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MORPHOMETRIC BRAIN MEASUREMENT OF PRENATAL ULTRASOUND-INDUCED RABBIT FETUS USING MICRO-COMPUTED TOMOGRAPHY (MICRO-CT)

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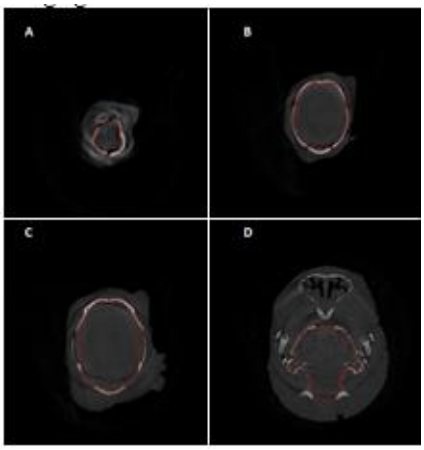
Graphical abstract



Abstract

Prenatal ultrasound is widely used and became an integral part of the clinical practice, particularly in obstetrics and gynecology. However, the advances in the capability of ultrasound equipment nowadays, trigger a greater image processing power, hence might as well increase the fetal exposure. Hence premises the morphometric brain measurement to be assessed in this study as an evidence of the ultrasound interruptions during the fetal neuronal development. This research randomly analyzed a total of 18 ultrasound-induced rabbit fetuses for fetal brain volume and surface after being exposed prenatally to the ultrasound exposure (duration=60 minutes; frequency = 7.09 MHz; spatial peak temporal average intensity (ISPTA) = 49.4 W/cm2; power = 56.0 W; thermal index (TI) = 0.2; mechanical index (MI) = 1.0). The fetuses were analyzed for morphometric brain measurement of brain volume and surface using Skyscan™ 1176 Micro-computed Tomography (Micro-CT). There were significant differences in the measurement of brain volume and surface at the 2nd and 3rd stage of gestation ($P < 0.05$). Results suggested that there are significant differences in the brain volume and surface between the controls and the 2nd and 3rd stage of gestation. There are also a significant reduction in the brain volume and surface in the exposed groups at all stages of gestation ($P < 0.05$).

Keywords: Brain, fetal, gestation, *in-utero*, micro-CT, morphometric, prenatal, ultrasound

1.0 INTRODUCTION

Prenatal ultrasound is widely accepted in clinical practice, where it has been found to reduce perinatal mortality due to early fetal malformations detection [1]. From the ultrasound scanning, plenty of fetal anomaly can be detected, including hydrocephalus, spina bifida, Down syndrome [2], [3] and other genetic and chromosomal abnormalities [3]. Certain stages of fetal development can be detected in a certain time frame during pregnancy.

As fetal development, fetal maturity is assessed through the several measurements, including gestational sac, bi-parietal diameter, head circumference, abdominal circumference, femur length and crown-rump length [4]–[8]. In this context, it premises the morphometric brain measurements which include the fetal brain volume and brain surface to be assessed in this study as an evidence of

the ultrasound interruptions during the fetal neuronal development. Moreover, the main cause of perinatal mortality is associated with early defects during the formation of central nervous system (CNS) [9], where the neuronal development in human embryo begins as early as in the 6th weeks of gestation [10] and develops until the 32nd week of gestation [11]. Besides, the neural tube defect could be caused by the malformation of the developing CNS during the neural tube closure and neural tube later growth [12]. Hence, these statements had driven this current research to study the effects of prenatal ultrasound exposure on fetal brain volume and surface throughout the gestational stages in a rabbit pregnancy.

As far as our concern, the advances in the capability of ultrasound equipment trigger greater image processing power to increase the quality of images produced and provide new information of the *in-utero* fetus [13]. However, the changes experienced by power and output capability in recent developments in ultrasound have intensified the need for revising the effects of the exposure; hence it became the main reason for this study to be carried out. This study aimed to assess the effects of ultrasound exposure on morphometric brain measurement of fetal brain volume and surface. The rabbit fetuses were insonated *in-vivo* with an ultrasound beam that had acoustic output parameters typical of those that could be emitted from clinical ultrasound scanner operating in pulsed wave (PW) ultrasound mode in 2D (2-dimensional) B-mode (brightness mode) ultrasound image display.

