SONOGRAPHIC STUDY OF GESTATION INRABBIT-DOE (*ORYCTOLAGUS CUNICULUS*) AND THE EFFECT OF ISONATION ON FETAL THYROID HORMONAL LEVEL

BY

Sheriff Yusuf IDRIS, DVM (ABU) 2011

(M.Sc./VET-MED/22913/2012-2013)

A DISSERTATION SUBMITTED TO THE POSTGRADUATE SCHOOL, AHMADU BELLO UNIVERSITY, ZARIA IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF DEGREE OF MASTER OF SCIENCE IN VETERINARY RADIOLOGY

DEPARTMENT OF VETERINARY SURGERY AND RADIOLOGY

AHMADU BELLO UNIVERSITY, ZARIA

NOVEMBER, 2015

DECLARATION

I declare that the work in this Dissertation entitled "**Sonographicstudy of gestation in rabbit-doe (***Oryctolaguscuniculus***) and the effect of isonation on fetal thyroid hormonal level**" has been performed by me in the Department of Veterinary Surgery and Radiology, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria under the supervision of Dr. S.T. Fadason and Dr. P.O. Ibinaiye.

The information from the literature has been duly acknowledged and a list of reference provided. No part of this Dissertation has been submitted elsewhere for a degree or diploma award at this or any Tertiary Institution.

Sheriff Yusuf IDRIS	…….	……..
Name of student	Signature	Date

CERTIFICATION

This dissertation titled "**SONOGRAPHIC STUDY OF GESTATION IN RABBIT-DOE (*ORYCTOLAGUS CUNICULUS)* AND THE EFFECT OF ISONATION ON FETAL THYROID HORMONAL LEVEL**" by SHERIFF YUSUF IDRIS meets the regulations governing the award of the degree of Master of Science of Ahmadu Bello University, and is approved for its contribution to knowledge and literary presentation.

Dr. S. T. Fadason	……	…….
Chairman, Supervisory Committee	Signature	Date
Dr. P.O. Ibinaiye	……	…….
Member, Supervisory Committee	Signature	Date
Dr. S. T. Fadason	……	…….
Head of Department	Signature	Date
Prof. Kabir Bala	……..	……..
Dean, School of Postgraduate Studies	Signature	Date

DEDICATION

This Dissertation is dedicated to my lovely mother in person of Mrs. Rakiya Idris for her endless love and immeasurable support.

ACKNOWLEDGEMENTS

All thanks go to Almighty Allah (SWT) for enabling me reach this phase of my life despite all obstacles encountered so far.

My profound gratitude and appreciation goes to my supervisors, Dr. S.T. Fadason, Dr. P.O. Ibinaiye for their patience, guidance and advice to see the success of this work. I want to thank Drs. M. Lawal, M. Bisalla, and M. Abdurrhmanfor their support during the course of this work, may Allah (SWT) bless them abundantly.

A special appreciation goes to Mr. Bitrus of histopathology laboratory, Mr. Yunusa of clinical pathology laboratory and Mr. Yohanna of NAPRI laboratory. To members of my team (Drs. Hassan, Latifat, Buhari, Hassana, Ochife, Aliyu, Taiwo, Felix and Mansur), may Allah continue to bless you all and reward you for all your efforts.

To the entire staff of the department of Veterinary Surgery and Radiology, thank you all for your collective effort. I cannot but also thank Arch. Diallo,Zainab, Jamila, Manir, Aliyu, Ahmed, Isah, and Hamidu for their collective contributions.

To my lovely parents Alh. & Mrs. Idris Yusuf and my siblings (Bashir, Rafiat, Khalid and Farida) may Allah (SWT) reward you immensely for the support you have given me.

ABSTRACT

Sixteen (16) mature non-gravid Does that had kittened at least once and at most thrice, had ultrasound (US)SV600® Ultrasound machine with a 6.5 MHz curvilinear transducer were used for the study. The experiment was designed to evaluate baseline information on the sonographic features of gestation in rabbit-doe and to investigate the effect of isonation on thyroid hormonal level of kittens upon parturition. All Does were scanned during the acclimatization period to ensure they were not gravid. They were all mated naturally by the introduction of a doe to a buck and then dividedrandomly into two groups A and B. Does in group B were scanned for an average of 5 minutes on the 5th day post coitus and thereafter on day 7, 12, 15, 20, 25, 27 and 29 while the procedure of scanning was mimicked in group A Does without isonation. After parturition, blood samples were collected from kittens of each litter via jugular venesection following light chloroform anaesthesia. Embryonic vesicles, seen as being small roundedanechoic (darkened) structures were first seen on day 7 of gestation. Hypoechoic structures within vesicles corresponding to embryo and placenta were seen on day 12 with an increase in size on day 15 of gestation. Bony formation, bi-parietal diameter (BPD), vertebral column, fetal heart and fetal heart rate were noted atadvanced gestation. The isonatedgroups were 100% pregnant at first to mid trimester. At term, only 12.5% remained pregnant. However, upon rebreeding 71.5% pregnancy success was achieved to parturition. Mean litter size and weight were 5.43 ± 0.9, 43.7 ± 4.3 g and 3.71 ± 0.7, 39.6 ± 2.1 g for the control and isonated group respectively. There was a statistical significant difference in the litter size and weight ($P < 0.05$). Mean Total Triiodothyronine (tT3) serum concentration was 1.60 ± 0.11 µg/dl and 1.58 ± 0.05 µg/dl respectively for control and isonated group while mean total Thyroxineserum concentration (tT4) was 1.44 ± 0.12 µg/dl and 1.39 ± 0.09 µg/dl for the control and isonated group respectively. The differences were not statistically significant ($P > 0.05$). There were

nohistopathologic changes on fetal thyroid tissue of both groups. In conclusion, this study demonstrated that ultrasound can be used effectively to diagnose pregnancy in rabbit-doe as early as the 7^{th} day of gestation. Frequent ultrasound exposure during pregnancy in does can result in an increase fetal death and reduce fetal size and weight. There is possibility of an increase threshold to ultrasound waves and ultrasound heating having effect on fetal hormone level especially in prolonged examination upon several isonation. This suggests that frequent isonation may not be entirely safe in pregnant does.

Table of Contents

Title Page.....I

Declaration.....II

Certification.....III

Dedication.....IV

Acknowledgement.....V

Abstract.....VI

Table of Contents.....VIII

List of Tables.....XIII

List of Figures.....XIV

List of Plates.....XV

List of Appendices.....XVI

Abbreviations……XVII

1.0 INTRODUCTION.....1

1.1 Background Information.....1

1.2 Statement of Research Problems.....3

1.3 Justification.....3

1.4 Aim of the Study.....4

1.5 Objectives of the Study.....4

1.6 Research Questions.....4

2.0 LITERATURE REVIEW.....5

2.1 Anatomy and Physiology of the Reproductive System.....5

2.1.1 Anatomy of the genitalia of rabbit……..5

2.1.2 Sexual receptivity……6

2.1.3 Oestrus cycle and behavior…….7

2.1.4 Ovulation…….8

2.1.5 Fertilization and gestation……...9

2.1.6 Pseudopregnancy……9

2.1.7 Oestrus synchronization……..9

2.1.8 Artificial Insemination……..10

2.2 The Thyroid Gland.....11

2.2.1 Development of the thyroid gland……11

2.2.2 Anatomy of the thyroid gland……...13

2.2.3 Histology of the thyroid gland……..……...15

2.2.4 Ultrasonography of the thyroid gland……...16

2.3Sonographic Imaging.....21

2.3.1 General principles.....21

2.3.2 Wavelength and frequency of ultrasound……23

2.3.3 Propagation of speed……23

2.3.4 Transducers……...24

2.3.5 Ultrasound interaction with tissue…….……24

2.3.6 Attenuation……27

2.3.7 Reflection, transmission, refraction, scattering and absorption……28

2.3.8 Scanning methods…….29

2.3.9 Resolution…….34

2.3.10 Artifacts……...36

2.3.11 Interpretative principles……..36

2.3.12 Echogenecity……...37

2.3.13 Safety……..38

2.4 Application of Ultrasound in Obstetrics……..39

2.4.1 Routine ultrasonography screening……..39

2.4.2 Pregnancy diagnosis…….40

2.4.3 Determination of fetal number and viability……40

3.0 MATERIALS AND METHOD ……41

3.1 Materials…….41

3.1.1 Experimental animals……41

3.1.2 Equipment and consumables……41

3.2 Methodology ……..42

3.2.1 Pre-mating scanning……..42

3.2.2 Natural mating……..42

3.2.3 Post-mating scanning……42

3.2.4 Blood collection ……43

3.2.5 Evaluation of thyroid gland……..43

3.3.6 Assay for thyroxine (T4) and triiodotyronine (T3)……...43

3.3 Data Analyses……...……..44

4.0 RESULTS……..45

4.1 Ultrasonography……..……..45

4.2 Litter Size and Weight……..…….54

4.3 Hormonal Assay.....56

4.4 Histopathology…..……...…60

5.0 DISCUSSION…….63

6.1 SUMMARY, CONCLUSION AND RECOMMENDATION……...66

REFERENCES…….67

APPENDICES……...……...…….76

LIST OF TABLES

Table	Title	Page
Table 4.1	Mean litter size and litter weight of control and isonated group of rabbit	55
Table 4.2	Mean tT3 and tT4 serum concentration in µg/dl for control and isonated group of kittens	57

LIST OF FIGURES

Figure	Title	Page
Figure 2.1	Genital apparatus of the female rabbit (non-pregnant genitalia)	6
Figure 4.1:	Variation of mean bi-parietal diameter with gestation age in Isonated group of rabbits	53
Figure 4.2:	Bar Chart showing tT3 serum concentration of Control and Isonated kittens	58
Figure 4.3	Bar Chart showing tT4 serum concentration of Control and Isonated kittens	59

LIST OF PLATES

Plate	Title	Page
Plate I	Sonogram of a doe (E7) taken on day 7 of gestation showing embryonic vesicles (EV)	47
Plate II	Sonogram of a doe (E2) taken on day 12 of gestation showing theconceptus (C) within amniotic fluid (AF)	48
Plate III	Sonogram of a doe (E2) on day 15 of gestation showing multiple Fetuses	49
Plate IV	Sonogram of a doe (E5) on day 25 of gestation showing bi-parietaldiameter measuring 18mm	50
Plate V	Sonogram of a doe (E2) on day 25 of gestation showing the vertebral column (VERT) and fetal heart rate (FHR) reading 125beats/minute	51
Plate VI	Sonogram of a doe (E2) on day 29 of gestation showing well developedvertebral column (VERT)	52
Plate VII	Photomicrograph of thyroid gland (controlled kitten)showing follicles (F)containing colloids surrounded by C-cells (C) (H&E stain x400 magnification)	63
Plate VIII	Photomicrograph of thyroid gland of an isonated kitten, showing follicles(F) containing colloids surrounded by C-cells (C) (H&E stain x400 magnification)	64

LIST OF APPENDICES

Appendix	Title	Page
Appendix I	Showing restraining of doe prior to scanning	76
Appendix II	Showing ultrasound scanning of the abdominal area of a doe in a transverse plane	77
Appendix III	Showing a process of natural mating	78
Appendix IV	Showing ultrasound screen capturing the image of a scan	79
Appendix V	Showing Accubind ELISA microwells kit for Thyroid hormone assay	80
Appendix VI	Showing result of haemogram of alldoes taken prior to mating	81

ABBREVIATIONS

AF	Amniotic fluid
AI	Artificial insemination
ALARA	As Low As Reasonably Achievable
BPD	Bi-parietal diameter
CT	Computerized tomography
CW	Continuous-wave
dB	Decibels
DNA	Deoxyribonucleic acid
dZ	Impedance mismatch
EV	Embryonic vesicle
FDA	Federal drugs administration
FH	Fetal heart
FHR	Fetal heart rate
FNA	Fine needle aspiration biopsy and cytology
GnRH	Gonadotrophin releasing hormone
HcG	Human chorionic gonadotrophin
H&E	Hematoxylin and eosin
HPT	Hypothalamic pituitary thyroid
KHz	Kilo hertz
LH	Leuteinzing hormone
MHz	Megahertz
MRI	Magnetic resonance imaging

PAS	Periodic acid schiff
pc	Post coitus
PGF2α	Postglandin F2α
PMSG	Pregnant mare serum gonadotrophin
pp	Postpartum
PW	Pulsed-wave
PZT	Lead zirconatetitanate
SE	Standard error
SPSS	Statistical package for social sciences
SPTA	Spatial peak temporal average
TGC	Time gain compensation
TSH	Thyroid stimulating hormone
T3	Thyroxine
T4	Triiodothyronine
US	Ultrasound

CHAPTER ONE

INTRODUCTION

1.1 Background Information

Ultrasound (US) is an oscillating sound pressure wave with a frequency greater than the upper limit of the human hearing range. Ultrasonic imaging (ultrasonography) is used in both veterinary and human medicine (Novelline, 1997). Today, US is one of the most widely used imaging technologies in medicine. It is portable, readily available, free of radiation risk, and relatively inexpensive when compared with other imaging devices, such as magnetic resonance and computedtomography. Furthermore, US images are tomographic, i.e., offering a "cross-sectional"view of anatomical structures (Edler and Lindstrom, 2004).

The frequencies used in medical imaging is between 2 and 10 megahertz (MHz), where 1 MHz is 1 million cycles per second, 50 times greater than the maximum frequency of audible sound by the human ear; hence the name ultrasound(Luc DesCoteaux, 2010). US imaging is based on the 'pulse-echo' principle in which a short burst of ultrasound is emitted from a transducer and directed into tissue (Burns, 2011).

Reflection of sound waves produces the echoes that result in the echographic image. Reflection occurs when the ultrasound wave meets an acoustic interface, which is the interface between two media with different acoustic impedances (Luc DesCoteaux, 2010). Reproductive ultrasonography has increased the knowledge of the changes during early pregnancy in different animals (Ginther and Pierson, 1984; Curran, 1986).With the use of a real-time, B-mode ultrasonography and 5 MHz transducer, pregnancy can be detected as early as $9^{th}$to 12^{th}days post artificial insemination into gestation (Ginther and Pierson, 1984; Curran, 1986).

USis 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). 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. 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).

Ultrasonography as an imaging tool in Veterinary Medicine is useful for diagnosis in many animal species. In rabbits, it has been utilized in early pregnancy detection (Ypsilantis and Saratsis, 1999). Ultrasonography has been widely applied for diagnosis of early pregnancy in domestic animals such as cow (White *et al*., 1984, Seratsis*et al*., 1993), mare (McKinnon *et al*., 1993), sheep (White *et al*., 1984), goat (Haibel, 1988), sow (Inaba*et al*., 1983), bitch and queen (Burke and Bardertscher, 1986). The useful technological innovation allows early, accurate and practical detection of pregnancy (Ypsilantis and Saratsis., 1999).

Rabbit pregnancy is traditionally diagnosed by palpation of the uterus through the abdominal wall on day 12-14 post coitus (p.c) or day 10-12 p.c by experienced practitioners (Hagen, 1974). ELISA kits for bovine progesterone (Ovucheck ELISA kit/Cambridge Life Sciences) have also been used to measure rabbit plasma or serum progesterone concentration on day 17-18 p.c in order to differentiate pregnant from pseudopregnant does (Morrell, 1990, 1993).

Hormonal activities are extremely sensitive to any changes in heat especially during fetal stage (Peavy, 2009).The amount of transport protein and total plasma hormone content are known to

change under certain physiological conditions, while altered hormone-receptor interactions may lead to endocrine abnormalities (Peavy, 2009).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(Sulaiman*et al*., 2013).

1.2 Statement of Research Problems

Although rabbit is an important experimental model in human reproduction studies, conclusive evidence of pregnancy is difficult to obtain in doe. Palpation under anesthesia may causebreeding errors and result in problems such as maternal or fetal death. Because of these considerations, ultrasonography could be a good instrument to detect and investigate cyesis in therabbit (Soroori*et. al*., 2008)

There is paucity of information on the sonographic features of the reproductive cycle of rabbit-doe in Nigeria. Even though ultrasound offers several clinical advantages, there is still a possibility on its adverse effects especially for highly proliferative cells, which then may speculate that this imaging modality cannot be completely regarded as harmless (Barnett, 2003).

1.3 Justification of the Study

Due to paucity of information on sonographic features of the reproductive cycle of rabbit-doe and increase in their economic uses, there is a need to utilize ultrasound to establish baseline information in pregnant does. Due to the increase use of ultrasound in the diagnosis of cyesis and other related diseases in veterinary medicine, it is necessary to study the possible adverse effect of ultrasound energy in fetuses exposed to ultrasound duringcyesis.

1.4 Aim of the Study

- To evaluate the sonographic features of gestationin rabbit-doe.
- To investigate the effect of isonationon thyroid hormonal level of kittens.

1.5 Objectives

- To sonographically study gestation in rabbit-doe
- To map out various sonographic features that occur during cyesis
- To evaluate the effect of isonation on thyroid hormonal level of kittens

1.6 Research Questions

- What are thesonographic changes in the gestational stages in rabbit-doe?
- Does ultrasound energy (heating) have effect on foetalthyroid hormonal level?
- Is there a significant difference in thyroid hormonal level of kittens exposed to ultrasound during gestation as compared to those not exposed?
- Is diagnostic ultrasound, as presently used in clinical practice, safe for rabbit fetus?

CHAPTER TWO

LITERATURE REVIEW

2.1 Anatomy and Physiology of the Reproductive System

2.1.1Anatomy of the Genitalia of Rabbit

Pair of ovaries is oval-shaped present on both left and right sides at same level and do not exceed 1-1.5cm. They are small and compact bodies. The ovaries lie behind the kidneys and attached to the dorsal wall of the abdominal cavity by the mesovaria. The oviduct is paired, located beneath the ovaries, made up of the duct, ampulla and isthmus. Though outwardly, the uterine horns are joined at the back into a single organ, there are actually two independent of about 7cm, opening separately through two cervical ducts into the 6–10cm median and highly muscular chamber-vagina. The urethra opens midway along the vagina at the vaginal vestibule. The vestibule opens midway along the vaginal vestibule. The vestibule opens outside by a longitudinal aperture, the vulva lying in front of the anus. The glands of Bartholin and the preputial glands can be identified. The whole is supported by the broad ligament attached at 4 main points under the vertebral column (Lebas*et al*., 2014).

