

**CORRELATION OF THE CYTOLOGIC, ULTRASONOGRAPHIC AND
HORMONAL CHANGES AT VARIOUS STAGES OF THE OESTROUS CYCLE
OF INDIGENOUS BITCHES**

BY

LAWAL, MARUF

DVM (2004) A.B.U, ZARIA

(M.Sc/ VET-MED/51884/2005-06)

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FACULTY OF VETERINARY MEDICINE,

AHMADU BELLO UNIVERSITY,

ZARIA, NIGERIA

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DECLARATION

I declare that the work in this thesis entitled “**Correlation of the cytologic, ultrasonographic and hormonal changes at various stage of the oestrous cycle of indigenous bitches**” has been performed by me in the Department of Veterinary Surgery and medicine, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria under the supervision of Dr A.Z. Hassan, Dr N. D. Chom and Professor A.B. Ogunkoya.

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

.....
Name of student

.....
Signature

.....
Date

CERTIFICATION

This thesis entitled **“CORRELATION OF THE CYTOLOGIC, ULTRASONOGRAPHIC AND HORMONAL CHANGES AT VARIOUS STAGES OF THE OESTROUS CYCLE OF INDIGENOUS BITCHES”** By Maruf Lawal meets the regulations governing the award of the degree of Master of Science of Ahmadu Bello University, Zaria and is approved for its contribution to knowledge and literary presentation.

.....
Dr A. Z. Hassan. DVM MSc, P.hD
Chairman, Supervisory committee.

.....
Date

.....
Dr N.D. Chom. MBBS, FWACS
Member, Supervisory Committee.

.....
Date

.....
Professor A. B. Ogunkoya. DVM. MSc, P.hD
Member, Supervisory Committee.

.....
Date

.....
Professor AKB Sackey, BSc Agric., DVM, MSc, P.hD
Head, Department of Veterinary Surgery and Medicine

.....
Date

.....
Prof. A.A. Joshua BA, MA, P.hD
Dean of Postgraduate School

.....
Date

DEDICATION

This thesis is dedicated to my family, Amie, Mujaheed, Rahama-Adama and in memory of Ahmatullah.

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ABSTRACT

Ten (10) mature bitches that had whelped at least once and at most twice were used the study was designed to provide baseline information correlating the cytologic, sonographic and hormonal changes that occur during the various phases of the reproductive cycle in Nigerian indigenous bitches. Baseline ultrasound data were obtained during the one month acclimatization period. Vaginal cytology, haematology and serum hormonal assay were carried out as described by Coles, 1986 and Nett *et al.*, 1975 respectively. Each bitch was given 1ml vial of Human Chorionic Gonadotropin (HCG) intramuscularly on day 1 and repeated after 48 hours. The sampling was done on days 1, 3 and 5 of the experiment. Ultrasound examination was conducted to visualize the internal genitalia and also measure the uterine luminal diameter throughout the study period. Following the first treatment, some of the bitches started showing characteristic physical signs of estrus. The bitches had a mean Packed Cell Volume of 37.93 ± 8.48 %. Mean White blood cell count of $4.85 \times 10^9 \pm 0.78 \times 10^9$ / L was recorded. Follicle stimulating hormone mean concentration was 9.33 ± 3.56 μ iu/ml. Mean concentration of Luteinizing hormone 7.91 ± 2.63 μ iu/ml. The result of the vaginal cytology showed all of them to be at diestrus on day 1, with large rhomboidal cells beginning to lose their nucleus, vaginal nucleated cells and neutrophils. At this time the internal genitalia was visualized only up to the uterus on ultrasonography, the mean uterine luminal diameter was 5.5 ± 0.70 mm. Three bitches (30%) were at early proestrus with neutrophils, parabasal cells and mucus while seven (70%) were at late proestrus cycle erythrocytes, superficial cells and absence of neutrophils on day 3. The internal genitalia up to the ovaries were visualized as being small anechoic (darkened) structures, and

luminal diameter was 7.5 ± 0.50 mm. All the bitches were at estrus on day 5, with keratinized superficial cells which were anucleated with angular margins. The same anechoic structures (as at proestrus) were observed with but were larger due to some fluid accumulation. The mean uterine luminal diameter was 8.6 ± 0.50 mm. At anestrus there was reduction in the size of the uterus (5.8 ± 0.4 mm). The ovaries were not visualized. In conclusion, this study demonstrated that there was good correlation between cytologic changes and ultrasonographic changes that occur during the different phases of the reproductive cycle in Nigerian indigenous bitches.

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CHAPTER 1

INTRODUCTION

1.1 Preamble

Sound waves are longitudinal waves. The particles of a tissue are brought to vibrate by the sound. The direction of the vibration corresponds to the direction of sonic propagation, which results in compression and rarefaction within the wave. Frequencies above 20 kHz are known as ultrasound (Nautrup, 2006).

Ultrasonography is defined as the use of ultrasound to make images of features that cannot be seen, especially for the purpose of medical examination or diagnosis (Jeanette, 1993). Medical ultrasonography is the application of high-frequency, low-intensity sound waves to various regions of the body (Jeanette, 1993, Anon I, 2008). The frequencies used in diagnostic ultrasound lie between 1-20 MHz and are considerably higher than the ultrasonic frequencies perceivable by animals such as cats, dogs or bats (Nautrup, 2006).

There have been considerable advances in the use of B-mode ultrasonography for imaging the reproductive tract in a number of species including dogs (England and Yeager, 1993). In the bitch, the early use of ultrasound was confined to the diagnosis of pregnancy (England and Yeager, 1993). More recently however, the full value of ultrasound imaging of the reproductive organs in dogs has been documented and the technology is now finding wide application in monitoring foetal development, timing gestation, predicting parturition, diagnosis and management of reproductive tract diseases as well as in supplementing breeding soundness examination (England and Yeager, 1993).

Females of most domestic animals species are sexually receptive (in heat or oestrus) every 17-21 days (Jeanette, 1993). Failure to return to oestrus after a successful breeding is often the earliest indication of pregnancy in these animals. In the bitch, the average interval between periods of oestrus is seven months regardless of whether the bitch becomes pregnant. Therefore, non-return to oestrus is not a reliable indicator of pregnancy in the canine (Jeanette, 1993).

Vaginal cytology is a simple technique that can be used by practitioners to help characterize stages of the reproductive cycle of the bitch or to evaluate certain diseases of the genital tract (Valerie *et al.*, 2003). This is used in conjunction with the clinical history, physical examination, vaginoscopy and hormonal assays to determine the stage of the reproductive cycle. This is especially important if artificial insemination is to be performed. Other uses of vaginal cytology and ancillary testing include, diagnosis of inflammation of the vagina, and identification of some types of neoplasia involving the vaginal vault and urethral orifice and determination of the whelping date (Valerie *et al.*, 2003; Josep, 2010).

The oestrous cycle of the bitch contains four distinct stages that can be followed cytologically. These stages include prooestrous, oestrus, dioestrus, and anoestrus. For some simplification and indication of which structures predominate throughout the cycle, these four stages may be grouped into the follicular phase (prooestrous and oestrus) or the luteal phase (dioestrus and anoestrus). The bitch frequently has been classified as monocyclic or having a reproductive cycle that lacks frequent, recurring periods of heat and has a long period of anoestrus regardless of pregnancy status (Valerie *et al.*, 2003; Josep, 2010).

1.2 Statement of Research Problems

There is little information on the sonographic changes of the reproductive tracts of the Nigerian indigenous breed of dog, and information on the vaginal cytological changes that occur during their different phases of the oestrus cycle. There is also paucity of data on hormonal responses to oestrus inducing agents whose use is important in controlled breeding and whelping date determination.

1.3 Justification

Due to the dearth of information on sonographic features of the reproductive cycle of Nigerian indigenous breed of dogs and the fact that the technology has come to stay; there is an urgent need to utilize ultrasonography to establish baseline information on the Nigerian local bitches as well as their various crosses which abound in various parts of the country.

1.4 Aim of the Study

This study aims to provide baseline information correlating the cytologic, sonographic and hormonal changes that occur during the various phases of the reproductive cycle in Nigerian indigenous bitches.

1.4 Specific objectives of the Study are to

1. Map out the various sonographic changes that occur during different phases of the reproductive cycle.
2. Study the normal non-pregnant reproductive tract of Nigerian local bitches cytologically.

3. Correlate the sonographic and cytological changes during the different phases of the reproductive cycle following induction of oestrus.

CHAPTER 2

LITERATURE REVIEW

2.1. Female Genital Organs

2.1.1. Anatomy

The female genital organs consist of ovaries, oviducts, uterus, vagina and vulva. The ovaries produce the female sex cells (ova), which are transported by the oviduct to the uterus or uterine horn, where implantation takes place if fertilization has occurred, and the embryo is developed. The vagina is a canal leading from the uterus to the external genitalia, the vulva (Christensen, 1964).

2.1.1.1. Ovaries

The ovaries are paired oval organs, located in the abdominal cavity caudal to the kidneys (Figure I). In its normal position, an ovary may be described as having cranial and caudal poles, medial and lateral borders and dorsal and ventral surfaces (Christensen, 1964). The ovary is divided into a medulla and a cortex. The medulla (*zona vasculosa*) contains blood vessels, nerves, lymphatics, smooth muscle fibers and connective tissue fibers. The cortex consists of a connective tissue stroma which contains a large amount of germ cells, follicle cells and graafian follicles (*folliculi oophorivesiculosi*). In the absence of germ cells, the development of interstitial cells appears to be correlated with the activity of the follicle or indifferent cells (Kingsbury, 1914). The ovary is supplied by the ovarian artery, which arises from the aorta approximately one-third to one-half the distance from the renal arteries to the deep circumflex iliac arteries. It is drained by the left and right ovarian veins (which have different terminations), the right drains into the posterior vena cava, while the left enters the left renal vein. The lymphatics drain into the lumbar lymph nodes (Polano

1903). Nerve supply is via the sympathetic division of the autonomic nervous system, they reach the ovaries through the renal and aortic plexuses (Kuntz, 1919a).

2.1.1.2. *Oviduct*

The oviduct transports the ova to the uterus. Each oviduct is located between the peritoneal layers of the mesosalpinx and connects the peritoneal cavity with the uterine cavity (Figure I). The ovarian extremity of the duct, the infundibulum, is located near the edge of the opening into the ovarian bursa. The infundibulum is a funnel-shaped dilatation of the lumen of the oviduct, which narrows into a minute opening, the abdominal ostium. It is fringed by finger-like processes (fimbriae) which are usually visible projecting out of the ovarian bursa (Dukes, 1947). The oviduct is covered by Tunica serosa which is composed of the peritoneum making up the mesosalpinx. The muscular layer of the duct is composed of primarily circular fibers with some longitudinal and oblique fibers present. Blood supply is by the ovarian and uterine arteries, the veins are satellites of the arteries. The lymphatics follow the ovarian lymph ducts to the lumbar lymph nodes (Ramsey, 1946). Nerves to the oviduct are derived primarily from the thoracolumbar (sympathetic) division of the autonomic nervous system and pass through the aortic and renal plexuses (Mitchell, 1938).

2.1.1.3. *Uterus*

This is a hollow muscular organ which serves as the habitation for the developing young. It gives attachment to the fertilized ovum and functions as source of fetal nourishment. It consists of a neck (cervix), a body (*corpus uteri*), and two horns (*cornua uteri*) (Figure I). The uterine horns are musculomembraneous tubes, slightly flattened dorsoventrally, which

unite at the body of the uterus. The body of the uterus usually extends from the point of convergence of the uterine horns to the cervix. The uterus is made up of three tunica serosa (peritoneum), muscularis (myometrium) and mucosa (endometrium) (Trautmann and Fiebiger, 1952). Blood supply to the uterus is via the ovarian and uterine arteries, blood is drained via uterine and ovarian veins. The lymphatics pass to the internal iliac and lumbar lymph nodes (Wislocki and Dempsey, 1939). Nerve supply is via sympathetic, visceral afferent fibers through the hypogastric plexus, parasympathetic and visceral afferent fibers via the pelvic nerves (Kollar, 1953).

2.1.1.4. *Vagina*

This is a musculo-membranous, highly dilatable canal, extending from the uterus to the vulva (Figure I). The term vulva is used to include the vestibule, clitoris and labia (Trautmann and Fiebiger, 1952). The Cranial portion of the vagina is covered dorsally by peritoneum which reflects unto the colon, forming the recto-uterine pouch (Trautmann and Fiebiger, 1952). Ventrally, this portion of the vagina has a peritoneal covering which reflects unto the bladder, forming the vesico-uterine pouch (Trautmann and Fiebiger, 1952). Laterally, the dorsal and ventral peritoneal coverings of the vagina fuse and become part of the broad ligament. The caudal half of the vagina is retroperitoneal, being connected dorsally to the rectum and ventrally to the urethra by means of loose connective tissue. Laterally, the caudal part of the vagina is related to the vaginal blood vessels and nerves to the urethra. The vaginal walls are made up of an inner mucosal layer, a middle smooth muscle layer and an external coat of connective tissues and peritoneum (cranially) (Trautmann and Fiebiger, 1952). Arterial blood is supplied to the vagina via the vaginal

artery, a branch of the urogenital artery. In addition to its vaginal distribution, the artery also supplies branches to the urethra and vestibule. Its urethral branches anastomose with the caudal vesicular artery and its vestibular branches (cranial vestibular) anastomose with caudal vestibular branches from the terminal part of the urogenital artery. The vaginal veins are satellites of the arteries and drain into the internal pudendal veins. The lymphatics drain into the internal iliac lymph nodes. The vagina is innervated by sympathetic and parasympathetic nerves from the pelvic plexus and by sensory afferent fibers via the pudendal nerve (Chritensen, 1964).

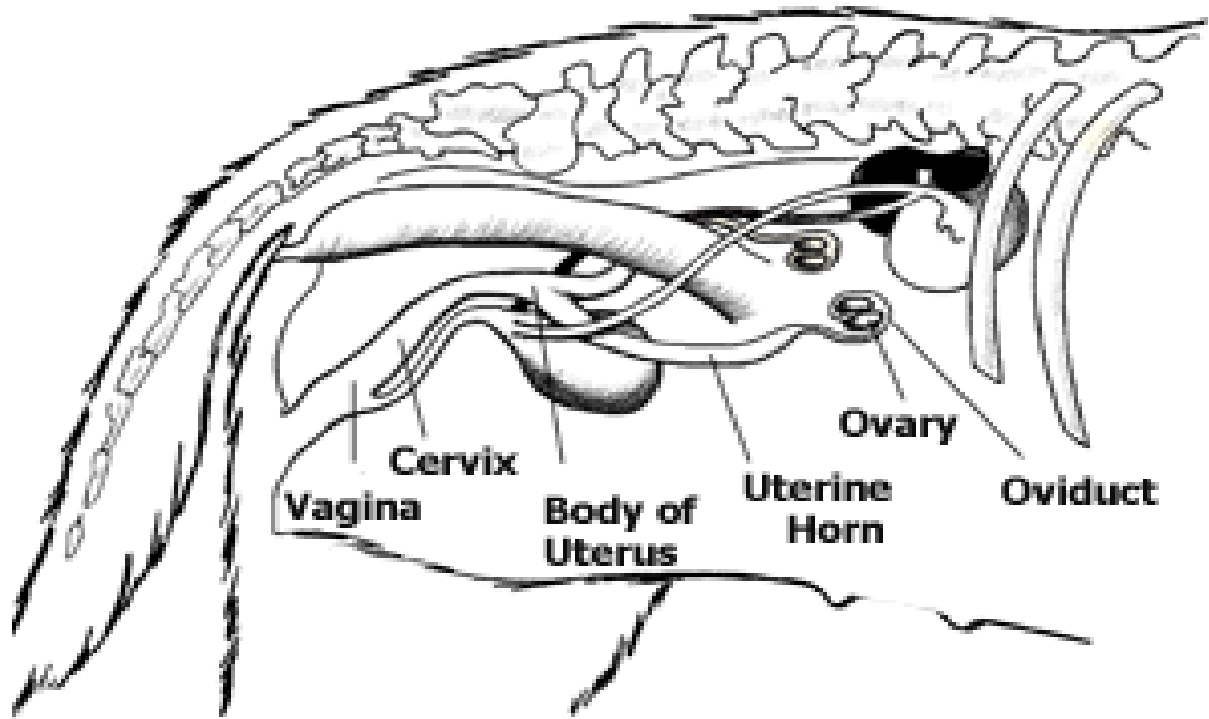


Fig. 2.1: Lateral View of the anatomy of the canine female (non-pregnant genitalia).

