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Effect of multiple in-utero insonation on rabbit fetal thyroid hormonal level

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Abstract

Due to the increased use of ultrasound in the diagnosis of cyesis, it is necessary to study the possible adverse effect of insonation on thyroid hormone in fetuses. Sixteen (16) adult rabbit does that had kindled at least once were used for the study and were scanned using a Medison S600V® Ultrasound machine with a 6.5MHz transcutaneous curve-linear probe. All does were mated naturally by the introduction of a doe to a buck and then divided randomly into two groups. The does that were scanned (insonated)-, were properly restrained on dorsal recumbency, liberally shaved; aquasonic gel applied and scanned using a transcutaneous curve-linear probe for 5 minutes on the 5th day post coitus and thereafter on days 7, 12, 15, 20, 25, 27 and 29. The restrain and scanning procedures were mimicked on the group of does that were not scanned (non-insonated). Upon parturition, blood was collected from kits and serum was extracted and used to assay for total thyroxine (tT4) and triiodothyronine (tT3). Fetal thyroid glands were also harvested for histologic study. Mean tT3 concentration was 1.60 ± 0.90 µg/dl and 1.58 ± 0.03 µg/dl while mean tT4 was 1.46 ± 0.10 µg/dl and 1.39 ± 0.07 µg/dl for the non-insonated and insonated groups, respectively. P values of 0.610 and 0.132 for tT3 and tT4 concentration respectively showed that there is no statistically significant difference in the values even though there was a numerical difference between the groups. No histopathologic change on fetal thyroid gland from both groups was observed. In conclusion, although the values were not significantly different, the numerical decrease of both tT3 and tT4 in the insonated rabbits suggest the possibility that insonation could alter their values in fetuses when the threshold of ultrasound waves are increased and insonation prolonged.

Keywords: Fetus, Insonation, Rabbit, Thyroid hormone, Ultrasound

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Introduction

Ultrasonography is widely accepted as a safe imaging technique and it has become a crucial procedure in theriogenology, particularly for the assessment of gestational stages and diagnosis of many foetal abnormalities (Abramowicz *et al.*, 2008). In rabbits, pregnancy can be confirmed as early as day 7 of gestation and there is good correlation between sonographic changes and gestational age (Idris *et al*., 2016). Due to its non-usage of ionising radiation (Abramowicz *et al.*, 2008), this imaging modality has been extensively applied in various specialties such as cardiology, vascular studies, ophthalmology, gastroenterology, etc. (Deanne, 2002; Barnett, 2003; Abramowicz *et al.,* 2008). The soft tissue images are obtained without the need to administer contrast agents and thus are not dependent on organ function (David, 1997). Furthermore, several studies have revealed that ultrasound could also be incorporated into clinical interventions such as in ultrasound heat therapy, distraction osteogenesis, and sono-thrombolysis procedures (Deanne, 2002; Barnett, 2003; Abramowicz *et al.,* 2008). The apparatus is relatively cheap and has the distinction of having become cheaper with technological advances (David, 1997).

The basic principle of ultrasound imaging involves transmitting small pulses of ultrasound echo from a transducer into the body. As the ultrasound waves penetrate body tissues of different acoustic impedances along the path of transmission, some are reflected back to the transducer (echo signals) and some continue to penetrate deeper (Chan & Perlas, 2011). The echo signals returned from many sequential coplanar pulses are processed and combined to generate an image (Chan & Perlas, 2011).

In fetal physiological development, formation of thyroid glands begins at the middle till the end stage of gestation (Terranova, 2009). Hence, any external disruption like ultrasound heating could possibly affect the development of the glands, thus its secretion (Dom *et al*., 2013).

