# TAMIL NADU VETERINARY AND ANIMAL SCIENCES UNIVERSITY





# Training Manual on

# APPLICATION OF DIAGNOSTIC IMAGING IN ANIMAL REPRODUCTION MANAGEMENT FOR VETERINARIANS

20.06.2022 to 22.06.2022

Sponsored by



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DEPARTMENT OF VETERINARY GYNAECOLOGY AND OBSTETRICS MADRAS VETERINARY COLLEGE, CHENNAI – 600 007

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# Organizing Committee for the

**Training on** 

# APPLICATION OF DIAGNOSTIC IMAGING IN ANIMAL REPRODUCTION MANAGEMENT FOR VETERINARIANS

20<sup>th</sup> – 22<sup>nd</sup> June 2022

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# VARIOUS IMAGING TECHNIQUES USED IN ANIMAL REPRODUCTION

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Adoption of various imaging procedures in the field of animal reproduction is a technological breakthrough that revolutionised knowledge of reproductive biology and pathology. Most imaging methods provide a lot of information by non-invasive and economical means. Each technique has its own advantages and disadvantages which may relate to cost, sensitivity, specificity and availability.

# Various imaging techniques

- ✤ X-rays (high-energy radiation)
- Ultrasonography(high-energy sound waves)
- Computed Tomography (CT)
- Mammography
- Magnetic Resonance Imaging (MRI)
- Endoscopy
  - □ Gynoscopy: Used in female reproductive system
  - □ Colposcopy: Examine the cervix, vagina and vulva
  - □ Hysteroscopy: Examine the uterine cavity
  - □ Falloposcopy: Examine the fallopian tubes using microendoscope
  - □ Fetoscopy: Surgical access to the fetus, amniotic cavity, umbilical cord and fetal side of the placenta

# X- Ray (Radiography)

Radiography is an imaging technique to create images to view the internal form of an object using X-rays, gamma rays or similar ionizing radiation and non-ionizing radiation called radiograph (black, white and gray images).



X-rays were discovered in 1895 by Wilhelm Conrad Roentgen who was a Professor at Wuerzburg University in Germany. Röntgen referred to the radiation as "X", to indicate that it was an unknown type of radiation.

Röntgen discovered X-rays' medical use when he made a picture of his wife's hand on a photographic plate formed due to X-rays. The first use of X-rays under clinical conditions by John Hall-Edwards in Birmingham, England on 11 January 1896, when he radiographed a needle stuck in the hand of an associate. Hall-Edwards used X-rays, for the first time, in a surgical operation on 14 February 1896.





W. C. Roentgen (1845-1923)



J. F.H.Edwards (1858 -1926)



Simple radiograph. *A*, X-ray machine; *B*, patient; and *C*, x-ray film. From Malarkey and McMorrow, 1996.



# Applications of x-ray radiography in animal reproduction:

- Pregnancy Diagnosis
- Estimation of number of foetus in pregnant animals
- Evaluation of foetal growth and gestational age by radiographic measurement of body parts
- Diagnosis of Pyometra/Endometritis
- Diagnosis of mass lesions growths, neoplasms, cysts etc



Pregnant uterus with foetuses



Mass lesions- growths, neoplasms, cysts

# **Contrast Vaginography**

 Positive contrast vaginography can be performed to further evaluate the vaginal vault and vestibule and the urethra – helps in diagnosis of ectopic urethra, recto-vaginal fistula, urethro-vaginal fistula, strictures, developmental anomalies etc



## Merits

- Cheaper compared to other advanced techniques like CT, MRI
- Radiography results are easier to read and evaluate compared to other sophisticated techniques like Ultrasonography which requires more skill to perform.
- Accurate tool for evaluation of foetal number and for detection of retained foetus after parturition
- ✤ Gives the exact location of the foetus/lesion inside the animal

## Demerits

- Emits hazardous radiation to the surroundings which is harmful to both the operators and animals
- During pregnancy diagnosis, there is a potential threat for radiation damage to the foetus undergoing this procedure
- Accurate pregnancy diagnosis is possible only after at least 45 days of gestation – does not help in early pregnancy diagnosis
- Reproductive organs of the bitch having the same radiographic density as surrounding soft tissues, making it more difficult to distinguish.

# Ultrasonography

Ultrasonography is the most commonly used imaging procedure in veterinary practices. Ultrasound is sound waves with frequencies higher than the upper audible limit of human hearing (20 kHz). Audible sound is in the range of 20-20000 HZ (Cycle/Sec.). Ultrasound is in the range of 1-10 MHZ (1MHZ -1000000 Waves/second). Echolocation in bats was discovered by Lazzaro Spallanzani in 1794. Pierre Curie in 1880 described the piezoelectric effect whereby mechanical distortion of ceramic crystals would produce an electric charge. The piezoelectric effect was discovered by Jacques and Pierre Curie in 1880, which was useful in transducers to generate and detect ultrasonic waves. Use of Ultrasonography in bovine practice began in the 1980's.



Lazzaro Spallanzani



Pierre Curie







Principle of Transducer

Vibrations of the crystals are produced by pulses of electric current. A proportion of sound waves reflected back to the transducer is converted to electric current and displayed as an echo on the ultrasound viewing screen.

- Black Anechoic
- Grey Hypoechoic
- White Hyperechoic

Therefore the transducer acts as both the sender and receiver of echoes. The most

commonly used frequencies in large animal reproduction are 3.5, 5.0 and 7.5 MHz where in small animals, it can be even higher.

#### **Classification of Ultrasonography**

#### 2 -D ultrasound

- Series of flat, two-dimensional cross section images
- Still standard for many diagnostic and obstetric situations

#### 3 - D Ultrasound

- Several two-dimensional images are acquired by the probes across the body
- The two-dimensional scans are then combined by specialized computer software to form 3-D images



MA



# 4 - D Ultrasound

- ✤ Fourth dimension, time, adds movement
- Creates the most realistic representation of all the structures
- Analyses of the dynamics of the foetal behaviour with morphological studies are directly reflecting development and maturation of the central nervous system.

# **Colour Doppler**

- Visualizing a foetus to assess its development, especially for observing abnormal development
- Visualizing blood flow in various organs or a foetus
- Doppler ultrasound measures the change in frequency of the echoes to calculate how fast an object is moving.
- Doppler ultrasound has been used mostly to measure the rate of blood flow through the heart and major arteries.
- a. Colour Doppler
  - Provides a more pronounced representation of blood flow speed and direction than traditional grayscale images.
- b. Power Doppler
  - Provides colour imaging of more sensitive and detailed blood flow measurements
  - Cannot indicate the direction in which blood is flowing
- c. Spectral Doppler
  - ✤ Can scan to determine both blood flow and direction
  - Displays this data in graphic form



**Types of Ultrasound** 



Diagnosis of Cleft palate in 4D Ultrasound



### Modes of ultrasound

- A-mode: A-mode is the simplest type of ultrasound. A single transducer scans a line through the body with the echoes plotted on screen as a function of depth. Therapeutic ultrasound aimed at a specific tumour or calculus is also A-mode, to allow for pinpoint accurate focus of the destructive wave energy.
- B -mode (brightness modality) most commonly used mode for real time scanning in both large and small animals is the b- mode (brightness modality) in which, the image is a two-dimensional display of dots (pixels), the brightness of the dots is proportional to the amplitude of the reflected echoes returning to the transducer.
- M-mode or "motion" mode is a form of ultrasound imaging that is of high clinical utility in the emergency department. It can be used in a variety of situations to evaluate motion and timing, and can document tissue movement in a still image.
- Doppler Ultrasonography which detects turbulence within blood vessels and direction of flow is also a useful diagnostic tool in animal reproduction. The Doppler phenomenon is the change in sound frequency of a moving object as perceived by a stationary observer.

# **Classification of probes**

#### Linear array

- Multiple elements arranged in a row-
- Designed for superficial imaging
- Crystals are aligned in a linear fashion
- within a flat head
- Produced sound waves in stright line
- Rectangular shape image
- ✤ Higher frequencies linear array (5-13MHz)
- ✤ Better resolution Less penetration





# Sectorial probe : Single element

- Sector shaped image
- ✤ Frequency : 01-08 MHz
- Greater penetration -Lesser resolution

### **Applications of Ultrasound**

Study the follicular dynamics and ovarian structures: growth and regression



Matured Corpus Luteum



Multiple follicles



Anovulatory follicles



Follicular Cyst





- Repeated, direct, non-invasive monitoring and measuring of follicles within the ovary
- Transvaginal follicular aspiration and oocyte recovery
- Complementary technology for embryo transfer
- To diagnose ovarian and uterine pathologies











Pyometra



Early pregnancy diagnosis with assessing foetal health and viability



Split hooves: 42-49 days





Twin pregnancy

- ✤ To estimate the age of the pregnancy
- To diagnose congenital abnormalities
- ✤ To evaluate the position of the foetus and placenta
- Foetal sex diagnosis







Foetal sex diagnosis

#### Contrast-enhanced ultrasonography

Contrast-enhanced Ultrasonography (CEUS) is a novel technique that uses micro bubble contrast agents injected intravenously or instilled into body cavities. Current ultrasound contrast agents (UCA) consist of small sized (1–10 mm diameter) micro bubbles of an inert, relatively insoluble gas, encapsulated by a protein, lipid, or polymer shell, disrupted at higher incident pressures.

# Merits

- Provides real-time images
- Does not involve the use of dangerous radiations/hazardous objects or chemicals
- One of the safest diagnostic tool and can be used in early diagnosis of pregnancy



- Only installation cost is needed. Minimal additional expense to run the diagnostic tool
- Comparatively smaller equipment and portable; can be carried to different locations for field study
- Does not require permissions and special licence from different agencies before installation.

# Demerits

- Requires high technical skill to perform Ultrasonography and interpret the results
- Not an accurate tool to estimate the number of foetus in pregnant animals
- Ultrasonography is sensitive for the detection of lesions, but it is not specific for the aetiology of a disease. Therefore, biopsy or fine-needle aspiration of the lesion may be necessary

# **Computed Tomography**

It is a modified as well as advanced form of conventional radiography.

The 1979 Nobel Prize in Physiology or Medicine was awarded jointly to South African-American physicist Allan M. Cormack and British electrical engineer Godfrey N. Hounsfield for the development of computerassisted tomography"

In computed tomography (CT), an x-ray tube moves around



the body and continuously projects a thin fan of x-rays through the body. As the X-rays pass through the patient, they are attenuated differently by various tissues according to the tissue density. A visual representation of the raw data obtained is called a sinogram, yet it is not sufficient for interpretation. Once the scan data has been acquired, the data must be processed using a form of tomographic reconstruction, which produces a series of cross-sectional images called slices

These cross-sectional images are made up of small units of **pixels or voxels**.

- Needs technically skilled people to operate
- The radiation used in CT scans can damage body cells, including DNA molecules, which can lead to radiation-induced cancer
- Reactions caused by intravenously injected radio contrast agents – nausea, vomiting, itching rash and rarely severe life-threatening reactions may occur

# Magnetic Resonance Imaging (MRI)

# Mechanism

It is a non-invasive imaging technology that produces three dimensional detailed anatomical images. Often used for disease detection, diagnosis, and treatment monitoring. It is based on sophisticated technology that excites and detects

the change in the direction of the rotational axis of protons found in the water that makes up living tissues.

In the late 1970s, physicists Peter Mansfield and Paul Lauterbur developed MRI-related techniques, like the echoplanar imaging (EPI) technique. Mansfield and Lauterbur were awarded the 2003 Nobel Prize in Physiology or Medicine for their "discoveries concerning magnetic resonance imaging".



PROCESSING





Allan M. Cormack



Hounsfield



## Application of MRI in animal reproduction

MRI is mainly involved in the studies of abnormal growths, masses and neoplasia in the reproductive organs, reproductive tracts and accessory glands.



## Merits

MRI works with powerful magnets

which are used to produce a strong magnetic field that forces protons in the body to align with that field. When a radiofrequency current is then pulsed through the patient, the protons are stimulated, and spin out of equilibrium, straining against the pull of the magnetic field. When the radiofrequency field is turned off, the MRI sensors are able to detect the energy released as the protons realign with the magnetic field.

The time it takes for the protons to realign with the magnetic field, as well as the amount of energy released changes depending on the environment and the chemical nature of the molecules. Physicians are able to tell the difference between various types of tissues based on these magnetic properties.

Contrast agents often containing the element Gadolinium) may be given to a patient intravenously before or during the MRI to increase the speed at which protons realign with the magnetic field. The faster the protons realign, the brighter the image. MRI scanners are particularly well suited to image the non-bony parts or soft tissues of the body

- Traditional MRI, unlike single photon emission computed tomography (SPECT) and positron emission tomography (PET), cannot measure metabolic rates. However, recent researches discovered a way to inject specialized compounds (eg: hyperpolarized carbon 13) into prostate cancer patients to measure the metabolic rate of a tumour
- This information can provide a fast and accurate picture of the tumour's aggressiveness.