(Source: Foster and Smith, 2006)

2.1.2. The normal reproductive cycle of the bitch

2.1.2.1. Oestrous cycle

Throughout the adult reproductive years of the female, the structural composition and hormonal activity of the ovaries are continually changing (Bowen, 1998). Gonadotropic hormones, produced by the anterior pituitary gland within the brain initiate such changes. During early development, prior to sexual maturity of the bitch, very little gonadotropic hormones are secreted and the ovaries, therefore, remain inactive. However, around the age of 6 months, the pituitary begins to secrete higher levels of the gonadotropic hormones called follicle stimulating hormone (FSH) and luteal hormone (LH) (Bowen, 1998; Pamela, 2000). The rise in FSH and LH in the bitch will initiate the sexual cycle sometime between the age of 6 months and 14 months. Cyclical increases and decreases in FSH and LH, in turn, control the cyclic ovarian changes and, as such, are responsible for the physiologic events in the normal reproductive cycle of the bitch (Bowen, 1998; Pamela, 2000).

The bitch has two ovaries that produce the ova (eggs). Within the ovaries, the ova are contained within follicles that grow toward the surface of the ovary. When FSH and LH from the pituitary gland begin to be secreted in high quantities during onset of sexual maturity, the ovaries and the follicles within them will begin to grow. Within these follicles, a follicular fluid hormone, secreted by the ovary, called estrogen, surrounds the ovum. This hormone is a biologic chemical that produces physiologic and

social/behavioral effects within the bitch that will signal a readiness to mate (Bowen, 1998; Pamela, 2000; Josep, 2010).

Two days prior to ovulation, there is a surge in the secretion of LH by the pituitary gland preceded by rapid swelling of the follicle. This LH surge is of critical importance because in its absence, even with the other hormonal physiologic effects taking place, ovulation will not occur (Pamela, 2000). Additionally, the LH surge causes the ovarian cells to switch over to secreting progesterone hormone rather than estrogen. As a result, there is an increase in progesterone levels, and a decrease in estrogen levels. Within two days of the LH surge, the follicle reaches the surface of the ovary and bursts, thereby releasing the ovum into a capsule that surrounds the ovary. This process is referred to as ovulation (Pamela, 2000). If the bitch is bred, then sperm will subsequently fertilize each of the released ova. The ova will then move down the oviducts, which connect each ovary to each of the two uterine horns, and the fertilized ova will eventually implant in the walls of the uterus where they will develop into fetuses. In the meantime, the ruptured follicles from which each ovum was developed will begin to produce a rapidly dividing mass of cells called luteal bodies, which will make up the corpus luteum. In addition to producing progesterone, which will maintain the pregnancy, the corpus luteum will also produce inhibin, the hormone that will signal the pituitary gland to decrease production of FSH and LH. When enough inhibin has eventually been secreted, this will end the mating period (Bowen, 1998; Pamela, 2000; Josep, 2010).

Though the above explanation mainly describes the mating stage of the bitch, the normal reproductive cycle of the bitch is comprised of four stages: proestrus, oestrus, dioestrus, and anoestrus (Bowen, 1998; Pamela, 2000; Josep, 2010).

1. Proestrus: (average duration = 9 days; range = 3-17 days). Swelling of the vulva, the external tissue of the vaginal opening, and bloody discharge marks the beginning of the proestrus stage, also known as the follicular stage. During proestrus, the ovarian follicles, each containing ova, increase in size. Increasing amounts of estrogen hormone, secreted by the ovarian follicles, cause the cells of the vaginal walls to take-on a distinctive shape, a process known as cornification. Both the level of estrogen and vaginal cornification are useful indicators of proestrus.
2. Oestrus: (average duration = 9 days; range = 3-21 days). Receptivity to mating marks the beginning of the oestrus stage. Physiologically, oestrus coincides with the predominant presence of cornified vaginal epithelial cells and an increase in serum progesterone levels to 2 ng/ml. Ovulation usually occurs 2 days following this increase in progesterone and hence, monitoring the levels of progesterone is an excellent indicator for timing breeding.
3. Dioestrus: (average duration = 2 months). Approximately 6 days after ovulation, the cornified vaginal epithelial cells will revert to a non-cornified state. This condition marks the beginning of dioestrus. This stage ends when progesterone levels fall to less than 1 ng/ml just prior to whelping in the pregnant bitch or approximately 2 months after ovulation in the nonpregnant bitch.

4. Anoestrus: (average duration = 4-4.5 months). The beginning of this stage is marked by the drop in serum progesterone levels to less than 1 ng/ml. The beginning of proestrual bleeding marks the end of this stage. Duration of anoestrus is quite variable among bitches and may be governed by both genetic and environmental variables (Bowen, 1998; Pamela, 2000; Josep, 2010).

2.2.2.2 Determining ovulation

The average bitch will experience the LH surge on Day 10 (where Day 1 is defined as the first day that bloody discharge is observed), will ovulate on Day 12, and will, therefore, optimally conceive on Day 14 (Bowen, 1998; Pamela, 2000; Josep, 2010). Traditionally, bitches were usually bred on the 14th day following onset of proestrus. This was because it was observed that most bitches would display "tail flagging", defined as the lateral deviation of the tail with elevation of the vulva, and "standing heat", defined as the bitches' behavior in allowing the male to mount and breed, at this point in time. Additionally, other physical changes such as a softening of the flesh of the swollen vulva were external signs indicating the onset of ovulation. Later, when it became the routine to perform multiple matings, the bitch was bred on Days 12 and 14 (for double service), or Days 11, 13, and 15 (for triple service). Though these schedules are still adequate for insuring optimal breeding and litter size in the average bitch, not all bitches ovulate on Day 12 following proestrus onset (Bowen, 1998; Pamela, 2000; Josep, 2010). Some may ovulate as early as Day 5 or as late as Day 25 in which case utilizing this standard mating schedule will result in breeding failure (Bowen, 1998; Pamela, 2000; Josep, 2010).

Using receptive behavior of the female as an indicator for ovulation and therefore, a method for determining mating schedule has many limitations because these approaches are not always clear-cut (Pamela, 2000). Some bitches may exhibit "phantom proestrous" (displaying little or no outward signs of bloody discharge, etc.) making it difficult to estimate the average date of ovulation, may "flag" and appear receptive to males throughout proestrous, or may remain unwilling to mate even following ovulation (Pamela, 2000). The differences observed from bitch to bitch in regard to mating signs and behavior as well as the fact that unsuccessful mating will result in a 6-month or longer wait to "try again" understandably leads to a sense of anxiety in many bitch owners (Pamela, 2000). Additionally, even cytological analysis, which can be used to determine onset of oestrus, is often a poor predictor for ovulation since LH surge, a key precursor to ovulation, may occur 3 to 5 days before to 5 days after the onset of oestrus. Therefore, a clinical screening test that accurately predicts ovulation in the bitch and therefore serves to optimize breeding schedule, is used frequently by many breeders (Pamela, 2000).

The serum progesterone enzyme-linked immunosorbent assay (ELISA) is an accurate predictor for ovulation (Pamela, 2000). For this assay, vaginal smears are examined periodically at the onset of proestrous to monitor cornification of the vaginal epithelial cells, which occurs as a result of increasing estrogen hormone. When the cells of the vaginal wall are approximately 60% cornified, as observed by microscopic analysis, testing with the serum-progesterone ELISA should commence. Blood samples are drawn, ideally, every day (though every 2 days may also be utilized), and whole blood or serum (depending on the test kit utilized) is added to a test indicator that has been treated with

monoclonal antibodies specific for progesterone. Late in proestrus, the level of estrogen will decrease and levels of LH will surge. This LH surge is concurrent with an increase in progesterone levels, which will rise above 1 ng/ml on the same day (Pamela, 2000). Therefore, detection of increased serum progesterone corresponds to the LH surge. This is an important indicator, since ovulation occurs 2 days following the LH surge. The serum progesterone ELISA manufactured by International Canine Genetics (ICG) a division of Synbiotics (Malvern, PA) provides a qualitative color change to indicate when the progesterone level in the bitches' serum rises (Pamela, 2000). Early in proestrus, the test indicator will produce a strong blue color indicating low levels of progesterone (between 0.0 to 1.0 ng/ml). For determination of LH surge, the first appearance of a fading of the test color (as compared to an internal "low-progesterone" control indicator) to a light blue (approximately 2.0 ng/ml) indicates the LH surge. Two days later upon ovulation, progesterone levels will further increase to 5.0 ng/ml or above, at which point the test color will appear white, confirming ovulation (Pamela, 2000).

Though different kits utilize different testing methods and procedures, the concept of each kit is virtually identical and provides the means for determining ovulation. However, limitations to the sensitivity of ELISA testing may sometimes result in false-positive and false-negative results. This is because the greatest inaccuracy in measuring serum progesterone levels occurs in the range of 1.5 to 3.0 ng/ml of progesterone, the concentration range of importance for determining the LH surge. Greater accuracy occurs in the high range of greater than 5.0 ng/ml. Occasionally, a test will indicate a "medium" level of progesterone one day (suggesting LH surge), but may indicate a "low" level of

progesterone when taken on the next day. This suggests that the former test demonstrated a false-positive because once progesterone levels rise, they should remain elevated and increase throughout ovulation. Therefore, to reduce incidence of inoptimal mating due to false-positives, two consecutive days of testing, whereby increased progesterone levels are indicated on both days, should be obtained prior to establishing the mating schedule. Additionally, a post-ovulatory testing should be performed on a day that mating is performed to confirm high levels of progesterone (5.0 ng/ml or greater), which indicates that ovulation has occurred (Pamela, 2000).

Alternatively, ICG also offers an LH ELISA that works on the same principle as the progesterone ELISA but which specifically detects levels of serum LH. Though the LH ELISA may be used alone for determining ovulation, the major limitation with the LH ELISA occurs as a result of the brief time in which the LH concentration is elevated in the serum. Unlike progesterone concentration, which continues to increase, LH peaks within 24 hours and then quickly dissipates. As such, it is possible to miss the LH surge if one does not test on a consistent, daily basis. For this reason, Synbiotics recommends combined testing with the progesterone ELISA and the LH ELISA, using the latter to reduce the possibility of false-positives and false-negatives occasionally encountered with the progesterone ELISA. For example, on the first day that the progesterone ELISA indicates a rise in serum progesterone levels, one may confirm the concurrent LH surge by re-testing serum using the LH ELISA. If both tests are positive, then there is less likelihood of false-positive results (Pamela, 2000).

Once the day of the LH surge is determined (to be considered Day 0), ovulation will occur on Day 2. Maximal litter size is achieved when the bitch is bred 2 days after ovulation (Day 4 following the LH surge). A single insemination 2 to 3 days following ovulation will result in pregnancy in the healthy reproductive bitch. The reason that optimal conception occurs 2 days following ovulation is because when ovulation occurs, the ova are immature (primary oocytes) and must undergo two meiotic divisions before they can be fertilized. These divisions can take up to 48 to 72 hours to occur. Once matured, the ova remain viable for another 2 to 3 days. Because normal sperm (spermatozoa) of the male delivered by natural insemination can live in the reproductive tract for at least 5 to 6 days, successful conception may occur if a bitch is bred from 2 days prior to ovulation to 4 days after ovulation (Pamela, 2000).

2.1.2.3. *Artificial insemination*

The use of artificial insemination in canine breeding offers a solution to a number of situations that may prohibit or complicate natural breeding. Such situations include anatomical deterrents (such as a narrow vulva and vagina in a virgin bitch), unwillingness to breed (dominant/aggressive behavior of the bitch or submissiveness in the male), weakness or pain in the spine or hind limbs (in geriatric studs still providing service), reducing risk of sexually transmitted disease to the stud (brucellosis), or geographical distance between stud and bitch (Pamela, 2000). When using the method of artificial insemination, timing of insemination is a critical factor for ensuring successful conception. Freshly collected or chilled semen should be introduced by means of intravaginal insemination at least 2 days after ovulation has occurred in the bitch (Table 2.1) for this

procedure, the semen is drawn into a sterile syringe and an insemination pipette designed for bitches (Synbiotics; some large breeds may require a larger pipette custom-made from pipettes used for bovine uterine infusions) is attached. A gloved finger, lightly lubricated with water, is inserted into the vagina and the pipette is guided over the finger, and extended into the vaginal passage as far as it will go. The semen is then expelled from the pipette followed by some air to clear any remaining semen from the tube. The pipette is withdrawn, but the finger remains to "feather" (gently scrape in a backward motion) the wall of the vagina. This procedure is very important as it simulates the gentle pulling action of the male penis within the vagina following the "tie" in natural breeding. This procedure will induce muscle contractions of the vaginal walls that will assist the locomotion of the sperm toward the awaiting ova. After several minutes of this stimulation, the gloved finger is removed and the bitch should be positioned with her hind-end in an elevated position. Care should be taken to ensure that no pressure is placed around the abdomen; therefore, lifting should be performed by gripping the bitch at her knees. After being in this elevated position for about 5 to 10 minutes, the bitch should be immediately crated for 30 to 60 minutes. She should not be allowed to urinate, and if it is required for her to be placed into a crate in a vehicle, she should be lifted by two people, with the person lifting the hind-end, gripping the knees and not around the abdomen to lift (Pamela, 2000).

Insemination using frozen-thawed semen requires a modified schedule for introduction of the sperm and more sophisticated techniques to ensure successful conception. First, the viability of frozen-thawed sperm is significantly reduced and as a result, compared to fresh

or chilled sperm that may live up to 5 or 6 days in the reproductive tract of the bitch, frozen-thawed sperm live only a few hours (Table 2.1). As such, the ova must be mature when frozen-thawed semen is introduced. Therefore, insemination with frozen-thawed semen is best performed 3-4 days following the LH surge (2-3 days following ovulation). Secondly, frozen-thawed sperm are insufficiently mobile to reach the ova if introduced by intravaginal insemination. For this reason, higher rates of conception with frozen semen have been achieved using intrauterine insemination. Several surgical methods have been developed for the purpose of delivering semen into the uterus. Laparotomy and laparoscopy utilize a small-gauge needle for delivery. Alternatively, transcervical catheterization is performed by passing a catheter by way of the vagina through the cervix and into the uterus for delivery of the semen. In regard to advantages of one intrauterine insemination method to another, there appears to be no significant difference in pregnancy rates or litter size (Pamela, 2000).

Table 2.1: Overall variability in pregnancy success rate for natural mating and artificial insemination methods in bitches.

Type of service	% success rate of conception
Natural service	80-90
Fresh AI, intravaginal insemination	62.3-100
Chilled AI, intravaginal insemination	59-80
Frozen AI, intravaginal insemination	52.6-60

2.2. Imaging of Female Reproductive Organs

2.2.1. Radiography

Contrast techniques, such as vaginography and hysterosalpingography, to visualize portions of the female genital system have been described but are not used routinely (Collery, 1956, Funquist *et al*, 1985). Vaginography has been used to demonstrate ectopic ureters. Iodinated contrast is injected into the vagina, allowing for the demonstration of flow into the urethra and into the ectopic ureter. This technique is also useful to identify vaginal clefts, vaginal strictures, vaginal masses, and vaginal lacerations (Jackson *et al* 1978, Gibbs and Lathan, 1984, Holt *et al* 1984 and Levelle and Atilo 1991).

2.2.1.1. Ovaries

Although not normally radiographically visible, the ovaries which are located just caudal to the kidneys may enlarge. Cysts (Plate I) and neoplasia are the more common causes of ovarian enlargement. The position of the adjacent kidney is an important clue to the presence of an ovarian mass because the kidney may be laterally displaced (Daniel, 2003).

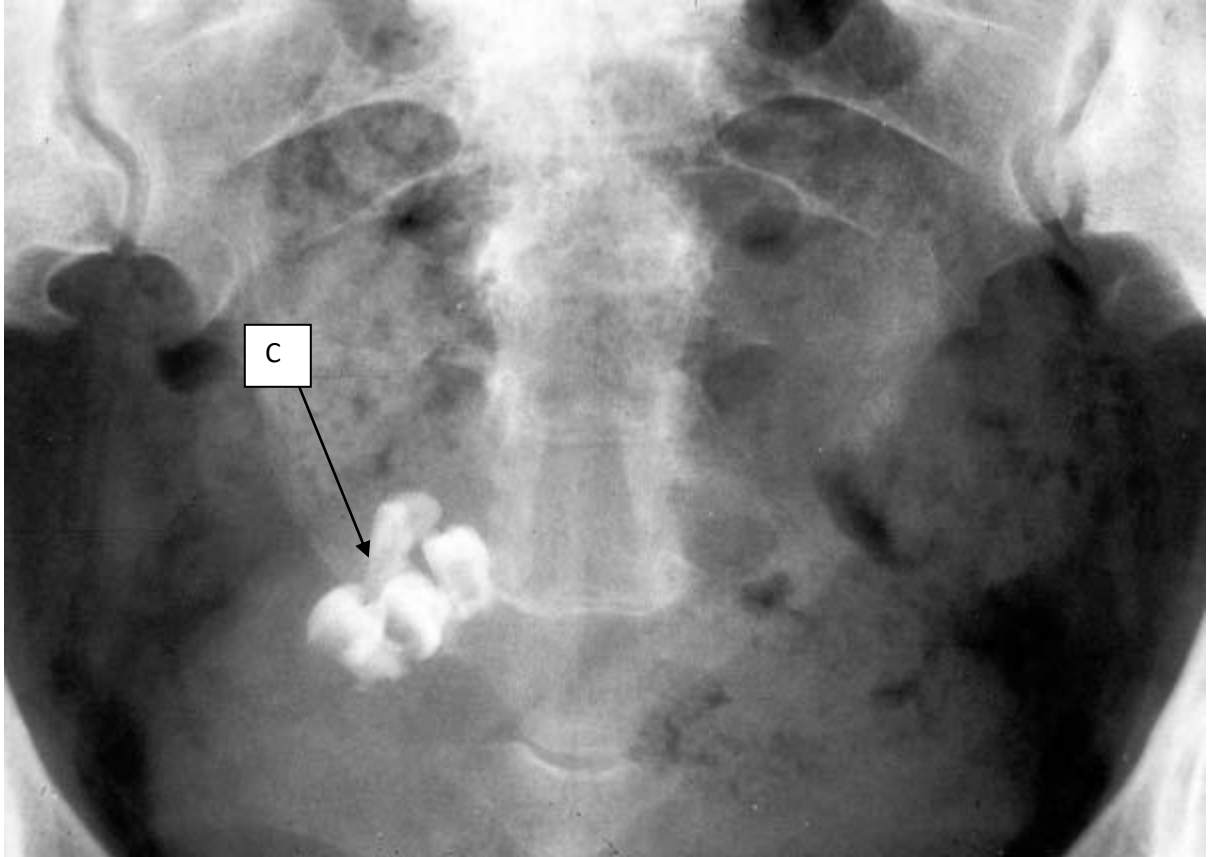


Plate I: Ventrrodorsal radiograph of the pelvic area showing cystic ovary (c) with arrow pointing to the lesion.

