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*M.V.M. Thesis*

# Ultrasonographic Studies on Early Bovine Pregnancy Diagnosis and Foetal Sexing

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Submitted for the degree of

Master in Veterinary Medicine

**Ultrasonographic Studies on Early Bovine Pregnancy Diagnosis and Foetal Sexing**

by

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## Abstract

This thesis is an attempt to further the knowledge of the use of real time B-mode ultrasound scanning in the management of reproductive events in cattle. There have been a number of reports of the use of ultrasonography in an attempt to identify early bovine pregnancy. However, this has resulted in a variety of results and statements about the efficiency of the technique, and the earliest day of pregnancy on which an accurate diagnosis can be achieved. This has been due to the use of a range of transducers of different frequency and a diversity of criteria quoted for establishing a positive diagnosis.

The first section of this thesis is designed to establish a technique with rigid criteria for pregnancy diagnosis, using a high frequency 7.5 MHz transducer and a scanner of good quality. The protocol was chosen so that adult cows were examined ultrasonographically for pregnancy three times, at each of three separate time windows, these being Days 16 to 20, Days 21 to 27 and Days 28 to 34 after breeding. For each of these time spans, criteria were evolved for the ultrasonographic appearance of the ovary, the uterine horn and the conceptus. In all cases, it was essential that the ovary exhibited an active corpus luteum of good echogenicity. The uterus was required to have a distended lumen with an anechoic centre and a smooth endometrial lining in all three time windows, but the degree of its distension varied according to the stage of the pregnancy. In the earliest period of pregnancy diagnosis, distension of the uterine horn was considered sufficient evidence, coupled with the necessary appearance of the corpus luteum and uterine lining, to give a positive identification. However the presence of an early embryo enhanced the diagnosis. In the second time windo it was necessary to establish the presence of an echogenic embryo with an heart beat as well as the necessary criteria for the corpus luteum and uterine lining. These criteria were also applied to the third time window, but here embryonic anatomical detail was available, and imaging of the placental membranes was obtained. The second and third group of scans proved to be 100% accurate when these criteria were rigidly applied. In the first phase errors were recorded, but they were explained by the occurrence of embryonic loss, which is discussed in the thesis.

The second section of the thesis is an evaluation of the efficacy of the more recently described techniques for foetal sexing, which have given a variety of parameters and dates for producing a diagnosis. A group of adult pregnant cows were examined during the period 48 to 62 days of pregnancy. Using embryological rationale and anatomical knowledge of foetal anatomy, it was possible to produce a high degree of accuracy for sex determination during the 51 to 54 day period. There was a distinct learning curve associated with the success and the pitfalls of the thechnique, and the possible sources of error are discussed in the thesis, which is the first in this area to attempt to elaborate on this technique using a high frequency transducer.

The findings of the work show that it is possible to use a high frequency transducer, with real time ultrasonography, to evaluate pregnancy with 100% accuracy from 20 days of gestation and within 88% accuracy from 16 days, as long as rigid criteria are adhered to. Foetal sexing is highly dependent on the skill and experience of the operator, which can achieve accurate results from 51 to 54 days of gestation. The technique has potential for wide application in the field of herd management.

Thesis Supervisor: Professor John S. Boyd
Title: Head of Department of Veterinary Anatomy

# Dedication

*Dedicated,*
*with love, to*
*Hermano,*
*Clarissa,*
*and Leonardo.*

# In Memorian

*In Memorian,*
*with love, to*
*Maria Etelvina (vovó Maria).*

# Canção do Exílio

*Minha terra tem palmeiras,*
*Onde canta o Sabiá;*
*As aves, que aqui gorjeiam,*
*Não gorjeiam como lá.*

*Nosso céu tem mais estrelas,*
*Nossas várzeas têm mais flores,*
*Nossos bosques têm mais vida,*
*Nossa vida mais amores.*

*Em cismar, sozinho, à noite,*
*Mais prazer encontro eu lá;*
*Minha terra tem palmeiras,*
*Onde canta o Sabiá.*

*Minha terra tem primores,*
*Que tais não encontro eu cá;*
*Em cismar — sozinho, à noite —*
*Mais prazer encontro eu lá;*
*Minha terra tem palmeiras,*
*Onde canta o Sabiá.*

*Não permita Deus que eu morra,*
*Sem que eu volte para lá;*
*Sem que desfrute os primores*
*Que não encontro por cá;*
*Sem qu'inda aviste as palmeiras,*
*Onde canta o Sabiá.*

*Gonçalves Dias, Coimbra, 1843.*

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# Chapter 1

# Introduction

## 1.1 Introduction

Since the discovery of piezoelectric properties, dated from 1880 (Ginther 1986), the use of ultrasound waves has developed rapidly. With its increasing application to a variety of aims, the search for better results, in the different research fields, led to the improvement of ultrasound technology. First reaching the field of human medicine, in the late 1940s, ultrasonography finally arrived in veterinary medicine in early 1950s (Omran 1989). Initially used to evaluate the reproductive status in some domestic animals, in early 1960s, transabdominal ultrasonography achieved its potential with the development of the grey scale in 1970s (Ginther 1986). Thereafter, the technique could be applicable to any body structure in different species. The appearance of real time ultrasound imaging, in the late 1970s, further enhanced the achievements of this technology, by transporting the live interior of the animal body to the screen of the ultrasound unit — the visualization of movements of the organs and structures.

The enhanced resolution provided by the available transrectal and, more recently, transvaginal transducer, has significantly improved the capability of ultrasound imaging to depict the ovarian structures and the pregnant uterus and its conceptus. Although ultrasound imaging may not detect all happenings in the structures of the reproductive tract, the majority of normal and pathological changes can be detected and evaluated with the use of a systematic approach for routine examination.

Ultrasound provides a safe non invasive means of evaluating reproductive structures and the conceptus. By assessing the reproductive tract of farm animals, an immense variety of procedures and reproductive occurrences can be ultrasonographically observed and analysed. Among those are the determination of the cyclicity period; evaluation of the viability of the conceptus; diagnoses of early pregnancy, embryonic loss and twin pregnancy; ovarian and uterine pathological conditions; determination of foetal age and sex; equine embryo reduction; ovarian follicular aspiration and embryo transfer.

In this thesis, a routine and systematic ultrasound approach for diagnosing early pregnancy and sexing the foetus in the cow, using a high frequency transrectal transducer (7.5 MHz) under field conditions, is defined and described.

## 1.2 Scope and Objective

Ultrasonography is, nowadays, widely used in veterinary medicine. Undoubtedly, its application in the veterinary field is far behind its use in human medicine. Nevertheless, it has already reached a large section of the veterinary field, having been applied to a variety of areas, such as clinical investigation, anatomical studies, embryology and reproduction. As in some other areas, the use of ultrasound in veterinary reproduction has provided great advances in research programmes.

Ultrasonographic visualization of the bovine reproductive tract and its conceptus, has immensely helped researchers to evaluate and understand the functioning and development of the reproductive organs and the conceptus. Detection of abnormal morphological and physiological functions, in this area, has improved animal production management, by correcting and treating the problem adequately.

In reproductive studies, the main application of transrectal ultrasonography in early pregnancy diagnosis in the bovine, is to detect the cows which did not conceive after insemination. Early recognition allows the cattle breeder to reinseminate the animal at the next expected oestrus. The next oestrus usually appears at about Days 19 to 24 after the previous one. Transrectal ultrasonography, performed at an early gestational stage, can provide an accurate diagnosis of the reproductive status of the cow before Day 24. Also using this examination route, the sex of the foetus can be determined in early stages of gestation. The anticipated knowledge of the foetal sex plays an important role in herd reproductive management, especially in dairy cows. It enables the cattle breeder to make progress in the management of the herd by being able to determine, in advance, the future use of the calf to be born.

This present research work intends to evaluate the efficiency of a 7.5 MHz transrectal transducer to diagnose early pregnancy and determine the sex of the foetus in the cow. An attempt to establish a standard approach and define the earliest day for achievement of a highly accurate result for both, pregnancy and sex determination, will be studied.

## 1.3 Motivation

The relative availability of the ultrasonographic technique has led to its widespread use in different clinical and surgery practices. Especially for obstetrical purposes in farm animals, ultrasound scanning has been more intensively used, therefore becoming an integral component of pregnancy diagnosis and animal production management.

Several studies have been carried out for diagnosing early pregnancy in cattle in both experimental and farm conditions. These experiments were performed using transrectal transducers of frequencies varying from 3.0 to 5.0 MHz (Pierson & Ginther 1984*a*, Chaffaux, Reeddy, Valon & Thibier 1986, Curran, Pierson & Ginther 1986*a*, Curran, Pierson & Ginther 1986*b*, Hanzen & Delsaux 1987, Kastelic, Curran, Pierson & Ginther 1988). However few studies are reported using a 7.5 MHz transrectal transducer for this purpose (Omran 1989, Kastelic, Bergfelt & Ginther 1991). These studies consisted of examinations during the first days after insemination of the animal and throughout the early stages of pregnancy. The results achieved in these experiments, revealed some divergence in pointing out the earliest day for accurately diagnosing early pregnancy in cattle with the aid of a high frequency transducer.

A desire to know how reliable this technique would be with a single examination of the cow from Days 16 to 20 after breeding, and wishing to determine which would be the realistic earliest day to offer the highest level of accuracy applied to farm conditions, led to the study of this subject in this present research work.

Determination of the sex of the foetus at an early gestational stage is one of the recent applications of transrectal ultrasonography. The sex of the early foetus can be determined by identification and location of the external genitalia in its early stage of differentiation. Relatively few works have been carried out in this field, for sexing bovine and equine foetuses under both experimental and farm conditions, using the 3.0 to 5.0 MHz transducers (Muller & Wittkowski 1986, Curran & Ginther 1989, Curran, Kastelic & Ginther 1989, Curran & Ginther 1991, Curran 1992).

It is known that the bovine foetal external genital organ is initially located between the hind limbs, in both male and female foetuses. Soon after the stage of differentiation of the gender has started, the genital tubercle, which is the embryonic structure that will differentiate into the male penis or the female clitoris, initially migrates toward the umbilicus in the male or toward the tail in the female (Day 49) (Inomata, Euguchi, Yamamoto, Asari & Kano 1982). The most feasible day for ultrasonographic differentiation of the genital tubercle, is the main point of the studies in this field. Reports revealed that identification of the genital tubercle was only possible on Day 50 after breeding, with reliable determination of the sex beginning on Day 55 and optimal age for sexing from Days 59 to 68 (Curran et al. 1989, Curran 1992).

The motivations that led to the study of this subject were, firstly, the challenge of the research work, as it was still growing and thus a standard routine technique, for successful fulfillment, was not yet fully defined. Secondly, to test the efficacy of a higher frequency transducer, 7.5 MHz. Finally, using a higher resolution transducer, to evaluate the earliest day possible to offer a high level of accuracy, in determining the sex of the bovine foetus under farm conditions in just one examination.

## 1.4 Organization

This thesis is basically divided into four sections.

The first section, Chapters 1 and 2, discusses the technology, giving basic information on its applications and describes the equipment used during the experimental period.

The second section, Chapter 3, is directed toward early pregnancy diagnosis. The routine approach used for examinations and the ultrasonographic appearance of the reproductive organs are described. The feasibility of the technique at different stages of gestation and the earliest advisable day for an accurate diagnosis are defined.

The third section, Chapter 4, describes the technique used for sexing foetuses and shows the difficulties encountered in interpreting the different planes of view achieved during the scans. Also it evaluates the different days for sexing and defines the earliest day in which the technique can be applied with accuracy.

Finally, Chapter 5 discusses the reliability of ultrasonographic techniques applied to both pregnancy diagnosis and sexing in the bovine under field conditions, in this current work. Possibilities for future work and enhancement of the technique are also briefly discussed.

There are two appendixes. Appendix A, contains the statistical results of the entire research work. Appendix B, provides more detailed data about the scan sessions.

# Chapter 2

# Ultrasonographic Principles

The use of ultrasound imaging techniques, for the purpose of reproductive studies in farm animals, has expanded greatly in recent years (Taverne & Willemse 1989). Organs that could only be analysed by palpation, can now be visualized and studied by means of ultrasonography (Ligtvoet, Bom & Gussenhoven 1989).

Firstly administered transcutaneously, and later intrarectally, the technique of ultrasound scanning has made further advancement with its application to the transvaginal route. In reproductive cattle management, ultrasonography is gradually replacing manual rectal palpation. Due to the size of the animal, the transducer can be manually held within the rectum of the cow, directly over the reproductive organs. Thus, structures of interest are ultrasonographically visualized, analysed and therefore an accurate diagnosis can be given.

Initially, ultrasonography appears to be a difficult technique, but after a period of training it is possible to acquire a high degree of competency. It is essential that the ultrasonographer be well qualified in reproductive and developmental anatomy, histology and pathology. Nevertheless, correct interpretation of the ultrasonographic images is a crucial point, since in ultrasonography they may not portray the authentic structures scanned and this is closely related to the knowledge of ultrasonographic principles. With this background, the ultrasonographer will be capable of relating the image obtained, on the monitor of the ultrasound scanner, to normal and abnormal structures and changes in the reproductive organs scanned.

Detailed descriptions of the acoustic physics and interpretation of ultrasonographic imaging, and origin and interpretation of artifacts are available (Rantanen & Ewing 1981, Pierson & Ginther 1984*b*, Ginther 1986, Pierson, Kastelic & Ginther 1988, Pierson & Ginther 1988, Omran, Ayliffe & Boyd 1988, Omran 1989, Vogelsang 1992*a*, Vogelsang 1992*b*).

The purpose of this chapter is to provide a brief understanding of the basic principles of sound propagation and interaction with body tissues, as well as scanning techniques and instrument adjustment necessary for a successful application of ultrasonography as a diagnostic aid in reproductive studies of farm animals.

## 2.1 Origin and Propagation of Sound Waves

Ultrasound is defined as any sound above a frequency audible to the human ear. The origin, propagation and echoing of ultrasound waves are similar to those of audible sound waves. Sound waves are propagated by the movement of the air molecules or piezoelectric crystals, in audible sound and ultrasound respectively. This movement is characterized by contraction and expansion of the crystals in response to electric signals (ultrasound), or of the air molecules as a result of a mechanical movement such as striking a drum or emitting a shout (audible sound). Actually, it is not the molecules or the crystals that propagate the sound itself; it is the space created in the medium, by the compression and rarefaction of these media, that is propagated as waves. Once produced, the sound will travel until it encounters a barrier (reflector surface) on its way. For audible sound, this reflector surface can be exemplified by a mountain, a wall, and in ultrasound it corresponds to body structures. When the sound wave (sound beam) strikes a reflector surface, part of the sound will continue travelling beyond the barrier, but most of it is reflected back to the sound source. The distance from the sound source to the reflector surface can be estimated by the time spent between propagation of the sound and reception of the echo. Such information is readily obtained when the velocity of sound propagation (audible sound in air 330 m/s and ultrasound in body tissue 1540 m/s (Barr 1990)) and the time spent from origin to reception of the wave are known. Sound wave propagation involves loss of mechanical energy dissipated along its way, so becoming weakened. In audible sound, if the reflector surface is situated far from the sound origin, the sound waves reaching it may be too weak to be reflected back.

Body structures have different tissue composition, this makes them heterogeneous as a medium for ultrasound propagation. This heterogeneous nature of body tissues is due to the difference in density of various layers, which results in *tissue interfaces.* When a sound beam strikes a tissue interface, a portion of the wave is reflected to the transducer as an echo, but the remaining portion passes through the tissue to interact with deeper interfaces. The amount of returning echo depends on the density of the tissue which is creating a resistance to the sound beam and this results in a variation of the strength of the echo. This gradation of resistance to the propagation of the sound wave is known as *acoustic impedance.* An interface with no acoustic impedance allows sound waves to pass through it without reflecting any of them. This free passage of a sound beam through an interface is ultrasonographically termed as *transmission* or *through transmission.* Non viscous fluid filled structures are examples of non-reflective interfaces (Figure 2.6). The difference in acoustic impedance of tissues makes the ultrasound wave become weakened (attenuated) by loss of energy, owing to absorption of the beam as it passes through interfaces. This process is referred to as *attenuation.*

In diagnostic ultrasonography, waves originate from the transducer, which is a device connected to the ultrasound unit, emitting high frequency waves throughout the body structures and receiving the returning echoes from these structures (Figure 2.1). The ability to carry out this function is due to the piezoelectric property of crystals within the transducer (Rantanen & Ewing 1981, Ginther 1986). These crystals are coated with a conducting material on the ventral part of the transducer (transducer face). When the ultrasound unit is switched on, the alternating polarity of the electric signals stimulates the crystals, resulting in their vibration and consequent production of sound waves. The reflected returning echoes are received by the transducer crystals causing them to vibrate producing electrical signals which are processed by the ultrasound scanner and an image with shades of gray is displayed on the screen.

## 2.2 Principals of Image Interpretation

Ultrasonographic images are usually displayed as white on a black background, where the appearance of the body structures is clearly observed by evaluating the grade of shades of gray, varying from black to white. Highly reflective structures are seen in white (bright),

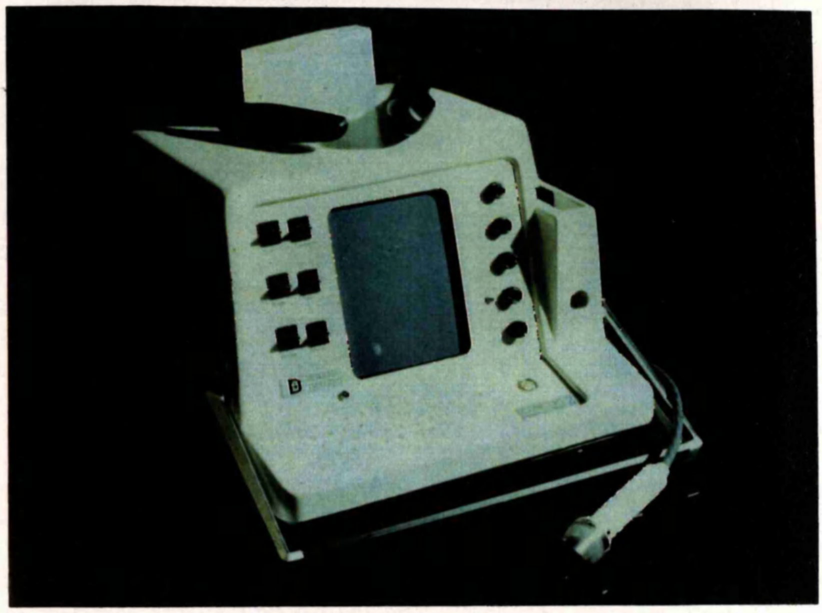


Figure 2.1: Transducer of various frequency, 3.5, 5.0, 7.5 MHz, connected to the ultrasonographic unit.

whereas poorly or non reflective structures are visualized in black (dark). For interpreting these images, specific terms are used to describe their echogenicity.

The ability of tissues to reflect sound waves is defined as *echogenicity*. Structures that have no acoustic impedance are said to be *anechoic*. They produce no echoes and are imaged as black. Structures filled by non viscous fluid, such as ovarian follicular fluid, cystic fluid and urine are examples of anechoic tissues (Figure 2.6). Structures that emit a weak echo, appearing in gray, are *hypoechoic*. Viscous fluid filled structures, e.g. certain cysts, abscess, are hypoechoic structures. *Hyperechoic* are structures that produce a strong echo because of total reflection of the sound waves. Consequently, a bright white image appears on the screen. Foetal bones, pelvic bones and gas filled structures (Figure 2.2) are examples of hyperechoic tissues.

Some hypoechoic or anechoic structures are used in ultrasonography as *acoustic windows*, as they absorb very few or no sound waves, permitting deeper free passage of the beam, reaching distant organs that will be imaged with greater intensity. The full urinary bladder and soft composition tissues such as spleen are examples of structures used as an acoustic window.

There are tissues that emit echoes similar to the ones emitted by surrounding tissues, having uniform echogenicity; these are termed *isoechoic* structures. *Echogenic* or *echoic*

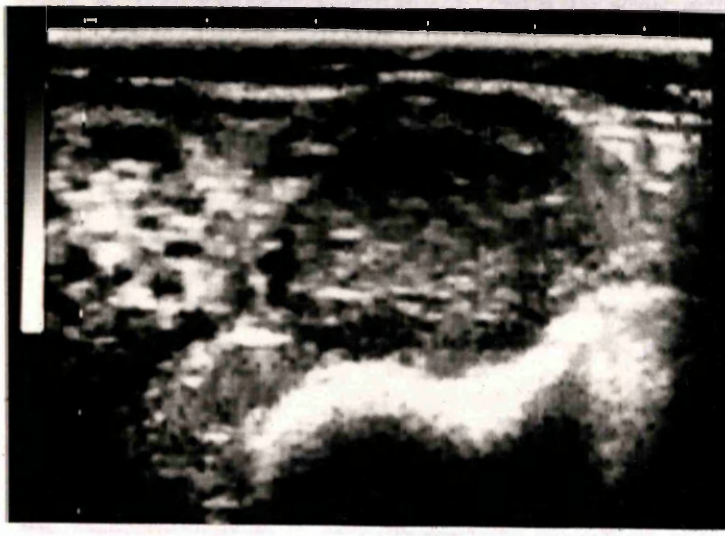


Figure 2.2: Hyperechoic structures. The bright surface represents the border of the gas filled intestine. The corpus luteum present in the ovary (center picture), is an echoic structure that exhibits the scattering artifact. Also, the shadowing artifact is present in highly reflective surface such as the gas filled bowel, and appears as a black region beneath the bright surface.

tissues are said to be those that have a relatively strong echo, such as corpus luteum, endometrium (Figure 2.2 and 2.3).

## 2.3 Image Artifacts and Interpretation

The principles of ultrasonography rely on the ability of sound waves to be conducted or reflected by tissues of different interfaces. The acoustic impedance of tissues can cause blockage of the sound beam, make it bounce back and forth, cause it to be diverted or become weak; these result in a variety of characteristic echoes being displayed as ultrasonographic images. These scan features that appear on the screen and which do not depict a true image of the structures being scanned are defined as *image artifacts.* Electronic interference and inappropriate connection of the equipment are, also, reasons for the appearance of artifactual images, but are not difficult to identify.

Image artifacts are frequently observed during scanning of the reproductive tract, owing to the presence of curved boundaries of fluid filled structures, intestinal gases and pelvic bones (Park, Nyland, Lattimer, Miller & Lebel 1981). They could be the reason for serious mistakes in ultrasonographic diagnosis, so that their identification and interpretation is important.

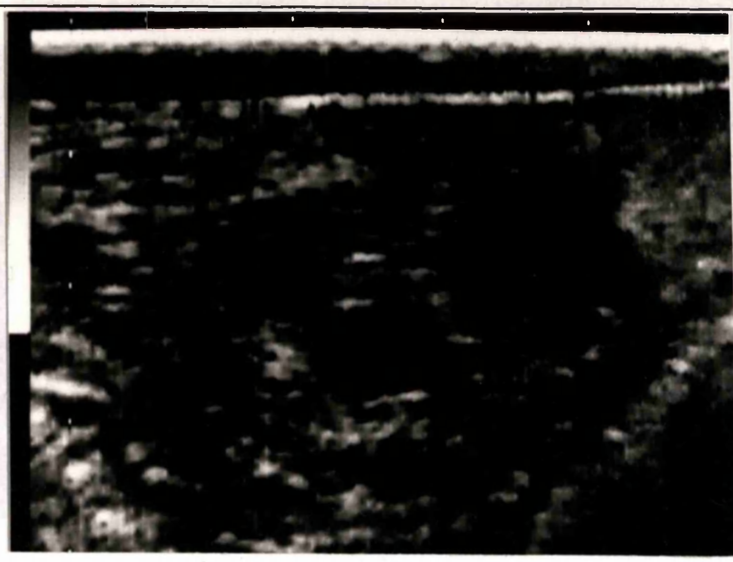


Figure 2.3: Specular reflection observed in an embryonic vesicle present in the uterine lumen.