There are two basic mechanisms, thermal and non-thermal, by which ultrasound is known to affect biologic materials (Ndumbe *et al*., 2008; Sheiner & Abramowicz 2008; Brendt, 1986; Sikov, 1986a; Sikov, 1986b; Laurel, 1993). The thermal mechanism is associated with the absorption of acoustic energy by tissue and the generation of heat. It appears to be the best understood, and analytic models have been developed to predict the possible temperature elevation in tissue (Sikov, 1986a). The non-thermal effect (mechanical effect) of US insonation causal to acoustic cavitation is defined as the production of bubbles in liquid that may exhibit behavioral collapse and contribute to sudden release of energy (Kremkau, 2002; Barnett, 2003). It might then disrupt the molecular bonds and release free radicals such as hydroxyl radicals and hydrogen that consequently interfere with DNA (deoxyribonucleic acid) of the cells and lead to chromosomal damage (Barnett, 2003; Wu & Nyborg, 2008).

Due to the increased use of ultrasound in the diagnosis of cyesis and related diseases in veterinary medicine, it is necessary to study the possible adverse effect of ultrasound energy in fetuses exposed to ultrasound and also evaluate the effect of insonation on thyroid hormone of kits.

Materials and Methods

Animals

Four (4) adult rabbit-bucks and sixteen (16) mature non-gravid Dutch breed of rabbit does that had kindled at least once and at most thrice were used in the study. They were purchased from a reputable rabbit breeder in Samaru Zaria, Kaduna state. Their average age was 1½ years and average weight of 2 kg. They were housed in individual wooden made hutches in the animal pen of the Department of Public Health and Preventive Medicine of Faculty of Veterinary Medicine, Ahmadu Bello University Zaria. They were fed with grower feed containing 15% crude protein, 7% fat, 10% crude fiber, 1% calcium, 0.35% phosphorus, 2550 kg metabolizing energy and water was provided *ad libitum*.

Equipment and consumables

Medison S600V® Ultrasound machine with a 6.5 MHz transcutaneous curve-linear probe, 2 ml syringes with 23 G needles, sample bottles, glass slides, dissecting kits, surgical gloves, formalin, T3 and T4 ELISA kits.

Natural mating

Mating was achieved by the introduction of a doe into the hutch of a buck and allowed together for an average of 30 minutes. Afterwards, the does were removed and returned to their respective clutches. Does were randomly divided into two groups comprising of 8 does each, as non-insonated (control) and insonated.

Ultrasound examination

Each doe was physically restrained properly and placed on dorsal recumbency. Furs from the level of the xyphoid cartilage down to the pelvic region were gently made wet with soaked cotton wool in water and antiseptic soap applied. The furs were then liberally shaved. The shaved region was cleaned thoroughly with dry cotton wool, swabbed with wool soaked in antiseptic solution, and aquasonic gel was applied on the skin. A portable ultrasound machine with a 6.5 MHz transcutaneous curve-linear probe was used to scan the abdomino-pelvic region using the bladder as a landmark. The probe was placed on gently on the skin transversely and tilted longitudinally until a descriptive echographic image is achieved on the screen. This process was carried out on the 5th day post coitus and thereafter on days 7, 12, 15, 20, 25, 27 and 29. Upon getting a descriptive echographic image, the freeze button on the keyboard is used to freeze the echographic image which is then printed on thermal paper for documentation. Does in the insonated group were scanned for an average of 5 minutes while the procedure of scanning was mimicked in the non-insonated group for the same period with the ultrasound machine put off.

Blood collection and hormonal assay

Upon parturition, litter size and weight of both groups were determined and recorded. Kits were sacrificed via jugular venesection and 2 ml of blood was collected into plain sample bottles, allowed to stand for a day then centrifuged at 100 rpm to obtain serum which was used to assay thyroxine (T4) and triiodothyronine (T3) in the National Animal Production Research Institute Laboratory at Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria.

Working reagents and substrate solution for both T3 and T4 were prepared. Microplate wells were formatted for each serum reference, control and samples to be assayed. Reagents were added to wells and plate swirled gently for 20 – 30 seconds to mix and later incubated at room temperature for an hour. Microplate content were discarded, washed and blotted, substrate solution added and incubated at room temperature for 15 minutes. 0.05ml stop solution was added to each well, mixed gently and their absorbance read at 450 nm.

