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# ULTRASOUND TREATMENT AS INTERVENTION IN INTRAUTERINE HYPERPARATHYROIDISM

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**Graphical abstract**

**Abstract**

Hypocalcemia during pregnancy reduced the normal foetal growth, skeletal mineralization and serum calcium concentration. Untreated maternal hyperparathyroidism associated hypocalcemia predisposed the foetal to intrauterine hyperparathyroidism resulting in adverse effect to skeletal mineralization. In vivo study has discovered that prenatal ultrasound exposure has the ability to reduce the foetal PTH level. Ten-month-old nulliparous New Zealand White (NZW) does were assigned to three different groups; Control (C), healthy NZW does and free from ultrasound exposure; hypoparathyroidism (HyPT), negative control groups having hypocalcemia condition established through external parathyroidectomy surgery and free from prenatal abdominal ultrasound exposure; treatment (T), experimental groups having hypocalcemia condition and receiving prenatal abdominal ultrasound exposure during pregnancy as intervention to hypoparathyroidism. In the treatment group, rabbits were exposed for 30, 60 and 90 minutes to parathyroid ultrasound on the 1st, 2nd and 3rd gestational stage accordingly. Following birth, foetal serum calcium (SCa), body weight (BW), crown-to-rump length (CRL), total body length (TBL), bi-parietal diameter (BPD) and femur length (FL) of the foetal were measured. Maternal hypocalcemia during pregnancy gave birth to litters with small to gestational age (SGA) and the reduction of BW, CRL, TBL, BPD, FL and SCa were also noted. Meanwhile, the outcome of ultrasound exposure given during the middle of 2nd gestational stage resulted in significant increase in progeny mean average BW, CRL, TBL, BPD FL and SCa compared to the HyPT group. Prenatal abdominal ultrasound helps to control the level of foetal parathyroid hormone and while still in the womb limits the postnatal complication.

*Keywords:* Micro-CT, bone morphology, bony trabecular structure, total porosity



## 1.0 INTRODUCTION

Calcium the most abundant mineral in the human body is essential for many biological path way and therefore a vital mineral for newborn infant [1]. During gestation, calcium is essential for embryo development and physiological functions [2], including cellular division and differentiation for various cell types [3, 4]. Thus, maintaining the normal calcium homeostasis is crucial for normal aerobic growth of the foetal. Foetal skeletal mineralization, development and growth too depend on the mother's calcium homeostasis status [5] as it has direct effect over the transplacental calcium flux [6]. Suppression of maternal calcium status during pregnancy causes calcium metabolic stress to the foetal, restrict the foetal longitudinal growth and produces litters with short body length [7].

Maternal hypoparathyroidism (HyPT) mother associated hypocalcemia will have the tendency of having babies with secondary hyperparathyroidism [8-10]. Animal study showed maternal

hypoparathyroidism associated hypocalcemia produced small foetal with low body weight and serum calcium concentration. Maternal hypocalcemia produced SGA litters with a 23 % reduction of body weight. Previous study also discovered 26-30% of low birth foetal experience hypocalcemia in postnatal life [11].

Neonatal HPT is a severe metabolic bone disease and often life-threatening [8]. At birth, severe bone deformities may be present reflecting impairment of mineralization and intense osteoclastic bone resorption [10]. Complications from untreated neonatal HPT can lead to serious consequences ranging from neonatal seizure [9], nephrocalcinosis, cardiac abnormalities, pulmonary problems, multiple intrauterinebone fractures and death [8]

Maternal hypocalcemia leads to intrauterine hyperparathyroidism condition which stimulates the increase in the foetal PTH and PTH related protein [12]. Meanwhile an in-vivo experimental study showed that prenatal ultrasound heating caused significant reduction of PTH level in newborn rabbits [13, 14].The present study will compare the outcome of prenatal abdominal ultrasound insonation administered as an intervention in maternal HyPT associated hypocalcemia condition in relation to foetal intrauterine growth and serum calcium concentration.

