



Alterations in haematological parameters of multiple *in-utero* insonated rabbits (*Oryctolagus cuniculus*)

SY Idris^{1*}, M Lawal², JS Enam¹, MS Muhammed¹, Y Muhammad³ & A Saleh¹

- ^{1.} Department of Veterinary Pathology, Ahmadu Bello University Zaria, Nigeria
- ^{2.} Department of Veterinary Surgery and Radiology, Ahmadu Bello University Zaria, Nigeria
- ^{3.} Department of Animal Health and Production, Binyaminu Usman Polytechnic Hadejia, Jigawa, Nigeria

*Correspondence: Tel.: +2347037865325; E-mail: drsheriffidris@gmail.com

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Abstract

The fact that ultrasound is generally perceived as a safe imaging modality due to its use of non-ionizing radiation encouraged its increasing use in the diagnosis of cyesis in Veterinary practice. Ultrasound heating during obstetric scans has the potential of increasing body temperature via absorption. The study was conducted to determine the effect of multiple prenatal ultrasounds scanning on haematological parameters of rabbit fetuses. The research involves *in vivo* experimental model using 16 pregnant does and exposing them to ultrasound for average of 5 minutes at day 7, 12, 15, 20, 25, 27 and 29 of gestation. They were divided into two groups as insonated and control, the restrain and scanning procedures were mimicked on the group of does that were not scanned (positive control). Upon parturition, blood samples were collected from the kits via jugular venesection into ethylene diamine tetra acetic acid containing tubes for haematology. There was a significant reduction ($p < 0.05$) of red blood cell count and haemoglobin concentration of insonated group. Values of packed cell volume and platelet were lower but not significant ($p > 0.05$) in insonated as against the control while a non-significant slight increase in total white blood cell count was seen in the control. Therefore, ultrasound heating from multiple *in-utero* exposure can possibly cause alterations in haematological values in Rabbits.

Keywords: Fetus, Haematology, Insonation, In-utero, Rabbit, Ultrasound

Introduction

Ultrasound has been widely accepted in clinical practice as a safe imaging technique particularly for pregnancy diagnosis, determination of gestational stages as well as early detection of fetal abnormalities (Saari-Kemppainen *et al.*, 1990; Abramowicz *et al.*, 2011). Its great considerable application beyond theriogenology include; cardiology, vascular studies, ophthalmology, gastroenterology (Deanne, 2002; Barnett 2003; Abramowicz *et al.*, 2011), surgery, medical imaging, physical and cancer therapy

(Shakeri-Zadeh *et al.*, 2001; Jafarian *et al.*, 2013; Shakeri-Zadeh *et al.*, 2013; Shakeri-Zadeh *et al.*, 2015). Some of the advantages of prenatal ultrasound include: obtaining an accurate due date, diagnosing missed miscarriages and any uterine abnormalities (Hande & Devi, 1992; Zelop *et al.*, 1994; Duggan *et al.*, 1995) and monitoring fetal development (Hande & Devi, 1992; Idris *et al.*, 2016). 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).

Despite its medical benefits, prenatal ultrasound has also been utilized for social and business purposes. Most pregnant women worldwide routinely have prenatal ultrasound scans, as they believe it can enhance parental-fetal bonding (Isa & Dom 2016). In veterinary practice, dog breeders for commercial purposes are not also left out in the routine prenatal ultrasound scan as they are interested in knowing status of their investment without considering its side effects. However, the increase of power outputs and extended time of scans along with weaker regulations for the use of ultrasound machine has caused it to become increasingly difficult to ignore the contribution of increasing fetal ultrasound exposure during recent decades (Miller *et al.*, 1998; Duck 2003; Church & Miller 2007). Animal studies have suggested that exposure to prenatal ultrasound could lead to low birth weight (Stolzenber *et al.*, 1980; Greshfeld & Murayama 1988; Md. Dom *et al.*, 2012; Idris *et al.*, 2017), reduced litter size (Idris *et al.*, 2017), changes in bone mineral density and parathyroid (Md Dom 2013), thyroid hormone (Idris *et al.*, 2017) and an increase in temperature in the fetal brain (Andreassi *et al.*, 2007).

The biophysical effects of ultrasound are traditionally divided into thermal and mechanical (non-thermal) (Ndumbe *et al.*, 2008; Sheiner & Abramowicz, 2008). The 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, 2003), it tends to absorb 60% or more of the incident ultrasound energy. 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, 2003). The non-thermal effect (mechanical effect) of ultrasound insonation causal to acoustic cavitation is defined as the production of bubbles in liquid that may exhibit behavioral of collapse and contribute to sudden release of energy (Kremkau, 2002; Barnett, 2003). Mechanical effect is also regarded as any biological effect from ultrasound

when accompanied by elevation of temperature less than 1°C above normal physiologic levels (Stratmeyer, 2008).

