ORIGINAL ARTICLE:

FR 50% in pregnancy results in different neuron and glial cell count (astrocytes, olygodendrocytes, and microglia) in the cerebrum and cerebellum of newborn *Rattus norvegicus*

Fitria Desky[1], Hermanto Tri Joewono[1]*, Widjiati[2]

[1]Department of Obstetrics and Gynecology, Faculty of Medicine, Universitas Airlangga, Dr Soetomo Hospital, Surabaya, Indonesia, [2]Department of Embriologi, Faculty of Veterinary Medicine, Universitas Airlangga, Surabaya, Indonesia

ABSTRACT

Objectives: To analyze the difference neuronal and glial (astrocytes, oligodendrocyte, microglia) cell count in cerebrum and cerebellum of *Rattus norvegicus* newborns with 50% food restriction and control group.

Materials and Methods: This was an analytical experimental study with single blind randomized post test only control group design using animals subjects *Rattus norvegicus*. This study was conducted at Animal laboratory, Veterinary Faculty, Universitas Airlangga. Animal subjects were divided into FR50% group and control. Neuron and glial (astrocytes, oligodendrocytes, microglia) counts were analyzed using comparison test, with CI 95%.

Results: There was a significant difference in cerebrum and cerebellum neuron cell count between intervention and control group (9.88±3.59 vs 16.88±2.553; p=0.000 and 7.5±1.789 vs 11.44±4.56; p=0.02). There was no difference in cerebrum and cerebellum glial cell count. There was a significant difference in cerebellum astrocyte between intervention and control group (80.94±24.255 vs 59.69±18.77; p=0.02) but no difference in cerebrum. There was a significant difference in cerebrum and cerebellum oligodendrocyte between intervention and control group (14.06±12.195 vs 25.13±8.609; p<0.000 and 13.63±6.712 vs 24.00±8.862; p=0.001), and there were significant difference in cerebrum and cerebellum microglia cell between intervention and control group (5.25±3.435 vs 4.94±3.838; p=0.620 and 8.81±4.119 vs 5.25±1.483; p=0.004).

Conclusion: Food Restriction 50% (FR50%) in *Rattus norvegicus* decreased cerebrum and cerebellum neuron cell and oligodendrocyte count and increased cerebrum and cerebellum microglial count.

Keywords: Food restriction 50%; prenatal; the number of neuronal cell and glia; *Rattus norvegicus*.

ABSTRAK

Tujuan: menganalisis perbedaan jumlah sel neuron dan glia (astrosit, oligodendrosit, mikroglia) cerebrum dan cerebellum *Rattus norvegicus* baru lahir pada kelompok dengan perlakuan FR50% selama kebuntingan dan tidak mendapat perlakuan.

Bahan dan Metode: Penelitian analitik eksperimental dengan desain single blind randomized post test only control group menggunakan hewan coba *Rattus norvegicus* di kandang hewan coba FKUA. Kelompok hewan coba dibagi dua yaitu kelompok kontrol tanpa diberi perlakuan, dan kelompok perlakuan yang mendapat perlakuan FR50% sejak dinyatakan bunting. Penelitian ini menggunakan uji komparasi dalam menganalisis jumlah sel neuron dan glia (astrosit, oligodendrosit, mikroglia)

Hasil: Didapatkan perbedaan bermakna jumlah sel neuron cerebrum (p:0.000) kelompok perlakuan (9.88±3.59) dibanding kontrol (16.88±2.553) dan cerebellum (p:0.02) kelompok perlakuan (7.50±1.789) dibanding kontrol (11.44±4.560). Tidak didapatkan perbedaan bermakna (p:0.05) jumlah glia cerebrum (p:0.612) pada perlakuan (99.19±26.234) dibanding kontrol (93.75±33.326) dan cerebellum (p:0.058) perlakuan (103.38±16.346) dibanding kontrol (88.94±24.255). Didapatkan perbedaan bermakna jumlah astrosit di cerebellum (p:0.002) perlakuan (80.94±16.270) dibanding kontrol (59.69±18.771), tidak bermakna di cerebrum (p:0.599) perlakuan (74.44±35.359) dibanding kontrol (69.13±18.726). Didapatkan perbedaan jumlah oligodendrosit di cereberum (P<0.000) perlakuan (14.06±12.195) dibanding kontrol (25.13±8.609) dan cerebellum (p:0.01) perlakuan (13.63±6.712) dibanding kontrol (24.00±8.862). Didapatkan perbedaan jumlah mikroglia di cerebrum (P: 0.620) perlakuan (5.25±3.435) dibanding kontrol (4.94±3.838) dan cerebellum (p:0.04) perlakuan (8.81±4.119) dibanding kontrol (5.25±1.483)

Simpulan: FR 50% selama kebuntingan menyebabkan lebih rendahnya jumlah sel neuron dan oligodendrosit cerebrum dan cerebellum, lebih banyaknya mikroglia di cerebrum dan cerebellum *Rattus norvegicus* baru lahir

Kata Kunci: Food restriction 50%; cerebrum; cerebellum; jumlah sel neuron dan glia; *Rattus norvegicus*

