ORIGINAL ARTICLE

Correlation Between Prenatal Doppler Ultrasound Exposure Durations and Newborn Rabbit's Body Weight

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ABSTRACT

Introduction: Doppler mode ultrasound is widely used in prenatal scanning and known to produce a higher acoustic output which later leads to higher heat energy conversion compared to other ultrasound modes. It has been reported that the use of Doppler imaging might increase the temperature of tissues, thus, when Doppler is used in combination with 2D ultrasound, the risks of bioeffects tend to increase more. It is also known that prolonged exposure to ultrasound during pregnancy can cause irreversible biological destructions to the fetus. Despite the benefits of using Doppler ultrasound, its potential adverse effects have received scant attention in the research literature. Therefore, this study aimed to examine a correlation between gestational stages (GS) and newborn rabbit's body weight at different prenatal Doppler ultrasound exposure durations. **Methods:** Twelve pregnant New Zealand white rabbits (NZWR) were exposed once using three different Doppler ultrasound exposure durations (30, 60, 90 minutes exposure) at three different GSs (1st, 2nd, and 3rd GS). After delivery, the mean weights of the 62 newborns were statistically analysed. **Results:** Strong negative and positive correlation between newborn's body weight at different GSs and Doppler ultrasound exposure durations with a significant result found in 60 minutes exposure ($p = <0.01$) and 90 minutes exposure ($p = <0.01$), respectively. **Conclusion:** It can be concluded that longer Doppler ultrasound exposure may lead to significant results onto the newborn rabbits' body weight.

Keywords: Bioeffects, Doppler ultrasound, Exposure duration, Rabbit, Weight

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INTRODUCTION

The role of ultrasound has received increased attention across number of disciplines in recent years as it is known for its non-ionising properties. Even though with the presence of quite a number of research on the pros and cons of ultrasound, there are still plenty of room for the discussion on the unidentified risks using ultrasound, especially in Doppler ultrasound. The heating effect of ultrasound is highly debated as it is proven that the elevation of both the mother and fetus temperature can result in irrevesible biological damages (1). The damages may include low birth weight, spontaneous abortion, stillbirth and premature contraction (2) that can occur anytime throughout the trimesters (3). A considerable amount of studies have already been done to explore the heating effect of Doppler ultrasound (4–6).

There is a growing body of literature that recognises the importance of Doppler ultrasound in monitoring and perceiving the development of fetuses in the obstetrics and gynaecology field (7). It aids in prenatal care as the Doppler ultrasound helps in the study of blood flow in the womb (8). Generally, ultrasound provides diagnostic images without ionizing radiation (9), however, the possible of its adverse effects cannot be foreseen. Food and Drug Administration (FDA) has made a general statement concerning the unknown long term effects of tissue heating by the frequent ultrasound visits and prolonged examination time (10). Even though there are no confirmed biological effects of Doppler ultrasound on human fetus, American Institute of Ultrasound in Medicine (AIUM) has stated that elevated fetal temperature by 4°C above normal for 5 minutes or more can potentially induced severe developmental defects (11). AIUM also has also made an official statement where prolonged and inappropriate use of Doppler ultrasound could give rise to ultrasound bioeffects, despite of its benefits (12).

It has been established that the maximum limits of spatial peak temporal average intensity (ISPTA) for fetal application is 720mW/cm^2 (13) and the ultrasound is safe when regulated at TI 1.0 (14). Previous studies have reported that the biological effects are significant once they reach or exceed the threshold or maximum limits as recommended (4,15,16). It is well known that the mechanism of ultrasound interaction is complex (17) since it involves three different modes of interaction;

thermal or heating, non-thermal or cavitation and direct effects (18). Apart from non-thermal and direct effect modes of interaction, the bioeffects of Doppler ultrasound are primarily concerning on the heat produced by the intensity of its exposure at where it travels (19,20).

It has been conclusively shown that there is no adverse ultrasound effect for fetal exposure using low frequency range for a temperature increase above the normal physiologic temperature ΔT, when $\Delta T < 4.5 - (\log_{10} t)/0.6$, where T is the exposure time in between 1 to 250 minutes, including off time for pulsed exposure (21). However, recent evidence has showed that the thermal index (TI) levels for Doppler mode could reach one point five (1.5) and higher as compared to B-mode ultrasound procedure (22). Supporting this statement, AIUM has also identified that the temperature upraises increasingly greater from B-mode to spectral Doppler mode (11). Therefore, to reduce the potential risk for ultrasound bioeffects, AIUM has come out with recommended scanning time for obstetric. The recommended time for thermal index (TI) 1.0 is less than 60 minutes while TI more than 1.0 must not exceed 30 minutes maximum (23). Therefore, this study aimed to look for these two critical exposure durations limit.

