

# THERMAL EFFECTS OF PRENATAL ULTRASOUND EXPOSURE ON PARATHYROID HORMONE SECRETION OF *ORYCTOLAGUS CUNICULUS* AND THEIR CORRELATION WITH SERUM BIOCHEMICAL REACTIONS AND BONE VOLUME

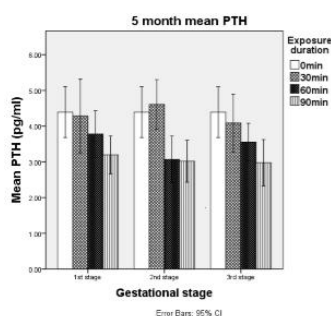
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## Graphical abstract



## Abstract

Calcium is the most essential element to provide energy to the body system and PTH secretion is responsible for the calcium regulations. PTH imbalance can cause serious medical conditions. Heat is one of the factors that is able to cause disturbance in the PTH secretion. As ultrasound has the potential to produce heat, its imprudent use during pregnancy may cause disturbance in the formation of the parathyroid gland. This study was done using young-aged *oryctolagus cuniculus* to evaluate PTH secretion and its correlation with the biochemical reaction and bone volume. New Zealand White rabbits were time-mated and exposed to 30, 60 and 90 minutes of prenatal ultrasound at 1<sup>st</sup>, 2<sup>nd</sup> and 3<sup>rd</sup> stage of gestation accordingly. The control group was left for full term delivery without receive ultrasound exposure. In 1 month-aged subject, the lowest PTH level which significantly different from the control was noted after exposed prenatally in the 1<sup>st</sup> stage for 90min ( $3.56 \pm 0.75$ ). Meanwhile, in the 5 months-aged subject, the lowest PTH level was noted in the 3<sup>rd</sup> stage group after 90min of exposure ( $2.98 \pm 0.62$ ). The reduction in PTH was found to affect the biochemical reactions, but not the bone volume.

**Keywords:** Parathyroid hormone, calcium balance, bone volume, ultrasound

## Abstrak

Kalsium adalah unsur yang paling penting untuk memberi tenaga kepada sistem badan dan rembesan PTH bertanggungjawab ke atas keseimbangan kalsium. Ketidakeimbangan PTH boleh menyebabkan masalah kesihatan yang serius. Haba adalah salah satu faktor yang dapat menyebabkan gangguan dalam rembesan PTH. Ultrasound berpotensi untuk menghasilkan haba. Jadi, penggunaan yang tidak sepatutnya semasa kehamilan boleh menyebabkan gangguan dalam pembentukan kelenjar paratiroid. Kajian ini dijalankan dengan menggunakan *oryctolagus cuniculus* pada usia muda untuk menilai rembesan PTH dan perkaitannya dengan reaksi biokimia dan status tulang akibat pendedahan kepada ultrasound semasa di dalam kandungan. Arnab *New Zealand White* yang mengandung didedahkan kepada ultrasound selama 30, 60 dan 90 minit pada peringkat 1, 2 dan 3 kehamilan mengikut kumpulan yang telah ditetapkan. Kumpulan kawalan tidak didedahkan kepada ultrasound sehingga beranak. Dalam kumpulan 1 bulan, tahap PTH di dapati paling rendah adalah selepas menerima 90 minit ( $3.56 \pm 0.75$ ) pendedahan pada peringkat 1 kehamilan. Sementara itu, dalam kumpulan 5 bulan, tahap PTH terendah dicatatkan selepas 90 minit pendedahan pada peringkat 3 kehamilan ( $2.98 \pm 0.62$ ). Pengurangan PTH didapati memberi kesan kepada tindak balas biokimia, tetapi tidak kepada status tulang.