*Correspondence: Hermanto T Joewono, Department of Obstetrics and Gynecology, Faculty of Medicine, Universitas Airlangga, Dr Soetomo Hospital, Jalan Prof dr Moestopo 6-8, Surabaya 60286, Indonesia. E-mail: hos_hermanto@yahoo.com or hermanto.tri@fk.unair.ac.id

INTRODUCTION

Prenatal period is the most important period in the fetal brain development phase, so pregnancy is the right time to prepare the fetal intelligence potential. There are two factors that influence fetal brain development, the nature (genetic) and nurture (environment) factors, in which nutrition of pregnant women is one of the important environmental factors in fetal brain development and influences an individual's short and long term intelligence.[1]

Intelligence is related to the speed of processing information. Human brain is one of the most complex organ systems. Cellular nervous system consists of two types of cells, the neurons and glia. Neuron and glial cells work in harmony so that the brain's computing abilities can run well. Neurons and glia can be stimulated to form by external stimulation. The higher the number of neurons, dendrites and synapses in an individual, the faster the information is processed, the smarter the individual is expected.[2,3]

Human brain development occurs from the age of two weeks of pregnancy and continues into adulthood.[4] The process begins with neurulation, differentiation/proliferation, migration, synaptogenesis and apoptosis. The two main cells that make up the brain are neurons and neuroglia which are maintained in balance through the process of neurogenesis and apoptosis.[5] This process is supported by neurotropin, a protein in the Central Nervous System (CNS) whose functions are to maintain neuron survival, axon and dendritic growth, and regulation of neuronal development, including synaptic formation and synaptic plasticity.[6]

Human nervous system is divided into two major groups, the central nervous system (CNS) and the peripheral nervous system. The development of an individual's CNS starts from the beginning of pregnancy until growing up. This period of development is divided into three, the embryonic period, the fetal period and the post natal period. The embryonic and fetal period is the main period of CNS development that starts from the beginning of the conception until the end of pregnancy.[4]

Fetal neuron cells do not increase since 32 weeks' gestation. Glial cells continue to proliferate until the post-natal period so they are the most numerous cells in human brain as compared to neurons with a ratio of ten to one. Higher increase of glial cell count than the neurons is associated with the development of higher brain functions.[2,3,7,8]

Poor nutrition during pregnancy affects the increase in neuronal cell apoptosis. Antonow (2010) in his research, found that 30% food restriction (FR 30%) in baboons during pregnancy reduced brain derived neurotrophic factor (BDNF) levels around 13.4%-26.3% and increased neuron cell apoptosis by 118% in baboons newborns.[9]

Prenatal period is a good time to increase brain growth and development early on. A study by Brent Logan, David Chamberlain, Rene van de Carr and Beatriz Manrique showed the influence of intrauterine environmental manipulation on fetal intelligence. Bures et al. and many other experts found that brain structures are formed by external stimuli known as "Stimulation induced morphological changes"[3,10]

Malnutrition in pregnant women will affect the intake of nutrients to the fetus. Martin-Gronert (2006) states that the fetus will immediately respond to the condition of maternal malnutrition by increasing the process of catabolism that can continue to cause changes in fetal metabolism in the form of decreased concentrations of IGF-1 and decreased glucose transport to the fetus.[11] An infant's mental development is the result of a complex multifactorial process. Nutrition forms the basis for proper nerve development and can have a long-term impact on mental development later on.

The cerebrum is the largest part and occupies about 85% of the human brain separated by two right and left hemispheres. Cerebrum is heavily involved in somato-sensory processes and motor information as well as intellectual awareness and function. Cerebellum is known as the center of motor and balance while the role of cerebellum in cognitive function is still being debated. Timman and Daum (2007) used PET (Positron Emission Tomography) and MRI (Magnetic Resonance Imaging) scanning to prove the involvement of cerebellum in the aspect of cognitive function, especially verbal memory which ultimately affects cognitive function although it is still a debate and further research is needed.[12,13]

This study is a continuation of a series of studies on intrauterine enrichment in order to optimize fetal brain development, which aims to determine the effect of nutritional deficiencies in neuron and glial cells count in cerebrum and cerebellum of newborn *Rattus norvegicus*. This study can later be used to identify the extent of cellular changes in neuron and glial cells count in the brain caused by 50% food restriction in *Rattus norvegicus* model.

MATERIALS AND METHODS

This study was an experimental analytic study with a single blind randomized post test only control group

design. The experimental animals used were *Rattus norvegicus* as a food restriction 50% (FR 50%) model which replaced pregnant women due to ethical consideration. The study subjects were divided into two groups randomly, the treatment group (P) comprising pregnant *Rattus norvegicus* receiving FR 50% and the control group (K) namely *Rattus norvegicus* pregnant without FR 50%.

The study was conducted in the Animal Cages and Pathology Laboratory of the Faculty of Veterinary Medicine, Airlangga University, Surabaya, during November 2016 - January 2017. The study inclusion criteria were 2-month-old healthy *Rattus norvegicus* broodstock, 130-160 grams body weight, had never given birth, and had newborns. The exclusion criteria for the study were *Rattus norvegicus* mothers with anatomic abnormalities.