 Monitoring disease progression can improve risk prediction, which is critical for prostate cancer patients who often adopt a wait and watch approach

### Demerits

- Traditional MRI, unlike single photon emission computed tomography (SPECT) and positron emission tomography (PET) cannot measure metabolic rates
- ✤ High cost of installation
- Requires technically qualified operators
- Cannot be used in unstable patients and patients with metal implants in the body



# INTRODUCTION TO ULTRASOUND IN VETERINARY PRACTICE

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An ultrasound examination, also known as ultrasonography, is a noninvasive imaging technique that allows internal body structures to be seen by recording echoes or reflections of ultrasonic waves. Unlike x-rays, which are potentially dangerous, ultrasound waves are considered to be safe.Ultrasound equipment directs a narrow beam of high frequency sound waves into the area of interest. The sound waves may be transmitted through, reflected or absorbed by the tissues that they encounter. The ultrasound waves that are reflected will return as "echoes" to the probe, and are converted into an image that is displayed on the monitor, giving a 2-dimensional "picture" of the tissues under examination.

The technique is invaluable for the examination of internal organs and was first used in veterinary medicine for pregnancy diagnosis. However, the technique is also extremely useful in evaluating heart conditions and identifying changes in abdominal organs.

Ultrasound examinations are of little value in examining organs that contain air. Ultrasound waves will not pass through air and therefore it cannot be used to examine normal lungs. Bone also stops ultrasound waves, so the brain and spinal cord are unable to be seen with an ultrasound study, and obviously, bones cannot be examined.

Depending on the images produced, ultrasound can take various forms. In veterinary work B-mode (brightness-mode) ultrasound, more commonly called 2-dimensional ultrasound is the most common form. This gives a two dimensional picture of the organ scanned. This is the type of ultrasound that is used to examine abdominal structures, perform pregnancy diagnosis, evaluate cardiac function and examine the eyes for certain eye diseases.

M-mode (motion-mode) is a type of B-mode in which a tracing of the motion of the structure being scanned is displayed. A combination of



M-mode and 2-dimensional ultrasound are used for examining the heart walls, chambers and valves to evaluate cardiac function. Cardiac ultrasonography is usually referred to as echocardiography. Doppler ultrasound is a specialized form of cardiac ultrasound in which the direction and speed of blood flow in the heart and blood vessels can be measured. Color-flow Doppler technology makes it even easier to observe the flow of blood through the heart and important blood vessels.

Anaesthesia is not usually needed for most ultrasound examinations, unless biopsies are to be taken. The technique is totally painless and most dogs will lie comfortably while the scan is being performed. Occasionally, if the dog is very frightened or fractious, a sedative may be necessary.

In most cases, the fur must be shaved to perform an ultrasound examination. Since ultrasound waves are not transmitted through air, it is imperative that the hand-held probe makes complete contact with the skin. In some cases, such as pregnancy diagnosis, it may be possible to get adequate images by moistening the hair with rubbing alcohol and applying a copious amount of water-soluble ultrasound gel. However, in all cases, the ultrasound images will be of better quality if the area to be examined is shaved.Since an ultrasound study is performed in real time, the results of what is seen are known immediately.

# **Physical Principles of Ultrasound**

Ultrasound is characterized by sound waves with a frequency higher than the upper range of human hearing, approximately 20,000 cycles per second (20 kHz). One cycle per second is 1 hertz; 1000 and 1 million cycles per second are 1 kilohertz (kHz) and 1 megahertz (MHz), respectively. Sound frequencies in the range of 2 to 10 MHz are commonly employed in diagnostic examinations. An ultrasound transducer (scan head) may emit sound waves of only one frequency or of multiple frequencies. The sonographer must select the appropriate transducer frequency according to the anatomic region to be examined.Ultrasound imaging is based on the pulseecho principle. This means that sound is produced by the transducer in pulses rather than continuously. The image is formed from the echoes returning to the transducer from the tissues after each pulse. Therefore, adequate time



must be allowed for all echoes to return before the transducer is pulsed again. Typically, sound is transmitted less than 1 % of the time; the transducer is waiting for all echoes to return more than 99% of the tin1e. When the crystal is pulsed, approximately two or three wavelengths are emitted in each pulse before a backing block in the transducer dampens the vibration.

A pulse of sound is emitted from the transducer after a special piezoelectric crystal contained within the scan head is vibrated and quickly dampened. The pulse-repetition frequency is the number of pulses occurring in 1 second, typically in the thousands of cycles per second. The frequency of sound emitted depends on a crystal's inherent characteristics. The crystal's vibrations are immediately dampened by a backing block so that only a short pulse length of two or three wavelengths is emitted.

The crystal then remains quiet while waiting for returning echoes reflected from tissues within the body. These echoes vibrate the crystal again, producing small-voltage signals that are amplified to form the final image. A timer is activated at the moment the crystal is pulsed so that the time of each echo's return can be determined separately and placed at the appropriate location on the video monitor.

#### Image interpretation and terminology

Ultrasonography is a technique that images anatomy in any desired tomographic plane. Therefore, the sonographer must be familiar with normal three-dimensional anatomy to recognize artifacts, interpret normal variations, and detect pathologic changes. Publications correlating normal sectional an atomy of the dog and cat with ultrasonography, computed tomography, and magnetic resonance images provide excellent references for interpretation. Specular echoes originate from interfaces at right angles to the beam. These echoes produce boundaries between structures much as they would appear in a gross anatomic cross section. For example, specular echoes may be seen at organ boundaries or at the walls of vessels. In contrast, nonspecular or scattered echoes usually sum with others to produce a detectable echo and do not dependon the orientation of the small structures with respect to the beam.



A dot on the screen does not necessarily represent a specific structure, and any relationship to the resulting image may be indirect. The parenchymal texture of organs is presumably related to the quantity and distribution of scattered echoes from the connective tissue framework. A mixture of specular and scattered echoes forms ultrasound images, but the complex mechanism of echo production within tissues is not yet fully understood. Blood or fluid that does not contain cells or debris is black on ultrasound images with a white-on-black display because few echoes are returned. As fluid gains viscosity from increased protein, cells, or debris, it becomes progressively more echogenic.

Normal parenchymal organs and body tissues are visualized as various shades of gray, which is fairly constant from animal to animal. Diseases that diffusely involve abdominal organs or tissues may alter the usual echogenicity relationship. Fat is generally thought to be highly echogenic, but low-level echoes are returned from fat in certain areas of the body, such as subcutaneous tissues of obese animals. Structural fat may be more echogenic than storage fat because of increased connective tissue content. Connective tissue usually appears highly echogenic, but certain uniform areas of fibrosis with few interfaces may actually appear relatively echo free. This is presumably because of the presence of relatively few interfaces at right angles to the beam. Regions distal to highly attenuating structures, such as bone or gas, appear dark on the image because of shadowing.

Artifacts such as shadowing must be differentiated from actual echopoor regions produced by fluid or necrosis. Areas distal to regions of low sound attenuation may appear bright owing to an artifact termed echo enhancement. This appearance must not be confused with regions of actual increased echogenicity. Terms that are used to describe the appearance of ultrasound images should relate to a tissue's echo intensity, attenuation, and image texture. These terms describe the ultrasound appearance relative to surrounding tissueor other structures. Areas of high echo intensity are referred to as echogenic, hyperechoic, or echo rich. Areas of low echo intensity may be properly termed echo poor or hypoechoic, whereas areas with no echoes are said to be echo free or anechoic.



Terms using density, such as high or low echo density, are best avoided because a tissue's echogenicity is not always related to its density. High attenuation and low attenuation are terms properly used to compare the appearance of a tissue or structure with surrounding echogenicity after the TGC controls have been adjusted. Specific terms, such as acoustic shadowing and distal acoustic enhancement, are commonly used to describe high and low sound attenuation by evaluating the echoes deep to a mass. Terms used to describe image texture are perhaps the most difficult to standardize because of the subjective nature of the interpretation. However, the size, spacing, and regularity of dots are important. The dots may be small, medium, or large, and they may be closely or widely spaced. In addition, size and spacing may be uniform (regular, homogeneous) or nonuniform (irregular, heterogeneous). Fine or coarse parenchymal texture refers to small or large dot size, respectively. A uniform texture suggests similar size and spacing of dots throughout the parenchyma. A heterogeneous texture suggests that the dot size, spacing, or both may vary throughout the parenchyma. Uniform and nonuniform (homogeneous and heterogeneous) can refer to either echogenicity or texture. Therefore, one should specify the echogenicity and texture separately. For example, one should specify heterogeneous parenchymal echogenicity or heterogeneous parenchymal texture, or both. Merely stating that there is a heterogeneous parenchymal appearance is confusing because echogenicity, texture, or both may be nonuniform.



# PRINCIPLES OF APPLICATION OF RADIOGRAPHY IN SMALL ANIMAL REPRODUCTION

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#### Introduction

The application of X-ray in diagnosing small animal reproductive disorders plays a invaluable role to field veterinarians. As Radiography is easily available tool at district level polyclinics it can be utilized for the diagnosis of various small animal reproductive disorders.

## Anatomical Landmarks of Male and Female Reproductive Tracts

# **Male Reproductive Tract**

Prostate penile urethra and penis are identifiable in normal abdominal radiographs of male dogs. Scrotum and testes are of same opacity and difficult to differentiate in normal dogs and in pathology as well. Prostate is identified in relation to bladder and colon surrounding it. The prostate lies in the pelvic canal and lies ventral to the colon and rectum and is caudal to the urinary bladder.

# **Female Reproductive Tract**

In normal dogs and cats uterus and ovary are not identifiable by radiographs. Mineralization (foacal) of ovarian pedicles can be seen in OHE done dogs as well as metallic clips if any applied while performing OHE can be visualized by radiographs. The rough area of vagina and vulva can be predicted in relation to rectum to which it is ventrally placed.



# **Exposure Factors/ Technique Chart**

S.No	Thickness of the animal abdomen	Кур	mAs	Grid Yes/No
	in Cm			
1	4	50	5	NO GRID
2	6	50	5	NO GRID
3	7	50	5	NO GRID
4	8	52	6	NO GRID
5	9	54	6	NO GRID
6	10	56	7	NO GRID
7	11	56	7	NO GRID
8	12	64	12	GRID
9	13	66	12	GRID
10	14	66	15	GRID
11	15	68	15	GRID
12	16	68	18	GRID
13	17	70	18	GRID
14	18	70	22	GRID
15	19	72	22	GRID
16	20	72	24	GRID
17	21	74	24	GRID
18	22	74	26	GRID
19	23	76	26	GRID
20	24	76	26	GRID
21	25	78	28	GRID
22	26	78	32	GRID
23	27	80	32	GRID
24	28	82	32	GRID
25	29	84	34	GRID
26	30	86	36	GRID
27	31	88	38	GRID
28	32	90	40	GRID



# **Principles of Interpretation**

#### Male Reproductive System

#### Testes

The testicular abnormalities that warrants radiographic examination may include orchitis, scrotal edema, and retained intra-abdominal testicles (Cryptorchidism). Orchitis and scrotal edema has same radiographic opacity to that of fluid. Intra abdominal testes can be differentiated as mass that persists cranial to bladder. If the testes is visible in radiograph as intra-abdominal mass mostly it is neoplastic in nature. These structures should be several cm large to be visualized in radiographs and are located between kidney and inguinal canal and cranial to urinary bladder.

#### Prostate

It is one of the major male reproductive structures to be visualized in a radiograph. Th prostate lies in the pelvic canal and lies ventral to the colon and rectum and is caudal to the urinary bladder. It is not visualized in neutered male dogs and difficult to be visualized in thin male dogs. The cardinal sign of prostatomegaly is the triangular region of fat between the bladder, prostate and ventral abdominal wall (Refer presentation). In addition it has a circular structure with soft tissue opacity seen caudal to bladder and there is dorsal displacement of colon and rectum when prostatic enlargement occurs. Neoplastic prostatic lesions are best identified by measuring the distance from the pelvic inlet to sacral promontory and if the prostate is 90 per cent of this distance it is assumed to be neoplastic and requires further investigations. A increase in size of 10 times to normal size is suggestive of prostatic hypertrophy and more than 20 times is suggestive of prostatic abscess or cyst. Special procedures like a positive contrast urethrogram can be performed for further assessment of the prostate. If the contrast agent passes through urethra and if urethra appears deviated it is suggestive of cyst or abscess and if the urethra appears straight it is more diffuse lesion of prostate such as hypertrophy.



# Female Reproductive System

## Ovary

Ovarian tumors that are visualized in radiographs lie caudal to kidney and are usually neoplastic. It can be unilateral and bilateral. If it is bilateral in nature it could be a epithelial tumor. Mineralization in form of calcification can occur in carcinomas and teratomas.

# Uterus

Uterus is visualized when there is physiological distension as it occurs in pregnancy as well as pathological conditions like pyometra, mucometra and uterine tumors. In pregnancy fetal calcification of bones occurs in 43 days in dogs and 38 days in cats and the fetus are best visualized counting the skull and spine. Radiograph gives the best visualization of number of fetus by counting the number of skull and spine. If there is superimposition of images orthogonal vies help in arriving at the fetal number. Contrast and uterus size acts as limiting factors for diagnosing the uterine pathology by radiography.

Fetal demise that occurs during dystocia is determined by accumulation of gas around the dead fetus. In dogs and cats dystocia caused by mal-position of fetus can also be identified by radiography.

In pyometra uterus gets distended and seen as homogenously distended tubular structure between the bladder and colon and in severe distension displaces the intestines cranially. It lies lateral to the colon on ventro-dorsal view and in lateral view lies caudal and ventral part of the abdomen. Mild distension is difficult to differentiate it from small intestines.

Uterine neoplasia is usually unilateral and appears as solid tubular strucure but should be differentiated from unilateral pyometra.

