

# THERMAL EFFECTS OF PRENATAL ULTRASOUND EXPOSURE ON THE GROWTH OF YOUNG-AGED ORYCTOLAGUS CUNICULUS

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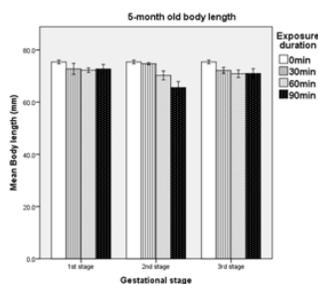
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## Graphical abstract



## Abstract

Bones are the living tissue that composed of collagen and minerals which can be considered as a framework for a child's growing body. As ultrasound examination during pregnancy is the most routine procedure, it is worrying that disruption of osteogenesis due to prenatal ultrasound may affect the bone at a young age. This ex-vivo experimental study was conducted using young-aged rabbits, to find the evidence of growth changes related to body length, femoral width and femoral length caused by prenatal ultrasound heating. Pregnant New Zealand White rabbits were exposed to 30, 60 and 90 minutes of prenatal ultrasound at 1st, 2nd and 3rd stage of gestation accordingly. Operating frequency was between 5 to 9 MHz with a free field ISPTA of 0.19 W/cm<sup>2</sup>, MI and TI of 1.0 and 0.2 respectively. Offspring were assessed at 1-month and 5-months of life. Minimal effects on body length, femoral width and femoral length were observed in both 1-month and 5-months old rabbits. Lowest animals' growth was recorded in the group exposed to ultrasound at the 2nd stage of gestation for 90 minutes.

Keywords: Prenatal ultrasound, thermal effects, bone, young age

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## 1.0 INTRODUCTION

Bones are the living tissue that composed of collagen and minerals which can be considered as a framework for a child's growing body. They provide both mechanical support and protection for vital organs. Building healthy bones are crucial for paediatric because at this stage, they are living an active lifestyle. In addition, bad start on adult health related to bone disorder could also be prevented because most adult bone mass is acquired during childhood and adolescence [1]. Even though consequences of bone disorder mostly occur during adulthood, it is actually a paediatric disorder. Currently, bone loss has become serious and common problems among children [2] due to improper dietary

intake, over consumption of steroid medicine or disruption in osteogenesis during foetal stage.

Osteogenesis is very sensitive to heat since bone is a poor conductor of heat [3]. Temperature rise during osteogenesis can lead to denaturation of the enzymatic and membrane proteins [3], bone tissue necrosis, bone microcirculation blockage and activation of bone marrow macrophages [4]. As ultrasound examination during pregnancy is the most routine procedure [5], it is worrying that disruption of osteogenesis can occur because of the ultrasound thermal effect. As we know, it is the main potential for an adverse biological effect which involves tissue heating from energy absorption of the ultrasound beam [6]. Heat is converted from absorbed sound [7] that leads to a temperature increase within the insolated tissue. Furthermore, the advancement of

the ultrasound has made the situation even worse because high frequency used for 3D and 4D viewing would increase the production of heat. An increasing number of researches done to investigate the ultrasound thermal effect on maternal or foetus have strengthened the fact that some consequences related to bone may arise from prenatal ultrasound heating [8-11]. Dom *et al.* [12] has proved that prenatal ultrasound has led to significance increase in foetal bone mineral density which increase the risk to develop osteoporosis.

To best of our knowledge, there is lack of research has been done to evaluate the ultrasound heating effect on bone in the young age. As mentioned preceding, bone status in childhood is an important key for healthy bone development. Sign and symptoms of certain bone disorders only emerge once it has been in a severe state. Normally, when this is happening, an epidemiological investigation will be done. Early detection of the bone changes during childhood is crucial as a precaution and treatment can be carried out immediately. Thus, it would be the major motivation for this study. This *ex-vivo* experimental study was conducted using young aged rabbits, to find the evidence of growth changes related to body length, femoral width and femoral length caused by prenatal ultrasound heating.