### 2.3.1 Reflection

Is defined as any returning ultrasound wave resulting from interaction through body tissues of differing acoustic impedance. Structures such as bone and gas reflect most of the sound beam, preventing visualization of deeper interfaces (Figure 2.2).When a beam strikes an interface that is broad and smooth, where the wave hits the structure at a right angle, most of echo will be reflected back. This interaction results in *specular reflection*. This artifact is observed as a highly echogenic region on the upper and lower surface of fluid filled structures. This artifact is often seen when scanning an embryonic vesicle or follicles (Figure 2.3). As these structures also give rise to through transmission artifact, the echogenic reflection on the lower surface might, sometimes, be obscured by the enhanced echo beyond the interface.

The result of the interaction of sound waves with interfaces less than one wavelength in size is known as *non-specular reflection* or *diffuse reflection* or *scatter reflection*. When the beam hits such interfaces, it is reflected in many directions, also named *scattering* of the sound beam; giving rise to a characteristic echotexture such as that commonly observed in a corpus luteum (Figure 2.2).

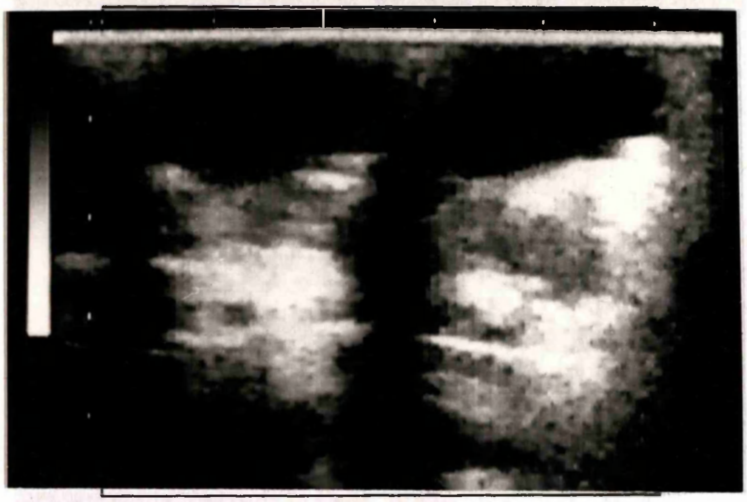


Figure 2.4: Blood vessels showing refraction. The sound bean is deviated when strikes the periphery of such curved boundary structure. It is characterized by the shadowing observed at the edges of the structures

### 2.3.2 Refraction

Refraction is a shadow or absence of echo as consequence of bending or refraction of a portion of sound wave as it strikes the periphery of a large curved boundary structure. This artifact is observed distal to the margin of rounded fluid filled structures such as follicles (Figure 2.4).

### 2.3.3 Reverberation

Reverberation occurs when the sound beam encounters a highly reflective interface and therefore most of the echo is reflected back to the transducer, which then re-emits sound waves to the interface and vice-versa. Because of the difference in acoustic impedance of the transducer face and the reflecting structure, the sound beam may keep bouncing back and forth a number of times until it is completely attenuated. The recorded image is represented by hyperechoic layers of echoes deeper to the reflector surface (Figure 2.5). Reverberation echoes can also happen internally between two highly reflective structures, such as bone and gas.

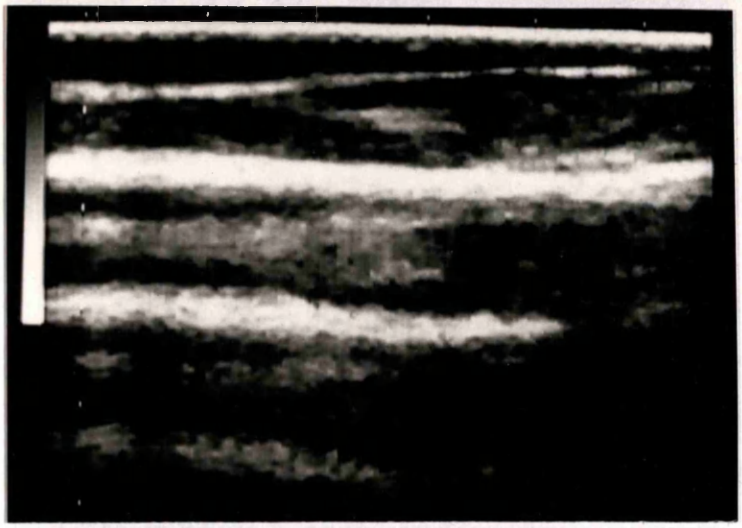


Figure 2.5: Reverberation artifact present in the pelvic region. The hyperechoic layers appear between the transducer and the pelvic bone, which does not appear in the image.

### 2.3.4 Enhancement or Through Transmission

This artifact is a result of minimal absorption of the ultrasound beam as it passes through an hypoechoic or anechoic structure. As the sound wave passes relatively unattenuated, compared with adjacent tissues, the region deep to the structure appears as a bright echo (enhanced). It is commonly seen in imaging of follicles, cysts and embryonic vesicles (Figure 2.6).

### 2.3.5 Shadowing

Acoustic shadowing is an artifact characterized by the lack of echo below an hyperechoic structure, due to maximal absorption of the sound beam as it hits such an interface. The hyperechoic structure blocks or deviates the sound waves, so that the area beneath it appears dark, restricting visualization of deeper tissues; whereas its surface is intensely echogenic. Shadowing is frequently encountered in ultrasonography of pelvic region structures, owing to the presence of bones, gas filled intestine (Figure 2.2 and 2.4).

## 2.4 Ultrasound Equipment

Nowadays, the ultrasound unit has a vast complexity of controls, but is basically formed by the scanner and transducer. Other ancillary pieces of apparatus can be connected to it, however are not vital to the functioning of the machine.

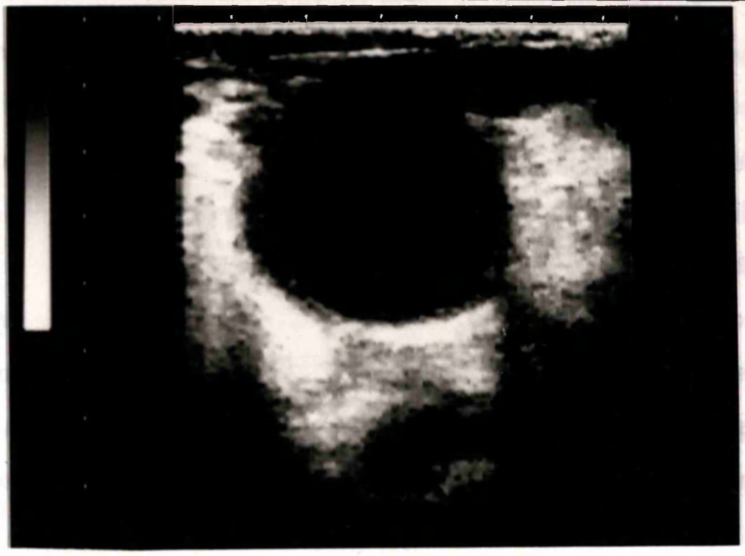


Figure 2.6: Enhancement or through transmission present in the image of a follicle. The follicle is the black rounded structure and the enhanced region is seen beneath the structure.

This section contains a description of the ultrasonographic equipment and some accessory instruments necessary for carrying out an ultrasonographic examination in farm animals.

### 2.4.1 Transducer

The transducer is the transmitter and receiver of sound waves in the ultrasonic equipment. It is connected to the ultrasound console by a coaxial cable, through which the electric signals are emitted, received and processed. Transducer can also be termed probe.

Transducer design depends on the route of examination to which they are applied. Based on that, they can vary in size and in format, however as part of their scanning purpose, transducers have to be waterproof, electrically insulated and smooth in contour, which provides safety in scanning and easy cleaning after use (Figure 2.1).

For a better understanding of certain physics terms used in ultrasonography, the following is a quick review:

The rate of recurrence of a vibration or cycle is referred to as *frequency*. This number of vibrations is measured in hertz (Hz) units, which is defined as one cycle per second. In the case of audible sound, frequency is the number of repetition of sound waves produced by the movement of the air molecules in a given time (seconds). Ultrasound frequency is determined by the number of vibration of piezoelectric crystals, per second. Ultrasound has high frequency waves, which means that more waves are produced per second, than for example, in audible sound. It is measured in megahertz (MHz), which is defined as one million cycles per second. Transducers are manufactured with various frequencies, with the most commonly used in the veterinary field being 3.5, 5.0, and 7.5 MHz (Figure 2.1). High frequency transducers (5.0 and 7.5 MHz) image an area of limited depth, but with great detail. They are used for the study of structures in the region close to the transducer face, mostly in ultrasonographic studies of the reproductive structures and early pregnancy diagnosis scanned transrectally. Low frequency transducer (3.5 MHz) image deeper areas, however with less resolution. They are usually intended for imaging large bovine and equine foetuses and foetal counting in sheep performed transcutaneously.

The capability of an ultrasound scanner to distinguish closely spaced interfaces is termed *resolution*. It is related to the sound waves length and width. If close distinct interfaces are separated by a space narrower than the beam width, they will be visualized on the scanner screen as a single structure; whereas interfaces separated by a space wider than the beam width will appear as distinct separate structures. This differentiation of tissues perpendicular to the axis of the sound wave is referred to as *lateral* or *side-to-side resolution*.

The narrower the beam width, the better the resolution. The ability of differentiating distinct structures situated along the longitudinal axis of the beam is known as *axial resolution*, which is determined by the length of the ultrasound wave. Thus, frequency is directly related to axial resolution. A high frequency transducer has short waves and therefore gives better resolution.

It is noteworthy to stress that the amount of absorption of the sound wave increases with the frequency of the transducer. The higher the frequency, the shorter the pulse of the beam, and the greater the absorption of the beam, producing a reduction in the penetration into body tissues; conversely to that, lower frequency sound waves penetrate further into tissue, but have poorer resolution.

Ultrasound transducers are manufactured in two main formats *linear* and *sector*, among which the most used are: the *linear array transducer*, which is commonly used in reproductive studies. It has the array of crystals arranged along its length, requiring a relatively large contact area with the region scanned. The image is produced with no mechanical motion of the probe and has a rectangular shape presented with no distortion of the frame;

and the *phased array sector transducer*, which face is relatively small and therefore requires a smaller area of contact with the region scanned. Mechanical motion in the probe is necessary to produce the image, which is characterized by a triangular or fan shaped view. It is also applied to reproductive scanning purposes.

### 2.4.2 Scanner

The scanner is a fundamental part of the ultrasonic equipment. It is a robust unit composed of a complex electronics, which generate the electrical pulse to the transducer, receive the echo from the transducer, process the electric signal and display the image of the scan in gray scale (Ginther 1986).

For veterinary use, mainly under farm conditions, ultrasound scanners are portable, relatively waterproof, easy to use and clean, and are kept in plastic cases appropriate for transport. They are mounted on a trolley of suitable size for holding video cassettes recorder and thermal printers, enabling the set to be readily moved.

Nowadays, most scanning units possess a keyboard; image adjustment controls; a video outlet that allows images to be recorded on video tapes and images to be printed on thermal copies; frame rate, allowing faster and slower rate for evaluation of moving structures; split frame, which permits the display of two images side-by-side and a foot control freeze frame facility. The keyboard has text annotation facilities for identification of the patient and a digital distance measurement mode, plus an outlet for use of a screen pen electronic measuring device that allows measuring of circumference area. There are scanners available with computerized foetal age calculation program for specific species. The panel control has freeze frame, brightness, contrast and magnification modes, as well as a storage memory and gain settings for near field, mid field and far field image adjustment control (Figure 2.1).

Fields are referred to as regions of scanning situated on the beam path (Rantanen & Ewing 1981). Near field corresponds to the first portion of the beam situated close to the transducer face, equivalent to the image that appears on the top of the screen. Far field is the region, on the wave path, farthest from the transducer and seen on the bottom of the screen. The mid field is called the region in between the far and near fields. The focussing of these regions can be changed as desired, according to the transducer frequency to be used.

On the screen of the ultrasound, the structures are usually imaged from left to right side. The transducer is moved forward and the structures situated at the end of the former will appear at the left side of the screen.

There are three basic modes of ultrasound display used in soft tissue imaging. They are termed *Amplitude Mode* or *A-Mode*, *Brightness Mode* or *B-Mode* and *Time motion (TM)* or *Motion Mode (M-Mode)* (Ginther 1986).

In A-Mode, the ultrasonic imaging is a one dimensional display of returning echo. It is seen as a peak, originated from a baseline, representing the returning echo. Each echo is indicated by a movement of the peak, the height of which represents the strength (amplitude) of the echo. This mode has a variety of uses, among them, evaluation of fat and lean proportion of meat animals.

M-Mode imaging is one dimensional display of dots, instead of peaks. The returning echoes are displayed as a sequence of dots along a baseline, where the former represent the depth of the reflecting interface and the brightness of them corresponds to their strength. If moving structures are scanned, the dots will move back and forth along the baseline on the screen as a resulting image. Heart valve movement is evaluated with this mode.

B-Mode has a two dimensional imaging display of dots, where the brightness of the shades of gray is proportional to the amplitude of the returning echo. It is used to study reproductive structures and the conceptus. The type of B-mode scanners available nowadays are named *Real Time*, in which the electronic information is displayed as it is received. The echoes are recorded continuously and hence, the motion of the imaged structure, such as an heart beating or foetal movement, can be observed as it occurs.

### 2.4.3 Data Recording Systems

The data recording system is a fundamental part of an ultrasonographic examination. Adequate documentation is essential for data control and so a record of the ultrasound image should be created. Details that may be imperceptible at the moment of the examination can be detected on the review of the recorded data. Besides, it gives the opportunity for future works based on the information collected, as well as the study of unusual cases.

The routine examination usually requires a video cassette recorder, with playback and freeze frame facilities, but sometimes a thermal copier can be used for printing frames at the time of the scan. Images should be appropriately labeled with the examination date,

Figure 2.7: Equipments used to record and review the ultrasonographic scanning. A video monitor, video cassette recorder and a thermal printer are seen.

patient identification, and if necessary, image orientation. To review the tapes, a video monitor possessing playback, slow/still, pause and forward facilities is an important tool for the study of the tapes.

The thermal printers have replaced the polaroid cameras and are easy to use. They are manufactured to print colour or black and white frames. The colour copier is expensive and therefore only used for printing images in which the colour will be needed for analysing the vascular system, such as the cardiovascular. The black and white printers are more accessible and conventionally used for printing images of most of the ultrasound scans. Printers can be connected to the scanner, at the moment of the examination, or to the monitor when reviewing recorded images. They are relatively small and waterproof, have a storage memory and double copy facilities, and some are provided with a remote control unit (Figure 2.7).

## 2.5 Ultrasound Techniques

The objective of this section is to describe the technique involved in the application of ultrasound in farm animals. However, most of the methods mentioned will be regarding to the use of the techniques in cattle.

For application of ultrasonography in animals, some important points have to be considered. Restraint of the patient is indispensable for the protection of the ultrasound equipment and also for the operator. It is rarely necessary to anaesthetise an animal for ultrasonographic examination. Restraining chutes are commonly used and are satisfactory for reduction of animal motion. However, some techniques, such as transvaginal, require not only a physical restraint, but also the use of epidural anaesthesia and sedation.

To perform ultrasonographic scanning, some precautions have to be taken for a successful technique. A careful preparation of the scanning area is indispensable, as the presence of air between the skin and transducer face interferes with the image obtained. Application of a coupling agent is indicated. The coupling medium is a mineral oil or a water soluble gel used on the transducer face or surface of the region to be imaged, avoiding any barrier to the transmission of the sound waves, therefore achieving an optimal image quality. For intrarectal scanning, there is not the same necessity for use of coupling gel, since the rectal wall is usually moist with mucus. The normal lubricants used for manual rectal examination are sufficient to act as a coupling media. The anal region, the operators arm, and the transducer must be lubricated before insertion into the rectum of the animal. The transducer must always remain in the ultrasonographer's hand. These precautions prevent rectal tearing and eliminate intervening air, ensuring efficient scanning.

The rectum of the animal under examination must be evacuated of faeces prior to scanning, because faecal material adherent to the transducer face can cause shadowing on the image obtained or even block the transmission of sound waves. In case of a small amount of faeces adhering to the transducer face, it can be removed by the operator by pressing and sliding a finger over the transducer face, without withdrawing it from the animal's rectum.

The ultrasound equipment should be mounted on a trolley, at a reasonable distance from the animal, allowing easy access to the ultrasonographer. The required transducer and the video cassette recorder should be connected to the scanner. It is recommended to place the equipment on the operator's side opposite to the scanning arm. Such a position permits the ultrasonographer to face the scanner and also to make more easy use of the panel control with the free hand. The animal identification, date and other relevant information should be typed on the screen before starting the scan. The panel controls can be altered as soon as the first images are obtained, with adjustment of brightness, contrast and gain. Rectal palpation is advisable before commencing the scan, to ensure approximate location of the organs of interest.

The pattern of movement of the transducer is of great importance in achieving good quality scans. Slow transducer movement are essential to obtain a detailed image of the region examined. Moving the transducer slowly back and forth, from side-to-side over the structure imaged, allows the operator an entire visualization of it and observation of both physiological and pathological changes.

During an ultrasonographic examination, the operator should utilize some anatomic points of reference, which might be helpful in locating and identifying structures of interest when reviewing the recorded images. The urinary bladder, for example, is used as a landmark to identify the left horn; for sexing foetuses, the beating heart serves to indicate the cranial end of the foetus, when a complete view of the latter is not possible.

The transducer should be cleaned, after its use in each animal, with a soft paper towel. At the end of the scans, it has to be washed with plain water and dried before being stored.

### 2.5.1 Transabdominal

Transabdominal scanning is generally used in animals that are too small for transrectal approach (Taverne 1989, Lavoir & Taverne 1989) When the transducer is to be applied to the skin, it is essential to clip the hair on the region of imaging and use a coupling gel or mineral oil. Sometimes, cleanliness of the skin is required to remove dirt or grease. This technique is used to study the reproductive tract of small animals and any conceptus, as well as for visualization of equine and bovine foetuses in late stages of gestation (after 100 days).

This is the common technique for diagnosing pregnancy and determining foetal number in ewes and goats. With the animal lying on dorsal position, the operator places the transducer face against the abdomen of the animal. The transducer is positioned over the inguinal region, cranial to the udder and begins to scan with slow lateral movements of the probe followed by movements towards the midline. This is repeated on both sides of the abdomen. The same technique can be applied to the animal on standing position (Russel 1989).

### 2.5.2 Transvaginal

For application of this technique, hygienic precautions must be taken, as well as sedation of the animal. Complete relaxation of the patient is essential for successful scanning.

The rectum of the animal has to be free of faeces and then the perianal region is washed with antiseptic solution. A coupling gel is applied to the transducer face and afterwards it is placed into a sterile condom. The transmission gel is also applied all over the covered transducer. The ultrasonographer holds the probe in one hand, and with the other free hand the vulva is opened to introduce the transducer, which is advanced to immediately caudal to the cervix. Once inside the vagina, the transducer is directed to locate the structure of interest.

Transvaginal ultrasonographic examination is rarely used to study uterus and ovaries in animals; perhaps because of the hygiene and chemical restraint that are required. It has been recently used in cattle for collection of oocytes by follicular aspiration, for the purpose of *in vitro* maturation and fertilization.

### 2.5.3 Transrectal

Transrectal imaging technique is used for observation of the oestrus cycle, detection of pathological states in the uterus and ovaries, pregnancy diagnosis and foetal sex determination. The transducer is inserted into the rectum facing the rectal floor just over the dorsal portion of the reproductive tract, which lies ventrally to the rectum.

The procedure of routine scanning varies according to the operator and the area of interest to be studied. However it is imperative that the ultrasonographer use a systematic routine in examining the reproductive tract. Manipulation of reproductive organs, as well as stimulation of foetal movement are occasionaly required, for some ultrasonographers, to obtain different viewing plane.

As the transducer is guided into the rectum, the vagina, cervix and uterine body are imaged in long axis. At the level of the uterine bifurcation, the horns are imaged by moving the probe laterally within the rectum. They are seen in cross sectional and longitudinal planes. Examination can start from this point, reaching one horn and the ipsilateral ovary, and then the contralateral horn and ovary. Another method of scanning can begin in the same way as the one mentioned, but first examining the horn and then ovaries. The sequence of the structures to be scanned using this technique depends on the operator; whether left or right structures of the reproductive tract are scanned first it is irrelevant, but a routine for each examination should be established. The procedure for transrectal technique used in this research is referred in Chapter 3.

# Chapter 3

# Early Gestational Diagnosis

One of the main applications of pregnancy diagnosis methods is to provide an early recognition of cows that have not conceived after service, owing to infertility problems, in order that they can be adequately treated, resulting in a minimum of production time lost. This early detection of non pregnant animals, allows a reduction of the interval between calving and conception, which is of considerable value to the cattle breeder and the dairy industry. In the reproductive management of cattle, different methods for indicating the establishment, or not, of pregnancy are available. These methods are intended for the improvement of economic management programmes of animal production.

The failure to return to oestrus, after fertilization, is an indication of pregnancy for most cattle breeders. On the other hand, the return of the animal to oestrus is indicative that conception has not occurred. However, this method is not reliable and is dependent on the efficiency of oestrus detection in the animals, which is profoundly influenced by the level of management utilized in the herd. Sometimes, cows and heifers show signs of oestrus during pregnancy, which could lead to incorrect negative pregnancy diagnosis (Donald 1943). Missed oestrus and long intervals between breeding can be highly prejudicial to the cattle breeder, since the calving interval will increase. The failure to conceive at the first time of breeding is also a potential cause of extending this interval. The variable effectiveness of visual signs of detection of oestrus, based on behavioural manifestation, led to the development and improvement of other techniques. Several methods for detection of oestrus (Lehrer, Lewis & Aizinbud 1992) and early pregnancy diagnosis, in cattle, have been used, among those, heat mount detectors, teaser animals, manual rectal palpation, laboratory tests and ultrasonography.

Recognition of the cows' reproductive status by rectal palpation of the uterus and ovaries, was one of the first techniques used for this purpose and it is still commonly applied. It is a rapid and relatively accurate method for diagnosing early pregnancy in cattle. The technique consists of detection of uterine horn enlargement (Figure 3.1), slipping of the chorioallantoic membrane and palpation of an amniotic vesicle, in early gestational stage (Figure 3.2), and palpation of placentomes or a foetus, in later gestational stage (Figure 3.3), within the uterine horn. Nevertheless, the reliability of the technique is related to the expertise of the palpator and the gestational age at the time of the examination. This method can be accomplished at Day 30 after breeding by an experienced palpator, but accuracy is considerably improved by palpation at a later stage (Day 35 to 40). It has been reported that the duration, frequency and nature of the procedure of rectal examination, may result in embryonic loss or even congenital defects (Paisley, Mickelsen & Frost 1977, Abbitt, Ball, Kitto, Sitzman, Wilgenburg, Raim & Seidel 1978, Pieterse, Szenci, Willemse, Bajcsy, Dieleman & Taverne 1990). At this stage of gestation, the heart is not enclosed within the body wall and as it is protruding and exposed it appears to be sensitive to relatively slight pressures (Rowson & Dott 1963). Therefore, palpation of the amniotic sac can be damaging at this stage (Day 30), unless performed with extreme gentleness.