An ethical approval was obtained from the Committee on Animal Research and Ethics (UiTM CARE) before the experiment was carried out.

2.0 MATERIALS AND METHODS

This study involves 3 pregnant New Zealand white rabbits exposed to 2D B-mode PW ultrasound using the ultrasound system (Philips HD3, Koninklijke Philips Electronics, N.V., Netherlands) once in the middle of a stipulated gestational stage (1st, 2nd, 3rd stage). The ultrasound acoustic output parameters are as follow; exposure duration=60 minutes; frequency = 7.09 MHz; spatial peak temporal average intensity (I_{SPTA}) = 49.4 W/cm^2; power = 56.0 W; thermal index (TI) = 0.2; mechanical index (MI) = 1.0. Meanwhile, another 3 pregnant rabbits served as the control group that was not exposed to ultrasound. The exposures were carried out during the gestational day (GD) 6-7, GD 17-18 and GD 28- 29 for the 1st, 2nd, 3rd gestational stages, respectively as described in a previous study [14].

All pregnant New Zealand white rabbits were sacrificed 48 hours after exposure to allow for biological effects to be detected. They were euthanized using Pentobarbital sodium (Doléthal, Ethical Agents Ltd., New Zealand) at a dose of 0.75 ml/kg body weight via intra-cardiac injection route. A surgical procedure, a laparotomy was performed and the ultrasound-induced rabbit fetuses from the mother's uterus in both right and left uterine horns were excised, thus became the subjects in this study. According to Lebas *et al*. [15] rabbit has an average of 7 to 9 kittens per litter in every pregnancy. Hence, a total of 18 ultrasound-induced rabbit fetuses (controls, n = 9; exposed, n = 9) were randomly analyzed for fetal brain volume and surface. The fetuses were fixed in 10% neutral buffered formalin (NBF) for 24 hours in the 4°C refrigerator by following the methods proposed by Metscher [16]. Then, the procedure is followed by a serial dehydration using 30%, 50%, 70% and absolute ethanol overnight in each solution. The fetuses were then immersed in a staining solution, an alcoholic phosphotungstic acid (EPTA) and left stained overnight.

The morphometric brain measurements of fetal brain volume and surface were carried out using a micro-computed tomography imaging system (Skyscan™ 1176 Micro-computed Tomography (Micro-CT), Bruker Micro-CT, N.V., Aartselaar, Belgium) as in Figure 1. This was carried out by selecting the regions of interest (ROI) on the cross-sectional micro-CT images as shown in Figure 2. The ROI was selected from the most superior part of the forebrain, which is the vertex until the most inferior part of the hindbrain which is the brainstem. Therefore, the micro-CT computed the values for the brain volume (μm^3) and brain surface (μm^2), then the results were statistically analyzed using statistical analysis software (Statistical Package for the Social Sciences (SPSS) version 21 (International Business Machines Corporation (IBM), N.Y., USA).