So far, some researchers seem to conclude that the Doppler ultrasound gives neither harm nor benefit to the human fetus, however, there has been little agreement about that. International Society of Ultrasound in Obstetrics and Gynecology (ISUOG) has recommended that the TI displayed should be less than 1.0 and the exposure duration to be kept as short as possible, not exceeding 60 minutes (24). There are also some contradictions in the results that can be seen in animal studies done by plenty of researchers as well. Many of the available literature on animal studies conducted on assessing Doppler ultrasound bioeffects have made several remarkable contributions toward further investigation (13,25–28).

In 2009, an experiment which has been done concluded that a prolonged prenatal exposure to Doppler ultrasound did affect the chick's cognitive function (28). They have found significant memory impairment on the chicks exposed to Doppler ultrasound after two days post-hatched. Later in 2011, another thorough study has proved that there is a linear correlation between the exposure index and the apoptotic activities of exposed liver tissues. In the same vein, there are several other studies that successfully drew our attention to the potential bioeffects of ultrasound (27). In 2013, a study has found that fluctuations in haematological analysis and fetal weight were significant after being exposed to the conventional two-dimensional (2D) ultrasound mode (29,30).

Therefore, this present study attempted to discover the heating effects of Doppler ultrasound on newborn rabbit's body weight. The aim of this study is to investigate the correlation between Doppler exposure durations at different GSs and newborn's body weight. Concomitantly, this will as well lead to fill the gaps in both previous and future literatures on the heating effect of Doppler ultrasound.

MATERIALS AND METHODS

Method of this present study was adopted and adapted from previous studies (29,31). Twelve pregnant New Zealand white rabbits (NZWR) were used as subjects and the animal ethical clearance was obtained prior to the study from the International Islamic University Malaysia Animal Care and Use Committee (I-ACUC) dated 21st April 2017. The NZWR was chosen since it possesses many benefits in teratology studies such as domestically available, high pregnancy rate and sensitive to teratogens (32). Other than that, some similarities of features between human and NZWR such as development of motor function, prolonged myelination maturity (33) and high degree of maternal-fetal bloodstream interaction (34) have been shown by previous studies.

The criteria for selecting the subjects were taken from general data by Quesenberry and Carpenter (2003). Thus, subjects aged between five to seven months and weighed approximately 2 to 6 kilograms were chosen for this study. The subjects were kept in 23.5 inch (length) x 19.5 inch (height) x 16.5 inch (width) iron steel cages in an animal house equipped with air-conditioner and wall-mounted exhaust fan to maintain the surrounding temperature (24°C to 27°C), ventilation and humidity at constant. They were given tap water and pellets ad libitum and timothy hay once a week. The animal house was cleaned weekly and the waste trays were cleaned every two days in order to keep the odours and bacterial infections at minimum. Twelve hours light and 12 hours dark photoperiodic illumination using ceiling-mounted fluorescent lights were created for the nocturnal nature of the subject as adapted from Dom and Zaiki (2018).

The subjects were divided equally into three main groups according to the designated gestational stages (GS); first (1st) GS, second (2nd) GS and third (3rd) GS. Each GS was then further divided into sub-group; control group and exposed group. The subjects in control group were not given any Doppler ultrasound exposure. Meanwhile, the subjects in exposed group were exposed to three different Doppler ultrasound exposure durations respectively; 30, 60 and 90 minutes.

The subjects were exposed to the Doppler ultrasound once at days eight to nine (8-9) (1st GS), eighteen to nineteen (18-19) (2nd GS) and twenty-nine to thirty (29-30) (3rd GS). The subject has been identified to have 32 days of gestational period (32) which can also vary between 30 to 33 days (Dom, 2011; Sirois, 2005). By adapting to the characteristic, the days of exposure given were selected after dividing the total number of the rabbit's gestational days into three equal lengths to reminiscence the human pregnancy trimesters.