Proteins produced by the placenta and the embryo secretory proteins, some also responsible for maintenance of the corpus luteum, are among the several proteins that may be found in the circulation of the pregnant animal and can be measured in the body fluids, allowing pregnancy detection. Laboratory tests such as plasma progesterone levels (Henricks & Dickey 1970), detection of oestrone sulphate (Hamon, Fleet, Holdsworth & Heap 1981) and progesterone concentration in the milk (Heap, Holdsworth, Gadsby, Laing & Walters 1976, Booth, Davies & Holdsworth 1979, Pieterse et al. 1990) are commonly used methods. Unfortunately, reports have shown that they are not efficient in early stages of pregnancy, due to the incidence of false positive pregnancy diagnosis (Booth et al. 1979). Oestrone sulphate can provide a reliable test, but this is for use in later pregnancy (Day 100). Presently, the milk progesterone assay gives the earliest test for diagnosing pregnancy, with satisfactory results achieved on Days 21 to 24 (77,5 to 85,8% for positive and 85,7 to 100% for negative pregnancy) (Heap et al. 1976).

**Figure 3.1: Gross anatomy of a bovine uterus. Note the enlargment of the right uterine horn, at the 58th day of gestation (white arrows). The luteal gland can be seen in the right ovary (black arrows).**

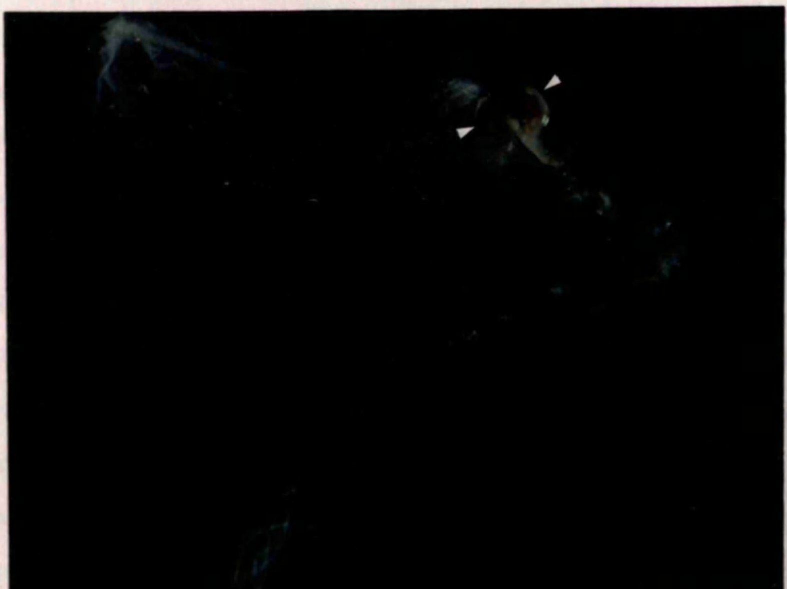


**Figure 3.2: A 33 day old bovine embryo with its associated membranes. The amnion is about the size of a pea (arrows).**

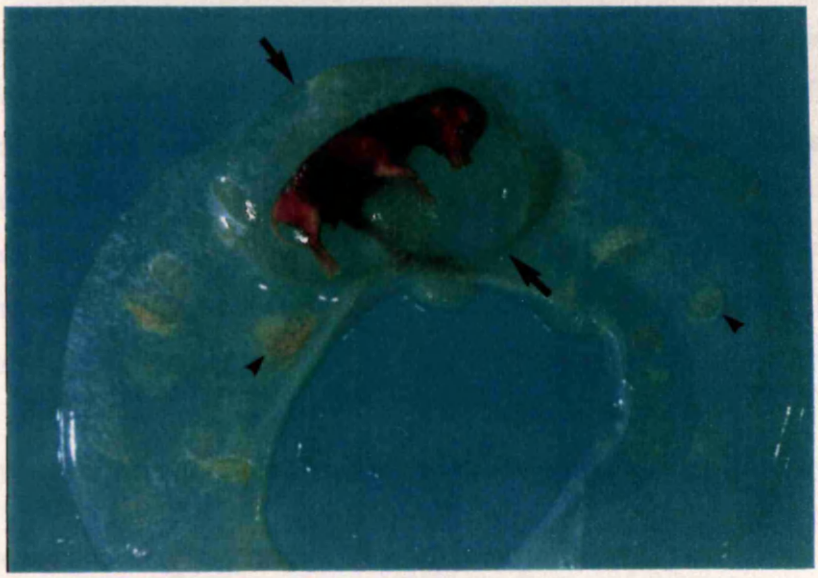


Figure 3.3: A 58 day old bovine foetus (arrows). The amniotic vesicle is evident encircling the foetus. The cotyledons are also visible over the chorio-allantois membrane (arrows head).

The use of ultrasound imaging of the reproductive tract of cows under farm conditions, for determination of the reproductive status, has increased. This technique is relatively easy to apply and gives an immediate result. Transrectal B-mode real time ultrasound scanning, with transducers of different frequencies, has allowed an accurate pregnancy diagnosis. Using a 5 MHz frequency transducer, discrete nonechogenic areas (conceptus) were first visible, within the uterus, between Days 12 and 14 after breeding; with first visualization of the embryo between Days 26 and 33 (Pierson & Ginther 1984*a*). Vesicles (conceptus) were first detectable on Days 10 (20%) and 12 (88%), with visualization of the embryo proper on Days 19 to 24 and detection of heart beat at Day 20 (Curran et al. 1986*a*). Badtram (Badtram, Gaines, Thomas & Bosu 1991) scanned cows between Days 16 to 31 and showed that scanning prior to 31 days, after insemination, did not prove to be highly accurate under field conditions.

Applying a 7.5 MHz transducer, nonechogenic areas were first visible at Day 9 after insemination and an accurate diagnosis could be achieved at Day 14, with 100% of accuracy by Day 20. However, this was with previous knowledge, by the operator, of the breeding history of the cow and an initial examination of the ovaries (Boyd, Omran & Ayliffe 1990). Working with both 5 MHz and 7.5 MHz transducers, Kastelic (Kastelic, Bergfelt

& Ginther 1991) reported that on Days 10 to 12 the embryonic vesicle was apparently observed, but not distinguished from free intrauterine fluid collection. His results showed that early pregnancy diagnosis in heifers was confounded by the presence of intrauterine luminal fluid. Thus, an accurate diagnosis was just possible after Day 16, but before this day, early pregnancy diagnosis was more of an opinion (50%).

This study intends to evaluate which should be the earliest feasible day for diagnosing pregnancy in cattle, under farm conditions, by transrectal ultrasonography of the reproductive tract and whether or not this is an accurate technique.

## 3.1 Materials and Methods

The following equipment was used in this experiment:

- A portable, approximately 14 kg, B-Mode Real Time Ultrasound Scanner — Dynamic Imaging Concept 2, Livingston, Scotland.
- A 7.5 MHz Linear Array Transrectal Transducer, with lateral and axial resolution of 1 and 0.5 mm respectively.
- A NV-G21 Digital Scanner Video Cassette Recorder.
- Fuji Super HG Double Coating, 180 minutes VHS Tapes.
- A Panasonic AG 500 VHS Monitor/Player.
- A Sony UP 850 Video Graphic Printer.
- A Sony UP 3000P Color Video Printer.

The experiment was carried out on 27 lactating multiparous Holstein Friesian cows, of between 3 and 12 years of age, over a 200 days period under farm conditions. The animals were housed indoors, in such a way that the normal cyclicity was known. The gestational age at the time of the scanning was calculated from the date of last service (Day 0), determined by computerized farm records and visual signs of oestrus reported by the herdsmen. The day of pregnancy diagnosis ranged from 16 to 35 post breeding. The examinations were usually done three times in each animal; the first scanning taking place on one of days 16 to 20, the second performed on one of days 21 to 27 and the last on one of days 28 to 34 after service. In some doubtful cases, other scans were required after Day 34, and also some animals were first examined on the last session of scans determined for diagnosing pregnancy in the experiment (Days 28 to 34). Scanning of the cows during the oestrous cycle and after service previous to Day 16 was not performed. There was no manipulation of any structure of the reproductive tract of the animals scanned during this experiment.

All the research data were recorded and stored for later assessment. For this purpose, three different recording systems were used – paper annotation, video cassette recorder and thermal copies.

A video cassette recorder was connected to the scanner and all the images obtained during the scanning were recorded on six tapes of 180 min each. The taped images were identified using the scanner keyboard annotation facilities. Thus, the identification of the animal under examination, the measurement of structures and their identification, as well as the date of the scan, could be read on the screen.

At the end of the scans, all the recorded information was assessed by playing back the tapes using a monitor, which possessed playback and slow motion facilities. The most representative images of each animal examined were printed using a thermal copier and after a detailed study of them, they were stored in folders for later study and reference. At the end of the research, the best images were selected and a higher quality thermal copier was used to print the final images.

The ultrasound equipment was mounted on a mobile trolley, positioned on the left hand side of the operator, opposite to the hand which held the transducer. The distance kept between the equipment and the operator allowed the former an easy access to the transducer and console and a safe position for both operator and equipment in case of an emergency (Figure 3.4). The cow shed lights were switched off to prevent screen glare, thus providing good visualization of the image. The ultrasound scanner and accessory equipment were connected and properly adjusted before the examination (Figure 3.5). A technician was in charge of the panel control, adjusting, enlarging and freezing images as requested and also taking notes of the findings (Figure 3.4 and Tables B.1 to B.6).

Firstly, the cows to be examined were identified in the herd and them separated from the other animals. Following that, the cows were led to a race within a cow shed. The first cow to be examined was restrained in a crush, while the others were kept in a chute, just

Figure 3.4: Ultrasonographic examination under farm conditions. The ultrasound unit is situated on the opposite side of the ultrasonographer's palpation hand. A technician is in charge of the panel control, close to the equipment. The cow under examination is restrained in a crush during the scanning.

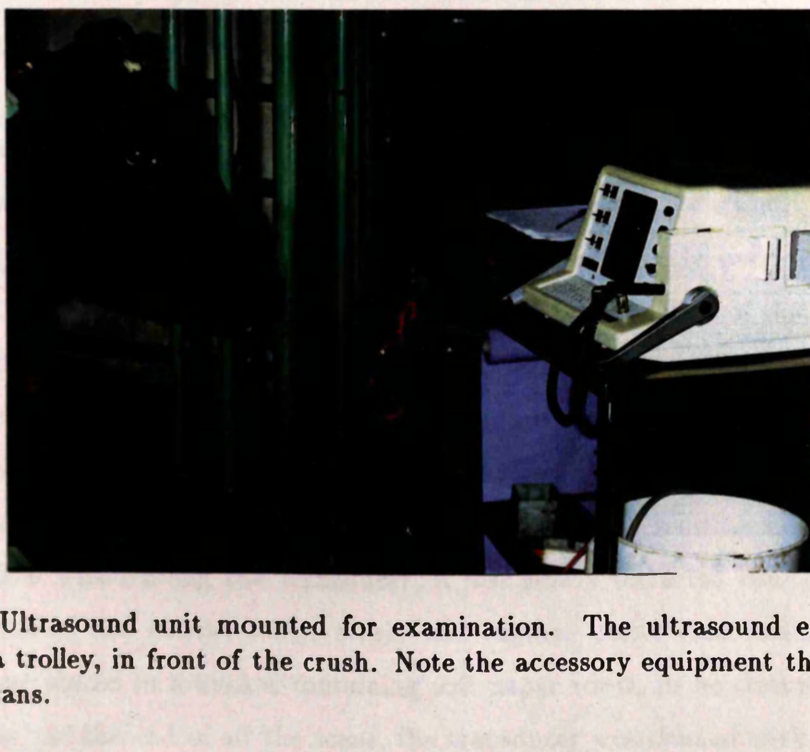


Figure 3.5: Ultrasound unit mounted for examination. The ultrasound equipment is mounted in a trolley, in front of the crush. Note the accessory equipment that was used during the scans.

Figure 3.6: The selected cows in a chute waiting to be scanned. The animals are situated just behind the crush where the animal under examination is restrained.

behind the operator (Figure 3.6). Wearing a glove, the operator lubricated her arm and the anal region of the cow. To perform the transrectal ultrasound scanning, faeces were evacuated from the rectum of the animal under examination. After removal of faeces, the reproductive tract was palpated and reproductive structures were identified and located. The transducer was, then, firmly held in the palm of the ultrasonographer's hand and covered with gel on its face. Subsequently, the transducer was inserted through the anal sphincter into the rectum, with its longitudinal axis parallel to that of the animal.

A standard scanning technique was established for the routine exam. Keeping the face of the transducer on the ventral part of the rectum, directly over the uterus,the examination began by moving the transducer along the dorsal surface of the reproductive tract, towards the right ovary and continuing on to the right uterine horn. Next, the transducer was led to the left ovary and then left uterine horn, following the same steps used on the right side of the reproductive tract. Visualization was achieved, on the scanner screen, by moving the transducer clockwise and anticlockwise relative to the structure scanned. When withdrawing the transducer, it was slowly retracted caudally, allowing a detailed view of the uterine body, cervix and vagina. After each examination, the transducer was placed in a bucket containing soft paper towel, to be cleaned before the next scanning. At the end of all the scans, the transducer was cleaned with warm water and dried to be stored.

As the transducer was inserted into the rectum, a longitudinal view of the vagina, cervix and uterine horn was acquired. Reaching the uterine body bifurcation, the transducer was deviated laterally along the right horn, which was seen in cross section, and then further lateral movement of the probe allowed imaging of the right ovary. As the latter was the starting point of the applied technique, the previously imaged structures were not thoroughly examined.

The ovaries were echogenic structures being readily discernible from the neighbouring tissues. The blood vessels that supply the ovary were visualized as anechoic rounded structures close to it, and could be confused with images of follicles. The ovarian veins were seen as 2 or sometimes 3 rounded nonechogenic areas similar to medium or large follicles. The veins could be distinguished from follicles by altering the plane of imaging, resulting in the appearance of an elongated anechoic view of the former. Ovarian arteries were visualized as a group of small nonechogenic rounded areas, generally imaged in cross section (Figure 3.7).

Ovaries could be completely imaged by rotating the transducer slowly clockwise and anticlockwise within the rectum. Placing the probe over the dorsal curvature of the ovary, a sagittal view of the structure was obtained, which showed the medial surface of the organ . Positioning the transducer face lateral to the organ, this was seen transversally. Obtaining a specific plane of the structure was not easy, because of its mobility. Ovarian ultrasonographic anatomy varied according to its cyclicity, owing to the presence of various structures emerging from its stroma (Pierson & Ginther 1988, Omran 1989). A prudent scanning of the organ was essential, otherwise important structures could be missed.

The follicles were seen as anechoic structures usually of asymmetrical shape and varying in size. Such irregular appearance of follicles is usually caused by compression on the walls between the adjacent follicles, by the corpus luteum, ovarian stroma or even by the transducer applied to the organ at the moment of imaging. The follicular walls in ovaries with multiple follicles sometimes are too thin to be visualized (Pierson & Ginther 1988) (Figure 3.8).

The echotexture of a corpus luteum was characteristically different from the ovarian stroma. The latter was slightly more echogenic and the corpus luteum was easily identified by its relatively smooth well defined outline. Occasionally, blood vessels supplying

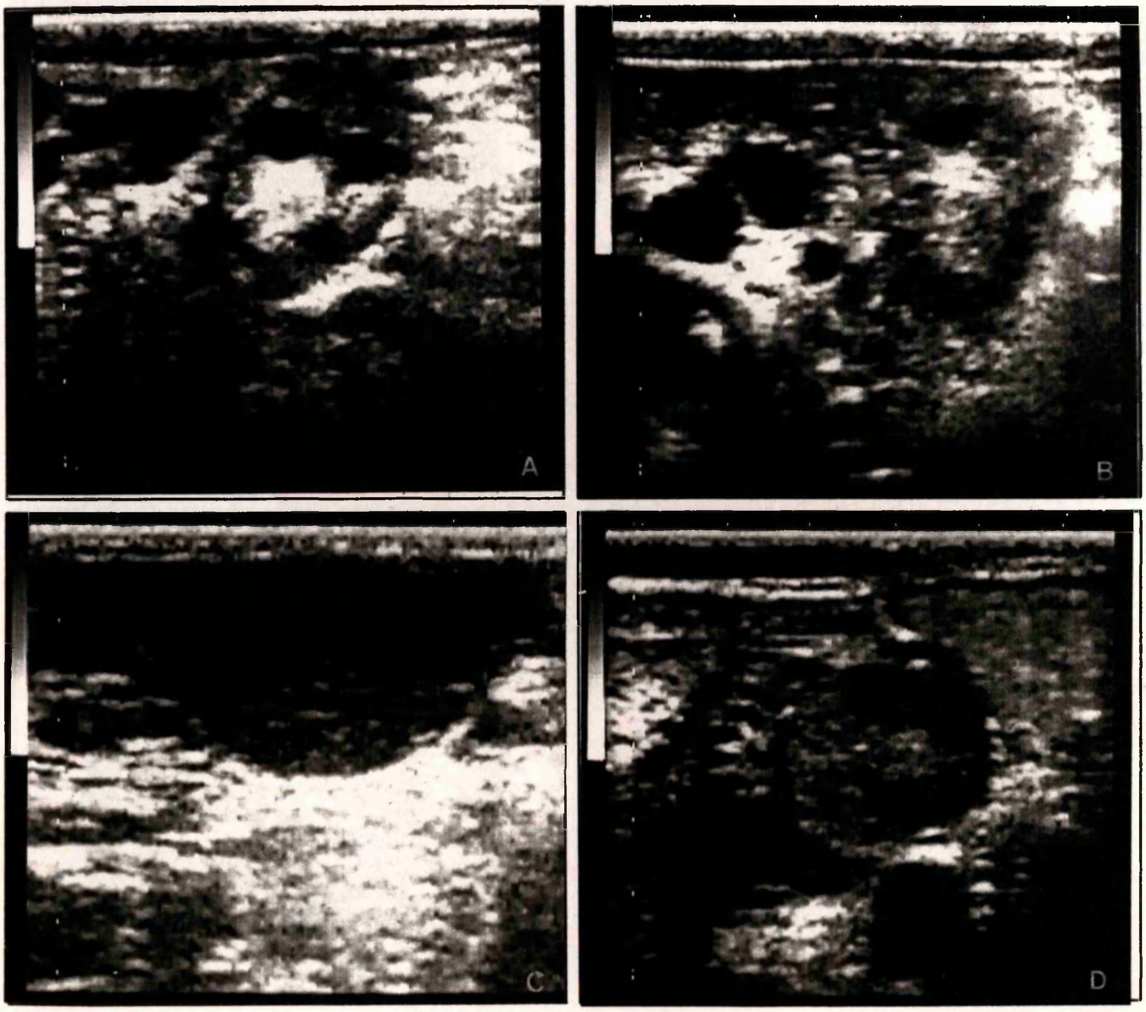


Figure 3.7: **A.** Ultrasonographic image of ovaries and blood vessels. Note the echogenic ovary discernible from the neighbouring tissues (central top field). The anechoic appearance of the ovarian blood vessels (top left) can be confused with the ovarian follicles (four central black areas in the ovary). **B.** The ovarian veins are seen as 2 or 3 rounded anechoic structures (top left) close to the ovary. **C.** Ovarian veins imaged as longitudinal nonechogenic areas (top field) at dorsal surface of the corpus luteum. **D.** Ovarian arteries are visualized as a cluster of small rounded black areas (central left field) lateral to the ovary (central field).

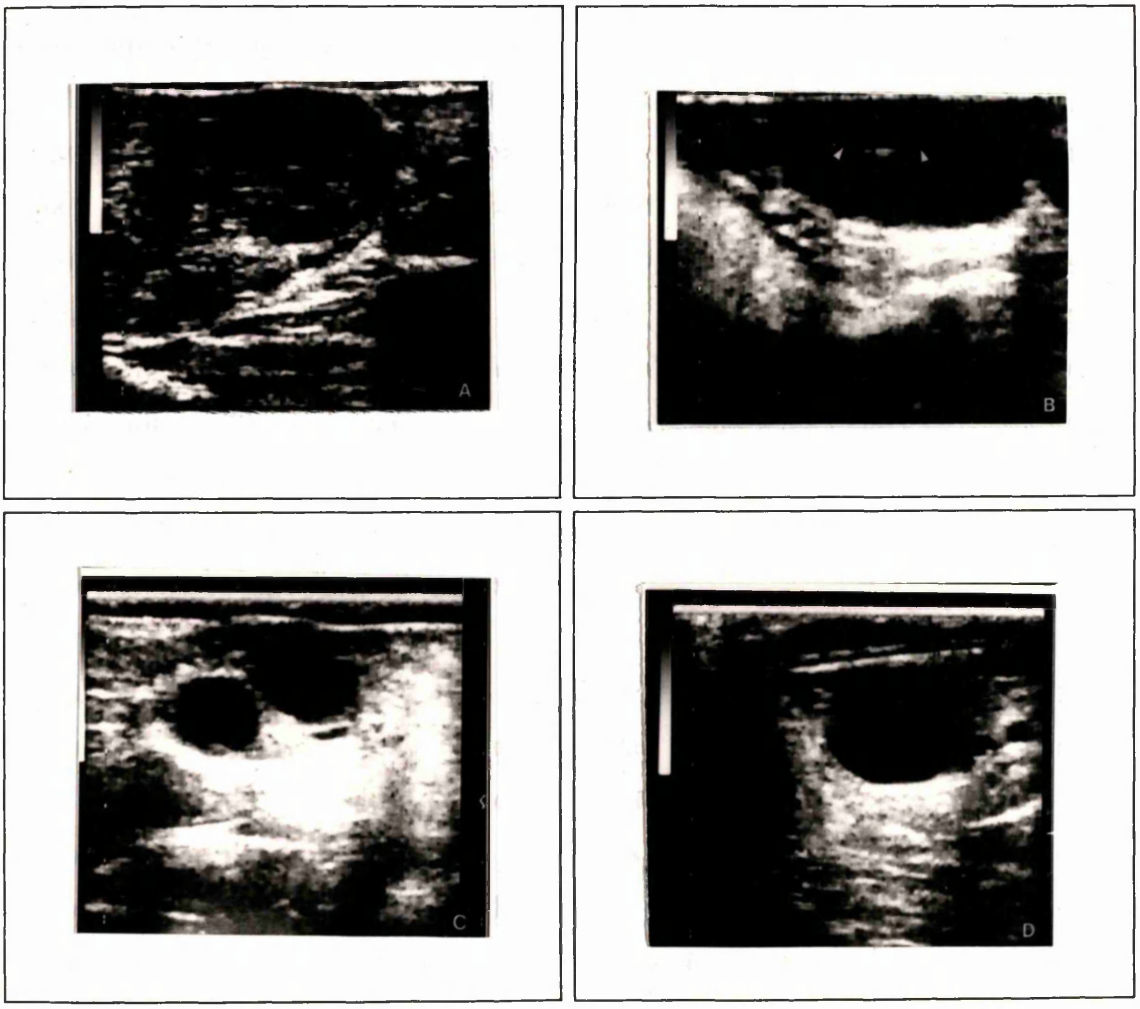



Figure 3.8: **A.** The entire ovary imaged (central field). Note a corpus luteum dorsally situated in the ovary central top). The central black area seen in the corpus luteum is a lacuna. The echogenic appearance of the ovarian stroma, the corpus luteum and the blood vessels are clearly distinguished in this transverse view. **B.** and **C.** The anechoic follicles show an asymmetrical shape due to compression on the walls between adjacent follicles and ovarian stroma. The thin follicular walls are almost imperceptible (arrows). **D.** A large follicle (20 mm) imaged in a non pregnant cow on Day 31 after breeding (central top).

the area were observed as an elongated anechoic region on the dorsal surface of the corpus luteum (Figure 3.7.C) Frequently, a corpus luteum exhibited a nonluteinized central portion (lacuna) seen as a black rounded structure. A lacuna could be mistaken for a follicle, but could be differentiated by its symmetrical shape lying within the surrounding echotexture of the luteal gland (Figure 3.9).