Evaluation of thyroid gland

Thyroid glands from sacrificed kits of both groups were harvested at necropsy and fixed in 10% formalin. After fixing, specimens were processed by embedding in paraffin, sectioned at 5µm thickness and stained with hematoxylin and eosin and viewed under the light microscope.

Data analysis

SPSS version 20 was used to analyze the data generated, mean and standard deviation of the mean for each variable was calculated. Student t-test was used to compare variables between the two groups. Values of $P \leq 0.05$ were considered significant.

Results

Mean litter size was 5.42 ± 0.7 and 3.71 ± 0.6 for the non-insonated and insonated groups respectively (Table 1). There was a statistically significant difference between the litter sizes of non-insonated group as against the insonated ($P < 0.05$). Mean litter weight was 43.8 ± 4.1g and 39.7 ± 2.0g for the non-insonated and insonated group respectively (Table 1). There was a statistically significant difference between litter weights of the non-insonated as against the insonated group ($P < 0.05$).

The total triiodothyronine (tT3) values were within 1.39 - 1.70 µg/dl with mean 1.60 ± 0.90 µg/dl for the non-insonated and 1.53 - 1.69 µg/dl with mean 1.58 ± 0.03 µg/dl for the insonated group (Table 2). There was a numerical difference between both groups, but the difference was not statistically significant ($P > 0.05$). Total thyroxine (tT4) values were within 1.28 - 1.60 µg/dl with mean 1.46 ± 0.10 µg/dl for the non-insonated and 1.28 - 1.54 µg/dl with mean 1.39 ± 0.07 µg/dl for the insonated group (Table 2). There was a numerical difference between both groups but the difference was not statistically significant ($P > 0.05$). There was no histopathologic change of the thyroid tissue under hematoxylin and eosin preparation of both groups. Follicles were seen to be closely packed and surrounded by rich capillary network with lumen containing colloids (Plate I).

Discussion

The result of litter size and weight showed a reduction in the insonated group as compared to the non-insonated. It is also plausible that frequent exposure to ultrasound may have influenced fetal number and growth. This is in agreement with Curto (1976) and O'Brien *et al*. (2008) where they reported an increased rate of neonatal death and reduced birth weight with no fetal malformations in animals exposed to continuous ultrasound. An investigation by Hollander (1972) using pulsed ultrasound of high frequency showed no adverse effects of any kind but Mannor *et al*. (1972) using continuous ultrasound in the same frequency range observed increased rates of malformations, fetal resorption and low birth weight, thus, pointing to hyperthermia as the most probable teratogenic mechanism. Ultrasound is absorbed by tissues and converted into heat energy (Schortinghuis *et al.*, 2003). Heating effects are the results of the absorption of ultrasound energy from an ultrasound beam (Gent, 1997). The heating effect is highly dependent on absorption coefficient of insonated tissue and because bones have high absorption coefficient values (Gent, 1997; Barnett *et al.*, 2010), they tend to absorb 60 percent or more of the incident ultrasound energy. Adult bone absorbs about 60 – 80 percent of the acoustic energy impinging on it (Sikov, 1986a). With fetal bone, there is a wide variation in absorptive behavior, depending on the degree of ossification. The clinical situation thus of greatest interest as far as thermal effects are concerned is a fetus in-utero with an ossified bone structure and a mother with a thin abdominal wall. In such a case there is little attenuation of acoustic energy because of the thin layer of intervening maternal tissue, yet there is a high degree of absorption associated with the fetal

Table 1: Mean litter size and mean litter weight of non-insonated and insonated group of rabbit

	Mean litter size	Mean litter weight (gram)
Non-insonated	5.42 ± 0.7	43.8 ± 4.1
Insonated	3.71 ± 0.6	39.7 ± 2.0

Table 2: Mean tT3 and tT4 concentration in µg/dl for non-insonated and insonated group of kits

	Mean tT3 conc. (µg/dl)	Mean tT4 conc. (µg/dl)
Non-insonated	1.60 ± 0.9	1.46 ± 0.10
Insonated	1.58 ± 0.03	1.39 ± 0.07

bone (Sikov, 1986a).