# Vagina and Vulva

Vaginal fibroma and leiomyoma are common in older dogs and contrast radiography using iodinated contrast agents can help to identify them better. A foley's catheter is passed into the vagina and kept at vestibule and vulva is kept closed with atraumatic forceps and contrast agent at 1ml per kg bwt is infused to visualize the structure.



# PREPARATION AND HANDLING OF FARM ANIMALS FOR ULTRASONOGRAPHY

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Ultrasonography is the second most common imaging technique practiced in veterinary diagnosis, next to radiography. As per history, Veterinary Gynaecology reserves the pride of using ultrasonography, as the first use of ultrasound as a diagnostic aid in veterinary medicine was for the detection of pregnancy in sheep. In fact the advancement and preference to ultrasonography to any other diagnostic technique is overwhelming in recent years owing to its ease of performance even at field level, its easy mobility, non invasiveness and instant interpretation. Ultrasonography can be used as diagnostic as well as therapeutic aid. In addition to pregnancy detection, ultrasound examination provides excellent information in determining the viability of the fetus, presence of multiple embryos, fetal age, calving date and fetal defects if any. In earlier days applications of ultrasound as a diagnostic aid in medicine utilised A-mode or amplitude mode. In later days, Apart from A mode, it has evolved enormously with introduction of B mode, M mode, Colour pattern and Spectral mode as advancement and has contributed to the precision of diagnosis and as an aid in interventional therapies.

#### Preparation of animal for ultrasonography

No sedation or tranquilizing is needed as the procedure is completely painless and non-invasive. No feed restriction or starvation is also indicated in basic ultrasonographic procedures. Otherwise preparation of animal differs slightly from procedure to procedure. Back racking or removal of dung from rectal passage is mandate to have better visualization of structures/ lesions during rectal probing.

Usually clipping or shaving of the entire side or area to be scanned is recommended in transabdominal or thoracic ultrasonography. But it may not be accepted or appreciated by the owner from the aesthetic point of view. But it becomes more compulsory in scanning small ruminants, as the hair coat is



more denser and curly, which may prevent proper placing of scanner probe and may lead to air spaces, even if is lubricated well with coupling gel.

The selection of conducting medium may differ from vet to vet, procedure to procedure, purpose of procedure and so on. Usually, a medical grade coupling gel is used, which is composed of a gelling agent comprising etherified hydroxyethylcellulose, an antimicrobial agent, preferably quaternary ammonium compound benzalkonium chloride, a solvent, 1,3-propanediol and water. Ultrasound coupling gel is normally considered as bacteriostatic. The other common conducting medium preferred by the author is surgical spirit. There also references for the usage of liquid paraffin as conducting medium. As per the opinion of author and few other studies, liquid paraffin can be preferred in time consuming scanning procedures and in case of student demonstrations; coupling gel in case of normal scanning procedures and surgical spirit in fast and short duration scanning.

Considering the species difference and sensitivity, conventional ultrasound gel will not last over the horse skin. Usually it liquefies and melts down in seconds. On the other hand horses are also more sensitive to spirit, owing to their sensitive skin. There are further advantages and disadvantages in each conducting medium.

#### Handling / Restraining a farm animal for Ultrasonography

Farm animals should be restrained properly to prevent injury to the handler, operator, damage to the ultrasound machine and probe, and injury to the cow itself and to the fetus if it is pregnant. Also, striking the probe against a hard object such as a trevis and dropping the probe on a hard surface can damage the probe resulting in loss of clarity in picture. Minimal restraining with head lock is sufficient for cooperative cows and for minor procedures. Standing posture is preferred for performing thoracic and abdominal ultrasound in farm animals, unless otherwise, the animal is recumbent due to any ailment.

Ventral or lateral recumbency is preferred in small ruminants, particularly for transabdominal pregnancy diagnosis and ventral hairless area is preferred for placing the probe. For performing ultrasound over ventral abdomen



in small ruminants, the fore limbs and hind limbs should be held by two attendants, one holding the fore limb together and the other holding the hind limbs, apart individually to facilitate better sliding of the probe and thereby better visualization of organs and structures. If standing posture is preferred, restraining can be done by an attendant, against a wall.

While performing rectal ultrasonography, extra care should be entertained as the internal structures are so delicate and any forceful insertion of probe or inadequate restraining of the animal will lead to tear or damage of the internal structures. While doing thoracic ultrasound in ruminants, forwarding of ipsilateral leg to the extent possible is indicated. Spending some time to calm the animal is worthy to make the animal co-operative for the procedure. Tail should be tied to leg or should be held by attendant to prevent tail slaps. Lifting one leg with the help of animal attendant is to be done in case of restless equine. Keeping the probe static over one place should be avoided, as the heat produced by the ultrasound wave may make the animal restless. This holds good for pregnancy diagnosis too.

Local anesthesia may be done prior to ultrasound guided interventions, after marking the site of intervention. Such local anesthesia may also be mandate in advanced scanning technique such as transcorneal ultrasonography in horses to prevent reflex blinking during evaluation of corneal defects.



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The use of ultrasound as a tool in small animal reproduction has expanded from its initial role in the evaluation of pregnancy in the femaleto its current use in monitoring fetal development, timing gestation and predicting parturition, diagnosis and management of reproductive tract disease and in supplementing breeding soundness examinations. In fact, B-mode ultrasonography has become an indispensable tool inveterinary practice.

The female reproductive tract consists of the ovaries, uterine tubes (oviducts), uterus, cervix, vagina, and vulva. Though the uterus and ovaries are well imaged with ultrasound; the vagina and vulva are usually better evaluated by direct visual inspection or by contrast radiography.

## PREPARATION OF THE ANIMAL

Ultrasound examinations are done with the patient in dorsal recumbency. However, many positions are possible for optimal scanning, including right or left lateral recumbency for scanning from the dependentor nondependent side and scanning with the animal standing. Theabdominal hair can either be clipped (which is standard protocol to obtain the best image) or matted down with alcohol before applying ultrasound gel. When a negative result is obtained while scanning without clipping the hair, the ultrasound examination should be repeated several weeks later to confirm a false- negative diagnosis. Midterm and late-term pregnancies, pyometra and otherconditions in which the reproductive tract is markedly enlarged can usually be diagnosed without clipping of the hair coat. The caudal pole of the kidney and the adjacent area are scanned in sagittal and transverse planes to locate the ovary. However, the ovaries in the dog and cat may not be visible due totheir small size, the fact that they are often surrounded by adipose tissue and interference by overlying bowel gas. A full urinary bladder makes scanningof the pelvic inlet region easier as it serves as an acoustic window. Diureticssuch as furosemide may be administered in low doses intravenously to produce urinary bladder



distension if needed. The uterus is usually close to midline but may be displaced to the right or left by the bladder. The uterine horns are sometimes seen cranially as they branch from the uterine body, butthey may be difficult to identify unless they are enlarged from pregnancy ordisease.

# THE NORMALNON-PREGNANT UTERUSAND OVARIES

Anormal, small and non-gravid uterus is usually not visualised but canbe differentiated from intestine from the lack of peristalsis, lack of luminal gas or fluid in the lumen and different layering of the wall. The uterine wallbecomes increasingly hypoechoic during proestrus and oestrus. A very smallamount of fluid may be seen in the uterine lumen during oestrus.

## UTERINE DISEASE

Abnormalities of the uterus that can often be diagnosed based onultrasound examination include cystic endometrial hyperplasia, pyometra, stump pyometra, stump granulomas, and uterine neoplasm.

# Cystic Endometrial Hyperplasia/ Pyometra

Cystic endometrial hyperplasia (CEH) is a common condition that is present in many middle aged-breeding bitches and occurs as a greater than normal hyperplastic and glandular response to normalchanges in estrogen and progesterone during the estrous cycle. The condition is also seen in bitches administered excessive amounts of progestins and in bitchesadministered estrogen during the luteal phase or following administration of a progestin. The spontaneous occurrence of CEH is typically not diagnosed unless it progresses to a more severe condition, and is usually an incidental finding. It may also involve a failure of the normally hyperplastic uterus of the cycle to undergo normal regression at the end of the luteal phase, such that a degree of hyperplasia persists, and ultimately the uterine wall becomes thickened and small cystic regions can be identified within the endometrium.

Depending on the size of the cysts, CEH may or may not be detected ultrasonographically, with the fluid-filled cysts appearing as small multiple 1 to 4 mm diameter anechoic regions within the uterus. Sterile luminal mucin fluid accumulation may occur, causing hydrometra or mucometra (the difference depends on degree of mucin hydration). Uterine infection leads topyometra.



Pvometra can be best diagnosed using ultrasonography and is usually performed to confirm a clinical diagnosis of pyometra based on presence ofvaginal discharge characteristic of open cervix pyometra or from systemic illness when the cervix is closed. Ultrasonographic imaging allows notonly the type of pyometra to be recorded (showing whether it is localized, segmental or uniform tubular in nature) but also integrity of uterine wall andcontent type.

Findings typically include an enlarged uterus with convoluted, tubular horns filled with anechoic to hypoechoic fluid. The luminal contents are usually homogenous, but the contents may also be echodense with slow, swirling patterns. The uterine wall may appear smooth and thin to thick and irregular. Segmentalvariations in wall thickness can occur.



Sonogram of two transverse sections of the uterine horn in a 6 year old cross bred bitchshowing marked enlargement uterine wall is mildlythick.

Sonogram of transverse sections of the uterine horn in an 8 year old Spitz bitch with closed pyometra. The lumen of the of the uterus with anechoic material The uterus was distended with echogenic material showing slow swirling patterns.

The wall may be more echoic than the uterine contents or be relatively hypoechoic. Additional differential diagnoses for a fluid-filled uterus include hydrometra and mucometra. These two conditions are less common than pyometra. They may be suspected if the luminal contents are anechoic (hydrometra) or echogenic (mucometra) and the clinical signs of pyometra are lacking. In massive uterine distension the uterine wall is usually thin, regardless of fluid type.

Ultrasonography may also be useful for monitoring medical treatment of cases of pyometra with prostaglandin F2a by measuring the reductionin



the diameter of the uterus. Treatment is best continued until there is an absence of fluid in all regions of the uterus.

### Uterine Stump Pyometra and Stump Granuloma

Stump pyometra may follow the surgical removal of a pyometra, or the administration of exogenous progesterone to an ovariohysterectomized bitch in which the uterus has not been entirely removed. Ultrasonographically stump pyometra appearance is similar tothat for pyometra, except that the lesions are only located at the pelvic inlet. In uterine stump granuloma, a mixed echogenicity appearance of the uterine stump along with hypoechoic regions representing accumulation of pus or edema, and hyperechoic regions representing fibrous tissue reaction will be seen rasonographically, uterine neoplasms appear as homogeneous



Pyometra in an 8 years old ovariohysterectomized spitz bitch mass lesions attached to the uterine wall and projecting into the lumen leading to uterine fluid accumulation. They may also be echogenic orhave a complex mixed echogenicity if they are necrotic or fibrotic.

#### PREGNANCY

Ultrasound imaging is not only used for the early detection of pregnancybut also for assessing fetalviability and the detection of pregnancy abnormalities. When ultrasonographic diagnosis of pregnancy is being performed it is important to remember that all events in canine pregnancy are related to day of the pre-ovulatory LH surge (day 0) or to the time of ovulation (which occurs 2 days after the LH surge) and not to the mating dates. Parturition typically occurs 64 to 66 days after the LH surge.



#### **Diagnosis of Pregnancy**

The gestational sacs or embryonic yolk sacs can be first imaged approximately 18 to 19 days after the LH surge and appear as spherical anechoic structures 1 to 2 mm in diameter within the lumen of the uterus. The embryonic heartbeat has been detected as a bright echogenic flickeras early as 23 days after the LH surge. The embryo has a bipolar shape by day 28 of pregnancy. The head region is identifiable as containing ananechoic area by day 30; limb buds are usually identifiable from day 32 to 34 onwards. The fetal skeleton is evident by day 34 of pregnancy. The bones of the head appear first, followed by those of the lower body. At this stage the hyperechoic heart valves can be imaged and are



Abdominal sonogram of a pregnant bitch 34 days after the LH surge, showing an anechoic (black), chorionic-fluid filled vesicle within a uterine horn (A) The uterine horn appears round in this transverse section, and is located in the right seen to bemoving, and the great vessels can be traced cranially and caudally.half of the image. The embryo appears as an echogenic bipolar (dumbbell shaped) mass

The zonary, circumferential placenta wraps around the central portion of the conceptus like a waistband, and is observed ultrasonographically between the fetus and the uterine walls in all planes. When imaged in the longitudinal plane, the placenta appears as two thick bands one on either side of thefetus, between the fetus and the uterine wall.

After Day 36 - 38 after the LH surge, it is possible to identify the fluidfilled fetal stomach caudal tothe liver in more than 90% of fetuses. A day or so later the fetal bladder is identifiable in the caudal abdomen and with careful examination the urachus may be imaged. These changes areobvious by Day 40 - 45 after LH surge. In late gestation the skeleton becomes more



obvious in late pregnancy and the skull, spinal column and ribs are easily identifiable

## Accuracy of Pregnancy Diagnosis with Ultrasound

Ultrasound examination in the first month after mating may lead to an inaccurate pregnancy diagnosis if the variation between mating time and ovulation time is not considered, and the examinationis scheduled too earlyin relation to the time of ovulation.