The ultrasonographic feature of the corpus luteum varied depending on its stage of development and whether or not pregnancy had occurred. The newly formed corpus luteum (corpus haemorrhagicum), which is accepted as difficult to image in its first days of existence, being observed as an echoic folded structure with a non-echogenic centre (Omran et al. 1988) was not observed in this study. This was attributed to the time of first scanning (D16). A regressing corpus luteum had a changed echotexture and a poorly defined border. When a lacuna was present in a regressing corpus luteum, it could occasionally be seen flattening (Figure 3.31.E). A mature corpus luteum was imaged as a large structure exhibiting an homogeneous echotexture and well defined border (Figure 3.9). After examination of the right ovary, the transducer was led to the right horn, which was subjected to close scrutiny.

Owing to its shape and location in the region of pelvic inlet, the horns were visualized in cross section when the transducer was maintained longitudinal to the axis of the body. A transverse plane was achieved by rotating the transducer laterally. The cross sectional image of the horn showed the lumen as a nonechogenic central area surrounded by the echoic endometrium and muscle layers (Figure 3.10.A). The longitudinal plane demonstrated an irregular anechoic line representing the lumen with normal uterine fluid passing in between the two layers of endometrium (Figure 3.10.B). The ultrasonographic appearance of the uterine tissue varies according to the period of the reproductive cycle (Pierson & Ginther 1988). In early diestrous the lumen can be seen as a highly echogenic line resulting from reflection of the folded surface of the organ.

By placing the transducer at the level of the bifurcation of the uterine body, with the probe laterally positioned relative to the organ, it was possible to obtain a cross section of both horns, as well as a longitudinal or cross section of the uterine body. The gravid or non gravid horn could be scanned by applying the transducer on its dorsal surface or also by scanning through the opposite horn. Once positioned on the dorsal portion of the horn, opposite to the one bearing the pregnancy, the probe was moved slightly lateral to

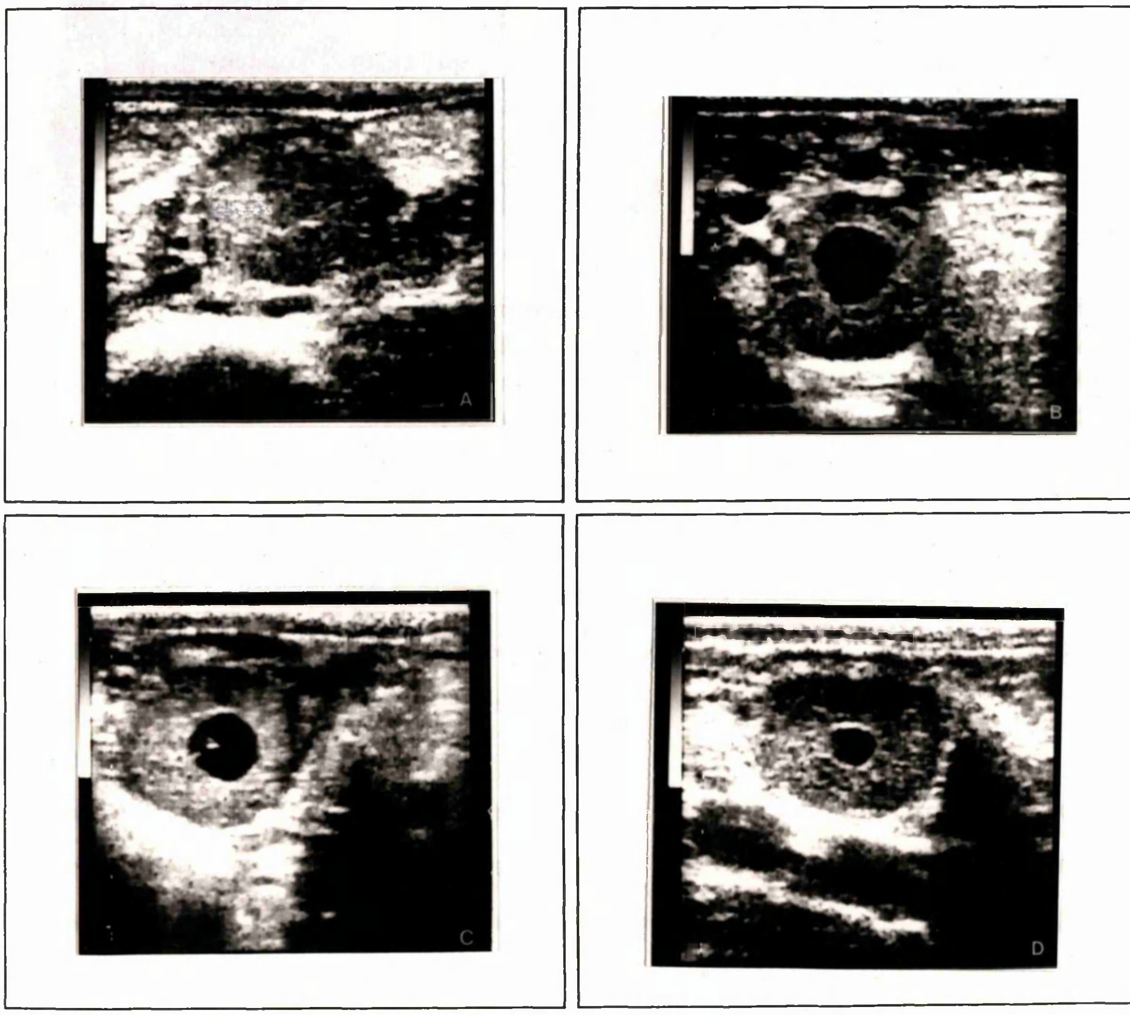
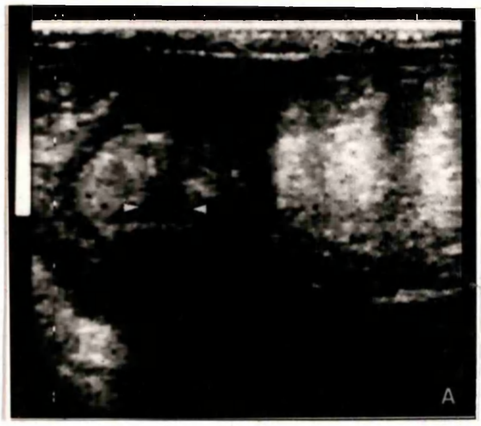


Figure 3.9: **A.** A large corpus luteum (28 x 30 mm) in a pregnant cow, on Day 26 after breeding. The smooth echotexture and outline of the luteal gland is noticed (central field). **B, C** and **D.** Corpus luteum with nonluteinized central portion, seen as nonechogenic structures, in different stages of development of the gland, on Days 19, 24 and 26, respectively (central field).



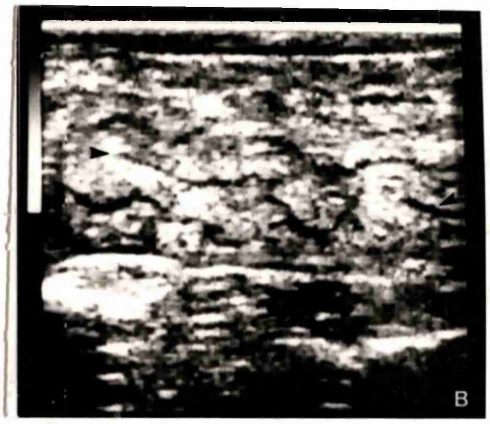




Figure 3.10: **A.** Cross section of an uterine horn (central left) showing the endometrium and uterine muscle layers surrounding the nonechogenic lumen (arrows). **B.** Longitudinal plane of an uterine horn (running from right to left through centre of field, arrows). Note the anechoic line representing the lumen (arrows), with the normal physiological amount of fluid, between the two folded endometrium layers.

its initial position. On the scanner screen, the gravid horn appeared lying beneath the non gravid horn (Figure 3.11). The left ovary and then the left horn, were examined using the same technique applied to the structures on the right portion of the reproductive tract. Moving the transducer caudally on its way out of the rectum, the cervix and vagina were imaged.

The cervix presents an hyperechoic ultrasonographic image. Usually viewed in longitudinal plane, it produced shadowing artifact (Figure 3.12). Lying ventrally to it, the bladder was seen as an anechoic structure with a distinct outline varying in conformation, depending on its degree of distension. The full urinary bladder, which is commonly deviated to the left side of the cow, can cause difficulty in visualization of the left ovary. Imaging of the ovary can also be obscured by fat or even part of the intestine (Figures 3.26.A and 3.32.A). On final withdrawal of the probe, the vagina was seen in close proximity as an hyperechoic structure in the longitudinal plane. Reverberation artifact was also very common in this region.

In this experiment, the main criteria for diagnosing pregnancy, as early as 16 to 19 Days after breeding, was the observation of structures in the ovaries of the cows. The presence of a well outlined corpus luteum, with its characteristic echotexture, was usually indicative of an established pregnancy (Positive Pregnancy Diagnosis). The absence of a corpus luteum

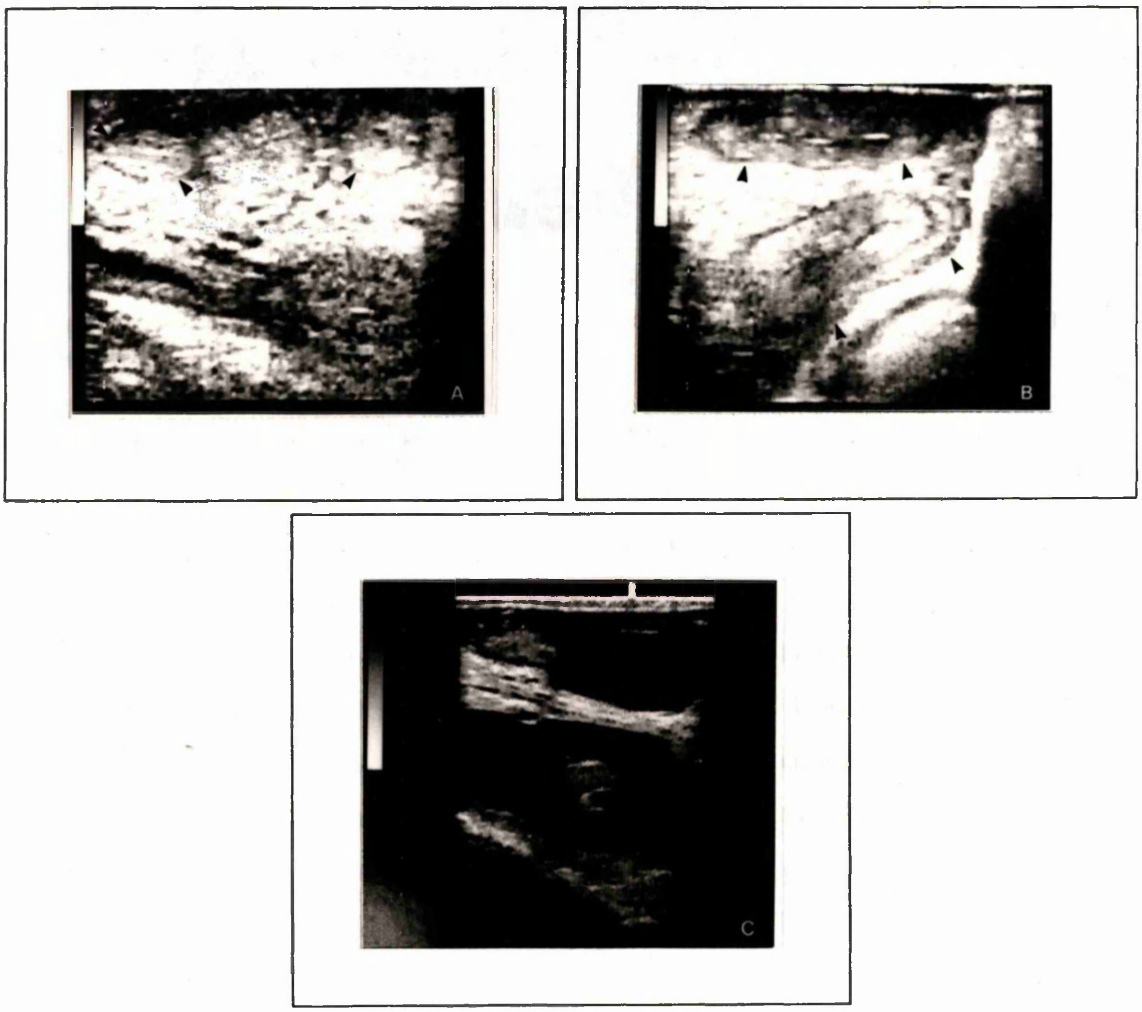



Figure 3.11: **A.** Cross section of the uterine body (central top image) seen between the two horns (arrows). **B.** Imaging of one horn through the opposite horn (arrows). Note the endometrium layer surrounding the lumen of the horns. **C.** Visualization of the uterine pregnant horn scanned through the opposite horn.

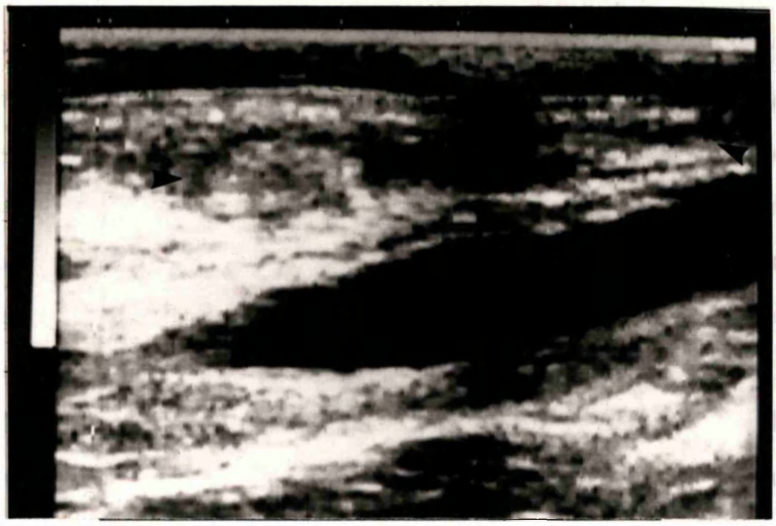


Figure 3.12: The vagina and cervix seen in longitudinal plane (arrows). Note the shadowing artifact present. The bladder is lying ventral to the cervix, and is seen as a large anechoic area.

or the presence of a regressing corpus luteum and follicles of different sizes, were suggestive of non pregnancy (Negative Pregnancy Diagnosis). The other criteria considered in this study for diagnosing pregnancy, was the observation of the lumen of the uterine horn. The presence of a noticeable amount of fluid in an irregular shape within the horn, which was exhibiting a smooth endometrium and usually ipsilateral to the ovary bearing the corpus luteum, was generally indicative of a pregnant animal (Positive Pregnancy Diagnosis). The amount of fluid within the lumen of the horn (Distended), which was sometimes visualized as a small dilatation of the nonechogenic lumen, in longitudinal plane, or as an irregular rounded area (Vesicle), in cross section, represented the early conceptus (Conceptus). With careful scanning, a faint echogenic structure (Early Embryo) could be seen within the nonechogenic distended lumen. The horn showing an absence of irregularly shaped fluid within the lumen or also containing a discrete collection of fluid within a lumen exhibiting a folded (oedematous) endometrium, was considered to be of a non pregnant animal (Negative Pregnancy Diagnosis).

The simultaneous observation of both criteria for positive pregnancy and also for negative pregnancy diagnoses led to an accurate diagnosis of the examined animals. However, in such an early gestational stage of pregnancy (Days 16 to 19), with no previous examination of the animals, some doubtful diagnoses arouse. The doubtful negative diagnosis arouse (Uncertain Negative Diagnosis) when a conceptus could be seen in the horn exhibiting an smooth endometrium and in some cases an early embryo was visible; but visualization of the luteal gland produced a lack of confidence in the appearance of the gland, this is, it seemed to have a slight change in its echotexture and border. Also considered as uncertain, was when a corpus luteum exhibiting an apparently normal echotexture and outline was present, but, in the ipsilateral horn, the nonechogenic intraluminal amount of fluid seemed to be greater than the expected size for this stage of gestation. Observation of the lumen of the contralateral horn was helpful in these uncertain cases, since depending on the gestational period, at the moment of the scanning, the conceptus could have reached part of the contralateral horn (Lumen), already completely filled it (Distended) or might not have achieved this stage (No lumen). As reported by Kastelic (Kastelic et al. 1988) and Omran (Omran 1989), the conceptus occupies all the ipsilateral horn at Day 16 and the contralateral horn at Day 19.

In this experiment, the uncertain negative diagnosis were accepted as relatively reliable, this is, more probable to be negative than positive; thus, it was considered to be a negative pregnancy diagnosis for the calculation of statistics results. It is worth stressing, that there was no manipulation of any structure of the reproductive tract of the animal scanned, during the course of the studies.

The criteria for diagnosing positive pregnancy at a later gestational stage (Days 23 to 35), was based on the presence of a corpus luteum and a horn containing an embryo proper and detectable heart beat. Failure to visualize a corpus luteum or imaging of a regressing luteal gland; and observation of a horn with a minor distension of the lumen, even exhibiting a smooth endometrium, were findings related to non pregnant cows. The presence of a corpus luteum with no evidence of regression and a horn exhibiting a large lumen, at this stage, was diagnosed as negative if an embryo proper and heart beat could not be detected. See Appendix B.

A correct positive pregnancy diagnosis (cpp) was defined as a cow ultrasonographically diagnosed as pregnant (pp), in an early gestational stage, and subsequently confirmed pregnant, in a later stage, by ultrasound or by producing a live calf. A correct negative pregnancy diagnosis (cnp) was defined as an animal diagnosed nonpregnant (np) by early ultrasound imaging, and later confirmed to be not pregnant by ultrasound or by return to oestrus. An incorrect positive pregnancy diagnosis (ipp) was defined as a cow ultrasonographically diagnosed pregnant (pp) and later found to be not pregnant, by ultrasound or return to oestrus at a later date. Incorrect negative pregnancy diagnosis (inp) was defined as an animal diagnosed as nonpregnant (np) with ultrasound, and subsequently confirmed to be pregnant by ultrasonography or by production of a live calf.

As the sample used in this study was not of great proportion, the data obtained was statistically analyzed by percentage calculation. The total accuracy (TA) of the achieved results, was determined by the percentage of the total number of correct pregnancy diagnosis (cpp + cnp), out of the total number of animals early diagnosed by ultrasound (pp + np). That is, TA = ((cpp + cnp)/(pp + np)) x 100. The negative predictive value (NPV) was determined as the percentage of the correct negative pregnancy diagnosis (cnp), out of the total of animals diagnosed as non pregnant (cnp + inp). That is, NPV = ((cnp)/(cnp + inp)) x 100. Positive predictive value (PPV) was defined as the percentage of the correct positive pregnancy diagnosis (cpp), out of the total of animals diagnosed as pregnant (cpp + ipp). That is, PPV = ((cpp)/(cpp + ipp)) x 100. The sensitivity (SE) of the method was calculated as the percentage of the cows correctly diagnosed as pregnant (cpp), out of the total of all animals really found to be pregnant (cpp + inp). That is, SE = ((cpp)/(cpp + inp)) x 100. Specificity (SP) was defined as the percentage of the cows correctly diagnosed as negative (cnp), out of the total of all cows really found to be not pregnant (cnp + ipp). That is, SP = ((cnp)/(cnp + ipp)) x 100. See Appendix A.

## 3.2 Results

The early conceptus was first observed on Day 16 post breeding, appearing on the monitor of the ultrasound, as an irregular fluid collection within the lumen of the pregnant horn. In the longitudinal plane of the horn, the early conceptus could be seen as a small dilatation (vesicle) of the elongated nonechogenic fluid (Figure 3.13). A discrete echogenic structure, usually visualized in comma or spot shape (early embryo), could be imaged, repeatedly within the vesicle. These images were obtained in the cross sectional view of the horn, by moving the transducer slowly over it (Figure 3.14).