## 2.0 MATERIALS AND METHODS

The research protocol was approved by University's Committee on Animal Research & Ethics and all experiments were carried out in Laboratory Animal Facility and Management, Faculty Pharmacies, UiTM.

### 2.1 Animals Preparation

Ten-month-old nulliparous New Zealand White (NZW) does were assigned to three different groups; control (C), healthy NZW does and free from ultrasound exposure; hypoparathyroidism (HyPT), negative control groups having hypocalcemia condition established through external parathyroidectomy (PTx) surgery [15, 16] and free from prenatal abdominal ultrasound exposure; treatment (T), experimental groups having hypocalcemia condition established through external PTx and receiving prenatal abdominal ultrasound exposure during pregnancy as intervention to hypoparathyroidism. In the treatment group, rabbits were exposed for 30, 60 and 90 minutes to parathyroid ultrasound exposure on 1st, 2nd and 3rd gestational stage accordingly (Figure 1). All does were mated with normal and healthy NZW bucks. The average gestational term for rabbit ranged between 30-33 days [17] and consisted of three stages.

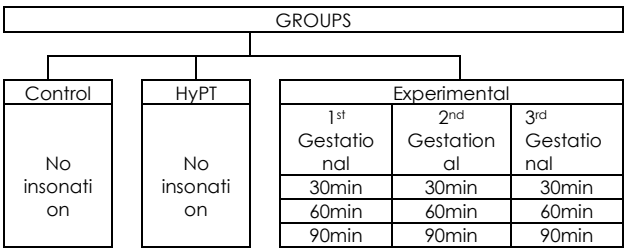


**Figure 1** Methodology chart of ultrasound insonation according to groups

### 2.2 Sample Collections

Following birth, foetal serum calcium (SCa), body weight (BW), crown-to-rump length (CRL), total body length (TBL), bi-parietal diameter (BPD) and femur length (FL) of the foetal were measured. Femoral bone were dissected and placed in 10% buffered formalin. Foetal BW were weighed using 6R-202 AND analytical balance. CRL were measured using the vernier scale, while the TBL were measured using standard measuring tape. Micro-computed tomography (micro-CT) was used to measure the BP and the femur length. Litters blood was withdrawn via cardiac puncture for serum calcium (SCa) evaluation. After 1 hour, the blood was centrifuged at 5000 rpm for 10 minutes and the serum was separated prior to storage at temperature of -80 °C and. The serum were analysed at the Faculty of Veterinary Medicine Universiti Putra Malaysia.

Results from the experimental groups were compared with the control and HyPT groups to evaluate the effects of ultrasound intervention during pregnancy in hypercalcemia condition. Data were analysed using ANOVA one-way analysis of variance followed by post hoc using Scheffe's test. Analysis were done using Statistical Pack-age for Social Sciences software (SPSS version 21).

## 3.0 RESULT

The value of the foetal body weight crown-to-rump length, total body length, bi-parietal diameter and femur length for Control and HyPT were tabulated in Table 1. Maternal hypoparathyroidism associated with hypocalcemia resulted in a significant reduction ($P<0.05$) of foetal average BW, CRL, TBL, BPD FL and SCa compared to maternal with normal calcium level condition.

### 3.1 Body Weight

Maternal HyPT associated hypocalcemia produced progeny with low body weight and small to gestational age (SGA). Mean body weight were not significantly different between the treatment and HyPT groups except for 2nd gestation with 60 minutes ultrasound exposure ($P<0.05$). Mean body weight in

the treatment groups showed a significant difference compared to the Control group except for 2nd gestation with 60 minutes ultrasound exposure (Figure 2). Sixty minutes ultrasound exposure administered in the middle of 2nd gestation improved the foetal body weight in maternal HyPT condition.