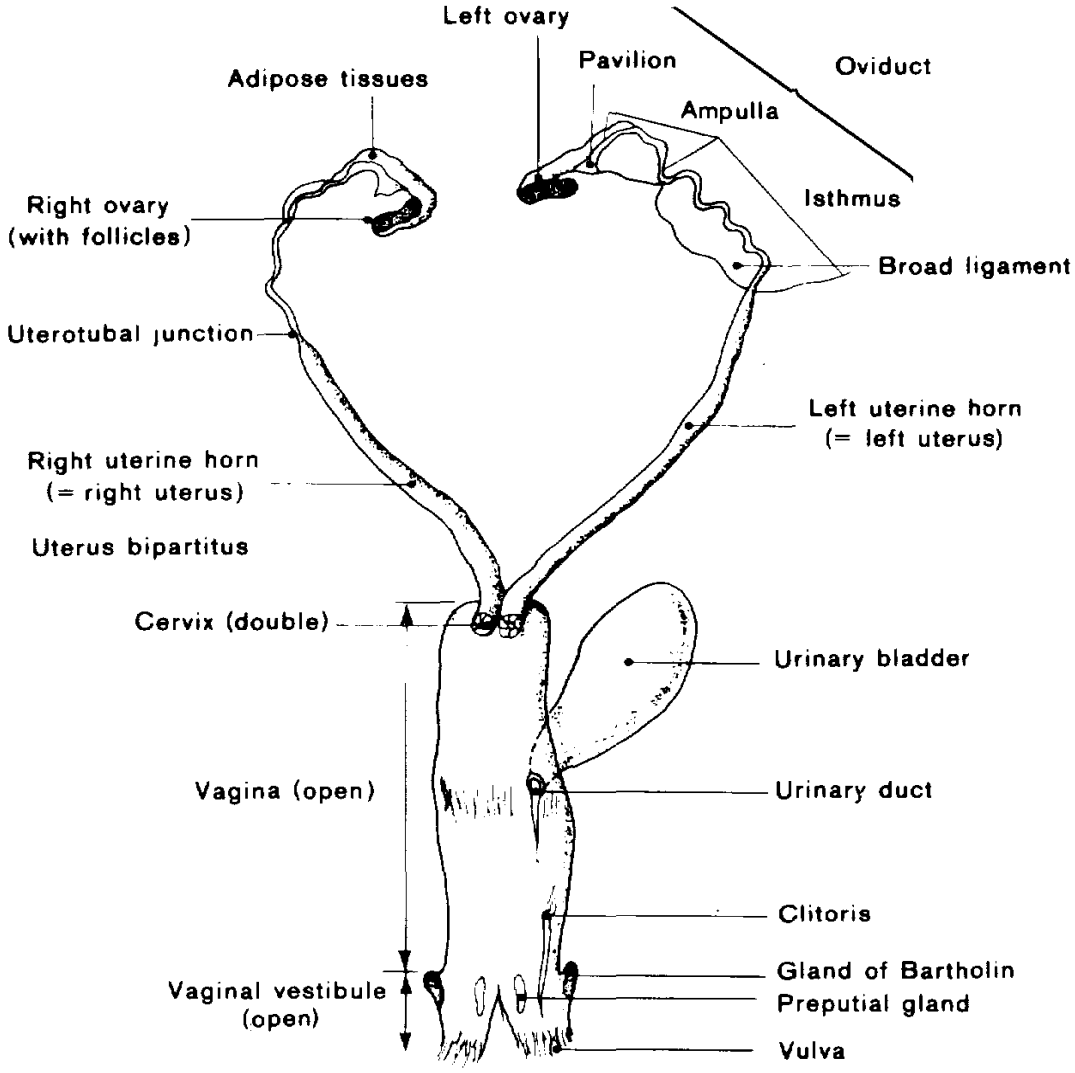


Fig. 2.1 Genital apparatus of the female rabbit (non-pregnant genitalia).
Source: http://www.fao.org/docrep/X5082E/X5082E0C.GIF

2.1.2 Sexual receptivity

A doe is regarded as sexual receptive if, in the presence of a buck she adopts a lordosis position and accepts the mate; the colour of the vulva is also a quite good indicator of receptivity and is easy to check (Maertens and Luzi, 1995). Sexual receptivity is the simplest index to detect the ovary status: a receptive doe generally exhibits a higher number of large follicles (Kermabon*et*

al., 1994) and a higher oestrogen level with respect to non-receptive ones (Rebollar*et al*., 1992; Ubilla and Rebollar, 1995).

Reproductive functions of rabbit does are not strictly related to sexual receptivity, and females can ovulate in every oestral phase,but the reproductive performance increases in receptive females (Theau-Clement *et al*., 1990). Sexual receptivity is highest immediately after parturition. This fact is probably due to the inversion of the oestrogen/progesterone ratio occurring after parturition. Successively, sexual receptivity decreases to a value of about 40% at 3-4 days postpartum (p.p.) and progressively increases 13-14 days p.p. The initial level of receptivity is reached only after the weaning of young rabbits (Theau-Clement *et al*., 1990). It should be considered that, apart from the first day p.p., receptivity is affected by many factors (milk production and/or number of suckling rabbits, general condition of doe, environmental conditions) and shows a considerable variability. The number of suckling rabbits exerts a negative action on the mating acceptance of the doe (Torres *et al*., 1987).

2.1.3 Oestrus cycle and behavior

In most domestic mammals, ovulation takes place at regular intervals when the female is in heat or oestrus. The interval between two periods of oestrus represents the length of the oestrus cycle (4 days for rats, 17 days for ewes, 21 days for sows and cows) (Lebas*et al*., 2014). Rabbits do not have well defined oestrus cycle and they are often, erroneously, considered to be permanently in oestrus. Sexual receptivity can be evaluated more accurately by the behavioral test in the presence of a vasectomised buck rather than by the colour of the vulva and its turgidity (Anon.III). Though, they have no estrus cycle, but rather a period of receptivity occurs every 5–6 days (Popesko*et al.*, 1990, Capello, 2005). A doe is said to be in heat when she accepts service and in dioestrus when she refuses (Lebas*et al*., 2014).

A doe in heat assumes a characteristic pose, called lordosis, with the back arched downwards and hindquarters raised,while a doe in dioestrus tends to crouch in a corner of the cage or exhibit aggression towards the buck (Lebas*et al*., 2014).

In post-partum period, does are highly receptive the first day after parturition and shortly (1 – 2days) after weaning (Theau-Clement *et al*., 2000). However, great variability exists among individual rabbit-does depending on parity, lactation stage, and other factors (Theau-Clement *et* al., 1990). Sexual receptive behavior in does is related to the presence of more large follicles on the ovary (Kremabon*et al*., 1994) and the higher plasma concentration of estrogens (Rebollar*et al*., 1992).

2.1.4 Ovulation

Like cats and ferrets, female rabbits are induced ovulators (Popesko*et al.*, 1990, Capello, 2005). Ovulation does notoccur spontaneously, but it has to beinduced through a neuro-hormonal reflex,which is initiated during mating. Does' egg is usually fertilized about an hour and a half after release of ovocytes (Quintela*et al*., 2004). Ovulation is normally stimulated by coitus and normally occurs 10-12 hours after mating (Lebas*et al*., 2014).In the absence of a buck, using artificial insemination (AI),ovulation has to be induced by artificialmethods. Ovulatory inducing methodmost frequently used is an intramuscularinjection of Gonadotrophin releasing hormone (GnRH) or its synthetic analogues(Quintela*et al*., 2004). Alternatively, luteinizing hormone (LH) or human chorionic gonadotrophin (hCG)canbe used to induce ovulation in rabbit-does, however, the repeated injection ofthese hormones can result in failure of thedoes to ovulate due to antibody formation (Quintela*et al*., 2004). Vasectomised males have been used toinduce ovulation in does submitted for AI (Khalifa*et al*., 1994; 2000).

2.1.5 Fertilization and Gestation

Ovocytes are fertilizable from the moment they are librated from the ovary, but they are not actually fertilized until about an hour and a half after release. The sperm is deposited by the buck in the upper part of the vagina. The spermatozoa makes their way upwards rapidly. Ovocytes reach the distal ampulla (fertilization area) 30minutes after coitus (Lebas*et al*., 2014). During their journey, the spermatozoa undergo a maturing process which enables them to fertilize the ovocytes. Implantation proper takes place 7 daysafter mating, at the blastocyte stage. From the $3^{rd}$to 15^{th} day after mating the progesterone rate continues to increase, then remains stationary and finally drops rapidly before parturition (Lebas*et al*., 2014).

2.1.6Pseudopregnancy

Librated ova which are not fertilized may occasion a pseudopregnancy lasting 15-17 days. At first the corpus luteum and uterus develop as in an ordinary pregnancy, but they do not reach the size or the level of progesterone production of the corpus luteum in pregnancy. Towards the 12^{th} day they regress and disappear under the action of a luteolytic factor secreted by the uterus, undoubtedly prostaglandin (Lebas*et al*., 2014). ELISA kits for bovine progesterone can be used to measure rabbit plasma or serum progesterone concentration on day 17-18 p.c. in order to differentiate pregnant from pseudopregnant animals (Morrell, 1990; 1993). The end of the pseudopregnancy is marked by the maternal behavior of the doe and nest making (Lebas*et al*., 2014).

2.1.7 Oestrus synchronization

The use of hormones to synchronize oestrus in rabbits is widely diffused because of its simplicity and efficacy. It also requires modifications of breeding operations. The treatment consists in administering different dose and type of hormone, 2-3 days before insemination (Alvarino*et al*.,

1995). Pregnant Mare Serum Gonadotrophin (PMSG) is the most widely used hormone in industrial rabbit breeding. The main action of PSMG is the rising of the oestrogen release of follicles present in the ovaries. PSMG also triggers immune response due to its proteic nature and high molecular weight (Adams, 1961; Heidbrink, 1980). Prostaglandin F2α (PGF2α) have also been used in rabbits for breeding purposes (McNitt, 1992) to reduce the functional life-span of corpora lutea in pseudo-pregnant does (Nava *et al*., 1992, Rebollar*et al*., 1992) and to induce parturition.

2.1.8 Artificial Insemination

Artificial insemination (AI) has been introduced in commercialrabbitriessince 1920's (Morrell, 1995) mainly to improve breeding management (Garcia *et al*., 1991).Conception rates after AI can be equivalent to or better thanthat achieved with natural breeding (Morrell, 1995). Natural mating in healthy individualscan result in conception rates of 85%. A single ejaculate can be split into 20-50 inseminationdoses and used for AI (Lavara*et al*., 2005).For the AI procedure, the female is placed into arestraining box, the tail is lifted and an insemination pipette with a bend approximately 8cmfrom the end is inserted into the vagina at an angle of 45º in order progress beyond thepelvic rim (Morrell, 1995).Semen is deposited intravaginally. Due to the fact that rabbitshave two separate uterine horns and cervices, intracervical AI is not performed as itwould be required to release semen into both cervices.As rabbits are induced ovulators, they always requireinduction of ovulation when AI is used (Morrell, 1995).

2.2 The Thyroid Gland

2.2.1 Development of the thyroid gland

The thyroid gland is the first endocrine gland to appear in embryonic development. Phase 1 consists of anatomic development andembryogenesis of the hypothalamic-pituitary-thyroid (HPT) system and occursduring the first trimester of gestation. Phase 2 ischaracterized by the maturation of the HPT system,including hormone production and control (Moore *et al*., 1998; Larsen, 1997).The thyroid gland develops from an endodermal thickening of cells that originatefrom the 3rd brachial pouch. Some cells originate from the 4thbrachial pouch. Incraniofacial development, the cells move to the base of the tongue to a point known asthe foramen caecum. This is where the main anlage of the thyroid develops (anaggregation of cells in the embryo indicating the first trace of an organ) (Fancy *et al*., 2010). From thebase of the tongue, the anlage descends as the thyroid diverticulum, leaving thethyroglossal duct, which is connected to the foramen caecum passing anteriorly tothe hyoid bone and thyroid cartilage. It settles as a bi-lobed organ just inferior to thethyroid cartilage, one lobe on each side of the trachea anterolaterally (Fancy *et al*., 2010). The gland liespartly on the cricoid cartilage. The two lobes are joined by an isthmus which unitesthe lobes over the trachea, anterior to the second and third tracheal rings (Fancy *et al*., 2010).

The next stage in the development of the thyroid gland is the appearance of thyroidfollicles which occurs between nine and ten weeks in the development of the human embryo (Moore *et al*., 1998; Larsen, 1997). The follicle consists of a layer of cells arranged around a big central cavity. Thecavity is filled with a colloid like material, consisting largely of the proteinthyroglobulin. Three types of cells become apparent. A cells, or perifollicularendothelial cells, are responsible for the blood supply to the follicles. B cells, orfollicular cells – the

predominant cells having an affinity for iodine and synthesiseiodothyronine hormones T3 and T4 (Moore *et al*., 1998; Larsen, 1997). A and B cells developed from the endoderm while C cellsor parafollicular cells produce calcitonin and develop from the ectoderm (Moore *et al*., 1998; Larsen, 1997).

The development of the thyroid gland is controlled by the thyroid stimulatinghormone (which is also known as thyrotropin). Thyroid stimulating hormone (TSH) or thyrotropin is a glycoproteinhormone. The hypothalamus produces and stores thyrotropin-releasing hormone. It isreleased into the hypothalamic-pituitary portal system and circulates to the anteriorpituitary. Here, TSH is stored which is consequently stimulated to be released (Fancy *et al*., 2010). TSH has a trophic effect on the gland stimulating its growth. The other effects of TSH onthe thyroid gland include: increase in release of stored thyroid hormones, and increase inthyroid hormone synthesis. As mentioned, these hormones are responsible forregulating the rates of metabolic processes throughout the body (Fancy *et al*., 2010).

Upon completion of development of the thyroid, the gland is surrounded by a thinfibrous capsule, which sends septa deeply into the gland. External to the capsule is aloose sheath formed by the visceral layer of the pre-vertebral deep cervical fascia.Dense connective tissue connects the capsule of the thyroid gland to the cricoidscartilage and the superior tracheal rings (Fancy *et al*., 2010).

Two pairs of parathyroid glands are normally present. These small glands usually lieexternal to the fibrous thyroid capsule on the medial half of the posterior surface ofeach lobe of the thyroid, but within its sheath. The number of glands can vary fromtwo to six. These glands produce parathormone also a regulator of serum calciumincreasing levels by resorption of bone (Fancy *et al*., 2010).

There are a few embryonic derangements that may occur during development. Thegland may retain its position at the base of the tongue, failing to descend from itsembryonic origin resulting in lingual thyroid gland. Alternatively there may beincomplete descent down the thyroglossal duct resulting in the thyroid gland beinglocated high in the neck or just inferior to the hyoid bone (Fancy *et al*., 2010).

Accessory thyroid tissuemay result in a pyramidal lobe, extending superiorly from the isthmus which developsfrom the remnants of the thyroglossal duct as do thyroglossal duct cysts (Fancy *et al*., 2010).

2.2.2 Anatomy of the Thyroid gland

The thyroid gland consists of two lobes joined by a thin band of tissue, the isthmus. The gland is closely attached to the anterior and lateral aspect of the trachea by loose connective tissue (Soliman*et al*., 2001). It is a highly vascular gland and has two blood supplies. It is suppliedby the superior and inferior thyroid arteries. These vessels lie between the fibrouscapsule and the pre-tracheal deep cervical fascia. The superior thyroid artery is usuallythe first branch of the external carotid artery (Popesko, 1990). It descends to the superior pole of eachlobe of the gland, piercing the pre-tracheal layer of deep cervical fascia and dividesinto anterior and posterior branches. The inferior thyroid artery is the largest branch ofthe thyrocervical trunk of the subclavian artery (Popesko, 1990). It runs superomedially, posterior tothe carotid sheath, to reach the posterior aspect of the thyroid gland. It divides intoseveral branches that pierce the pretracheal fascia to supply the inferior pole of thegland. There are anastomoses across the midline between the left and right arteriesand also between the superior and inferior thyroid arteries, explaining the vast supplyto the gland (Popesko, 1990).

The thyroid gland has three pairs of veins draining the venous plexus on the anteriorsurface of the gland. The superior thyroid veins drain the superior poles of the thyroidgland; the middle thyroid veins drain the middle of the lobes; the inferior thyroidveins drain the inferior poles(Popesko, 1990). The superior and middle thyroid veins drain into theinternal jugular veins whilst the inferior thyroid veins drain into the brachiocephalicveins, posterior to the manubrium of the sternum (Popesko, 1990).

The thyroid gland is a ductless gland. This is because it is an endocrine gland. Thus, the hormones secreted are directly introduced into the blood (Capello, 2005). The lymphatic vessels of the thyroid gland run in the interlobular connective tissue,often associated with the arteries, communicating with a capsular network oflymphatic vessels. The lymphatic drainage can occur via many nodes, includingsuperior deep cervical, inferior deep cervical, pre-tracheal, pre-laryngeal andpara-tracheal (Capello, 2005).

The nerve supply to the thyroid gland derives from the superior, middle and inferiorcervical sympathetic ganglia. They reach the gland through plexuses that accompanythe thyroid arteries. The fibres are vasomotor causing the constriction of the bloodvessels. There is no secretomotor supply, since the gland is hormonally controlled viaTSH as previously discussed (Popesko, 1990).

Posterior to the gland is the cricoid cartilage and the superior trachea as well as theparathyroid glands. Also medially to the gland lie the trachea, recurrent laryngeal nerve,larynx and oesophagus. The gland lies between the levels C5 and T1. The gland liesdeep to the strap muscles (ansacervicalis) (Popesko, 1990). The two main muscles are sternothyroidand sternohyoid. (The attachment of sternothyroid muscle to the oblique line on thethyroid cartilage has consequences, since it limits the movement of the glandupwards. Lying relatively closely

laterally to the gland is the internal jugular vein,common carotid artery and vagus nerve, enclosed within a deep cervical fascial layerknown as the carotid sheath (Popesko, 1990).

The two most important relations to the thyroid gland clinically are the recurrentlaryngeal nerve and the external laryngeal nerve, both branches of the vagus nervewhich run behind the gland in the groove between the oesophagus and trachea. Therecurrent laryngeal nerve is intimately related to the inferior thyroid artery and itsbranches near the inferior pole. The external laryngeal nerve is related to the superiorthyroid artery (Capello, 2005).

2.2.3 Histology of thyroid gland

In hematoxylin and eosin preparations, the gland appears to be enclosed in a connective tissue capsule which penetrates it forming trabeculae. The gland is composed of closely packed follicles, which vary considerably in size, and are surrounded by a rich capillary network (Soliman*et al*., 2001). The walls of the follicles consist of one layer of simple cuboidal epithelium (follicular cells) which rests upon a thin basement membrane. The lumen of the follicles contains a clear gelatinous material (colloid) which stains positively with periodic acid-Schiff (PAS) (Soliman*et al*., 2001). In addition to the follicular cells, the gland contains a small population of parafollicular cells, or C cells. In sections stained with hematoxylin and eosin, these cells appear polygonal with a granular, weakly eosinophilic cytoplasm, which is paler and larger than that of the follicular cells. They are located individually or in small groups, both within the follicular wall and in the interfollicular spaces (Soliman*et al*., 2001). When found in the follicular wall, they are situated immediately adjacent to the basement membrane and without contact with the follicular lumen (Soliman*et al*., 2001).

2.2.4 Ultrasonography of the thyroid gland

Ultrasonography is the most common and most useful way to image the thyroid gland and its pathology, as recognized in guidelines for managing thyroid disorders published by the American thyroid Association (Cooper *et al*., 2009 and Blum, 1990). In addition to facilitating the diagnosis of clinically apparent nodules, the widespread use of US has resulted in uncovering a multitude of clinically unapparent thyroid nodules, the overwhelming majority of which are benign (Blum, 1990).