In those bitches which have been mated based on ovulation timing, thendetection fetal heart movements can be taken as the criterion of pregnancy and the ultrasound examination should be scheduled about 26to 30 days after the estimated day of ovulation (i.e., Day 28 to 32 after the LH surge) in those bitches.

The age at first appearance of ultrasonographic features of early pregnancy typically Ultrasonographic parameters observed in the bitch, expressed as days after the pre- ovulatory LH surge and days after ovulation. The ultrasound status of embryos or fetuses can be used to estimate gestational age. The gestational age can be used to predict the day of parturition based on parturition occurring 65 + 1 day after the LH surge in the vast majority of bitches.

Ultrasonographic Parameters Observed with a 5.0 or 7.5 MHz Transducer	Days after Ovulation	Days afterLH Surge
Detection of the conceptus as a 1 - 2 mm uterine vesicle	17 – 18	19 - 20
Presence of the embryonic mass within vesicle, at periphery	21 – 22	23 – 24
heartbeat	22 - 23	24 - 25
Identification of the yolk sac membrane	23 - 25	25 - 27
Identification of the allantoic membrane	25 - 29	27 - 31
Placenta develops zonary shape	25 - 27	27 - 29
Bipolar embryo shape	24 - 26	26 - 28
Differentiation of the head with focal anechoic area	25 - 28	27 - 30



Chorionic cavity exceeds the size of the yolksac	26	28
Collapsing of the elongated yolk sac	29 - 32	31 - 34
Detection of dorsal tubular spinal column	28 - 34	30 - 36
Detection of formation of limb buds	31 - 33	33 - 35
Detection of formation of the axial skeleton	31 - 32	33 - 34
Anechoic stomach and urinary bladder become visible	34 - 36	36 - 38
Hyperechoic lung distinct from liver	36 - 38	38 - 40
Trunk diameter exceeds diameter of head	36 - 38	38 - 40
Trunk diameter exceeds 50% of chorionic cavity diameter	36-40	38 - 42
Crown-rump length exceeds length of placenta	36-40	38 - 42
Detection of kidneys	38 - 44	40 - 46
Detection of eyes	38 - 44	40 - 46
Detection of individual cardiac chambers	40	42
Trunk diameter exceeds 50% of uterine outside diameter	44 – 46	46 - 48
Detection of intestines	56 - 60	58 - 62

If timing of examination is based on observed or suspected breeding alone, then it is better for the examination to be conducted 31 - 33 days after that breeding, since bitches may become pregnant following a breeding that occurs as early as 5 days before ovulation and 3 days before the LH surge. False negative diagnoses may also be produced by overlooking a conceptus, or due to acoustic artifacts produced by gas or fecal material hiding a conceptus.

False positive diagnoses may be the result of the confusion of empty loops of small intestine with early pregnancy. However, the intestine canbe shown to be tubular by imaging in two planes, whereas early pregnancy involves the segmental occurrence of individual vesicles in the uterinelumen.


Resorption of an entire litter may be missed if the initial ultrasound exam is delayed much beyond 30 days, and inaccurately reported as a failure to conceive.

### **Estimation of Fetal Numbers**

The accuracy of detecting absolute fetal numbers is generally poor. To acertain extent the fetal numbers can be estimated with greater accuracy when examinations were done before Day 30 after the LH surge. Generally, the numbers of fetuses are underestimated, with the error being associated with overlooking fetuses, mistaking them as already counted or due to acoustic artifacts. Fetal resorption may also produce a disparity between the number of fetus imaged and the number of offspring born.

# **Determination of Gestational Age and Prediction of Parturition Using Ultrasound**

Gestational length is most accurately determined by using either the LH surge or ovulation. Parturition occurs  $65 \pm 2$  days after the LH surge. However, where LH estimations are not possible the use of fetal measurements or the timing of the first ultrasonographic appearance of certain organs may be useful for the estimation of gestational age and prediction of parturition. For example, the kidneys are usually only visible within the last 20 days of gestational age and parturition dates were obtained when fetuses were measured atday 30. The decline in prediction accuracy during late gestationis probably due to variation in fetal growthrates across breeds.

The gestational age of the embryos determined at the time of an ultrasound exam conducted to confirm or diagnose pregnancy, typically performed 4 to 5 weeks after mating, can be estimated by measuring 3 ormore of the following parameters for each of 2 or more fetuses:

- 1. Gestational sac (or chorionic cavity) diameter in early pregnancy
- 2. Crown-rump length
- 3. Body (abdominal) diameter at the level of the liver and stomach.
- 4. Biparietal diameter after mid-gestation



#### Fetal measurements and estimation of fetal age

- ✤ For Pregnancy < 40 days, GestationalAge (GA) is: GA= (6 xGSD) + 20 where GSD is GestationalSac Diameter</li>
- ✤ For Pregnancy > 40 days Gestational Age (GA) is: GA = (15 x HD) + 20 where HD is HeadDiameter

#### **Days Before Parturition (DBP) = 65-GA**

From days 20 to 37, inner chorionic cavity or gestational sac diameter (GSD) was the best predictor gestational age. From days 38 to 60, head or biparietal diameter (BPD) was the most accurate predictor. Another parameter that is useful to evaluate when ultrasound is conducted shortly after mid gestation is crown-rump length in relation to the length of the placental girdle. The crown-rump length (CRL) is typically less than the length of the placenta before Day 38 of pregnancy, and then becomes



Day 53 bi-parietal head diameter. Abdominal sonogram of a Day53 pregnant bitch showing the head and neck of a fetus. The developing bones of the of the calvarium appear as hyperechoic elements at the top of the skull (white arrows). The vertebrae of the neck (V) are well developed. The biparietal diameter of the headmeasured by the electronic calipers (+ --- +) is 1.9 cm. The scale on the right is marked at 5 mm intervals.

Longer than the placenta so as to very clearly exceed the length of the placenta from Day 40 - 42 onward. When measuring the gestational sacs, two transverse plane measurements should be taken at  $90^{\circ}$  angles to each other and the values averaged before using the above formulas. Head and body diameters are measured to the transverse planes. When taking measurements of fetal or extra-fetal structures at least two distinct fetuses or gestational sac should be measured whenever possible and the measurements averaged before applying them to formulas. This becomes difficult if it is a singleton



fetus and measurement of multiple features such as GSD, HD, CRL or body diameter may be carried out to increase the accuracy.

Accuracy of predicting the whelping date was independent of litter size. The method was less accurate for toy, miniature and giant breeds. A correction factor of + 1 day should be applied to the gestational ageprediction for small body weight (<9 kg) bitches and - 2 days for giant body weight (>40 Kg) bitches.

## Assessment of Fetal Stress

Ultrasonography can also be used to assess fetal stress. Normal fetal heart rate is 220 to 240 bpm while rates of < 180 bpm were indicative of fetal distress due to hypoxia. One should take care to remember that uterinecontractions over a fetus may cause a temporary reduction in heart rate which would return to normal within 1-2 minutes and would remain within the normal range if there is no fetal distress.

# **Gestational Abnormalities**

# **Embryonic resorption**

Should embryonic death occurbefore 35 days after ovulation, there is usually complete resorption of the conceptus. This can occur withoutvaginal discharge as late as Day 30. The sonographic aspects of a resorption are generally a reduction in the volume of the conceptus, an increased echogenicity of the embryonic fluid (sometimes particles may be identified free-floating within the allantoic fluid), an



Fetal resorption at late gestation. sac



Absence of the embryonic heartbeat, disintegration of the embryonic mass and ultimately collapseof the conceptus with inward bulging of the uterine wall. The uterus often remains slightly enlarged in this region and there may be a small volume offree luminal fluid; the uterine wall often appears moderately hyperechoic.

## Fetal abortion

Death of fetuses occurring after Day 35 of pregnancy is usually followed by abortion and vaginal discharge and is associated with expulsion of fetal material and fluid. The early features of fetal abortion are an increase in echogenicity of the allantoic and amniotic fluid often with echogenic particles, followed by an absence of the fetal heartbeat and sometimes a thickening ofthe uterine wall. After expulsion, the uterus assumes an appearance that is similar to that observed in the postpartum bitch.

## Fetal abnormalities

It is uncommon to detect fetal abnormalities in the bitch, since there are usually multiple fetuses and it is difficult to fully examine each. However, a number of striking abnormalities have been detected, some of which have necessitated delivery of the litter by caesare an operation. Such abnormalities include hydrocephalus, fetal anasarca, herniation of the ventral abdominal wall and fetal monsters.

## Foetal distress and Foetal death:

At the time of a prolonged or difficult parturition, fetuses may becomehypoxic. The fetal response is to become bradycardic. Fetal heart rate can be measured using B or M mode ultrasonography, and a decline in fetal heart rate to less than twice the maternal heart rate is indicative of fetal distress (hypoxia).Fetal distress is considered severe with values < 180 BPM at Day58 - 62 of pregnancy.

Signs of fetal death detected by ultrasound include the absence of heartbeat, lack of fetal movement, reduced volume and increased echogenicity of fetal fluid, and accumulation of gas within the fetal stomach, other fetal cavities or the uterus. The latter should not be confused with artefacts produced by overlying intestine.



#### **Postpartum Abnormalities**

The presence of retained fetuses may be suspected clinically by the animal's behaviour and a persistent vaginal discharge. Fetuses may be readily diagnosed with ultrasound by the presence of echogenic skeletons. Fetal viability may be assessed as previously discussed. However, in the event of fetal death and emphysema, it is better to go for a radiography as there is always the possibility of failureto detect the fetus due to surroundinggas. Persistence of soft tissue debris or blood clots within the uterusafter parturition may be indistinguishable from remnants of placental tissue.

#### The Ovary

The ovaries of the bitch are difficult to examine with ultrasound due to their small size, the fact that they are surrounded by a bursa, which may be fatty in older bitches, and their rather superficial location. The ovaries are located adjacent to the caudal pole of the kidney, level with the fifth lumbarvertebrae. They are positioned mid-abdominally in the standing bitch close to the lateral abdominal wall. Imaging of the ovary is therefore best achieved with the bitch either in the standing position after clipping the hair from the lateral abdominal wall, or with the bitch in dorso-lateral recumbence, with the transducer placed over the ventro-lateral abdomen. During estrus the position of the ovaries may vary slightly and they are often located more caudally and ventrally.

#### Abnormalities of the Ovary, Ovarian cysts

True ovarian cysts originate from the ovarian stroma or follicles. Theyare usually multiloculated, with frequent irregular septae. Unfortunately in some cases the ultrasonographic appearance is bizarre. It is not possible to distinguish these structures from ovarian neoplasms. Follicular cysts may beassociated with persistent or prolonged estrus, whilst luteal cysts are more commonly found in older bitches and may be associated with a persistent haemorrhagic vulval discharge. Luteal cysts may also be identified inbitcheswith pyometra.



#### **Ovarian neoplasm**

The most common neoplasm is the granulosa cell tumor which may become large and cystic indistinguishable from cystic ovarian disease Ultrasonography may revel hyperechoic fibrous tissue, heterogeneous neoplastic tissue and anechoic cystic regions. The clinical signs of these tumors may include persistent estrus or cystic endometrial hyperplasia if they secrete estrogen or progesterone, respectively. Training Manual on APPLICATION OF DIAGNOSTIC IMAGING IN ANIMAL REPRODUCTION MANAGEMENT FOR VETERINARIANS



### **REPRODUCTIVE ULTRASONOGRAPHY IN LARGE ANIMALS**

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Ultrasound examination of the bovine female reproductive tract opened the gateway for the use of ultrasound equipment on farms in the mid-1980s. Since then ultrasound examinations of the reproductive tract offers practitioners one of the most rapid, precise, and cost-effective means of diagnosis in bovine reproductive medicine. This diagnostic tool has a wide variety of applications for herd and reproductive management applications in cattle and diagnostic significance in small animals. Nowadays, practitioners and researchers using this technology to understand ovarian physiology better using color Doppler ultrasound, the specific applications of ultrasonography in embryo transfer programs, and the potential use of ultrasound examination in reproduction synchronization protocols for dairy cattle.

#### PREPARING THE ANIMAL, RESTRAINT, REQUIRED MATERIALS:

- The cow must be adequately restrained to avoid injury and ensure that the user is in a comfortable position to carry out the examination.
- Manual emptying of the rectum is usually necessary to obtain the high-quality images needed for ovarian and uterine evaluations, early diagnosis of pregnancy, and fetal sexing.
- A 5-MHz linear rectal probe is the most versatile and commonly used probe for bovine reproductive examinations. Some practitioners prefer probes with a higher frequency (7.5–10 MHz), especially when examining the ovaries and for early diagnosis of pregnancy. The more recent ultrasound units often include multifrequency probes that function between 5 and 10 MHz.
- ✤ A systematic and methodic examination of the reproductive tract should be performed in all cases. The authors recommend examining the ovaries first to help interpret the examination of the rest of the reproductive tract (eg, probability of pregnancy,



diagnosis of twins, ovarian cyst) and to develop better diagnostic precision.

#### Ultrasound examinations of the ovaries

Bovine ovaries are dynamic organs that produce anovulatory and ovulatory follicles and form corpora lutea (CLs) at regular intervals. In dairy cows, the estrus cycle lasts between 18 and 24 days, and this allows two to three (and sometimes four) follicular waves to develop; at the end of these, a single dominant follicle 12 to 15 mm in diameter emerges.