## 2.0 MATERIALS AND METHODS

The animal use protocol was approved by the Universiti Teknologi MARA Committee on Animal Research and Ethics (UiTM CARE). Malaysian breeding New Zealand white rabbits (*Oryctolagus cuniculus*) were time-mated to allow the use of pregnant does of known gestation. Does were mated when the red vulva was noted, signalling they accept mating and ovulate [13]. The full term gestational period for a doe ranged between 30-33 days [14] and consists of three stages which each stage has 11 days. Pregnant does were grouped according to the ultrasound exposure given at different gestational stage for different duration of exposure. The experimental groups were received ultrasound exposure for 30 minutes, 60 minutes and 90 minutes at the middle of gestational stage

The control group was free from exposure throughout pregnancy. The abdominal region of pregnant does of the experimental group was shaved to ensure proper transducer application. The exposure was given onto as many foetuses as possible by applying several transducer manoeuvres such as sliding, rotating and fanning. Phillips HD3 system (Phillips Electronics E.V., Germany) fitted with a 5-9 MHz linear-array transducer (L9-5, Philips Electronics E.V., Germany) was used for the B-mode exposure. The settings of ultrasound machine such as mechanical index (MI), thermal index (TI), transducer frequency

and focal distance were maintained during the entire study period accordingly.

**Table 1** In-house normal range for femur width and length based on control group data

Group	Measurement (mm)	Mean ( $\bar{x}$ )	Standard deviation (SDs)	Standard error
1 month	Body length	48.95	2.291	0.724
	Femoral width	7.431	1.286	0.407
	Femoral length	45.418	4.551	1.439
5 months	Body length	75.4	1.022	0.323
	Femoral width	14.753	0.583	0.184
	Femoral length	98.228	1.958	0.619

The linear transducer used was able to transmit frequency between 5 MHz to 9 MHz, which complemented to the focal depth of the subject (5.5cm). MI and TI displayed 1.0 and 0.2 respectively. Previous characterization of the transducer found the spatial peak temporal average ( $I_{SPTA}$ ) and output power were varied from 0.13 to 0.19 W/cm<sup>2</sup> and 0.4 W to 0.7 W respectively [15]. The offspring of all does were used for data collection at 1-month and 5-months age corresponded to the young age.

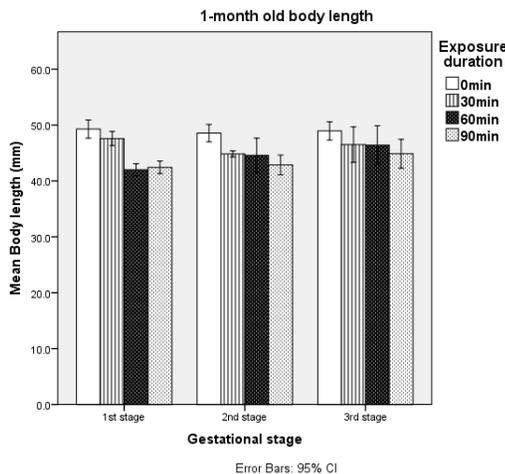
During the period, the studied animals were given *ad lib* water supply. The pelleted feed was measured as 5% of its body weight [16] so that external factors that might affect validity of the outcome can be eliminated. The studied animals were anaesthetised and euthanized [17]. A dose of ketamine hydrochloride (50 mg/kg body weight) and xylazine hydrochloride (10 mg/kg body weight) were administered intramuscularly to provide anaesthesia. A dose of pentobarbital sodium (100 mg/kg body weight) was administered via intravenous injection to euthanised the animals. The body length of the studied animals was measured using standard measuring tape. Femoral bones were dissected using a scalpel and scanned via micro-computed tomography (micro-CT) for width and length measurements. The Skyscan 1176 (Skyscan 1176, SkyScan bvba, Aartselaar, Belgium) was the modality used since it is well-known for its high performance and low dose micro-CT scanner. Data were analysed by analysis of variance (ANOVA) for group differences and Pearson's correlation to correlate parameters via Statistical Package for the Social Sciences, SPSS version 21.0.

### 3.0 RESULTS

An in-house normal reference range of body length, femoral width and femoral length were developed using the outcome of the control groups since there was no reference value of these parameters for 1 and 5 months old rabbit. Table 1 displayed reference range that was used to compare with results from the experimental group. The Q-Q plots for the animals' growth (body length, femoral width and femoral length) had shown normal distribution where the data plots fell approximately in a straight line. Statistical analysis revealed differences in certain experimental groups when compared to the control.