Sample size was calculated using Federer's formula and the number of samples for each group was 16. Each *Rattus norvegicus* mothers was pregnant, from each two newborns with the heaviest weight were taken. Immediately after birth, the *Rattus norvegicus* newborns' brain tissue was taken for histochemical preparations and BDNF expression was assessed using Remmele Scale Index. Results were compared between groups using statistical tests. Ethical eligibility was obtained from the Research Ethics Commission of the Faculty of Veterinary Medicine, Universitas Airlangga, Surabaya, Indonesia.

RESULTS AND DISCUSSION

Rattus norvegicus gestational age

All *Rattus norvegicus* newborns were born in the normal pregnancy, ranging 19-21 days. The highest percentage of 20-day gestational age of the *Rattus norvegicus* was obtained, which was 68.75% in control group and 62.5% in the treatment group.

Rattus norvegicus mothers's body weight

This study used *Rattus norvegicus* pregnant mothers in control and treatment groups with initial weight range between 144-152 grams measured before the FR 50% study since they were found to be pregnant and randomized into 2 groups, each of 16 rats. The treatment group received FR 50% while the control group did not. There were no deaths in *Rattus norvegicus* mothers' in either treatment or control groups. Most *Rattus norvegicus* had body weight ranging from 141-150 grams, which was 87.5% in control group and 87.5% in treatment group. In this

study no mother's body weight measurements were taken after treatment or before delivery so that the effect of FR 50% during pregnancy could not be identified.

Rattus norvegicus newborns' weight

All newborns of *Rattus norvegicus* in both control and treatment groups were weighed, then two with heaviest weights were chosen to be sacrificed and the brain was removed. Two *Rattus norvegicus* brains were made as one preparation and stained with Hemotoxilin-Eosin staining and the neuron and glial (astrocytes, oligodendrosites, microglia) cells were counted. The characteristics of *Rattus norvegicus* newborn based on the highest body weight ranged from 5.00-5.99 grams in control group was 62.5% (range 5.00-5.99 grams), and 43.75 (range 4.00-4.99) in the treatment group.

Neuron and glial cell count

In the cerebrum, the lowest neuron cell count was 6, and the highest was 17 in the treatment group. Whereas, in control group the lowest neuron cell count was 13 and and the highest was 21. The lowest glial cell count 46, the highest was 173 in treatment group. In control group the, lowest glial cell count was 63 and the highest was 155. The lowest number of astrocytes was 27, and the highest was 109 in treatment group. Whereas, in control group the lowest number of astrocytes was 44 and the highest was 105. The lowest oligodendrocytes coount was 6, and the highest was 58 in treatment group. Whereas, in the control group oligodendrocytes count was at least 14 and the highest was 39. The lowest microglial count was 2, and the highest was 13 in treatment group. Whereas, in control group the lowest microglial count was 1 and the highest was 13.

In the cerebellum, the lowest neuron cell count was 5, the highest was 11 in treatment group. Whereas, in control group the lowest number of neuron cells was 5 and the highest was 21. The lowest number of glia was 78, the highest was 135 in the treatment group. Whereas in control group, the number of glia was at least 48 and the highest was 142. The lowest number of astrocytes was 59, and the highest was 114 in the treatment group. In control group the least amount of astrocytes was 33 and the highest was 99. The lowest number of oligodendrocytes was 6, and the highest was 29 in the treatment group. In control group, the number of oligodendrocytes was at least 10 and the highest number was 46. The lowest number of microglia was 4, the most was 20 in the treatment group. Whereas in the control group the number of microglia was at least 3 and the highest was 8.

Analysis of *Rattus norvegicus* weight data

Data analysis on *Rattus norvegicus* weight was calculated using descriptive statistics to fin the average body weight. Normality test was performed using the Shapiro-Wilk test. Table 1 shows the average weight of *Rattus norvegicus* in each group.

Table 1. *Rattus norvegicus* mothers' and newborns' average body weights in control and treatment groups

Groups	Mothers' BW (gram)		Newborns' BW (gram)	
	Mean	$\pm$S/D	Mean	$\pm$S/D
Control	147.19	$\pm$2.344	5.08	$\pm$0.508
Treatment	147.31	$\pm$2.414	4.21	$\pm$0.658

To determine whether the samples had normal distribution, we carried out normality test using the Shapiro-Wilk test.

Table 2. Shapiro-Wilk normality test for *Rattus norvegicus* mothers' and newborns' body weights

Body weight (gram)	Groups	Shapiro-Wilk		
		Statistic	*df*	*p*
Mothers	Control	.927	16	.218
	Treatment	.9355	16	.288
Newborns	Control	.968	16	.803
	Treatment	.966	16	.762

Based on the Shapiro-Wilk test results obtained p> 0.05, which means that the weight of the mother and newborn *Rattus norvegicus* distribution is normal.

Analysis of data on the cerebrum

The Shapiro-Wilk test was performed as a normality test for the number of neuron cells, glial cells, astrocytes, oligodendrocytes, and microglia in each group. Then we carried out the analysis with descriptive statistics. The results of normality tests on neurons, oligodendrocytes and microglia showed abnormal data distribution (p<0.05), so we went on by using non-parametric Mann-Whitney U test, and for normal data distribution we used the parametric test independent t-test.