(Source: www.thewelltimedperiod.blogspot.com. 01 September 2010)

2.2.1.2. *Uterus*

The uterus is not readily visualized by radiography unless it becomes enlarged (Cobb and Archibald, 1959; Ackerman, 1981). In a fat dog, the uterine body may be visible dorsal to or superimposed upon the bladder and ventral to the colon on lateral radiograph. When enlarged, the uterus becomes visible as a tubular soft tissue structure that displaces the small intestine cranially, dorsally, and towards the midline. The uterus folds upon itself and appears to be oval or sausage shaped. Although fluid-filled distended loops of the may create similar appearance, the presence of gas within the intestines is an important feature that helps to distinguish between an enlarged uterus and distended intestines (Brown, 1974; Herbert, 1979; Biller and Haibel, 1987; Freeman, 1988; Montgomery, 1989). Diffuse enlargement of the uterus may be due to pregnancy, post partum, hemorrhage, infection (pyometra and endometritis), accumulation of secretions (mucometra and hydrometra), subinvolution or neoplasia (Dunn and Foster, 1977; Dillon and Henderson, 1981; DeGeer, 1987; Abel, 1990; Charan *et al*, 1994; Cockcroft, 1995; Arnold *et al*, 1996).

2.2.1.3. *Vagina*

Unless they are quite large, lesions affecting the vagina are radiographically apparent only when contrast is used. In most cases, the vagina lends itself to thorough physical or endoscopic examination and radiography is rarely needed. Pneumovaginography or positive contrast vaginography may be helpful in outlining the cranial extent of a vaginal mass when an endoscope cannot be passed beyond a mass or if clear delineation of an

anomaly cannot be from vaginoscopy (Daniel, 2003).

2.2.1.4. *Pregnancy*

The definitive determination of pregnancy is not possible until approximately the forty-second day pregnancy, when the fetal skeletons mineralize sufficiently to be visualized (Plate II). Before this fetal calcification, the visible uterine enlargement is nonspecific. A symmetric segmentation of the pregnant uterus may be observed at 25-30 days of gestation. The canine uterus involutes by 21 days postpartum. It can be detected radiographically up to 12 days postpartum (Pharr and Post, 1992; Feretti *et al*, 2000). Radiography is superior to ultrasonography for the determination of litter size in the bitch (Bondestam *et al*, 1984; and Toal *et al* 1986). Pelvimetry (radiographic measurement of the pelvic canal) can be used to assess likelihood of dystocia, though described is very rarely used (Eneroth, 1999). In addition radiography is useful in assessing dystocia in that is if there is no fetus lodged in the birth canal, the likelihood of uterine inertia is much greater (Eneroth, 1999).

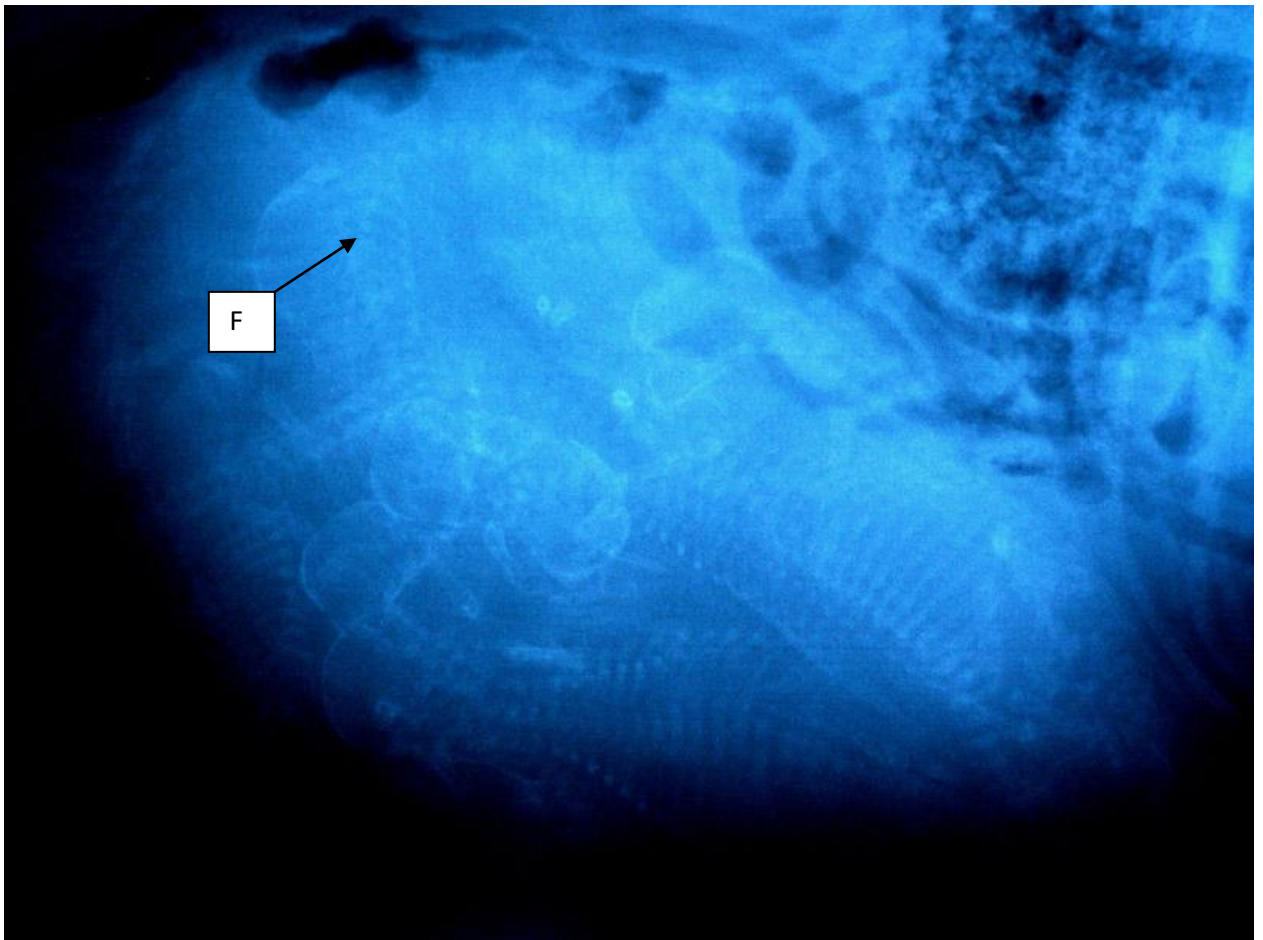


Plate II: Lateral abdominal radiograph showing normal pregnancy (F= fetus) in a bitch.

(Source: www.yourownvet.com. 01 September 2010)

2.2.2. Magnetic Resonance Imaging (MRI)

MRI an imaging technique that uses electromagnetic waves to obtain images of the body's soft tissues, e.g. the brain and spinal cord. In MRI the body is subjected to a powerful magnetic field, allowing tiny signals from atomic nuclei to be detected and then processed and converted into images by a computer (Encarta, 2008).

2.2.2.1. Technique

Pelvic anatomy is particularly well visualized on T2-weighted (T2W) images and it is recommended that these are obtained in the sagittal plane for evaluation of the midline structures. Supplementary images in the axial or coronal plane optimally demonstrate the adnexa and pelvic sidewall. Some degree of tissue characterization can be obtained by the use of multiple pulse sequences: the presence of fat or haemorrhage can be suggested on T1-weighted (T1W) sections and confirmed by the use of frequency-selective fat and water suppression techniques (Kier *et al.*, 1992) or opposed-phase gradient echo sequences. A STIR sequence can also be utilized to provide high contrast images in which the signal intensity of fat and other short T1 substances is suppressed. Flow-sensitive gradient echo sequences are occasionally useful in assessing vessel patency (Yamashita, 1994). Motion artefacts arising from respiration, peristalsis and vascular flow may be troublesome and are not obviated by scanning the patient in the prone position. Peristaltic artefacts are reduced

by avoiding oral intake for several hours, emptying the bladder immediately prior to scanning and by the administration of anti-peristaltic agents such as hyoscine butylbromide (Buscopan^(R) — Boehringer, Ingelheim) or glucagon (Lilly^(R) — Novo Nordisk). Both agents have a short duration of action and should be administered immediately prior to image acquisition. Slow intravenous injection of glucagon (0.5–2 mg over 1 minute) is preferred to intramuscular (Stevens *et al.*, 1993) administration, resulting in a more predictable onset of action whilst minimizing adverse effects (Chernish, *et al.*, 1990). Vascular flow artifacts, which are particularly prominent on scans obtained following intravenous administration of contrast medium, can be reduced by the use of pre-saturation pulses or gradient moment nulling techniques.

2.2.2.2. Ovaries

The ovaries are most readily recognized on T2W images by the presence of hyperintense follicles, which are more consistently observed when multicoil arrays and fast spin echo (FSE) pulse sequences are used (Smith, *et al.*, 1992). On T1W images, the ovaries are of uniformly low signal intensity though enhancement of the ovarian stroma immediately following contrast injection highlights the non-enhancing follicles (Hricak and Kim, 1993). Ultrasound (US), computerized tomography (CT) and MRI are usually considered to be of approximately equal efficacy for ovarian identification, yet in a recent study normal ovaries were prospectively identified by transvaginal ultrasound (TVUS) in only 76% of pre-menopausal women and 20% of post-menopausal women owing to the restricted field-of-view (DiSantis *et al.*, 1993). Using MRI, which does not suffer from this limitation, the

reported success rate of MRI for ovarian identification in women of reproductive age is as high as 95% (Dooms *et al.*, 1986).

2.2.2.3. Uterus

On T2W images a characteristic trilaminar zonal architecture of the adult uterus is evident. The normal endometrium and endometrial cavity are depicted by a high signal intensity central endometrial stripe. The surrounding low signal intensity junctional zone represents an inner layer of myometrium containing increased numbers of myometrial cells and an increased density of compact muscle fibres (Scoutt *et al.*, 1991). More peripheral is the outer myometrium which is of intermediate signal intensity and in which arcuate vessels (branches of the ovarian artery) can be seen on high resolution MR images. The boundaries of these zones are different from those observed on US images (Mitchell *et al.*, 1991).

The uterine size and zonal anatomy seen on MR images reflect the hormonal milieu. Cyclical changes result in a maximum endometrial thickness of 5–10 mm during the mid-secretory phase. Myometrial thickness and signal intensity also increase at this time (McCarthy *et al.*, 1986) though the thickness of the junctional zone (typically 3–5 mm) does not vary significantly during the menstrual cycle. Prior to menarche and post-menopausally, the uterus is smaller and the zonal anatomy is less evident (Hricak and Kim, 1993). On T1W images, the uterine corpus is of homogeneously low signal intensity though inconsistent zonal differences in enhancement are observed on sections taken shortly after administering contrast medium (Hricak and Kim, 1993).

The uterine cervix is predominantly hypointense on T1W images. On T2W images, a central hyperintense stripe representing the endocervical canal with contained mucus is visible. High-resolution images reveal a lower signal intensity zone immediately surrounding the endocervical canal, probably representing the mucosa and infolded plica palmatae. More peripherally, a zone of low signal intensity fibrous stroma is seen, which is continuous with the junctional zone of the uterine corpus. The outermost layer is of intermediate signal intensity and represents an outer layer of the fibrous stroma with reduced cellular density. This blends imperceptibly with the myometrium of the uterine corpus (Hricak and Kim, 1993).

Following intravenous contrast-medium administration, marked enhancement of the endocervical canal and outer cervical wall are apparent, with only modest enhancement of the intervening fibrous stroma. In some instances, possibly due to inflammatory disease, the fibrous stroma also enhances and the zonal anatomy is then indistinct (Hricak and Kim, 1993).

The normal anatomy of the vagina is clearly delineated on T2W images; central hyperintensity of the mucosa and secretions contrast with the low signal intensity fibromuscular wall, which in turn is highlighted by the surrounding hyperintense perivaginal venous plexus (Hricak and Kim, 1993).

2.2.3. Nuclear imaging

2.2.3.1 Introduction

Radiography and nuclear imaging share in common the use of ionizing radiation. Radiographic images are produced by recording the differential absorption of X-rays by body tissues. Nuclear medicine images are obtained by mapping the distribution of radioactivity of an administered radiopharmaceutical within the body (Alan *et al.*, 2003).

In order to produce a nuclear medicine image, several steps must be completed. First, a pharmaceutical with appropriate biological behavior must be chosen. This compound must be successfully bound to some radioactive material without changing the biological behavior of the original pharmaceutical. The resulting radioactive compound is referred to as a radiopharmaceutical (Alan *et al.*, 2003).

Once administered to the patient, radiation detectors are used to record the spatial and temporal distribution of the radiopharmaceutical within the body. In most nuclear medicine departments today, a scintillation camera is used to record the internal distribution of the radiopharmaceutical. The spatial distribution (of the radiopharmaceutical) yields anatomical information. Additional information can also be obtained by recording the temporal changes in the concentration of the radiopharmaceutical. Computer processing of both the spatial and temporal data yields unique physiological or metabolic information currently not attainable from other imaging modalities. (Alan *et al.*, 2003).

The detection of electromagnetic radiation depends on its ultimate interaction with matter. Photons interact with matter only through electrical or magnetic forces. There are three forms of photon interaction with matter which are most important in nuclear medicine. These include photoelectric absorption, Compton scattering and pair production (Alan *et al.*, 2003).

In a photoelectric interaction, an atom absorbs all of the energy of the incident photon. The photon ceases to exist but the energy is transferred to an inner-shell electron which is ejected and is then referred to as a photoelectron. This creates a vacancy in an orbital shell which eventually leads to emission of a characteristic X-ray (Alan *et al.*, 2003).

In a Compton interaction, a photon interacts with an orbital electron and loses some of its energy which is then transferred to the electron. The photon as a result is 'scattered' or deflected off in a new direction with a lower energy (Alan *et al.*, 2003). In pair production, high energy photons interact with the electric field of a nucleus: the photon transfers its energy to create a positron and an electron. Since each particle has the rest mass of an electron, 0.511 MeV, this requires a minimum photon energy of $2 \times 0.511 = 1.022$ MeV. Radionuclides with photons exceeding 1.02 MeV are not generally used in nuclear medicine (Alan *et al.*, 2003).

2.2.3.2. *Imaging equipment*

The Scintillation Camera; this is the primary imaging equipment in nuclear medicine. The detection of radioactivity is dependent on the interaction of radiation with matter. The detector most commonly used in nuclear medicine is the sodium iodide (NaI) crystal. When a sodium iodide crystal is struck by a photon of X- or gamma rays, it 'scintillates' or gives off energy as a flash of light. In the NaI crystal, an energized electron will fluoresce as it relaxes to a ground state. This results in emission of visible blue light (Holden and Walker, 1976).

The amount of light produced in scintillation is proportional to the energy deposited in the crystal by the incident photon. By measuring the amplitude of each flash of light, one can select photons with specified energies. This relationship between the energy of the gamma ray and the amount of light produced is critical to the production of good quality nuclear medicine images. Gamma rays which have undergone scattering within the body before interacting with the crystal lose energy. Such scattered photons can degrade image quality and are rejected by the detection system (Holden and Walker, 1976).