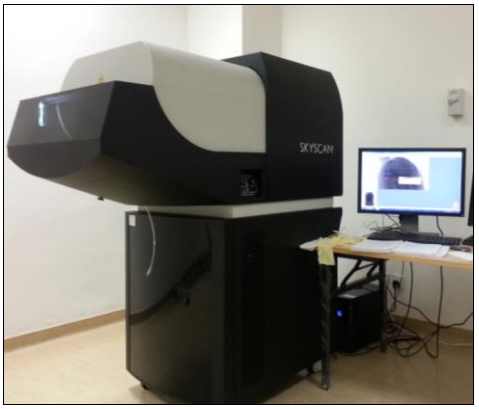


Figure 1 Rabbit fetal brain scanning using Skyscan™ 1176 micro-CT imaging unit

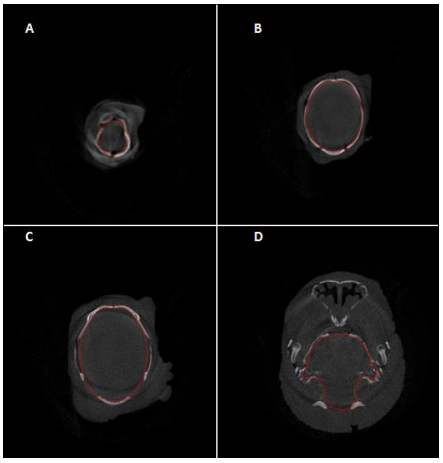


Figure 2 Cross-sectional micro-CT images of rabbit fetal brain showing the selected regions of interest (ROI) (red lines); begins from vertex (A) until brainstem (D) for a morphometric brain measurement

3.0 RESULTS

The t-tests for the brain volume and surface have shown significant differences in the 2nd and 3rd stage of gestation with p-values less than 0.05 ($P < 0.05$), Table 1. The results indicated that there are significant differences in the brain volume and surface between the control and exposed groups during the 2nd and 3rd stage of gestation, Figure 3.

Table 1 T-tests for the brain volume and surface

Stage of gestation	Brain volume (μm³)	Brain surface (μm²)
	P-value	
1st stage	0.05	0.05
2nd stage	0.01*	<0.001*
3rd stage	<0.001*	0.01*

Table 2 Correlation tests for the brain volume and surface

Stage of gestation	Brain volume (μm³)	Brain surface (μm²)
	P-value (coefficient)	
1st stage	0.01*(-0.90)	0.01*(-0.93)
2nd stage	0.01*(-0.94)	<0.001*(-0.97)
3rd stage	<0.001*(-0.99)	0.01*(-0.94)

Note: *P<0.05 shows a significant difference/association in mean values between the control and exposed.

