THERMAL EFFECTS OF PRENATAL DOPPLER ULTRASOUND ON NEWBORNS OF *ORYCTOLAGUS CUNICULUS*

BY

UMI NADRAH BINTI AMRAN

A thesis submitted in fulfilment of the requirement for the Master of Health Sciences (Medical Imaging)

Kulliyyah of Allied Health Sciences
International Islamic University Malaysia

MARCH 2020

ABSTRACT

Despite the usefulness of Doppler ultrasound in complementing prenatal care, its usage had also been reported to increase the temperature of tissues. Prolonged exposure to Doppler ultrasound may increase the risk of bioeffects. Therefore, the aim of this study was to investigate the influence of Doppler ultrasound on the body weight, brain size and bone mineral density (BMD) of newborn *Oryctolagus cuniculus* throughout three gestational ages (GA). The study utilised twelve (12) pregnant *Oryctolagus cuniculus* or New Zealand white rabbits (NZWR) (Control Group = 3; Exposed Group = 9). By utilizing Siemens Model ACUSON X150 ultrasound machine, linear array transducer VF10-5 (5.0 to 10.0 MHz) the exposed group was exposed once to Doppler ultrasound at a stipulated gestational day (GD) of their GAs (1^{st} GA, GD 8-9; 2^{nd} GA, GD 18-19; 3^{rd} GA, GD 29-30). They were exposed to three different Doppler ultrasound exposure durations (ED) (30, 60 and 90 minutes). Acoustic output parameters such as frequency = 5.2 MHz, focal distance = 4.5 cm, Db/DR = 55/50 and mechanical index = 0.7 - 1.0 were kept constant. A total of 64 newborns (control, n = 17; 30 minutes ED, n = 17; 60 minutes ED, n = 12; 90 minutes ED, n = 18) were analysed for body weight, brain size and BMD measurements. The data were statistically analysed using Statistical Package for Social Sciences (SPSS) version 20. In the newborn's body weight, significant differences were perceived for 1^{st} GA ($p = <0.01$) and 2^{nd} GA ($p = 0.02$). No significant correlations were found for all GAs. For the newborn's brain size, significant correlation was found for 1^{st} GA (brain surface, $r = -0.44$; brain volume, $r = -0.42$) and 2^{nd} GA (brain surface, $r = -0.57$; brain volume, $r = -0.52$) only. Significant differences were also found for 1^{st} GA (brain surface, $p = <0.01$; brain volume, $p = <0.01$ and 2^{nd} GA (brain surface, $p = <0.01$; brain volume, $p = 0.01$). In terms of BMD, significant correlation was found for all GAs (1^{st}, $r = 0.47$; 2^{nd}, $r = -0.64$; 3^{rd}, $r = 0.50$) but significant differences were only found in BMD for 2^{nd} GA ($p = 0.01$), and 3^{rd} GA ($p = 0.02$). In conclusion, the results highlighted the heating effects of Doppler ultrasound on *Oryctolagus cuniculus* newborns with respect to body weight and brain size in early GAs (1^{st} & 2^{nd}), whereas BMD in later GAs (2^{nd} & 3^{rd}). The study could suggest that Doppler ultrasound ED should be limited to 30 minutes as significant results were observed in all GAs in order to reduce the potential heating effects on the newborns. The findings presented in this thesis fortified the understanding on the heating effects of Doppler ultrasound besides facilitating the establishment of guidelines on using Doppler ultrasound safely in the future.