**Kata kunci:** hormone parathyroid, keseimbangan kalsium, status tulang, ultrasound

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## 1.0 INTRODUCTION

Calcium is the most essential element to provide energy to the nervous and muscular system, and also to provide strength to the skeletal system. Parathyroid gland acts as the regulator for the calcium balance. The function of the gland is to secrete parathyroid hormone (PTH) which responsible for calcium regulations in the blood and bone. PTH imbalance can cause serious medical conditions, including hyperparathyroidism and hypoparathyroidism. PTH disturbance occurs for a number of reasons, but can include chronic kidney disease, removal of the gland, autoimmune disease and extensive radiation therapy. In addition, elevation in core body temperature by the exogenous factor is also able to induce hormonal disturbance [1] including the PTH. Exposure to heat will initially increase the hormone production and it declines after a prolonged exposure [2].

Ultrasound exposure has the potential to produce a temperature rise in the insolated tissue. Formation of parathyroid gland begins in middle of the gestational stage during the organogenesis where susceptibility to teratogens is maximal [3]. Therefore, external disturbance like the ultrasound heating at this stage in pregnancy is a concern to affect the formation of the glands and thus affect its secretion. The issue of imprudent use of ultrasound has lured many researchers to evaluate its potential risks to the foetus on various aspects. However, the relationship between thermal effects of prenatal ultrasound and the hormonal responses that occur during postnatal life is not well documented. Thus, the present study was done to find the evidence of hormonal changes and its effect on serum biochemical and bone volume.

## 2.0 MATERIALS AND METHODS

Prior to the investigation, all procedures for the animal were approved by the Universiti Teknologi MARA Committee in Animal Research and Ethics (UiTM CARE). All Malaysian breeding New Zealand white rabbits (*Oryctolagus cuniculus*) were housed and maintained under controlled temperature (14°C – 28°C) and light (16L:8D) conditions with a humidity range between 34% and 40% [4]. BioGS air purifier was used to provide clean environments in the animal house from harmful gases released by the animals such as ammonia and carbon dioxide [5]. The rabbits were given ad lib water supply and the pelleted feed was measured as 5% of its body weight [6] to ensure the outcomes were not affected by any other external factor.

Female (does) and male (bucks) rabbits, 5 to 8 months of age, were time-mated. The 0 day of

pregnancy was taken when does succeed at the servicing for the second time. The pregnant does were divided into 4 groups where 4 does in a control group and 18 does in individual experimental groups. The control group was free from ultrasound exposure and left for full term delivery. The experimental group were received one time exposure throughout the gestational period.

The full term gestational period for a doe was between 30-33 days [7] and consists of 3 stages with 11 days on each stage. The ultrasound exposure were given in the middle of each gestational stage; 1<sup>st</sup> stage (Day 6), 2<sup>nd</sup> stage (Day 17) and 3<sup>rd</sup> stage (Day 28) for 30 minutes, 60 minutes and 90 minutes according to the assigned group. A Philips HD3 system (Philips Electronics E.V., Germany) fitted with a 5-9 MHz linear-array transducer (L9-5, Philips Electronics E.V., Germany) was used to provide B-mode exposure. The transducer used was able to operate with a focal depth of 5.5cm which correspond to the location of the foetus in the pregnant does. The maximum frequency used in each exposure was 9 MHz. The thermal index (TI) and mechanical index (MI) recorded were 0.2 and 1.0 respectively. Based on previous characterization of the transducer, the calculated spatial peak temporal average (ISPTA) 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 [8]. All of these exposure factors were maintained throughout the investigation.

In order to ensure that all foetuses were exposed to the exposure, several transducer manoeuvres such as fanning, rotating and sliding were applied. The abdominal region of pregnant does was shaved beforehand for proper transducer application. Control and experimental animals were left to complete gestational period in an individual laboratory cage. The kits were weaned at 1 month of age and were kept in separated cage once they reach 2 months of age. At 1 and 5 months of age, the kits were subjected to biochemical analysis, hormonal test and bone volume analysis. The blood was collected for serum collection from marginal ear vein venipuncture directly into plain VACUTAINER® containing no anticoagulant. The serum was separated following centrifugation for 10 minutes at room temperature with 5000 r.p.m. Any haemolysed samples were discarded. The serum were kept in separated Microvette® CB 300 blood collection system and stored at -20°C for analysis. The biochemical test was done for calcium and alkaline phosphatase (ALP) while hormonal test was done for parathyroid hormone (PTH). The serum was sent to the Department of Veterinary Laboratory Diagnostics, Universiti Putra Malaysia (UPM) for analysis. Table 1 shows reference values for parameters at 1 and 5 months of age.