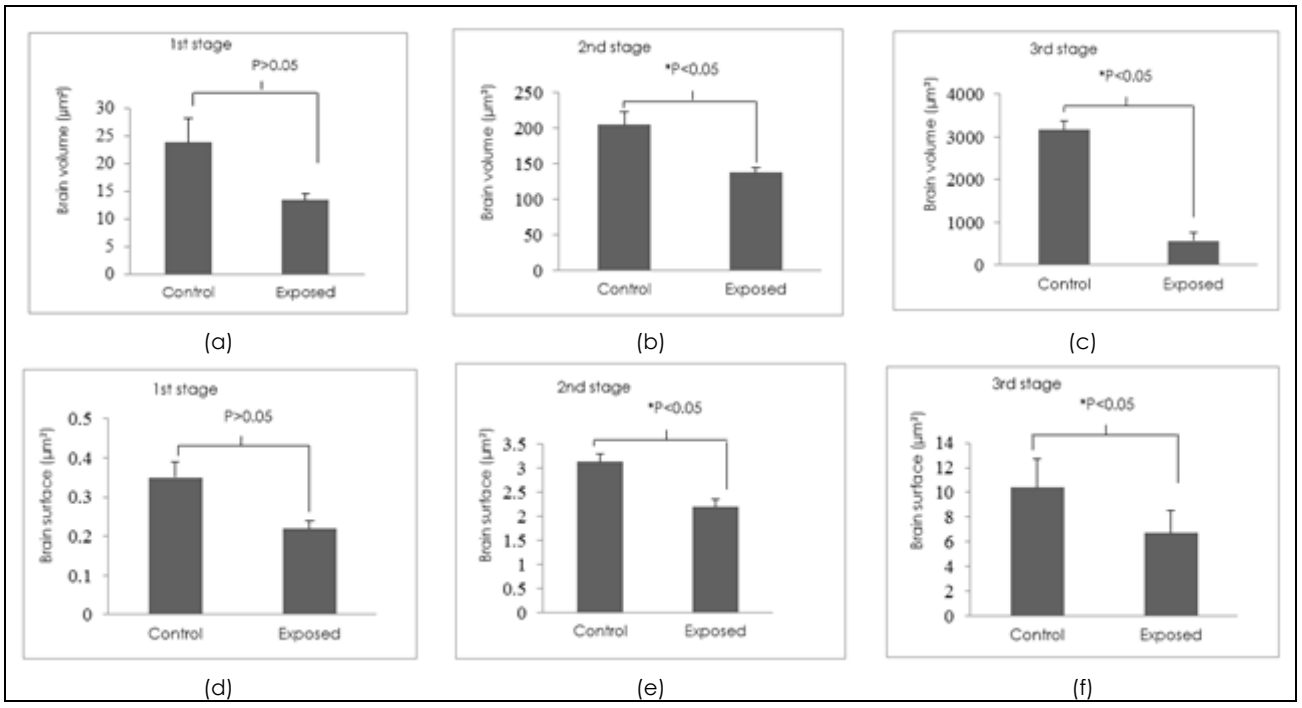


Figure 3 The comparison of the brain volume (a) - (c) and surface (d) - (f) between control and exposed groups at 1st, 2nd, and 3rd stage of gestation. *P<0.05 shows a significant difference

Further statistical analysis, the correlation test was carried out to correlate both dependent and independent variables. The results showed a negative association between the exposure and the brain volume and surface with p-values less than 0.05 ($P < 0.05$), Table 2. Hence, there are significant reductions in the brain volume and surface in the exposed groups at all stages of gestation as

compared to the control groups.

4.0 DISCUSSION

The results indicated that there are significant differences in brain volume and surface between the controls and the 2nd and 3rd stage of gestation. Reported that the fetal activity is more pronounced during the middle and the late stage of gestation where the formation of major body structures and growth of the structures in those stages [10], [17]. Hence, the cerebral tissue damages in prenatally exposed fetal brain in this animal modeling study showed remarkable changes in the morphometric measurements of brain volume and surface during the 2nd and 3rd stage of gestation indicating the neuro-sensitivity of these stages to the ultrasound interruptions during the fetal neuronal development.

The significance of these morphometric findings is supported by previous research that found morpho-physiological changes in the brain tissue exposed to hyperthermia. The changes in the brain include damages in the cerebral cortex and showing a thrombosis of the cerebral vessels, simultaneously [18]. Reviewed by Sharma [19] the magnitude of brain damage due to hyperthermia could be much related to the alterations in the blood parameters. Moreover, the state of stress was created when the oxygen level that reached the fetal brain tissue is low [20] which could be evident by the enhancement in the release of neurotransmitter [19], [20]. Concerning to the total blood count, prior research has proven the decrement in the total red blood count in rabbit's newborns when longer ultrasound exposure time was given prenatally [21]. If the same condition is observed in this current study, the occluded blood vessels and dwindling in the total red blood count can be postulated as the cause in the cessation of oxygen from reaching the fetal brain tissue *in-utero* leading to brain ischemia and stress.

The morphometric measurements for both brain volume and surface in this current study demonstrated a significant reduction in the exposed groups as compared to the control groups at all stages of gestation. Cardinally, the interaction of ultrasound beam with biological tissue creates heat [22]–[24] and the way of rabbit's thermoregulation system dissipates heat is mainly via a large arteriovenous anastomotic system [25]. With regard to this current study, when the occlusion occurred to the only vessel that connects the fetus to maternal circulation, the umbilical cord, which is responsible for a transportation of oxygen and nutrients, the fetal thermoregulation may decrease since few chances of heat exchange can occur.

Furthermore, the decreased in the blood flow to the placenta and fetus is believed to cause embryonic and fetal growth retardation [17]. The possibility of the reduction in the brain volume and surface upon ultrasound exposure in this present study attributing to the fetal growth restriction cannot be totally excluded. This is because, a significant reduction in fetal weight was observed by previous research after exposure to the prenatal ultrasound [14], [26], which could explain the contribution of these morphometric brain measurement findings to the total fetal body weight. Further studies on these relationships are underway.

5.0 CONCLUSION

As a conclusion, in line with previous research that evaluated the effects of prenatal ultrasound to a fetus, this study has revealed the remarkable changes in the morphometric measurements of brain volume and surface indicating the neuro-sensitivity of the fetus to the ultrasound interruptions during the fetal neuronal development.

Acknowledgement

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