In a scintillation camera, the NaI crystal usually contains a small amount of thallium (Tl) which improves the crystal's ability to scintillate. The components of a scintillation camera are shown in Figure 6.2. An array of electronic photomultiplier tubes (PMTs) detect the light produced in the NaI (Tl) crystal. Each PMT generates a signal with amplitude proportional to the amount of light which is thus a measure of the energy of the incident photon (Holden and Walker, 1976).

The PMTs require a voltage supply which is very stable as small variations in the voltage on each tube can cause errors in determining the energy of the incident photon. Voltage pulses produced by the PMTs are small and must be amplified before processing (Holden and Walker, 1976).

When light is given off in the NaI (Tl) crystal, it spreads out in all directions. In a scintillation camera, a hexagonal array of PMTs is arranged facing the crystal. Old, small-field-of-view cameras have as few as 19 PMTs. Today, large-field-of-view cameras may have as many as 91 PMTs to cover the surface of the crystal. Early cameras used mineral oil or lucite light pipes to couple the PMTs to the scintillation crystal. Today optical

coupling grease is used for more efficient transmission of the light from the scintillation site in the crystal to the PMTs (Holden and Walker, 1976).

The location of scintillation in the crystal is determined by a position-decoding matrix. This is an electronic circuit which simultaneously compares the amplitude of the output from all PMTs. (Holden and Walker, 1976).

The PMT closest to the site of the scintillation will receive the greatest amount of light. By weighting the signal strength from all PMTs, the decoding matrix generates x- and y-coordinates which correspond to the site of origin of the scintillation in the crystal. The signals from all PMTs are also processed by an addition circuit which sums the output from all tubes for each scintillation. The summed output signal is called the Z pulse and is proportional to the total energy absorbed by the crystal. The amplitude of the Z pulse is then measured by a pulse height analyser (PHA) which allows only pulses within a specific range or energy 'window' to be displayed (Holden and Walker, 1976).

For most nuclear medicine imaging purposes, the window is set to accept photons which have undergone some scatter. Typically, when imaging ^{99m}Tc which has a gamma ray energy of 140 keV, the window is 20%. This allows gamma rays with energies from 126–156 keV to be counted. Using a wider window permits scattered photons to be counted but this reduces image quality although it decreases imaging time (Holden and Walker, 1976).

2.2.4. Ultrasonography

2.2.4.1. General principles

Ultrasonography is the second most commonly used imaging format (second only to

radiography) in veterinary practice. It uses ultrasonic sound waves in the frequency range of 1.5-20 megahertz (MHz) to create images of body structures based on the pattern of echoes reflected from the tissues and organs. Several different types of image formats can be displayed. The most familiar one (and the one that creates the actual anatomic image of) is B-mode, or grayscale scanning. The sound beam is produced by a transducer placed in contact with the animal. An ultra short pulse of sound is directed into the animal, after which the transducer switches to the receive mode. Echoes occur as the sound beam changes velocity while passing through tissues of varying density. The greater the change in velocity the greater the strength of the echo. The transducer then reconverts the echoes to electrical impulses recorded by the computer in the ultrasound machine. The strength of the echo, the time required for the echo to return after the pulse, and the direction the sound beam was sent is recorded. Using the information from multiple echoes, the machine creates an image that represents the appearance of the tissues when cut in that same plane on an anatomic specimen. In modern scanning systems, the sound beam is swept through the body many times per second, producing a dynamic, and real time image that change as the transducer is moved across the body. This real-time image is easier to interpret and allows the examiner to scan continuously until a satisfactory image is obtained. (Anon i, 2008).

Ultrasound has become established as an important modality for tomographic imaging of soft tissues. It can also be used to quantitate the movement of structures such as cardiac valves 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. As far as is known, ultrasound used at diagnostic intensity

levels does not cause damage to tissues. It has shown no sign of reaching the limits to its development, and continued improvements as well as new techniques are emerging. The apparatus is relatively cheap and has the distinction of having become cheaper with technological advances (David, 1997). Ultrasound has found very important applications in the abdomen as well as in the neck, heart and peripheries and the neonatal brain, but its major clinical impact is in obstetrics, where the unique combination of safety and tomographic imaging of the fetus has rendered it indispensable (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). Thus ultrasound is not generally useful for the lungs or in the head — except in the neonate, when the open fontanelle provides an excellent window. In other areas, overcoming the barrier caused by the bony skeleton and gas-containing viscera requires considerable technical expertise. Ultrasound is also subject to many artefactual signals which complicate interpretation and add to the operator skills required (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, a major advantage for emergency uses. In addition, mobile ultrasound systems that can be taken to the bedside are available (David, 1997).

Ultrasound is easily employed as a means of guiding biopsy needles and catheters, which can be followed directly in real time during insertion because of the interactive nature of real time scanning (David, 1997).

2.2.4.2. Nature of ultrasound

Ultrasound is a coherent, mechanical vibration at high frequencies. In diagnostic applications, frequencies in the 1–20 MHz (megahertz = million cycles per second) range are used, corresponding to wavelengths of 1–0.1 mm in tissue (Nautrup, 2006).

2.2.4.3 Ultrasonic transducers

Ultrasound is generated by piezoelectric materials which have the property of changing thickness when a voltage is applied across them. The piezoelectric effect derives from movements 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. Lead zirconate titanate (PZT) is widely used and piezoplastics show promise. The crystal is mounted in a conveniently shaped holder which contains the electrodes and any associated electronics as well as the lenses and matching layers required to improve the beam shape. The whole assembly is known as the probe or transducer, though strictly the latter term should be reserved for devices that change one form of energy into another, in this case electrical to acoustic energy (David, 1997; Nautrup, 2006).

2.2.4.4. Propagation in tissue

Ultrasound travels through tissue as a beam which in most clinical applications is focused

to around 1 mm in diameter at the focal zone. It propagates as a sequence of compression and rarefaction waves which are transmitted by virtue of the elastic forces between adjacent tissue particles. The particles move in the same direction as the wave — thus ultrasound is a longitudinal wave in contrast to the transverse wave that occurs at the surface of water where the particles move up and down as the wave travels horizontally. The frequency of the oscillations is inversely proportional to the wavelength ($f = c/l$, where f is frequency, c is the velocity of ultrasound and l is wavelength) (Nautrup, 2006). The constant speed of ultrasound in soft tissues allows the depth of reflectors to be calculated from measurements of the delay in the return of echoes. This is the essence of the pulse-echo method used in both ultrasound imaging and sophisticated forms of Doppler ultrasound. However, the position of reflectors across the scanned plane is determined in a quite different manner, by the direction in which the ultrasound beam is transmitted (David, 1997; Nautrup, 2006).

2.2.4.5 Attenuation

Provided that the constituent particles of a tissue are small enough to move as a single entity, the acoustical vibrations are transmitted in an orderly and efficient manner. However, when very large molecules are involved, the vibrations become disorganized, one part of the molecule responding more or less than another. While coherent vibration is what we know as sound, disorganized vibration is heat. This loss of coherence is the major mode of dissipation of ultrasound energy. It is known as absorption and is approximately proportional to the number of large molecules present, and this correlates fairly well with viscosity. Absorption is also highly dependent on the ultrasound frequency, higher

frequencies being more strongly absorbed. For average soft tissues the loss amounts to approximately 1 dB per cm tissue depth for each MHz. Thus, when using a 3 MHz probe, for every 2 cm of tissue penetration there will be a loss of 6 dB, which is a halving of the signal strength. The noise floor (produced by random vibrations in the tissue and the transducer as well as by imperfections in the scanner electronics) lies some 60 dB below the peak signal so the penetration of such a probe would be limited to 20 cm depth and, for a 6 MHz probe, to 10 cm (David, 1997).

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 mechanisms 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). Frequencies as high as 20 MHz can be used when only a few mm of tissue are to be traversed, such as for eye scanning and intravascular ultrasound (IVUS). For superficial tissues, such as the thyroid, breast and scrotum, 7–10 MHz is appropriate (David, 1997). For the heart, abdomen and second and third trimester obstetrics, 3–5 MHz is optimal, while for some difficult applications, such as the abdomen in obese subjects, and for transcranial studies (most of which use Doppler), one has to resort to 1.5 or 2.5 MHz (David, 1997).

Obviously a way to compensate for this rapid reduction in signal intensity is required if the image is to display similar reflectors as equal in brightness over a range of tissue depths. This is achieved by applying progressively increasing amplification (gain) to later echoes

in proportion to their depth using a time-varying amplifier that is triggered when each ultrasound pulse is sent. This is the TGC (time gain compensation), an important user control that must be set to equalize the image brightness for superficial and deep structures (David, 1997).

2.2.4.6 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 beam is reflected. The direction of the reflection of a wave hitting an interface perpendicularly is the same as that of the incident wave (Nautrup, 2006). The nonreflected wave continues in the second medium without changing direction (transmission). If the interface hits at an angle, the angle of reflection is the same as the incident angle. The transmitted wave is refracted (refraction). 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 a full-strength 'echo' because this part of the rope cannot move at all (Nautrup, 2006).

2.2.4.7 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 of sound in tissue to convert from time to depth; the same principle is used in radar and sonar (David, 1997).

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). A two-dimensional tomographic ultrasound image is formed by sweeping the beam through a slice of tissue and mapping the echogenicity of the reflectors as shades of grey to form a B-mode scan (B for brightness), also known as a 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 (David, 1997).

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 triangular 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 the 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 (David, 1997).

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 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 separate electrical connections and the trigger pulses to each element are serially delayed from one end of the array to the other. 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 (David, 1997).

2.2.4.8. *Resolution*

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. 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. The change-over is the transition between the near (Fresnel) and far (Fraunhofer) 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 centre 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 rarefaction 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 an 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 centre. Thus interference patterns are set up across the sound field that emphasize the centre region of the ultrasound beam and cancel ultrasound waves that otherwise would spread laterally. 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 interfaces. This

dynamic focusing optimizes resolution over the entire depth of the scan (David, 1997)

2.2.4.9. *Artifacts.*

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 per cent 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) (McAllister, 200)

2.2.4.10. *Interpretative 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 deeper tissues, bounded by the ultrasound beam lines which are parallel for a linear transducer and radiating for a sector transducer (McAllister, 2000).

Absorption and reflection are the two main causes of shadowing. If a portion of the tissue being imaged absorbs ultrasound faster than the background, the chosen TGC will be insufficient to compensate properly and therefore tissues deeper to the highly attenuating region are undercorrected and appear darker than adjacent tissue. Fibrous tissue and, to a lesser extent, fat attenuate at a higher rate than the usual $1 \text{ dB MHz}^{-1} \text{ cm}^{-1}$ 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 tissue: 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 artefacts 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; McAllister, 2000).

2.2.4.11. *Echogenicity*

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 fibrous tissue (often in the form of the perivascular microkeleton) and fatty tissue. Thus, while uniform regions of fiber or 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 a 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 nephronia in reflux nephropathy (David, 1997; McAllister, 2000).

2.2.4.12. *Safety*

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 development of cataracts, hearing loss or childhood malignancies. 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 behaviour, 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 Achievable), 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).

2.2.5. Developments in Ultrasonography

2.2.5.1. High frequencies, fast computers, 3D

Recent trends in ultrasound technology that can be expected to develop further are the use of higher frequencies, often linked with intracavitary transducers, and exploitation of the power of modern microprocessors. Probes operating at frequencies as high as 20 MHz are being developed for specialized purposes such as intravascular ultrasound, intraductal probes for the pancreas and biliary tree and for skin scanning (David, 1997).

Fast microprocessors allow better control of beam profiles and open the way to encode pulses so that their echoes can be allowed to overlap but still be separated out for display, overcoming the conventional limitations to the pulse repetition frequency (PRF). This will increase the frame rate and allow multiple focusing to be applied on transmit, as well as make it possible to run Doppler and imaging simultaneously at fast frame rates. More sophisticated image processing methods could be applied in real time. Examples include automatic time gain compensation, speckle reduction and clutter suppression. 3-D reconstruction, at present restricted to off-line processing because of the time taken to collect and process the data, could become a real-time operation thanks to the combination of 2D arrays and fast signal processors (David, 1997).

2.2.5.2. Contrast agents (ultrasound enhancing agents)

An important new development is the introduction of agents to enhance ultrasound signals. Several approaches have been tried, of which the most promising exploits the very high reflectivity of tissue–gas interfaces. It uses microbubbles made small enough to cross the lung capillary bed so that systemic (including myocardial) ultrasound enhancement can be achieved following an intravenous injection. A major technical difficulty with bubbles in the 2–10 μm range is that their surface tension is high so that they collapse and dissolve within a few seconds of formation. To produce enhancement of useful duration, the microbubbles must be stabilized. This can be achieved by adding a surfactant to lower their surface tension or by encapsulating them in a membrane (David, 1997). Products under development increase the reflectivity of blood by 10–20 dB and this fades over 5–10 minutes as the microbubbles disintegrate. Usually this degree of increase in echogenicity can only be detected with Doppler ultrasound, but the next generation of agents is expected to give sufficient enhancement to be obvious on grey scale (B-mode) ultrasound. Enhancers will extend to ultrasound many of the features of angiography, such as demonstrating tissue perfusion where infarction is a clinical question, and demonstrating bleeding points (the microbubbles are confined to blood vessels unless there is haemorrhage). In addition, measuring time–echogenicity curves analogous to those used in dynamic radioisotope studies will be possible for the first time, launching ultrasound into a new functional era of great potential (David, 1997).

2.2.5.3. Harmonic Imaging

An interesting development of microbubbles technology, known as 'harmonic imaging',

exploits the fact that bubbles of specific diameter resonate when insonated by ultrasound of tuned frequency. As they resonate, they emit ultrasound at harmonics of the original frequency, for example at 5 MHz if a 2.5 MHz beam was being used. By using appropriate filters in the receiver circuitry, a harmonic image that originates exclusively from the microbubbles can be formed. Because of the removal of clutter interference by tissue echoes, harmonic imaging will be capable of improving the quality of images, and of Doppler and timing measurements (David, 1997).

2.2.5.4. Ultrasound Therapy

Ultrasound seems likely also to find a role in tissue ablative therapy, particularly in oncology. Therapy ultrasound uses the thermal effects of high-power ultrasound beams that are very tightly focused so that small target tissue regions can be heated and the tissue coagulated. The general approach is very similar to radiotherapy, but ultrasound therapy has the advantages of being much more precise, rapid (the entire process could be completed in one session), easy to handle and not damaging adjacent tissues. It is also possible to monitor its effects in real time with an ultrasound scanner. The physical limitations to accessible anatomical regions are the same as for diagnostic ultrasound (David, 1997).

2.2.6. Obstetric Applications of Ultrasound

2.2.6.1. 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 bioeffects 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 (National Institute of Child Health and Human Development Report, 1984).

2.2.6.2. Instrumentation and scanning techniques

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

- a) Transabdominal real-time sonography forms the foundation for most obstetrical evaluations. This technique allows delineation of the fetus in real time, thereby affording optimal delineation of the fetus, uterus, and adnexal areas. Transabdominal sonography 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

Transabdominal sonography is usually performed with real-time transducers and according to certain protocols as established by the American Institute of Ultrasound

in Medicine and American 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.

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

- b) Transvaginal/transrectal sonography can be performed using a variety of transducer types. These include a curved linear multi-element transducer, a single-element mechanical sector transducer, and a multi-element phased array transducer. Transvaginal sonography can utilize higher frequencies than transabdominal sonography, 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.
- 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.

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 cm^{-2} , 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).

1.5 Materials**3.1.1 Experimental animals**

Ten (10) matured female Nigerian indigenous breed of dogs that have parturated at least once and at most twice were used for the study. They were acquired from local dog owners around Samaru and environs of Kaduna state, Nigeria. Their ages ranged from 2 ½ - 3 ½ years, with weight between 12-18 Kg. The bitches were housed in the Canine Research Kennels of the Small Animal Unit of the Veterinary Teaching Hospital, Ahmadu Bello University, Zaria Kaduna state Nigeria. They were fed once a day and provided water *ad libitum*.

3.1.2. Equipment

Medison S600V^(R) ultrasound machine with a 3.5MHz transcutaneous probe, a Sony videographic^(R) thermal printer, Ultrasound bed (Appendix i). Other equipments used were, Olympus light microscope, Casio Exilim 10.1mega pixel digital camera Ex-S10^(R) and Tiger electricity generator.

3.1.3. Consumables

The consumables used in the study include, thermal printing paper, aquasonic gel, glass slide, lighter, razor blades, immersion oil, tissue paper, 5ml syringes (with 21 gauge needles). Others are petrol, human chorionic gonadotropin (Appendix ii), Pasteur's loop, Giemsa reagent, methylated spirit.