Previously, imaging of the thyroid required scintiscanning to provide a map of those areas of the thyroid that accumulate and process radioactive iodine. The major premise of thyroid scanning was that thyroid cancers concentrate less radioactive iodine than healthy tissue (Butch *et al*., 1995). Although, scintiscanning remains of primary importance in patients who are hyperthyroid or for detection of iodine-avid tissue after thyroidectomy for thyroid cancer, US has largely replaced it for the majority of patients who require a graphic representation of the regional anatomy because of its higher resolution, superior correlation of true thyroid dimensions with the image, smaller expense, greater simplicity, and lack of need for radioisotope administration(Blum 1990, Ishigaki*et* al., 2004).The other imaging methods, computerized tomography (CT) and magnetic resonance imaging (MRI) are more costly than US, are not as efficient in detecting small lesions, and are best used selectively when US is inadequate to elucidate a clinical problem (Blum 1990, Ishigaki*et* al., 2004).

Sonography depicts the internal structure of the thyroid gland and the regional anatomy and pathology without using ionizing radiation or iodine containing contrast medium rather images are produced via high frequency sound waves(Butch *et al*., 1995; Leopold, 1990). The procedure is safe, does not cause damage to tissue and is less costly than any other imaging procedure.The

patient remains comfortable during the test, which takes only a few minutes, does not require discontinuation of any medication, or preparation of the patient (Rago*et al*., 2006).There is considerable potential for improving ultrasound images of the thyroid by using ultrasound contrast agents. These experimental materials include gas-filled micro-bubbles with a mean diameter less than that of a red blood corpuscle and Levovist, an agent consisting of granules that are composed of 99.9% galactose and 0.1% palmitic acid. They are injected intravenously, enhance the echogenicity of the blood, and increase the signal to noise ratio (Jakobsen, 2001; Menna*et al*., 1999).

Dynamic information such as blood flow can be added to the signal by employing a physics principle called the Doppler effect, which is that the frequency of a sound wave increases when it approaches a listener (the ear or, in the case of ultrasonography, a transducer) and decreases as it departs. The Doppler signals, which are superimposed on real time gray scale images, are extremely bright in black and white images and may be color coded to reveal the velocity (frequency shift) and direction of blood flow (phase shift) as well as the degree of vascularity of an organ (Clark *et al*., 1995).

The depth penetration and resolution power of ultrasound depends greatly on frequency (Leopold, 1990). Depth penetration is inversely related and spatial resolution is directly related to the frequency of the ultrasound. For thyroid, a frequency of 7.5 to 10 or 14 megahertz is generally optimal for all but the largest goiters. Using these frequencies, nodules as small as two to three millimeters can be identified (Leopold, 1990).It is informative for orientation to survey the entire thyroid gland with a low-energy transducer before proceeding to 10-14 megahertz equipment to delineate the fine anatomy. Protocols have been devised to assemble a montage of images to encompass an unusually large lobe or goiter. For an overview, panoramic ultrasound,

which is a variation of conventional US has been reported to produce images with a large anatomic field of view, displaying both lobes of the thyroid gland on a single image (Shapiro, 2003).

2.2.4.1 Sonography of the normal thyroid gland and its region

The thyroid gland is slightly more echo-dense than the adjacent structures because of its iodine content. It has a homogenous ground glass appearance. Each lobe has a smooth globular-shaped contour and is no more than 3 – 4 centimeters in height, 1 – 1.5 cm in width, and 1 cm in depth (Butch *et al*., 1995). The isthmus is identified, anterior to the trachea as a uniform structure that is approximately 0.5 cm in height and 2 – 3 mm in depth. The pyramidal lobe is not seen unless it is significantly enlarged. In the female, the upper pole of each thyroid lobe may be seen at the level of the thyroid cartilage, lower in the male (Butch *et al*., 1995). The surrounding muscles are of lower echogenicity than the thyroid and tissue planes between muscles are usually identifiable. The air-filled trachea does not transmit the ultrasound and only the anterior portion of the cartilaginous rings is represented by dense, bright echoes. The carotid artery and other blood vessels are echo-free unless they are calcified. The jugular vein is usually in a collapsed condition and it distends with a Valsalva maneuver (Butch *et al*., 1995). There are frequently 1-2 mm echo-free zones on the surface and within the thyroid gland that represent blood vessels. The vascular nature of all of these echoless areas can be demonstrated by color Doppler interrogation to differentiate them from cystic structures (Clark *et al*., 1995). Lymph nodes may be observed and nerves are generally not seen. The parathyroid glands are observed only when they are enlarged and are less dense ultrasonically than thyroid tissue because of the absence of iodine. The esophagus may be demonstrated behind the medial part of the left thyroid lobe, especially if a sip of water distends it(Clark *et al*., 1995).

2.2.4.2 Sonography of an enlarged thyroid gland (goiter)

US may be used in a goiter to identify for biopsy of non-palpable thyroid nodules, or those that have enlarged or become harder to palpation. The value of aspirating a selected nodule in a goiter is under current scrutiny. At this time, the data seems persuasive that the incidence of cancer in a particular nodule in a goiter is independent of the number of sonographically identified nodules, in distinction to prior belief. Therefore, this practice seems worthwhile (Marques *et al*., 2000; Tollin*et al*., 2000). It will be useful to know the ultrasonic appearance of a dominant nodule in a goiter, a tender spot, a region of focal hardness because it might give a clue about pathology (Cooper *et al*., 2009).Sonography can identify one region in a goiter whose echo pattern is distinct from the rest of the goiter suggesting a second type of pathology, especially if the region is surrounded by an incomplete and irregular sonoleucent rim, has punctate microcalcifications or Doppler examination reveals internal vascularity (Cooper *et al*., 2009).Among the lesions that have been demonstrated in goiters using US are neoplasms and lymphoma. Other uses of sonography in goitrous patients include: differentiation of thyroid enlargement from adipose tissue or muscle, identifying a large unilateral mass in distinction to an asymmetric goiter, confirming substernal extension, providing the correct interpretation to varying clinical impressions among several examiners, and objectively documenting volume changes in response to suppressive therapy with thyroid hormone, which may be particularly useful information when patients change physicians (Butch *et al*., 1995).

An interesting public health use of US in underdeveloped countries has been to objectively identify goiter as a screen for iodine deprivation. Furthermore, in the epidemiological setting, with proper ultrasound equipment, assessment of thyroid volume and prevalence of thyroid nodules, but not echogenicity or echographic pattern, are comparable among different observers

(Knudsen *et al*., 1999). Sonography is useful to monitor patients undergoing long-term treatment with lithium for bipolar, major depressive and schizoaffective disease. Their total thyroid volume in one investigation was significantly greater (23.7 ml vs. 13.6 ml) in the lithium-treated group (Giovagnorio*et al*., 2005) than among controls (96 gender- and age-matched control subjects). Furthermore, US detects thyroid enlargement with greater accuracy and sensitivity than palpation (Bauer *et al*., 2007).

2.2.4.3 Sonography of the thyroid nodule

The most frequent use of US is to refine the diagnosis of a thyroid nodule. US can identify thyroid nodules, which are a very common disorder, even when nodules are too small to palpate. Sonography can demonstrate nodules that have an enhanced risk of malignancy with the best sensitivity of any non-invasive technique, but with only fair specificity (Ishigaki*et al* ., 2004).

In addition, US can be used clinically to enhance the clinical value of fineneedle aspiration biopsy and cytology (FNA) of nodules (Tollin*et al*., 2000). Thyroid nodules can be identified by sonography because they distort the uniform shape or echo pattern of the thyroid gland. Thyroid nodules may be large or small. They may distort the surrounding thyroid architecture or may dwell within a lobe and be unobtrusive. They may be solid tissue or consist of solid areas interspersed with echo-free zones that represent fluid-filled hemorrhagic or straw-colored degenerative zones.A smooth, globular area without echoes generally represents an epithelial-lined cyst, which is a rare benign lesion (Simeone, 1982). Most thyroid nodules have a less dense ultrasound appearance than normal thyroid tissue and some are more echo-dense (Butch *et al*., 1995). A sonolucent rim, which is called a halo, may be present around a nodule. A halo represents a capsule or another interface, such as inflammation or edema, segregating the nodule

and the rest of the gland. Doppler interrogation may demonstrate increased vascularity within a nodule or in halo (Clark *et al*., 1995).

The ultrasonic appearance of a thyroid nodule does not reliably differentiate a benign thyroid lesion and cancer (Cooper *et al*., 2009; Butch *et al*.,1995) but it does offer strong clues that help the clinician in the process of triage. Never the less, sonography cannot identify a specific kind of tumor such as a Hurthle cell lesion (Maizlin*et al*., 2008). The most reliable sonographic indicator that a nodule is malignant is observing vascular invasion by tumor, which is rarely seen. However, there are distinctions in echo-density, calcifications, distortion of the rim, and vascularity that favor a benign or malignant diagnosis among thyroid nodules (Capelli*et* al., 2007; Moon *et al*., 2008).

2.3 Sonographic Imaging

2.3.1 General principles

The use of ultrasonography has revolutionized the medical and industrial fields. Ultrasound was first used as a medical diagnostic tool in the 1940's and the first veterinary application, the detection of ovine pregnancy was reported in 1966 (King, 2006). Since then, improvements in imaging quality combined with an increased awareness of the benefits of ultrasound as a clinical tool has led to the widespread use in the veterinary field for reproductive management and other applications (King, 2006). Ultrasound has become established as an important modality for tomographic imaging of soft tissues. It can also be used to quantify the movement of structures such as cardiac valves (M - mode) and, using the Doppler mode, the patterns of blood flow (David, 1997). The soft tissue images are obtained without the need to administer contrast agents and thus are not dependent on organ function. The apparatus is relatively cheap and has the distinction of having become cheaper with technological advances (David, 1997).

The parts of the body that can be imaged with ultrasound are however, limited because of the means by which it penetrates tissue. Ultrasound does not cross a tissue-gas or tissue-bone boundary and so not only is it limited to imaging the soft tissue components around gas-containing and bony structures, but structures lying deeper to them are obscured (David, 1997). Patients readily accept an ultrasound scan because the procedure requires only light pressure on the skin. Patient preparation is minimal: bladder filling is required for obstetric and pelvic scanning and fasting is necessary for the gall bladder, but otherwise the patient may be scanned as and when convenient, which is a major advantage for emergency uses (David, 1997).

The basic principle of ultrasound imaginginvolves transmitting small pulses ofultrasound echo from a transducer into the body. As the ultrasound waves penetrate bodytissues of different acoustic impedances along the path of transmission, some are reflectedback to the transducer (echo signals) and some continue to penetrate deeper (Chan and Perlas, 2011). The echosignals returned from many sequential coplanar pulses are processed and combined togenerate an image.Thus, an ultrasound transducer works both as a speaker (generatingsound waves) and a microphone (receiving sound waves). The ultrasound pulse is in factquite short, but since it traverses in a straight path, it is often referred to as an ultrasoundbeam (Chan and Perlas, 2011).The direction of ultrasound propagation along the beam line is called the axialdirection, and the direction in the image plane perpendicular to axial is called the lateraldirection (Hangiandreou, 2003).Usually only a small fraction of the ultrasound pulse returns as a reflected echoafter reaching a body tissue interface, while the remainder of the pulse continues along thebeam line to greater tissue depths (Chan and Perlas, 2011).

2.3.2 Wavelength and Frequency of ultrasound

The wavelength and frequency of US are inversely related, i.e., ultrasound of highfrequencyhas a short wavelength and vice versa (Otto, 2000).The frequency of an ultrasoundwave consists of the number of cyclesor pressure changes that occur in one second. The units are cycles per second orHertz.Frequency is determined by thesound source only and not by the mediumin which the sound is traveling (Aldrich, 2007). Ultrasoundis actually sound with a frequency in excessof 20 kHz, which is greater than the upper limit for audible humanhearing (Lawrence, 2007; Aldrich 2007). Typical ultrasound frequenciesused for clinical purposes are in the 1MHz to 20 MHz range.Proper selection of transducer frequencyis an important concept for providing an optimal image resolution in diagnostic andprocedural ultrasound.High-frequency ultrasound waves (short wavelength) generate images of high axial resolution. However, high-frequency waves are more attenuatedthan lower frequency waves for a given distance; thus, they are suitable for imaging mainlysuperficial structures (Lawrence, 2007). Conversely, low-frequency waves (long wavelength) offer images oflower resolution but can penetrate to deeper structures due to a lower degree of attenuation (Chan and Perlas, 2011). For this reason, it is best to use high-frequency transducers (up to10 - 15MHz range) to image superficial structures (such as for stellate ganglion blocks) andlow-frequency transducers (typically 2 - 5 MHz) for imaging the lumbar neuraxial structuresthat are deep in most adults (Chan and Perlas, 2011).

2.3.3Propagation Speed

Propagation speed isthe speed at which sound travel througha medium and is typically considered 1540m/sec for soft tissue. The speed is determined solely by the medium characteristics, especiallythose of density and stiffness (Aldrich, 2007).

2.3.4 Transducers

The beam of ultrasound comes from asmall device called a transducer. Ultrasoundtransducers convert electrical tomechanical energy and work on the piezoelectricprinciple (Aldrich, 2007). The piezoelectric effect derives from movement of a heavy charged atom that is loosely bound within a complex crystal; when an electrical field is applied, the atom moves and distorts the crystal (David, 1997; Nautrup, 2006).Piezoelectric materialshave the property that if they aremechanically deformed, they produce anelectric field that tries to restore theshape. Conversely, if an electric field isapplied to a piezoelectric material, in theform of a voltage pulse (typically a fewhundred volts) then the material willphysically deform (Aldrich, 2007).

2.3.5Ultrasound wave interaction with tissue

Asa beam of ultrasound travels through amaterial, various things happen to it. Areflection of the beam is called an echo, acritical concept in all diagnostic imaging.A reflectionoccurs at the boundary between two materialsprovided that a certain property of thematerials is different. This property isknown as the acoustic impedance and is theproduct of the density and propagationspeed (Aldrich, 2007). If two materials have thesame acoustic impedance, their boundarywill not produce an echo. If the differencein acoustic impedance is small, a weak echowill be produced, and most of the ultrasoundwill carry on through the secondmedium. If the difference in acoustic impedanceis large, a strong echowill be produced. If the difference in acousticimpedance is very large, all the ultrasoundwill be totally reflected. Strong reflectionsor echoes show on the ultrasound image aswhite and weaker reflections as gray (Aldrich, 2007).

2.3.5.1 *Mechanism of Interaction*

There are two basic mechanisms, namely, thermal and non-thermal, by which ultrasound is known to affect biologic materials (Ndumbe, 2008; Sheinerand Abramowicz, 2008; Brendt, 1986; Sikov, 1986a; Sikov, 1986b; Laurel, 1993).Non-thermal mechanisms include cavitational and non-cavitational effects, which are associated with certain mechanical aspects of the acoustic or ultrasonic field. These aspects can be described in terms of second-order phenomena, such as radiation pressure, radiation force, radiation torque, and acoustic streaming, and are comprehensively reviewed in other sources (Sikov, 1986b; Laurel, 1993).

2.3.5.2 *Thermal Effects of Ultrasound*

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).As an ultrasound beam traverses tissue layers, the rate of energy deposition is determined by the factors defined by the operational characteristics of the imaging system and the physical parameters of the tissue being imaged. The system's operating characteristics are functions of the mode (imaging and/or Doppler) being used, as well as of the focal characteristics of the transducer and its frequency (Sikov, 1986a). As an example of the way these characteristics influence energy transfer, one can note the difference in energy distribution between scanned modes such as B-mode and color-Doppler and un-scanned modes such as pulsed-wave (PW) Doppler. More specifically, B-mode energy is distributed over a large volume, whereas in un-scanned modes, such as PW Doppler, the acoustic energy is aimed along a single line (Sikov, 1986a).Similarly, a highly focused transducer has the potential for a highly concentrated energy deposition, whereas weakly focused

transducers tend to spread the energy over larger volumes (Sikov, 1986a).For example, there is almost no absorption in liquids such as amniotic fluid, blood, and urine. However, an adult bone absorbs about 60 - 80% of the acoustic energy impinging on it.With fetal bone, there is a wide variation in absorptive behavior, depending on the degree of ossification. The clinical situation 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 bone(Sikov, 1986a).

In vivo, US is absorbed by tissues and converted into heat energy (Schortinghuis*et al.*,2003). Heating effects are the results of the absorption of US energy from an US beam (Gent, 1997). The heating effect is highly dependent on absorption coefficient of isonated tissue and because bones have high absorption coefficient values (Gent, 1997; Barnett*et al.*, 2010, Anon I), it tends to absorb 60 percent or more of the incident US energy. During 3rd trimester of pregnancy, fetal bones are almost completed since it has reached the final stage of organogenesis. The surrounded soft tissues are the responsible area for an inevitable rise of temperature during isonation (Gent, 1997) which can go up to a factor of five (Church and Miller, 2007). It is considered hazardous when embryo and fetus have been exposed to US 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). Large body of evidence has also proven that heat stimulates the abnormalities in studied animal and the sensitivity varies throughout gestational stages. For example, neuronal tube closure and neurogenesis of central nervous system have been noted to become hypersensitive to thermal injury (Miller *et al*,. 1998, Edwards*et al*., 2003; Church and Miller, 2007) and even 2 °C elevation of temperature may develop anomaly or defect (Anon IV).

2.3.5.3 *Non-thermal Effects of Ultrasound*

Where thermal effects are negligible, for instance when applying US at low frequencies and intensities, US is most likely to deliver its biological effects through non-thermal mechanisms. The non-thermal effect (mechanical effect) of US isonationleads 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).The term cavitation refers to phenomena associated with the vibration and motion dynamics of small gaseous bodies (bubbles) when exposed to an ultrasound field (Ndumbe*et al*., 2008). If the bubble does not collapse during the ultrasound exposure, the condition is referred to as stable cavitation, in contrast to inertial (collapse) cavitation (formerly known as transient cavitation), during which the vibration amplitude of the bubble wall increases so much that the bubble implodes. This implosion generates highly localized shock waves and is also associated with extremely high local temperatures (up to 10,000K) (Abbott, 1999). Mechanical effect is also regarded as any biological effect from US when accompanied by elevation of temperature less than 1°C above normal physiologic levels (Stratmeyer*et al.*, 2008). It might then disrupt the molecular bonds and release free radicalssuch as hydroxyl radicals and hydrogen that consequently interfere with DNA (deoxyribonucleic acid) of the cells and lead to chromosomal damage (Anon. III; Barnett, 2003; Wu and Nyborg, 2008).

2.3.6 Attenuation

The loss of intensity (or energy) of the forward propagating wave due to reflection, refraction, scattering and absorption is denoted attenuation. The intensity is a measure of the power through a given cross-section; thus the units are W/m^2.Although the challengeof overcoming the problem

of weakly reflectedpulses is significant in itself, theintensity of the ultrasound beam is furtherreduced by attenuation due to variousprocesses such as reflection, refraction,scattering, and absorption. All theseprocesses divert energy from the mainbeam.Reflection and refraction occur atsurfaces that are large compared with thewavelength of the ultrasound (Aldrich, 2007).

