 22850218].
9. Scholl TO, Johnson WG. Folic acid: influence on the outcome of pregnancy. *Am J Clin Nutr.* 2000;**71**(5 Suppl):1295S–303S. [PubMed: 10799405].
10. Joubert BR, den Dekker HT, Felix JF, Bohlin J, Ligthart S, Beckett E, et al. Maternal plasma folate impacts differential DNA methylation in an epigenome-wide meta-analysis of newborns. *Nat Commun.* 2016;**7**:10577. doi: 10.1038/ncomms10577. [PubMed: 26861414].
11. DeVilbiss EA, Gardner RM, Newschaffer CJ, Lee BK. Maternal folate status as a risk factor for autism spectrum disorders: a review of existing evidence. *Br J Nutr.* 2015;**114**(5):663–72. doi: 10.1017/S0007114515002470. [PubMed: 26243379].
12. Hogeveen M, Blom HJ, van der Heijden EH, Semmekrot BA, Sporken JM, Ueland PM, et al. Maternal homocysteine and related B vitamins as risk factors for low birthweight. *Am J Obstet Gynecol.* 2010;**202**(6):5721–6. doi: 10.1016/j.ajog.2010.01.045. [PubMed: 20400059].
13. Krishnaveni GV, Veena SR, Karat SC, Yajnik CS, Fall CH. Association between maternal folate concentrations during pregnancy and insulin resistance in Indian children. *Diabetologia.* 2014;**57**(1):110–21. doi: 10.1007/s00125-013-3086-7. [PubMed: 24162586].
14. Koning IV, Groenenberg IA, Gotink AW, Willemsen SP, Gijtenbeek M, Dudink J, et al. Periconception Maternal Folate Status and Human Embryonic Cerebellum Growth Trajectories: The Rotterdam Predict Study. *PLoS One.* 2015;**10**(10):0141089. doi: 10.1371/journal.pone.0141089. [PubMed: 26491876].
15. van Uitert EM, Steegers-Theunissen RP. Influence of maternal folate status on human fetal growth parameters. *Mol Nutr Food Res.* 2013;**57**(4):582–95. doi: 10.1002/mnfr.201200084. [PubMed: 23213022].
16. Sutton M, Mills JL, Molloy AM, Troendle JF, Brody LC, Conley M. Maternal vitamin levels in pregnancie affected by congenital malformations other than neural tube defects. *Birth Defects Res A Clin Mol Teratol.* 2012;**91**:610–5.

17. Yajnik CS, Chandak GR, Joglekar C, Katre P, Bhat DS, Singh SN, et al. Maternal homocysteine in pregnancy and offspring birthweight: epidemiological associations and Mendelian randomization analysis. *Int J Epidemiol.* 2014;**43**(5):1487–97. doi: 10.1093/ije/dyu132. [PubMed: 25052622].
18. Molloy AM, Daly S, Mills JL, Kirke PN, Whitehead AS, Ramsbottom D, et al. Thermolabile variant of 5,10-methylenetetrahydrofolate reductase associated with low red-cell folates: implications for folate intake recommendations. *Lancet.* 1997;**349**(9065):1591–3. doi: 10.1016/S0140-6736(96)12049-3. [PubMed: 9174561].
19. Guerra-Shinohara EM, Paiva AA, Rondo PH, Yamasaki K, Terzi CA, D'Almeida V. Relationship between total homocysteine and folate levels in pregnant women and their newborn babies according to maternal serum levels of vitamin B12. *BJOG.* 2002;**109**(7):784–91. [PubMed: 12135215].
20. Tamura T, Goldenberg RL, Chapman VR, Johnston KE, Ramey SL, Nelson KG. Folate status of mothers during pregnancy and mental and psychomotor development of their children at five years of age. *Pediatrics.* 2005;**116**(3):703–8. doi: 10.1542/peds.2004-2189. [PubMed: 16140711].
21. Schlotz W, Jones A, Phillips DI, Gale CR, Robinson SM, Godfrey KM. Lower maternal folate status in early pregnancy is associated with childhood hyperactivity and peer problems in offspring. *J Child Psychol Psychiatry.* 2010;**51**(5):594–602. doi: 10.1111/j.1469-7610.2009.02182.x. [PubMed: 19874428].
22. Steenweg-de Graaff J, Roza SJ, Steegers EA, Hofman A, Verhulst FC, Jaddoe VW, et al. Maternal folate status in early pregnancy and child emotional and behavioral problems: the Generation R Study. *Am J Clin Nutr.* 2012;**95**(6):1413–21. doi: 10.3945/ajcn.111.030791. [PubMed: 22572645].
23. Ars CL, Nijs IM, Marroun HE, Muetzel R, Schmidt M, Steenweg-de Graaff J, et al. Prenatal folate, homocysteine and vitamin B12 levels and child brain volumes, cognitive development and psychological functioning: the Generation R Study. *Br J Nutr.* 2016:1–9. doi: 10.1017/S0007114515002081. [PubMed: 26794411].