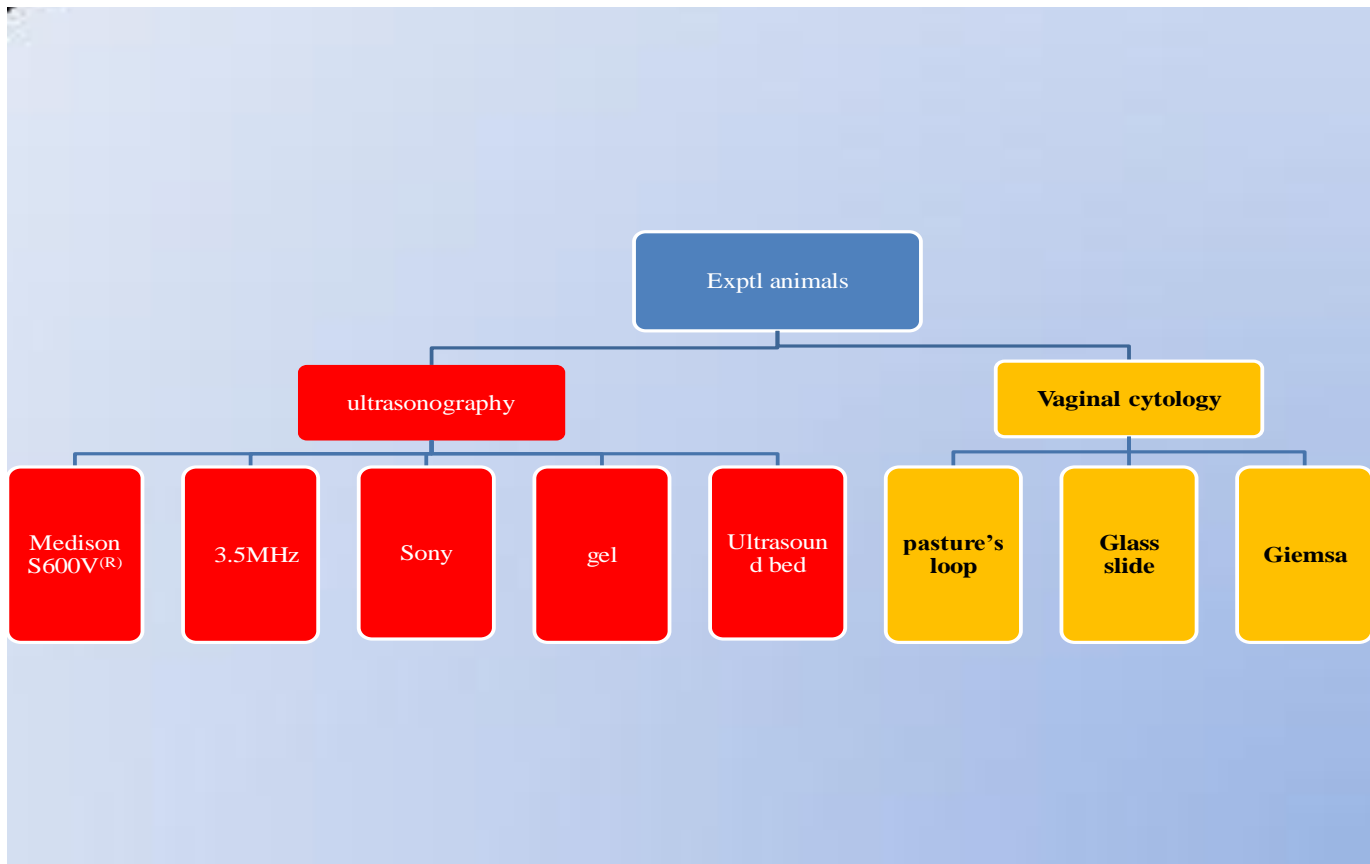


Fig. 3.1:List of Experimental Materials

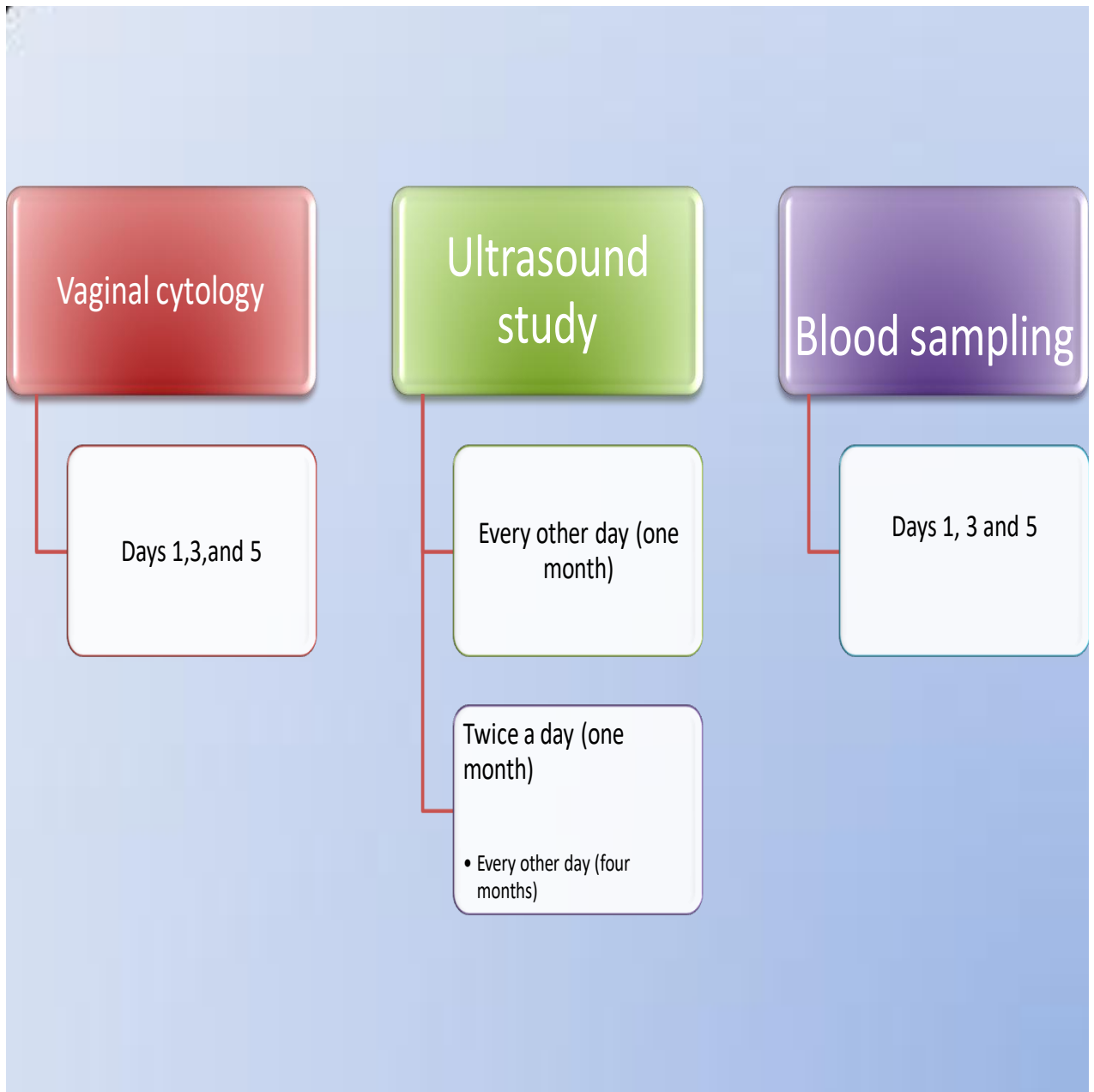


Fig. 3.2: Experimental timeline for the experimentally induced bitches.

3.2.1 Methodology

3.2.1.1 Vaginal cytology

The bitches were adequately restrained in the kennel and Methylated spirit was used to clean the vulva area; Pasteur's loop was then sterilized (using flame from a lighter) and allowed to cool. The loop was then inserted into the vagina of the bitch (Appendix iii); a scrape was taken from the vaginal wall and placed on a clean glass slide, which had a drop of water placed on it. The smear was air-dried and then stained with Giemsa. The stained smear was then observed under the microscope with x100 magnification and oil immersion. This was to determine the phase of oestrous cycle each bitch was on. This process was then repeated on days 3 and 5.

3.2.1.2 Induction of oestrus

The bitches were treated with 1ml each of the reconstituted Human Chorionic Gonadotropin (Diclair^(R) HP-HCG), which is equivalent to 5,000.I.U. of purified HCG. One ml vial of Human Chorionic Gonadotropin (HCG) containing 5,000 i.u. of the active ingredient was reconstituted with its diluents in a five (5) ml syringe for each bitch. The bitches were then adequately restrained and the HCG administered intramuscularly to each bitch. The process was repeated after 48 hours. The bitches were thereafter observed for signs of oestrus.

3.2.1.3 Blood collection and sampling

Five (5) ml of whole blood was taken using a 5ml syringe (21 Gauge needle), from the cephalic vein. It was divided into two parts. Two (2) ml was put into a sample bottle

containing anticoagulant (EDTA) for haematological studies as described by Coles, 1986. The sampling was repeated on days 3 and 5. Three (3) ml of whole blood was put into sample bottle free of anticoagulant for serum hormonal assay using the method described by Nett *et al* 1975.

3.2.1.4 Breeding

The bitches were bred via natural mating, that is done by taking the bitches to kennels containing adult males (in the ratio of two females per male). This was done when signs of the bitches coming on heat were noticed. They were left with the males for three weeks.

3.2.1.5 Ultrasonographic examination

The ventral abdominal area of the bitch was liberally shaved from the level of the xyphoid cartilage to the pubic area using razor blade, soap and water. The shaved area was then cleaned thoroughly with cotton wool soaked in water and swabbed with methylated spirit. The bitch was then restrained on dorsal recumbency on the ultrasound table. A portable ultrasound machine (Medison Ultrasound SV 600 manufactured by Krusse of Denmark) with a 3.5MHz linear transcutaneous probe was used to examine the pelvic area of the bitch after application of aquasonic gel to the probe (Appendix iv). The urinary bladder was used as landmark for the examination of the internal reproductive organs; the uterine luminal diameter was also measured. This process was carried out every other day for one month prior to induction of oestrus, then twice a day for one month and once every other day for the next four months.

4.1 Vaginal cytology.

The result of the vaginal cytology taken of the bitches prior to induction of oestrus showed that all of them were at dioestrus on the first day of taking the samples (Table 4.1). Forty-eight (48) hours after the first administration of the hormone Human Chorionic Gonadotropin (HCG) that is at day three of the experiment, the result showed that 30% of them (3 out of 10 bitches) were in early proestrous while 70% (7 out of 10 bitches) were in late proestrous. By day five (48 hours after second administration) of the experiment all the bitches were in oestrus Table 4.1. Plate III shows a photomicrograph of a bitch on day one of the experiment, with the bitch on dioestrus with few leucocytes and vaginal nucleated cells. Large rhomboidal cells are present but starting to lose their nucleus. Vaginal cytology samples taken on day three (Plates IV and V) showed the bitches in proestrous phase of the reproductive cycle erythrocytes, superficial cells and absence of neutrophils. Vaginal cytology samples taken at day five of the experiment () showed the bitch in oestrus phase of the cycle with keratinized superficial cells which are anucleated with angular margins

Table 4.1: Summary of vaginal cytology results for induced bitches.

Dog	Day1	Day3	Day5
	Dioestrus	Early Prooestrous	Oestrus
2	Dioestrus	Late Prooestrous	Oestrus
3	Dioestrus	Early Prooestrous	Oestrus
4	Dioestrus	Late Prooestrous	Oestrus
5	Dioestrus	Late Prooestrous	Oestrus
6	Dioestrus	Late Prooestrous	Oestrus
7	Dioestrus	Early Prooestrous	Oestrus
8	Dioestrus	Late Prooestrous	Oestrus
9	Dioestrus	Late Prooestrous	Oestrus
10	Dioestrus	Late Prooestrous	Oestrus

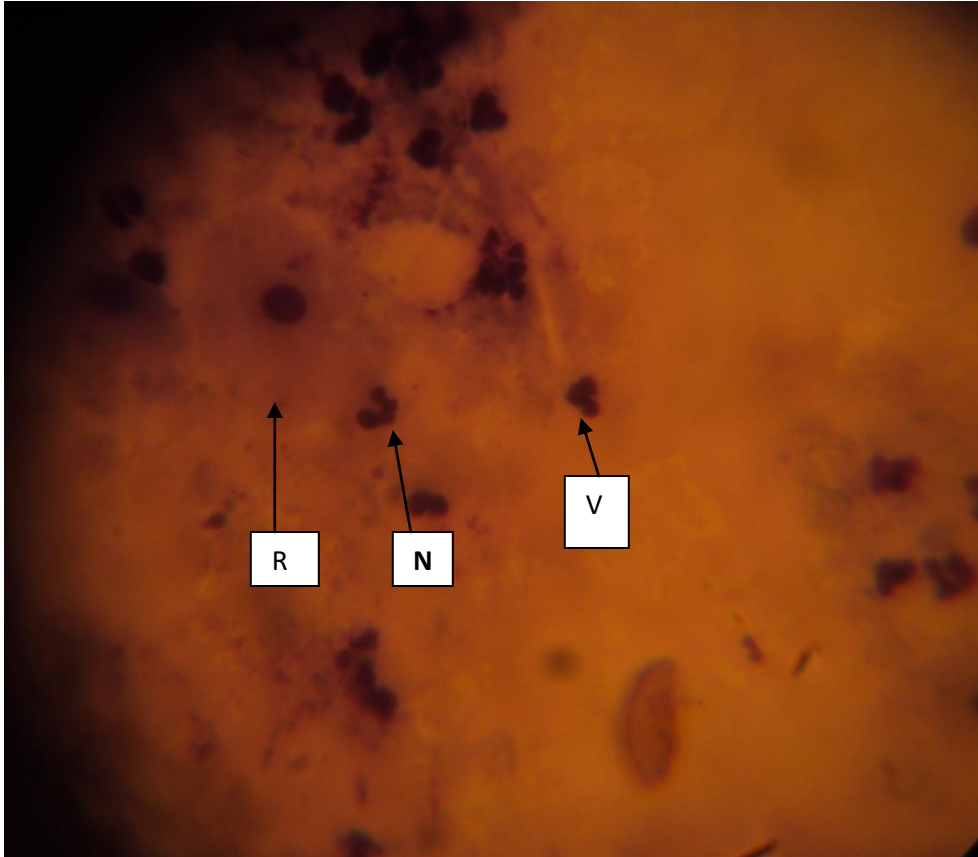


Plate III: Photomicrograph of the vaginal smear taken on day one showing features of the dioestrus phase of the cycle, with large rhomboidal (R) cells beginning to lose their nucleus, vaginal nucleated cells (V) and neutrophils(N) (Giemsa stain, at x100 magnification).

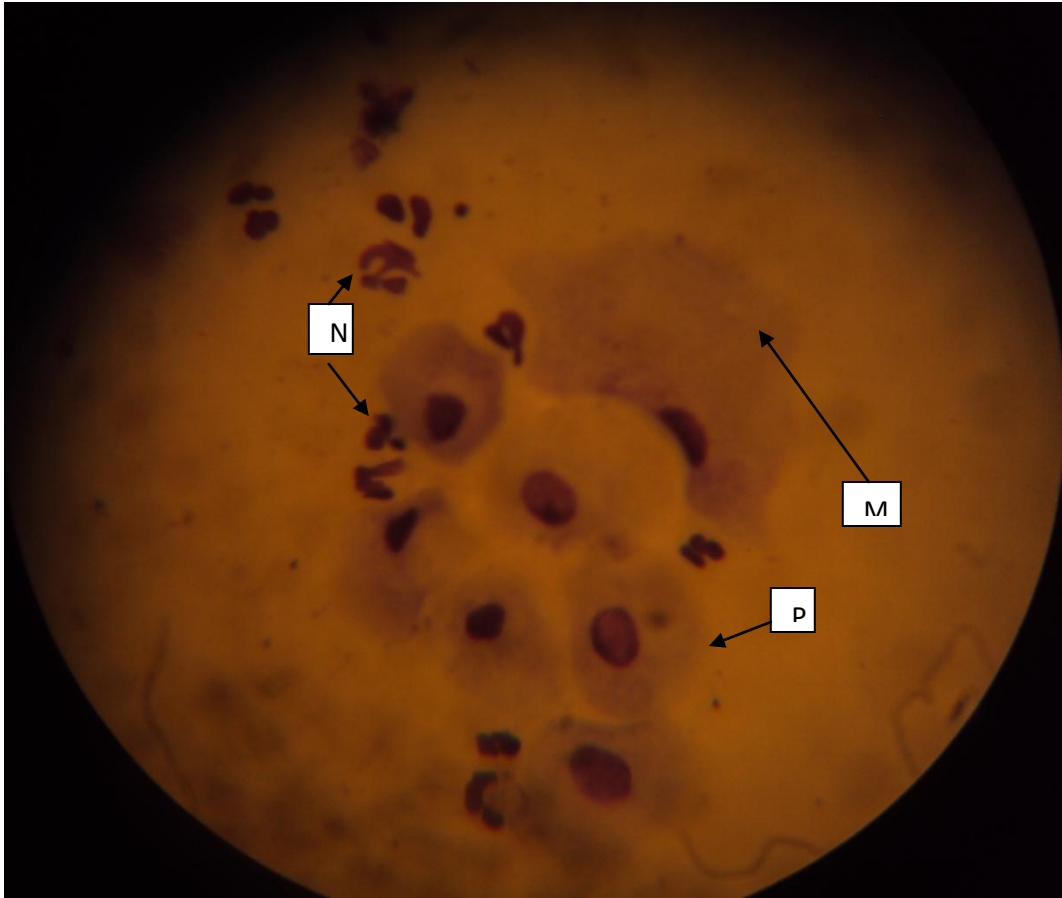


Plate IV: Photomicrograph of the vaginal smear taken on day three showing the bitch in early proestrus with neutrophils (N), parabasal cells (P) and mucus (M) (Giemsa stain, at x100 magnification).