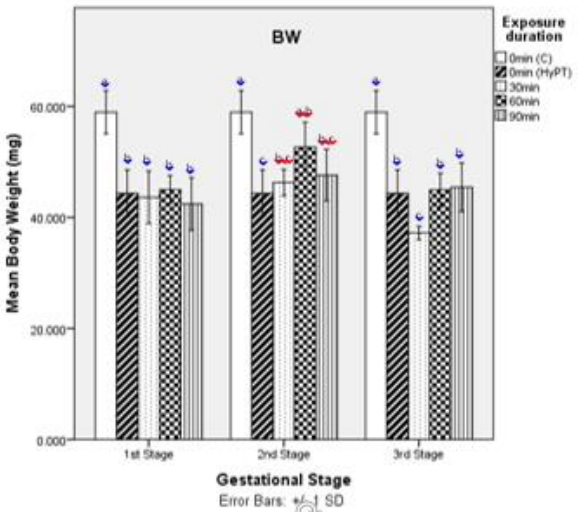


**Figure 2** Significant increase in body weight were noted in 2nd gestation with 60 minutes exposure

### 3.2 Longitudinal Growth

Control progeny mean average CRL were significantly different with HyPT and treatment groups (Figure 3). Meanwhile, litters mean TBL were significantly different between control and treatment groups except for the 2nd gestational with 90 minute exposure duration which also showed a significantly higher TBL compare to maternal HyPT ($P<0.05$).

### 3.3 Biometric Parameter

Statistically, there is no significant difference in BPD between Control and treatment groups except in the 1st gestational with 30 and 90 minutes ultrasound exposure and 3rd gestation with 30 minutes exposure. No significant differences were detected in BDP between treatment

### 3.4 Serum Calcium Concentration

No significant differences were detected in SCa concentration between Control and treatment groups except in the 1st gestational with 30 minutes ultrasound exposure and 3rd gestation with 30 minutes ultrasound exposure. Serum calcium were significantly increased in treatment groups from 2nd gestation stage and 3rd gestation stage with 90 minute ultrasound exposure compared to maternal HyPT (Figure 4).

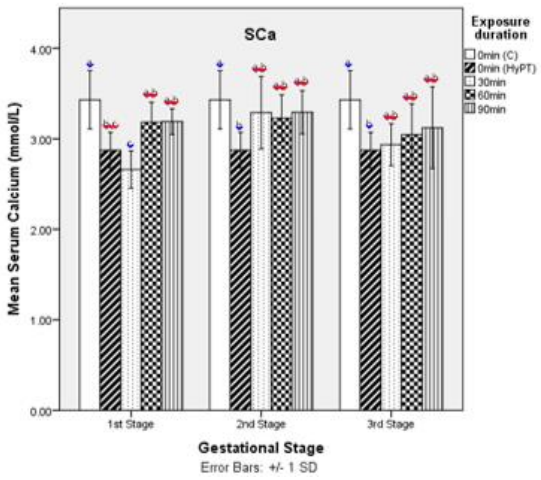


**Figure 4** Significant increase in SCa were noted in 2nd gestation compare to maternal HyPT

## 4.0 DISCUSSIONS

Foetal birth weight is the visible parameter to predict the sufficiency of maternal nutrient supply across the placenta and the condition of intrauterine environment [18]. Alteration in maternal calcium metabolism during pregnancy is a possible lead to foetal growth restriction (FGR) which is harmful to the foetal growth [19]. Animal study carried out proved that maternal hypoparathyroidism associated hypocalcemia gave birth to low birth-weight litters, small in size and hypocalcemic [20] with slightly marked increased in parathyroid hormone which is therefore inclined to the present study of the foetal physiological outcome. Litters' serum calcium concentration, birth weight, crown to rump length (CRL), total body length (TBL), bi-parietal diameter (BPD) and femur length (FL) were measured to evaluate the effects of prenatal ultrasound exposure under maternal hypocalcemia condition.

The progeny body weights were significantly increased after the prenatal ultrasound exposure in the middle of 2nd gestation with 60 minutes duration. Alteration in maternal serum calcium can possibly lead to placental insufficiency resulting in preeclampsia (PE) [21]. Epidemiological and clinical studies s illustrated that alteration in maternal calcium mineral metabolism play an important role in the pathogenesis of preeclampsia [21]. Besides, there exists a strong relationship between foetal body weight and placenta nutrient transfer suggesting that low foetal body weight is a result of placenta insufficiency [22].

