THE THERMAL BIOEFFECTS OF PRENATAL ULTRASOUND ON RABBIT NEWBORNS: BODY WEIGHT ANALYSIS

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ABSTRACT

Introduction: The Doppler mode is associated with higher acoustic output which leads to a greater conversion of energy into heat. This is due to the fact that the Doppler ultrasound beam is focused and localized at a particular area of interest. The energy in an area is converted to heat which accumulates and increases the tissue temperature at the localized area. Since heat is considered as a teratogen in pregnancy, any thermal bioeffects can be fatal to fetuses. **Aim:** This study aims to investigate the thermal bioeffects of using the Doppler ultrasound on the weights of newborn rabbits. **Method:** Twelve pregnant New Zealand White rabbits were exposed once at three gestational stages using three different exposure durations. After delivery, the mean weights of the newborns were analysed using the Statistical Package for the Social Sciences (SPSS). **Results:** This study found that longer periods of fetal exposure to the Doppler ultrasound resulted in thermal bioeffects in which a decrease in newborn body weight can be seen in the early (1st and 2nd) gestational stages (GS) prolonged Doppler exposure. Longer periods of exposure to the Doppler ultrasound increased the risk of thermal bioeffects. **Conclusion:** It is recommended that health practitioners limit fetal exposure to Doppler ultrasound to minimise the potential bioeffect risks.

KEYWORDS: *bioeffects, Doppler, prenatal, rabbit, hyperthermia, ultrasound*

INTRODUCTION

A key aspect of the Doppler ultrasound is to serve as a complimentary method for monitoring and detecting the development of a fetus in the obstetrics field (Chau, 2002). Despite its obvious usefulness, specifically in the study of fetal blood flow (Alfirevic, Stampalija, & Dowswell, 2017), the higher thermal index associated with the Doppler ultrasound cannot be ignored. In recent years, there has been an increasing interest in the bioeffects of the Doppler ultrasound as reflected by the vast number of research studies done on the subject (Pekkafalı & Kara, 2015; Pooh et al., 2016; Sheiner & Abramowicz, 2012).

Previous studies have reported that the biological effects are significant once it reaches its threshold (Barnett & Maulik, 2001; D. L. Miller, 2008; Pooh et al., 2016). They also have highlighted that significant bioeffects can be seen when the maximum operating setting is used during scanning. The bioeffect of the Doppler ultrasound is primarily the heat produced by the intense exposure to the area at where the ultrasound beam travels (Bushong, 1993; Kremkau, 2006).

However, previous investigation has concluded that the Doppler ultrasound gives neither harm nor benefit to fetuses(Alfirevic, Stampalija, & Medley, 2015; Bricker & Neilson, 2000).A 20 year follow-up on human trials after multiple prenatal ultrasound scan had shown that the frequent used of spectral Doppler has no significance influence on ocular development (Forward et al., 2014). Albeit with the presence of a quite number of researches on the pro and cons of ultrasound, the heating thermal effects of Doppler has yet to be extensively investigated. The available literature on animal studies conducted to assess the Doppler ultrasound bioeffects has provided several remarkable contributions to fuel further investigation.

To date, several animal cell studies have been conducted to verify the hypothetical assumptions made by researchers around the globe. One of the bioeffects of Doppler ultrasound was discovered in an analysis on the effects of the Doppler ultrasound on the myocardial cell apoptosis of fetal rats. The researchers in that study had found a highly significant difference in the myocardial cell apoptosis of the fetal exposed group compared to the unexposed group. A higher significant difference was also found for the fetal exposed group compared to the control group (Jia et al., 2005). Later, a study on the effect of pulsed Doppler ultrasound on the ductus venosus in rat fetuses showed a linear relationship between the exposure index and the apoptotic activities of exposed liver tissues (Pellicer et al., 2011).

Therefore, this present study attempted to identify the thermal bioeffects of the Doppler ultrasound on newborn rabbits' body weight. The main aim of this study is to determine the differences between newborn rabbits' body weight according to different Doppler ultrasound exposure durations throughout several gestational stages. This study offers some insight into the lack of evidence on the thermal bioeffects of the Doppler ultrasound and provides some guidance for further research.

METHODS

Twelve pregnant New Zealand White Rabbits were used in this study. Ethical clearance was obtained from the International Islamic University Malaysia Animal Care and Use Committee (I-ACUC) dated 21st April 2017. The rabbits were divided equally depending on their gestational stages (GS). Gestational day (GD) 8-9 served as the 1st GS, while GD 18-19 served as the 2nd GS and GD 29-30 served as the 3rd GS. The range of GDs was designated by dividing the gestational length of a rabbit's pregnancy into three as identified by Palmer (1968) in order to mimic the human pregnancy trimesters.