Considering the easy access and frequent use of ultrasound vis-à-vis the clinical importance of bone marrow hematopoietic and peripheral circulatory cells, it is necessary to determine the effects of insonation on haematological parameters in-utero.

Materials and Methods

Study 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 and weight were 1½ years and 2 kg respectively. 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 and fed with commercial 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*.

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 (Medison S600V®) 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 gently on the skin transversely and tilted longitudinally until a descriptive echographic image was 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. 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 haematological analysis

Upon parturition, kits were sacrificed via jugular venesection and 2 ml of blood was collected into green capped EDTA (Ethylene Diamine Tetraacetic Acid) tubes and taken to clinical pathology laboratory for analysis. A total of 41 blood samples were analysed. Red blood cell (RBC), total white blood cell (TWBC) and platelets (PLT) were counted using haemocytometers. Packed cell volume (PCV) was determined using the microhaematocrit method while the haemoglobin concentration (Hb) was measured by the cyanmethaemoglobin method (Drabkin, 1945). From the values obtained the erythrocytic indices; mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentrations (MCHC) were calculated (Zinkl, 1986). Differentiation of white blood cells into segmented neutrophils, band cells, lymphocytes, eosinophils, monocytes and basophils was carried out by microscopic examination of Giemsa-stained thin blood smears; 100 cells were counted to determine the differential cell count.

Data analysis

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

Results

Table 1 shows the mean haematological variables of control and insonated kittens while Table 2 shows their ranges. The haematocrit indicated a non-significant ($p > 0.05$) lower value in the insonated (29.4 ± 0.52) as against the control (30.3 ± 0.62). Haematocrit has a range of 27 - 36% and 26 - 34% for the control and insonated group respectively.

Red blood cell count showed a reduction of 15.3% ($p < 0.01$) in insonated group compared with the control. There was also significant difference ($p = 0.015$) observed in haemoglobin concentration of insonated in comparison with that of control (Table 1). RBC values were within $4.9 - 7.4 \times 10^{12}/L$ with mean of $6.0 \pm 0.13 \times 10^{12}/L$ and $4.3 - 6.4 \times 10^{12}/L$ with mean of $5.2 \pm 0.12 \times 10^{12}/L$ while Hb concentration values were within 12 - 14 g/dL with mean of 13 ± 0.2 and 11 - 14 g/dL with mean of 12 ± 0.12 for both control and insonated group respectively (Table 2).

From the erythrocytic indices calculated, no significant difference ($p > 0.05$) was observed with exception of a significant increase ($p = 0.02$) in MCV of insonated as against the control group. Mean values of MCV, MCHC, and MCH for control and insonated group respectively were 51 ± 1.7 fl, 43 ± 0.67 g/dL, 22 ± 0.76 pg and 58 ± 1.8 fl, 42 ± 0.15 g/dL, 24 ± 0.68 pg (Table 1).

There was no significant difference ($p > 0.05$) observed in both PLT and TWBC count of both groups. However, much lower PLT was observed in insonated when compared to control group. PLT values were within $380 - 660 \times 10^9/L$ with a mean of $500 \pm 17 \times 10^9/L$ and $330 - 650 \times 10^9/L$ with a mean of $460 \pm 21 \times 10^9/L$ while TWBC values were within 5.1 - 6.5 x

Table 1: Mean \pm SD haematological parameters for control and insonated group of kittens (*Oryctolagus cuniculus*)

Parameter	Control	Insonated	p value
PCV (%)	30.3 ± 0.62	29.4 ± 0.52	0.28
RBC ($\times 10^{12}/L$)	$6.0 \pm 0.13^{**}$	$5.2 \pm 0.12^{**}$	< 0.01
Hb (g/dL)	$13 \pm 0.2^*$	$12.0 \pm 0.17^*$	0.015
PLT ($\times 10^9/L$)	500 ± 17	460 ± 21	0.22
MCV (fl)	$51 \pm 1.7^*$	$58 \pm 1.8^*$	0.02
MCHC (g/dL)	43 ± 0.67	42 ± 0.15	0.12
MCH (pg)	22 ± 0.76	24 ± 0.68	0.08
TWBC ($\times 10^9/L$)	5.9 ± 0.12	6.0 ± 0.09	0.78
Lymphocytes ($\times 10^9/L$)	4.1 ± 1.3	4.6 ± 1.5	0.11
Neutrophils ($\times 10^9/L$)	1.3 ± 0.9	1.1 ± 0.4	0.30
Monocytes ($\times 10^9/L$)	0.2 ± 0.05	0.1 ± 0.02	0.06
Eosinophils ($\times 10^9/L$)	0.1 ± 0.02	0.0 ± 0.00	0.2
Band cells ($\times 10^9/L$)	0.3 ± 0.01	0.2 ± 0.01	0.13
Basophils ($\times 10^9/L$)	0.00 ± 0.00	0.00 ± 0.00	-