#### 3.1 Animals' Growth for 1-Month Old

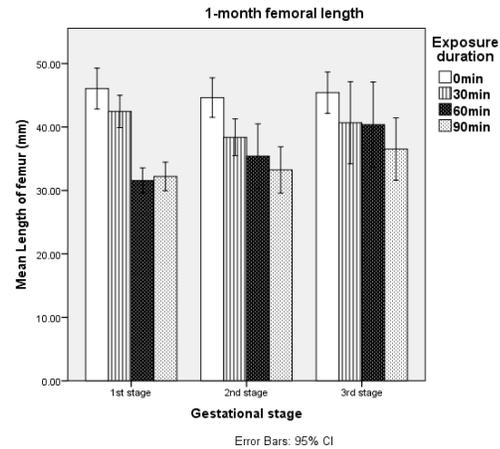
Statistically significant reductions were found in body length between control and experimental groups ( $p < 0.05$ ) (Figure 1a). Body length was significantly lower in treated animals after 30, 60 and 90 minutes exposure at the 2<sup>nd</sup> gestational stage. At 1<sup>st</sup> and 3<sup>rd</sup> stage, it is significantly lower after 60 and 90 minutes, and 90 minutes exposure respectively. There were also statistically significant differences noted at all gestational stages for femoral width ( $p < 0.05$ ) (Figure 1b). Further analysis revealed exposure at 1<sup>st</sup> gestational stage for 60 and 90 minutes differed significantly to the control showing reduced means. This difference also noted at 2<sup>nd</sup> and 3<sup>rd</sup> stage of gestation for 90 minutes of exposure. For femoral length, exposure at 2<sup>nd</sup> stage yielded significant differences for all durations of exposure ( $p < 0.05$ ). There were significant difference noted at 1<sup>st</sup> and 3<sup>rd</sup> stage for 60 and 90 minutes, and 90 minutes of exposure respectively (Figure 1c).



**Figure 1(a)** Body length of 1-month old rabbit. Note that statistically significant differences of all groups at 2<sup>nd</sup> stage when compared to the control group



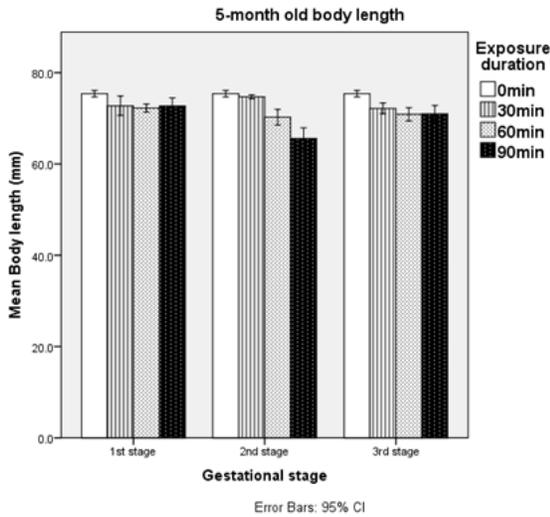
**Figure 1(b)** Femoral width of 1-month old rabbit. 90 minutes of exposure at 2<sup>nd</sup> and 3<sup>rd</sup> stage showed significant reduction of femoral width as compared to the control group. At 1<sup>st</sup> stage, significant different were noted after 60 and 90 minutes of exposure



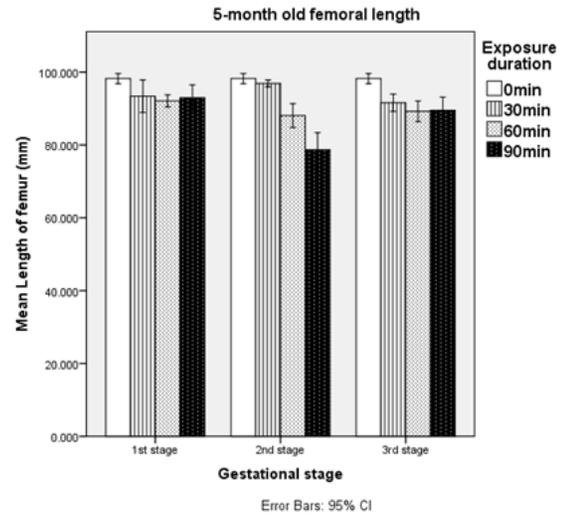
**Figure 1(c)** Femoral length of 1-month old rabbit. Differences were noted at all gestational stage after 90 minutes of exposure