A total of 84 ultrasonographic scans, for diagnosing pregnancy, were performed in 27 cows from Days 16 to 42. The animals were scanned several times during the course of the research, in average 3 times each animal. Thus, the total for animals used in the different days of examination, is not the same as the total number of animals used in the

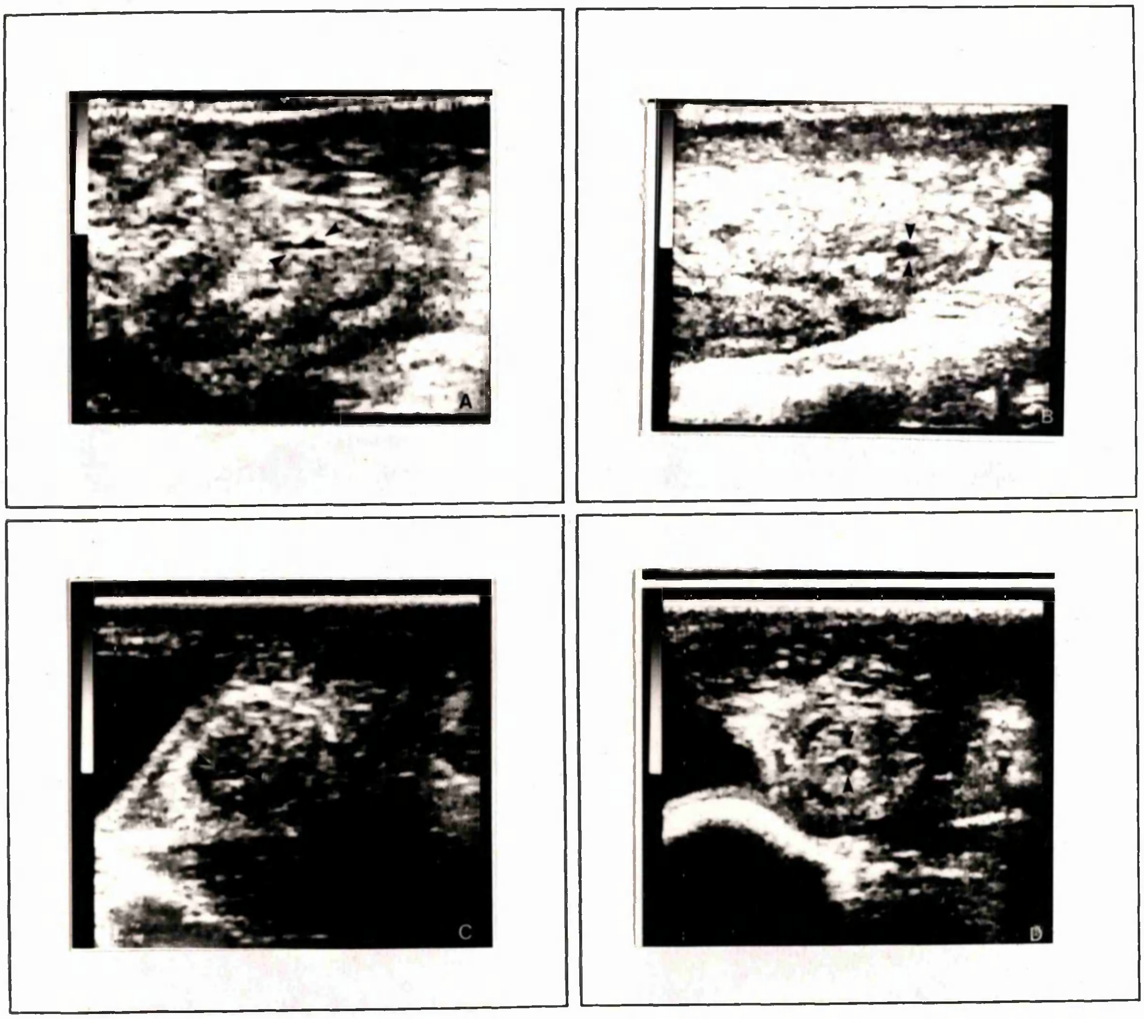



Figure 3.13: **A.** Cross section plane of a pregnant horn 19 days after oestrus (central frame). Note the vesicle as a black distended region (arrows)in the central portion of the uterine muscles. **B.** Uterine horn on Day 19 after breeding, in cross section view (central right field). The irregular nonechogenic vesicle (arrows) is surrounded by the uterine endometrium and muscle layers. **C.** The large nonechogenic area (left top picture) is the cranial portion of the full bladder. The horn seen close to the bladder is the left horn; the former is used as a reference to locate the left pregnant horn. The horn in seen in cross section and a distended lumen is observed (arrows). **D.** The uterine horn is viewed in cross section (central picture) and shows a distended lumen (arrows). The cranial portion of the bladder is visible (left top picture) and the hyperechogenic line beneath the bladder is the pelvic bone.

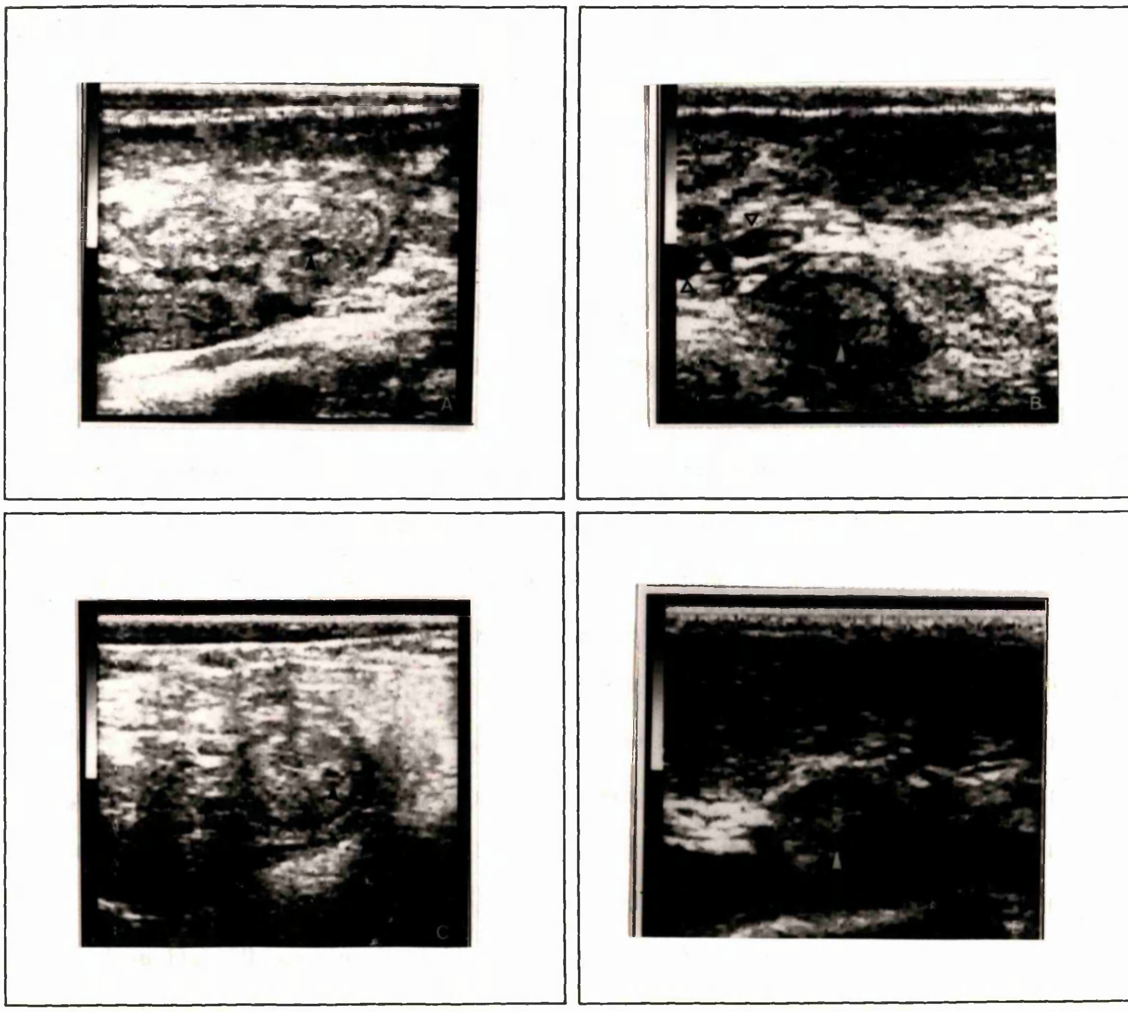



Figure 3.14: **A.** The horn is seen in cross section and shows a vesicle. The early embryo, Day 19 is visualized as an echogenic structure, comma shape, within the vesicle (arrow). **B.** The distended nonechogenic lumen exhibits an echogenic structure within it, an 18 day old embryo (arrow). The nonechogenic areas close to the horn are blood vessels that supply the region and can be confused with the uterine lumen (open arrows). **C.** Oblique plane of an uterine horn, 17 days after breeding, showing a vesicle and discernible early embryo within it (arrow). **D.** A Day 17 early embryo, visible within the vesicle in a cross section plane of a horn.

research; and therefore, the total of scans per day, in some cases, is more than the number of animals scanned in that day (see Table A.1).

Some animals that were diagnosed as non pregnant and returned to oestrus, came back to be rescanned. There were a few cases of animals examined twice in the same period of the scanning sessions, e. g., Day 16 and then Day 18, due to problems in the recording of the scans. Thus, the total of animals used in the different days of examination, is not equal to the number of animals used in the research; and also, the total of scans per day, in some cases, is more than the number of animals scanned in that day.

The percentage calculation was based on the total of examinations performed during each of the determined periods designated for diagnosis in this research (Days 16 to 34), and not on the total of animals used in the experiment. In the early gestational stage (Days 16 to 19), 24 scans were performed in a total of 21 animals. During this period, 6 cows were diagnosed as non pregnant (25%) in 6 examinations performed; one cow was scanned twice (Day 16 and 18), because the first scan could not be recorded. Ten early conceptuses (vesicles) where seen in 9 scans performed in 9 cows; one cow was carrying twins. Nine early embryos were detected in 9 scanned cows, two of which were first diagnosed as non pregnant, but after a second oestrus they conceived (see Table B.1 to B.6). Considering the detection of twins as one examination, the percentual of early conceptus observed in this period of scanning was 37,5%, and the detected early embryos was also 37,5%. From the total of animals diagnosed as positive, the figures would be 50% for observation of both early embryo and conceptus. The embryo and the embryonic heart beat were first detected on Day 20, and in all viable embryos from this day (100%).

A careful scan during this period of early gestation, Days 16 to 19, was essential to avoid erroneous diagnoses. The amount of fluid present in a pregnant horn on Day 16 can be confused with the amount seen in an oestrous uterus (cycling cow). A detailed observation of the endometrium is indispensable to identify the smooth appearance of the pregnant uterus, compared with the folded or oedematous endometrium of a cycling cow (Figure 3.10.D).

Ultrasonographic visualization of nonechogenic areas in the region of the uterus was common, due mainly to the blood vessels that supply that region. A careful scan, during this period of early gestation (Days 16 to 19), was essential, to preclude the possibility of erroneous identification of anechoic structures as an early conceptus (Figure 3.14B). It was important to ensure that the ultrasonographic nonechogenic lumen of the horn was surrounded by the endometrial layer of the organ (Figure 3.13 and Figure 3.14).

During the period of examination determined for the studies (Days 16 to 34), presumptive embryonic losses were observed in 5 individuals (6.41%). They were considered presumptive, because no heart beat was detected at a prior examination, just observation of an early embryo. The non detection of a heart beat was related to the early gestational age at the time of the first scan.

All pregnancy diagnoses were 100% accurate from Day 20. On Day 19, there was one cow diagnosed as pregnant and later found to be a non pregnant animal, this was thought to be an early embryonic death. The accuracy on Day 18 was a 100% for both positive and negative pregnancies. Among the animals examined on Day 17, there was one cow diagnosed as pregnant and later found to be non pregnant; this was attributed to an early embryonic death. An uncertain negative pregnancy was observed in this day of examination, which was also related to death of embryo. On Day 16 two cows were examined. One cow was diagnosed as negative and the other was diagnosed as positive, but later found to be negative, what was attributed to a presumptive embryonic loss.

Uncertain positive diagnosis was not observed during the experiment. Three uncertain negative diagnoses (12,5%) were present in the early stage of diagnosis (Days 16 to 19). Incorrect negative diagnosis did not occur in the experiment. Incorrect positive diagnoses were observed in three cows (12,5%) at early gestational stage, which were attributed to early presumptive embryonic losses.

Embryonic structures could be clearly seen in some images obtained in scans from Day 20 onward (see Figures 3.15 to 3.20). The forelimb buds could be observed on the Days 24 to 26 embryos and the forming hindlimb buds could be visualized on the Day 26 embryo (Figure3.15.B and Figure 3.16.B). The amnion was observed encircling the embryo on Day 24 (Figure 3.16.A). The amnion and chorio-allantois fusion with the endometrium was clearly perceptible on the Day 26 embryo (Figure 3.17).

Details about the structures seen in each cow scanned in different days are given in Appendix B.

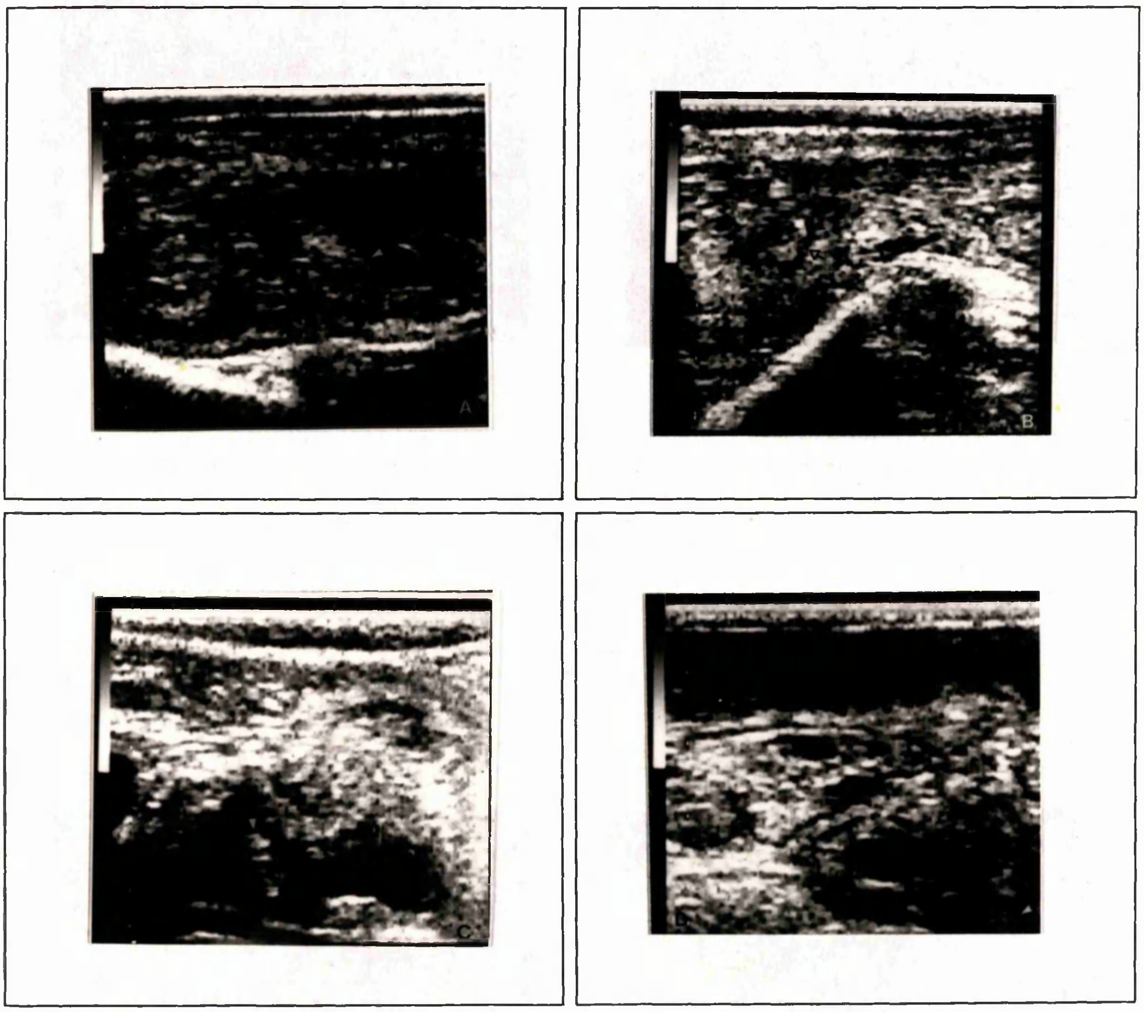


Figure 3.15: Embryos and embryonic structures on Days 23 and 24. **A.** A Day 23 embryo in longitudinal plane. The region of the heart beat was clearly detected. The embryo is lying with cranial portion to the right side of the picture (arrow). **B.** Uterine horn (central left picture) showing a 24 day old embryo. The head is visible and seen as a hyperechogenic structure (arrow head). The forelimb buds are visible (open arrows). **C** and **D.** 24 days old embryo. Note the body structure. The head is seen (arrow).

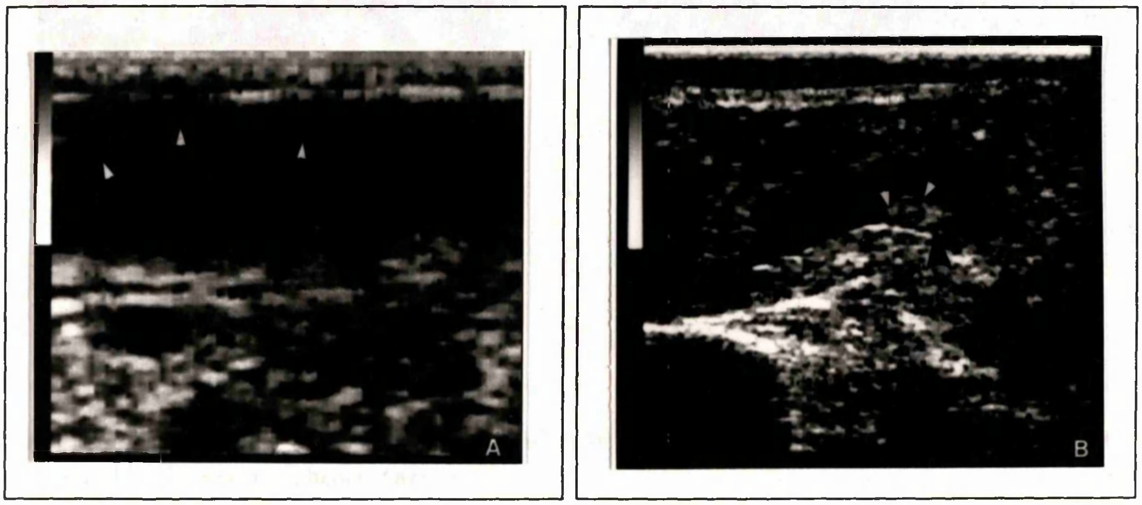



Figure 3.16: **A.** Embryo Day 24. The amnion is visible (white arrows). The head is on the right side of the image (arrow). **B.** Day 26 embryo. The fore limbs and the forming hind limb buds are visible (white arrows). The head is also discernible (black arrow).

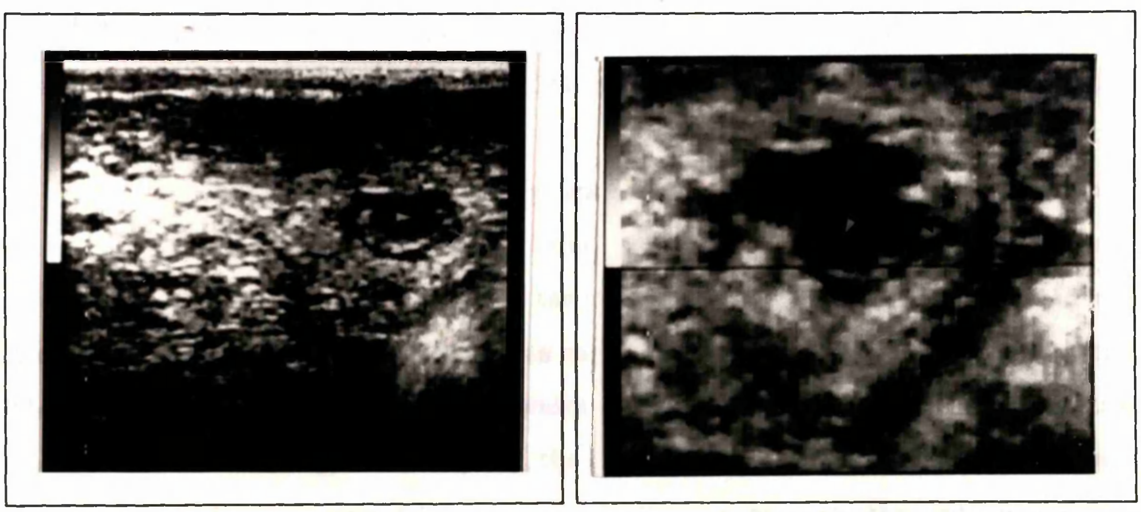


Figure 3.17: Embryo on Day 26 of gestation. Note the amnion and chorio-allantois fusion with the endometrium clearly perceptible (arrows).

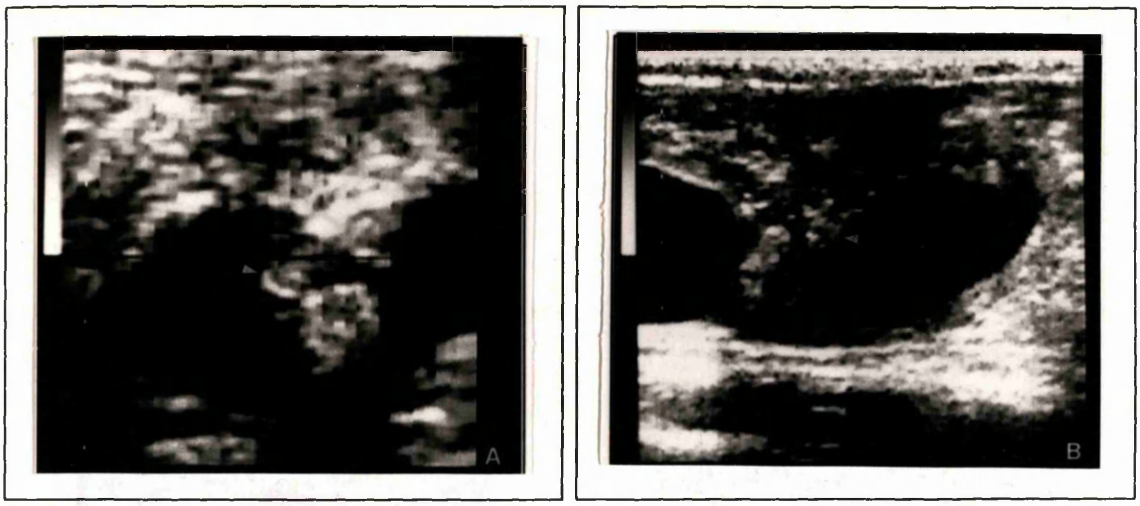



Figure 3.18: Day 29 (A) and 30 (B) embryos. Note the embryonic structures clearly visible. The heads are shown (arrows).

### 3.2.1 Abortions

During the course of the experiment there was one abortion observed by the herdsman. The animal (C20) was scanned four times, on Days 17, 24 and 31 after last standing oestrus, for pregnancy diagnosis purpose. Another scan, but for sex determination of the foetus, which was part of another experiment (Chapter 4), was performed on Day 52 after breeding. The early embryo was imaged on the first day of scanning and its heart beat was detected at the time of the subsequent examinations, on Days 24 and 31 (see Figures 3.21 and 3.22). The abortion occurred on Day 180 of gestation. The cause of the late abortion was unknown.

