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Prenatal Ultrasound Heating Influences on Fetal Weight Assessment of Oryctolagus cuniculus Throughout Pregnancy

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Abstract. The aim of this *in vivo* experimental study is to determine the association between ultrasound exposure time and *Oryctolagus cuniculus's* fetal weight. A total of 14 pregnant does were exposed to ultrasound heating for 30, 60, and 90 minutes at each gestational stage. Meanwhile, another group of 4 pregnant does served as control, making it a total of 136 fetuses' weight that were analyzed. There were significant differences in fetal weight during 1st, 2nd and 3rd trimester of pregnancy between groups with different length of ultrasound exposure time. There were also fair correlation and association between groups with different length of ultrasound exposure time and fetal weight during 1st and 3rd trimester of pregnancy. This study suggested that the heating effects of ultrasound exposure might affect the *Oryctolagus cuniculus's* fetal weight. Therefore, there should be a rising concern in the awareness towards the negligible effects produced from prenatal ultrasound exposure as it is widely applied during pregnancy in humans.

Keywords: Fetal weight, prenatal, ultrasound heating.

1 Introduction

Ultrasound is widely accepted in clinical practice, where it has been found to reduce perinatal mortality due to its ability in early detection of fetal malformations [1]. Prenatal ultrasound has four basic usage, namely; assessment of normality or abnormality of the 1st trimester, assessment of gestational age approximately implemented below 22 weeks of gestation which is around 2nd trimester of pregnancy and fetal number such as in multiple pregnancy, assessment of structural abnormalities and assessment of growth and fetal well-being throughout pregnancy [2]. Fetal abnormalities are usually detected earlier in pregnancy while fetal growth become a major factor in fetal size estimation later in pregnancy [2, 3]. Regarding this issue, the official statements made by the American Institute of Ultrasound in Medicine (AIUM), the institute advocates the prudent use of prenatal ultrasound and it should be strongly restricted for nonmedical usage as such for entertainment purposes including fetal viewing, obtaining fetal images or even fetal gender screening without clinical indications [4].

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As an ultrasound wave propagates through the human body, it interacts with the human anatomy, therefore creating diagnostic images. However, throughout the process, it is being attenuated, reflected, scattered and also absorbed by human body mainly as heat. A large body of literature have investigated and reviewed the embryos of many mammals and found it to be susceptible to heat damage. Embryonic development consists of highly ordered sequences of cell proliferation, cell differentiation, cell migration and apoptosis (programmed cell death). If the process are interfered by the presence of heat, they are prone to incidence of cranio-facial defects and microencephaly in animals [5]. It was also reported that the embryo in 1st trimester consists of actively dividing tissue and is more susceptible than mature tissue to damages caused by any external agent [6, 7] including physical injuries by ultrasound heating [8].Their sensitivity are somehow dependent on the stages of gestation [5]. In fact, hyperthermia has been recognized as a teratogen in animals in numerous studies, therefore, it is logical to suspect that the same teratogenic effect could occur in humans as well [9]. As the safety issues of ultrasound are still debatable, hence, this study is designed to assess whether there is an association or relation between ultrasound exposure time and *Oryctolagus cuniculus's* fetus weight during 1st, 2nd and 3rd trimester of pregnancy.

2 Methods

This *in-vivo* experimental study involved the use of 18 female New Zealand White rabbits (*Oryctolagus cuniculus*). 4 does (female rabbit) served as controlled group while the rest were categorized as treated group. The animal ethical approval was obtained from Committee on Animal Research and Ethics (UiTM CARE). The courtship and mating sessions of does were arranged systematically. The does' egg will be fertilized about an hour and a half after release of ovocytes. Ovulation is normally stimulated by coitus and normally occurs 10-12 hours after mating [10]. Due to these characteristics of accurately timed ovulation and fertilization of the *Oryctolagus cuniculus*, the first day of conception and the middle of each trimester can be correctly determined.