#### Follicles

Because of the several follicular waves and because follicles greater than 8 mm in size are nearly always present throughout the entire estrus cycle (except for the first few days), it is difficult, even in a research context with daily ultrasound examinations, to identify the dominant follicle, and thus predict when ovulation is going to occur. This difficulty is even greater in the field, with a single ultrasound examination that could take place at any point during the cycle. In this situation, to identify cows that are edging toward heat (proestrus and estrus), the practitioner must rely on other signs, such as changes in the echotexture of the uterus, uterine tonus, and endometrial secretions (see section on ultrasound of the nongravid uterus), in addition to changes in behavior.



Ultrasound image of a cow's ovaries in proestrus. This image shows two 0.5-cm follicles

(1) on the left ovary and one 2.3-cm dominant follicle (2) on the right ovary. The CL is difficult to identify on the left ovary because it is isoechogenic with the surrounding ovarian stroma. <, edge of the corpus luteum.



#### Corpus luteum (CL)

The presence of a CL on one of the ovaries provides evidence of sexual maturity in heifers and estrus cycling in cows In an inseminated cow, the position of the CL on one of the ovaries should alert the practitioner to the possibility of pregnancy in the ipsilateral uterine horn, which should be examined with special attention. The presence of a double ovulation in an inseminated cow should cause the practitioner to suspect a twin pregnancy, and this cow should be re-examined at a later date because of the increased risk for embryonic loss. It might be possible to estimate the age of CLs using ultrasound along with an examination of the uterus. Without a record of recent reproductive history, however, accurate diagnosis is a challenge because CLs can vary in morphology and ultrasonographic appearance. Transportable and portable ultrasound units used in bovine practice allow us to identify the contours of young CLs, starting from the third or fourth day of the estrus cycle. As they age, the CLs become hypoechogenic (darker or blacker) compared with the surrounding ovarian stroma because of the greater concentration of blood vessels feeding this structure, which continues to develop until luteolysis occurs. The subtle signs of this on the ultrasound monitor are sometimes difficult to recognize in field conditions and in a well-lit environment. For the first 10 days of the estrus cycle, between 30% and 50% of CLs are indented with a cavity that can easily be identified on the ultrasound image. A CL with a central cavity (CLc) is usually a young functional one, in spite of the fact that it can also be found, albeit rarely, after day 30 of gestation. CLcs are normal structures that produce a normal amount of progesterone. They do not change the length of the estrus cycle in cows, they do not reduce the probability of pregnancy, and they do not alter the risk for embryonic death in pregnant cows.



Training Manual on APPLICATION OF DIAGNOSTIC IMAGING IN ANIMAL REPRODUCTION MANAGEMENT FOR VETERINARIANS



Comparison of a CL on days 6, 9, and 11 of the estrus cycle in a cow (8-MHz linear probe, depth of 4 cm). Note that the CL from day 6 that was nearly isoechogenic with the adjacent ovarian stroma becomes hypoechogenic (darker) as it matures. CL on day 6 (A), CL on day 9 (B), and CL on day 11 (C)



Ultrasound images of a CLc between days 8 and 10 of the estrus cycle (10-MHz linear probe, depth of 4 cm).

#### **Ovarian Pathologic Conditions**

#### **Ovarian inactivity**

Anovulatory anestrus occurs in between 10% and 64% of lactating dairy cows, with a mean of 20%.9,10 The ovaries in these cows do not contain a CL or any large follicles; instead, small follicles less than 2 to 4mm in diameter are present.

Training Manual on APPLICATION OF DIAGNOSTIC IMAGING IN ANIMAL REPRODUCTION MANAGEMENT FOR VETERINARIANS





Ultrasound images of an inactive ovary (A) and an ovary in proestrus (B). On the image to the right, note the presence of a 1-cm dominant follicle and some small follicles at the edges of the ovarian stroma



4-cm follicular cyst (A) along with its ultrasound image (B) obtained using an 8-MHz linear probe (depth of 5 cm). Note that the follicular cyst is divided into two cavities.

#### **Ovarian cyst**

Ovarian cysts are traditionally defined as follicular structures larger than 25 mm in diameter that persist for more than 10 days in the absence of a CL. More recently, a diagnosis of ovarian follicular cyst was based on a diameter greater than 16 or 17 mm. The follicular cyst that undergoes partial luteinization becomes a luteal cyst that responds to prostaglandin F2a injection. Differentiation between a luteal cyst and a CLc is generally based on the thickness of the structure's luteal wall: it is generally, but not exclusively, greater than 3 mm in a CLc and less than 3 mm in a luteal cyst. This difference can be difficult to evaluate in the field with certain portable ultrasound units. This definition is mainly a theoretic one, however, because it is possible to encounter a CLc that is greater than 25 mm in diameter and does not persist longer than a normal CL. It should be noted that the ability to make a precise diagnosis of this type of ovarian structure does not involve any changes in the treatment plan for synchronizing ovulation in these cows.



#### Ultrasound examination of the nongravid uterus

Ultrasound examination of the uterus provides practitioners with one of the most rapid, most precise, and least invasive methods of evaluating uterine health. This section presents the principal ultrasound images of the uterus that can be obtained during the estrous cycle.

#### Periestrus

This period extends from approximately 3 days before until 4 days after estrus and includes the phases of proestrus, estrus, and the beginning of metestrus. It is characterized by high estrogen levels in circulation and an increase in blood flow to the uterus. On transrectal palpation, the uterus is turgid and the horns are less curved than during diestrus. The cervix is partially opened, allowing the mucus produced by the endometrial glands to escape when estrus occurs. These changes in the uterus are viewed by ultrasound as reduced uniformity in the gray tones and a swollen appearance of the uterine wall. A more extensive surface of the uterus with dark anechoic zones corresponds to the edema and increased blood vessel activity under the endometrium that are also characteristic of this phase. During estrus, the mucosa of the endometrium becomes more echogenic, and the interface between the endometrium and myometrium reveals a more extensive vascular bed lying between these two layers. Additionally, a greater accumulation of endometrial liquid can be seen in the uterine lumen during estrus; this can be confused with early pregnancy examinations and lead to errors in diagnosis.



Ultrasound images of transverse (A) and longitudinal (B) sections of the uterus during proestrus. Note the significant nonuniform echogenicity and the swollen appearance of the uterine wall, particularly the endometrium. The anechoic vascular bed is easily identified under the endometrium of the transverse section of the uterus (black ring around the endometrium). 1, endometrium; 2, vascular zone; 3, myometrium





Ultrasound image of an oblique longitudinal uterine section with a hyperechogenic endometrial mucosa and slight accumulation of mucus in the uterine lumen during estrus (8-MHz linear probe, depth of 4 cm). The prominent vascular zone under the endometrium and the swollen appearance of the uterine wall should also be noted. 1, endometrium; 2, myometrium; 3, accumulation of mucus in the uterus; <, vascular zone beneath the endometrium



Ultrasound image of a longitudinal section of the uterus with significant accumulation of endometrial liquid in the lumen during estrus (8-MHz linear probe, depth of 4 cm). 1, endometrium; 2, vascular zone; 3, myometrium; 4, uterine liquid

#### Diestrus

During diestrus, high levels of circulating progesterone indicate the dominant hormonal state. The uterine wall is thinner than during the preceding period, and the uterus can be more difficult to view completely with sweeps of the ultrasound probe unless additional manipulations are performed using free fingers, because the horns are normally folded over ventrally during this phase. The uterus is normally empty of endometrial liquid, and the ultrasound scan shows a more uniform appearance.





Ultrasonogram of transverse sections of the uterus (white circle) during diestrus (8-MHz linear probe, depth of 4 cm). Note the highly uniform echogenicity of the uterus during this phase

#### **Uterine Pathologic Conditions**

The main pathologic conditions of the uterus that can be viewed using ultrasound are infectious disorders. These include puerperal metritis, clinical metritis, clinical and subclinical endometritis, and pyometra. Another noninfectious pathologic condition that can be diagnosed with ultrasonography is a mucometra.

#### Puerperal and clinical metritis (less than 21 days in milk)

A diagnosis of acute puerperal metritis (abnormally enlarged uterus and a fetid watery red-brown uterine discharge associated with signs of systemic illness [decreased milk yield, dullness, or other signs of toxemia] and fever >39.5 \_C) is generally reserved for the first 10 to 15 days of lactation, although it is possible until 21 days in milk. Ultrasound confirmation is not necessary for this acute infectious problem. Ultrasound examination of clinical metritis (cow is not systemically ill but has an abnormally enlarged uterus and a purulent uterine discharge detectable in the vagina within 21 days post partum) usually shows a thick uterine wall with an extensive blood vessel network, without cotyledons; the liquid in the lumen has varying degrees of echogenicity (showing gray tones on the monitor) and contains many hyperechogenic particles.

Ultrasound images of clinical metritis in a cow between days 10 and 15 after calving. Note the liquid with variable echogenicity on the image to the



left, the presence of many hyperechogenic particles (<), and a thick uterine wall. 1, endometrium; 2, vascular zone; 3, thickened myometrium (B) with an extensive vascular network (A)

## Endometritis (clinical and subclinical) and pyometra

Clinical endometritis is characterized by the presence of purulent uterine discharge detectable in the vagina 21 days or more after parturition. In the absence of purulent discharge, an infectious uterus with accumulation of abnormal contents can be diagnosed as subclinical endometritis. Pyometra is defined as the accumulation of purulent material within the uterine lumen in the presence of a persistent CL and a closed cervix. This uterine infection is generally encountered beyond the voluntary waiting period (>50 days) in dairy cows. Ultrasound examination of this condition shows a variable accumulation of purulent liquid that is nonuniform in echogenicity, with hyperechogenic particles present. The diameter of the infected uterine horn generally varies between 5 and 20 cm.



Ultrasound image of pyometra in a cow on day 55 of lactation. Note the nonuniform echogenic appearance of the uterine contents, which include several hyperechogenic particles

## Ultrasound examination of the gravid uterus

Early diagnosis of pregnancy in cows using ultrasound is a rapid, safe, and cost-effective procedure for dairy producers who would like to improve the reproductive performance of their herd. Ultrasound diagnosis of bovine pregnancy can be done with an excellent level of precision starting on day 27 after insemination.



### **Embryonic Period**

The embryonic period is defined as the period between fertilization and the end of organogenesis on the 42nd day of gestation. Starting on the 26th day of gestation, ultrasound of early pregnancy shows a uterine lumen containing a variable quantity of anechoic liquid produced by the conceptus. The following ultrasound images show a few indicators of pregnancy that can be seen between days 28 and 42. Before day 27 or 28, there may not be enough liquid in the lumen of the uterus to confirm the diagnosis. Visualization of the embryo in the amniotic liquid can be delayed until days 28 to 30 of gestation because it may be hidden behind endometrial folds. Starting on the 30th day, the amnion can be viewed as a highly echogenic envelope that produces specular reflections. Many ultrasound units come with a program that allows the practitioner to estimate the age of the bovine embryo or fetus using specific measurements. The distance between the crown of the head and the rump, the "crown-rump length" (CRL), is the easiest and most precise method of estimating gestational age up to day 55.

#### Fetal sexing

This examination can take place between days 56 and 100 of gestation, but the ideal time frame to facilitate ultrasound diagnosis is between days 60 and 70. The genital tubercle, which is the fetal structure that develops into the penis or the clitoris, is visible starting on day 45 but does not reach its definitive location until somewhere between 55 and 58 days after insemination. The genital tubercle in the male and female cow is a highly echogenic structure that appears on the monitor as a two-lobed structure with an echogenicity resembling that of bone tissue. The position of the tubercle relative to the fetal umbilicus and tail determines the diagnosis of fetal gender. The genital tubercle is located just caudal to the umbilicus in the male fetus, whereas in the female fetus, its final position is just under the tail. After 70 days of gestation, the genital tubercle is covered by urogenital folds that eventually develop into the labia minora or the prepuce, which diminishes its echogenicity. At this point, the ultrasound image reveals a four-lobed structure After day 70 of gestation, the term genital tubercle should therefore be replaced by external genital organs. Other indicators of



fetal gender can also be identified. Among these are the teats that become visible on the female fetus beginning on days 75 to 80 of gestation. These structures remain rudimentary in the male fetus.



Ultrasound images of a gravid uterus between days 28 and 42. (A) Pregnancy on day 28 with accumulation of liquid only, because the embryo is not visible. (B) Embryo, aged 32 days (1.2 cm long), is well hidden behind the endometrial folds. (C) Embryo, aged 40 days (1.8 cm long), is surrounded by the amnion. The limbs and the head are visible. (D) Embryo, aged 42 days (2.4 cm in length) with an extremely echogenic amniotic membrane is shown. 1, allantoic liquid; 2, endometrial folds; <, amniotic membrane; X-X, the embryo lies between the two "X" marks



Estimation of embryonic age by measuring the CRL. (A) CRL 5 0.7 cm (estimated age: 30 days). (B) CRL 5 2.7 cm (estimated age: 42 days) Ultrasound images of the fetus between days 45 and 53. (A) Fetus, aged 45 days, with all four limbs visible (thoracic limb [TL] and pelvis limb [PL]), the abdomen, and the head (H). (B) Fetus aged 53 days (CRL 5 4.5 cm) with visible placentomes (P), the amnion (<), the limbs (TL and PL), and the head (H) in a longitudinal section



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Ultrasound images of a male fetus (A) and a female (B) fetus (10-MHz linear probe, depth of 4 cm). (A) Male 67-day-old fetus. Note the presence of the genital tubercle (1) caudal to the umbilicus (2). (B) Female 56-day-old fetus with genital tubercle (1) located under the tail (3). H, head; PL, pelvic limb; TL, thoracic limb



Ultrasound image of a transverse section at penis level of a 73-day-old male fetus (8-MHz linear probe, depth of 4 cm). Note that an oblique longitudinal section of the umbilical cord can be seen (2) just to the left of the external male genital organs (1). This fourlobed hyperechogenic structure is composed of the penis in the center surrounded by the prepuce

#### Other applications

Ultrasound examination has become an essential tool in making important decisions relating to the specialized programs and techniques used in bovine reproduction. This section contains a brief presentation of the programs that benefit from judicious use of ultrasound examination to improve reproductive performances. The details and ultrasound images that illustrate the application of these techniques are beyond the scope of this review and can be consulted in the specialized references.