Intrauterine hyperparathyroidism secondary to maternal hypoparathyroidism has been reported [23]. The postpartum complication of intrauterine hyperparathyroidism secondary to maternal hypocalcemia leads to foetal hypocalcemia and tetany in the early days of life [9]. An in-vivo experimental study discovered that prenatal

ultrasound heating caused significant reduction of PTH level in newborn rabbits [13, 14]. The progeny serum calcium showed a significant increase after perinatal ultrasound treatment was introduced in the middle of 2ndgestation with 30, 60 and 90 minutes and 3rd gestation with 90 minute duration.

Foetal skeletal mineralization, development and growth depend on the mother's calcium homeostasis status as it has direct effect over the transplacental calcium flux [15]. Maternal hypoparathyroidism associated with hypocalcemia exposed the foetal to intrauterine hypercalcemia condition which stimulate the increase in foetal PTH and PTH related protein resulting in poor bone mineralization and smaller foetal cortical envelope [12, 24]. Prenatal ultrasound is also proven to reduce the PTH level in newborn rabbits [13]. Thus, exposing the foetal to prenatal ultrasound insonation helps to reduce the foetal PTH level in maternal hypocalcemia condition. Foetal TBL and femoral too were significantly increased after the ultrasound insonation.

**Table 1** Reference values for litters from control and maternal hypoparathyroidism.

| | Unit | n | Control | | Hypoparathyroidism | |
|---|---|---|---|---|---|---|
| | | | Mean ($\bar{x}$) | SEM | Mean ($\bar{x}$) | SEM |
| **Maternal Ca** | mmol/L | 12 | 3.370 | 0.019 | 2.383 | 0.065 |
| **SCa** | mmol/L | 12 | 3.430 | 0.093 | 2.856 | 0.427 |
| **BW** | mg | 12 | 58.981 | 1.118 | 44.341 | 1.220 |
| **CRL** | cm | 12 | 10.250 | 0.917 | 9.192 | 0.125 |
| **TBL** | cm | 12 | 16.258 | 0.099 | 14.925 | 0.217 |
| **BPD** | µm | 12 | 16.293 | 0.227 | 15.411 | 0.123 |
| **FL** | µm | 12 | 18.872 | 0.293 | 17.167 | 0.235 |

a) Abbreviations: n, no. of sample; SEM, standard error; SCa, serum calcium level; BW, body weight; CRL, crown to rump length; TBL, total body length; BPD, biparietal diameter; FL, femoral