Ultrasound exposures of 30 minutes, 60 minutes and 90 minutes were used as a teratogen, and were given prenatally to pregnant does at the middle of each gestational stage. The exposures were carried out during gestational day (GD) 6-7, GD 17-18 and GD 28-29 for 1st, 2nd and 3rd trimester of pregnancy respectively. Gestational period varies between 31-33 days [11]. For control group, no ultrasound exposure was given. This study used Philips HD3 ultrasound machine from Koninklijke Philips N.V, Netherlands 2D operating at B-mode pulsed-ultrasound. With an exception of the lengths of exposure time (eg: 30, 60, 90 minutes), ultrasound parameters such as transducer frequency, focal distance, thermal index (TI) and mechanical index (MI) were kept constant. Transducer of linear array, L 5-9 with the ability to transmit frequency between 5 MHz until 9 MHz was used. Focal distance was kept constant at 4.5 cm throughout the experiments. The TI and MI displayed by the output display throughout the experiment showed the value of 0.2 and 1.0 respectively. The output power and spatial peak temporal average intensity (I_{SPTA}) of

ultrasound varies from 0.4 W to 0.7 W and 0.13 to 0.19 W/cm^2 respectively when measured in water for the durations of 30, 60 and 90 minutes of ultrasound exposures. All pregnant does were sacrificed after being euthanized using Pentobarbital sodium, Doléthal 250ml from Ethical Agents Ltd. NZ, 1ml /kg body weight by intravenous injection or 0.75 ml/kg intra-cardiac injection. Fetuses were taken out during laparotomy. A total of 136 fetuses (1st trimester, n=34; 2nd trimester, n=28; 3rd trimester, n=74) were analyzed for fetal body weight using A & D Ek610i digital scale weighing balance from A & D Corporation, USA. An average of 3 readings were recorded. Statistical Package for the Social Sciences, SPSS version 21.0 was used to analyze the data.

3 Results

Table 1 showed the fetal weight characteristics of 136 fetuses throughout the gestational stages. The calculated mean showed the declined of fetal weight at 1st, 2nd and 3rd trimester of pregnancy as the durations of ultrasound exposure was increased. The mean fetal weight at 1st, 2nd and 3rd trimester of pregnancy were documented lowest in 90 minutes ultrasound exposure group. Statistical tests of fetal weight in each gestation between different duration of ultrasound exposure were shown in table 2. P-values are 0.00 ($p<0.05$) in 1st, 2nd and 3rd trimester of pregnancy. 95 % confident interval does not cross 0. The results suggest that there are significant differences in fetal weight at 1st, 2nd and 3rd trimester of pregnancy in different duration of ultrasound exposure. Further analysis of results also shows negative correlation between different durations of ultrasound exposure and fetal weight at 1st and 3rd trimester of pregnancy ($p<0.05$ and $r = -0.40$, $p<0.05$ and $r = -0.23$ respectively). Therefore, longer ultrasound exposure time is associated with lower fetal weight. There is also significant linear relationship between the durations of ultrasound exposure and fetus weight at 1st and 3rd trimester of pregnancy ($p<0.05$). Regression coefficient, r^2, were 0.15 and 0.04 respectively, indicating poor relationship between the durations of ultrasound exposure and fetus weight. Across the durations of ultrasound exposure time, there are variations of 15% and 4 % in fetus weight at 1st and 3rd trimester of pregnancy respectively. However, there is no significant correlation and relationship between the duration of ultrasound exposure and fetus weight at 2nd trimester of pregnancy ($p>0.05$, $r = 0.10$, $r^2 = 0.00$).

Table 1. Fetal weight characteristics throughout the gestational stages

Gestational stage	Group	n	Mean fetal weight (g)	SD (±)
1st trimester	No exposure	9	0.30	0.05
	30 min exposure	6	0.33	0.05
	60 min exposure	10	0.33	0.05
	90 min exposure	9	0.21	0.03

2nd trimester	No exposure	6	2.55	0.05
	30 min exposure	10	2.62	0.18
	60 min exposure	5	2.84	0.05
	90 min exposure	7	2.53	0.11
3rd trimester	No exposure	16	52.67	7.60
	30 min exposure	19	51.93	11.06
	60 min exposure	16	56.07	5.43
	90 min exposure	23	46.44	6.94
TOTAL		**136**		

Table 2. Statistical tests of fetal weight in each gestational stage between groups of different duration of ultrasound exposure time