Table 3. Mann-Whitney test results on neuron cells in the cerebrum

Groups	Neuron cells (Mean$\pm$S/D)	P value
Control	16.88$\pm$2.553	0.000
Treatment	9.88$\pm$3.59	

Table 4. Mann-Whitney test results on oligodendrocytes in the cerebrum

Groups	Olygodendrocytes (Mean$\pm$S/D)	P value
Control	25.13$\pm$8.609	0.000
Treatment	14.06$\pm$12.195	

Table 5. Mann-Whitney test results on microglia cells in the cerebrum

Groups	Microglial cells (Mean$\pm$S/D)	P value
Control	4.94$\pm$3.838	0.620
Treatment	5.25$\pm$3.435	

Data with abnormal distribution revealed that the average number of neurons in the control group was 16.88$\pm$2.553 and in the treatment group was 9.88$\pm$3.59. Mean oligodendrocytes in the control group was 25.13$\pm$8.609 and in the treatment group it was 14.06$\pm$12.195. Mean microglia in the control group was 4.94$\pm$3.838 and in the treatment group 5.25$\pm$3.435.

Data with normal distribution revealed the average number of glial cells in the control group was 93.75$\pm$33.326 and in the treatment group was 99.19$\pm$26.234. Mean astrocytes in the control group was 69.13$\pm$18,726 and in the treatment group was 74.44$\pm$35,359.

Table 6. Independent t-test results on glial cells in the cerebrum

Groups	Glial cells (Mean$\pm$S/D)	P value
Control	93.75$\pm$33.326	0.612
Treatment	99.19$\pm$26.234	

Table 7. Independent t-test results astrocyte cells in the cerebrum

Groups	Astrocytes (Mean$\pm$S/D)	P value
Control	69.13$\pm$18.726	0.599
Treatment	74.44$\pm$35.359	

Tables 6 and 7 show the independent parametric t-test on normal data distribution (glia cells and astrocytes). The results revealed p value >0.05 in the parameters of glia and astrocyte cells. This means that there was no significant decrease in the number of glia cells and astrocytes in the cerebrum.

The tables show Mann-Whitney parametric test with abnormal data distribution on neurons, oligodendrocytes and microglia. The obtained p value was <0.05 in the parameters of neuron and oligodendrocyte cells. This means that there is a significant decrease in the number

of neuron and oligodendrocyte cells in both groups in the cerebrum. The p value of the microglia was > 0.05, indicating no significant increase in microglia in both groups in the cerebrum.

Analysis of data on the cerebellum

To determine data distribution on the number of neuron, glia, astrocytes, oligodendrocytes, microglia data, we performed normality test using Shapiro-Wilk test. The results showed significance of p=0.470 for astrocytes, p=0.069 for oligodendrocytes, and =0.925 for glia. The above results showed that all data distribution on the cerebellum was normal (p> 0.05).

Table 8. Mann-Whitney Test on neuron cells in the cerebellum

Groups	Neuron cells (Mean±S/D)	P value
Control	11.44±4.560	0.02
Treatment	7.50±1.789	

Table 9. Mann-Whitney Test on microglia cells in the cerebellum

Groups	Microglial cells (Mean±S/D)	P value
Control	5.25±1.483	0.04
Treatment	8.81±4.119	

Table 8 and 9 show the Mann-Whitney parametric test on the distribution of abnormal data (neuron cells and microglia). The obtained p value was <0.05 in neuron and microglia cell parameters. This means that there is a significant decrease in the number of neuron cells and a significant increase in microglia in both groups in the cerebellum. After finding that the data had normal distribution, we proceeded to use the independent parametric t-test.

Table 10. Independent T-Test on glia cells in the cerebellum

Groups	Glial cells (Mean±S/D)	P value
Control	88.94±24.255	0.058
Treatment	103.38±16.346	

Table 11. Independent T-Test on astrocytes in the cerebellum cells

Groups	Astrocytes (Mean±S/D)	P value
Control	59.69±18.771	0.002
Treatment	80.94±16.270	

Table 12. Independent T-Test Results on Cerebellum Oligodendrocyte Cells.

Groups	Olygodendrocytes (Mean±S/D)	P value
Control	24.00±8.862	0.01
Treatment	13.63±6.712	

Tables 10 to 12 show the results of independent parametric t-test on data with normal distribution (glia cells, astrocyte cells, oligodendrocyte cells). The obtained p value was <0.05 on the parameters of astrocytes and oligodendrocytes. This indicates a significant increase in the number of astrocytes and a significant decrease in the number of oligodendrocytes in the cerebellum. P value of >0.05 was obtained on glia cells, showing no significant increase in the number of glia cells in the cerebellum.

This research aimed to observe cellular changes in the brain due to 50% Food Restriction. The developing brain has plasticity properties or ability to adapt, but the vulnerability to nutritional deficiencies is greater than the plasticity properties of the brain so that the consequences of nutrient deficiency do not only occur at this time but also afterwards. Nutrition and growth factors regulate brain growth during fetal and early postnatal periods. Nutrition plays an important role in the maturation and functional development of the central nervous system because nutrition provides the energy and substances needed for the development of cell structure and various metabolic functions. Malnutrition can have adverse effects on various aspects of brain development and function. However, because it is related to ethics, there have not been many studies on the effect of prenatal nutrition on brain development.[14]

Obstetrics, gynecology and women's health department of the University of Missouri at Columbia (2010) conducted observations on hunger that occurred in the Netherlands during the winter of 1980. This study was focused on how hunger in pregnant women at that time affected the health of their offspring. There was no difference in the birthweight of the babies from mothers starving throughout their pregnancy. Pregnant women who were starving from the middle of pregnancy to the end gave birth to babies with significant birthweight loss. Whereas, mothers who suffered from hunger only at the beginning of pregnancy gave birth to babies with normal birthweight, but those who were obese during adulthood were more than those who were born from mothers who started starving from mid-pregnancy to the end. So there was no difference in birthweight in pregnant women who experienced food restrictions during pregnancy.