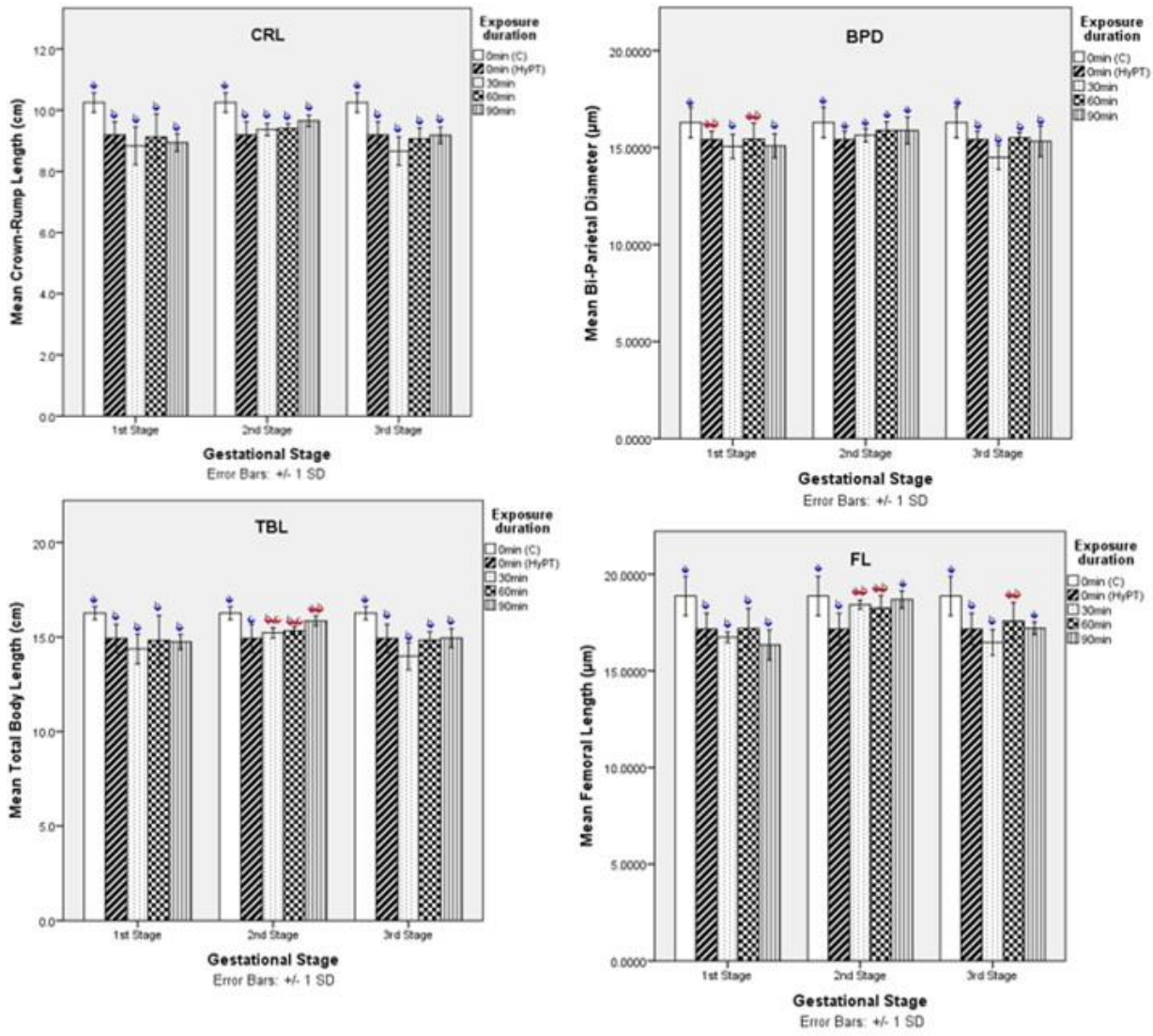


**Figure 3** TBL in treatment groups yield a significant increase following ultrasound exposure in 2nd gestation and 3rd gestation. Increase in FL were noted in 2nd gestation and the BPD were also increase following ultrasound treatment

Present study demonstrated that ultrasound intervention given during middle 2nd gestational stage resulted in a significant increase in progeny mean average BW, CRL, TBL, BPD FL and SCa. Foetal development differs in each gestation stage. Hence, the outcome of the prenatal ultrasound exposure in maternal hypocalcemia over physiological development also varies depending on the critical period of gestational sensitivity [25]. Middle of 2nd gestation is the early foetal development or organogenesis phase [26]. In rabbit, the osteogenesis occurs on the 16th day of gestation with the earliest centres of calcification present in the clavicle and mandible area [27]. Rapid appearance of calcification centre and progressive osteogenesis were observed during this time [27]. During this phase, embryo developed robustly which made it vulnerable to any changes induced during this time [25]. Therefore exposing the foetal to ultrasound as an intervention during the middle 2nd gestation is vital for the improvement of the foetal physiological status as a whole.

## 5.0 CONCLUSION

The intrauterine environment during foetal life is an important instrument to detect and determine the risk of future growth delay and health impairment. Maternal hypocalcemia during pregnancy exposes the foetal to intrauterine hypercalcemia condition which evokes the increase of foetal PTH and PTH related protein and causes an adverse result to skeletal mineralization. Exposing the foetal to ultrasound insonation helps to reduce the foetal PTH level while still in the womb thus reducing postnatal complications.

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