The results of the hormonal assay of total triiodothyronine (tT3) and total thyroxine (tT4) showed a numerical reduction but not statistically significant in the insonated group as compared to the non-insonated; ultrasound heating may have some effect on thyroid hormonal level. Dom *et al*. (2013) reported a significantly lowered parathyroid hormone level in the insonated group as against the non-insonated. This could possibly be because there was an increase in the time of exposure (60minutes) as against the time of exposure in this study (5 minutes). Also T4 concentration was lower than that of T3 within each group. This is can be attributed to the fact that T4 functions as a pro-hormone for the production of the more biologically and most metabolically active hormone T3 via deiodination of T4 in peripheral tissues (Klee, 1996).

During 3rd trimester of pregnancy, fetal bones are almost completed since they have reached the final stage of organogenesis. At this stage, the surrounding soft tissues are the responsible area for an inevitable rise of temperature during insonation (Gent, 1997), which can go up to a factor of five (Church & Miller, 2007). It is considered hazardous when embryo and fetus have been exposed to ultrasound for five minutes or more, as this will elevate both embryonic and fetal temperature to 4 °C above the normal body temperature (Barnett *et al.*, 2010). Hormonal activities are known to be extremely sensitive to any changes in heat especially during fetal stage and the amount of transport protein and total plasma hormone content can change under certain physiological conditions such as heat.

Hematoxylin and Eosin stained thyroid tissue appeared normal in both groups. Follicles filled with colloids were seen to be closely packed and surrounded by rich capillary network. The normalcy can be associated to the fact that the ultrasound heating is not focused directly on the foetal thyroid gland as ultrasound at sufficiently high intensities can generate cavitational activity that completely destroys cells (Kaufman *et al*., 1977; Moore & Coakley, 1977). Also, high intensity focused ultrasound (HIFU) have been reportedly used to treat patients with thyroidal nodes; exposed tissues became necrotic that later disintegrated and later shrank (Huedayi *et al*., 2014).

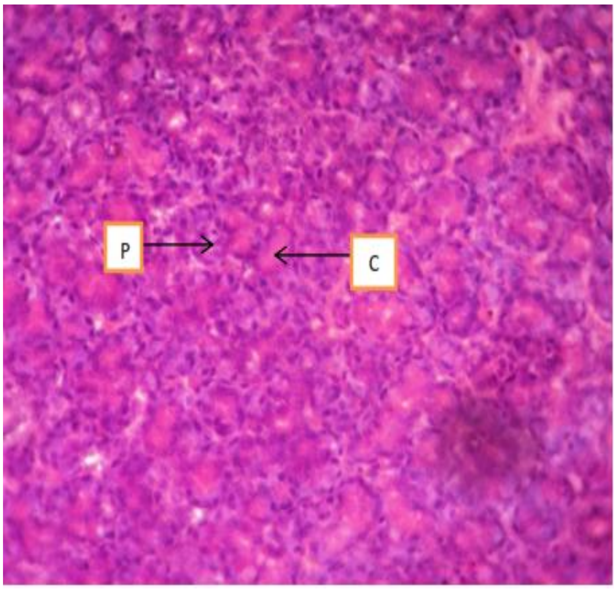

Plate I: Photomicrograph of normal thyroid gland, showing follicles containing colloids (C) surrounded by parafollicular cells (P) (Hematoxylin and Eosin stain, at x400 magnification)

In conclusion, although the results of the hormonal assay were not statistically significant, there were indications of a possible side effect of ultrasound heating due to the numerical differences observed. It is therefore recommended that further studies should be carried out on the possible effect of ultrasound on other measureable parameters in other species of animal. It is advised that repeated ultrasound imaging examination should be restricted only to clinical benefit to prevent possible side effects. Also, there is a need to further study the effect of insonation on neonates for some weeks after parturition.

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