خلاصة البحث

ABSTRACT IN ARABIC

وعلى الرغم من فائدة الموجات فوق تخطيط صدى القلب الدوبلري في استكمال الرعاية ما قبل الولادة، فقد تم الإبلاغ عن استخدامه أيضًا لزيادة درجة حرارة الأنسجة. وقد يزيد التعرض لفترات طويلة إلى الموجات فوق تخطيط صدى القلب الدوبلري من خطر الآثار الحيوية. لذلك، كان الهدف من هذه الدراسة هو التحقق من تأثير الموجات فوق تخطيط صدى القلب الدوبلري على وزن الجسم وحجم الدماغ والكثافة المعدنية للعظام لحديثي الولادة أوريكتولاغوس كونيكلس طوال ثلاثة عمر الحمل. واستخدمت الدراسة اثني عشر (12) حامل لأوريكتولاغوس كونيكلس أو الأرانب البيضاء النيوزيلندية (المجموعة الضابطة = 3 ؛ المجموعة المكشوفة = 9). ومن خلال استخدام جهاز الموجات فوق الصوتية ACUSON X150 من سيمنز، محول الصفيف الخطي 5.0) 5-VF10 إلى 10.0 MHz)، تعرضت المجموعة المكشوفة مرة واحدة إلى الموجات فوق تخطيط صدى القلب الدوبلري في يوم الحمل المنصوص عليه (GD) من GA الخاصة بهم (GA الأولى، GD 8-9 ؛ GA 2nd، GD 18-19 GA 3rd ، GD 29-30). وتعرض لثلاث فترات مختلفة من التعرض بالموجات فوق تخطيط صدى القلب الدوبلري (30) (ED و 60 و 90 دقيقة). وتم الحفاظ على معلمات الإخراج الصوتي مثل التردد = 5.2 ميجاهرتز، والمسافة البؤرية = 4.5 سم، Db = 55/50 DR / والمؤشر الميكانيكي = 0.7 - 1.0 ثابت. وتم تحليل ما مجموعه 64 مولودًا جديدًا (التحكم ، n = 17 ؛ 30 دقيقة ED، n = 17 ؛ 60 دقيقة ED، n = 12 ؛ 90 دقيقة ED، n = 18) لتحليل وزن الجسم وحجم الدماغ وقياسات الكثافة المعدنية للعظام. وتم تحليل البيانات إحصائيًا باستخدام الحزمة الإحصائية للعلوم الاجتماعية (SPSS) الإصدار 20. وفي وزن جسم المولود الجديد، تم إدراك اختلافات كبيرة لـ GA الأول ($p = <0.01$) و GA الثاني ($p = 0.02$). ولم يتم العثور على ارتباطات مهمة لجميع عمر الحمل. وبالنسبة لحجم دماغ الوليد، تم العثور على ارتباط كبير لـ GA الأول (سطح الدماغ ، $r = -0.44$ ؛ حجم الدماغ ، $r = -0.42$) و GA الثاني (سطح الدماغ، $r = -0.57$ ؛ حجم الدماغ ، $r = -0.52$) فقط. وتم العثور أيضًا على اختلافات كبيرة لـ GA الأول (سطح الدماغ ، $p = <0.01$ ؛ حجم الدماغ، $p = <0.01$ و GA الثاني (سطح الدماغ ، $p = <0.01$؛ حجم الدماغ، $p = 0.01$). ومن حيث الكثافة المعدنية للعظام (بي.ايم.دي)، تم العثور على ارتباط كبير لجميع عمر الحمل (1 ، $r = 0.47$ ؛ الثاني ، $r = -0.64$ ؛ الثالث، ص = 0.50) ولكن تم العثور على اختلافات كبيرة فقط في الكثافة المعدنية للعظام (بي.ايم.دي) (GA 2 ($p = 0.01$) ، و GA 3 ($p = 0.02$). وفي الختام، سلطت النتائج الضوء على تأثيرات التسخين بالموجات فوق تخطيط صدى القلب الدوبلري على حديثي الولادة أوريكتولاغوس كونيكلس فيما يتعلق بوزن الجسم وحجم الدماغ في جميع عمر الحمل (الأول والثاني)، في حين أن بي.ايم.دي في عمر الحمل لاحقا (الثاني والثالث). ويمكن أن تقترح الدراسة أن تخطيط صدى القلب الدوبلري (اي.دي) يجب أن يقتصر على 30 دقيقة حيث لوحظت نتائج مهمة في جميع عمر الحمل من أجل تقليل تأثيرات التسخين المحتملة على الأطفال حديثي الولادة. وعززت النتائج المقدمة في هذه الأطروحة الفهم حول تأثيرات التسخين بالموجات فوق تخطيط صدى القلب بالإضافة إلى تسهيل وضع إرشادات حول استخدام الموجات فوق تخطيط صدى القلب بسلام في المستقبل.

APPROVAL PAGE

I certify that I have supervised and read this study and that in my opinion, it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a thesis for the Master of Health Sciences (Medical Imaging)

……..