One cow (C28) diagnosed pregnant by scans on days 19 and 26, subsequently died. At the first examination an active corpus luteum was observed (23 x 27 mm) in an ovary. The horn ipsilateral to the ovary bearing the corpus luteum contained fluid in its lumen. A cross section view of the organ showed an early embryo within the nonechogenic vesicle. The findings led to a positive diagnosis, which was enhanced by the presence of the early embryo. In the following scan, Day 26, the corpus luteum was exhibiting echotexture characteristics of a well formed luteal gland. In the uterine horn, the embryo could be visualized and the embryonic heart beat was detected (Figure 3.23). Seven days later the cow died suddenly. The cause of death was unknown, but the post morten examination showed no damage to the rectum, which excluded the possibility of have been caused by

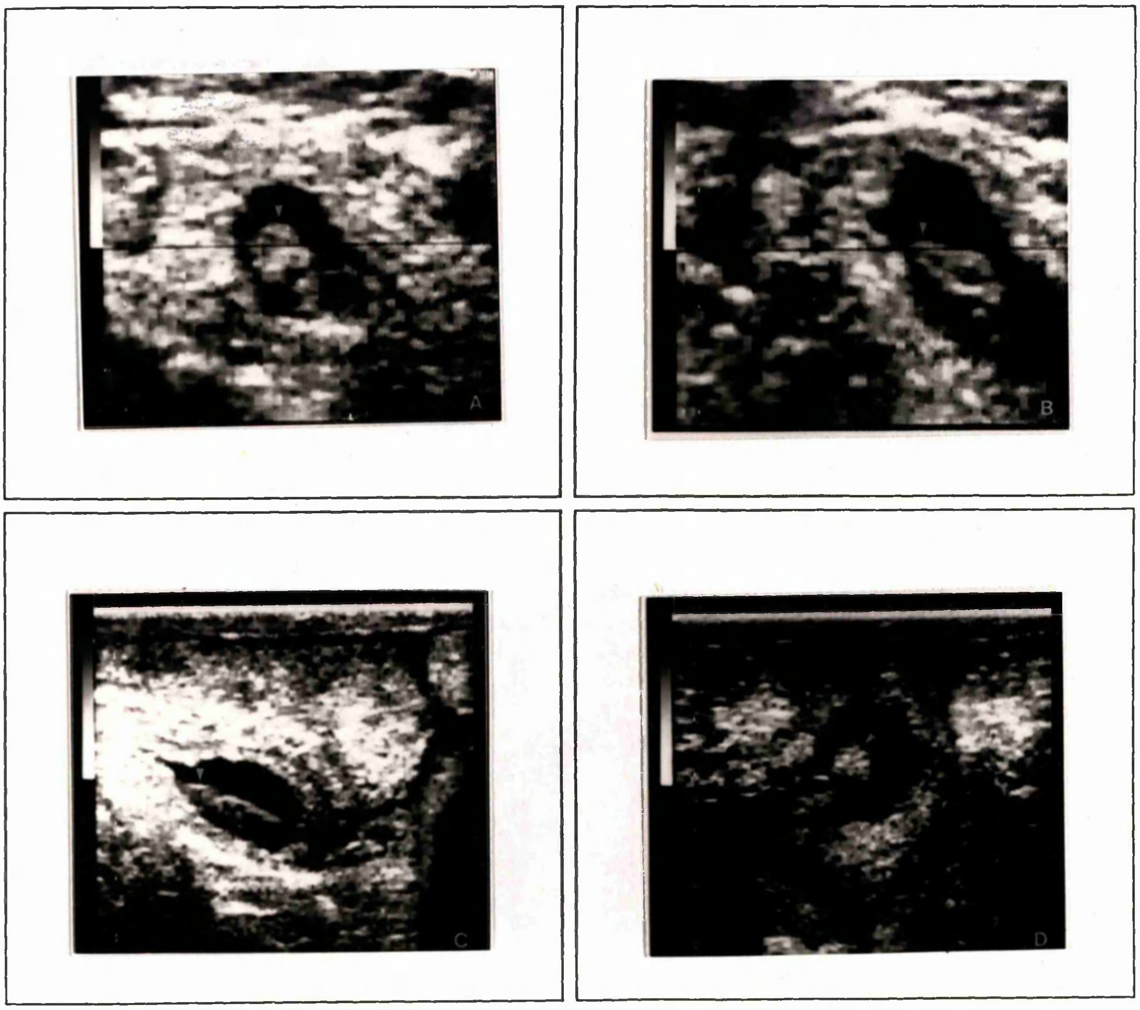



Figure 3.19: Embryos at Day 31 of gestation in various planes: dorsal and sagittal planes. The embryonic structures are distinguished; the heads (arrows).

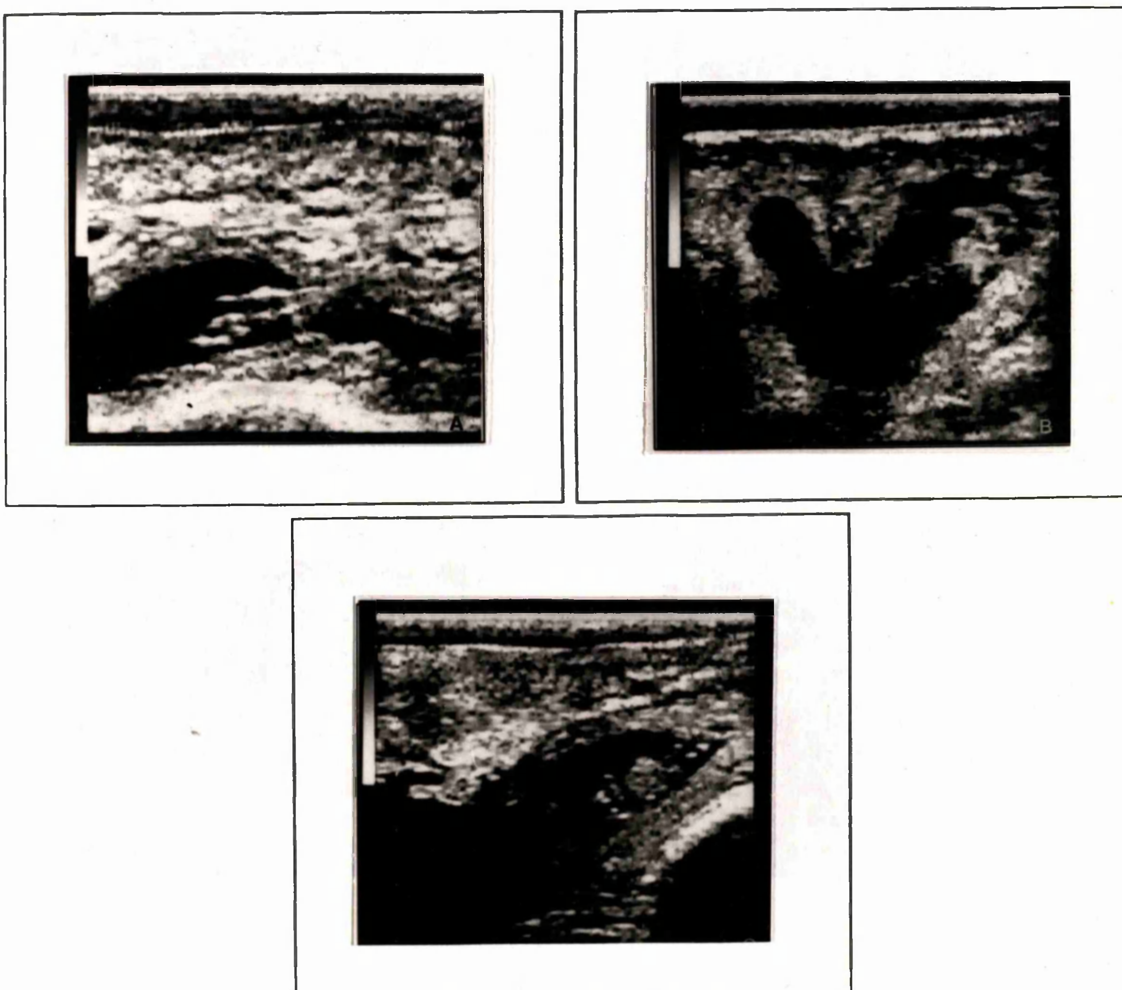



Figure 3.20: Embryos on Days 33 (A), Day 34 (B) and Day 40 (C) of gestation. Note the body structures easily distinguished. The heads are located on the right side of the images.

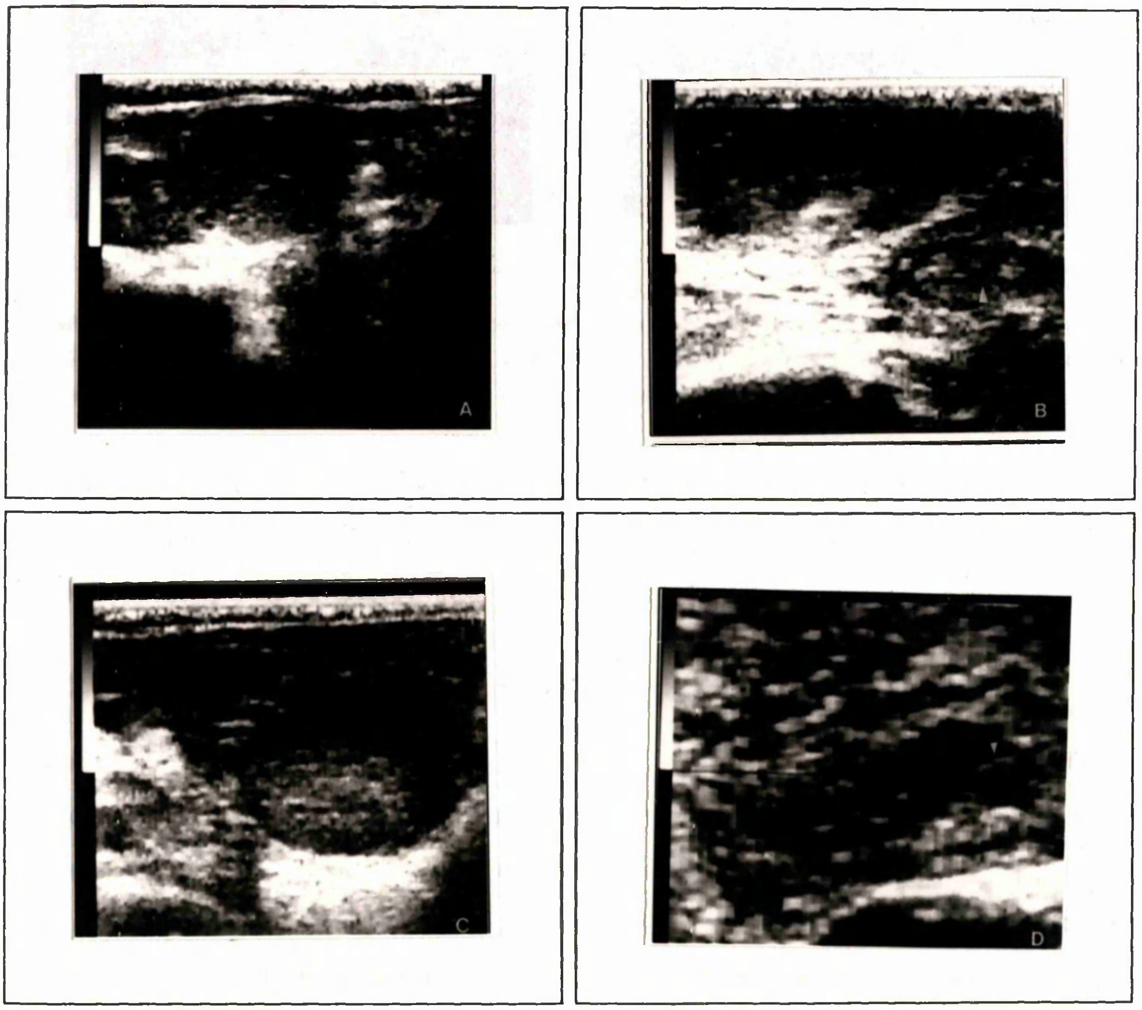



Figure 3.21: Cow 20 on Days 17 and 24 of gestation. **A.** Corpus luteum (25 x 27 mm) on Day 17 after breeding (top center), presenting good echotexture and outline. **B.** The horn (right center) showing a distended lumen, on Day 17, exhibiting an early embryo (arrow). **C.** Day 24. The luteal gland has increased in size (23 x 25 mm) and shows an even greater echotexture and border. **D.** The pregnant horn, Day 24, is large and the embryo is clearly visible. Heart beat was detected during the scanning. The head of the foetus is well defined (arrow).

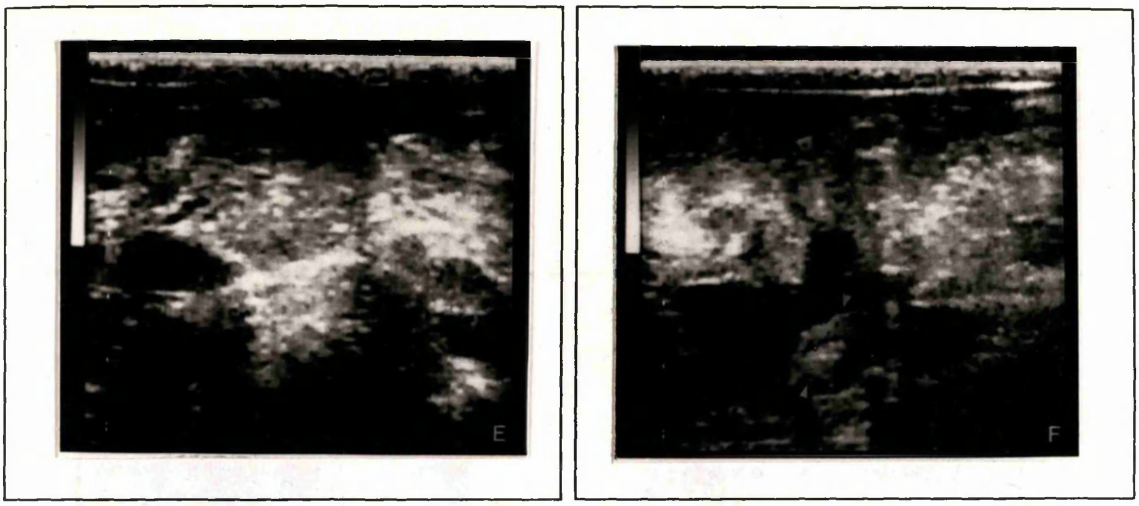


Figure 3.22: **E** and **F**. Day 31 after breeding, showing a corpus luteum 25 x 25 mm. The gland presents a normal echotexture and border. The uterine horn shows the embryo within the lumen (arrows). The heart beat was detected during the scanning.

the transrectal scanning procedure applied. The conceptus was recovered from the uterus. The embryo and the embryonic membranes presented normal development and appearance for the gestational age (Figure 3.2).

### 3.2.2 Presumptive Early Embryonic Losses

Among the five observed presumptive embryonic losses, in the course of the experiment, four of these embryonic deaths were observed during the first session of scans, early stage of gestation (Days 16 to 19); and the other was seen in the second period of scans, in a later stage of gestation (Day 21). Four of these presumptive embryonic death occurrences will be discussed in this subsection, since they were positively diagnosed. Another will be discussed in the next subsection, uncertain diagnosis, as this was uncertainly diagnosed as not pregnant.. Three of these cows, that presumably underwent embryonic loss, were diagnosed as positive pregnant animals at the time of first scanning. These cases will be discussed in this section. A fourth cow (C11) returned to oestrus twice after the first session of scanning in the course of the experiment. It showed embryonic loss after the two first breedings. After the first breeding, the cow was scanned and correctly diagnosed as non pregnant (Day 21). After the second breeding, the animal was rescanned and diagnosed as uncertain pregnant (Day 17). In the scanning performed after the third breeding (Day 17), the cow showed a positive pregnancy, and subsequently calved. The

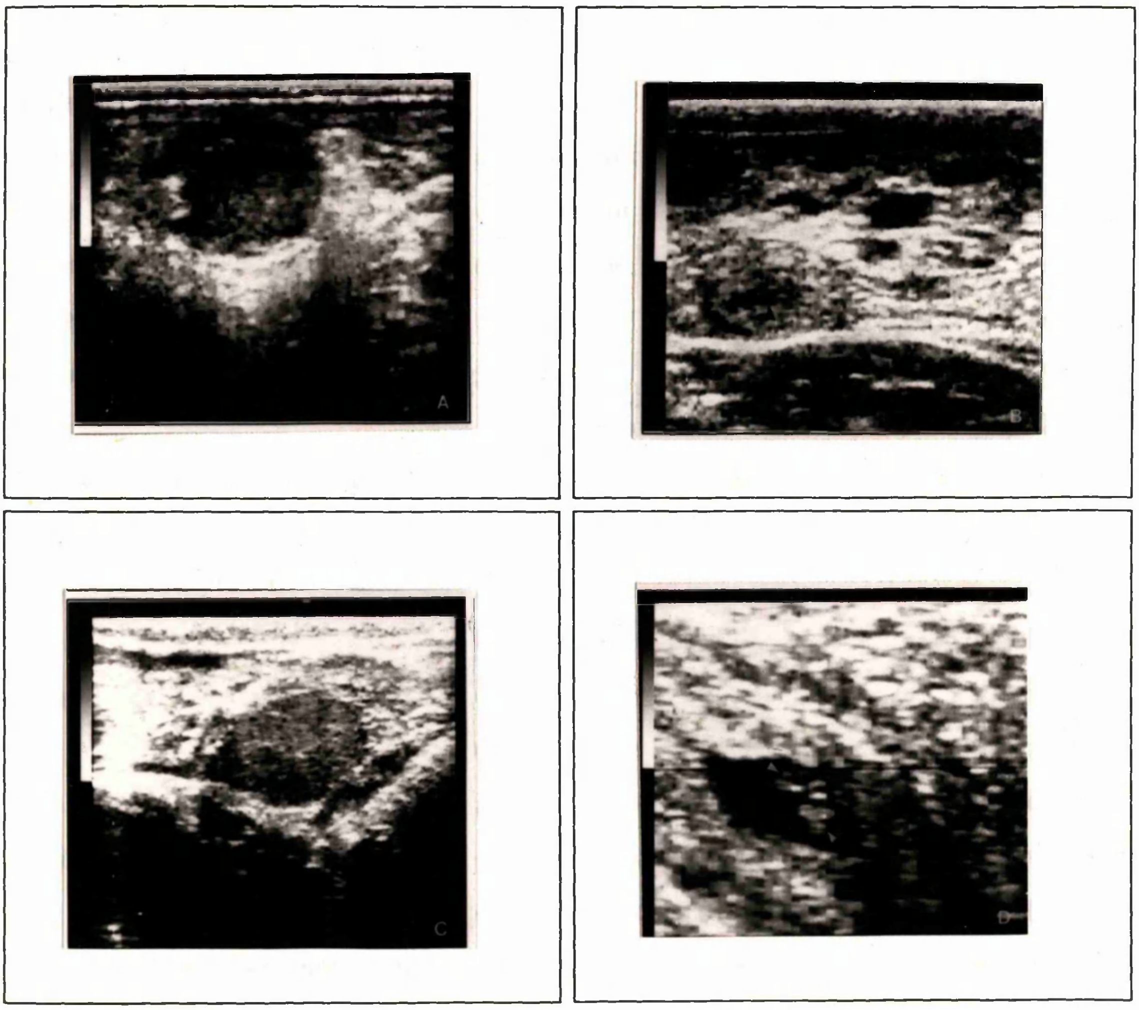


Figure 3.23: Cow 28, Days 19 and 26 after breeding. **A** and **B**. Cow 28, Day 19 after breeding. The ovary presents normal echotexture and well defined border (top center picture). The image shows the horn with a distended lumen and an early embryo is visible (arrow). **C** and **D**. Day 26 after breeding. The luteal gland is active and well defined (central picture). The embryo is visible (arrows) and heart beat was detected.

first scan session will be discussed in this section, but the second will be discussed in the following section for uncertain diagnosis. The breeding after the last standing oestrus, which led the animal to conceive, will be not discussed, since there was no doubt about the diagnosis.

### Cow 31

At first examination, on Day 16, the cow showed an apparently active corpus luteum (19 x 19 mm) with a lacuna. The luteal gland showed a normal echotexture and well defined border. The cross section of the ipsilateral horn exhibited a discernible early embryo within the vesicle. The findings were within the criteria used for positive diagnosis and hence the animal was considered to be pregnant. Nine days later, the cow was supposed to be scanned, but due to rectal bleeding, at the first insertion of the ultrasonographer's hand to evacuate the faeces of the animal, the examination was postponed. On Day 28 after breeding, a second scan was performed. The corpus luteum was larger than previously (27 x 26 mm) and the echotexture of the gland was apparently normal, but the lacuna was deforming. The horn presented a larger lumen, however small for the gestational age. The embryo and the embryonic heart beat were not visualized. At this stage (Day 28), under normal conditions, the embryo is clearly seen and its heart beat is easily detected, thus the absence of both led to a negative diagnosis. The last scan was performed on Day 32. The lumen was reduced but still distended, and hence, considered to be small for gestational age. Embryo and embryonic heart beat were not present. The corpus luteum still showed an apparently normal echotexture and had slightly varied in size (24 x 30 mm). The lacuna was not exhibiting the unusual appearance seen in the prior scan, but seemed to be flattening. The diagnosis was negative based on the size of the lumen and the absence of an embryo and its heart beat. The cow return to standing oestrus 11 days after this last scan. The interval between the first and the last standing oestrus was 43 days (see Figures 3.24 to 3.25).

### Cow 13

This cow was first scanned on Day 17, after last standing oestrus. The ovary showed a well formed corpus luteum with a lacuna (30 x 28 mm) and the presence of a large follicle.

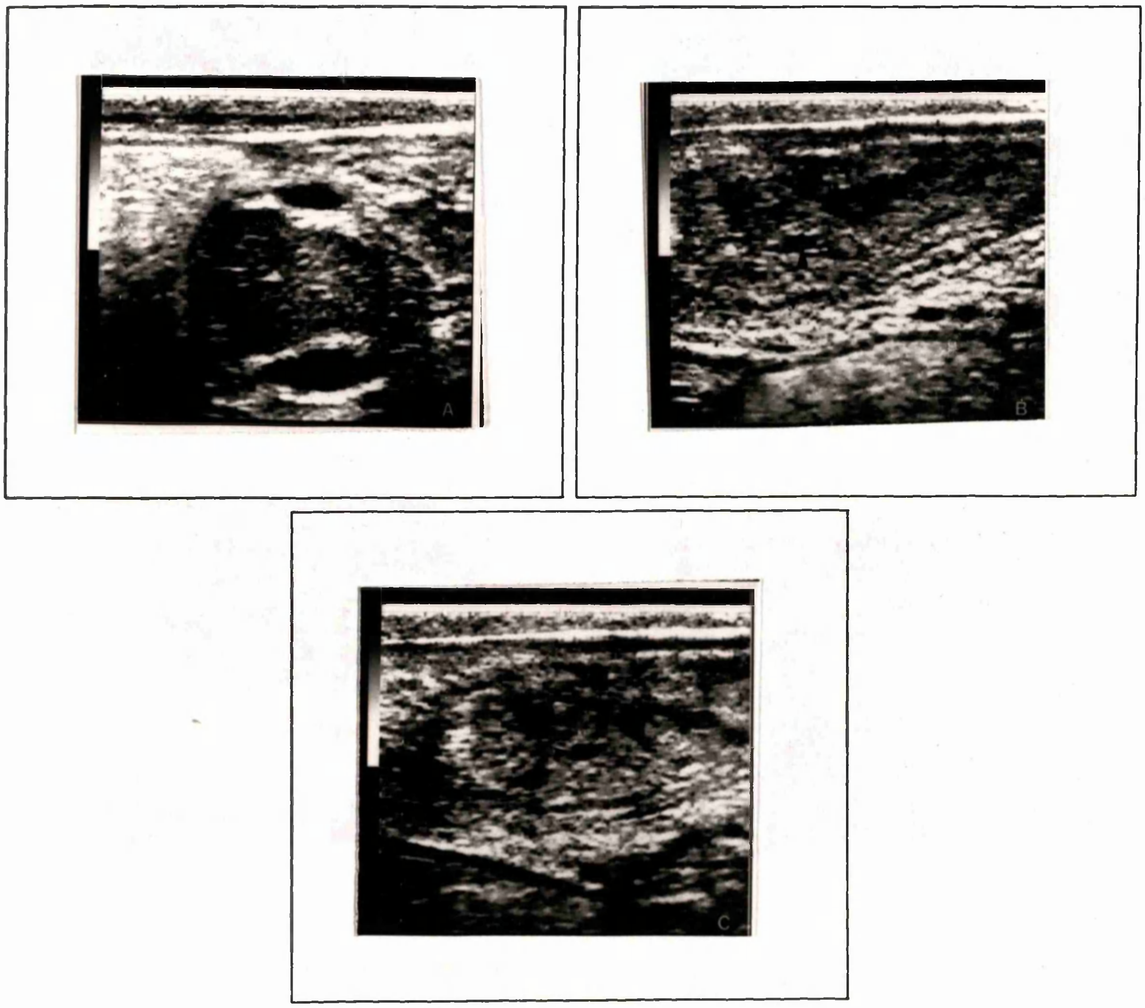


Figure 3.24: Cow 31 at Day 16 after breeding. **A.** Luteal gland showing a normal echotexture (center picture). **B** and **C.** A 16-day uterine horn. The early embryo is seen within the nonechogenic vesicle as a faint echogenic structure (arrows).