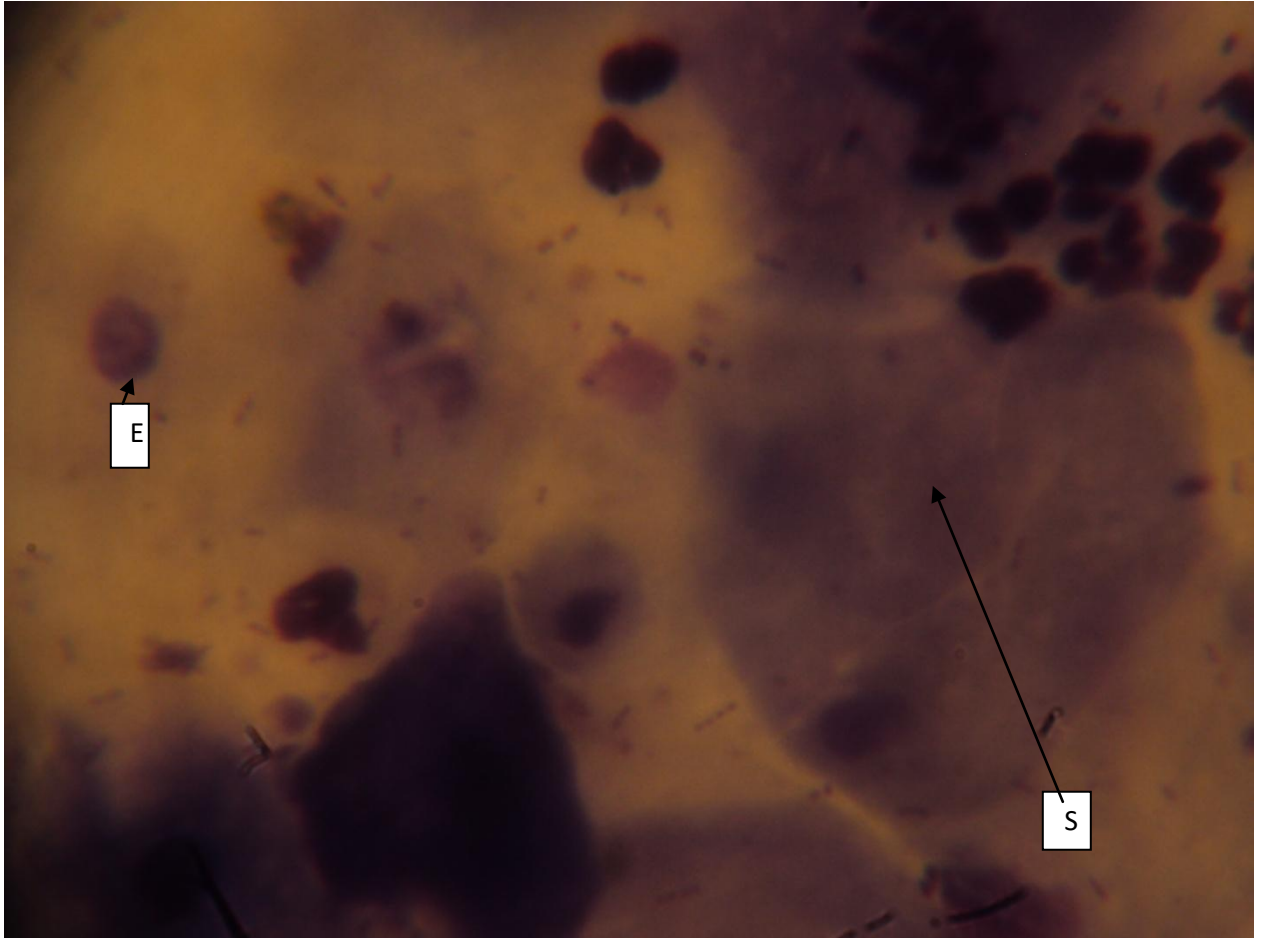


Plate V: Photomicrograph of the vaginal smear taken on day three with the bitch at late proestrous phase of the cycle erythrocytes (E), superficial cells (S) and absence of neutrophils (Giemsa stain, at x100 magnification).

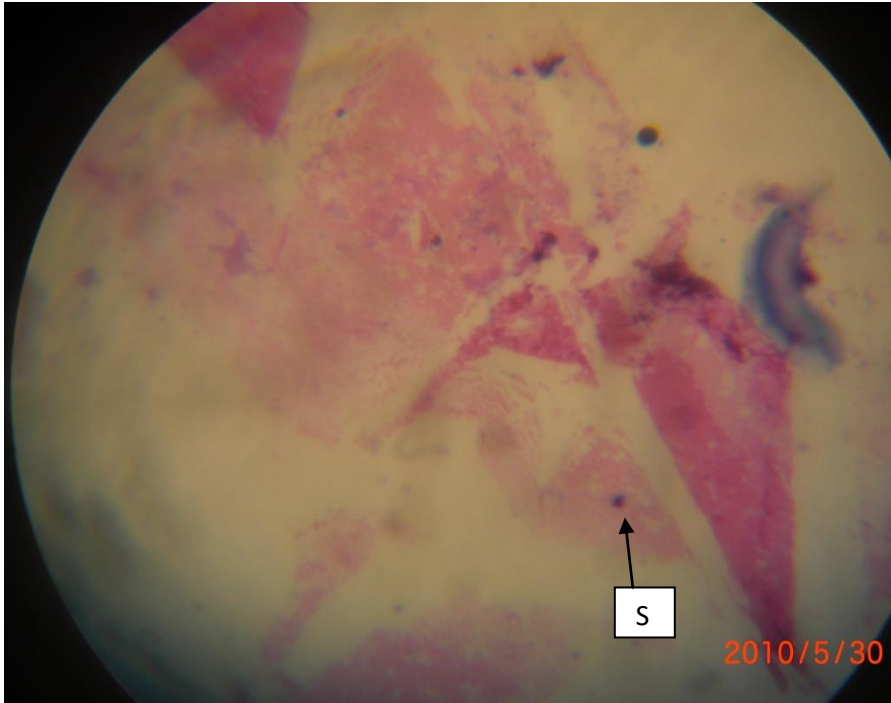


Plate VI: Photomicrograph of the vaginal smear taken on day five with the bitch at the oestrus phase of the cycle, with keratinized superficial cells (S) which are anucleated with angular margins (Giemsa stain, at x100 magnification).

4.2. Oestrus Induction

Following the first treatment with HCG on day three, there was evagination of the vulva and less resistance to handling (they became more amenable to handling). By day five (forty-eight hours following second HCG treatment) of the experiment, some of the bitches showed characteristic physical signs of heat (such as raising of the tail, attempting to mount each other Plate VII and in some cases clear discharges from the vulva).



Plate VII: Two bitches in a research kennel, with one raising the tail and following the other in an attempt to mount.

4.3. Blood collection and sampling

The results of the packed cell volume (PCV) on the various days for individual bitches are shown on appendix 4.2. The bitches had PCV ranging from 25-58 % with a mean PCV of $37.93 \pm 8.48\%$ figure 4.1 shows the graphical representation of the results. Appendix 5 shows the distribution of the PCV of each bitch taken during the induction phase of the experiment. The result of the white blood cell count (appendix 6) taken during the induction phase of the experiment showed total count ranging from $3.6-7.2 \times 10^9/L$, with a mean WBC count of $4.85 \times 10^9 \pm 0.78 \times 10^9/L$. Figure 4.2 graphical representation of the result.

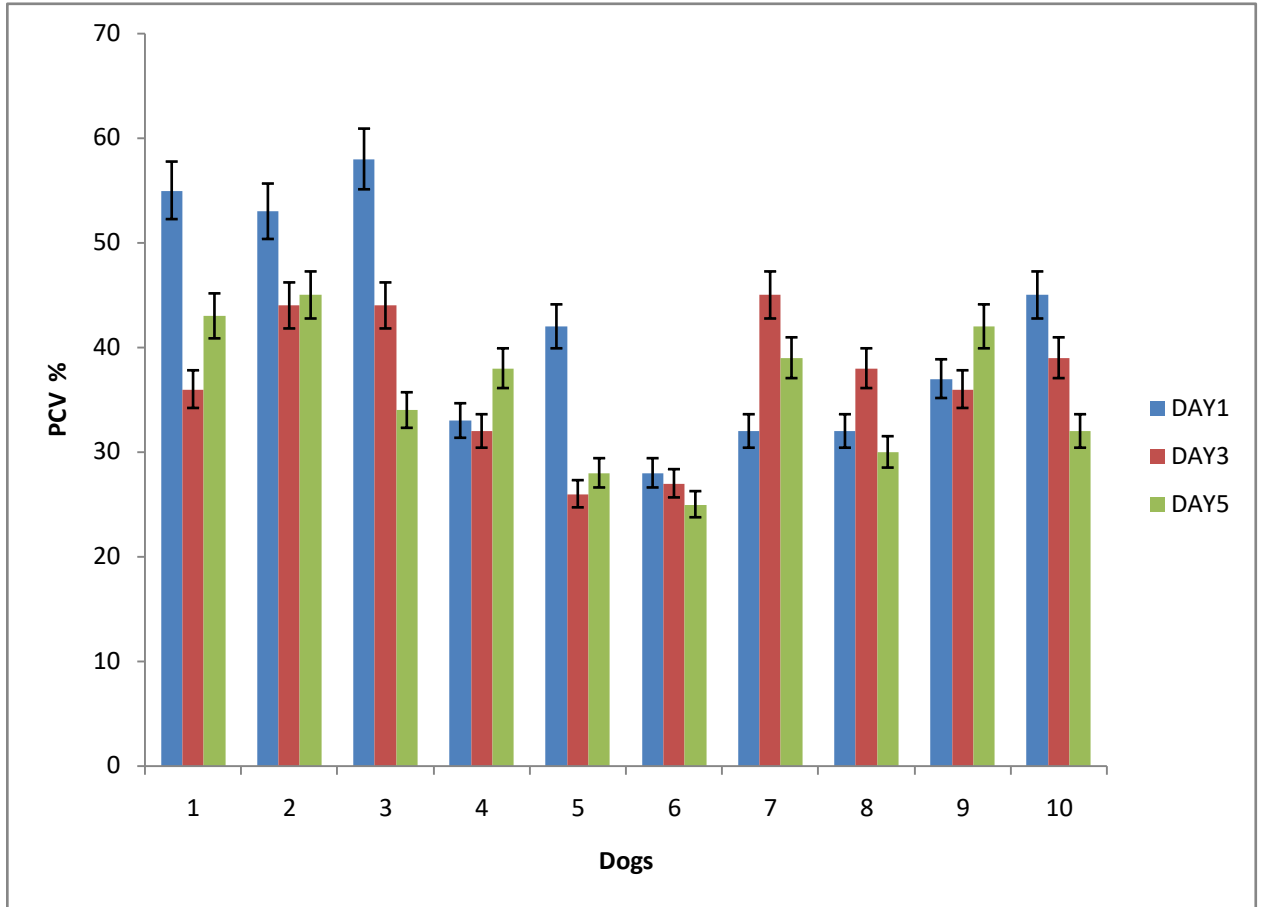


Figure 4.1: Packed cell volume (PCV) of the experimental bitches taken during the induction phase.

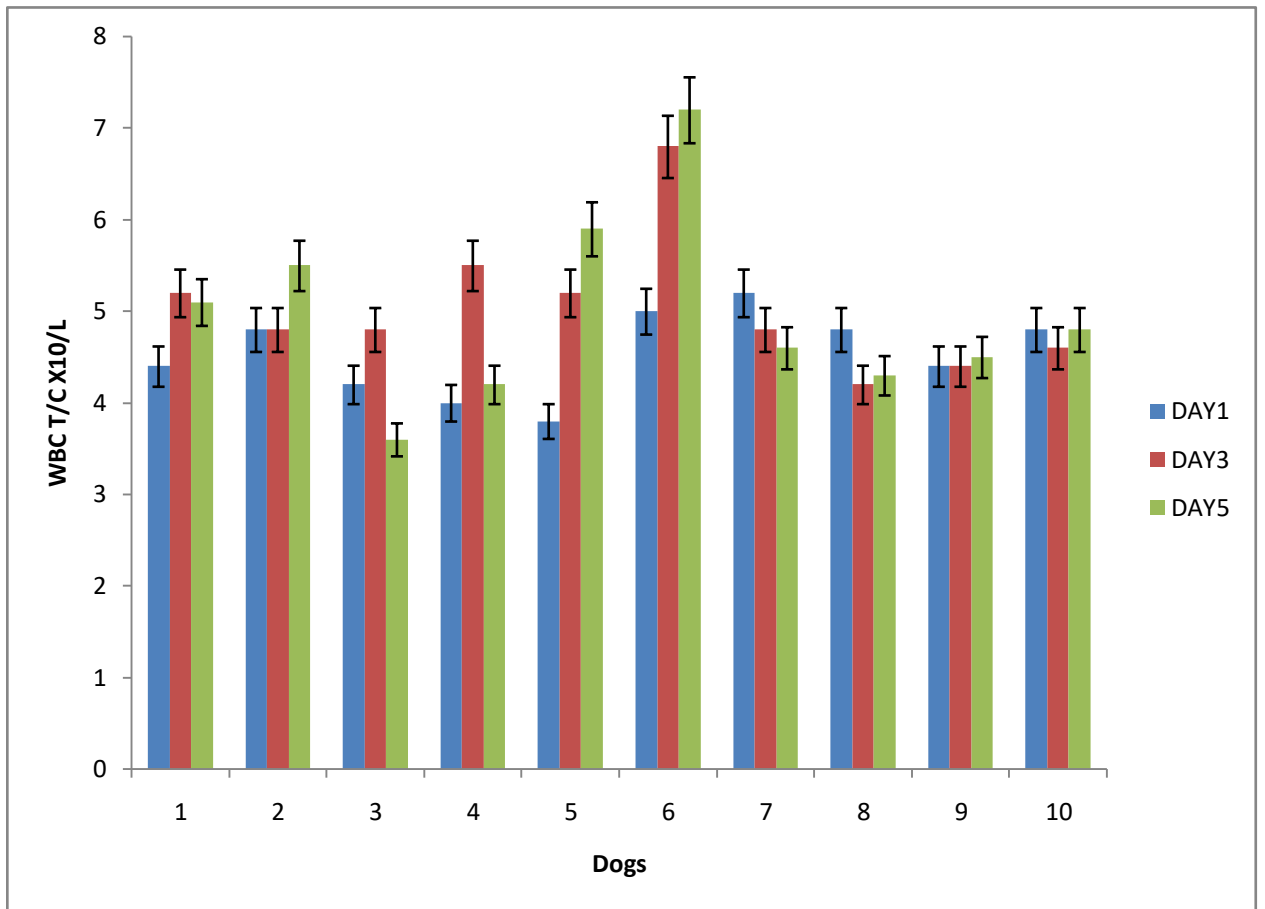


Figure 4.2: White blood cell (WBC) of the experimental bitches taken during the induction phase of the experiment.

4.4 Hormonal Assay

The results of the Follicle stimulating hormone (FSH) assay on the various days for individual bitches are shown on Appendix 7. The bitches had FSH values ranging from 5.1-18.3 $\mu\text{iu/ml}$ with a mean $9.33 \pm 3.56 \mu\text{iu/ml}$. Figure 4.3 is a column graph showing the distribution of the FSH concentration of each bitch taken during the induction phase of the experiment. The result of the luteinizing hormone assay taken during the induction phase of the experiment showed concentration of LH ranging from 4.6-14.2 $\mu\text{iu/ml}$, with a mean concentration of $7.91 \pm 2.63 \mu\text{iu/ml}$. Appendix 8 and Figure 4.4 are tabular and graphical representation of the result of the results respectively.