For objectsthat are small in comparison with thewavelength, energy is scattered in manydirections, and the eventual fate of theultrasound is to be absorbed as particlevibration and the production of heat. Theamount of attenuation varies with thefrequency of ultrasound. A high-frequencybeam will be attenuated morethan a lower frequency (Aldrich, 2007).In most situations involving diagnosticultrasound, the absolute intensity ofthe beam is not important. However, the relative intensitiesof the initial pulse and its subsequentreturned echoare what are important.The variation of the attenuation ofultrasound varies with frequency, and itis approximately true that attenuation(decibels) per centimeter is the same asthe ultrasound frequency (megahertz).Therefore a 3MHz beam will be attenuated3dB in the first centimeter (decreasedto 50%), another 3dB in the nextcentimeter, and so on. The depth of penetrationis defined as the depth of tissuerequiredreducing the intensity by 50%and is given by the simple relationship3/frequency (megahertz). For example, a6MHz beam will be reduced in intensityby 75% in 1cm of tissue. After passingthrough 20cm of tissue and being reflectedback to the transducer, the intensityof the ultrasound beam will be reducedby a factor of more than a million (Aldrich, 2007).

Ultrasound energy is also lost to the receiving transducer if it is reflected or refracted away from the returning line of sight or if the beam diverges. The total loss from all these mechanism is called attenuation. Using high frequencies of ultrasound gives better resolution because of their shorter wavelength, but the rapid attenuation of high frequency ultrasound by tissue is the

limiting factor to the maximum frequency that can be used in any given clinical application (David, 1997)

2.3.7 Reflection, transmission, refraction, scattering, and absorption

When sound waves pass interfaces between two tissues with different wave propagation velocities or with different acoustic impedance, a part of the sound is reflected. The direction of the reflection of a wave hitting an interface perpendicularly is same as that of the incident wave (Nautrup, 2006). The non-reflected wave continues in the second medium without changing direction (transmission). If the interface hits at an angle, the angle of reflection is same as the incident angle the transmitted wave is refracted. Irregular acoustic interfaces cause reflection of the sound wave in all direction (scattering). In addition, sound beam is absorbed as it passes through tissues (absorption) (Nautrup, 2006). The phenomenon is similar to that occurring in a rope that is fixed at one end and made to oscillate by shaking the free end up and down provided the rope is of uniform construction; waves travel along it until they die out or reach the fixed end. But if a part of the rope is thicker or more rigid (or the reverse), then the waves are partly reflected back when they reach this 'obstruction'. At the fixed end the same effect occurs but to a greater extent, all of the vibrational energy being returned as full-strength 'echo' because this part of the rope cannot move at all (Nautrup, 2006).

2.3.8 Scanning methods

The Pulse-Echo Method: In the conventional pulse-echo ultrasound imaging process, signals are recorded at a depth calculated from the time delay between transmission and receipt of the echoes, using the value of the speed and sound in tissue to convert from time to depth; the same principle is used in radar and sonar (David, 1997). Excitation of a piezo-electric transducer by an

electronic pulse creates an ultrasonic wave, the wavelength of which is determined by the fundamental frequency of the transducer and the velocity of sound in the present medium. The pulse-wave can be as short as several wave-lengths if the transducer has adequate damping (Bom*et al*, 1992).While travelling through the adjacent tissue, the US wave scatters or reflects on small particles and boundaries, where a change of the acoustic impedance of the material occurs.Part of the scattered and reflected acoustic energy comes back onto the transducer and forms the echo-signal. The distance between the transducer and the reflecting structure is known from the sound velocity in the present medium and the time-interval between emission of the pulse-wave and reception of the echo (Harm and Geboren, 1993).

In the simplest system, only the depth of the echo-producing interface is determined. It is displayed as a vertical deflection on a monitor and is known as an A-scan (A for amplitude). It has one spatial dimension and the strength of the echoes is indicated by the height of the deflection (David, 1997).

A simple way to record these changes is to modulate the intensity of the spot on the monitor in proportion to the intensity of the echoes and then to sweep the line of echoes across the screen. The resultant trace, showing depth versus time, is known as an M-mode display (M for movement; sometimes also referred to as TM, for time-motion scan) and is especially useful in echocardiography for evaluating the rapid movement of valve leaflets (David, 1997). The A-mode and M-mode signals represent information obtained along a single sound beam (Bom*et al*, 1992). A two-dimensional tomographic ultrasound image is formed by sweeping the beam through a slice of tissue and mapping the echogenicity of reflectors as shades of grey to form a B-mode scan (B for brightness), also known as grey scale scan. While the depth of the reflectors is determined by the delay in the return of the echoes to the transducer (the pulse-echo principle),

their lateral position is determined by the direction in which the ultrasound beam was sent (Harm and Geboren, 1993).

A second way to create a two-dimensional image is by positioning more transducers in line and establishing electronically, sequential adjacent excitation of subgroups of transducer elements (linear array) (Harm and Geboren, 1993). The resulting wave-front of the array of transducers can be manipulated by differentially delaying (phase shifting) the excitation pulses to the elements. An electronic sweep of the over-all beam can be performed this way covering a sectorial area (phased array) (Bom*et al*, 1992).

Beam Steering: The direction of the beam can be determined mechanically or electronically. Mechanical steering systems are simpler and commonly use a single crystal transducer which is mechanically swept through an arc either by spinning it on a wheel or by oscillating it to and fro. The resulting image has a triangle or sector shape; this approach has the practical advantage of requiring only a small skin contact area (or footprint) but it provides only a limited display of superficial tissues (David, 1997). The transducer must be housed in a fluid bath, usually containing mineral oil, to avoid direct skin contact with the moving parts. Difficulties with electrical connections and maintaining mechanical integrity over long periods of use have been overcome in modern transducer designs (Bom*et al*, 1992).

Electronic sector systems (also known as phased arrays) control the beam direction by building up interference patterns between the waves transmitted from an array of a large number of transducer elements. The usual arrangement consists of numerous lead zirconatetitanate (PZT) crystals (128 in state-of-the-art systems), each 1 mm or less in width and 5-10 mm in length, stacked up in a block. Each has a separate electrical connection and the trigger pulses to each

element are serially delayed from one end of the array to the other (Harm and Geboren, 1993). These minute differences in timing occur within the time needed to form an individual pulse and produce interference patterns in which the troughs and peaks of the pressure waves add where they coincide and subtract where they are out of phase (hence the title 'phased array') (David, 1997). This results in a beam that is directed away from the straight-ahead direction. For the next pulse, a slightly different set of delays is applied to adjust the steering position. The operation of electronic linear array transducers is somewhat simpler. They are longer than phased arrays and the beam is moved across the scanned plane by firing the elements in groups, starting from one end of the array and stepping along to the other (David, 1997). This produces an image with a rectangular shape, best suited for the superficial structures that are of prime interest in small parts scanning. In a simple variant, the linear array is shaped in a curve so that the field of view is trapezoidal, as with a sector scanner, but with a longer skin line. This compromise is particularly useful when both superficial and deeper structures need to be imaged, for example in obstetrics (Harm and Geboren, 1993).

There are three major types of ultrasound examinations in obstetrics. These include transvaginalsonography for early pregnancies, transabdominalsonography for mid and late trimester pregnancies, and Doppler evaluations of the placental and fetal circulations (Platt, 1988).

a) Transvaginal/transrectalsonography can be performed using a variety of transducer types. These include a curvilinear multi-element transducer, a single element mechanical sector transducer, and a multi-element phased array transducer. Transvaginalsonography utilizes higher frequencies than transabdominalsonography, since the region of interest is nearer to the probe. However, it may be difficult initially for the sonographer to get used to the

limited field of view of this type of probe. One must establish that certain structures should be documented, such as uterus and adnexa, with this technique (Platt, 1988).

b) Transabdominal real-time sonography forms the foundation for the most obstetrical evaluations. The technique allows delineation of the fetus in real time, thereby affording optimal delineation of the fetus, uterus, and adnexal areas. Transabdominalsonography is performed after the patient drinks water freely to attain a distended urinary bladder, so as to displace bowel loops out of the pelvis and provide an acoustic standard for assessing the echogenicity of surrounding structures. (Platt, 1988).

   Transabdominalsonography is usually performed with real-time transducers and according to certain protocols as established by the America College of Radiology. These transducers can have various configurations, including multi-element linear array that is either electronically or phase-activated, single-element wobbler transducers, and multiple rotating transducers. Each of these has a specific 'footprint' and is therefore best utilized for certain specific applications (Platt, 1988).

   A curved linear multi-element transducer affords the best means for evaluation of obstetric patient in the second and third trimester since it allows a relatively large field of view with the flexibility of a smaller transducer (Platt, 1988).

c) Doppler transducers can either be incorporated into real-time transducers (a Duplex scanner) or be an 'outrigged' relative to the main transducer elements. Doppler scanners can either image with continuous-wave (CW) or range gated or pulsed imaging (PI). Pulsed imaging requires greater intensities than with CW Doppler (Platt, 1988).

   One should be aware of the relative intensities of each type of transducer as expressed in spatial peak temporal average (SPTA). Pulsed Doppler transducers may emit relatively

high intensity waves, approximately 100 mW/cm², and are currently under restriction by the Federal Drugs Administration (FDA) in the USA. Doppler transducers are used for evaluation of fetal blood flow and have been incorporated in colour Doppler systems for visual delineation of flow patterns in the fetus and placenta (Platt, 1988).

2.3.9 Resolution

The resolution of an ultrasound imaging system can be defined as the minimum distance between two objects, at which they are identified separately. For an US transducer three components of the resolution can be recognized: the axial resolution in the US beam direction and two lateral resolutions perpendicular to the US beam (Bom*et al*, 1992). Ultrasound resolution must be considered separately for the two dimensions, along and across the beam. Depth or range resolution is simply determined by the length of the ultrasound pulse, which is kept as short as possible by placing a sound-absorbing backing block behind the piezo material and by shortening the electronic shock with damping circuitry. These devices result in an emitted pulse some two wavelengths long; obviously this equates to a shorter physical pulse if higher frequencies can be used, the main reason for the improved resolution of high frequency systems (David, 1997). Lateral resolution, in contradistinction, depends on the width of the ultrasound beam. Two means of controlling this have been exploited: use of large aperture transducers and the addition of mechanical or electronic lenses (David, 1997).

The ultrasound emitted from a point source spreads in a hemisphere but, as the source is enlarged, it forms a beam with near parallel sides for a few centimeters of depth before the beam diverges again (Bom*et al*, 1992). The change-over is the transition between near and far fields. In fact it represents the point beyond which the ultrasound waves from the edge of the transducer

arrive less than a wavelength later than those from the transducer center and this is why larger aperture transducers have a longer near field. The beam is actually formed by the same interference effects that are exploited to steer the beam in electronic transducers and occur because the compression and refraction phases of the ultrasound pulse add in the direction of the beam but cancel elsewhere (David, 1997).

Adding a converging lens (usually a curved plastic layer bonded to the front of the piezo material), or simply shaping the transducer face into a shallow dish, acts to narrow the beam. This can be thought of as working in the same way as optical lens, i.e. by beam refraction (David, 1997). A more useful concept is based on the fact that the lens conducts ultrasound more rapidly than tissue, so that waves emanating from the transducer edge are fractionally ahead of those from the center. Thus interference patterns are set up across the sound field that emphasize the center region of the ultrasound beam and cancel ultrasound waves that otherwise would spread laterally (Harm and Geboren, 1993). The same effect can be produced with a multi-element array by sending the transmit pulses to the outer elements fractionally ahead of those to more central elements this is the electronic equivalent of shaping the transducer surface into a dish. Focusing also occurs on receipt of the echoes, in a reciprocal fashion (David, 1997). While electronic focusing is complex, it confers two benefits. First, the focal position can be altered by changing the delays within the transmit pulse so that the probe can be optimized for each clinical situation with a mechanical probe the focus is fixed and so the entire probe must be exchanged if the focal zone needs to be altered. In addition, with electronic focusing on receipt of the echoes, initially the focus can be set close to the transducer to optimize resolution of superficial tissues and then progressively re-focused deeper into the body as the train of echoes

returns from deeper interferences. This dynamic focusing optimizes resolution over the entire depth of the scan (David, 1997).

2.3.10Artifacts

This refers to the display of information that does not accurately reflect the true image of the area under examination. The speed of ultrasound varies by only a few percent in watery soft tissues but fat conducts approximately 20% more slowly. This means that the depth of echoes arising beyond large fatty regions is over-estimated and this distorts the image of deeper lying structures so that they are depicted as further away from the skin than they actually are. It may be seen as a shelf in the diaphragm, if, for example, a fatty tumor is found in the liver (e.g. a lipoma) (David, 1997).

2.3.11Interpretative principles

Shadowing and Enhancement: Acoustic shadowing and enhancement are important components of the ultrasound image. Shadowing occurs when little or no ultrasound can penetrate an interface and results in a dark band over the deep tissues, bounded by the ultrasound beam lines which are parallel for a linear transducer and radiating for a sector transducer (David, 1997). Absorptionand reflection are the two main causes of shadowing. If a portion of the tissue being imaged absorbs ultrasound faster than the background, the chosen time gain compensation (TGC) will be insufficient to compensate properly and therefore tissues deeper to the highly attenuating region are under corrected and appear darker than adjacent tissue (Harm and Geboren, 1993). Fibrous tissue and, to a lesser extent, fat attenuate at a higher rate than the usual 1dB MHz-1 and are common causes of acoustic shadowing, for example in a fatty liver, behind scars and behind scirrhous breast carcinomas. High attenuation also partly accounts for the

shadows seen deep to calcific lesions such as biliary and renal stones, but here intense reflection is also a factor (David, 1997). Whatever proportion of the incident ultrasound is reflected is not available to continue through for imaging. For stones, this amounts to about 50% of the incident energy but for tissues: gas interfaces almost all the incident ultrasound is reflected and these produce dense shadows. In this case, the shadows are often partially filled in by reverberant echoes which form because of the efficiency of these gases: tissue boundaries as reflectors, so that reverberation artifacts commonly occur. The noise in gas shadows has given rise to their designation as 'dirty shadows' in stones, as compared to the 'clean shadows' behind stones, and this may be a useful differential diagnostic feature (David, 1997).

2.3.12Echogenecity

The prime determinant of the strength of ultrasonic echoes is the impedance mismatch (dZ) between adjacent tissue components. At the risk of being somewhat simplistic, in practical clinical terms this may be understood as interfaces between tissues of different densities. The larger the mismatch the stronger the echo, so that interfaces between soft tissues and bone, for example, give very strong echoes and, within soft tissues, the most significant components are the fibrous tissue (often in the form of the perivascular microskeleton) and fatty tissue. Thus, while uniform regions of fiber of fat are echo poor (an example is retroperitoneal fibrosis), admixtures between them and watery tissues give stronger echoes (David, 1997). A second important factor is the concentration of the scatterers: for given impedance mismatch, a region that contains a large number of scatterers is more echogenic than one where they are spread out. Commonly the 'dilution' of scatterers is caused by an increase in water content. The low reflectivity of the congested liver in right heart failure is a common example. Malignant tumors are a general case. Until they grow large enough to undergo necrosis or calcification (which

produces new reflectors) they tend to be echo poor. Similarly, the edematous tissues in acute inflammation give low-level echoes; examples include the echo poor pancreas in acute pancreatitis and the segmental echo-poor regions of acute lobar nephropathy (David, 1997).

2.3.13Safety

An important feature of diagnostic ultrasound, especially in obstetric applications, is its apparent safety. This is evidenced by the many studies that have failed to show any damaging effect of pulsed ultrasound at diagnostic intensities when applied to the intact animal fetus in utero. These studies include follow-up of growth and the growth and the development of cataracts, hearing loss or childhood malignancies (Harm and Geboren, 1993). A Canadian finding that in utero ultrasound led to delayed speech in children could not be reproduced in a second, Norwegian, study and a detailed epidemiological study of this may be warranted (David, 1997). There have, however, been several reports of biochemical alterations to cells in suspension, including changes to DNA and in surface membrane behavior, both of which are obvious at high intensities but are demonstrable also (at least in some studies) at diagnostic powers. It is debatable whether these are significant for the intact animal and it is notable that many of them have proved elusive when repeat studies have been attempted. They do, however, inject a note of caution and reinforce the commonsense point of ALARA (As Low As Reasonably Achievement), i.e. that one should always use the lowest power that will give the diagnostic information required, especially in obstetrics and gynaecology where one might expect the greatest tissue sensitivity. The entire subject of safety is being kept under continuous review (David, 1997).

2.4 Application of Ultrasound in Obstetrics

The ability of ultrasound to distinguish fluid from soft tissue and differentiate between soft tissues based on their composition makes it better than radiography for examining soft tissue structures (Nyland and Mattoon, 2002). US therefore provide a non-invasive alternative to many radiographic contrast procedures, though the two techniques are still considered as complimentary. It may also often provide information that was previously only available through exploratory laparotomy. Further applications of ultrasound include identifying pregnancy, its viability and foetal number determination (White and Russel, 1987). It also permits foetal sexing (Ribadu and Nakao, 1999). As a pregnancy diagnostic method, ultrasonography is accurate and rapid, and the outcome of the test is knownimmediately at the time the test is conducted. The rate of embryonic mortality and the efficacy of strategies to rebreed at various stages post breeding also play a role in determining the advantages and disadvantages of the timing of pregnancy diagnosis and re-synchronisation (Fricke *et al*., 2003).

2.4.1 Routine ultrasonography screening

One important issue in addressing the efficacy of diagnostic sonography in obstetrics is whether routine screening is efficacious. It can be established that routine sonography detects clinically unsuspected conditions such as twins, placenta praevia, fetal anomalies, etc, that have important obstetrical implications on the other hand, the lack of conclusive proof that there are no long-term bio-effects of ultrasound, and a cost-benefit analysis may work against the concept of routine screening of all pregnant patients. In the United States, the National Institutes of Health (NIH) consensus panel established 28 indications for obstetrical sonography (Anon II, 2010).

2.4.2 Pregnancy diagnosis

Early pregnancy diagnosis can improve reproductive performance by decreasing the interval between successive artificial insemination services and coupling a non-pregnancy diagnosis with an aggressive strategy to rapidly rebreed the animal (Fricke, 2002). Pregnancy diagnosis can be achieved by ultrasonography. In this, the foetus appears as an echogenic structure inside a non-echogenic structure (Pierson and Ginther, 1984).To compensate for embryonic mortality, animals diagnosed pregnant early post breeding must undergo one or more subsequent pregnancy examinations to identify and rebreed those that experience embryonic mortality.This applies to all methods for early pregnancy diagnosis including transrectal palpation conducted before the rate of embryonic mortality decreases (Mohamed and El-Aty, 2010).

2.4.3 Determination offetalnumberandviability

The abilitytoidentifymultiple fetuses withreal-timeultrasonography isaclearadvantageoverothertechniques (Mohamed and El-Aty, 2010).Fetalnumbervia ultrasonography is detectable at day 11 of pregnancy in rabbits (Soroori*et al*., 2008) day 40 in goats(Medan *et al*., 2004) and day 55 in cows (Davis and Haibel, 1993).Determination offetalnumberin dairy production wouldallowproducerstoseparateanimals carrying singles,twinsortripletsfordifferentialmanagement (Davis and Haibel, 1993). Determination of fetalviabilityisaclearadvantageofultrasoundoverothermethods ofpregnancydiagnosis.Theheart beatscanbeseen between theribs duringexamination (Mohamed and El-Aty, 2010).