Food restriction in early pregnancy is considered to increase obesity, change lipid profile, and lead to cardiovascular disease. Conversely, food restructuring in mid-pregnancy causes reduction in specific kidney function. Rooij et al showed that food restrictions in early pregnancy had an impact on selective attention testing at the age of 56-59 years. Although exposure to food restriction during early pregnancy, which has been known since 35 years ago, is very important influence on subsequent health, the causes are still not fully understood.[15]

In our study, there was no significant difference in body weight between *Rattus norvegicus* newborns from mothers receiving 50% FR exposure and *Rattus norvegicus* newborns' from mothers who did not get 50% FR exposure. From our study, we found a significant difference in the number of neurons between the groups receiving FR50% treatment and control group, where the number of *Rattus norvegicus* newborns' cerebrum and cerebellum neuron cells was less in the treatment group compared to the control. This indicates that number of neuron cells in cerebrum and cerebellum is less in *Rattus norvegicus* newborn from pregnant mothers with FR50% exposure than without exposure.

The underlying mechanism that affects neuron cells is related to nutritional deficiencies and the availability of appropriate neurotropic factors in sufficient quantities.[16] Embryonic period, during which organogenesis occurs, including brain development at 24 to 42 weeks' gestation, is very susceptible to nutritional deficiencies because the brain is in rapid growth, where the process of neuron formation, gliogenesis, cell migration, and initial differentiation occur, including formation synapses and myelination.[14]

The initial fetal period, which lasts until the middle of pregnancy, is an important period in which the growth of cortical neurons and migration of neurons and the formation of brain tissue occur to process information. Disturbances in the early stages of this development will cause innervation patterns. If they occur in the prepubertal period, it will cause functional changes that are more adaptive.[4,17]

Neural progenitor cell reserves are very small to be able to accommodate the production of large neurons, so the initial stage for the formation of neuron cells is to multiply neural progenitor cells. Neuron cells are post mitotic cells, where once formed they are no longer able to divide and produce new cells. After the end of the gastrulation process until day 42, neural progenitor cells divide symmetrically and produce two identical neural progenitor cells so that the number of cells increases.

On day 42 of the embryonic period, cleavage changes from symmetrical cleavage to asymmetric. In this asymmetrical division, two kinds of cells are formed, ie. neural progenitor cells and neuron cells. Neural progenitor cells will continue to divide while neuron cells stop proliferation and migration towards the neurocortex.[4]

During this proliferation phase, it is estimated that there will be a growth of new neurons of 250,000 neurons per minute. Thus the number of neurons in the human brain can reach 10^{11}. After that nerve cells will undergo a process of migration to the definitive location. Neurons do not increase or stop proliferating at the time of when the baby has not been born at 32 weeks.[18] Some of the studies below supports our conceptual framework.

A study conducted by Roy, Sable, et al (2013) find that an imbalance of micronutrients (folic acid and vitamin B12) during pregnancy will cause an increase in oxidative stress which results in reactive oxygen species (ROS). If the condition continues, the defense mechanism by superoxide dismutase (SOD) in brain neuron cells cannot compensate for the ongoing oxidative stress and decreased SOD, resulting in cellular energy decreases with the final result the decrease in the number of neuron cells.[19-21]

Another study conducted by Bedi in 1991 showed that malnutrition during early life caused a significant deficit in the total number of gyrus dentata granule cells. Bearing this in mind, Sani and Bedi believe that the increase in the number of apoptotic cells observed in their study strongly shows that most of these micro neurons have experienced cell death due to lack of nutrients.[16]

In previous studies, micronutrient deficiencies such as folic acid and vitamin B12 can increase brain MDA levels in both the rats' mother and offspring at birth. Roy, et al. also hypothesized that impaired intake/ metabolism of micronutrients during pregnancy could increase oxidative stress which would risk neuronal development abnormalities in the future.[22]

The results of a study conducted by Craciunescu, et al (2010) show that folate deficiency and vitamin B12 inhibit methionine regeneration and have an adverse effect on cells by allowing the accumulation of homocysteine, a potentially toxic substance produced by methionine demethylation. Homocysteine induces DNA damage in neuron culture by a mechanism that might involve a decrease in DNA transmetilation.[23]

Neuron cell death program in the fetus in the womb consists of two processes, namely the pathological

necrosis process and the physiological apoptosis process. The program is influenced by the expression of several genes and can be prevented by absorption of several neurothropic factors. Neuron cells will stop multiplying at birth. Thus, the neuron cells will stop multiplying before we are born.[24] Neurons are produced in very large numbers and this number of cells will be regulated by cell death that occurs during periods of connectivity to the target tissue. Naturally apoptotic neurons are useful so that neurons form the right connections with their targets. Growing neurons compete to obtain a limited number of neurotropic factors produced by tissue targets. From the hypothesis, neurons that successfully form a network with a target means that they get access to neurotropic factors and will survive while cells that fail to make the right tissue mean they fail to obtain neurotropic factors and will die.[25]