**Table 1** Reference values for parameters at 1 and 5 months of age

Age	Measurement	( $\bar{x}$ )	SD	SEM	Minimum	Maximum
1 month (n=10)	Ca (mmol/L)	3.63	0.18	0.06	3.35	3.99
	ALP (U/L)	163.81	28.96	9.16	124.6	196.2
	PTH (pg/ml)	6.04	0.9	0.28	4.57	7.51
	BV/TV (%)	62.1	0.87	0.27	60.6	63.85
5 months (n=10)	Ca (mmol/L)	3.97	0.39	0.12	3.23	4.51
	ALP (U/L)	92.02	21.46	6.79	65	131
	PTH (pg/ml)	4.39	0.99	0.31	3.56	6.25
	BV/TV (%)	68.93	0.79	0.25	66.94	69.94

a) Abbreviations: n, no. of sample;  $\bar{x}$ , mean; SD, standard deviation; SEM, standard error; Ca, calcium level; ALP, alkaline phosphatase level; PTH, parathyroid hormone level; BV/TV, bone volume fraction

After done with the blood collection, the kits were anaesthetised and euthanized [9] for bone volume analysis. Ketamine hydrochloride (50 mg/kg body weight) and xylazine hydrochloride (10 mg/kg body weight) were administered intramuscularly to provide anaesthesia. For the euthanasia, a dose of pentobarbital sodium (100 mg/kg body weight) was administered via intravenous injection. Femoral bone were dissected and scanned via low-dose micro-computed tomography (Skyscan 1176, SkyScan bvba, Aartselaar, Belgium) for bone volume analysis [10].

Analysis of variance (ANOVA) was used to analyse the data for group differences and Pearson correlation was done to correlate parameters using Statistical Package for the Social Sciences, SPSS version 21.0. All differences were assumed statistically significant at  $P \leq 0.05$  and mean  $\pm$  SD were reported. Pearson correlation was evaluated between parameters and gestational stage, parameters and duration of exposure and among parameters for 1 and 5 months of age.

### 3.0 RESULTS

The details of serum biochemical parameters and bone volume of *Oryctolagus cuniculus* in the control group for 1 and 5 months were presented in Table 1. These results served as in-house normal reference range and used to compare with the result of the experimental groups. The Q-Q plots for all parameters had shown normal distribution.

#### 3.1 Hormonal, Biochemical and Bone Volume Analysis of 1 Month Aged Rabbits

PTH was decreased significantly in the 1<sup>st</sup> stage (90 min:  $3.56 \pm 0.75$ ), 2<sup>nd</sup> stage (90 min:  $4.35 \pm 0.95$ ) and 3<sup>rd</sup>