CHAPTER THREE

3.0 MATERIALS AND METHOD

3.1 Materials

3.1.1 Experimental animals

Four (4) adult bucks and Sixteen (16) mature non-gravid female Dutch breed of rabbits that had kittened at least once and at most thrice were used for the study. They were purchased from a reputable rabbit breeder in Samaru Zaria, Kaduna state. . They aged between 1 – 2 years and weighed 1.7 – 2.3 kg.They were housed in individual clutches in the animal pen of the Department of Public Health and Preventive Medicine, fed with grower mash (15% crude protein, 7% fat, 10% crude fibre, 1% calcium, 0.35% phosphorus, 2550kg metabolizing energy) and water provided ad libitum.

3.1.2 Equipment and Consumables

Equipment and Consumables used were: Medison SV600®Ultrasound machine with a 6.5MHz curvilinear probe and a Sony videographic thermal printer with printing paper, aquasonic gel, tissue paper, antiseptic, packets of tiger razor blade, 2ml syringe with 23G needle, glass slides, cover slips, chloroform, dissecting kit, sample bottles, surgical gloves, formalin, T3 and T4 ELISA kits.

3.2 Methodology

3.2.1 Pre-mating scanning

Experimental rabbits were allowed to adapt in their new environment for two weeks, after which all does were placed on dorsal recumbency, liberally shaved from the level of xyphoid cartilage to the pelvic area. Shaved area was cleaned thoroughly with cotton wool soaked with antiseptic and aquasonic gel applied. Their uteri were all scanned for pregnancy diagnosis using the bladder as a landmark with a 6.5MHz transcutaneous probe and a MedisonSV600®Ultrasound machine.

3.2.2 Natural mating

Natural mating was achieved by the introduction of a doe to a buck and allowed together for an average of 30 minutes. After successful mating, does were divided randomly into two groups (A and B) of eight does each. Group A served as control and group B as experimental.

3.2.3 Post-mating scanning

Scanning was carried out on the 5^{th} day post coitus and thereafter on day 7, 12, 15, 20, 25, 27 and 29 (Gutierrez *et al.*, 2004) using same technique. Rabbit-does in Group B were scanned for an average of 5 minutes, while thecontrol group had same procedure without isonation.

3.2.4Blood collection

Upon parturition, two (2) kittens per litter were sacrificed via jugular venesection following light chloroform anaesthesiaand 2ml of blood was collected into plain sample bottles and then centrifuged at 1,000 revolutions per minute (rpm) for 10 minutes to obtain serum. The serum was used to assay Thyroxine (T4) and triiodotyronine (T3)

3.2.5 Evaluation of Thyroid gland

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

3.2.6 Assay for Thyroxine (T4) and Triiodotyronine (T3)

The assay was carried out in the National Animal Production Research Institute Laboratory, Ahmadu Bello University Zaria.

Working reagents for both T3 and T4 were prepared by diluting T3/T4-enzyme conjugate 1:11 with T3/T4 conjugate buffer in a suitable container. The working substrate solution was prepared by pouring the contents of the amber vial labeled Solution 'A' into the clear vial labeled Solution 'B', mixed properly and stored at 2 - 8ºC. All reagents, serum references and controls were brought to room temperature (20 - 27ºC) before proceeding to assay. Microplates' wells were formatted for each serum reference, control and patient specimen to be assayed for. 0.005 ml (50 µl) of the appropriate serum reference, control and specimen were pipetted into the assigned well. 0.1 ml (100 µl) of working reagent A and T3/T4 enzyme reagent were added to all wells. Microplate was swirled gently for 20 – 30 seconds to mix and was later incubated for 60 minutes at room temperature. Contents of the microplate was discarded by decantation and blotted with absorbent paper. 350 µl of washed buffer was added and the plate decanted. This was repeated

two (2) additional times for a total of three (3) washing. The working substrate solution, 0.1 ml (100 µl) solution was added to all wells and incubated at room temperature for 15 minutes. The stop solution, 0.05 ml (50 µl) was added to each well and gently mixed for 15 – 20 seconds. The absorbance of each well was read at 450 nm in a microplate reader (Following manufacturer's recommendation).

3.3 Data Analyses

Sonographs were printed on thermal printing paper and results obtained were subjected to statistical analysis. SPSS version 20 was used to analyze the data generated and Mean ±SE 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.

CHAPTER FOUR

RESULTS

4.1. Ultrasonography

Pre-mating scanning

Pre-mating ultrasound scan of all the does showed no evidence of pregnancy.

Post-mating scanning

Pregnancy was first detected on the 7th day post breeding. Ultrasound examination for the isonated group confirmed a 100% pregnancy rate at first trimester. Pregnancy rate dropped to 12.5% at second trimester.100% pregnancy rate to parturition was observed in control group. Upon rebreeding of the isonated group of rabbits, 100% pregnancy rate was seen at first trimester which later dropped to 71.5% at second trimester.

There was no sonographicsign suggestive of pregnancy at gestational day 5. However, embryonic vesicles (EV) were seen at gestation day 7 (Plate I). These structures appear as fluid filled round anechoic (darkened) structures with surrounding isogenic area. On day 12 of gestation, hypoechoic structures were seen to occupy one-third of the embryonic vesicles which corresponded to placenta formation and embryo and the remaining two-third appear anechoic which corresponded to amniotic fluid nourishing the embryo (Plate II). Hypoechoic structures and amniotic fluid within the vesicles occupied about two-third and one-third the vesicle respectively on day 15 of gestation (Plate III). Also, at this stage individual vesicles can be isolated and number of fetus (es) estimated. Bi-parietal diameter (Plate IV) and fetal heart (Plate V) were visible at day 20 and 25 of gestation respectively. Also fetal heart rate was picked and estimated at same stage. As gestational age increased, structures (fetal heart, bi-parietal diameter, vertebrae column) became more visible with an increase in bi-parietal diameter and a decrease in

fetal heart rate (Day 25 – Plate IV, Day 29 – Plate VI). Figure 4.1 is a line graph showing variation of BPD with gestational age.

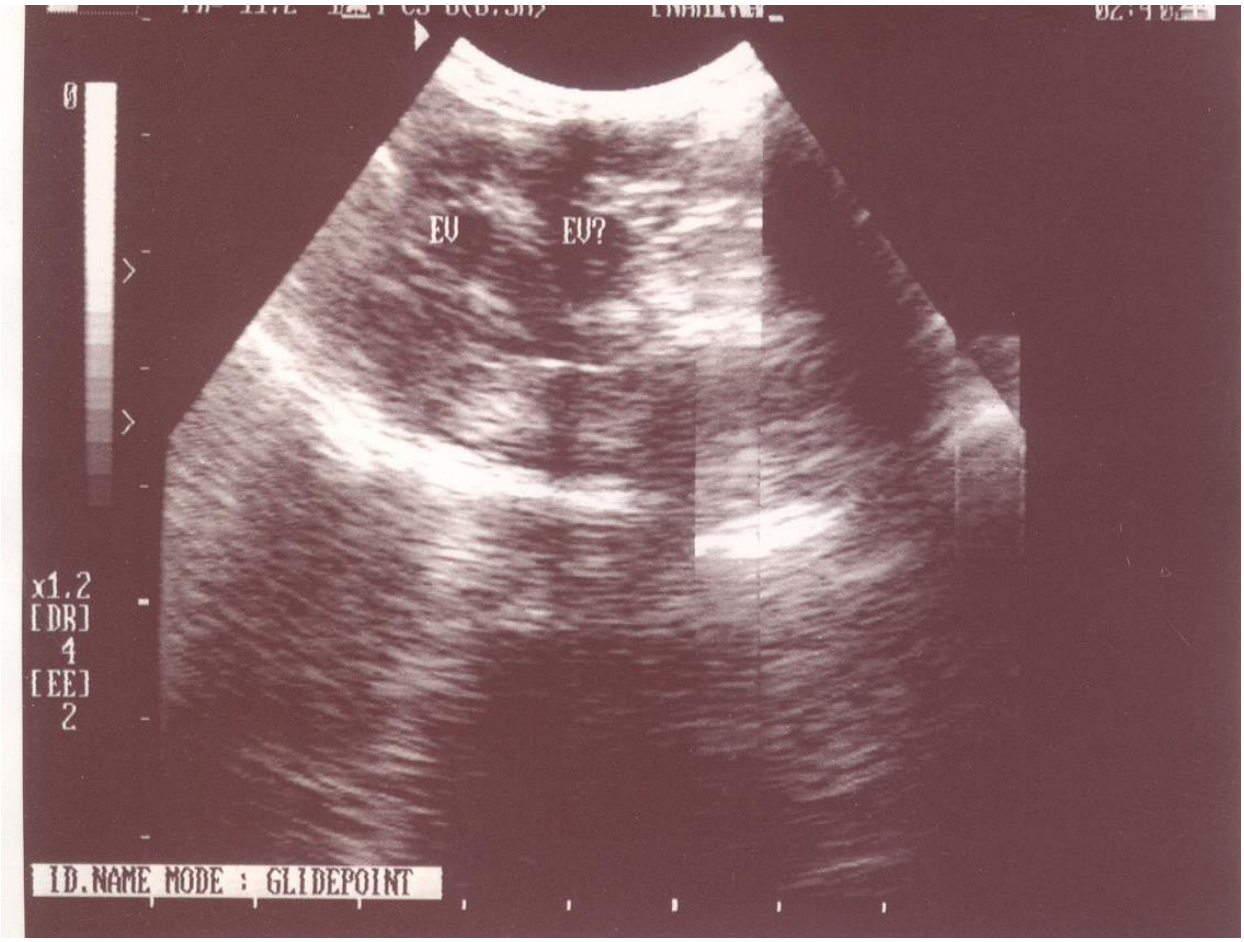


Plate I: Sonogram of a doe (E7) taken on day 7of gestation showing embryonic vesicles (EV).

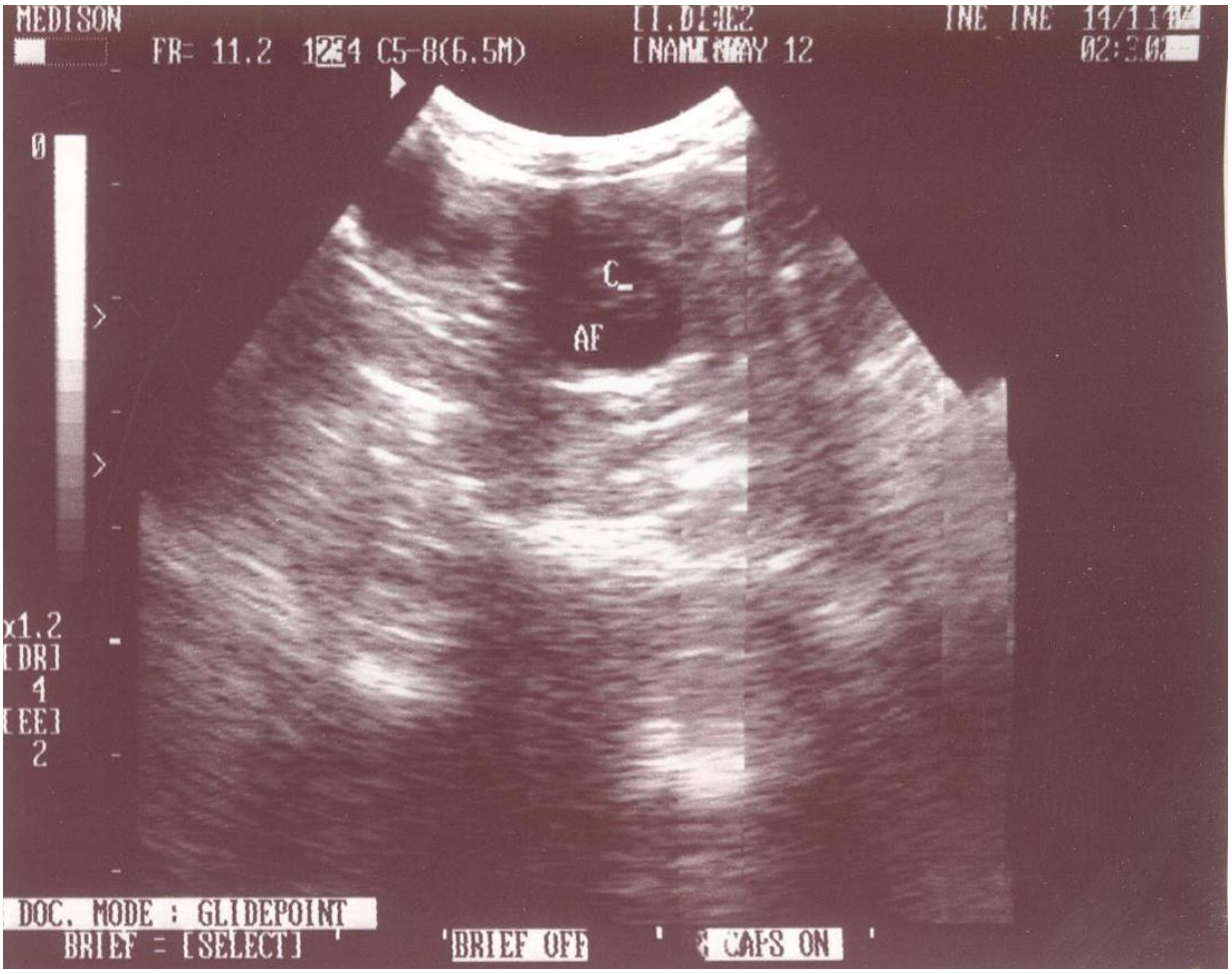


Plate II: Sonogram of a doe (E2) takenon day 12 of gestation showing the conceptus(C) and amniotic fluid (AF).

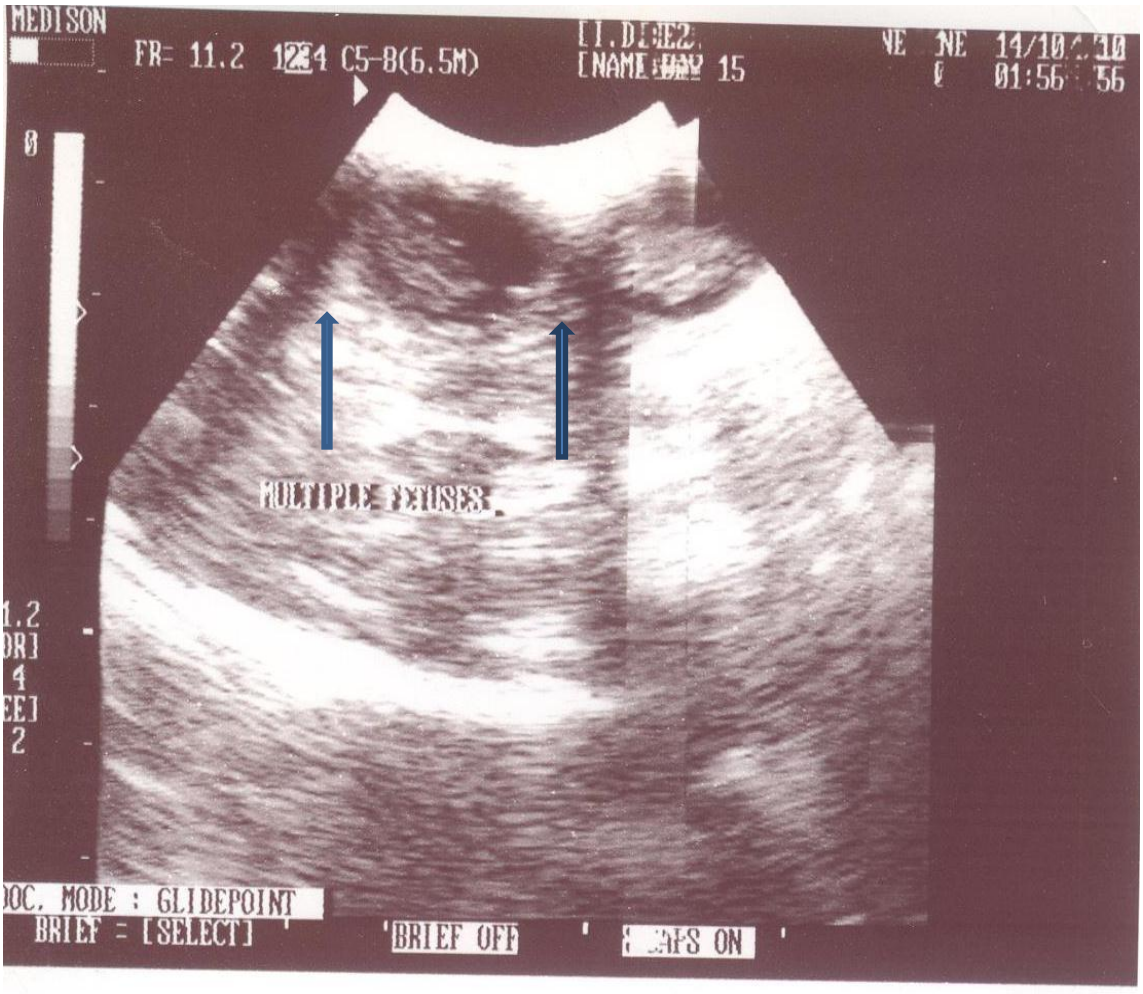


Plate III: Sonogram of a doe (E2) on day 15 of gestation showing multiple fetuses (arrowed).

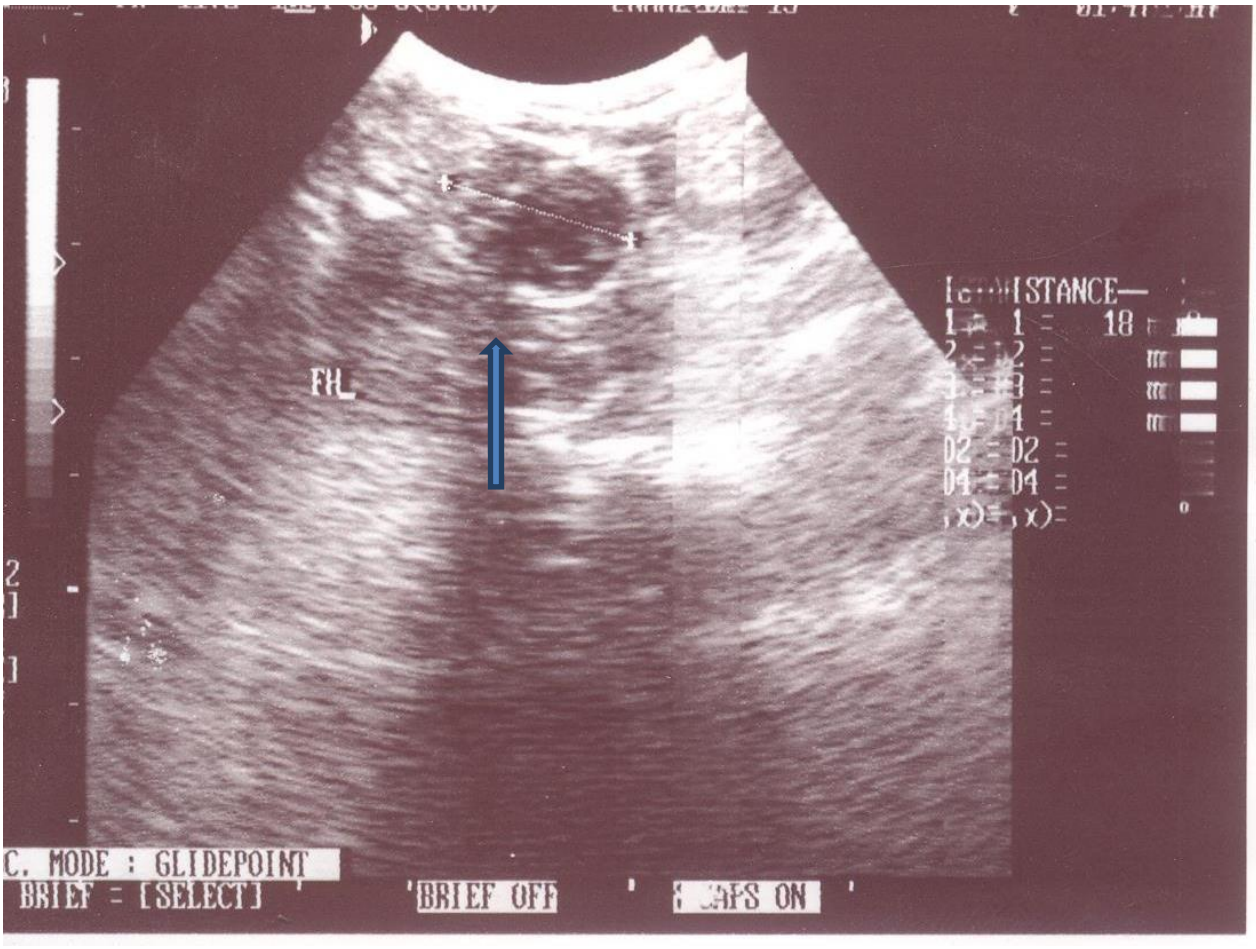


Plate IV: Sonogram of a doe (E5) on day 25 of gestation showing bi-parietal diameter measuring 18mm.