Bures et al. (1988) state that brains that grow in an stimulus-rich environment have thicker cortex, larger nucleus neuron cells and more glia cells. Brain neurons also found that grow in a stimulus-rich environment have more dendritic sites, allowing more synapses to form. Rees also stated that the number of cells undergoing apoptosis depends on synapses, the more synapses the less apoptosis occurs. The richer the neuron cells on the dendritic site, the more the synapses formed so that the number of cells undergoing apoptosis will also be reduced. Brains that grow in a stimulus-rich and nutritional environment will experience less apoptosis, thereby increasing the brain's capacity.[26]

A previous study conducted by Hapsari A (2017), showed that the administration of FR50% to the mother *Rattus norvegicus* during early pregnancy led to an increase in apoptotic index in the cerebrum and the newborn *Rattus norvegicus* cerebellum.[27] A similar study carried out by Fauzi A (2017) to see dendritic densities, obtained lower dendritic densities in the cerebrum and cerebellum *Rattus norvegicus* newborn who received FR50% treatment compared to controls.[28]

Scharfman et al in 2004 stated that BDNF administration would significantly increase the neurogenesis process in the dendate gyrus and hippocampus. This was indicated by the addition of various numbers of various neuron cells in the brain. BDNF has the ability to defend and the potential to activate neuronal growth.[29]

Previous research conducted by Anggraini E, (2017) comparing BDNF cerebrum and newborn *Rattus norvegicus* cerebellum in a mother given FR50% at the start of pregnancy showed that BDNF was lower in the treatment group than in the control group.[30]

Another study conducted by Coupe, et al. in 2008 showed that BDNF levels in rat fetuses are very sensitive to maternal nutritional status. Pregnant mice with FR50% showed a decrease in BDNF concentrations in the hippocampus of offspring mice. Several studies have shown that maternal nutritional disorders have a short-term effect on BDNF levels in the fetal brain. Research by Coupe, et al. suggested that BDNF plays an important role in brain development, where a 50% reduction in maternal nutrition (50% Food Restriction) during embryonic times will affect BDNF levels in rat fetuses in the hippocampus and hypothalamus sections. Antonow (2010) in his research also stated that Food Restriction 30% (FR30%) in baboons during pregnancy will reduce BDNF levels around 13.4%-26.3% and increase neuron cell apoptosis by 118% in baboon's newborns.[9,31]

From the results of our study of *Rattus norvegicus* cerebrum and cerebellum glaucid cells from the mother receiving 50% food restriction from the start of pregnancy, we did not obtain a significant difference in the number of glia cells in the treatment group compared to the control. The number of neuroglia in the brain is about 10 times the number of neuron cells in the brain. The greater the brain mass, this ratio will increase. Therefore, in rats, smaller comparisons are obtained, where the neuroglia's number is higher 0.3-0.4 times the number of neurons.[5] Neuroglia has main functions including maintaining the position of neurons, providing the supply of nutrients and oxygen to neurons, maintaining the environment and metabolism needed by neurons, limiting neurons from other neurons, forming myelin, taking and storing neurotransmitters produced by synapses around them and destroying pathogens and get rid of dead neuron cells. In general there are 3 types of glia, the astrocytes, oligodendrocytes, and microglia.[32]

A study conducted by Clos et al (1977), in malnourished 35-day-old rats with quantitative restrictions on the mother's diet from 6 days gestation, wet weight and cerebellum DNA content were slightly lower than cerebrum. Cell growth (estimated from DNA concentration and from the ratio of RNA and protein to DNA) is significantly affected by food deprivation only in the cerebellum. In the cerebellum cortex, the number of Purkinje, Golgi and stellate cells does not change. The number of other cell types is affected to various extents: there are fewer granules and basket cells per Purkinje cell, and hypoplasia is more pronounced than glia involving glia cells from the molecular layer, and astrocytes from internal granular and Bergmann cells from Purkinje cell layers. Finally, the number of glia cells in the cortex decreases 44% compared to 13% for neurons. The effects of malnutrition on cell acquisition

in the brain, and on the cellular composition of the cerebellum, contrast with thyroid deficiency.[33]

Astrocytes are the most numerous types of glia cells and have various cell forms in the CNS that can function to replace damaged neurons, oligodendrocytes or microglia. Some of the important functions of astrocytes include stem cells (radial glial cells), brain microarchitecture, ie. forming brain parenchymes, especially gray matter (protoplasmic astrocytes), regulating local blood flow to the brain, regulating synaptogenesis, maintaining synapses that have formed and maintaining homeostasis of several processes important in the brain.[34,35]

From the results of our study of the number of cerebrum astrocytes and cerebellum of *Rattus norvegicus* newborns from mothers receiving 50% food restriction from the start of pregnancy, we obtained more cerebellum astrocytes than those in controls. While in the number of cerebrum astrocytes did not show significant difference in the treatment group compared to the control. Previous studies showed that healthy brain cells have a small amount of astrocytes. Astrocytes will increase if there is damage to neurons where astrocytes will proliferate and fill the space to form 'glial scar', repairing damaged areas by transforming into neuron cells. Active astrocytes can synthesize and excrete neurotrophic factors which will indirectly reduce apoptosis, which in turn will increase the number of the surviving neurons.[34,35]