#### Embryo Collection and Transfer Programs and In Vitro Fertilization

Bovine practitioners who collect and transfer embryos or perform specialized in vitro fertilization techniques need the information provided by ultrasound examinations of the genital tract to improve the reliability of their services. Much practical research has been devoted to ultrasound evaluations in this context, and ultrasound is certainly justified when doing follow-up checks on superovulation protocols, on the day of insemination, and when the embryos are collected. Ultrasound examination of cows receiving the embryos leads to the selection of the most suitable cows and improved gestation rates for the transferred embryos.

#### **Color Doppler Ultrasound**

The color Doppler ultrasound technique shows hemodynamic changes in local ovarian circulation throughout the estrus cycle in addition to pathologic conditions. Examination of the uterus using this technique during the estrus cycle, during gestation, and in the postpartum period could possibly improve diagnostic precision and guide better treatment choices. The added value of this supplemental ultrasound diagnostic method for the bovine uterus remains to be demonstrated. It could be valuable tool for avant-garde practitioners interested in developing the use of this type of ultrasound in the near future.

#### Ultrasound use in Reproduction Synchronization Protocols

Ultrasound can be used to great advantage to improve results in synchronization programs by estimating the proportion of uterine problems and anovular cows before and at breeding time, thus indirectly assessing the early lactation nutritional balance at a herd level, by recommending rational synchronization protocols based on the presence or absence of CLs and by proposing early pregnancy examinations to resynchronize open cows efficiently.



#### Summary

Ultrasound is an economically valuable tool readily available to all veterinarians and producers. The ability of ultrasound to identify pregnant versus nonpregnant animals earlier allows nonpregnant animals to be returned to the breeding pool more quickly. More accurate identification of ovarian structures has become especially important with the advent of synchronization protocols. High rates of embryonic and fetal loss, particularly in dairy cattle, make the ability of ultrasound to evaluate fetal viability critical.



## DEMONSTRATION OF ULTRASONOGRAPHIC IMAGING OF REPRODUCTIVE ORGANS AND PREGNANCY ASSOCIATED STRUCTURE

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The application of real-time B-mode ultrasonography to bovine reproduction has grown rapidly in the last two decade. Ultrasonography has provided answers to a number of unanswered questions regard to bovine reproductive cycle and its concurrent disorders. One of the main advantages of ultrasonography is that it is totally non-invasive and so repeated examinations of an animal's reproductive tract can be performed without affecting its breeding potential. Ultrasonography is used for the following clinical purposes

- Monitoring follicles for diagnosis or assessing treatments.
- Establishing time or failure of ovulation.
- Monitoring the corpus luteum.
- Estimating the stage of estrous cycle.
- Collecting oocytes by transvaginal aspiration technique and ultrasound-guided centesis.
- Selection of donor animals and monitoring superovulatory response in embryo transfer.
- Detecting and studying the early embryo.
- Diagnosing twins during the later fetal stage.
- Determination of fetal sex.
- ✤ Assessing fetal viability.
- Diagnosing ovarian pathologic conditions such as luteal and follicular cysts, peri- ovarian cysts, ovarian tumours, and hemorrhagic follicles.



- Diagnosing pathology of the tubular organs such as hydrosalphinx, pyometra, uterine cysts, collection of intraluminal uterine fluid and fetal debris.
- Monitoring of postpartum genital resumption,
- ✤ Male genital ultrasonography.

#### Advantages

- Improve accuracy of diagnosis.
- Ultrasound provides better economic value because there are less mistakes made.
- In the cow, the examination is performed in a way similar to that of the rectal palpation. Generally, all commonly available ultrasound scanners (linear, sector and convex) can be used for transrectal sonography in cattle. The only condition for their use is that one must be able to manipulate the chosen ultrasound probes inside the rectum without causing damage.
- After the rectum has been evacuated and the internal genitalia have been palpated in the usual manner, the hand holding the ultrasound probe is introduced through the anus and then advanced cranially along the rectal floor.
- The cervix of the non-pregnant cow can be found at the level of the urinary bladder .The cervical structures that can be identified include the cervical rings and a central, hyperechoic line which represents the cervical canal. Immediately cranial to the cervix, usually in the midline, appear the body and horns of the uterus.
- After scanning the uterus, the probe can be rotated further laterally in order to visualize the ovaries. In their normal position, they can usually be reached by the sound beam and any additional digital fixation or repositioning of the ovaries is not necessary.
- Care should be taken to allocate each identified ovary to the correct side.



## **OVARIAN STRUCTURES IN THE COW**

## Follicles

- The sonographic image of bovine ovarian follicles is characterized by the anechoic, circular area of the follicular lumen as their fluid content usually contains no reflections.
- Each dominant follicle has a growing phase and a static phase; each lasting for about 5–6 days. The dominant follicle of the first wave is anovulatory. It remains dominant for 4–5 days, and generally by day 11 or 12 of the estrous cycle, it loses its dominance and begins to regress which lasts for 5–7 days. In the meantime, the second wave of follicles has been recruited and selection of the second wave dominant follicle has occurred, this dominant follicle goes on to ovulate. In a three wave cycle, this second dominant follicle regresses, making way for yet another group of follicles, with the third dominant follicle ovulating.

## **Corpora Lutea**

- The sonographic section of luteal tissue appears as a roughly granular, gray- structured oval area on the monitor.
- Developing CL poorly defined, irregular, greyish-black structure with echogenic spots all within the ovary.
- Mid-cycle CL -well defined granular, greyish echogenic structure with a demarcation line visible between it and the ovarian stroma.
- Regressing CL the demarcation line is faint, owing to the slight difference in echogenicity between the tissues.

## **Ovarian Cysts**

- By ultrasonography, a follicular cyst appeared as a uniformly nonechogenic ovarian structure >25 mm in diameter with a wall thickness <3 mm.</p>
- Luteal cysts, on the other hand, appeared as nonechogenic structure
  >25 mm in diameter with grey patches within the antrum or along the inner cyst wall and a wall thickness >3 mm.



 Cystic corpora lutea are usually no larger than 3 cm in diameter and the wall is about 5–10 mm thick. Corpora lutea are rarely spherical usually presenting an oval shape on the screen.

# Uterus during the Estrous Cycle

- The ultrasound image of the uterus showed a distinctly echogenic structure with different layers of the uterine horn reflected by differing echotextures.
- Uterine echotexture was characteristically dark during the follicular phase (estrus) reflecting an extensive degree of edema of the endometrium. The uterine horns were maximally curled during luteal dominance but were less curled during follicular dominance.

## Pregnant uterus

## Day 25 to 30 of Pregnancy

- On day 25 of pregnancy the embryonic vesicle of the bovine reaches a diameter of 10 mm at the point of its largest expansion
- The course of the pregnant uterine horn with its dorsal segment, the ventral bend and the caudally directed portion is best demonstrated if the probe is positioned above the uterus with its sound plane oriented along the longitudinal axis of the cow's body and the beam directed dorso-ventrally with a slight lateral deviation.

# Day 31 to 40 of Pregnancy

- The crown-rump-length (CRL) of the embryo reaches 12 mm around day 30, 15 mm by day 35 and 20 mm by day 40. The placentomes also become visible for the first time between days 30 and 40
- The first signs of placentomes are usually noticeable in the area near the embryo.

## Day 41 to 90 of Pregnancy

 Around day 40 a stage is reached when the sonographic examination of the pregnancy can be extended to include the demonstration of embryonic or fetal structures, respectively



The only organ that is available at this stage of pregnancy is the beating heart. In contrast, after day 40 the outline of the fetus with its head, extremities and umbilical cord become visible.

## Second and Third Trimester of Pregnancy

- In advanced pregnancy the sonographic examination of the fetus gains importance. During the second and third trimesters of pregnancy they are frequently surrounded by a 1 to 2 mm thick, very hyperechoic border.
- In many cases the placentomes lie so close to each other that many of them can be seen on a single sonographic image whereas the amniotic fluid remains anechoic during the first trimester of pregnancy.
- As the pregnancy progresses these reflections may assume a snowstorm-like appearance and turbulences within the amniotic fluid may become very obvious.
- The very prominent, thin echo line of the amnion can nearly always be recognized as a floating membrane drifting within the dark placental fluids.

## **GESTATIONAL ABNORMALITIES**

## **Embryonic Resorption**

- Should embryonic death occur before 35 days after ovulation, there is usually complete resorption of the conceptus. This can occur without vaginal discharge as late as Day 30.
- The sonographic aspects of a resorption are generally a reduction in the volume of the conceptus, an increased echogenicity of the embryonic fluid (sometimes particles may be identified freefloating within the allantoic fluid), an absence of the embryonic heartbeat, disintegration of the embryonic mass and ultimately collapse of the conceptus with inward bulging of the uterine wall.

## **Fetal Abortion**

 The early features of fetal abortion are an increased echogenicity of the allantoic and amniotic fluid often with echogenic particles, followed by an absence of the fetal heartbeat and sometimes a thickening of the uterine wall.

Signs of fetal death detected by ultrasound include the following: absence of heartbeat, lack of fetal movement, reduced volume and increased echogenicity of fetal fluid, and accumulation of gas within the fetal stomach, other fetal cavities or the uterus.

### **Uterine abnormalities**

Ultrasonographic appearance of inflammatory conditions of the uterus were characterized by distended lumen filled to varying degrees with partially echogenic, diffuse, flaky reflections. The degree of echogenicity depended on the consistency of the fluid.

In fetal maceration, the fetal bones were identified as echogenic structures in the uterine lumen suspended in nonechogenic fetal fluids with a thickened uterine wall.

Fetal mummification, on the other hand, appeared as poorly defined mass (fetal mummy) with complete absence of uterine fluids.

## Conclusions

The use of ultrasonography has contributed immensely to the understanding of follicular dynamics, pregnancy diagnosis, fetal sex determination, folliculocenteses, amnio and allantocentesis, reproductive tract pathology, monitoring of normal and abnormal postpartum interval, diagnosis and evaluation of treatment of ovarian cysts, mammary ultrasonography, male reproduction; ultrasonography has proved to be a useful clinical and research tool. Ultrasonography is quite helpful for individuals who are inexperienced in palpation per rectum as palpation skills are acquired quickly while at the same time making accurate diagnosis via ultrasonography. One of the greatest constraints limiting the widespread use of ultrasonography in bovine reproduction is the high cost of the ultrasound machine. In future, availability of several types of scanners (from different manufacturers) and improvements in the quality of scanners, the price may be affordable to a large number of veterinarians and research scientists and this will enhance its application in clinical as well as research studies of



bovine reproduction. Ultrasonography handling skills are usually obtained during university training or through continuous professional development courses organized for such purposes. Moreover, experience can be gained through the use of the ultrasound scanner repeatedly.





Uterus during proestrus 1, endometrium; 2, vascular zone; 3, myometrium

Mid-cycle CL



2-1.5-cm follicle; 3- CL



3.7-cm luteal cyst with a wall varying between 2 - 3 mm thick



73-day-old male fetus -umbilical cord (2) male genital organs (1)



72-dayold female fetus - two pelvic limbs (PL) and two of the teats (T)





Embryonic death on day 45



Fetal death identified on day 68

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# REPRODUCTIVE MANAGEMENT IN DAIRY CATTLE AND BUFFALO

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Getting dairy cattle rebred in a timely fashion is critical for dairy farm profitability. With the intensive selection of dairy cows for higher and higher milk production, there is clear evidence from many parts of the world that fertility is in decline. Final goal of the reproduction in a dairy farm is to obtain a calf per year to obtain a pregnancy rate of 90%, thus a 365-day calving index. In this paper we will see in detail about the different reproductive management techniques that helps to achieve better reproductive efficiency.

## I. SELECTION OF DAIRY ANIMALS

Selection is always done to improve a particular trait usually milk yield. A selection criterion available early in age is useful to select and use the animal for breeding. A score card with following scores is used to Judge a good dairy animal

Sl. No.	Characters	Marks
1.	General appearance	30
2.	Dairy conformation	20
3.	Body capacity	20
4.	Mammary systems	30
	100	

## 1. General appearance

Animal should be attractive. It should possess feminine look and vigor. Different parts of body blends harmoniously.

Head	:	Clean cut, proportionate to body.		
Muzzle	:	It is moist in cattle. Should be broad with large nostrils.		