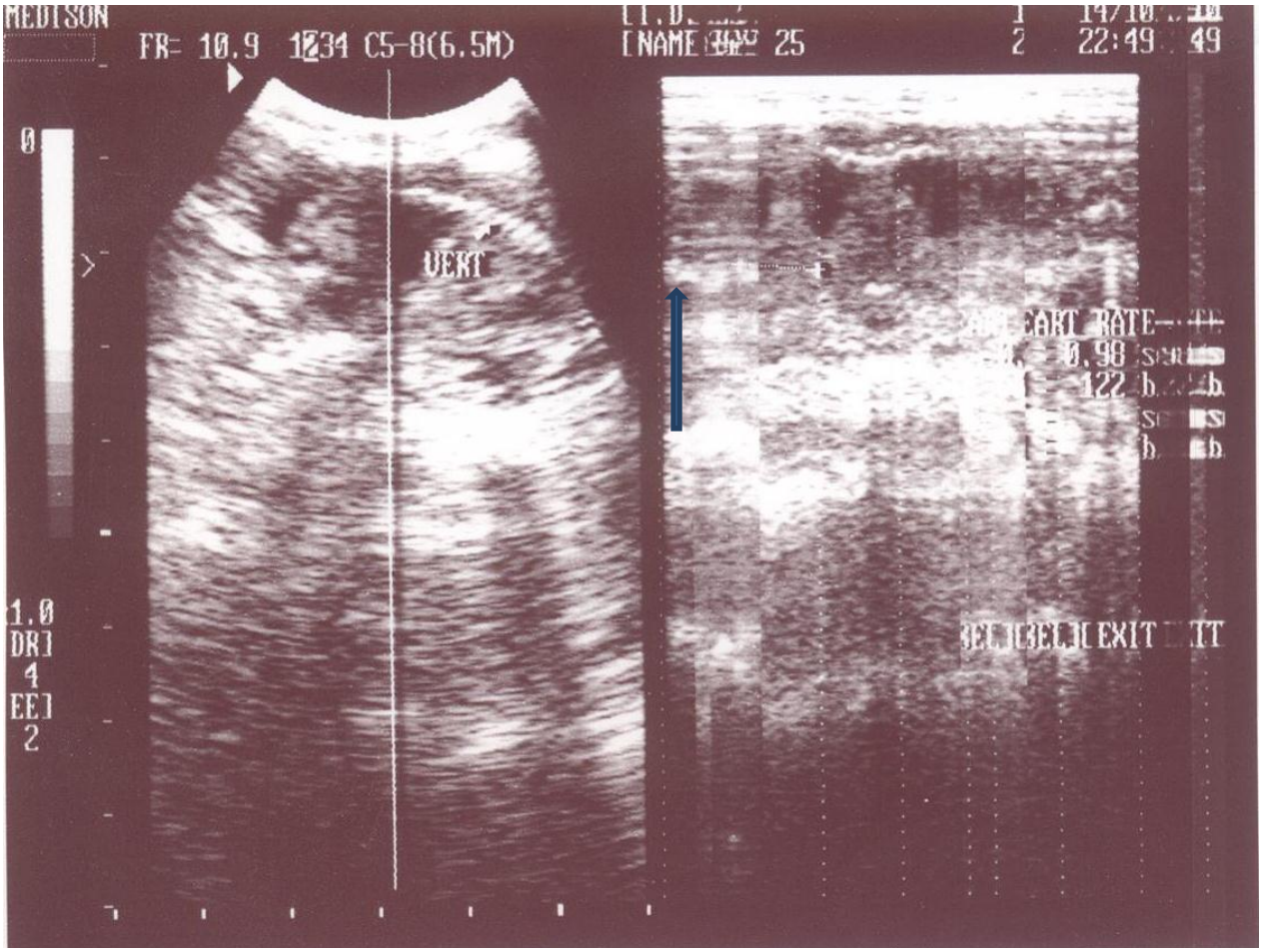


Plate V: Sonogram of a doe (E2) on day 25 of gestation showing the vertebral column (VERT) and fetal heart rate (FHR) arrowed, reading 125beats/minute.

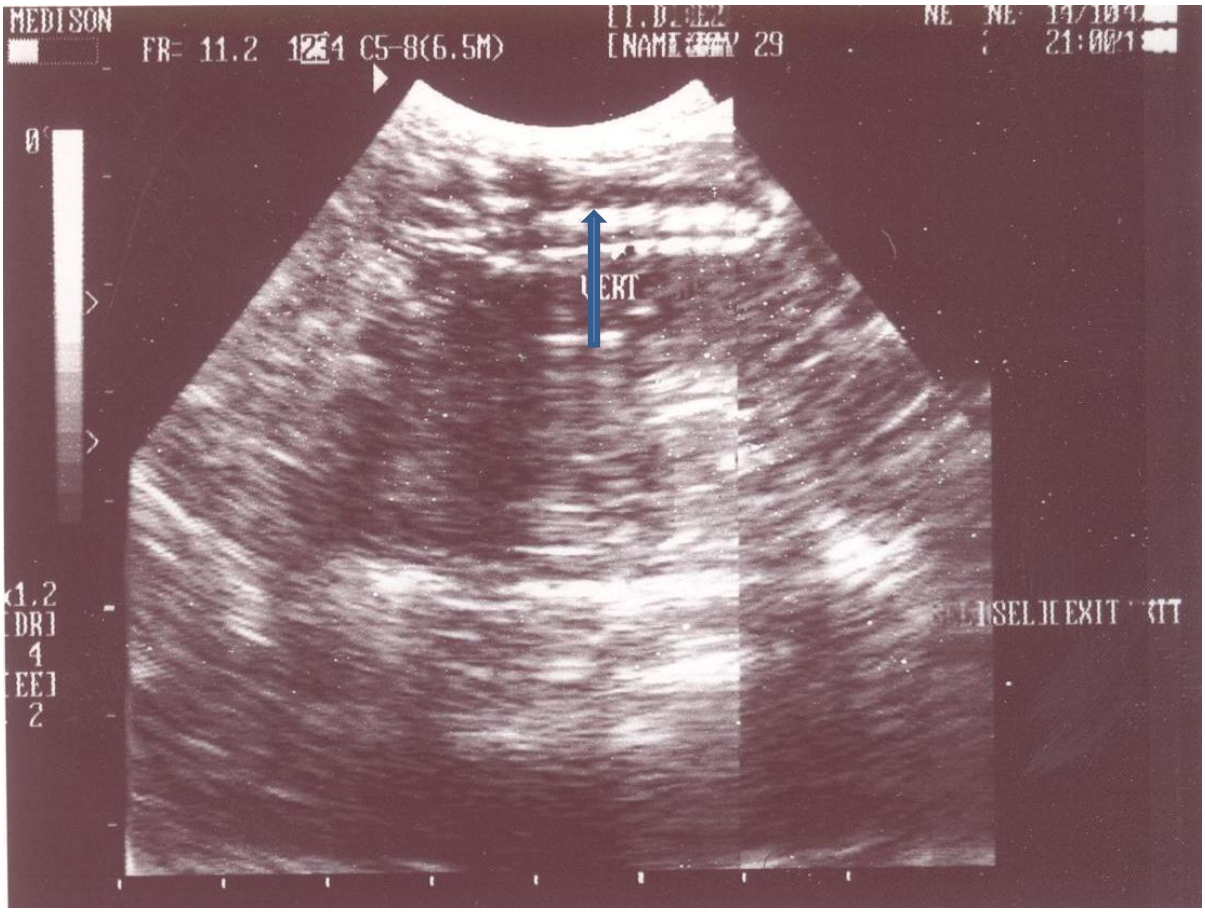


Plate VI: Sonogram of a doe (E2) on day 29 of gestation showing a developed vertebral column (VERT) (arrowed).

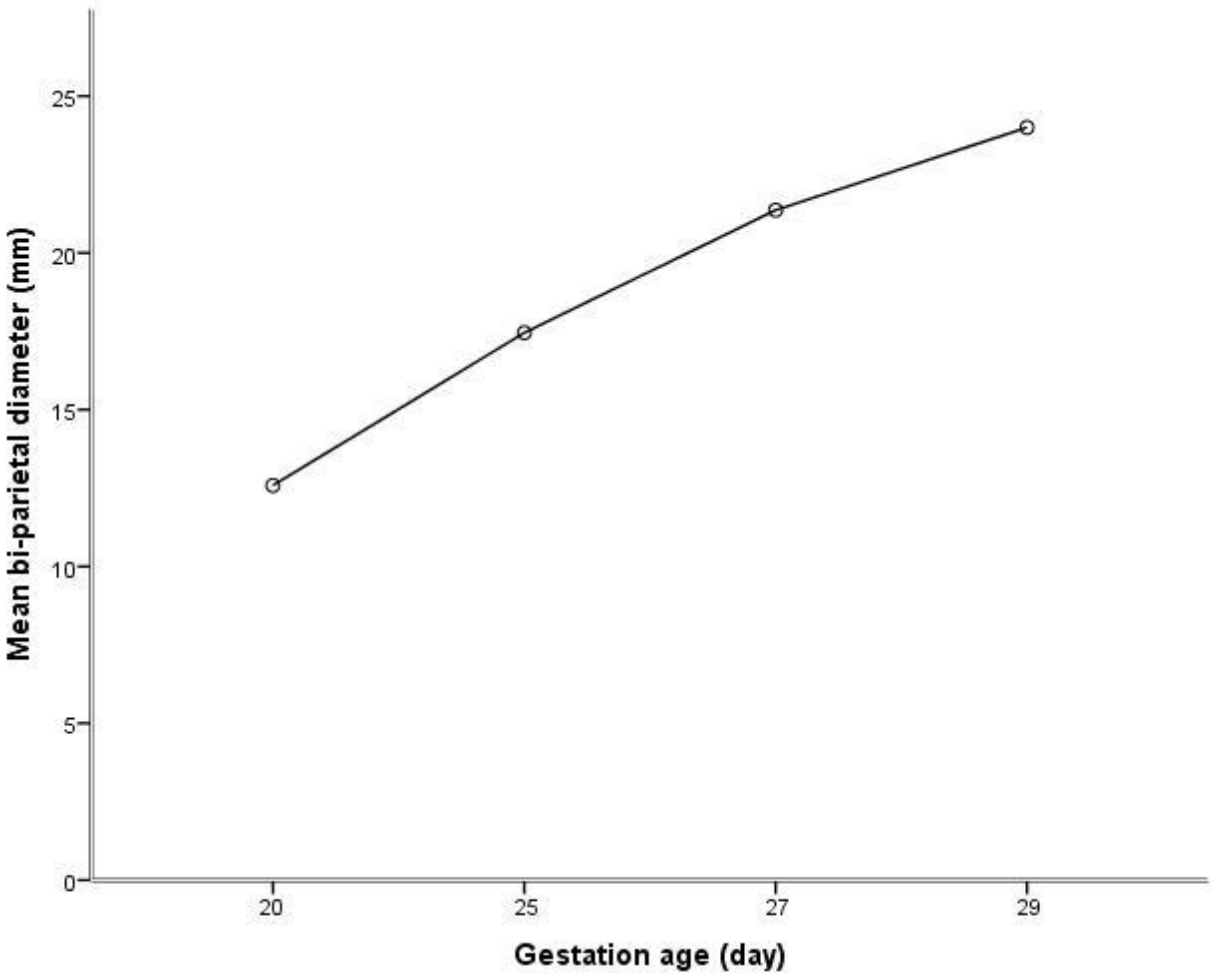


Fig. 4.1: Variation of mean bi-parietal diameter with gestation age in Isonated group of rabbits

4.2. Litter size and weight

Mean litter size was 5.43 ± 0.9 and 3.71 ± 0.7 for the control and isonated group respectively (Table 4.1). There was a statistical significant difference between the litter sizes of control group as against the isonated ($P < 0.05$). Mean litter weight was 43.7±4.3 gand 39.6±2.1 g for the control and isonated group respectively (Table 4.1). There is a statistical significant difference between litter weights of the control as against the isonated group ($P < 0.05$).

Table 4.1: Mean litter size and weight of Control and Isonated group of rabbit

	Mean litter size	Mean litter weight (g)
Control	5.43 ± 0.9	43.7 ± 4.3
Isonated	3.71 ± 0.7	39.6 ± 2.1

4.3. Hormonal Assay

The Total Triiodothyronine (tT3) serum concentration values ranged 1.39 -1.70 µg/dl with mean 1.60 ± 0.11 µg/dl for the control and 1.53-1.69 µg/dl with mean 1.58±0.05 µg/dl for the isonated group. There was a numerical difference between both groups but the difference was not statistically significant (P > 0.05). Figure 4.2 is a bar chart showing tT3 serum concentration of both groups. Total Thyroxine (tT4) values ranged1.28- 1.60 µg/dl with mean 1.44 ±0.12 µg/dl for the control and 1.28-1.54 µg/dl with mean 1.39±0.09 µg/dl for the isonated group. There was a numerical difference between both groups but the difference was not statistically significant (P> 0.05). Figure 4.3 is a bar chart showing tT4 serum concentration of both groups.

Table 4.2: Mean tT3 and tT4 serum concentration in µg/dl for control and isonated group of kittens

	Mean tT3 conc. (µg/dl**)**	**Mean tT4 conc. (**µg/dl**)**
Control	1.60 ± 0.11	1.44 ± 012
Isonated	1.58 ± 0.05	1.39 ± 0.09

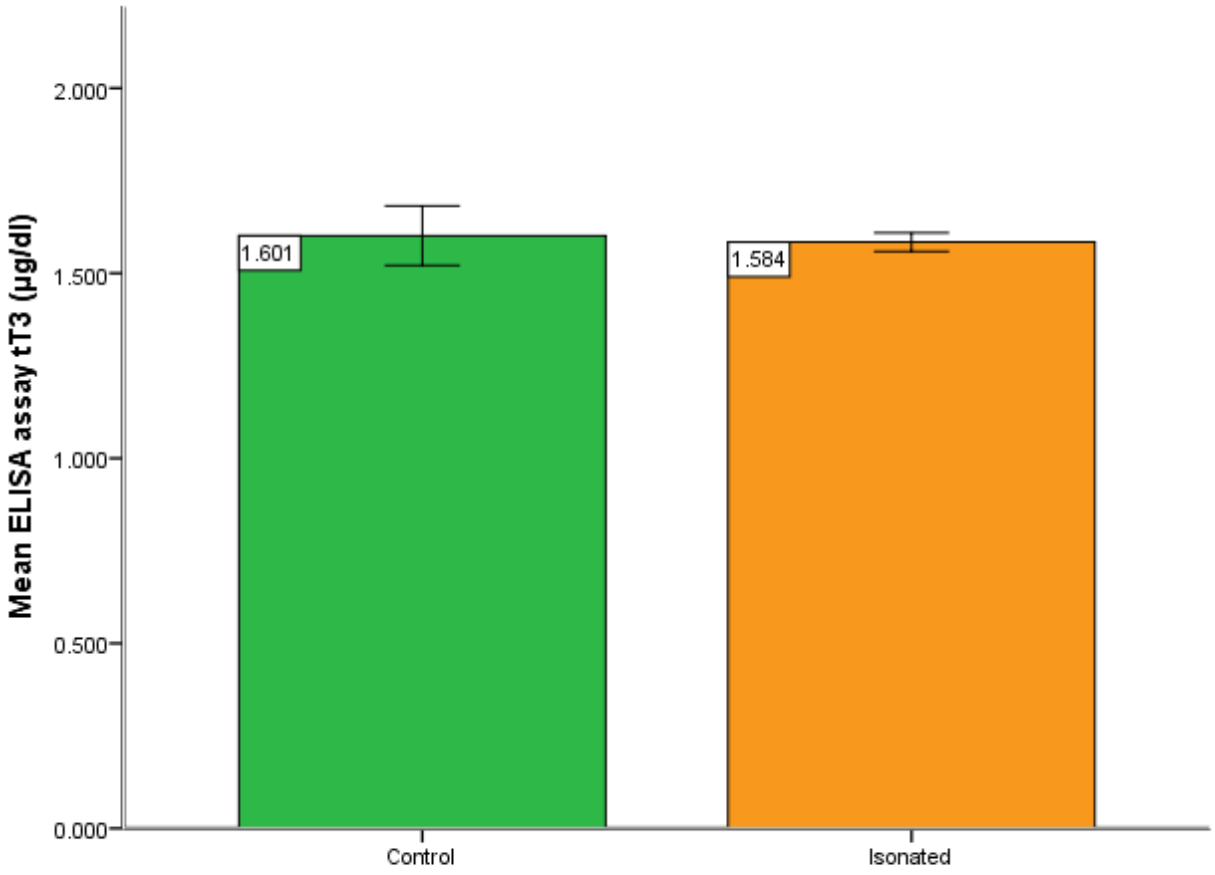


Fig. 4.2: Bar Chart showing tT3 serum concentration of Control and Isonated kittens