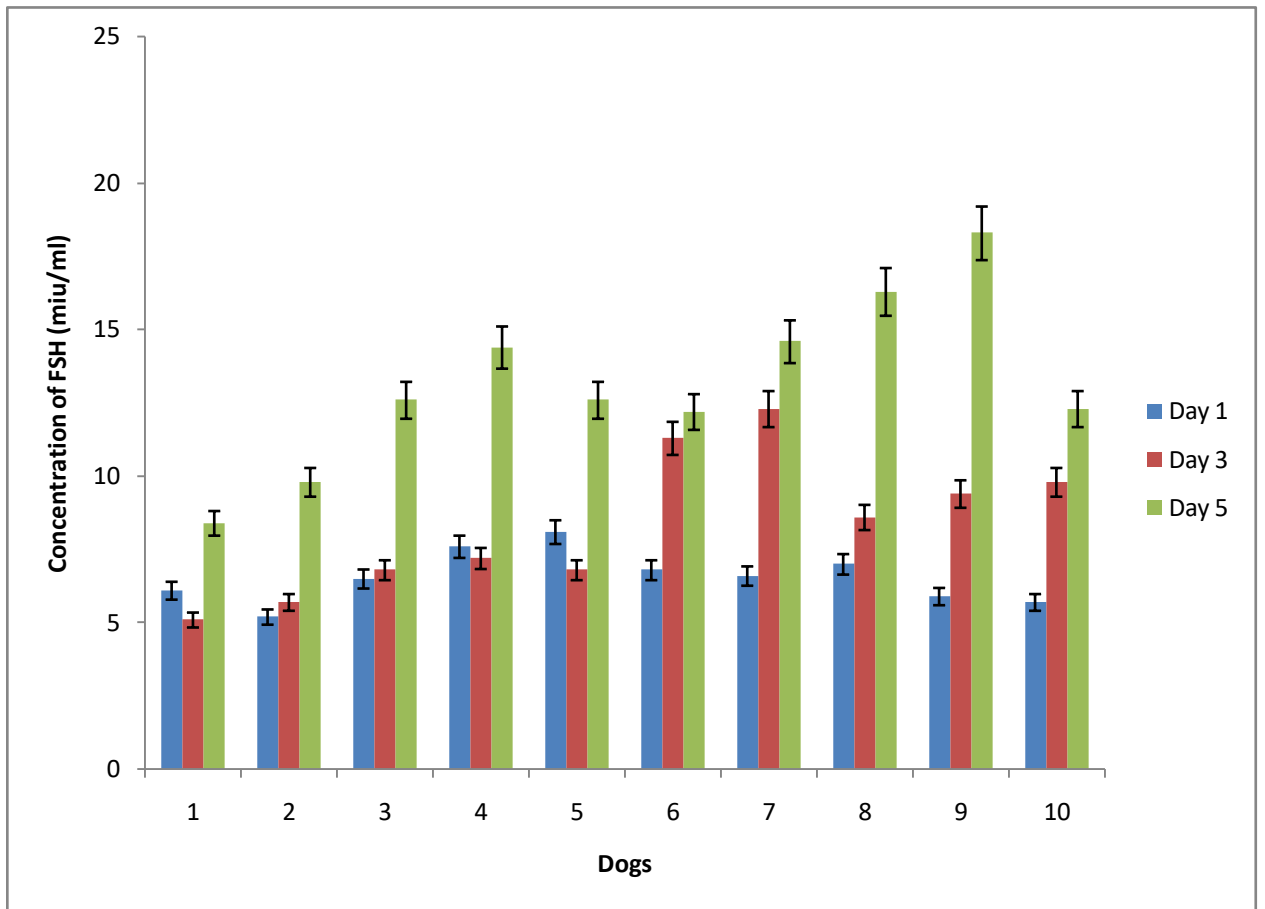


Figure 4.3: Follicle stimulating hormone (FSH) concentration of the experimental bitches taken during the induction phase.

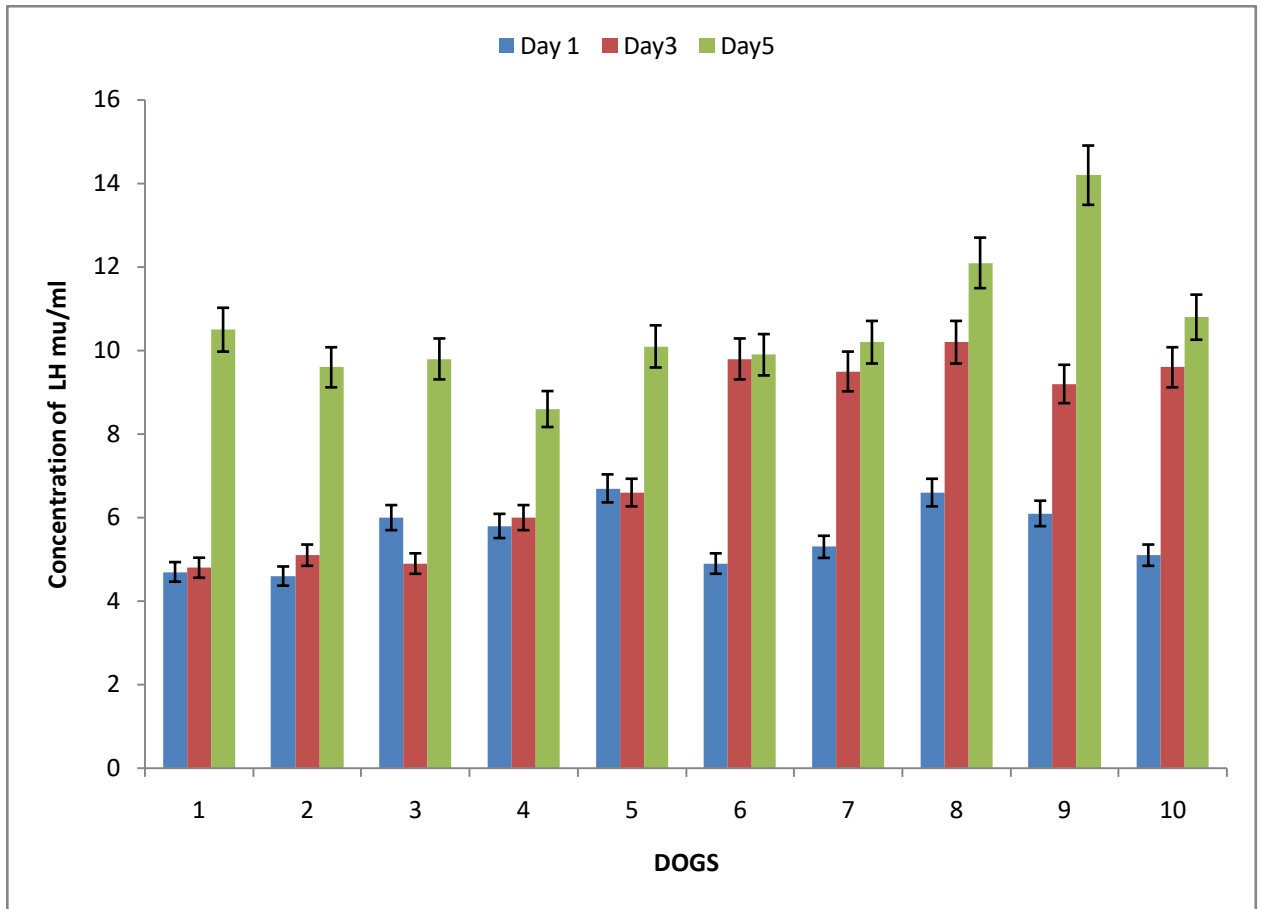


Figure 4.4: Luteinizing hormone (LH) concentration of the experimental bitches taken during the induction phase.

4.5. Ultrasonography

Serial ultrasound examination carried out every other day on the bitches during the first one month of the study revealed that examination of the urinary bladder had thickened wall when not filled with urine (Plate VIII) and that this made abdomino-pelvic scanning much more difficult.

Prior to induction of oestrus, the bitches were found to be at late dioestrus, the internal genitalia were visualized up to the point of the uterus (Table 4.6 and Plate VII). But the ovaries were not visualized ultrasonographically at that phase of the cycle. The diameter of the lumen was measured during the study and at dioestrus the mean luminal diameter was 5.5 ± 0.70 mm (Table 4.3). At late dioestrus (Plate IX) there were corpora lutea which contain lots of fluid initially before complete regression occurs.

During prooestrus, the internal genitalia up to the ovaries were visualized as being small anechoic structures (Plate XI). The mean luminal diameter was 7.5 ± 0.5 mm (Table 4.3).

During oestrus, the same anechoic structures were observed with some fluid accumulation (Plate XII). Measurement of the luminal diameter (Table 4.3) taken at this phase of the oestrus cycle showed a mean luminal diameter increment to 8.6 ± 0.50 mm.

During anoestrus, there was reduction in the size of the uterus (Table 4.3) the ovaries were not visualized. Measurement of the uterus showed slight reduction in lumen at this phase of the reproductive cycle (Plate XIV). The mean luminal diameter at anoestrus was 5.8 ± 0.4 mm.

Table 4.2: Structures visualized by ultrasonographic study of the reproductive cycle of Nigerian local bitches.

Phase of the cycle	Ovary	Oviduct	Uterus	Cervix	Vagina
Dioestrus	-	-	+	+	+
Prooestrus	+	+	+	+	+
Oestrus	+	+	+	+	+
Anoestrus	-	-	+	+	+

Key

- = cannot be visualized
- + = can be visualized

Table 4.3: uterine diameter during the various phases of oestrous cycle in the induced experimental bitches

Dog no	Measurement At dioestrus (mm)	Measurement At prooestrus (mm)	Measurement At oestrus (mm)	Measurement At anoestrus (mm)
1	5	7	8	6
2	6	7	8	6
3	6	7	9	6
4	5	8	9	5
5	6	8	8	6
6	6	8	9	6
7	7	8	9	6
8	6	8	8	6
9	5	7	9	6
10	5	7	9	5
MEAN_±SD	5.7_±0.7	7.5_±0.5	8.6_±0.5	5.8_±0.4

Key

SD=STANDARD DEVIATION

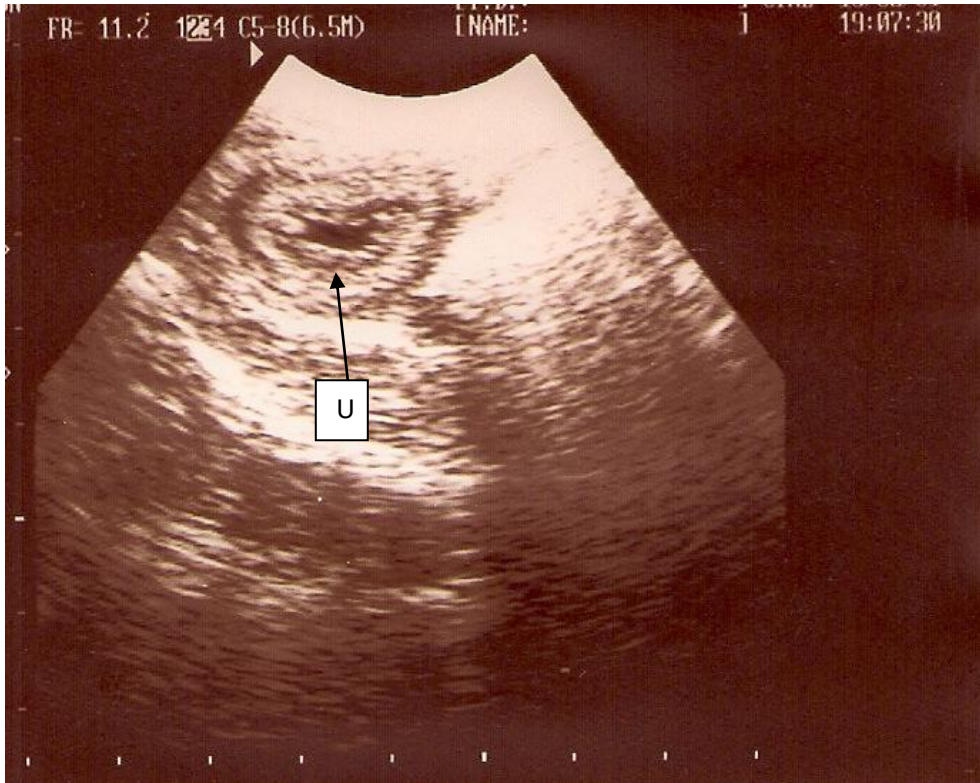


Plate VIII: Sonograph of an empty urinary bladder (U) with thickened wall and absence of urine during the ultrasound scanning.

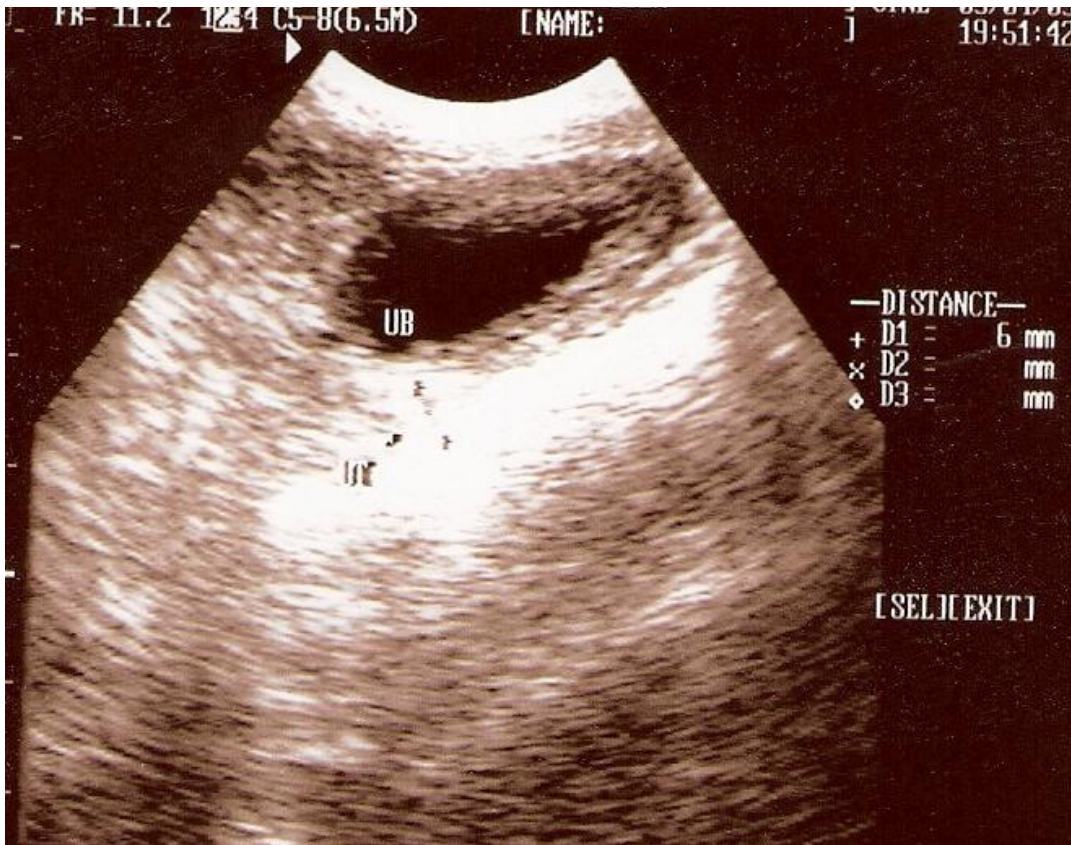


Plate IX: Sonograph of a bitch (no 4) taken at the late dioestrus phase of the reproductive cycle showing the urinary bladder (U) the uterus (UT) and the luminal diameter of the uterus to be 6mm.