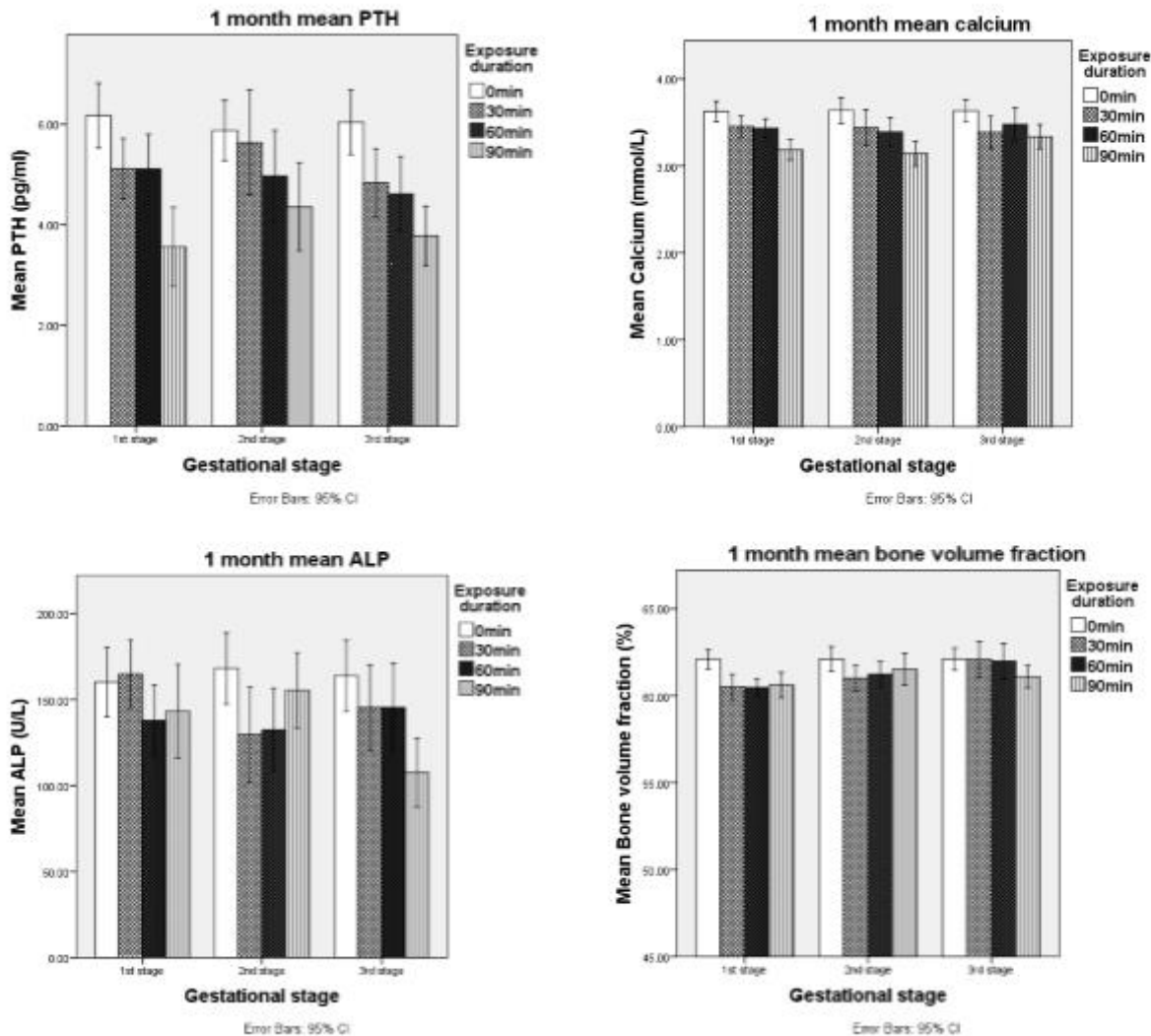
stage (30 min:  $4.83 \pm 0.65$ , 60 min:  $4.62 \pm 0.71$ , 90 min:  $3.78 \pm 0.71$ ). Exposure on 1<sup>st</sup> stage ( $3.19 \pm 0.11$ ), 2<sup>nd</sup> stage ( $3.14 \pm 0.16$ ) and 3<sup>rd</sup> stage ( $3.34 \pm 0.17$ ) caused the calcium level to significantly decrease after 90 min of exposure. In terms of ALP level, significant differences were noted only in the 3<sup>rd</sup> stage after 90 min of exposure ( $107.63 \pm 23.78$ ) where no different noticed in the 1<sup>st</sup> and 2<sup>nd</sup> stage ( $p > 0.05$ ). Bone volume was only significantly lower in the experimental group after 30 min ( $60.49 \pm 0.8$ ), 60 min ( $60.47 \pm 0.54$ ) and 90 min ( $60.62 \pm 0.71$ ) of exposure in the 1<sup>st</sup> stage. The results were shown in Figure 1. Gestational stage was negatively correlated with ALP levels ( $r = -0.38$ ,  $p = 0.01$ ) and PTH level ( $r = -0.39$ ,  $p = 0.01$ ) which explained low level occurred after exposure at a later stage. There were significant negative correlations noted between duration of exposure with calcium level ( $r = -0.60$ ,  $p = 0.01$ ), ALP level ( $r = -0.33$ ,  $p = 0.01$ ) and PTH level ( $r = -0.65$ ,  $p = 0.01$ ), with longer exposure caused decreases in the parameter reading. Bone volume showed no significant correlation with the calcium level, ALP level and PTH level ( $p > 0.05$ ).