Asst. Prof. Dr. Farah Wahida Ahmad Zaiki

Supervisor

……..

Assoc. Prof. Dr. Hj. Sulaiman Md Dom

Co-Supervisor

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a thesis for the Master of Health Sciences (Medical Imaging)

……..

Assoc. Prof. Dr. Sayed Inayatullah Shah

Internal Examiner

……..

Asst. Prof. Dr. Noor Shafini Mohamad

External Examiner

This thesis was submitted to the Department of Diagnostic Imaging and Radiotherapy and is accepted as a fulfilment of the requirement for the Master of Health Sciences (Medical Imaging)

……..

Assoc. Prof. Dr. Sayed Inayatullah Shah

Head, Department of Diagnostic Imaging and Radiotherapy

This thesis was submitted to the Kulliyyah of Allied Health Sciences and is accepted as a fulfilment of the requirement for the Master of Health Sciences (Medical Imaging)

……..

Prof. Dr. Suzanah Abdul Rahman

Dean, Kulliyyah of Allied of Health Sciences

DECLARATION

I hereby declare that this thesis is the result of my own investigations, except where otherwise stated. I also declare that it has not been previously or concurrently submitted as a whole for any other degrees at IIUM or other institutions.

Umi Nadrah binti Amran

Signature..... Date.....

INTERNATIONAL ISLAMIC UNIVERSITY MALAYSIA

DECLARATION OF COPYRIGHT AND AFFIRMATION OF FAIR USE OF UNPUBLISHED RESEARCH

THERMAL EFFECTS OF PRENATAL DOPPLER ULTRASOUND ON NEWBORNS OF *ORYCTOLAGUS CUNICULUS*

I declare that the copyright holders of this thesis are jointly owned by the student and IIUM.

By signing this form, I acknowledged that I have read and understand the IIUM Intellectual Property Right and Commercialization policy.

Affirmed by Umi Nadrah binti Amran

……..…….. ……..
Signature Date

DEDICATIONS

I dedicated this thesis to myself for being brave, patience and strong enough to complete this unexpected master's journey. This is for the sacrifices, tears, blood, and sweat that accompany me all along.

ACKNOWLEDGEMENTS

Bismillahirrahmannirrahim. In the name of Allah the most Benevolent and the most Merciful. First and foremost, praise to Allah S. W. T, with His blessings, I able to finish this study after a few ups and downs throughout my master's journey. I would like to express my countless gratitude to my supervisor, Asst. Prof. Dr. Farah Wahida Ahmad Zaiki and co-supervisor, Assoc. Prof. Dr. Hj. Sulaiman Md Dom for their advice, beneficial critics, patience, and kindness through this whole time guiding me without fail.

I shall thank all the lecturers, staffs from Diagnostic Imaging and Radiotherapy Department (DDIR), as well as other lecturers and staffs from Kulliyyah Allied Health Sciences (KAHS) IIUM, Department of Orthopaedics from Jalan Hospital Campus (JHC) and Centre of Medical Imaging, Faculty of Health Sciences, UiTM Puncak Alam for their unconditional assistance and suggestions. Not forgotten to my lab partner, Nadzirah Mohamad Radzi and fellow friends who walk by my sides, together holding me up, always reminding me to stay strong and never once failed to offer help whenever I need to have one.

Lastly, my special appreciation would be to my both parents, Amran bin Zakaria and Siti Rohana binti Asmuni, my fiancé, Engku Fathul Shah Nor Haqim bin E Shahrunizad and my families for always believe and being thoughtful for every single thing I have done to complete this journey. I would not be able to put my two legs at where I stand today if there are no prayers from them. Thank you for always have faith in this one human, unwearyingly waited for her to complete her long study journey and for every penny you spent for her educational fees and well-being. May each and every single person who involves in this journey, directly or indirectly, is granted with His blessings for this life and hereafter.

Thank you.