Gestational stage	**Group**	**95% CI**	**P-value[a]**	**P-value(r)[b]**	**P-value(r^2)[c]**
1st trimester	No exposure	0.26 to 0.34	0.00*	0.02* (-0.40)	0.02* (0.15)
	30 min exposure	0.28 to 2.55			
	60 min exposure	0.30 to 0.37			
	90 min exposure	0.15 to 0.27			
2nd trimester	No exposure	2.49 to 2.61	0.01*	0.60 (0.10)	0.82 (0.00)
	30 min exposure	2.49 to 2.75			
	60 min exposure	2.77 to 2.91			
	90 min exposure	2.43 to 2.63			
3rd trimester	No exposure	48.62 to 56.72	0.00*	0.04* (-0.23)	0.04* (0.04)
	30 min exposure	46.60 to 57.26			
	60 min exposure	53.18 to 58.96			
	90 min exposure	43.44 to 49.44			

[a] ANOVA ; [b] Correlation test ; [c] Prediction test
*$p<0.05$; statistical tests were performed by a parametric or a non-parametric test

4 Discussion

This study showed that the fetal weight at each gestational stages, the 1st, 2nd and 3rd trimester of pregnancy were statistically significant among ultrasound exposed and non-exposed group. In prenatal development, the sensitivity to a teratogen, heat, varies between gestational stages, the state of maturation of the structures and also

varies considerably between species. A major developmental period in rabbit includes pre-implantation stage (GD 0-5) where there is fertilization and implantation of embryo into uterine wall; organogenesis stage (GD 6-15), is when the formation of major body structures; and finally a fetal stage (GD 16-31.5) where there is growth of the structures [5]. Throughout these 3 gestational stages, present research had found the differences in fetal weight among ultrasound exposed and non-exposed group. Furthermore, when the duration of ultrasound exposure was increased, a decrease in fetal weight was noted. This indicates the possibility of ultrasound heating in causing defect in studied animal. The decreased in fetal weight at longest ultrasound exposure time groups suggests the heating effect of ultrasound exposure may act as a noxious agent that promotes intra-uterine growth restriction (IUGR) in developing fetus.

A randomized controlled trial study done by Newnham and Evans [12] found that increased frequency of ultrasound exposure and Doppler flow imaging during 18 to 38 weeks of gestation (2^{nd} and 3^{rd} trimester) elevates about one third of IUGR in developing fetus as compared to single ultrasound exposure. Another study also reported that ultrasound exposure in utero could potentially cause hematopoiesis and reduced body weight in macaque (monkey) offspring [13]. Meanwhile, Bukowski et al. [14] and Smith et al. [15] reported that routine ultrasound measurement that shows small fetal size at early pregnancy is indicative of reduced birth weight. This indicated that fetus is at risk of poor growth. Moreover the IUGR had found to be prominent even at very early of pregnancy [15]. This condition is evident in this present study where results showed an association of 1^{st} and 3^{rd} trimester fetal weight upon an exposure to ultrasound heating. During the 1^{st} trimester of pregnancy, mainly embryonic developments occur whereas the 3^{rd} trimester of pregnancy involves the growth of a fetus. Once the embryonic developmental period had been altered by means of ultrasound heating, it can cause growth restriction later in pregnancy. In another *in vivo* study by Dom et al. [16] the differences in fetal parathyroid hormone level in ultrasound exposed and non-ultrasound exposed group could lead to a possible result in alteration of physiological developments of fetus. This will cause various physiological variables alteration, not limited to reduction in fetal weight which was reveled in this study.

5 Conclusion

This fundamental *in vivo* experimental study suggests that the heating effect of ultrasound exposure might be related to disturbance in the physiology of studied animal, *Oryctolagus cuniculus*. Therefore, there should be a rising concern in the awareness towards the negligible effects produced from prenatal ultrasound exposure as it is widely applied during pregnancy in humans. Although *in vivo* experiments conducted in animal cannot ethically be replicated in humans, the role of fetuses in intrauterine environments in animal model is estimated to mimic the gestational stage of human pregnancy [17]. The results, when applied onto human, will therefore ensure that pregnant women will receive professional care and consultation to undergo prenatal ultrasound during pregnancy, which will also bring out medical

benefits higher than that of risk while at the same time contributes to the health of the next generation of human capital.

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