The choice of three different exposure durations were determined according to the maximum time limit suggested by British Medical Ultrasound Society (BMUS) (Dom & Zaiki, 2018). BMUS has suggested the exposure with TI less than 1.0 should not exceed 60 minutes (39). Then, 50 percent (%) increment and decrement from the maximum exposure duration suggested were calculated to design the less and exceed exposure durations respectively at TI 1.0. Another baseline that was also referred by this current study is the recommendation by American Institute of Ultrasound in Medicine (2016), stated that the recommended time for TI 1.0 is less than 60 minutes while for TI more than 1.0 must not exceed 30 minutes maximum (23).

Prior to the exposure, all subjects in exposed groups were shaved clean at the abdominal area using a commercialised electric shaver, PRITECH Rechargeable Hair Trimmer (Model No.: PR-1040). This process was crucial to ensure that the ultrasound probe was in close contact to the skin during scanning to eliminate air layer in between them and facilitate the sound passage into and out of the body. During the shaving and scanning processes, the manual restraining method of tenderness, pamper and care (TPC) to calm the subjects was adapted throughout the study. Sedation was omitted during all the processes. There was no procedure done on the control subjects except maintaining their food intakes and husbandry until the litters were delivered.

Following the preparation, Siemens model Acuson X250 ultrasound machine was used together with a linear array ultrasound probe VF 10-5 with a transmitting frequency of 5-10 MHz for scanning. The acoustic output parameters were set at constant; focal distance = 4.5 cm, frequency = 5.2 MHz, thermal index = 0.9 and mechanical index = 0.7-1.0. Then, the brightness mode (B-mode) was used to rule out the possibility of pseudopregnancy and to confirm the presence of the fetuses. Once confirmed, the probe was placed in stationary at the lower middle of the abdomen during the exposure durations. The time was counted down using a mobile phone's stopwatch application once the Doppler mode was activated.

After the completion of exposure, all the subjects were then allowed for a normal delivery. A total of 62 newborns (control; n= 18, 30 minutes exposure; n= 15, 60 minutes exposure; n = 14, 90 minutes exposure; n= 15) were taken and weighed three times using a Mini Portable Electronic Kitchen Scale (Model: YYC VOYAGE-Electronic Kitchen Scale) right after delivery. However, deceased litters were excluded from the data. All the data were recorded and the average mean for each group were calculated. Statistical Package for the Social Sciences (SPSS) version 20, International Business Machines Corporation (IBM), N. Y., USA. was used to find the correlations between the exposure durations and the newborns' body weight throughout the GSs were statistically analysed.

RESULTS

Bivariate Pearson product moment correlation was used to identify the strength and direction of the relationship between different GS and newborn rabbit's weight at different exposure durations. Preliminary analyses were performed to exclude any violation of linearity and normality assumption and the results showed all variables were linearly and normally distributed. Table I show the results of the Pearson correlation.

The results of correlation were reported individually according to the exposure time given as follows. For the control group, there was a weak negative correlation between two variables; $r = -0.20$, $n = 17$, $p = 0.44$ with a high level of newborn body weight associated with lower level of GS. There was no significant statistical difference as $p > 0.05$.

For the 30 minutes- exposure duration group, there was a moderate positive correlation between two variables; $r = 0.44$, $n = 17$, $p = 0.08$ with a high level of newborn body weight associated with higher level of GS. There was no significant statistical difference as $p > 0.05$.

For the 60 minutes-exposure duration group, there was a strong negative correlation between two variables; $r = -0.78$, $n = 12$, $p = <0.01$ with a high level of newborn body weight associated with lower level of GS. There was a significant statistical difference as $p < 0.05$. This indicated the weightier newborns' body weight at the lower gestational stages.

For the 90 minutes-exposure duration group, there was a strong positive correlation between two variables; $r = 0.78$, $n = 18$, $p = <0.01$ with a high level of newborn body weight associated with higher level of GS. There was a significant statistical difference as $p < 0.05$. This

Table I: Bivariate Pearson product moment correlation results

Exposure duration	Weight, n (mean ± SD)	Pearson Correlation, r	Sig.(2-tailed) value, p	Coefficient Determination, r^2 (%)
Control	n = 17 (41.65 ± 4.76)	-0.20	0.44	4.04
30 minutes	n = 17 (41.53 ± 11.53)	0.44	0.08	19.10
60 minutes	n = 12 (46.00 ± 9.49)	-0.78	<0.01*	60.06
90 minutes	n = 18 (38.11 ± 8.67)	0.78	<0.01*	60.06

*a significant result at level p<0.05, SD = standard deviation indicated the weightier newborns' body weight at the higher gestational stages.