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Jaws	:	Should be strong and perfect		
Eyes	:	Large and bright		
Forehead	:	As per breed standard		
Nasal bridge	:	Straight except in Jersey where it is dished		
Ears	:	Medium size and alert, varies with breed.		
Shoulder blades	:	Set smoothly and tightly against body.		
Back	:	Strong and straight.		
Loin	:	Broad and nearly flat or leveled.		
Rump or croup	:	Long and wide.		
Tail head	:	It should be set in line with back without any evidence of coarseness.		
Tail	:	Long and slender.		
Legs	:	Bone is flat and strong. Pastern should be short and long.		
Hock	:	Clean and moulded.		
Feet	:	Short, compact, well rounded off with deep heel and elevated sole.		
Foreleg	:	Medium and straight, wide apart, squarely placed.		
Hind leg	:	Perpendicular from hock to pastern from the side view and straight from rear view.		

### 2. Dairy Confirmation

The angularity of animals gives a true picture about dairy conformation of animals. Dairy conformation is usually represented in terms of triple wedge.

## i. Dorsal wedge

Joining imaginary lines from withers to the point of hip on either side and a line joining the two points constitutes it. The dorsal wedge line indicates the accommodation of reproductive tract of animals. Training Manual on APPLICATION OF DIAGNOSTIC IMAGING IN ANIMAL REPRODUCTION MANAGEMENT FOR VETERINARIANS





### ii. Lateral wedge

It is constituted by drawing an imaginary line from withers to point of hip and withers to the flap of flank and an imaginary line from flank and hip which indicates capacity of digestive tract.



#### iii. Front wedge:

Imaginary line from point of shoulders on either side to withers and a line joining the point of shoulders. It gives an indication about the respiratory tract.



## 3. Body capacity

- Proper length, width and depth. Ribs should be well arched.
- ✤ Barrel should possess rounded appearance
- ✤ Lack of depth, width, length, straight ribs are undesirable.
- Muzzle and mouth strong, large enough to give indication about the animals consumption of coarse dry fodder and green fodder.

## 4. Mammary System

- i. Wide, strong attachment. Fore quarters extended well forward.
- Udder should possess an excellent width of rear view. Udder should be pliable, soft and spongy and collapse completely after milking.



- iii. Teats should be uniform in size and squarely spaced.
- iv. Prominently branched milk vein should be seen on the udder as well as on the body barrel.

# II. TARGET FOR REPRODUCTIVE MANAGEMENT IN DAIRY CATTLE AND BUFFALO

Particulars	Indigenous	Exotic / Crosses	Buffaloes	
Age at puberty	24 months	12-15 months	24-30 months	
Age at first breeding	30 months	18-20 months	30-36 months	
Optimum weight at first mating	250 kg	180-275 kg	300-350 kg	
Dry period	80 -90	60 - 70	90 - 120	
Calving to first heat	40 days	40 days	40 days	
Calving to first service	60 – 75 days			
Days open	115-125 days			
Calving interval	12 - 13 months			
Services per conception	< 2.5			

## **III. BREEDING**

- The duration of breeding period is a function of the estrus detection rate and the level of individual cow fertility.
- The percentage of cows detected in estrus depends on the efficiency of detecting estrus in all cows, while the level of cow fertility depends upon a number of factors, including the fertility of the service sire, correct thawingand handling of semen, AI-breeding technique, and timing of insemination.

## **Detection of estrus**

- The greatest limiting factor to successful fertilization is detection of estrus.
- Approximately 50% of the estrous periods go undetected on the average dairy farm.



### Signs of estrus

- Cow in estrus will be the first cow to rise in the morning.
- The cow become restless does not eat and frequently bellows and seldom ruminates.
- Sudden drop in milk production.
- Searching for male.
- Traits of homosexuality is shown in which the cow will attempt to mount other cows while other females not in estrus tend to mount the estrus cow which she permits.
- The cow is receptive to the act of mating and will stand when the bull mounts her.
- The behaviour of standing quietly while being mounted by the bull or other cow is referred to as the 'standing heat' which is the surest sign of estrus.
- This extends for 14-16 hours and shows other symptom like bellowing, nervousness, anorexia, reduction in milk yield.
- Mucous discharge may be found sticking to the tail.
- In early heat the discharge is watery and copious in mid heat (standing heat) it becomes thick and sticky and in late heat it will be scanty and discoloured.
- Bulling: The best indicator of oestrus is when any cow or heifer repeatedly stands and accepts mounting by one of her herd mates. Unfortunately, they do not do this on demand. Those responsible for oestrus detection must watch for this behaviour and combining what they see with their own previous knowledge/experience, to decide whether to inseminate or not.

# Heat detection in buffaloes

Cows do mount over other cows when they are likely to come in heat and stand for mounting when they are in good heat. This is not seen in buffaloes. Buffaloes neither mount on other buffaloes nor other buffaloes mount on buffaloes in heat. In buffaloes copious



ropy hanging discharge is not seen on the contrary it gets suddenly dropped and is not noticed by the owner and the discharge is scanty. Some buffaloes do not bellow and show silent heat, especially high yielding buffaloes.

- ✤ The main heat symptoms of buffaloes are as follows.
- The vulva becomes edematous, swollen. The lower portion of vulva looks oily. The gap is seen between vulvar lips and slight opening is seen. The wrinkles which are present in anoestrus buffalo become shallow or vanish.
- ii. The mucous membrane of vulva becomes reddish, moist and glossy.
- iii. Mucus discharge which is not seen normally can be seen before or after oestrus spontaneously.
- iv. The colour, consistency and fern pattern of mucus help in determination of correct oestrus.
- v. Engorgement of teats in lactating buffaloes which is due to holding of milk following increased estrogen level in blood is seen when they are in heat.
- vi. Frequent urination. The urine coming in spurting action wetting the part of skin below vulva and above udder (perineum). The drying of the urine leaves white mark on skin.
- vii. Buffaloes in heat remain restless, off feed, raising head in a typical fashion.
- viii. Local non-descript buffaloes bellow, become restless and remain off feed. Milk yield is reduced. The bellow is sharp and for longer duration.
- ix. The buffaloes expose their teeth while bellowing which is very characteristic.
- x. The mucus discharge, in buffaloes is seen in about 49% cases. It is thin on the day of heat, become thick as the time passes and changes the colour from clear to white.



- xi. 60-70 % of the buffalo come in heat from 6 pm to 6 am (after sunset and before sunrise) and this should be borne in mind and attendant should watch the buffaloes in the evening and early morning for expression of heat symptom.
- xii. Teaser bull (Vasectomised bull), can be used for parading in buffalo barn for detection of heat

## Estrus-detection aids

Ideal estrus detection system has the following characteristics: 1) continuous surveillance of the cow; 2) accurate and automatic identification of the cow in estrus; 3) operation for the productive lifetime of the cow; 4) minimal labor requirements; and 5) high accuracy and efficiency (95%) for identifying the appropriate physiological events that correlate with estrus, ovulation, or both.

# IV. KNOWING THE REPRODUCTIVE STATUS OF DAIRY ANIMALS

#### Criteria: 1

- ✤ 18 hours (an average of 12-30 hours) of oestrus signs—Normal
- Less than 12 hours / absence of oestrus signs-abnormal (anoestrus)

#### Reasons

- ✤ Failure to detect oestrus signs.
- Suboestrus, weak or silent oestrus.
- ✤ A low plane of nutrition- lack of energy and protein, deficiency of minerals namely P, Co, Fe, Cu, I, Mn and Vitamin A
- ✤ Failure to recognize that an animal is pregnant.
- Anoestrus due to uterine pathology such as pyometra, mummified foetus, foetal maceration, mucometra and hydrometra and
- Insufficient hormonal stimuli.

#### Management

 Unobserved oestrum may be due to managerial deficiencies and short period of oestrus.



- The dairy animals should be observed for heat signs at least three times a day.
- Wall charts, breeding wheels, herd monitors and individual cow records may be used for identify the oestrus.
- Teaser bulls (vasectomized or by applying apron) are useful in identifying heat in large number of animals especially buffalo cows.
- Provision of adequate lighting to improve oestrus detection.
- Silent / weak / Suboestrus are most common in buffalo cows and common in postpartum period. In this cyclical changes in the genital organs occurs but the signs of heat are not exhibited or not observed. This requires rectal examination by qualified veterinary doctor.
- Extra feeding of a concentrate mixture or grains like maize, Cholam, kambu. Etc., and at least small amount of green fodder along with other roughages.
- Mineral mixture should be properly supplemented
- After breeding the animals should be checked for pregnancy within 45-60 days by qualified veterinary doctor.
- Uterine pathology and hormonal stimuli should be handled by qualified veterinary doctor.

# Criteria: 2

- Animal always in oestrus signs / sexually aggressive-abnormal (Bullers)
- Reasons
- Development of single or multiple anovulatory follicles, on one or both ovaries.
- Hereditary, high protein diet, postpartum uterine infection and high milk production may predispose this condition.
- This animals also exhibit upward displacement of coccygeal vertebrae known as "Sterility Hump"
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In this condition prolonged period accept riding of another cow and frequent attempts to mount on other cows popularly known as "Bullers".

#### Management

- This condition needs rectal examination of ovaries by qualified veterinary doctor.
- Prognosis is good in early cases and poor in long standing cases.
- Consult with nearest qualified veterinary doctor

### Criteria: 3

- ✤ Oestrus cycle at 18-21 days interval- Normal
- Oestrus cycle at less than 18 days interval- Abnormal (Short cycle)
- Oestrus cycle at more than 21 days interval-Abnormal (Long cycle)
- Animal conceived within three services- Normal
- Animal not conceived for more than three services- Abnormal (Repeat breeders)

### Reasons

- Due to deficient luteinizing hormone release, delayed ovulation or failure of ovulation may leads to fertilization failure.
- Defective ovum or ageing of ovum may leads to fertilization failure.
- Inability of the sperm to fertilize a viable ovum.
- Inability of gametes to reach one another.
- The organisms Trichomonas fetus, Campylobacter fetus, Brucella abortus and IBR-IPV which may cause early embryonic death.
- Deficiency of Selenium and Vitamin E may cause early embryonic death.
- Long period of feeding estrogenic forages may affect the embryo survival.
- Environmental stress during first week after breeding may lead to early embryonic death.



### Management

- Bring the animal into positive nutritive balance.
- Mineral mixture supplementation should be done to breeding animals.
- Do Artificial Insemination twice at each oestrus preferably at 12 or 24 hrs intervals.
- Skipping of AI and intrauterine infusions may be considered for uterine pathology.
- Diseased bulls should not be allowed for breeding.
- By avoiding diseased breeding bulls the pathogenic organisms causing abortion may be controlled.

### V. MANAGEMENT OF CALVING INTERVAL

Maximal reproductive efficiency requires management of the calving interval. This consists of three major components:

- 1. Post-partum period (elective waiting period),
- 2. the breeding period, and
- 3. gestation (including the dry period) period.

#### 1. Post-partum period or elective waiting period

- This period varies from 40 to 70 days on most farms.
- Part of its duration is based on the physiological need for the reproductive tract of the cow to undergo a healing process or involution.
- Generally when cows calve without complication, this healing process requires no more than 40 days.

#### Peri-parturient period

- Parturition in the cow is a process that requires attention, care, and cleanliness.
- ✤ A multitude of calving-related disorders predispose cows to ill health, loss in milk production, and reduced reproductive efficiency.



- Whatever can be done to reduce one or more of these disorders will result in the reduced incidences of other disorders because of their strong interrelationships.
- Cows that consume less DM than their contemporaries will have delayed first ovulation and first estrus after parturition, produce less milk, and less fertile

### **Calving pattern**

- For the better reproductive efficiency a compact calving period is of 2 monthsf should be managed at farmlevel.
- This ensures that calves are of similar age and weight at weaning, improves their overall health and reduces calf mortality by ensuring that late-born calves do not acquire infection from older, earlierborn animals.
- Cows should calve at the best time of year to utilise the available feed, thus in spring, winter and autumn but not summer.
- Ideally, heifers should be served so that they calve 2–3 weeks before the cows in the herd, to provide the opportunity for a longer calving–conception interval.

### 2. Breeding period

### Inter – service interval

- The inter-service interval also is sometimes called the interbreeding interval or re-insemination interval.
- It is common for herds to have inter-service intervals of more than 40 days.
- But achieving a tighter interval will help to increase a herd's pregnancy rate, decrease average days open and shorten calving intervals.
- Approximately 50% of the estrous periods go undetected on the average dairy farm. Programmed breeding may be employed to improve estrus detection and conception.
- Programmed breeding is a method to schedule and control the insemination program of lactating cows in the herd.



- Potential Benefits of the control breeding technique are as follow
  1). Improve efficiency of heat detection 2). Control timing of first service postpartum 3). Reduce variation of calving intervals 4).
  Reduce reproductive culling 5). Improve reproductive performance.
- To shorten your inter-service interval, heat detection three weeks after insemination is useful, even in a herd relying primarily on timed AI.
- Loss of BCS between parturition and AI may negatively influence conception, because cows with BCS with more days open and delayed intervals to first service.
- Increased feeding frequency and better feed management to maintain a fresh, adequate supply of feed and multiple sources of clean are critical for stimulating appetite and maximal DMI.

### 3. Gestational and dry period

- The third component of a calving interval is gestation, including the dry period.
- The duration of gestation is fairly constant and cannot be shortened significantly without adversely affecting the health or viability of the newborn.
- Dry period is a critical component to subsequent performance of dairy cows.
- Nutrients required during thisperiod include the maintenance and growth of the cow plus that required by the developing fetoplacental unit.