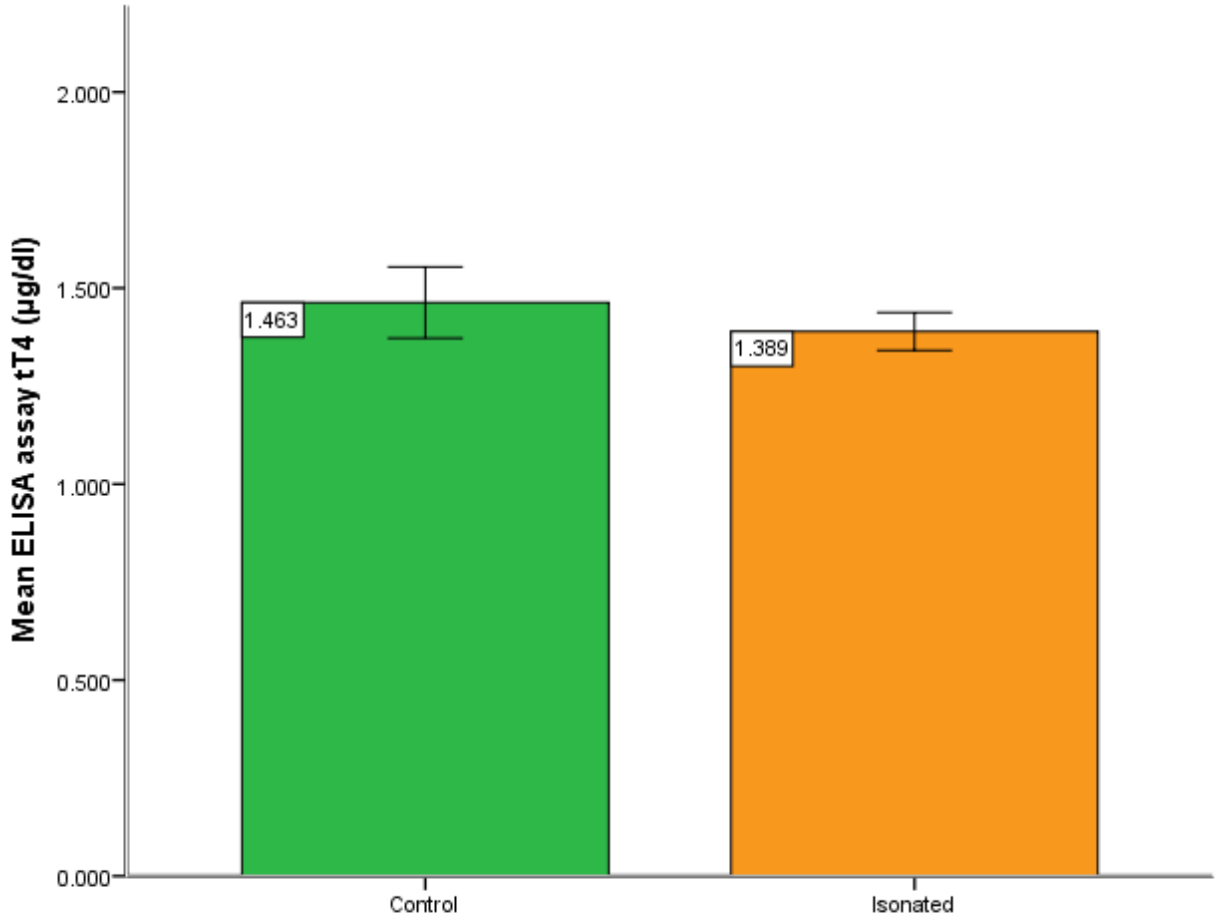


Fig. 4.3: Bar Chart showing tT4 serum concentration of Control and Isonated kittens

4.4. Histopathology

There were no histopathologic changes 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 VII).

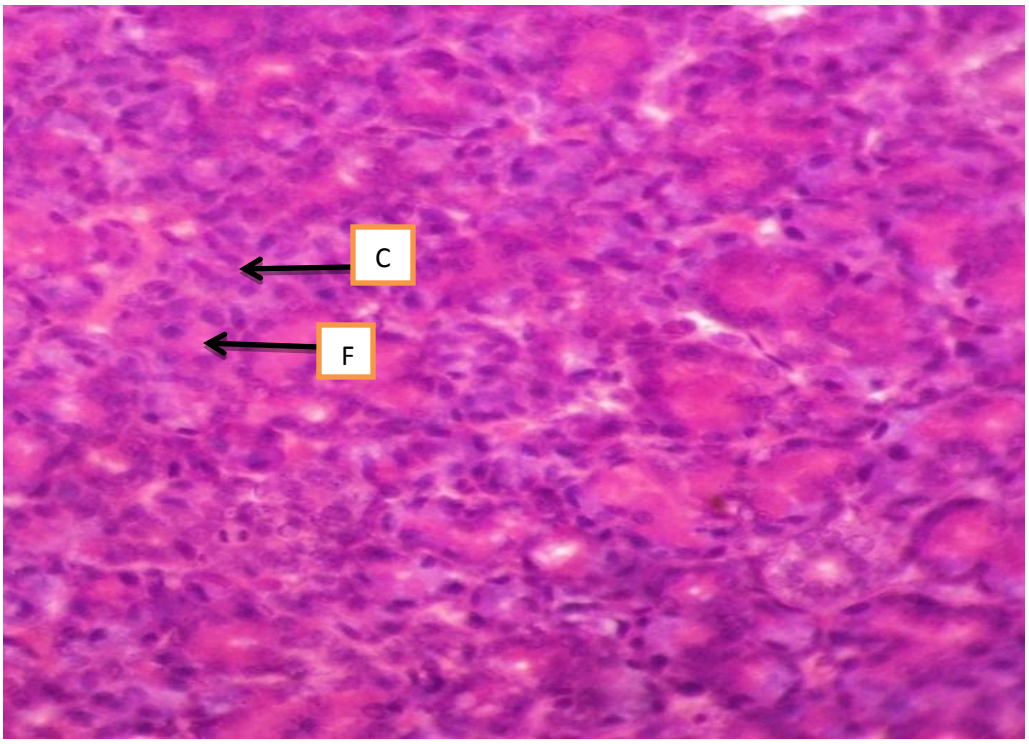


Plate VII: Photomicrograph of thyroid gland (controlled kitten), showing follicles (F) containing colloids surrounded by C-cells (C) (Hematoxylin andEosin stain, x400 magnification).

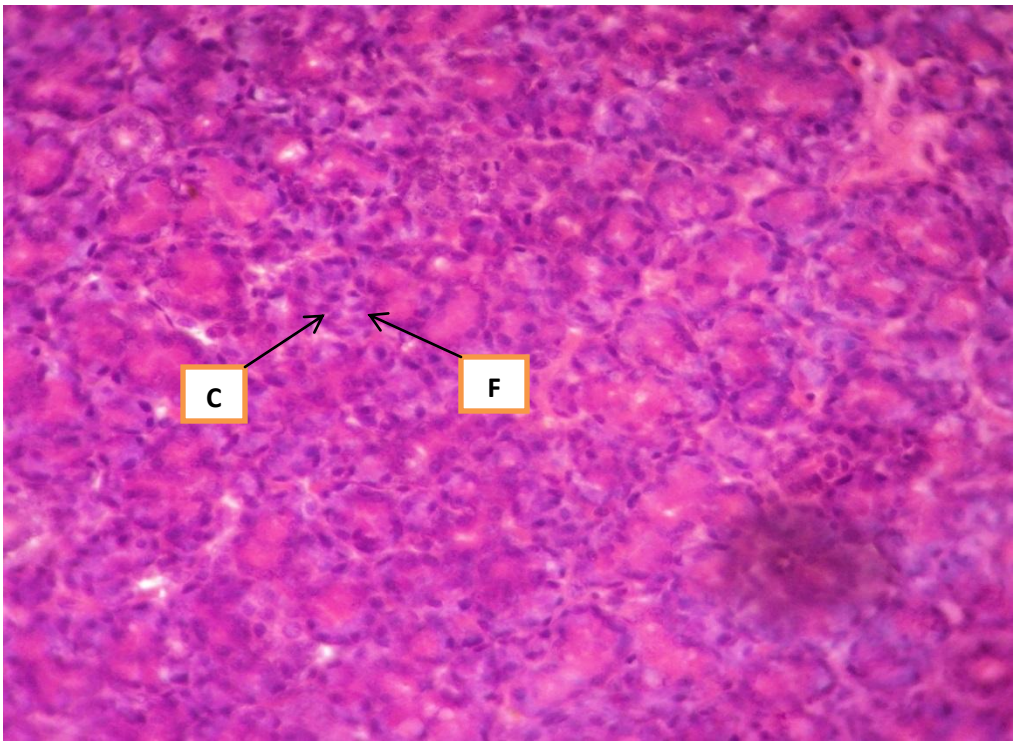


Plate VIII: Photomicrograph of thyroid gland (Isonated kitten), showing follicles (F) containing colloids surrounded by C-cells (C) (Hematoxylin and Eosinstain, x400 magnification).

CHAPTER FIVE

5.0 DISCUSSION

The study was designed to evaluate the sonographic features that occur during the three gestational stages in rabbit-doe and to see the possible effect of ultrasound exposure to fetal thyroid hormone level. The total time needed for sonographic examination (restrain, clipping and examination) of a doe was between 5 – 10minutes. Although, clipping took few minutes longer when non-lactating does were to be examined. This was because more hair was present on the ventral abdomen (Guteirrez and Zamora, 2004).

The 100% pregnancy rate of the isonated group at first trimester which later dropped to 12.5% at second trimester as against the control group of 100% can be associated to fetal exposure to frequent ultrasound exposure which agrees with previous studies of Curto 1976 and O'Brien *et al* 2008 where they all reported an increase neonatal death in animals exposed to continuous ultrasound. This work suggests that frequent isonation can lead to neonatal death in rabbits.

The result of thisultrasound showed that the earliest time for detection of pregnancy in rabbit was on the 7^{th} day of gestation where round anechoic(dark) areas surrounded by isogenic area denoted embryonic vesicles which were the first suggestive signs of pregnancy. This agrees with findings of previous workers (Ypsilantisand Saratsis, 1999; Guteirrez and Zamora, 2004; Soroori*et al*., 2008)where they found embryonic vesicles as early as day 7 of gestation measuring 7 - 9 mm which enabled them to confirm pregnancy at this stage.

Embryonic vesicles characterized the first trimester in does. Second trimester however was characterized by placenta formation and development of the embryo within the amniotic fluid at the early stage. The beginning of bone formation which appears as hyperechoic structures in-utero marked the late stage of the second trimester. Most appreciable observable

sonographicchanges were seen during the third trimester because of the rapid growth and development at this stage. An increase in size of bi-parietal diameter, vertebral column and appendages marked this stage of the cycle with an increase in gestational age. The increase in bi-parietal diameter with an increase fetal age agrees with studies of Soroori*et al*., 2008 and Nwaogu*et al*., 2010 in rabbit and goats respectively where they both observed high significant correlation between bi-parietal diameter and fetal age. It was difficult to associate structures to individual fetuses. This was due to an increase in fetal movement, thus overlapping of osseous structures such as vertebral column and appendages as seen in previous work by Guteirrez and Zamora (2004).

The result of litter size and weight showed a reduction in the isonated group as compared to the control. While it is possible that this finding was a chance effect, it is also plausible that frequent exposure to ultrasound may have influenced fetal growth and number. 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 rangeobserved an increased rates of malformations, fetal resorption and low birth weight. Thus, pointing to hyperthermia as the most probable teratogenic mechanism.

The result of the hormonal assay oftotal Triiodothyronine (tT3) and total Thyroxine (tT4) showed a numerical reduction but not statistically significant in the isonated group as compared the control, this shows that the effect of ultrasound heating on thyroid hormone level was an indication of hormonal interruption. This finding disagrees with the work of Sulaiman*et al*.(2013)where there was a significantly lowered parathyroid hormone level in the exposed

group as against the control. This could possibly be because there was an increase in the time of exposure (60minutes) as against this research (5 minutes) and also parathyroid hormone was assayed for as against thyroid hormone of this study (Sulaiman*et al*., 2013).

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 and C-cells indicative of an active thyroid gland. The normalcy can be associated to the fact that the ultrasound was not focused directly on the foetal thyroid gland as ultrasound at sufficiently high intensities could generatecavitational activity that completely destroys cells (Kaufman *et al*., 1977; Moore and Coakley, 1977). Also high intensity focused ultrasound (HIFU) has been used to treat many patients with thyroidal nodes; exposed tissues became necrotic that later disintegrated and shrank (Huedayi*et al*., 2014).

CHAPTER SIX

6.0 SUMMARY, CONCLUSION AND RECOMMENDATIONS

6.1 Summary

Ultrasound waseffectively used to study pregnancy in rabbits and gestational age was correctly approximated when evaluated by a trained individual. Early pregnancy diagnosis in rabbits using ultrasound was achieved as early as the 7^{th} day after coitus. The fetal morphology was recognisedat advanced stage of pregnancy. The heart beat was picked from day 25. Frequent exposure to ultrasound couldaffect fetal development, maturation as well as cause fetal resorption. Although the hormonal results were not statistically significant, there were indications of ultrasound heating having effect on fetal hormone level.

6.2 Conclusion

This study has shown that pregnancy in does can be confirmed as early as the 7^{th} day post coitus and also there was a good correlation between ultrasonographic changes with gestational age. Although hormonal assay results were not statistically significant, there were indications of a possible side effect of ultrasound heating on fetal thyroid gland.

6.3 Recommendations

1. Modern ultrasound equipment should be used in pregnancy evaluation in does to ensure accurate results.
2. Further studies should be carried out on the possible effect of ultrasound on other measureable parameters inother species of animal.
3. Repeated ultrasound imaging examination should be restricted only to diagnostic purpose to prevent possible side effect or using ALARA.
4. There is a need to further study thelateeffect of isonation on neonatepostnatal.

References

Abbott, J.G. (1999).Rationale and derivation of MI and TI-a review: *Ultrasound in Medicine and Biology* 25: 431.

Abramowicz, J.S., Lewin, P.A. and Goldberg, B.B. (2008).Ultrasound bioeffects for the perinatologist.*Global library women's medicine*.DOI 10.3843/GLOWM.10204.

Adams, C. (1961). Artificial insemination in the rabbit.*Journal of Reproduction and Fertilization*2, 251-254.

Anon.I (2000).Rapid Response Group, Executive Board of the International Society of Ultrasound in Obstetrics and Gynecology Safety statement, 2000. International Society of Ultrasound in Obstetrics and Gynecology (ISUOG).*Ultrasound Obstetrics and Gynecology* 2000; 16: 594-6. [PubMed] (Accessed Feb. 15, 2014 12:32pm).

Anon. II (2002). National Council on Radiation Protection and Measurements (NCRP), Diagnostic Ultrasound Safety: A summary of NCRP technical report no. 140: Exposure Criteria for Medical Diagnostic Ultrasound: II. Criteria Based on all Known Mechanisms, 2002 (Online). Available online: http://www.ncrponline.org/Publications/Reports/Misc_PDFs/UltrasoundSummary--NCRP.pdf (Accessed Feb. 23, 2014 11:12am).

Anon. III (2005). Recommendation and guidelines for applied reproduction trials with rabbit does. *World Rabbit Science* 13; 147-164.

Anon.IV (2010). Health Protection Agency-Radiation, Chemical and Environmental Hazards, "Health effect of exposure to ultrasound and infrasound: Report of the independent advisory group on non-ionising radiation", 2010 (Online). Available online: http://www.hpa.org.uk (Accessed Feb. 14, 2014 03:43pm).

Aldrich, J.E. (2007). Basic physics of ultrasound imaging.*Critical Care Medicine*; 35; S131-S137.

Alvarino, J.M.R., Rebollar, P.G., Arco, J.A., and Torres, R. (1995). Control de la reproduccion en cunicultura: tratamientoshormonales. *Boletin de Cunicucultura*77, 18, (1) 43-46.

Barnett, S. B. (2003). Key issues in the analysis of safety of diagnostic ultrasound; *Ultrasound Bulletin*;6:41-3.

Barnett, S. B., Abramowicz, J. S., and Ziskin, M. C. (2010).Symposium on Safety of Non-medical Use of Ultrasound.*Ultrasound Medical Biology*; 36: 1209-12.

Bauer, M., Blumentritt, H., Finke, R., Schlattmann, P., Adli, M., Baethge, C., Bschor, T., Muller-Oerlinghausen, B., and Berghofer, A. (2007).Using ultrasonography to determine thyroid

size and prevalence of goiter in lithium-treated patients with affective disorders.*Journal of Affective Disorders* 104:45.

Blum, M. (1990). Evaluation of Thyroid Function; Sonography, Computed Tomography and Magnetic Resonance Imaging. In: Becker KL, ed. Principles and Practice of Endocrinology and Metabolism. Philadelphia, Pa.: Lippincott Co.; pp. 289-93.

Bom, N., Lancee, C.T.,Gussenhoven, E.J., Li, W., Hoff, H. (1992). Basic principles of intravascular ultrasound imaging, In: Intravascular ultrasound imaging, Tobis J.M., Yock P.G., eds., Churchill Livingstone, New York, Pp. 8.

Brendt, R.L. (1986).The effects of embryonic and fetal exposure to X-ray, microwaves, and ultrasound.*Clinics in Perinatology* 13: 615.

Burke, T.J. and Badertscher, R.R. (1986).Pregnancy diagnosis. In; Burke T. J. (*ed*). *Small animal reproduction and infertility.*A clinical approach to diagnosiss and treatment. Lea and Febiger, Philadelphia, pp. 85-91.

Burns, P.N. (2011). Introduction to the physical principles of Ultrasound. *Fundamentals in Medical Biophysics* , pp. 2-3.

Butch, R.J., Simeone, J.F., and Mueller, P.R. (1995).Thyroid and parathyroid ultrasonography.*Radiologic Clinics of North America* 23:57.

Cappelli, C., Castellano, M., Pirola, I., Cumetti, D., Agosti, B., Gandossi, E., Agabiti Rosei, E. (2 007).The predictive value of ultrasound findings in the management of thyroid nodules.*Quarterly Journal of Medicine* 100:29.

Capello, V. (2005).Surgical techniques for neutering the female pet rabbit.*ExoticDVM*;7(5):15–21.

Chan, V. and Perlas, A. (2011).Basics of Ultrasound imaging.In *Atlas of Ultrasound-Guided Procedures in International Pain Management*. Pp. 13-19.

Church, C. C., and Miller, M. W. (2007).Quantification of risk from fetal exposure to diagnostic ultrasound.*Programmed Biophysics in Molecular Biology*; 93: 331-53.

Clark, K.J., Cronan, J.J., and Scola, F.H. (1995). Color Doppler sonography: anatomic and physiologic assessment of the thyroid. *Journal of Clinical Ultrasound* 23:215.

Cooper, D.S., Doherty, G.M., Haugen, B.R., Kloos, R.T., Lee, S.L., Mandel, S.J., Mazzaferri, E.L., McIver, B., Pacini, F., Schlumbeger, M, Sherman, S.I., Steward, D.L., and Tuttle, R.M. (2009).Revised American Thyroid Association management guidelines for patients with thyroid nodules and differenciated thyroid cancer.*Thyroid* 19: 1167-214.

Curran, S.P. (1986). Ultrasonographic appearance of the. *Journal of America Veterinary Medical Association,* 189, 1295

Curto, K.A. (1976). Early postpartum mortality following ultrasound radiation. In: White, D.N., Barnes R (eds) *Ultrasound in Medicine*, 2: 535-536.

David, O. C. (1997). Ultrasound: General Principles: In *Diagnostic Radiology a Textbook of Medical Imaging*. Grainger and Allison's (Electronic) Edition. Folio Bound Views. Pp. 102-28.

Davis, M.E., and Haibel, G.K. (1993).Use of real-time ultrasound to identify multiple fetuses in beef cattle.*Theriogenology*;40(2):373–82.