24. Schmidt RJ, Tancredi DJ, Ozonoff S, Hansen RL, Hartiala J, Allayee H, et al. Maternal periconceptional folic acid intake and risk of autism spectrum disorders and developmental delay in the CHARGE (CHildhood Autism Risks from Genetics and Environment) case-control study. *Am J Clin Nutr.* 2012;**96**(1):80–9. doi: 10.3945/ajcn.110.004416. [PubMed: 22648721].
25. Suren P, Roth C, Bresnahan M, Haugen M, Hornig M, Hirtz D, et al. Association between maternal use of folic acid supplements and risk of autism spectrum disorders in children. *JAMA.* 2013;**309**(6):570–7. doi: 10.1001/jama.2012.155925. [PubMed: 23403681].
26. Pannia E, Cho CE, Kubant R, Sanchez-Hernandez D, Huot PS, Harvey Anderson G. Role of maternal vitamins in programming health and chronic disease. *Nutr Rev.* 2016;**74**(3):166–80. doi: 10.1093/nutrit/nuv103. [PubMed: 26883881].
27. Middaugh LD, Grover TA, Blackwell LA, Zemp JW. Neurochemical and behavioral effects of diet related perinatal folic acid restriction. *Pharmacol Biochem Behav.* 1976;**5**(2):129–34. [PubMed: 996047].
28. Pourie G, Martin N, Bossenmeyer-Pourie C, Akchiche N, Gueant-Rodriguez RM, Geoffroy A, et al. Folate- and vitamin B12-deficient diet during gestation and lactation alters cerebellar synapsin expression via impaired influence of estrogen nuclear receptor alpha. *FASEB J.* 2015;**29**(9):3713–25. doi: 10.1096/fj.14-264267. [PubMed: 26018677].
29. McKay JA, Mathers JC. Maternal folate deficiency and metabolic dysfunction in offspring. *Proc Nutr Soc.* 2016;**75**(1):90–5. doi: 10.1017/S0029665115004280. [PubMed: 26621202].
30. McKay JA, Xie L, Harris S, Wong YK, Ford D, Mathers JC. Blood as a surrogate marker for tissue-specific DNA methylation and changes due to folate depletion in post-partum female mice. *Mol Nutr Food Res.* 2011;**55**(7):1026–35. doi: 10.1002/mnfr.201100008. [PubMed: 21520493].
31. Kulkarni A, Dangat K, Kale A, Sable P, Chavan-Gautam P, Joshi S. Effects of altered maternal folic acid, vitamin B12 and docosahexaenoic acid on placental global DNA methylation patterns in Wistar rats. *PLoS One.* 2011;**6**(3):17706. doi: 10.1371/journal.pone.0017706. [PubMed: 21423696].
32. Sable P, Dangat K, Kale A, Joshi S. Altered brain neurotrophins at birth: consequence of imbalance in maternal folic acid and vitamin B(1)(2) metabolism. *Neuroscience.* 2011;**190**:127–34. doi: 10.1016/j.neuroscience.2011.05.010. [PubMed: 21640168].
33. Koz ST, Gouwy NT, Demir N, Nedzvetsky VS, Etem E, Baydas G. Effects of maternal hyperhomocysteinemia induced by methionine intake on oxidative stress and apoptosis in pup rat brain. *Int J Dev Neurosci.* 2010;**28**(4):325–9. doi: 10.1016/j.ijdevneu.2010.02.006. [PubMed: 20188811].
34. Roy S, Kale A, Dangat K, Sable P, Kulkarni A, Joshi S. Maternal micronutrients (folic acid and vitamin B(12)) and omega 3 fatty acids: implications for neurodevelopmental risk in the rat offspring. *Brain Dev.* 2012;**34**(1):64–71. doi: 10.1016/j.braindev.2011.01.002. [PubMed: 21300490].
35. Ferguson SA, Berry KJ, Hansen DK, Wall KS, White G, Antony AC. Behavioral effects of prenatal folate deficiency in mice. *Birth Defects Res A Clin Mol Teratol.* 2005;**73**(4):249–52. doi: 10.1002/bdra.20111. [PubMed: 15744731].
36. Whitley JR, O'Dell BL, Hogan AG. Effect of diet on maze learning in second generation rats; folic acid deficiency. *J Nutr.* 1951;**45**(1):153–60. [PubMed: 14880969].
37. Jadavji NM, Deng L, Malysheva O, Caudill MA, Rozen R. MTHFR deficiency or reduced intake of folate or choline in pregnant mice results in impaired short-term memory and increased apoptosis in the hippocampus of wild-type offspring. *Neuroscience.* 2015;**300**:1–9. doi: 10.1016/j.neuroscience.2015.04.067.