### Other criteria that affects breeding efficiency

Preventive veterinary practices at farm: Appropriate preventive herd health programs including a vaccination program for cows and replacements, deworming of animals on pasture, mastitis control, hoof care, reproductive visits, and other diagnostic procedures applied to blood and tissue samples resulting from abortions and other unexplained illnessesare critical for better reproductive efficiency.



- Stall level management of animals: For maximum comfort and milk yield in dairy cows, they must stand to eat, stand to be milked, and lie down to ruminate and rest. Therefore, tie-stall or free-stall comfort is critical to increased milk yields and acceptable conception rates.
- Fertility and season:During the active AI-breeding period, heat stress reduces uterine blood flow oocyte quality, embryo development, luteal function, and endometrial function; and milk yields and overall reproductive performance. Modifications in free-stall or loose housing with shade, cooling under shades, forced ventilation with fans, sprinklers and wallowing for buffaloes prevent hyperthermia and its harmful effects on the dairy animal.
- Weight at maturity: A dairy heifer reaches maturity and is ready for insemination as it reaches 2/3 of the adult body weight (Approximately 200kg). It is not advisable to breed the animals before this even when heat symptoms are observed.
- Records: Accurate record kept is very important in ensuring reproductive efficiency in the herd. Their production details like date of estrus, date of service and calving should be maintained properly. This data should be used to predict the probable date of heat, such animal should be watched carefully in the morning and evening for signs of heat. Complete breeding history, past performance and difficulties of an individual cow should also be maintained.
- Pregnancy diagnosis: Cow should be examined for pregnancy 45 to 60 days after service so that if they are non-pregnant, steps can be taken to re-breed them at the earliest opportunity.



Training Manual on APPLICATION OF DIAGNOSTIC IMAGING IN ANIMAL REPRODUCTION MANAGEMENT FOR VETERINARIANS

### ACCELERATED REPRODUCTIVE TECHNIQUE IN SMALL RUMINANTS

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#### Introduction

The success of sheep and goats operation depends on the number of lamb and kids raised, weaned and marketed each year. The percentage of ewes, does, ewe lambs, doelings conceived early in breeding season: the lambing, kidding and weaning percentages: and the percentage of ewes, does, ewelambs and doelings lambing and kidding unassisted are some of the most important factors influencing profits in the sheep and goat business.

In India majority of the farmers are marginal and they depend upon small ruminants like sheep and Goat for their livelihood. Sheep and goat husbandry in India is characterized by extensive rearing systems depending mainly on common property resources (CPRs) for meeting their feed and fodders requirement. The population of small ruminants in general and sheep in particular has almost reached a saturation point. However, the need for meat from small ruminants is increasing is tune with steadily increasing human population and change in lifestyle of people. The options are either to increase the number of small ruminants or by increasing the per animal productivity; considering the shrinking land resources for small ruminants there is little scope for increasing the number of small ruminants. Hence, the only option available is to increase the productivity of animals.

Considering the above said facts the sheep/goat farmers shall try any one/two or all the following reproductive techniques based on their resources and size of their flock.



#### 1. Body condition scoring (BCS)

This is a simplebut efficient tool for the reproductive management of all ruminants. Body Condition Scoring (BCS) is an estimation of muscle and the fat development of animal and is correlated with the direct measurement of back fat depth or the proportion of fat in the animal body. The body condition sore has to be done based on 1-5 score scale with an internal range of 0-5 (Rugiel et.al 1969). The body condition score of an animal is assessed by the palpation of lumbar region specifically around the backbone in the loin area, immediately behind the last rib and above the kidneys to examine the degree of sharpness.



Figure 1.—Feel for the spine in the center of the sheep's back, behind its last rib and in front of its hip





Figure 2.—Feel for the tips of the transverse processes.





Figure 3.—Feel for fullness of muscle and fat cover.











Some suggested (optimum) condition score values for the various stages of the production cycle are:

Production stage	Optimum score
Breeding	3–4
Early–Mid Gestation	2.5–4
Lambing (singles)	3.0–3.5
Lambing (twins)	3.5–4
Weaning	2 or higher

### 2. Early pregnancy diagnosis

This technique shall be followed in organized state owned farms or farmers involved in sheep/goat farming in a large scale commercial level. Here the major objective is to diagnosis does/ewes during their earlier stage of pregnancy say within 30 days after mating. The main advantage is that animals which have not became pregnant shall be segregated and based on their condition scoring they shall be supplemented with extra feeding to revive their reproductive cycle. The main pre requisite for this technique in proper maintenance of mating breeding records and ultrasonography for early pregnancy diagnosis.

#### 3. Oestrous synchronization using the exogenous hormones

Oestrous synchronization in does/ewes is used as a management tool to control the time of ovulation and parturition to harvest maximum number of weaned kids/lambs.Oestrous synchronization allows the does/ewes to be bred within a short period of time and subsequent kidding/lambing will happen correspondingly in a shorter period.

Day	Day	Day	Day	Day	Day	Day	Day	Day	Day	Day	Day
0	1	2	3	4	5	6	7	8	9	10	11
Progesterone Sponge insertion		Observation of the ewes						PGF2α injection (7.5 mg)		Sponge removal, PMSG injection (200 IU)	

Oestrous	synchronization	protocol	for	ewes
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### **Oestrous synchronization protocol for does**

Day	Day	Day	Day	Day	Day	Day	Day	Day	Day	Day	Day	Day
0	1	2	3	4	5	6	7	8	9	10	11	13
Progesterone Sponge Observation of the does insertion							PGF2a injection (7.5 mg)	Sponge removal, PMSG injection (200 IU)				

### Advantages of estrus synchronisation

- Saves labour and time in detecting estrus
- Simplify the use of AI
- Permit fixed time AI
- Efficient feeding of animals in groups
- Compact and predictable lambing/kidding with specific time limits
- Avoid loss of young ones at birth by providing care and supervision
- Uniform group of young ones reared to market age
- Strict control of disease cleaning after each batch

## Disadvantage

- High Ewe/doe: Ram/Buck ratio in natural service
- Cost
- Depend on nutritional status
- No change in lambing percentage

# 4. Oestrous synchronization using buck/ram effect

It has been long recognized that rams, under certain circumstances, can stimulate estrus and ovulation in females, with this phenomenon being termed "the ram effect". This effect is exploited in both sheep and goat management to stimulate the onset of both puberty and estrus, and when employed at the beginning of the breeding season, to facilitate synchronization of both estrus and ovulation. Although the degree of response obtained varies with genotype and latitude, it represents a valuable management tool under most circumstances. The mechanism by which this occurs is considered to be largely pheromonal and mediated via the female vomero-nasal organ (VNO). The ram effect can also be used successfully, both to induce ovulation and to



synchronize estrous cycles. Ram exposure results in increased LH production in receptive ewes, followed by ovulation without estrus (silent ovulation) within a few days.

The male effect is an interaction male-females, which promote the induction of ovulation in anoestrous females after male introduction in flock (proportion of one buck per 12 goats). Traditionally, females should be isolated from males at least 40 days before male introduction. These does were isolated for a period of one month at a distance of 100 meters away from the sight, smell and sound of buck. The pre-ovulatory LH peak occurs normally between 1 and 3 days after male introduction and goats ovulate approximately 22 hours after LH peak. An intense sexual behavior by male goats is necessary to induce LH preovulatory surge and ovulation. The previous male sexual experience, recent sexual stimulation with females, novelty of the stimulus are described also as factors that improve the ovulation response. Although a silent ovulation can occurs at first time, inversely to ewes, goats mainly presents estrus behavior. However, normally this first ovulation in goats is followed by a short estrous cycle and a fertile second ovulation event occurs 5 to 7 days after the first one, accompanied by estrus behavior. In order to increase the synchronization of fertile ovulations, and even to reduce the occurrence of short estrous cycles, a progestogen priming before or at the same time of male introduction can be applied.

Day 0	Day 0 - 30	Day 30
Doe/Ewe	Isolation period	Buck/Ram
separation	(30 days)	introduction

### 5. Focused feeding

An energy cost is attached to the physiological and behavioural components of the reproductive process as illustrated by the poor reproductive success observed in sheep and goats raised under conditions where nutritional inputs are limited. It is possible to improve the reproductive performance of sheep and goats by using short, targeted feeding regimes, 'focused feeding'. Successful focused feeding targets the underlying mechanisms that have evolved to allow sheep and goats to reproduce in changeable environments.





Fig 2. Targets for focus feeding that are known to affect reproductive output in small ruminants. Timing, quantity and quality of supplements are not indicated because they vary between sheep and goats, and among genotypes and environments. Redrawn after Martin et al. (2004).

Feeding supplements to rams and bucks for 8 weeks before mating ensures that their testicular size and sperm production are maximal. Feeding of concentrate at 250g/ ewe/day during 30 days before breeding, 30 days after breeding, 30 days during last stage of gestation and 30 days after lambing will facilitate better ovulation, better survival of embryos, improved growth of the fetus, better lactation and lamb survivability respectively.

#### Advantage

- Boost sperm production before mating
- Maximize potential litter size
- Avoid early loss of embryos
- Program the future productivity of developing fetus
- Maximize postnatal survival and development

#### 6. Early weaning

In ewes lactation peak is reached in the first weeks of lactation and thereafter production decreased rapidly being very low after 8 weeks of lactation. Hence, lambs can be weaned at an early age of two months. Further, early weaning facilitates faster return to estrus in ewes than conventional weaning. However, early weaning didn't reduced the days to estrus post kidding in does as the have a longer lactation period than ewes.



### 7. Advancing puberty

Reproductive efficiency of sheep and goats could be improved if the ewe/doe lambs go through puberty early and produce their first lamb at one year of age, rather than the more traditional 2 years. The onset of puberty is linked to the attainment of critical biomass. Puberty generally occurs in ewe/ doe lambs when they attain 50-70% of their expected mature body weight. Accelerating the rate of growth and accumulation of muscle and fat will advance puberty and improve reproductive success. Age at first parturition depends on the growth rate, genetics and feed availability. The objective is to mate at 7 - 8 months and to get first lambing at 1 year. The critical criteria for advancing puberty is attainment of 60 - 65% adult body weight within 7 - 8 months. Strategies for genetic improvement of body composition, particularly rapid muscle accumulation will likely also advance puberty and improve overall reproduction rate of the flock and life time production performance of each ewe.

### 8. Artificial insemination

Artificial insemination offers the best means of distributing germplasm from nucleus breeding flock to many small flocks within each ecosystem. Fresh as well as frozen semen is used. The speculum method of insemination is used for ewes and does. Generally, artificial insemination leads to lower reproductive rate than natural service and frozen semen gives even much low pregnancy rate that is around 40%. Cervical insemination is generally followed for better conception rate. Artificial insemination (AI) is increasingly being used by goat producers because it allows for both dissemination of valuable genetics and control of sexually transmitted diseases. Proper heat detection and/or hormonal synchronization of the oestrous cycle are essential and may lead to increased labour and costs. Ovulation in does occurs toward the end of standing estrus; therefore, insemination must occur around this time to be effective. The AM: PM rule is generally used: if the doe is first noticed to be in standing heat in the morning, AI should be performed in the evening (or vice versa). However, breed-specific estrus duration should be considered when deciding the best time to inseminate. Vaginal (pericervical deposition) or cervical (intracervical deposition) insemination techniques



are inexpensive and easy to perform and can result in acceptable pregnancy rates if fresh semen is used. However, if frozen semen is used, transcervical or laparoscopic intrauterine insemination techniques must be used, which are more expensive and require more skilled personnel.

### 9. STAR lambing system

The single most important factor contributing to pounds of lamb marketed per ewe per year is the number of lambs born. With a gestation length of approximately 146 days, it is possible for ewes to produce more than one lamb crop per year. However, when considering implementation of an accelerated lambing system, it is critical to remember that if one cannot or does not do a good job of managing ewes to lamb once a year, one will not do a good job of managing ewes to lamb more than once a year. With the Star system, the calendar year is depicted in a circular fashion and segmented into 5 intervals of 73 days each, which is also one half the gestation length of most ewes ( $146 \div 2 = 73$  days). Each of the points of the star coincides with the start of that time period when a given group of ewes is exposed to rams. Each of the limbs emanating from the point coincide with when that group of ewes is scheduled to lamb (first limb) and is subsequently re-exposed to rams (the second limb). For example, if a ewe is bred on the first day of a 30-day breeding period, she should lamb on the first day of the lambing season two periods later. Concomitantly, while one group of ewes is being bred, another group of ewes is lambing. Contingent upon when they lamb, ewes lactate for 36 to 63 days before being weaned (over a 7- day interval) and subsequently are re-exposed to rams for 30 days. Ewes that fail to conceive during the 30day breeding period are re-exposed at a subsequent breeding period, which occurs every 73 days until ewes conceive or are culled. In an ideal scenario, ewes will conceive or lamb at every third point of the star.

The advantages cited for the Star system, as with any accelerated system of production, include the following:

Greater reproductive performance by the ewes, permitting ewes to lamb up to five times in 3 years. In addition, this system also facilitates the identification of those ewes that will breed out of season, as well as those with a short postpartum anestrous interval.



Fewer rams are needed because only one fifth to one third of all ewes will be bred at any time.

More effective or less seasonal use of labor and facilities. Although the lambing barn need not be as large as that required to accommodate the entire flock, it must permit efficient feeding and waste removal. Good management skills are of the utmost importance.

Improved cash flow as the result of a more steady supply of marketable lambs.