Oligodendrocytes are cells that act to form the myelin sheath for the CNS. Formation of myelin in mice begins immediately after birth and the process is complete approximately 2 months after birth. Oligodenrocytes do not have the ability to regenerate, so damage to the CNS often results in permanent disability. Disorders of protein connexins can cause hypomyelinization and pathological conditions.[32]

From our study we found that the number of cerebrum oligodendrocytes and cerebellum of *Rattus norvegicus* newborns had significant difference between the groups receiving FR50% treatment and the control group, where the number of cerebrum and cerebellum oligodendrocytes was less in the treatment group compared to control.

A study conducted by Mallard, et al (2000) observing the effects of intrauterine growth restriction (IUGR) on the development of myelinating oligodendrocytes and astrocytes in the brain and spinal cord of guinea pigs fetus for myelin base protein, found that myelination levels in spinal cord, cerebral cortex, corpus cellosum and cerebellum were reduced in fetuses that were IUGR

compared to controls. As for glial fibrillary acidic protein, there was no significant difference between control and IUGR in the expansion or distribution of astrocyte cells at 52 or 62 days in the cerebellum. However, in the cerebral cortex at 62 days there is a marked proliferation of astrocytes around cortical blood vessels in fetuses that have IUGR. Ultrastructural studies have shown that at 52 days, myelination of the corticospinal canal has begun in control but is almost absent in fetuses that are IUGR. On 62 days, the total amount of myelinated fiber in the IUGR fetus was significantly reduced by 56% (P <0.01) compared to the control fetus. However, there was no difference between groups in the total amount of fiber in the corticospinal tract where myelin fibers and myelin sheaths are disproportionately reduced relative to the axon diameter. Thus, at the IUGR there are delays in initiation and in the degree of myelination. This can be caused by a reduced amount of myelinating glia that forms and a limited capacity to produce myelin.[36]

Oligodendrocyte cells can trigger the process of axons myelinizationin neurons and express genes in the process of myelinization. There was a significant decrease in the number of oligodendrocytes in the cerebrum and cerebellum in the treatment group. This is thought to occur because of the presence of factors that increase apoptosis by 50% food restriction. Oligodendrocytes affect the proliferation of neurons through the production of several neurothropic factors. Good myelinization increases the speed of impulses between nerve cells in Schwann cells and oligo-dendrocytes. Oligodendrocytes not only play a role in the myelinization, but also play a role in supporting axons in neuronal cells, even the death of oligodendrocyte cells will be followed by axon degeneration. The level of Intelligence Quotient (IQ) in humans is associated with good myelinization.[8]

Microglia are part of the immune system for the CNS against various pathogens. Microglia will experience a resting phase if there are no pathogenic factors. In this phase microglia are the fastest moving cells in the brain to check for the presence of pathogens. If a pathogen threat is found, microglia will gather around damaged neurons, multiply and destroy damaged cells.[32]

From the results of our study on the number of cerebrum microglia and cerebellum of *Rattus norvegicus* newborns from the mother with 50% food restriction from the start of pregnancy, we found that number of microglia cerebrum and cerebellum was higher than those in controls. This is also thought to occur because of the factor that increases apoptosis, the 50% FR which causes microglia that contribute to phagocytosis to become more numerous.[37]