Values with the same superscript asterix within the same row are statistically significant with $p \leq 0.05$
p values ≤ 0.05 are significantly different

Table 2: Range values of haematological parameters for control and insonated group of kittens (*Oryctolagus cuniculus*)

Parameter	Control	Insonated
PCV (%)	27 – 36	26 – 34
RBC ($\times 10^{12}/L$)	4.9 – 7.4	4.3 – 6.4
Hb (g/dL)	12 – 14	11 – 14
PLT ($\times 10^9/L$)	380 – 660	330 – 650
MCV (fl)	41 – 71	42 – 76
MCHC (g/dL)	41 – 52	41 – 43
MCH (pg)	17 – 33	18 – 31
TWBC ($\times 10^9/L$)	5.1 – 6.5	5.4 – 6.4
Lymphocytes ($\times 10^9/L$)	3.9 – 4.3	4.2 – 4.7
Neutrophils ($\times 10^9/L$)	0.8 – 1.5	1.0 – 1.3
Monocytes ($\times 10^9/L$)	0.1 – 0.3	0.1 – 0.2
Eosinophils ($\times 10^9/L$)	0.0 – 0.1	0.0 – 0.0
Band cells ($\times 10^9/L$)	0.1 – 0.4	0.0 – 0.3
Basophils ($\times 10^9/L$)	0.0 – 0.0	0.0 – 0.0

$10^9/L$ with a mean of $5.9 \pm 0.12 \times 10^9/L$ and $5.4 - 6.4 \times 10^9/L$ with a mean of $6.0 \pm 0.09 \times 10^9/L$ (Table 2).

Discussion

When mammalian blood is heated above normal temperature, spontaneous hemolysis of the red blood cells occurs (Gershfeld & Muranyama, 1988). Energy absorption of the ultrasound beam (Ahmad *et al.*, 2013; O'Brien, 2007) can lead to thermal effects, which is the main potentially adverse biological effect (Isa & Md Dom 2016). The thermal mechanism is associated with the absorption of acoustic energy by tissue and the generation of heat (Abramowicz *et al.*, 2011), this heating during obstetric scans has the potential of triggering heat stress in pregnant does. RBCs of multiple insonated kittens in this study showed a significant reduction which is in agreement with previous reports on neonates subjected to prenatal ultrasound (Matics *et al.*, 2013; Isa & Md Dom, 2016; Mehrpou *et al.*, 2016). This could be attributed to haemolysis that might occur due to temperature increase resulting from insonation. As supported by Karle (1968; 1969), a small increase in temperature associated with pyrexia is sufficient to induce haemolysis in erythrocytes of rabbits and human. Developmental hematopoiesis in mammals is initiated within the yolk sac of the embryo and is taken over by the bone marrow at a later stage of gestation (Tarantal *et al.*, 1993). 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 a greater percentage of the incident ultrasound energy. Heat absorption by bone as a result of ultrasound exposure at crucial

developmental stages might result in arrest in maturation of the myeloid lineage of blood cells (Tarantal & Hendrickx, 1989).

The significant decrease in Hb in the insonated group is probably suggestive of cells destruction due to ultrasound heating that creates blood cells haemolysis. When RBCs are destroyed, Hb escapes into the plasma and causes iron depletion (Rother *et al.*, 2005), this is further supported by the decrease in MCHC observed in insonated group. This finding is in agreement with studies of Zaiki *et al.*, 2013 and Isa & Md Dom, 2016. As at this study we could not ascertain if the observed haematological alterations is due to heat and/or cavitation as this will require a more define in vivo research with state-of-the-art equipment. In utero ultrasound exposure from this study did not show significant difference in WBC and PLT. This is contrary to leukocytosis reported by Tarantal *et al.*, 1993 and Isa & Md Dom, 2016. However, the reason for the leukocytosis remain unclear to them, they postulated inability of the progenitor population in fetal bone marrow to mature and/or a delay in formation of stromal supporting cells as possible explanation of the alterations.

It can however be concluded that repeated in-utero insonation can probably result in alterations in haematological parameters specifically on red blood cells count and haemoglobin concentration due to haemolysis. Therefore, further studies are recommended to observe the haematological dynamics weeks post parturition. It is also advised that in-utero insonation be restricted only to clinical benefits and ALARA (as low as reasonably achievable) adopted at all situations.

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Conflicts of Interest

The authors declare no conflict of interest.

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