Umi Nadrah binti Amran

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF EQUATIONS

LIST OF SYMBOLS / SI UNITS

%	Percent
”	Inch
±	Precision of an approximation
°	Degree
µm	Micrometre
μm^2	Micrometre squared
μm^2	Square micrometre
μm^3	Cube micrometre
μm^3	Micrometre cubed
µs	Microsecond
A	Amplitude
℃	Degree Celsius
c	Velocity
cm	Centimetre
d_f	Doppler shift
f	Frequency
g	Gram
$g.cm^3$	Gram per cubic centimetre
h	Hour
Hz	Hertz
kg	Kilogram
kV	Kilovoltage
M	Mean value
m/s	Metre per second

M_1	Molarity of concentrated solution
M_2	Molarity of diluted solution
Md	Median value
MHz	Megahertz
ml	Millilitre
ml/kg	Millilitre per kilogram
mm	Millimetre
ms	Millisecond
mW/cm	Milliwatt per centimetre
n	Number of samples
Na_2HPO_4	Diphasic sodium phosphate
NaCL	Sodium chloride
p	P-value
r	Pearson correlation coefficient
T	Period (time)
U	Mann-Whitney U value
V_1	Volume of concentrated solution
V_2	Volume of diluted solution
W/kg	Watt per kilogram
z	Z-value
λ	Wavelength
$\bar{x}$	Mean

LIST OF ABBREVIATIONS

2D	Two dimensional
3D	Three dimensional
3Rs	Replacement, reduction and refinement
AIUM	American Institute of Ultrasound in Medicine
ALARA	As low as reasonably achievable
ANOVA	Analysis of variance
BMD	Bone mineral density
B-mode	Brightness mode
BMUS	British Medical Ultrasound Safety
CE	Conformité Européene / European Conformity
CPU	Central Processing Unit
CT	Computed tomography
CTAn	Computed tomography analyser
ED	Exposure duration
FDA	Food and Drug Administration
GA	Gestational age
GD	Gestational day
I-ACUC	Animal Care and Use Committee
IBM	International Business Machine Corporation
IIUM	International Islamic University Malaysia
IPCC	Intergovernmental Panel on Climate Change
I_{SPTA}	Spatial-pulse-temporal-average intensities
KAHS	Kulliyyah of Allied Health Sciences
LCD	Liquid Crystal Display

MI	Mechanical index
Micro-CT	Micro-computed tomography
M-mode	Motion mode
MRI	Magnetic resonance imaging
MRM	Magnetic resonance microscopy
mRNA	Messenger-ribonucleic acid
NIR	Non-ionizing radiation
NRecon	NReconstruction software
NZWR	New Zealand white rabbit
PET	Positron emission tomography
PTA	Phosphotungstic acid
ROI	Region of interest
SAR	Specific absorption rate
SD	Standard deviation
SPECT	Single photon emission computed tomography
SPSS	Statistical Package for Social Sciences
TI	Thermal index
TPC	Tenderness, pamper and care
UiTM	Universiti Teknologi MARA

CHAPTER ONE

INTRODUCTION

1.1 BACKGROUND OF THE STUDY

Ultrasound has been long known as the safest imaging modality as it involves no ionising radiation and is used frequently in prenatal care for decades. Doppler ultrasound plays a role in the obstetrics and gynaecology field to serve as a complementary mode in a standard prenatal scan (Chau, 2002). It aids in investigating foetus blood flow in expectant mothers' wombs, which is usually for those who come with pregnancy complications (Alfirevic, Stampalija, & Dowswell, 2017; Schellpfeffer, 2013).

After the invention of ultrasound in the late 1950s, it has continued to develop throughout these decades (Chau, 2002). Today, Doppler ultrasound has been commercially applied by private companies and healthcare institution for prenatal care. Doppler ultrasound's technology advancement has improved the service of standard prenatal ultrasound scanning. The practicality of Doppler effects by the motion or direction of blood flow helps practitioners to evaluate and estimate blood circulation abnormalities of the foetus in a better view (Oglat et al., 2018).

As of current practice, only an expectant mother with high potential for complication gets the privilege of having Doppler ultrasound to check on her foetus (Hill, 2016). As reported by Hill (2016), the percentage of stillborn rate can be reduced if Doppler ultrasound is implemented as one of the standard prenatal scanning procedure. In an interview conducted, a mother who had lost her unborn child believed that her child could be saved if Doppler ultrasound has been made as a standard practice in the health institution. She said Doppler ultrasound should be done on every pregnant woman since standard prenatal ultrasound could not give any information whether the foetus had gotten enough oxygen, nutrients and blood supply from the placenta to grow healthy in the womb.