The subjects were exposed once to 3 different exposure durations in each GS according to the GDs described above. The choosing of three different exposure durations of 30, 60, and 90 minutes chosen was adopted and adapted from previous studies done by Zaiki and Dom(2014). The rabbits which received exposure were grouped into the exposed group, while the subjects that did not receive any exposure served as the controls.

Prior to the exposure, the subjects' abdominal fur was shaved using a commercialized electric shaver, Pritech Rechargeable Hair Trimmer (Model No.: PR-1040) and cleaned off. This process was done to ensure close contact between the transducer (ultrasound probe) and the abdominal area during scanning. No euthanasia was given to the subjects during both the shaving and scanning processes. Instead, a manual restraining method together with gentle touch, pampering and tender care were adopted throughout the study.

A Siemens model Acuson X250 ultrasound machine was used together with a linear array transducer VF 10-5 with transmitting frequency of 5-10 MHz for scanning. The focal distance was set at a constant of 4.5 cm, while frequency was set at 5.2 MHz and the mechanical index was set at 0.7-1.0. To rule out the possibility of pseudopregnancy, the brightness mode (B-mode) was used to confirm the existence of fetus in the rabbits' womb. After confirmation, the transducer was placed at the lower middle of the abdomen in a static manner throughout the exposure duration. Time was measured using a stopwatch application once the Doppler mode was activated.

A total of 64 newborns (control = 17, 30 minutes = 17, 60 minutes = 12, 90 minutes = 18) were taken and weighed using a Mini Portable Electronic Kitchen Scale (Model: YYC VOYAGE-Electronic Kitchen Scale) right after delivery.

The data were then tabulated and analyzed using the Statistical Package for the Social Sciences (SPSS) version 20, International Business Machines Corporation (IBM), N. Y., USA. Significant differences between the newborns' weights throughout the GS were statistically analyzed.

RESULTS

Statistical analysis was done to compare the effects of different exposure durations on the newborns' body weight throughout the GS. Prior to the data analysis, all variables were subjected to a normality test and the result suggested a normal data distribution of newborn body weight for all groups.

A one-way ANOVA was conducted to compare the effect of different exposure durations on the newborn body weight at different GS. Post hoc test using Tukey HSD test procedure was later conducted to compare every means of the research variables that showed significant results. The reference values for all the statistical test in this study was the control group, thus, the exposed groups were compared to the reference values to determine significant differences between them. Table 1 summarizes the results from the ANOVA, meanwhile, Table 2 summarizes the post-hoc test respectively.

For 1st GS, there was statistically significant difference at the $p<0.05$ level for the four exposure duration groups (control, n = 5; 30 minutes exposure, n = 7; 60 minutes exposure, n = 5; 90 minutes exposure, n = 6); Welch $_{(3,\ 22)}$ = 37.39, p = <0.01. Despite reaching statistical significance, the actual difference in mean scores between the groups was intermediate. The effect size, calculated using ETA squared, was 0.61. Post-hoc comparisons using the Turkey HSD test indicated that there was no significant result found at all gestational ages when compared to the reference values (control group).

For the 2nd GS, there was a statistically significant difference at the $p<0.05$ level for the four exposure duration groups (control, n = 6; 30 minutes exposure, n = 4; 60 minutes exposure, n = 5; 90 minutes exposure, n = 6); F $_{(3,\ 20)}$ = 4.46, p = 0.02. Despite reaching statistical significance, the actual difference in mean scores between the groups was small. The effect size, calculated using ETA squared, was 0.44. Post-hoc comparisons using the Turkey HSD test that there was no significant result found at all gestational ages when compared to the reference values (control group).

For the 3rd GS, there was no statistically significant difference at the $p<0.05$ level for the four exposure duration groups (control, n = 6; 30 minutes exposure, n = 6; 60 minutes exposure, n = 2; 90 minutes exposure, n = 6); $F_{(3, 19)} = 1.04$, $p = 0.40$. The effect size, calculated using ETA squared, was 0.16. This showed that the actual difference in mean scores between the groups is small. There was no statistical difference found between the groups, thus, no post-hoc test was carried out.

In summary, statistically significant results were shown in the 1st and 2nd GSs. Since no significant differences were found between the control and the exposed groups, therefore, the exposure duration that showed the highest decrement of newborn body weight could not be determined.