#### 3.2 Animals' Growth for 5-Months Old

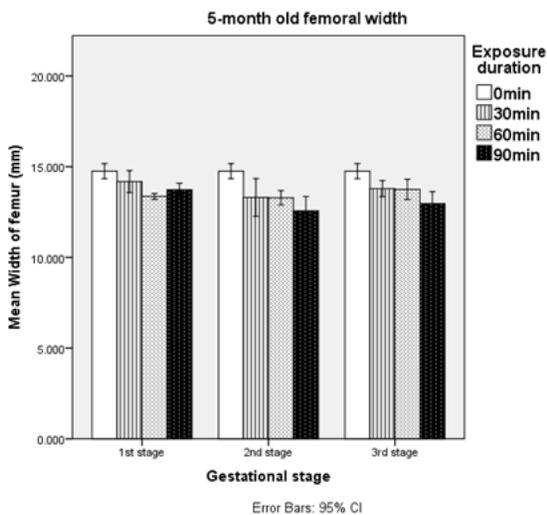
Exposure at 1<sup>st</sup> and 2<sup>nd</sup> stage of gestation showed significant reduction in body length after 60 and 90 minutes of exposure ( $p < 0.05$ ). Meanwhile, exposure at 3<sup>rd</sup> gestational stage yielded significant different in body length for all duration (Figure 2a). There were significant differences in femoral width of all groups ( $p < 0.05$ ) (Figure 2b) as compared to the control group. Further analysis revealed a lower mean at 1<sup>st</sup> and 2<sup>nd</sup> stage for 60 and 90 minutes of exposure. Meanwhile, significant differences were noted at 3<sup>rd</sup> stage of all duration. Similar differences were also noted for the femoral length (Figure 2c).



**Figure 2(a)** Body length of 5-months old rabbit. Exposure at all gestational stage showed significant different where the lowest mean body length was at 2<sup>nd</sup> stage after 90 minutes of exposure (65.571)



**Figure 2(c)** Femoral length of 5-months old rabbit. Lowest mean femoral length was recorded at 2<sup>nd</sup> stage after 90 minutes of exposure



**Figure 2(b)** Femoral width of 5-months old rabbit. Exposure for 60 and 90 minutes at all gestational stage caused significant difference in femoral width as compared to the control

### 3.3 Pearson Correlations

Pearson correlations were evaluated between animals growth and gestational stage, and animals' growth and exposure duration for 1-month and 5-months old. Weak correlations were found between gestational stage and animals' growth (body length,  $r=-0.01$ , femoral width,  $r=0.02$ , femoral length,  $r=-0.02$ ), where gestational stage was not associated with animals' growth of 1-month old. However, there were fair negative correlations between gestational stage and animals' growth (body length,  $r=-0.33$ , femoral width,  $r=-0.3$ , femoral length,  $r=-0.35$ ), with exposure at later stage of gestation associated with reduction of animals' growth of 5-months old. Analysing the correlations between exposure duration and animals' growth at 1-month old, strong negative correlations were found (body length,  $r=-.59$ , femoral width,  $r=-0.54$ , femoral length,  $r=-0.61$ ), with longer exposure associated with lower animals' growth. Strong negative correlations were also found in 5-months old data (body length,  $r=-0.6$ , femoral width,  $r=-0.55$ , femoral length,  $r=-0.6$ ). Pearson correlations were also evaluated relationship between animal's growth variables for 1-month and 5-months old.

There were strong positive correlations between body length and femoral width ( $r=0.86$ ), and between body length and femoral length ( $r=0.99$ ) of 1-month old, with longer body length associated with wider femoral width and longer femoral length. Similar strong positive correlations were also found in 5-months old data (femoral width,  $r=0.55$ , femoral length,  $r=0.99$ ).