REFERENCES

1. Hermanto TJ. Bersujud Dalam Rahim 2. Mencerdaskan Janin Sejak dalam Rahim dengan Kombinasi Stimulasi 11-14 Musik Karya Mozart dan Nutrisi". Surabaya: Global Persada Press; 2013.
2. Story L. A head start in life? Prenatal parenting and discourse of fetal stimulation. Atlantis, 2003;41-48.
3. Hermanto TJ. Smart babies through prenatal university mission impossible? Majalah Obstetri dan Ginekologi Indonesia, 2004;14.
4. Stiles J, Jernigan TL. The basics of brain development. Neuropsychol Rev, 2010;20:327-48.
5. Hines T. Anatomy of the Brain. Mayfield Brain and Spine (MayfieldClinic.com); 2016.
6. Reichardt LF.. Neurotrophin-regulated signalling pathways. Philosophical Transaction of The Royal Society. 2006;1545–64.
7. Hermanto TJ, Estoepangesti ATS, Widjiati. The influence of various musical compositions exposure to pregnant *Rattus norvegicus* to the number of rat offspring brain cells. The 3rd Scientific Meeting on Maternal Fetal Medicine and AOFOG Accredited Ultrasound Workshop. Surabaya. 2002.
8. Rajkowska G, Hidalgo JJM. Gliogenesis and glial pathology in depression. CNS Neurol Disord Drug Targets. 2007;219–33.
9. Antonow-Schlorke I et al. Vulnerability of the fetal primate brain to moderate reduction in maternal global nutrient availability. PNAS. 2010;3011-16.
10. Lee M, Chang H, Lee T. Influence of prenatal noise and music on the spatial memory and neurogenesis in the hippocampus of developing rats. Brain Development. 2006;20-26.
11. Martin-Gronert MS, Ozanne SE. Maternal nutrition during pregnancy and health of the offspring. Biochem Soc Trans. 2006;34(Pt5):779-82.
12. Timmann D, Daum I. Cerebellar contributions to cognitive functions: a progress report after two decades of research. Cerebellum. 2007;6(3):159-62.
13. Marieb E & Hoehn K. Human Anatomy and Physiology. 2007;14
14. Morgane PJ, Austin-LaFrance RJ, Bronzino, JD Tonkiss J, Galler JR. Prenatal malnutrition and developing central nervous system. In: The Vulnerable Brain and Environment Risks, Volume I. New York: Plenum Press; 1993. p. 33-9.
15. Schulz LC The Dutch hunger winter and the developmental origins of health and disease. Proceedings of the National Academy of Sciences. 2010;107(39):16757-8.
16. Sani J, Bedi KS. Undernutrition during early life increases the level of apoptosis in the dentate gyrus but not in the CA2+CA3 of the hippocampal formation. Brain Research. 2007;60-9.
17. Andersen SL. Trajectories of brain development: point of vulnerability orwindow of opportunity? Neuroscience and Biobehavioral Reviews. 2003;3-18.
18. Volpe, Joseph J, Neurology of The Newborn. WB Sauders, Philladelphia, USA. 2001; p. 45-99
19. Roy S et al. Effect of maternal micronutrients (folic acid and vitamin b12) and omega 3 fatty acids on indices of brain oxidative stress in the offspring. Brain Development. 2014;36(3):219-27.
20. Sable P, Kale A, Joshi A, Joshi J. Maternal micronutrient imbalance alters gene expression of BDNF, NGF, TrkB and CREB in the offspring brain at an adult age. Intl. J. Devl. Neuroscience, 2014;24-32.
21. Kundakovic M & Jaric I. The epigenetic link between prenatal adverse environments and neurodevelopmental disorders. Genes. 2017;104.
22. Sanyal T et al. Effect of prenatal loud music and noise on total number of neurons and glia, neuronal nucleae area and volume of chick brainstem auditory nuclei, field 1 and hippocampus: A stereological investigation. Int J Devl Neuroscience. 2013;31:234-44.
23. Mattson MP, Shea TB. Folate and homocysteine metabolism in neural plasticity an neurodegenerative disorders. Trends in Neurosciences. 2003;26 (3).
24. Rodeck C, Whittle M. Fetal Medicine. Basic Science and Clinical Practice, 2nd Edition. Churchill Livingstone, 2008
25. Gordon N. Apoptosis (Programmed Cell Death) and other reasons for elimination of neurons and axons. J. Brain Development. 1995;17(1). P: 73-77
26. Rees S, Walker D. Fetal growth and development. In: Harding R, Bocking A. editors. Nervous and Neuromuscular Systems. Cambridge: Cambridge University Press; 2001. p.154-85.
27. Hapsari, A., Hermanto & Widjiati. Pengaruh Food Restriction 50% Selama Kebuntingan Terhadap Indeks Apoptosis Sel Neuron pada Cerebrum dan Cerebellum Otak *Rattus norvegicus* Baru Lahir. Unpublished. 2017.
28. Fauzi A, Hermanto TJ, Widjiati. Pengaruh food restriction 50% selama kebuntingan terhadap kepadatan dendrit cerebrum dan cerebellum otak *rattus norvegicus* baru lahir. Unpublished. 2017
29. Scharfman HE, Goodman J, Macleod A, Phani S, Antonelli C, Croll S. Increased neurogenesis and the ectopic granule cells after intrahippocampal BDNF infusion in adult rats. Experimental Neurology. 2005;192:348–56.
30. Anggraini EP, Hermanto TJ, Widjiati. Pengaruh food restriction 50% selama kebuntingan terhadap ekspresi brain derived neurothropic factor cerebrum

dan cerebellum *rattus norvegicus* baru lahir. Unpublished. 2017.

31. Coupe B. et al. Perinatal undernutrition modifies cell proliferation and brain-derived neurotrophic factor levels during critical time-windows for hypothalamic and hippocampal development in the male rat. Journal of Neuroendocrinology. 2008;21:40-8.

32. Kettenmann, H, Hanisch UK, Noda M, Verkhratsky A. Neuroglia: Definition, classification, evolution, numbers, development. In: Verkhratsky A, Butt A. editors. Glial Physiology and Pathophysiology. John Wiley & Sons; 2013. p. 73-104.

33. Clos J et al. Effect of undernutrition on cell formation in the rat brain and specially on cellular composition of cerebellum. Neuroscience. 1977; 123:13-26.

34. Santello M, Volterra A. Synaptic modulation by astrocytes via Ca2+-dependent glutamate release. Neuroscience. 2008;158(1):253–9.

35. Sofroniew M, Vinters HV. Review: Astrocytes: biology and pathology. Acta Neuropathol. 2010; 119:7-35.

36. Mallard C et al. Reduced number of neurons in the hippocampus and the cerebellum in the postnatal guinea-pig following intrauterine growth-restric-tion. Neuoroscience. 2000;100(2):327-33.

37. Nayak F, Roth TL, McGavern DB. Microglia development and function. Annual Review of Immunology. 2014;32:367-402