Table 1 summary of ANOVA of newborn weight

GS	Exposure duration	n	Levene's statistic (p-value)	Mean ± SD	ANOVA / Welch, (p-value)	ETA squared
1st	Control	5	0.03	43.60 ± 6.58	<0.01*	0.61
	30 minutes	7		34.00 ± 14.00		
	60 minutes	5		56.00 ± 3.39		
	90 minutes	6		29.00 ± 4.69		
2nd	Control	6	0.40**	40.50 ± 4.42	0.02*	0.44
	30 minutes	4		49.75 ± 7.23		
	60 minutes	5		38.20 ± 3.11		
	90 minutes	6		40.33 ± 5.28		
3rd	Control	6	0.31**	41.17 ± 3.54	0.40	0.16
	30 minutes	6		44.83 ± 3.54		
	60 minutes	2		40.50 ± 6.36		
	90 minutes	6		45.00 ± 6.57		

Note: (**) indicates p-value more than 0.05 for Levene's test indicates no violation of the assumption of homogeneity of variance. (*) indicates significant difference is assumed when $p<0.05$.

Table 2 Post-hoc test results of newborn body weight

GS	Exposure duration (I)	Exposure duration (J)	Mean Difference (I-J)	p-value
1st	Control	30 minutes	9.60	0.29
		60 minutes	-12.40	0.16
		90 minutes	14.60	0.06
2nd	Control	30 minutes	-9.25	0.05
		60 minutes	2.30	0.87
		90 minutes	0.17	1.00

DISCUSSION

These results partially match research that has been claimed in the earlier study. Zaiki and Dom (2014) have shown that fetal weight was statistically significant at 30, 60 and 90 minutes exposure duration groups at each GS (1st, 2nd, and 3rd). However, they studied on fetal weight, not the newborn weight as in this present study and the conventional 2D ultrasound was used in their study. It was expected that the effect of longer exposure duration to Doppler ultrasound could be worse at birth for this study since due to higher energy involved. However, the present study showed no evidence to support this hypothesis.

Even though the results were contrary to expectations, the decrement in newborn's weight might be due to the result of prolonged exposure to Doppler ultrasound. Bushong (1993) holds the view that the thermal effect occurs when the heat produced by intense ultrasound exposure can lead to undesirable effects and any risks may increase as ultrasound technology continues to advance. The previous study also has highlighted the differences between Doppler and other ultrasound modes, in which pulsed-wave Doppler ultrasound has the highest thermal index (TI) (Sheiner et al., 2007).

Other than that, since the ultrasound probe was kept stationary during the Doppler exposure, it eventually can alter the fetal exposure (M. W. Miller et al., 2002). The static maneuver during Doppler scanning can result in accumulation of heat at only one particular area which would increase the temperature at that region. Theoretically, as the ultrasound waves propagate through tissues, they attenuate and cause the temperature to rise (Kremkau, 2006). The thermal effects due to the increase in temperature, or also known as hyperthermia is widely considered teratogenic during pregnancy. In the hyperthermia analysis done in 2003, irreversible damages to the fetus are observed which include abortion, retardation of growth, developmental defects and also embryonic death (Edwards, Saunders, & Shiota, 2003).

Another evidence that could be served in this current study finding is the sensitivity and vulnerability of fetus on the exposure to the Doppler ultrasound. This explains why a significant reduction in newborn body weight at 1st and 2nd GS but the newborn body weight increased in the 3rd GS. Dziuk (1992) who claims that animals' embryos and fetuses also develop in an orderly manner in the same way as humans, implies that the effects of Doppler exposure to animals could be the same to human as well. He explains that environmental factors such as high temperatures can influence the rate of growth of the animal fetus, where the shunting of blood away from the uterus to the periphery in order to maintain its body temperature would cause a reduction in nutrient supply to the fetus in the womb.

The statement by Dziuk (1992) above is then supported years later byZiskin and Morrissey(2011) which likewise, stated that the human embryo and fetus are vulnerable at different rates depending on their development stages. They have also concluded that Doppler ultrasound devices are capable in raising the fetal temperature up to several degrees.

It seems possible that these results may be explained by another fact that a doe can carry a different number of litters in one pregnancy. The body weight of the newborns could be different from one doe to another doe depending on the number of litters occupied in the doe's womb. The doe with a smaller number of litters might have weightier newborns than the doe with a larger number of litters in the womb. Dziuk (1992) again supports the above statement as he has ruled out that the number of litter in uterine space may be one of the contributing factors for the newborn's body weight.

CONCLUSION

In conclusion, these findings suggest that the bioeffects of the Doppler ultrasound exposure could be seen in both earlier GS (1st and 2nd). Even though there is no evidence of potential Doppler heating effect during the 3rd GS, it is recommended for practitioners to limit fetal Doppler exposure and adhere to the concept of 'as low as reasonably achievable' (ALARA).

Whilst this study has yet to find evidence on humans, it does give some insight that the bioeffects of the Doppler ultrasound should not be ignored. Hopefully, this study opens doors for further research either on animals or human studies.

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