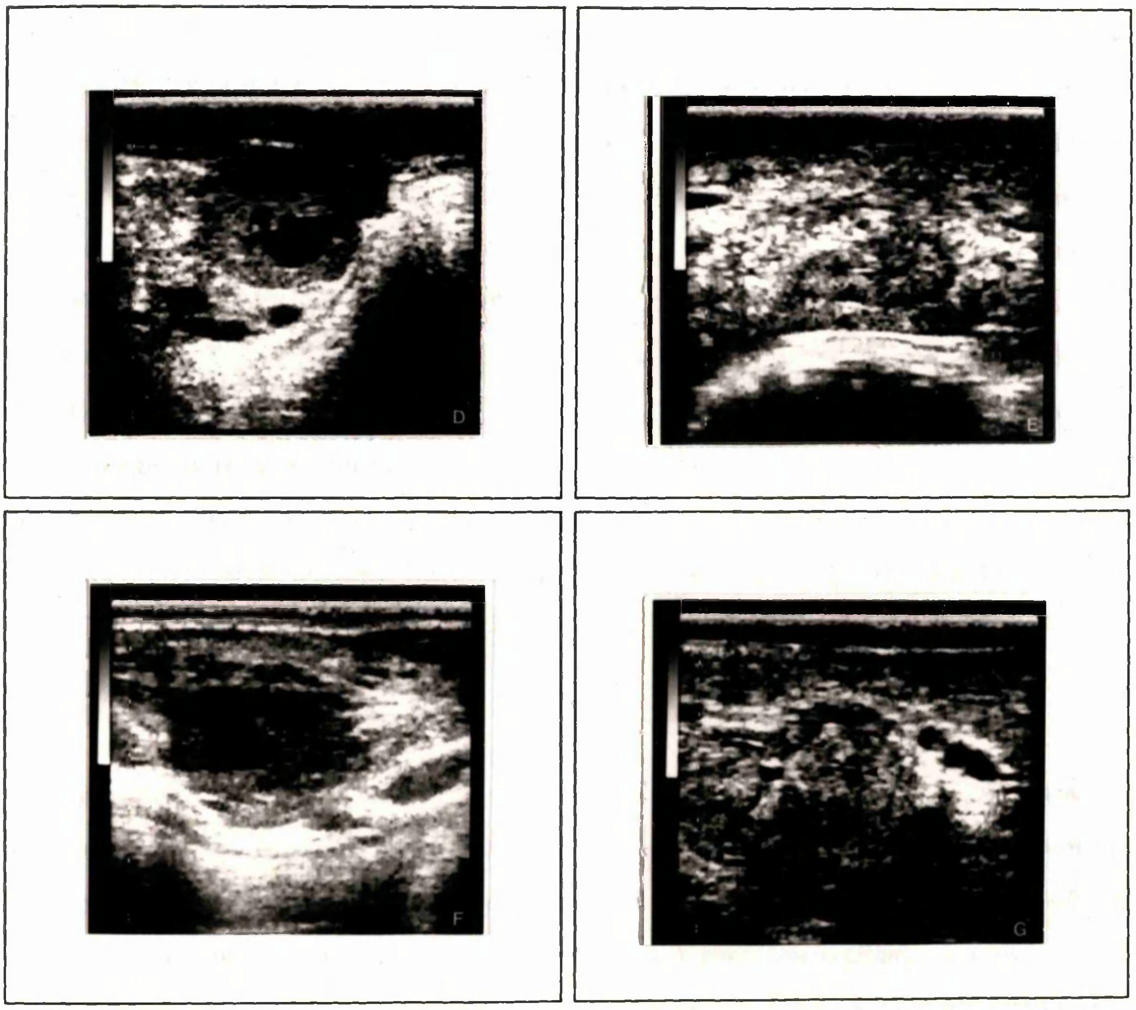



Figure 3.25: Cow 31 on Days 28 and 32 after breeding. **D.** The luteal gland at Day 28. Note the unusual lacuna apparently deformed. The echotexture of the gland seems to be a normal feature. **E.** The pregnant horn on Day 28. Note an increase in the size of the fluid within the lumen, however considered to be a small amount of fluid for the gestational period (center picture). An early embryo was not visible. **F.** The corpus luteum shows a lacuna slightly deformed (center image). **G.** The horn presents a distended lumen, small for gestational age (arrow).

The longitudinal view of the uterine horn, ipsilateral to the corpus luteum, presented a nonechogenic lumen, and the cross section plane exhibited a vesicle. The early embryo was not clearly visible, except for the presence of an echogenic structure within the lumen, seen in longitudinal plane. As the endometrium was smooth and the amount of fluid within the horn was compatible with the amount observed at this gestational stage, even though an early embryo was not seen, the diagnosis was positive. Scanned on Day 24, the image of the corpus luteum showed a reduction in the size of the lacuna , but an increase in the size of the luteal gland (31 x 32 mm), which showed a well discernible border and echotexture. The horn showed a small lumen, but larger than observed at last scanning. Embryo or embryonic heart beat were not detected, which led to a negative diagnosis. On Day 31 the ovary showed a regressing luteal gland (24 x 20 mm) and the lacuna was small. The lumen was discretely distended and there was no obvious presence of a conceptus. The following scans imaged a reduced corpus luteum (13 x 14 mm), and a discrete lumen and folded endometrium visualized in cross section. The cow returned to oestrus 29 days after last scanning; the interval between the two standing oestrus was of 69 days (see Figures 3.26 to 3.27).

## Cow 26

The cow was scanned on Day 19 after standing oestrus. The image of the ovary bearing the corpus luteum was not easily obtained, and the frames achieved showed a corpus luteum (44 x 42 mm) with a very large lacuna. Then, a detailed examination of the ipsilateral horn presented a distended nonechogenic lumen (conceptus). A cross section plane of the horn showed an early embryo within the vesicle. The presence of a discernible early embryo, even though the corpus luteum was not entirely visualized, led to a positive pregnancy diagnosis. On Day 26, the corpus luteum with lacuna was clearly undergoing regression, with change in its echotexture and outline (36 x 38 mm). The uterine lumen was slightly larger than observed at the last scanning, but with less fluid than expected for the gestation stage. Embryo or embryonic heart beat were not observed. According to the criteria used for diagnosing pregnancy, the cow was diagnosed as not pregnant. On the following scan, Day 33, the corpus luteum with lacuna was reduced in size (32 x 28 mm). The uterus exhibited a discrete lumen with a folded endometrium. The findings confirmed the negative pregnancy and the cow return to standing oestrus 28 days after

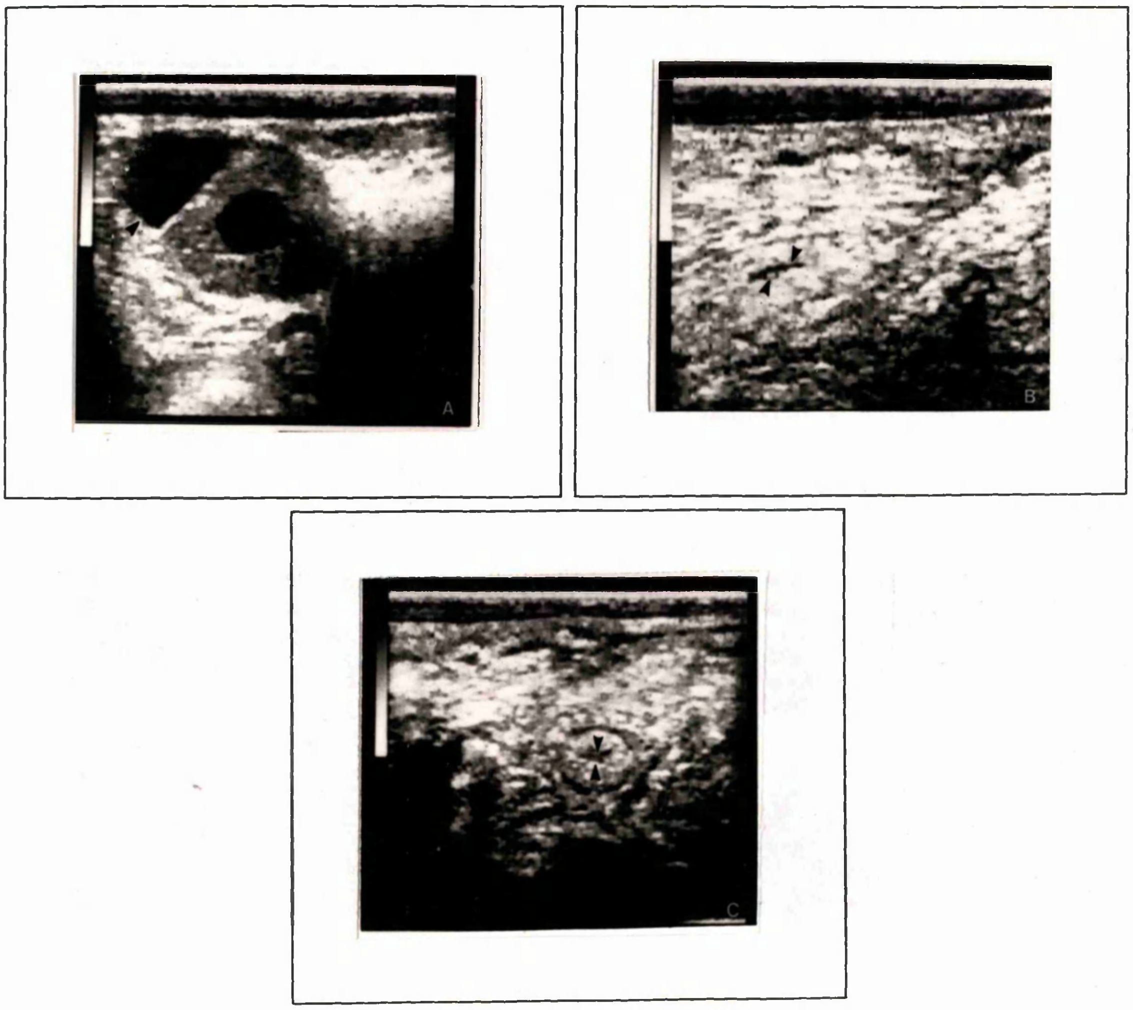



Figure 3.26: Cow 13 on Day 17. **A.** The ovary, exhibits a corpus luteum (30 x 28 mm) with lacuna (center field). A large follicle is also seen on the left side of the luteal gland (arrows). The gland shows normal echotexture and border. **B.** Longitudinal view of the horn. Note the distended lumen exhibiting an echogenic structure (arrows) within it. **C.** Cross section plane of the horn. The nonechogenic vesicle is clearly seen with an echogenic structure within it (arrows).

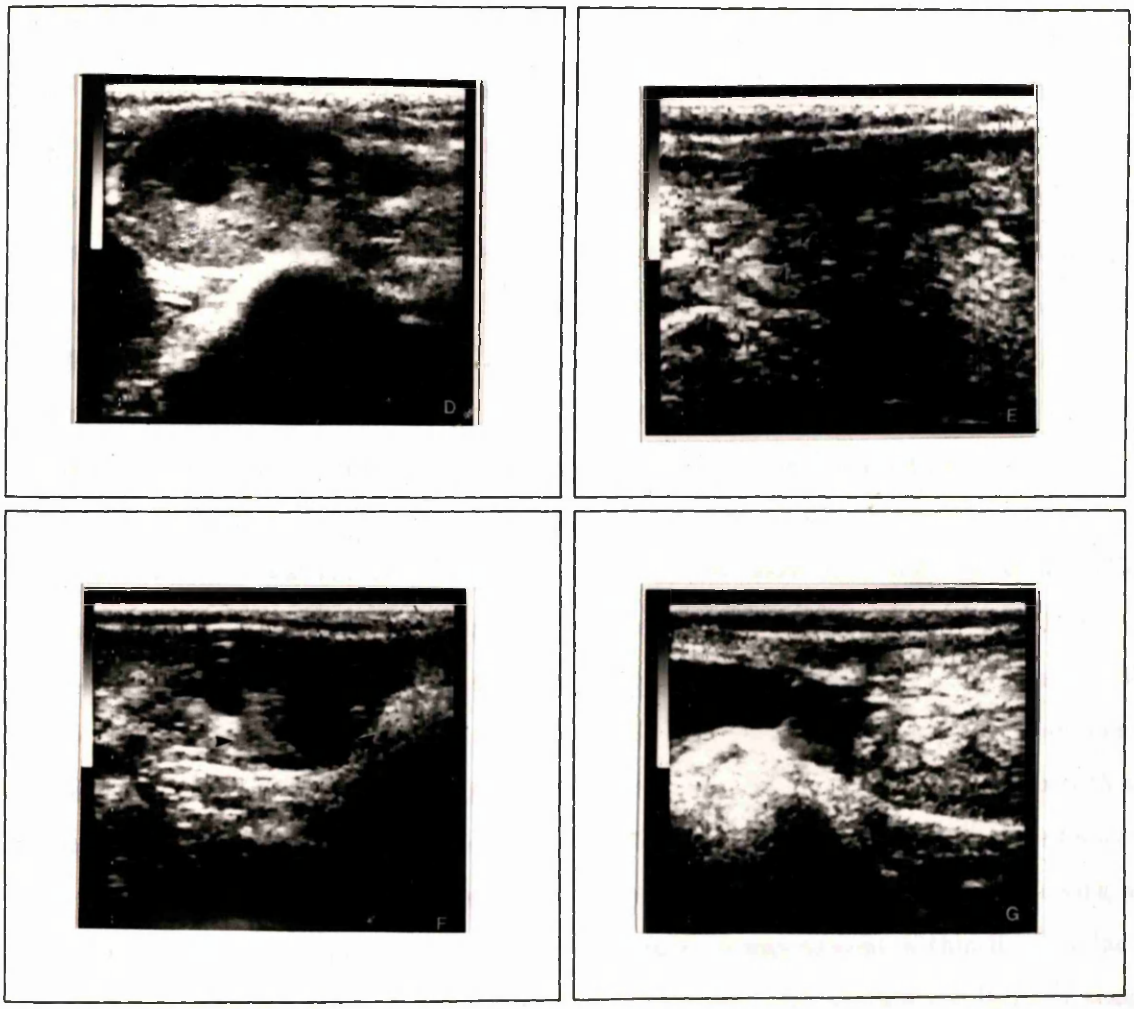



Figure 3.27: Cow 13 on Days 24 and 28 after breeding. **D.** Day 24 after breeding. The luteal gland exhibits a good echotexture and well defined border. It has increased in size (31 x 32 mm) and its lacuna is smaller. **E.** The ipsilateral horn, on Day 24, shows an increase in the amount of the intrauterine fluid, but no viable embryo is visible. **F.** Day 28. The luteal gland is still seen (arrows). The gland shows a poor echotexture. The lacuna is flattening and small. Follicles are seen emerging from the ovarian stroma. **G.** Day 28. The horn is presenting a folded endometrium (center right).

the last scanning. This last oestrus, with an interval of 61 days to the prior oestrus, also did not result in pregnancy, but after 19 days a third standing oestrus was observed and the animal became pregnant (see Figures 3.28 to 3.29).

### Cow 11

This cow returned to standing oestrus twice after the first oestrus at which the animal was first scanned for diagnosing pregnancy. The first examination was performed on Day 21. A corpus luteum with lacuna was visualized and showed an apparently well formed gland (24 x 28 mm). The horn was visualized in cross section and, within its lumen, an embryo like structure was seen, however, no heart beat was detected. At this stage, visualization of the embryo and non detection of a heart beat can be indicative of embryonic death. Thus, the diagnosis was negative for pregnancy. At the following scan, the corpus luteum showed a slight increase in its size, change in its echotexture and reduction of the lacuna. The lumen had increased and echogenic structure could be seen, in some images, lining the endometrium. Neither an embryo nor an embryonic heart beat were detected. The findings confirmed the negative pregnancy diagnosed at the first scan. Scanned on Day 28, the ovary showed a reduction in the size of the corpus luteum and lacuna. The presence of a discrete lumen, with no echogenic structure within it, was seen. Other scans were done to follow the regression of the luteal gland. One on Day 35, when the corpus luteum with a flattening lacuna and change in its echotexture (25 x 27 mm) was observed. A large follicle was seen (20 x 15 mm) close to the regressing corpus luteum. The horn was showing a discrete nonechogenic lumen and no echogenic structure was present within it. The last scan, on Day 42, revealed the luteal gland about the same size as observed in prior scan and a flattening lacuna. The cross section of the horn exhibited a discrete nonechogenic lumen and a slight change on the endometrial layer, which was becoming folded. The animal returned to oestrus three days later, with an interval of 45 days between the two oestrus (see Figures 3.30 to 3.31).

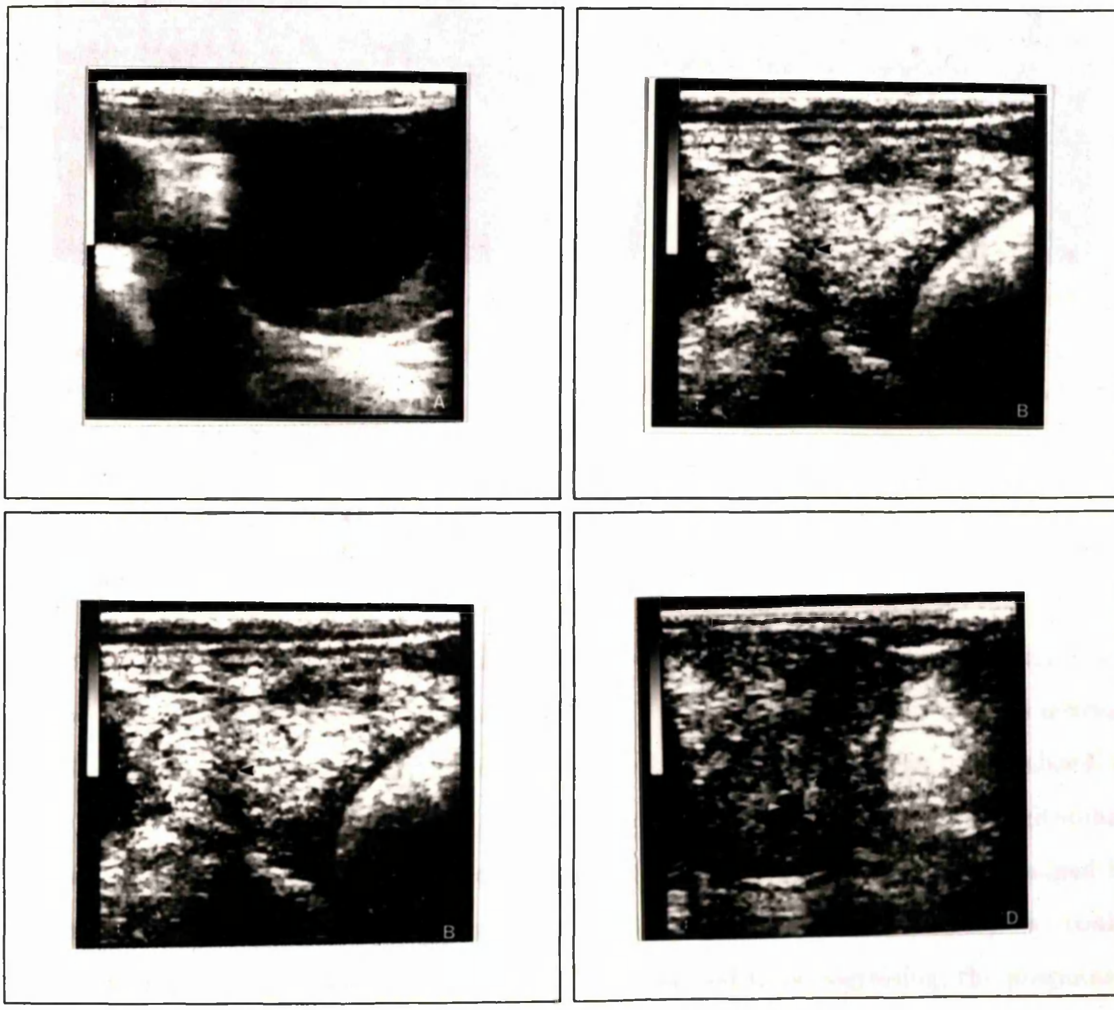



Figure 3.28: Cow 26 on Days 19 and 26 after breeding. **A.** Day 19 after breeding. A large corpus luteum (44 x 42 mm) bearing an unusual size of lacuna is seen (center field). The luteal tissue is seen in small quantity, due to the large size of the lacuna, which occupies a great expanse of the gland. **B.** The cross section of the uterine horn on Day 19 (left center). The early embryo is visible within the vesicle (arrow). **C.** Day 26 after breeding. The luteal gland shows evident change in its echotexture (center picture) and slight reduction on its size (36 x 38 mm). The lacuna is still large. **D.** The lumen, on Day 26, shows a small size for the gestational stage (arrows) and no viable embryo is seen.

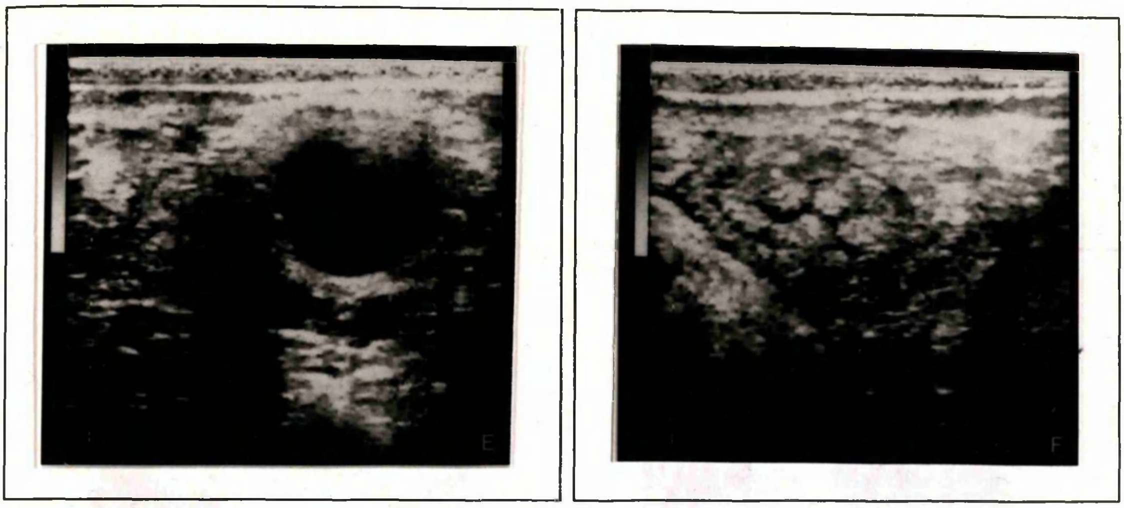


Figure 3.29: Cow 26 on Day 26 after breeding. **E.** The corpus luteum exhibits a poor echotexture and no defined outline. Its size has reduced. **F.** The horn is showing a folded endometrium and a normal physiological amount of fluid within the lumen.