Deanne, C. (2002). Safety of diagnostic ultrasound in fetal imaging: Diploma in fetal medicine & International Society of Ultrasound in Obstetrics and Gynecology educational series. *Doppler in Obstetrics*. Pp. 73-76.

Edwards, M. J., Saunders, R. D., and Shiota, K., (2003). Effects of heat on embryos and foetuses.*International Journal of Hyperthermia*; 19: 295-324.

Edler, I. and Lindstrom, K. (2004).The history of echocardiography.*Ultrasound in Medicine and Biology* 30;1565–1644.

Fancy, T., Gallagher, D., and Horing, J. (2010). Surgical Anatomy of the Thyroid and Parathyroid gland.*Otolaryngologic Clinics of North America* 43; 221-227.

Fricke, P.M. (2002).Scanning the future- ultrasonography as a reproductive management tool for dairy cattle.*Journal of Dairy Science*;85(8):1918–26.

Fricke, P.M., Caraviello, D.Z., Weigel, K.A., and Welle, M.L. (2003). Fertility of dairy cows after resynchronization of ovulation at three intervals following first timed insemination. *Journal of Dairy Science*;86(12):3941–50.

Garcia-Ximenes, F. Vicente, J.S., and Santacreu, M.A. (1991). Embryo transfer in lactating rabbit does by laparoscopy. *Animal Reproductive Science*24(3-4); 343-346.

Gent, R., (1997). Biological effects and safety of diagnostic ultrasound. In: *Applied Physics and Technology of Diagnostic Ultrasound*, 1st ed. Australia: Milner: pp. 301-16.

Ginther, O.J. and Pierson, R.A. (1984). Ultrasonic evaluation of the reproductive tract of the mare ovaries.*Journal of EquineVeterinary Science* 4; 11–16.

Giovagnorio, F., Drudi, F.M., Fanelli, G., Flecca, D., and Francioso, A. (2005).Fatty changes as a misleading factor in the evaluation with ultrasound of superficial lymph nodes.*Ultrasound in Medicine and Biology* 31: pp. 1017.

Gutierrez, H.E., and Zamora, F.M.M. (2004) Ultrasonography study of rabbits pregnancy. Proceedings - 8th World Rabbit Congress –September 7-10, pp. 276-280.

Haibel, G.K. (1988). Real time ultrasonic fetal head measurement and gestational age in dairy goats.*Theriogenology,* 30, 1053-1058.

Hagen, K.W. (1974). Colony husbandry. In; Weisbroth, S. H., *et al*., (eds). The biology of laboratory rabbit.Academic press, London, pp. 23-49.

Hangiandreou, N. (2003). AAPM/RSNA physics tutorial for residents: topics in US. B-mode US: basicconcepts and new technology. *Radiographics*23;1019–1033.

Harm, T.H. and Geboren, T.B. (1993). Scanning mechanisms for Intra vascular ultrasound imaging; a flexible approach.*Medical Introduction.* Pp. 15-32.

.

Heidbrink, G. (1980). Artificial insemination in commercial rabbit production - Bulletin 573S Collorado State University.

http://www.fao.org/docrep/X5082E/X5082E0C.GIF Accessed 09/23/2014 07:27PM.

Huedayi, K., Niklas, F. Micheal, S., Christain, H., and Frank, G. (2014). Early assessment of high-intensity focused ultrasound treatment of benign thyroid nodules by scintigraphic means. *Journal of Therapeutic Ultrasound* 2; 18.

Inaba, T., Nakazima, Y., Matsui, N., and Imori, T. (1983).Early pregnancy diagnosis in sows by ultrasonic linear electronic scanning.*Theriogenology* 20; 97-101.

Ishigaki, S., Shimamoto, K., Satake, H., Sawaki, A., Itoh, S., Ikeda, M., Ishigaki, T., and Imai, T**.** (2004). Multi-slice CT of thyroid nodules: comparison with ultrasonography. *Radiation Medicine* 22;346.

Jakobsen, J.A. (2001). Ultrasound contrast agents: clinical applications. *European Journal of Radiology* 11;1329.

Kaufman, G.E., Miller, M.W., Griffiths, T.D., Ciaraino, V. and Carstensen, E.L. (1977).Lysis and viability of cultured mammalian cells exposed to 1MHz ultrasound. *Ultrasound Medical Biology* 3; 21-25.

Khalifa, R.M. (1994). Mating frequency and artificialinsemination in rabbit using vasectomizedbucks.*World Rabbit Science*2; 1–5.

Khalifa, R.M., El-Alamy M.A., and Beshir M.A., (2000).Vasectomized buck gave better reproductiveresults in artificial insemination techniques inrabbit than GnRH or HCG. 7th World RabbitCongress, Valencia, Spain.Pp. 28.

King, A. M. (2006). Development advances and applications of diagnostic ultrasound in animals. *Veterinary Journal* 171; 408-420.

Knudsen, N., Bols, B., Bulow, I., Jorgensen, T., Perrild, H., Ovesen, L., and Laurberg, P. (1999).Validation of ultrasonography of the thyroid gland for epidemiological purposes.*Thyroid* 9: pp. 1069.

Kremabon A.Y., Belair L., Theau-Clement M., Salesse R., and Djinane J. (1994).Effects of an oestrus and bromocryptine treatment on the expression of prolactin and LH receptors in the rabbit ovary during lactation.*Reproductive fertility*102;131-138.

Kremkau, F. W., (2002). Performance and safety. In: *Diagnostic Ultrasound Principles and Instruments*, 6th ed. Philadelphia, USA: Saunders: pp. 335-51.

Laurel, M.D. (1993). Bioeffects and Safety of Diagnostic Ultrasound.*American Institute of Ultrasound in Medicine*,pp. 1-40.

Larsen, W. (1997).*Human Embryology*, 2nded. New York: Churchill Livingstone, pp. 347-374.

Lavara, R., Mocé, E., Lavara, F., de Castro, M.P.V., and Vicente, J.S. (2005). Do parameters ofseminal quality correlate with the results of on-farm inseminations in rabbits. *Theriogenology*, 64;1130-1141.

Lawrence, J.P. (2007).Physics and instrumentation of ultrasound.*Critical Care Medicine* 35; 314–322.

Lebas, F., Coudert, P., Rouvier, R., and Rochambeau, H. D. (2014)."The Rabbit: Husbandry, Health and Production," in Food and Agriculture Organization (FAO) Animal Product and Health Series, [Online].
Available: http://www.fao.org/docrep/X5082E/X5082E00.htm Accessed 09/23/2014 7:29PM.

Leopold, G.R. (1990).Ultrasonography of superficially located structures.*Radiologic Clinics of North America* 18;161.

Luc DesCôteaux, G.G. (2010). *Practical Atlas of Ruminant and Camelid Reproductive Ultrasonography.* Wiley-Blackwell. Pp. 32.

Maertens, L. and Luzi, F. (1995). Effect of diluent and storage time of rabbit semen on the fertility of does reared under two different lighting schedules. *World Rabbit Science* 3; 27-34.

Maizlin, Z.V., Wiseman, S.M., Vora, P., Kirby, J.M., Mason, A.C., Filipenko, D., and Brown, J.A. (2008).Hurthle cell neoplasms of the thyroid: sonographic appearance and histologic characteristcs. *Journal of Ultrasound in Medicine* 27; 751..

Mannor, S.M., Serr, D.M., Tamari, I., Meshorer, A., and Frei, E. (1972).The safety of ultrasound in fetal monitoring.*America Journal of Obstetrics and Gynecology* 113; 653-661.

Marqusee, E., Benson, C.B., Frates, M.C., Doubilet, P.M., Larsen, P.R., Cibas, E.S., and Mandel, S.J. (2000).Usefulness of ultrasonography in the management of nodular thyroid disease.*Annals of Internal Medicine* 133;696.

McKinnon, A.O., Voss, J.L., Squires, E.L., and Carnevale, E.M. (1993).Diagnostic ultrasonography. In; McKinnon, A. O. and Voss, J. L. (*eds*). *Equine Reproduction*. Lea and Febiger, Philadephia, London, pp. 266-302.

McNitt, J.I. (1992). Endocrinological approaches for commercial rabbit production. *Journal of Applied Rabbit Research,* 15; 364-397.

Medan, M., Watanabe, G., Absy, G., Sasaki, K., Sharawy, S., and Taya,K.Early pregnancy diagnosis by means of ultrasonography as a method of improving reproductive efficiency in goats.*Journal of Reproduction and Development*50(4);391–7.

Menna, S., Di Virgilio, M.R., and Burke, P. (1999). Ultrasonography contrast media Levovist and power Doppler in the study of the breast. Methodology, vascular morphology and automatic enhancement quantification with wash-in and wash-out curves.*RadiologiaMedica* 97;472.

Miller, M. W., Brayman, A. A., Abramowicz, J. S., (1998). Obstetric ultrasonography: a biophysical consideration of patient safety--the "rules" have changed. *America Journal of Obstetrics and Gynecology* 179; 241-54.

Moon, W.J., Jung, S.L., Lee, J.H., Na, D.G., Baek, J.H., Lee, Y.H., Kim, J., Kim, H.S., Byun, J.S .,Lee, D.H., (2008). Benign and malignant thyroid nodules: US differentiation – multicenter retrospective study. *Radiology* 247;762.

Moore, J.K. and Coakley, W.T. (1977). Ultrasonic treatment of Chinese hamster cells at high intensities and long exposure times. *British Journal of Radiology* 50; 46-50.

Moore, K. and Persaud, T.V.N. (1998). The Developing Human: *Clinically Oriented Embryology*, 6th ed. Philadelphia: WB Saunders, pp. 230-232.

Morrell, J.M. (1990). Use of an ELISA for plasma progesterone to facilitate rabbit husbandry. *Veterinary Record* 127; 521-524.

Morrell, J.M. (1993). Preliminary investigation of an ELISA kit as a qualitative assay for rabbit progesterone.*Veterinary Record* 132; 434-436.

Morrell, J. M. (1995). Artificial insemination in rabbits.*British Veterinary Journal*151(5); 477-488.

Nautrup, C.P. (2006). An introduction to the Physical-Technical basics of X-ray and Ultrasound Diagnostics.*Anatomy of the Dog* (an illustrated Text) 4thEdition.Schlutersche. Pp. 158-172.

Nava, G.A., Brivio, R., Batocchio, M., Cairoli, F., and Soldano, F. (1992).Utilisation du Tiaprosten chez la lapinesuivantune insemination infeconde.*Cuni-Science* 7; 107-110.

Ndumbe, F.M., Navti, O., and Chilaka, V. N.(2008).Prenatal diagnosis in the first trimester of pregnancy.*Obstetrical and Gynecological Survey* 63; 317.

Novelline, R. (1997). *Squire's Fundamentals of Radiology* (5th ed.). Harvard University Press. pp. 34–35.

Nwaogu, I. C., Anya, K. O. and Agada, P. C. (2010).Estimation of foetal age usingultrasonic measurements of different foetal parameters in red Sokoto goats (*Caprahircus*).*VeterinarskiArhiv* 80; 225-233.

Nyland, T.G., and Mattoon, J.S. (2002).Small Animal Diagnostic Ultrasound. 2nd ed. Philadelphia: WB Saunders Pp. 71.

O'Brien, W.D.Jr., Deng, C.X., Harris, G.R., Herman, B.A., Merrit, C.R., Sanghvi, N. and Zachary, J.F. (2008). The risk of exposure to diagnostic ultrasound in post natal subjects: thermal effects. *Journal of Ultrasound in Medicine* 27; 517-535.

Otto, C.M. (2000).Principles of echocardiographic image acquisition and Doppler analysis. In: *Textbookof Clinical Ecocardiography*. 2nd ed. Philadelphia, PA: WB Saunders; 2000: pp. 1–29.

Peavy, D.E. (2009).Endocrine Control Mechanisms.*Medical Physiology, Part IX, Endocrine Physiology*31; 234-57.

Pierson, R.A., and Ginther, O.J. (1984).Ultrasonography of the bovine ovary.*Theriogenology*;21(3); 495–504.

Platt, L.D. (1988). New look in ultrasound: the vaginal probe. *Technology* pp. 99-101.

Popesko, P., Rajtovà, V., and Horak, J. (1990).*A Color Atlas of Anatomy of Small Laboratory Animals. Vol. 1, Rabbit, Guinea Pig, Vol. 2: Rat, Mouse,* Hamster. London, UK: Wolfe Publishing Ltd. Pp. 16.

Quintela, L.A., Pena, A.I, Vega, M.D., Gullon, J. Prieto, M.C., Barrio, M., Becerra, J.J., Maseda, F., and Herradon P.G. (2004).Ovulation induction in rabbit does submitted to artificial insemination by adding buserelin to the seminal dose. *Reproductive Nutrition Development* 44; 79-88.

Rago, T., Bencivelli, W., Scutari, M., Di Cosmo, C., Rizzo, C., Berti, P., Miccoli, P., Pinchera, A ., and Vitti, P. (2006). The newly developed three-dimensional (3D) and two-dimensional (2D) thyroid ultrasound are strongly correlated, but 2D overestimates thyroid volume in the presence of nodules. *Journal of Endocrinological Investigation* 29;423.

Rebollar, P.G., Ubilla, E., Rodrigues, J.M., Illera, J.C., and Silvan, G. (1992).Induction of luteolysis by administration of a synthetic prostaglandin F2α analogue in pseudopregnant rabbits.*Journal of Applied Rabbit Research* 15; 456-461.

Ribadu, A.Y., and Nakao, T. (1999).Bovine reproductive ultrasonography: a review. *Journal of Reproduction and Development*45(1);13–28.

Saratsis, P.H., Andersen, P., and Rexha, S. (1993). Early pregnancy diagnosis and examination of the ovaries of cows by means of ultrasound tomography.*Bulletin of the Hellenic Veterinary Medical Society*, 44(4); 253-263.

Schortinghuis, J. Stegenga, B., Raghoebar, G. M., and Bont, L. G. M., (2003).Ultrasound stimulation of maxillofacial bone healing.*Critical Review in Oral Biology and Medicine* 14(1);63-74.

Shapiro, R.S. (2003).Panoramic ultrasound of the thyroid.*Thyroid* 13:177.

Sheiner, E. and Abramowicz, J.S. (2008). Clinical end users worldwide show poor knowledge regarding safety issues of ultrasound during pregnancy. *Journal of Ultrasound in Medicine* 27;499.

Sikov, M.R. (1986a).Effect of ultrasound on development. I. Introduction and studies in inframammalian species. *Journal of Ultrasound in Medicine* 5; 577.

Sikov, M.R. (1986b).Effect of ultrasound on development. II. Studies in mammalian species and overview. *Journal of Ultrasound in Medicine* 5; 651.

Simeone, J.F., Daniels, G.H., Mueller, P.R., Maloof, F., vanSonnenberg, E., Hall, D.A., O'Conne ll, R.S., Ferrucci, J.T.Jr., and Wittenberg, J. (1982) High-resolution real-time sonography of the thyroid.*Radiology* 145; 431.

Soliman, E.B., Zahran, M.W., and El-Bakary, A.H. (2001).Histological structure and hormonal profile of pituitary and thyroid glands affected by castration and iodine supplementation in male rabbits. Proceedings of the 1st International Conference: *Egyptian Journal of Biology* 3; 38-47.

Soroori, S., Dehghan, M., and Molazem, M. (2008).Ultrasonographic assessment of gestational age in rabbit. Proceedings- 15th Congress of Federation of Asian VeterinaryAsssociation; pp. 367-368.

Sulaiman, Md.D. Hairil, R.A. Farah, W.A.Z. Nurul, H.S., Khairunnisa, A.M., Iza, N.C.I.,andUmmi, F.H.(2013). Ultrasound exposure during pregnancy affects rabbit foetal parathyroid hormone (PTH) level.*QuantitativeImaging in Medicine and Surgery* 3 (1); 49-53.

Stratmeyer, M.E., Greenleaf, J.F., Dalecki, D., Salvensen, K.A. (2008). Fetal ultrasound: mechanical effects. *Journal of Ultrasound in Medicine* 27 (4); 597-605.

Terranova, P.F. (2009). Fertilization, Pregnancy, and Fetal Development:*Medical Physiology, Part X, Reproductive Physiology*39;684.

Theau-Clement, M., Bolet, G., Roustan, A., and Mercer, P. (1990).Comparaison de differents modes d'induction de l'ovulation chez les lapinesmultipares en relation avec leurstadephysiologiqueset la receptivite au moment de la misea'la reproduction. 5emes *Jour. Rech.Cunicole,* Paris, Comm. 6.

Tollin, S.R., Mery, G.M., Jelveh, N., Fallon, E.F., Mikhail, M., Blumenfeld, W., and Perlmutter, S. (2000). The use of fine-needle aspiration biopsy under ultrasound guidance to assess the risk of malignancy in patients with a multinodular goiter.*Thyroid* 10; 235.

Torres, L.C., Pla, M., and Garcia, F. (1987).Sovrapposizionetragestazione e lattazionenellaconiglia Riv. Li Coniglicoltura 24 (4); 37-39.

Ubilla, E. and Rebollar, P.G. (1995). Influence of the post-partum day on plasma estradiol 17B levels, sexual behavior and conception rate in artificially inseminated lactating rabbits. *Animal Reproduction Science* 38 (4), 337-344.

White, I.R., Russel, A.J.F., and Fowler, D.G. (1984).Real time ultrasonic scanning in the diagnosis of pregnancy and the determination of fetal numbers in sheep.*Veterinary Record,* 115; 140-143.

White, I.R., and Russel, A.J.F. (1987).Pregnancy diagnosis and fetal number detemination. In: Fayez, I.,Marai, M., and Owen, J.B., editors. *New Techniques in Sheep Production*. London: Butterworth & Co. Ltd. pp. 207–20.

Wu,J.andNyborg W.L. (2008).Ultrasound, cavitation bubbles and their interaction with cells.*Advanced Drug Delivery Reviews* 60; 1103–16.

Ypsilantis, P., and Saratsis, P.H. (1999).Early pregnancy diagnosis in the rabbit by real time ultrasonography.*World Rabbit Science* 7; 95-99.

APPENDIX I

Restraint of doe prior to scanning



APPENDIX II

Scanning of the abdomino-pelvic area of the doe.



APPENDIX III

Natural mating in rabbits



APPENDIX IV

Ultrasound screen capturing the image of a scan



APPENDIX V

Accubind ELISA microwells kit used for thyroid hormone assay.





APPENDIX VI

Haemogram ofRabbit-does taken prior to mating.

DOE	PCV	HB	T. PROTEIN	WBC	RBC
1	34	134	6.0	18.3	4.9
2	37	167	5.2	8.5	5.8
3	35	144	5.0	5.9	5.2
4	38	108	4.0	8.8	6.7
5	46	163	4.2	15.2	8.2
6	40	162	5.6	11.8	7.4
7	39	167	5.0	12.6	7.0
8	35	170	4.0	10.8	4.7
9	38	122	3.2	14.1	6.0
10	40	162	4.0	13.0	5.9
11	48	168	3.8	10.4	4.5
12	38	160	5.6	9.8	6.0
13	45	153	5.0	11.5	6.1
14	35	132	4.6	9.8	2.6
15	36	107	4.2	8.7	6.0
16	44	155	4.0	8.2	6.4