#### 3.2 Hormonal, Biochemical and Bone Volume Analysis of 5 Months Aged Rabbits

PTH yielded significant differences in the 1<sup>st</sup> stage (90 min:  $3.19 \pm 0.69$ ), 2<sup>nd</sup> stage (60 min:  $3.07 \pm 0.62$ , 90 min:  $3.02 \pm 0.63$ ) and 3<sup>rd</sup> stage (90 min:  $2.98 \pm 0.62$ ). Calcium level was decreased significantly in all gestational stages as compared to the control. In the 1<sup>st</sup> stage, significant difference was noted only after 90 min ( $3.43 \pm 0.22$ ) of exposure while in the 3<sup>rd</sup> stage noted after 60 min ( $3.27 \pm 0.32$ ) and 90 min ( $3.31 \pm 0.30$ ) of exposure. In the 2<sup>nd</sup> stage, all duration exposures caused differences in the calcium level (30 min:  $3.28 \pm 0.20$ , 60 min:  $3.33 \pm 0.32$ , 90 min:  $3.12 \pm 0.22$ ). However, the ALP level shown no significant difference at all duration of exposure ( $p > 0.05$ ). Exposure on the 1<sup>st</sup> stage (30 min:  $62.20 \pm 0.75$ , 60 min:  $67.46 \pm 0.66$ , 90 min:  $60.60 \pm 0.70$ ), 2<sup>nd</sup> stage (30 min:  $64.04 \pm 0.63$ , 60 min:  $64.09 \pm 0.68$ , 90 min:  $62.84 \pm 0.72$ )

and 3<sup>rd</sup> stage (60 min:  $65.57 \pm 0.96$ , 90 min:  $65.15 \pm 0.68$ ) had shown lower bone volume compared to the control. The results were shown in Figure 2. Gestational stage only showed significant negative correlation with the changes in calcium level ( $r = -0.46$ ,  $p < 0.01$ ), which decrease in calcium associated with exposure on a later stage. Meanwhile, duration of

exposure significantly correlated with a calcium level ( $r = -0.48$ ,  $p < 0.01$ ), PTH ( $r = -0.57$ ,  $p = 0.01$ ) and bone volume ( $r = -0.61$ ,  $p = 0.01$ ), with longer exposure duration lead to decrease in the parameter level. Bone volume was only shown a significant correlation with the calcium level ( $r = 0.43$ ,  $r = 0.01$ ).



**Figure 1** PTH yielded a significant difference as compared to the control in all gestational periods. The decreased calcium level and fluctuation in the ALP level were noted to have correlation with the level of PTH. However, the bone volume shown no correlation