In contrary to the conventional two dimensional (2D) ultrasound, the Doppler ultrasound beam is focused at only one point. This may lead to heat accumulation in the area, thus increasing the temperature. Therefore, concerns arise regarding the safety of Doppler ultrasound mode's prolonged use on the foetus. It is proven that temperature elevations on both mother and foetus contributed to numerous adverse outcomes (Strand, Barnett, & Tong, 2011). These include low birth weight, spontaneous abortion, stillbirth and premature contraction (Goldenberg, Culhane, Iams, & Romero, 2008; Salvesen et al., 2011).

The possible harm of ultrasound including Doppler is uncertain since it is well-known to be the safest imaging modalities among others (Barnett & Maulik, 2001). Nonetheless, ultrasound still has quite a number of possibilities to cause heat (thermal) and mechanical (non-thermal) effects. The bioeffect risk increases as the technology of Doppler ultrasound advances through the decades. It has been reported that potential cavitation can happen when Doppler is used together with three dimensional (3D)

ultrasound (Pooh et al., 2016). Other literature has also stated that the Doppler's acoustic outputs are relatively sufficient to result in obvious biological effects when maximum operating settings are used (Barnett & Maulik, 2001).

1.2 STATEMENT OF THE PROBLEM

Years before the trend set, USA Today (2004) has reported that the American Institute of Ultrasound in Medicine (AIUM) notifies expecting parents about possible harms in having an unregulated ultrasound for entertainment purposes ("Parents ignoring FDA warning against prenatal portraits," 2004). Even though there are no confirmed biological effects from the regulated prenatal ultrasound, unregulated prenatal ultrasound takes longer time and uses more energy compared to regulated ones. Food and Drug Administration (FDA) has made a statement concerning the unknown long-term effects of tissue heating by frequent visits and prolonged examination time (Pawlowski, 2014; Romm, 2014). Therefore, experts have stated that ultrasound using Doppler should only be done on expectant mothers when there is a medical purpose to perform it. If it is performed on an expectant mother without any diagnostic purpose, it begs the question of whether it is safe and justified for the foetus.

In recent years, there are increasing interests in exploring the bioeffects of Doppler ultrasound. Previous studies have reported several results on Doppler ultrasound bioeffects in various animals. Jia et al. (2005) have found that the insonification fetal group has a higher significant difference in myocardial apoptosis compared to the control group. Later in 2009, the finding of Schneider-Kolsky et al. (2009) confirmed that exposure to Doppler ultrasound may result in an impairment towards a mammal's cognitive function. They found significant memory impairment

after 2 days post-hatch following the Doppler exposure to the foetal chick for several minutes on day 19 of the incubation period.

In 2011, a study on the effect of pulse Doppler examination on ductus venosus in rat foetuses showed a positive result where there was a positive linear correlation between Doppler exposure time and apoptotic activities of exposed liver tissues (Pellicer et al., 2011). Helmy, Bader, Koch, Tiringer, and Kollmann (2015) have undertaken an *in-vitro* study in measuring the thermal output of Doppler ultrasound. The energy output of the ultrasound transducer was investigated using the water bath model. In the study, they found that the activation of Doppler ultrasound in water bath increased the temperature of water in one minute. Thus, they come into a conclusion that Doppler ultrasound can induce a thermal effect to the foetuses, especially in early pregnancy.

In spite of several new findings of the bioeffects of the Doppler ultrasound, several other studies have been done to investigate the heating effects of prenatal ultrasound without using Doppler mode as well. In 2013, a study of fluctuations in haematological analysis and foetal weight was statistically found a significant difference in the newborn of *Oryctolagus cuniculus* after being exposed to prenatal ultrasound (Zaiki, Dom, Razak, & Hassan, 2013; Zaiki & Dom, 2014). Zaiki and Dom (2016) later found the heating effect during prenatal scanning does interfere with the foetal neuro-development. In 2016, Isa and Dom (2016) found that the lowermost *Oryctolagus cuniculus* growth is recorded when they are exposed to 2D ultrasound for 90 minutes long at the 2^{nd} gestational age.