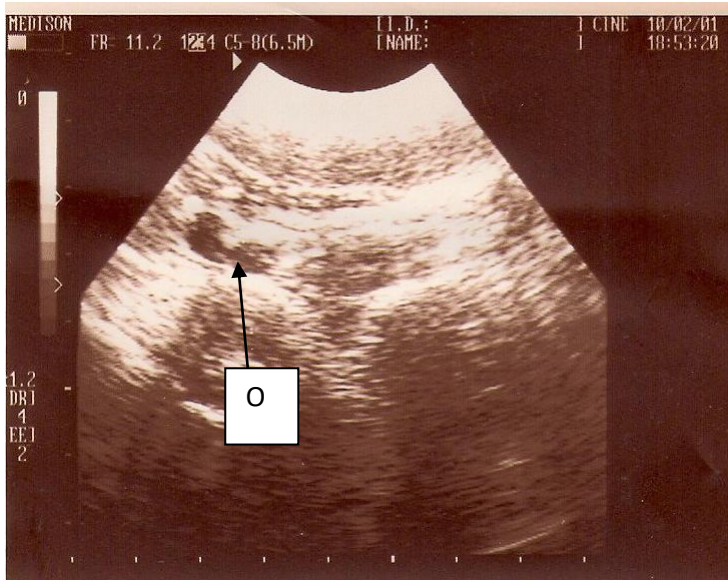


Plate X: Sonograph of a bitch (no 8) taken at the late dioestrus phase of the reproductive cycle showing the ovary (O) to be small anechoic structures

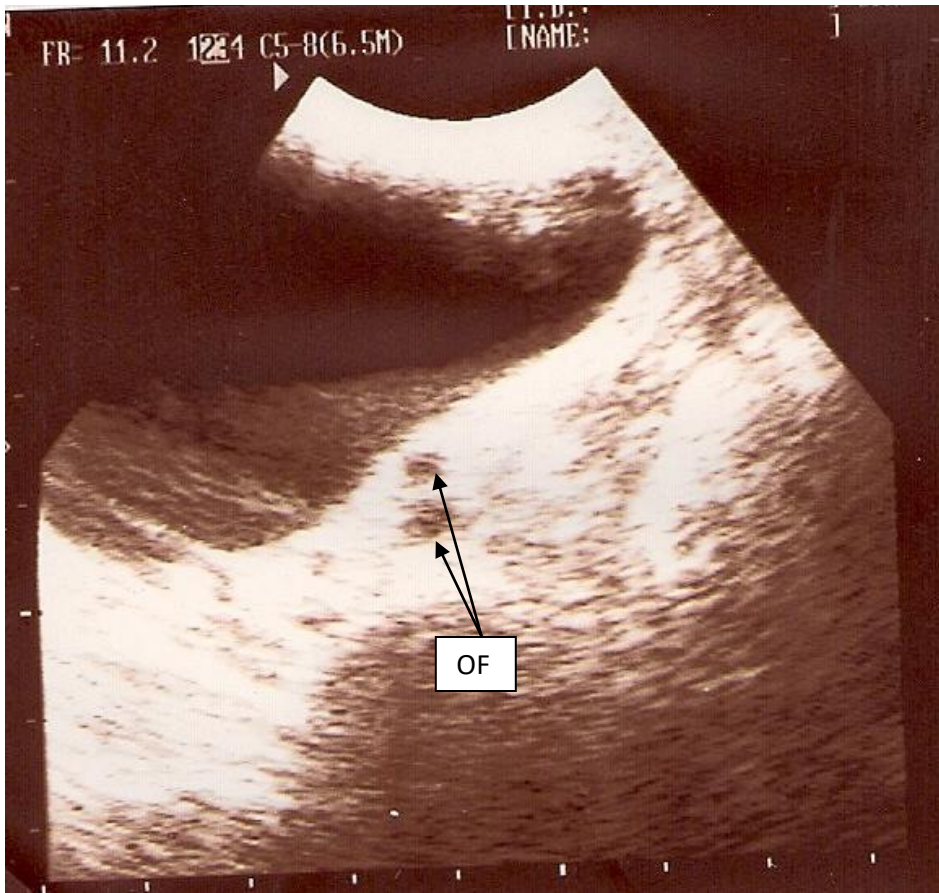


Plate XI: Sonograph of a bitch (no 5) at the proestrous phase of the reproductive cycle showing the ovarian follicles (OF) on one ovary.



Plate XII: Sonograph of a bitch (no 3) at the oestrus phase of the reproductive cycle showing the luminal diameter of the uterus to be 9mm.

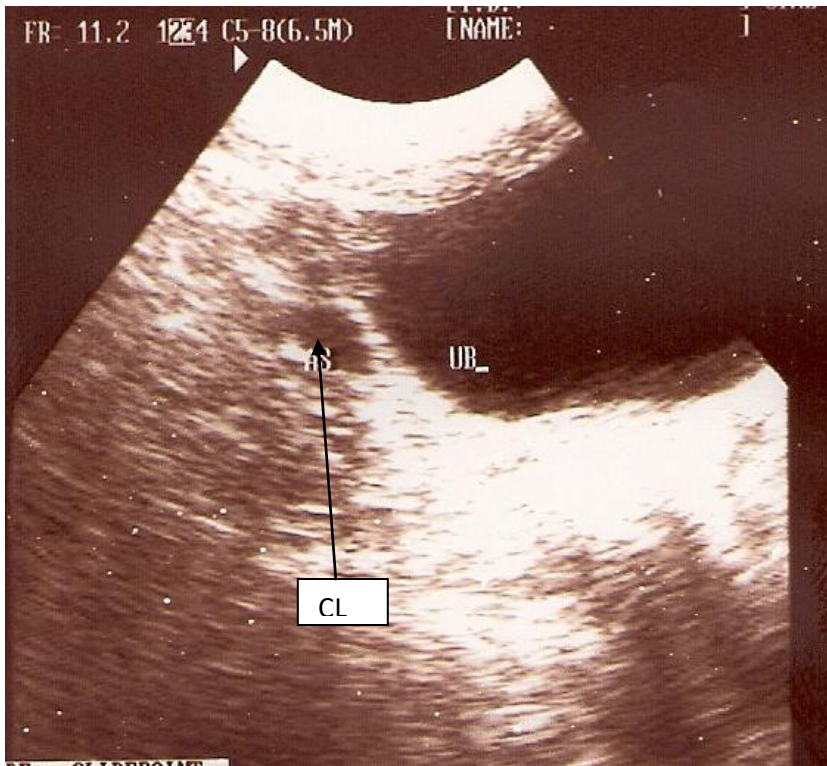


Plate XIII: Sonograph of a bitch (no 1) at the oestrus phase of the reproductive cycle showing a corpus luteum(CL) on one ovary with the urinary bladder.

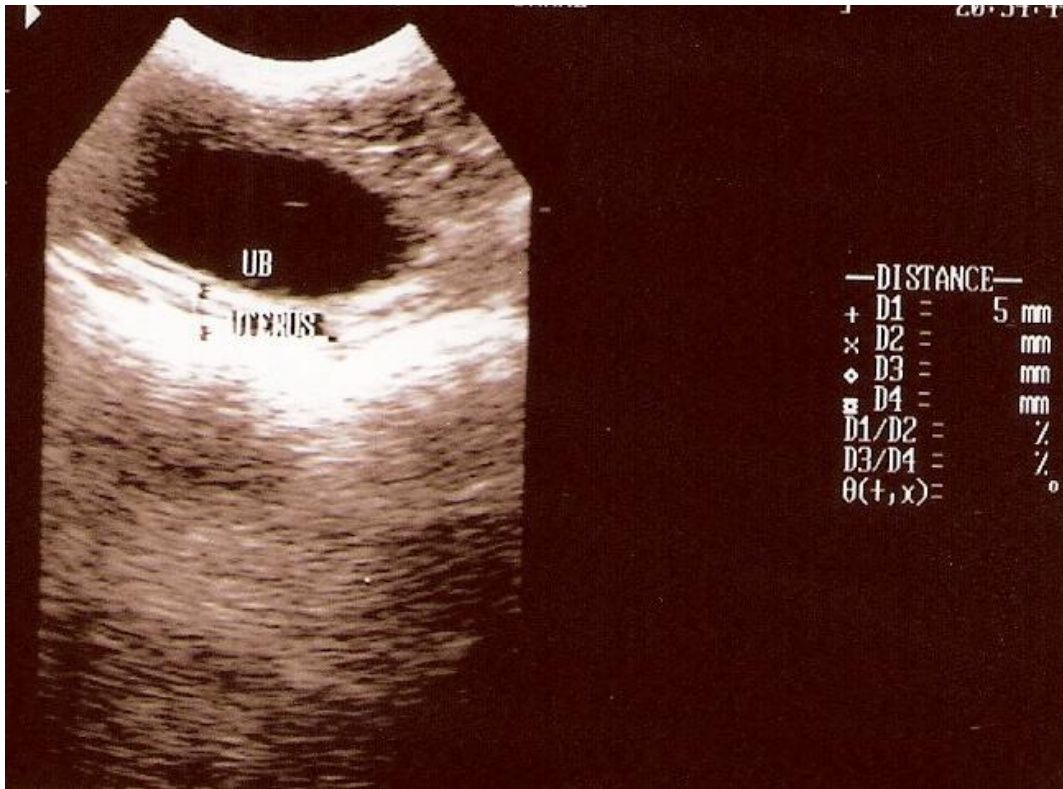


Plate XIV: Sonograph of a bitch (no 9) at the anoestrus phase of the reproductive cycle showing luminal diameter of the uterus to be 5mm.

CHAPTER 5

DISCUSSION

Considering the fact that bitches are classified as being monocyclic having the same cycling interval (6-7 months between cycles), the only way to study them as a group sonographically is to ensure they are all in the same phase of the reproductive cycle. This was achieved by in this study by inducing oestrus in the bitches.

The study was designed to establish the sonographic changes that occur during the different phases of the reproductive cycle and correlate them with both cytologic and possibly hormonal changes. The result of the cytologic study showed that the bitches were all initially at the dioestrus phase of the reproductive cycle; during this phase of the reproductive cycle, cell populations changed abruptly, superficial cells decrease by 20% and smaller intermediate cells increase in number. Neutrophils often reappeared and often contained phagocytosed erythrocytes and bacteria the result of the cytology studies agrees with the finding of previous workers (Valerie, 2003; Josep, 2010).

The result at proestrous showed that predominant cell populations in early to mid proestrous included nondegenerate neutrophils and a mixture of parabasal, intermediate, and superficial epithelial cells. The background of the slide contained an abundance of mucus. Variable numbers of extracellular bacteria represent normal flora. In late proestrous, the number of neutrophils declined and superficial cells began to predominate this finding agreed with the works of Valerie, 2003.

In oestrus, the cell population consisted of ~ 90% superficial cells and < 5% parabasal or intermediate cells. Less mucus was present in the background of the preparation.

Endogenous regulation of this transition was not well understood, but can be affected by exposure to oestrus pheromones and possibly other external stimuli. It apparently required prolonged withdrawal of progesterone, based on the ≥ 2 month obligate anoestrus seen following the end of the 2 month luteal phase or progestin contraception. It likely also involves prolactin and or changes in dopaminergic activity in the hypothalamus, based on the fore-shortening of anoestrus by prolactin-lowering doses of dopamine agonists. The role of LH was probably greater than that of FSH in initiating the transition, based on induction of oestrus and/or prooestrous by administration of highly purified LH alone, but not by FSH alone (Verstegen, 1997). The present study confirmed the effectiveness of the treatment regimen using HCG for inducing oestrus in the bitch during dioestrous phase as described by other workers such as Archbald, 1980, Nakao, 1984, whose result showed greater than 80% success rate when oestrus was induced using PMSG or/and HCG. There was also marked reduction in the length of the prooestrous phase of the oestrus cycle and an absence of anoestrus as compared to spontaneous oestrus (where dioestrus is followed by anoestrus then prooestrous).

During the anoestrous period, the ovaries are not always easy to locate. They have a small size, and appear a little bit heterogeneous, especially in post-pubertal bitches, where remnants of anterior corpora lutea can be visualized.

In pro-oestrous bitches, the shape of the ovaries was easier to see. The ovaries were often found in a more caudo-ventral position from the kidneys. They were found to contain several small circular anechoic follicles, surrounded by a thin echoic wall, less than 1 mm in thickness. The size of the ovaries increases just before oestrus, due to the large amount of anechoic fluid within the follicles, they become really easy to visualize. These findings

agree with the result from other researchers who worked on other breeds of dogs (Xavier and Alain, 2007). At the time of ovulation, in some cases a complete disappearance of the follicular cavities (“follicular collapsus”) can be visualized.

CHAPTER 6

Summary, Conclusions and Recommendations

6.1 Summary

Oestrus can be induced in dogs using two doses of Human Chorionic Gonadotropin (HCG) at 5,000 i.u. intramuscularly forty- eight hours apart. Vaginal cytology is also a very efficient and rapid field method of determining the phase of oestrous cycle tin a bitch. It can be done with minimal need for advanced equipments. Ultrasound was an effective method of following the changes that occur during the various phases of the oestrous cycle of the bitch, when evaluated by trained individual.

6.2 Conclusion.

This study has shown that there is a good correlation between vaginal cytologic changes, serum hormonal changes and ultrasonographic changes that occur during the different phases of the reproductive cycle in Nigerian local bitches.

6.3 Recommendations.

1. Further studies should be carried out in the field (veterinary ultrasonography).
2. There should be use of more modern ultrasound equipment to ensure that more accurate results will be gotten from future studies.
3. A longer time of study should be undertaken to map out the sonographic and cytologic changes that occur at spontaneous oestrus, to compare the results with those of induced oestrus.

4. There is the need to further explore the application of ultrasonography in the study of the female genitalia/ reproductive activity.
5. Ultrasonographic changes in internal genitalia post partum following natural whelping.

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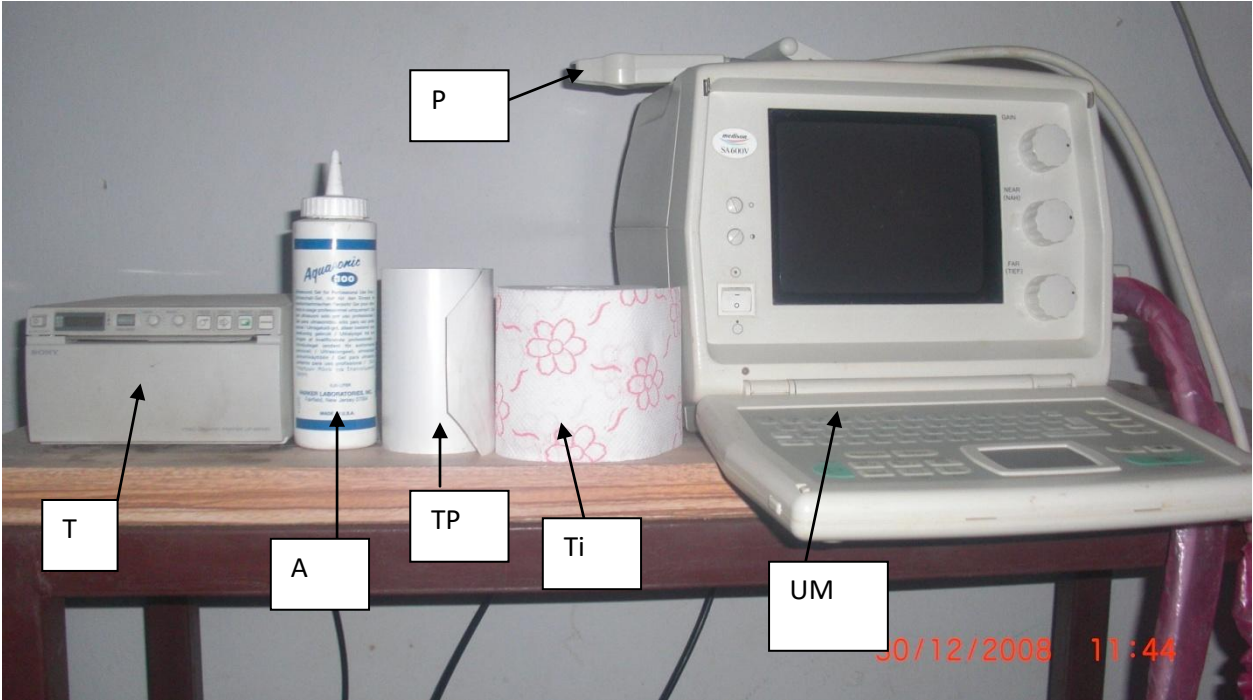
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APPENDIX I

Ultrasonography equipment and consumables. Thermal Printer (T), Ultrasound jell (A), Thermal printing paper (TP), Tissue paper (Ti), Ultrasound machine (UM), Ultrasound probe (P).



APPENDIX II

Hormonal preparation used for estrus induction.



APPENDIX III

Pasteur's loop being inserted into the vagina (arrowed) for collection of vaginal smear



APPENDIX IV

Scanning of the ventral abdominal area of the bitch.



APPENDIX V

PCV of the bitches taken prior, during and after oestrus induction.

DOG	DAY1	DAY3	DAY5
1	55	36	43
2	53	44	45
3	58	44	34
4	33	32	38
5	42	26	28
6	28	27	25
7	32	45	39
8	32	38	30
9	37	36	42
10	45	39	32

APPENDIX VI

WBC of the bitches taken prior, during and after oestrus induction.

DOG	DAY1	DAY3	DAY5
1	4.4	5.2	5.1
2	4.8	4.8	5.5
3	4.2	4.8	3.6
4	4	5.5	4.2
5	3.8	5.2	5.9
6	5	6.8	7.2
7	5.2	4.8	4.6
8	4.8	4.2	4.3
9	4.4	4.4	4.5
10	4.8	4.6	4.8

APPENDIX VII

FSH concentration taken prior, during and after oestrus induction phase of the experiment

Dog	Day 1	Day 3	Day 5
1.	6.1	5.1	8.4
2.	5.2	5.7	9.8
3.	6.5	6.8	12.6
4.	7.6	7.2	14.4
5.	8.1	6.8	12.6
6.	6.8	11.3	12.2
7.	6.6	12.3	14.6
8.	7	8.6	16.3
9.	5.9	9.4	18.3
10.	5.7	9.8	12.3

APPENDIX VIII

LH concentration taken prior, during and after oestrus induction phase of the experiment

DOG	Day 1	Day3	Day5
1	4.7	4.8	10.5
2	4.6	5.1	9.6
3	6	4.9	9.8
4	5.8	6	8.6
5	6.7	6.6	10.1
6	4.9	9.8	9.9
7	5.3	9.5	10.2
8	6.6	10.2	12.1
9	6.1	9.2	14.2
10	5.1	9.6	10.8

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