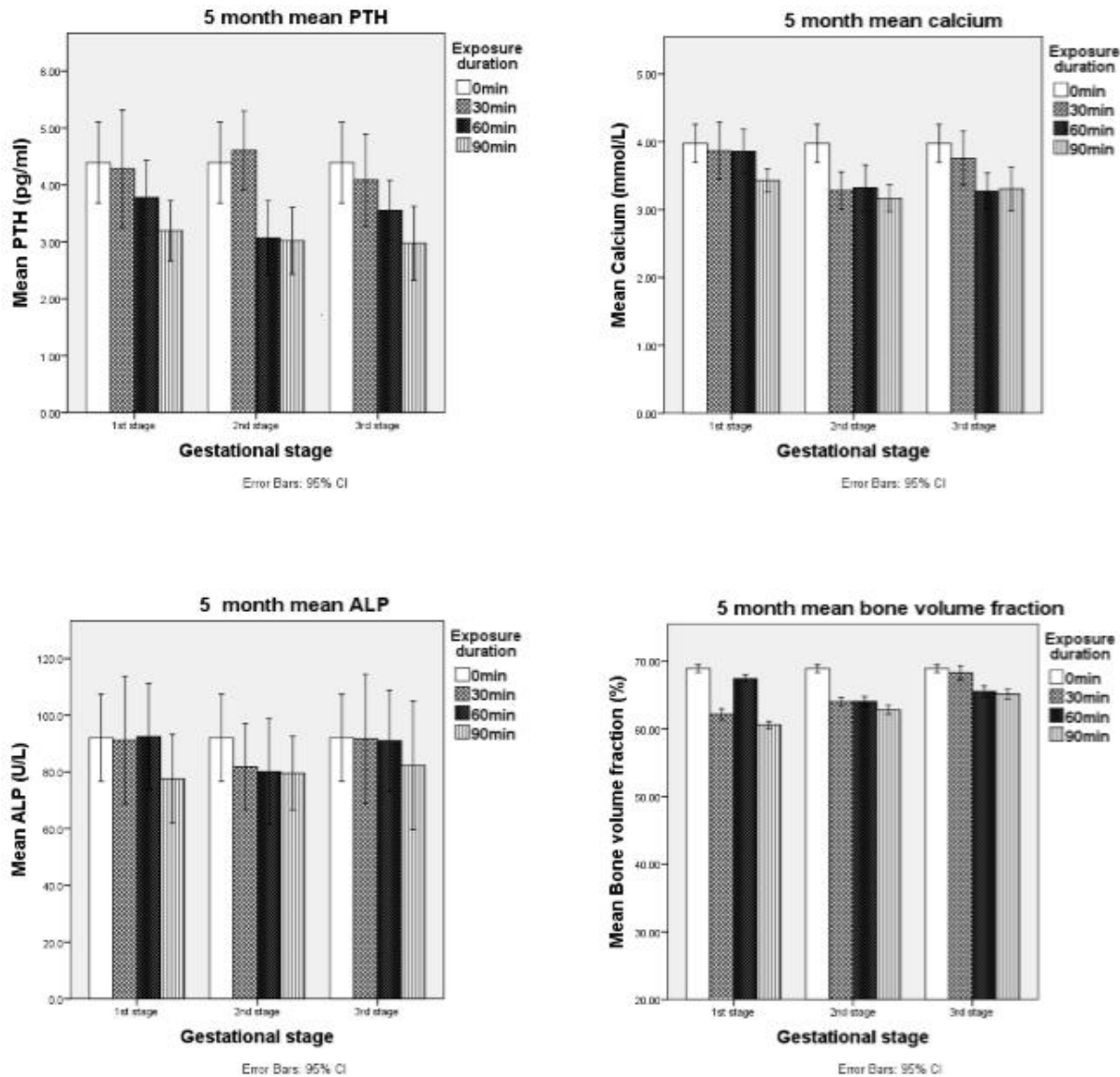
## 4.0 DISCUSSION

The results of the present study indicated that one time exposure to diagnostic levels of ultrasound during intrauterine life in *oryctolagus cuniculus* can disturb parathyroidal activity which also affected serum biochemical parameters and bone volume at the young age. Endocrine glands are subtle to both short and long term environmental heat and in turn affected the hormone including the PTH. During ultrasound exposure, the core temperature of the

body is able to elevate as proven by laboratory approaches [11; 12]. This temperature rise is due to energy absorption of the ultrasonic beam by the insolated tissue. In the present study, PTH level was recorded lower in all experimental groups (1<sup>st</sup>, 2<sup>nd</sup> and 3<sup>rd</sup> stage) of 1 and 5 months of age after exposing to the ultrasound prenatally for different duration of exposure. In a previous study, low PTH level was also found in rabbit newborns after prenatal ultrasound exposure given in different gestational stage [13]. These findings are in accord