In summary, strong correlations between newborn body weight and GS can be seen when longer exposure durations were given (60 and 90 minutes exposure durations). The data also showed significant differences in higher exposure durations groups with p-value of less than 0.05.

DISCUSSION

The present study was designed to determine the correlation between different GS and the newborn rabbits' weights at different exposure durations. The current study found that despite the direction of the correlation, there was generally moderate to strong correlations between the two variables being analysed.

In this study, exposure to Doppler ultrasound were found to have some correlation between the variables at 30 minutes, 60 minutes and 90 minutes exposure. Significant results were found in 60 minutes and 90 minutes exposure groups. These results could suggest that newborn's weight could be affected by the prolonged exposure to the Doppler mode at 60 minutes at any GS. It is also possible to consider that continuous 30 minutes exposure to Doppler ultrasound as a threshold level before any significant bioeffects arise.

These results matched those claimed in earlier study. Zaiki and Dom (2014) has showed that fetal weight was statistically significant at 30, 60 and 90 minutes exposure duration groups at each gestational stages (1st, 2nd, and 3rd). However, that study was done on fetal weight, not the newborn weight as in this present study and they were using conventional 2D ultrasound. Nevertheless, this current study believed that, if the effect could occur on fetal weight due to ultrasound exposure, the effect might also be expressed at birth. Perhaps, the effect could be worse since the Doppler mode was used due to higher energy involved.

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 on where the waves travel to and the undesirable effect risk increases as the ultrasound technology advances. Heat effect 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 (40).

Another evidence that could serve the current study findings is that the sensitivity and vulnerability of different GS on the exposure to the Doppler ultrasound. Human embryology is divided into three stages where period of embryological development starts to occur during 1st trimester, followed by development of body system in 2nd and 3rd trimesters (41). According to Edwards et al. (2003), the incidence rate for ultrasound thermal effects to happen are most probably related to the stage of gestation. This could explain the reduction of newborns' weight in 60 minutes exposure duration group throughout the GS with a statistically significant correlation.

It seems possible that these result 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 supported 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 weight. This could explain why newborn body weight significantly differs during 60 minutes exposure as compared to control.

Even though the understanding on the biological effects of Doppler ultrasound exposure to humans was very little, a review done in 2017 able to show the possible effects of the Doppler ultrasound used in high-risk pregnancies (8). Previous study also highlighted the differences between Doppler and other ultrasound modes, in which pulsed-wave Doppler ultrasound has the highest thermal index (TI) (22). Other than that, since the ultrasound probe was kept stationary during the Doppler exposure, it eventually altered the fetal exposure (43).

However, the findings in this study were subjected to several limitations. First, the current study had only examined a small number of animal subjects; thus, the findings might not be transferable into any other larger scale animal practice. Secondly, other influencing factors that could contribute to the reduction in newborn body weight should be taken into consideration. The influencing factors might include the pregnant rabbit's husbandry including dietary nutrition, temperature and housing, the number of fetuses, the number of fetuses occupied in each uterine horn, and the psychological behaviour (44). However, the approaches to keep the influencing factors at minimum have been described in the method section.

CONCLUSIONS

In conclusion, prolonged exposure duration to Doppler ultrasound has shown significant results on newborn's body weight. These findings could indicate that the usage of the Doppler ultrasound during prenatal scanning should be limited to 30 minutes maximum throughout all GSs to reduce the potential bioeffect risks of the Doppler ultrasound. Since the application of 'as low as reasonably achievable' (ALARA) can be found in radiology with respect to reducing radiation risk, this concept also can be directly adopted and adapted towards reducing heat effects in ultrasound. Therefore, it is suggested for practitioners or experts to limit fetal exposure to Doppler ultrasound and follow the ALARA concept in the animal setting.

Notwithstanding those limitations discussed above, this present study was able to provide additional evidence with respect to the bioeffects of Doppler ultrasound in animal studies. It is recommended that further research on humans is undertaken in the following matters so that a greater degree of accuracy and a better understanding related to the human setting can be established.

Although this study has yet to find proof on humans, it did partially give an understanding that the bioeffects of the Doppler ultrasound should not be eradicated in higher or developed subjects. Eventually, this matter opens plenty of rooms for future research; either through animals or humans, in-vivo studies as well as in-vitro studies.

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