### 3.4 Development of Body Length, Femoral Width and Femoral Length

The percentage differences of body length, femoral width and femoral length, acquired at 1-month and 5-months data, were calculated. These differences regarded as the gap of development occurred between 1-month to 5-months when comparing the control with the exposed groups. Control group recorded higher rate of development than the exposed group. The difference in body length, femoral width and femoral length were 3.56%, 7.37% and 10.93% respectively.

## 4.0 DISCUSSION

This study was done to scrutinize the effects of prenatal ultrasound on animals' growth observed at 1-month and 5-months old. The study observed the effect at the young age because sometimes the effects may become obvious at later stage of life. The results indicated that a single exposure throughout gestational period can induced minimal effects on body length, femoral width and femoral length. The damage occurred were depended on duration of exposure as well as developmental stage at which exposure given.

In the present study, significant differences were noted at all gestational period for all duration of exposure for both 1-month and 5-month group. These observations were in agreement with previous reports. A study conducted by Hande and Devi had shown significant reduction in body length of the mice foetus after exposed to ultrasound prenatally at Day 6.5 [18]. In another study, Day 8.5 Doppler-exposed mice also showed small reduction in body length at 3 weeks after birth [19]. The exposure in these studies including the current study was given during the major events in organogenesis occurred. Organogenesis stage of a mouse is from Day 6 to Day 13 while organogenesis stage of a rabbit is within 2<sup>nd</sup> gestational stage (Day 6- Day 15).

Organogenesis and cell division period are very sensitive. Direct heating of the embryo during organogenesis will disrupt formation of the major structure, such as bone and its developmental processes as it may causes damage to the blood vessels and the placenta [20]. According to Barnett [21], worse-case heating will occur in late stage of pregnancy which is in the 2<sup>nd</sup> stage and 3<sup>rd</sup> stage. During these stages, the bone mineralisation is increased which make it thicker and denser, thus increasing its acoustic absorption coefficient. The absorption coefficient of bone is about 10 dB /cm/MHz [22] which is 30 times higher than any other structures. Bigelow *et al.* has mentioned that thermal risk increase with exposure time, however, the TI formulations does not related to exposure time [23].

Therefore, the risk of thermal damage is higher at a lower TI with longer exposure than a higher TI for a shorter exposure. Longer exposure required smaller temperature increase to produce biological effects [24]. In the current study, the TI recorded was 0.2 which is considered low. The effects were noted significantly at the longest exposure time (90 minutes) and followed by 60 minutes. Ultrasound heating has altered the embryonic development period, which causes intrauterine growth restriction (IUGR) throughout pregnancy.

This is evident from the present study where animals' growth in both 1 and 5-months old were lower and significantly different from the control after exposed to ultrasound at 3<sup>rd</sup> gestational stage. This stage was when the foetal growth takes place. The adverse consequences of IUGR are not only affected during infancy and childhood, but spans over life time [25]. The ability of temperature elevation to produce growth retardation has been discussed in the literature, but using other sources of heat. In a study, pregnant cotton-eared marmoset was exposed to heat by placing them in a bacteriological incubator preset at a temperature of 42°C [26]. In comparison to the control group which placed in the incubator at room temperature, skeletal changes were noted in the exposed group. In another study, pregnant ewe was delivered intrauterine growth-retarded lamb after exposed to heat-stressed during pregnancy [27]. In studied animal, it is noted that certain gap of development occurred where the development was lower in the exposed group compared to the control. The growth and development of the skeleton requires a tolerable supply of various different nutritional factors. Since this study measure the food intake by the rabbit, it can be assume that the results observed were due to prenatal ultrasound effects.

## 5.0 CONCLUSION

The findings of this *ex vivo* experimental study suggest that there are minimal effects on the general growth of young-aged *Oryctolagus cuniculus* related to body length, femoral width and femoral length after exposed to the prenatal ultrasound especially at 2<sup>nd</sup> and 3<sup>rd</sup> gestational stage for 90 minutes. By adding this information to current spectrum knowledge, early precaution and awareness regarding obstetric ultrasound can be applied in the clinical field to improve the quality of life for both mother and child.

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