with the mechanism of high-intensity focused ultrasound (HIFU) to treat primary hyperparathyroidism (HPT). In HIFU, high temperature is maintained which able to cause protein denaturation, cellular destruction and tissue stiffening [14; 15]. These conditions will further cause cell death and ceases the metabolic activity [16]. As foetal and neonate tissue are less tolerated with the heat, a small temperature rise is able to cause disturbance in

the tissue and organ development. Thus, during exposure given in 2<sup>nd</sup> and 3<sup>rd</sup> stage, parathyroid gland may abnormally develop and thus affected the PTH secretion. Without proper treatment, the endocrine abnormality will span over a life time. This explains why the low PTH level was noted in the 5 months group as the subjects was maintained under similar environments for all groups.



**Figure 2** At 5 months of age, the lower PTH level noted in all gestational stage and correlated with the low calcium level. The low calcium level was also noted to have significant correlation with the decreasing of bone volume

In a normal condition, parathyroid glands maintain a normal calcium balance in the blood. Low PTH can cause calcium level to drop. This can be noticed in hypoparathyroidism condition, which able to develop hypocalcemia. Trauma to the parathyroid gland prevent PTH secretion to the level required to elevate the low serum calcium concentration, and thus develop hypocalcaemia

[17]. The lower level of serum calcium was noted in the present study along with the low PTH level. However, there was a low calcium level noted which is not in accord with the PTH level. Tojo and Huston [18] found that calcium levels in pullets kept at 35°C had significantly lower calcium. In another study, calcium level of dromedary camels was found decrease during the summer months [19]. These

results suggested that low level of calcium can be attributed secondarily to heat stress rather than primarily to low level of PTH.

The highest concentration of the serum ALP is found in the liver and bone, which elevated reading suggest a potential defect either in the liver or bone. However, clinical interpretation of the bone and liver ALP is unclear as both are coded by a single gene [20]. Hence, several researches were done to develop assay that is only sensitive to either liver or bone via different technique such as heat inactivation, wheat germ lectin and inhibition by amino acids and urea [21-23]. Bone alkaline phosphatase is the bone-specific isoform of the ALP which imitates the biosynthetic activity of bone-forming cells. It is a sensitive and reliable marker for bone metabolism. There were slight decreases in the ALP level of the experimental group with significant different only noted at 1 month subject that was exposed on the 3rd stage for 90minutes. This may be due to common ALP test that was used rather than bone-specific ALP test. Thus, the difference noted may be assumed occurred by chance.

As PTH is one of the bone metabolism regulators, its deficiency will leads to decrease in bone resorption and thus cause reduction in new bone formation [24]. PTH is required for either osteoclasts final maturation or initiation of resorption through the osteoblast or both [25]. Thus, lack of PTH cause resorption activity to slow down after the disappearance of osteoclasts. In a study of hypoparathyroidism dog, treatment with PTH was able to increase the number of bone cells, but bone activity was reduced [26]. Rubin *et al.* found that hypoparathyroid subject had significantly higher bone volume [27]. However, this finding was not in accord with the current finding where bone volume was noted significantly decreased almost in every group as compared to the control. Bone is a poor conductor of heat, which make the osteogenesis is very sensitive to heat [28]. The drawbacks that could arise from exposure to heat are bone tissue necrosis, enzyme denaturation [28], blockage of bone microcirculation and bone marrow macrophage activation [29]. Thus, the noted significant decreased in bone volume in the present study is may be due to direct effect of heat on osteogenesis because ultrasound exposure is capable to produce heat.

## 5.0 CONCLUSION

The study suggests that ultrasound exposure given prenatally to the pregnant does may affect the kits postnatally and even spans when they reach young age. Even though it was not proven by means of the clinical trials, precaution and awareness should be taken seriously for early prevention.

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