### 3.2.3 Uncertain Negative Pregnancy

#### Cow 11

After last standing oestrus, the animal returned to be reexamined on Day 17. Visualization of the ovary bearing the corpus luteum (22 x 20 mm) was not easy, the cow was moving constantly and hence the images seemed to be out of focus. The horn was visualized in both longitudinal and cross sectional planes, but was not very clearly seen in longitudinal plane. The nonechogenic lumen showed a distinct region (vesicle). The vesicle imaged in cross sectional plane showed an apparent early embryo within it. As the luteal gland could not be properly imaged, and hence, evaluated, but seemed to be regressing, the pregnancy diagnosis was considered to be uncertain negative. On Day 24, the corpus luteum was apparently regressing and the lacuna was about the same size it was on Day 17. The uterine horn was exhibiting a large lumen and the embryo-like structure increased in size and was clearly observed, even though considered to be small for the age. The presumptive embryo was visible but its heart beat could not be detected, suggesting embryonic death. A negative pregnancy diagnosis was achieved based on the findings and failure to detect a viable embryo (see Figures 3.32 to 3.33) The cow was observed in standing oestrus 54 days after last scanning, showing 78 days interval between observed standing oestrus. The animal was subjected to another session of scans and pregnancy was diagnosed on Day

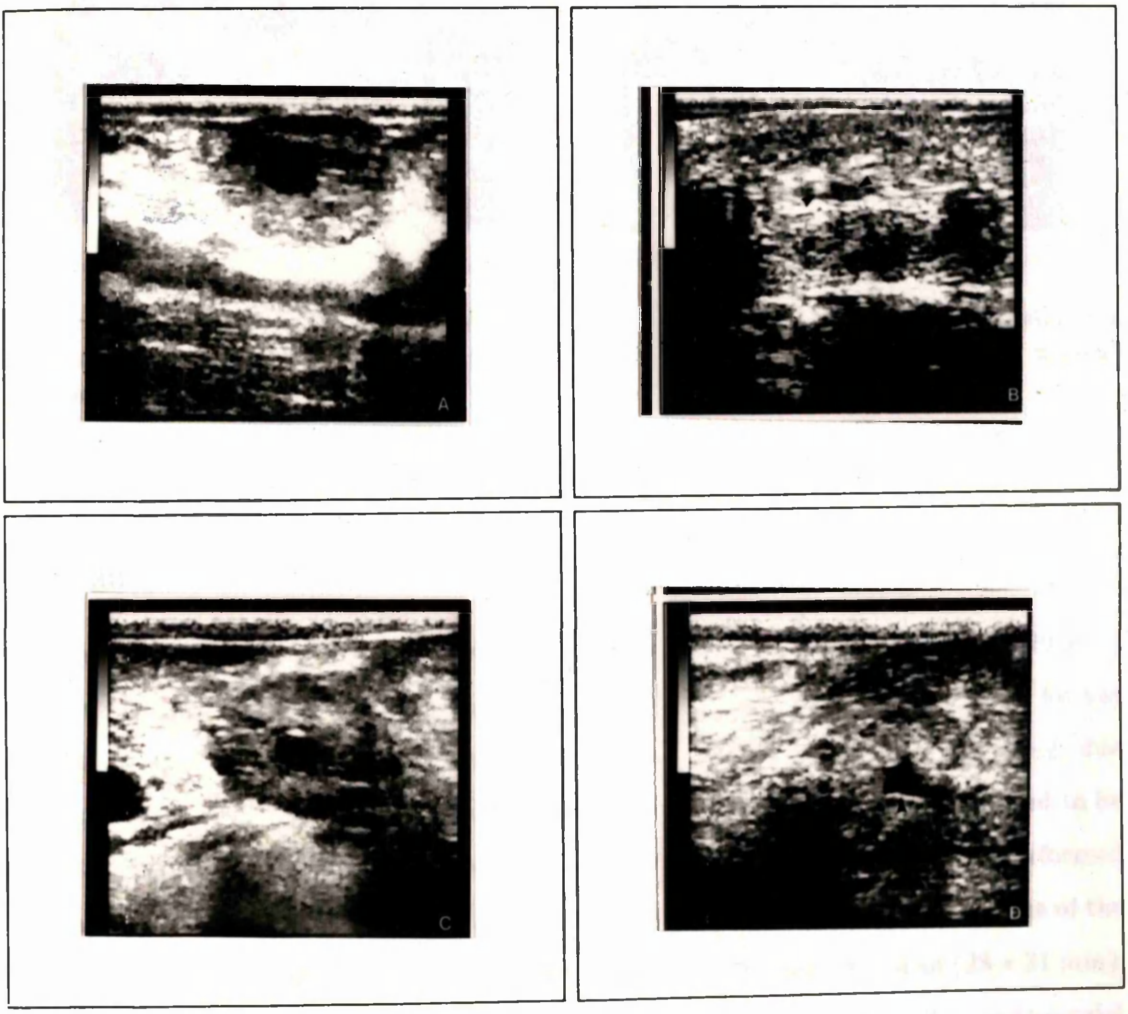



Figure 3.30: Cow 11, on Days 21 and 25. **A** and **B**. Day 21 after breeding. The corpus luteum presents a good echotexture (24 x 28 mm) and a lacuna. The uterine horn shows an echogenic structure within the lumen (arrow). **C** and **D**. Day 25 after breeding. The corpus luteum is showing changes in its echotexture, but has slightly increased in size. The uterine horn is distended and exhibits echogenic structures lining the endometrium (arrow).

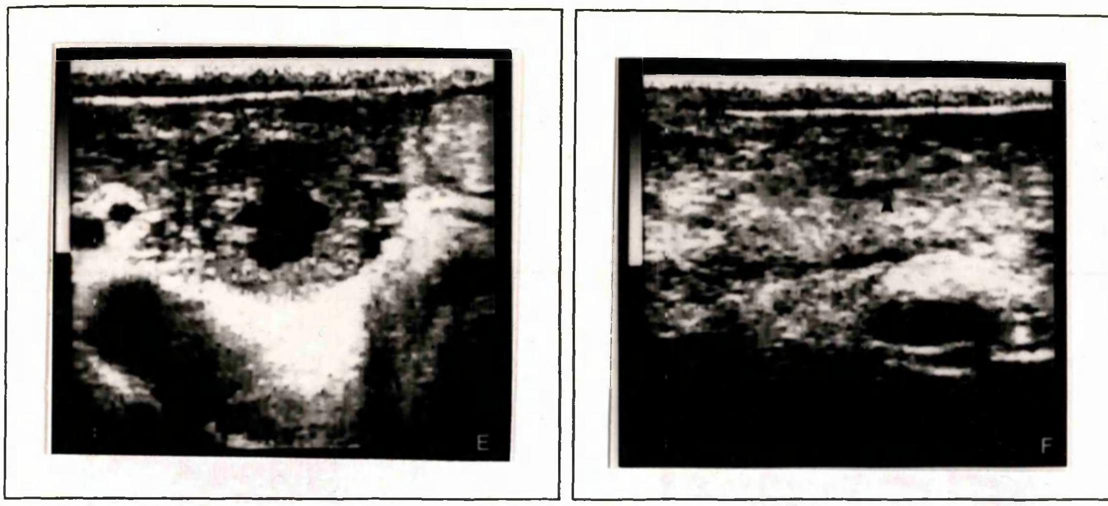



Figure 3.31: Cow 11 on Day 35 after breeding. **E** and **F.** The luteal gland shows a flattening lacuna. The uterine horn shows a discrete lumen and no embryo is present (arrow).

17, 24 and 31, with embryo and heart beat detected.

### Cow 30

First scanned on Day 16, the animal showed a well formed luteal gland (29 x 29 mm) in one ovary. The horn had a distended lumen, but with a large amount of fluid for the gestational age. The scanning performed in this day was not permanently recorded, due to inappropriate connection of the outlet of the video. The diagnosis was considered to be a doubtful negative based on the size of the uterine lumen. Another scan was performed on Day 18. The corpus luteum had changed its outline, which was observed in some of the images obtained. Its size was about the same observed at the previous scan (28 x 31 mm). The lumen had reduced in size, but was still large for the gestational age. The endometrial layer was not well defined. The negative pregnancy diagnosis was then confirmed, based on the criteria applied. Another examination was performed on Day 23. A small lacuna was seen and the corpus luteum had slightly reduced in its size (28 x 22 mm). The lumen was reduced and not clearly nonechogenic. It was considered to have a small amount of fluid for the gestational stage, and no embryonic heart was detected; this enhanced the negative diagnosis. In the last scanning, on Day 28, the corpus luteum, was apparently regressing and a small lacuna could be visualized. The lumen had increased in size and exhibited a lumen which was slightly turbulent. The cow returned to standing oestrus 7

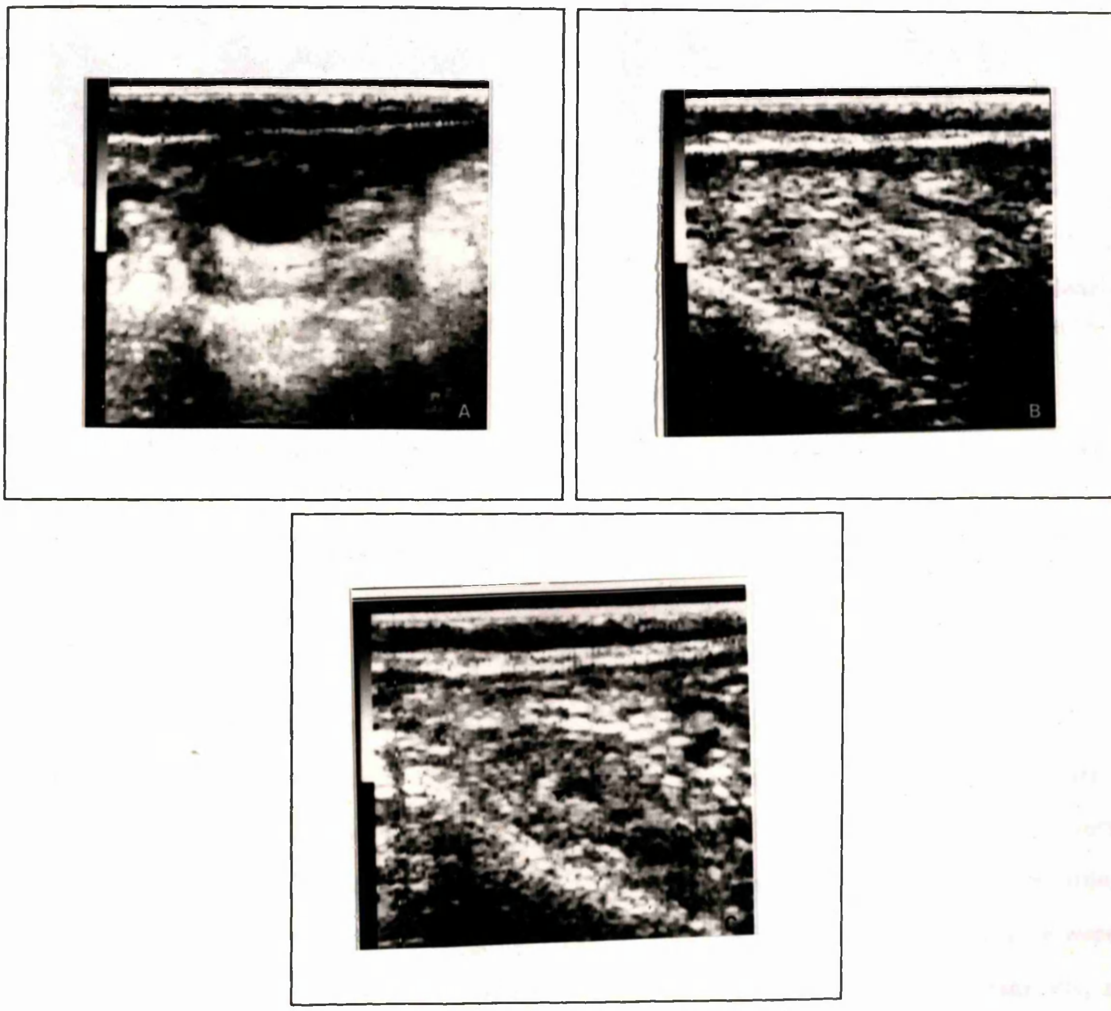



Figure 3.32: Cow 11, Day 17 after breeding. **A**, **B** and **C**. Note the ovary showing a lacuna. The ovary is not clearly imaged. The horn, in longitudinal section shows a lumen with a discrete dilatation (arrow). The horn in cross section shows a vesicle with an echogenic structure within it. This structure resemble an early embryo (arrow).

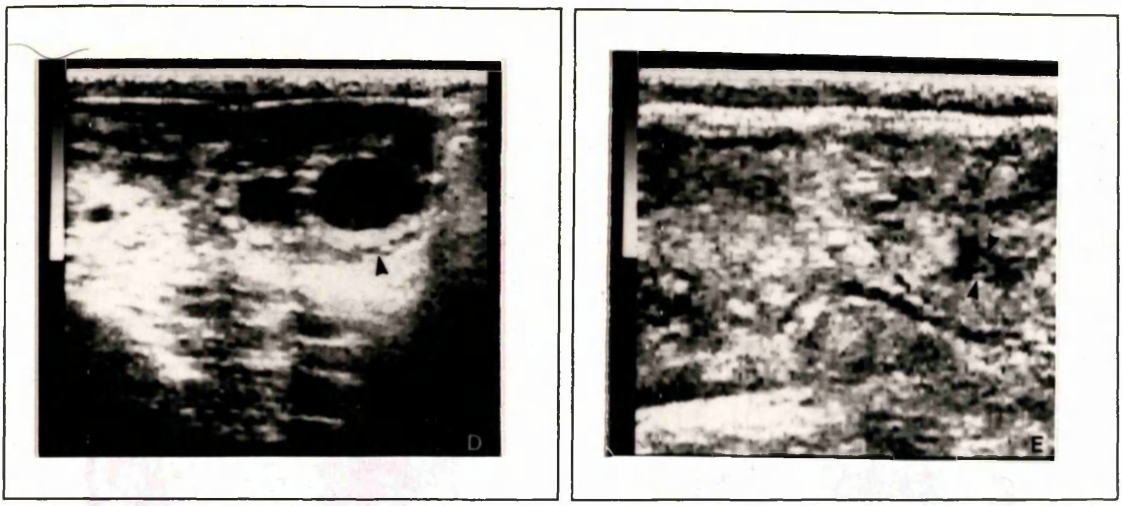



Figure 3.33: Cow 11, Day 24 after breeding. **D** and **E.** The corpus luteum is not clearly visible (arrow). The uterine horn shows a small lumen with an embryo-like structure within it (arrows). No heart beat was detected.

days after the last scan and then conceived. Later, it was found that the cow had been treated for an extreme mucopurulent vulvar discharge prior to the scanning. The interval between the two oestrus records was 37 days (see Figures 3.34 to 3.35).

### Cow 29

Examined on Day 18 after oestrus, the luteal gland did not have a clearly defined appearance. A lacuna was present in the luteal gland, which in some images achieved, appeared to be undergoing changes to its outline. The horn seemed to be small, as the muscles layers were not easily visible. The lumen was distended, but no early embryo was discernable. As imaging of the corpus luteum suggested that it was regressing, the uterine layers were not well discernible, and the early embryo was not present to enhance the diagnosis, a doubtful negative pregnancy was concluded. On Day 25, the animal was examined. The luteal gland had increased in size and the lacuna reduced. Due to erroneous connection of the video outlet, the animal was rescanned on Day 27. The images showed an apparent regressing corpus luteum (20 x 22 mm) exhibiting a very small lacuna. The lumen was distended and an echogenic small structure was seen within it. However the amount of fluid was less than that expected for the gestational age and embryonic heart beat was not detected. The cow was observed to return to standing oestrus 5 days after the last scan; with 32 days interval between oestruses. The later oestrus led to conception (see

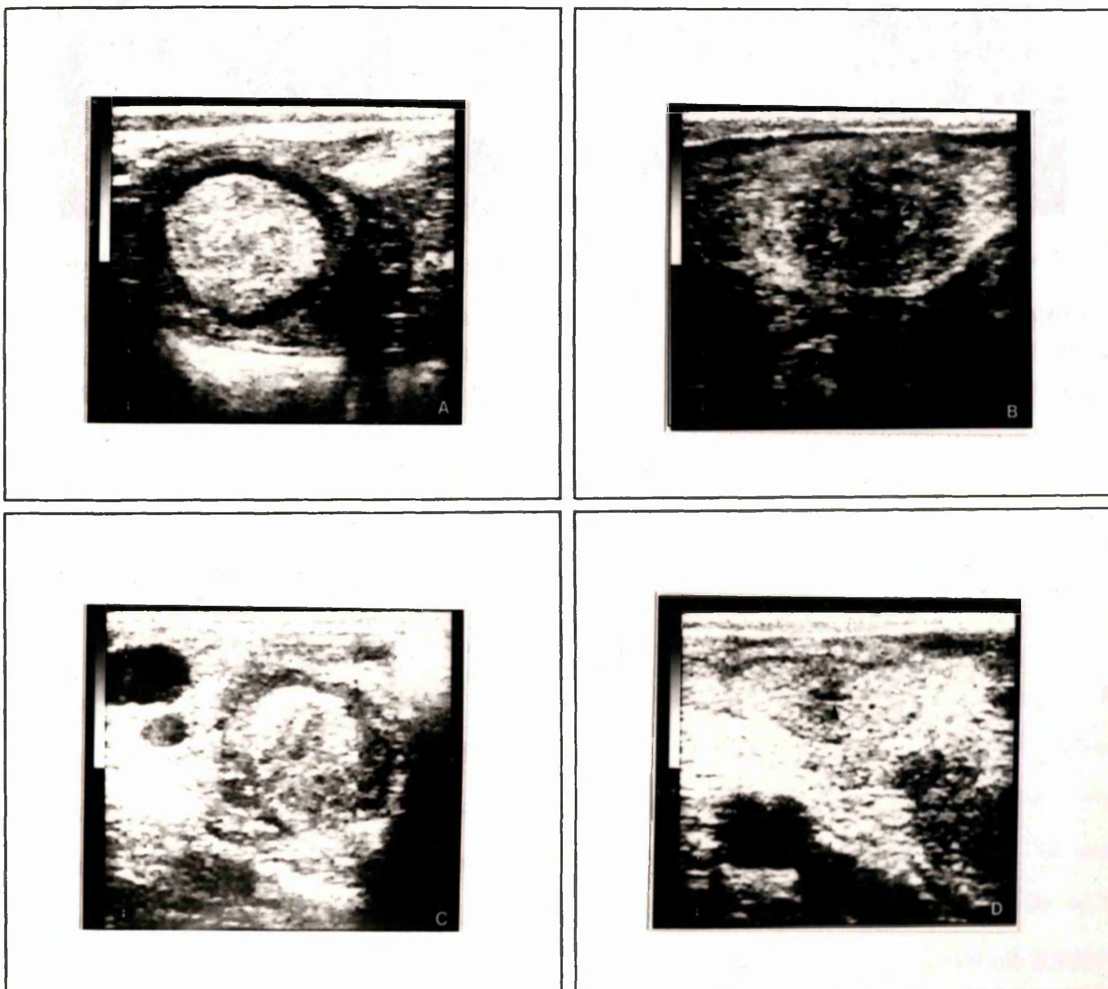



Figure 3.34: Cow 30 on Days 18 and 23 after breeding. **A.** Day 18. The corpus luteum has a hyperechoic appearance. **B.** Day 18. The uterine horn shows a small lumen, considered to be large for the gestational age and has an endometrium not well defined (arrows). No early embryo or vesicle is seen. **C.** The corpus luteum on Day 23. The echotexture and outline of the gland has changed. **D.** Day 23. The horn has a distended lumen (arrow) and no detectable embryo.

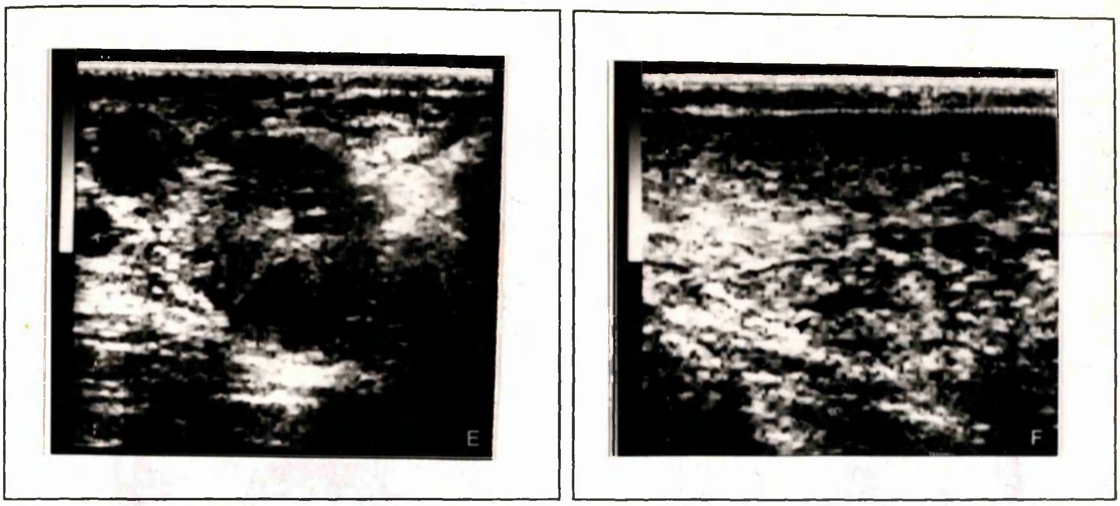


Figure 3.35: Cow 30 on Day 28 after breeding. **E.** The corpus luteum is clearly regressing with no defined border (central picture). **F.** The uterine horn shows a distended lumen with a turbulent fluid (arrows). No embryo detected.

Figures 3.36).

### Twin Pregnancy

Twin pregnancy was diagnosed in one cow (C6), which was first examined on Day 18. The scan of the ovaries showed one ovary bearing two corpora lutea. Both corpora lutea could not be imaged in the same frame, but could be noted as two distinct structures (20 x 20 mm, 20 x 15 mm), by slowly rotating the transducer. Both uterine horns were distended, exhibiting a nonechogenic lumen. A vesicle (early conceptus) could be seen in each horn and also some faint echogenic structures within it, however a discrete early embryo was not discernible. The diagnosis was positive and probably a twin pregnancy, as the contralateral horn also showed a distended lumen.

Six days later (Day 24), the cow was submitted to a second scan. The two corpora lutea were then imaged together on the screen of the ultrasound. Echotexture and border of both luteal glands showed a normal appearance (about 20 x 20 mm). In both horns a small lumen could be seen. The embryos were not clearly visible due to their small size. Both embryos could be seen, but obtaining a still image of the embryos was very difficult, since they easily disappeared and appeared on the screen of the monitor. Due to their size, any movement of the cow would make their visualization harder. The heart could be confidently seen in one of the embryos. The other embryo showed a rhythmic movement

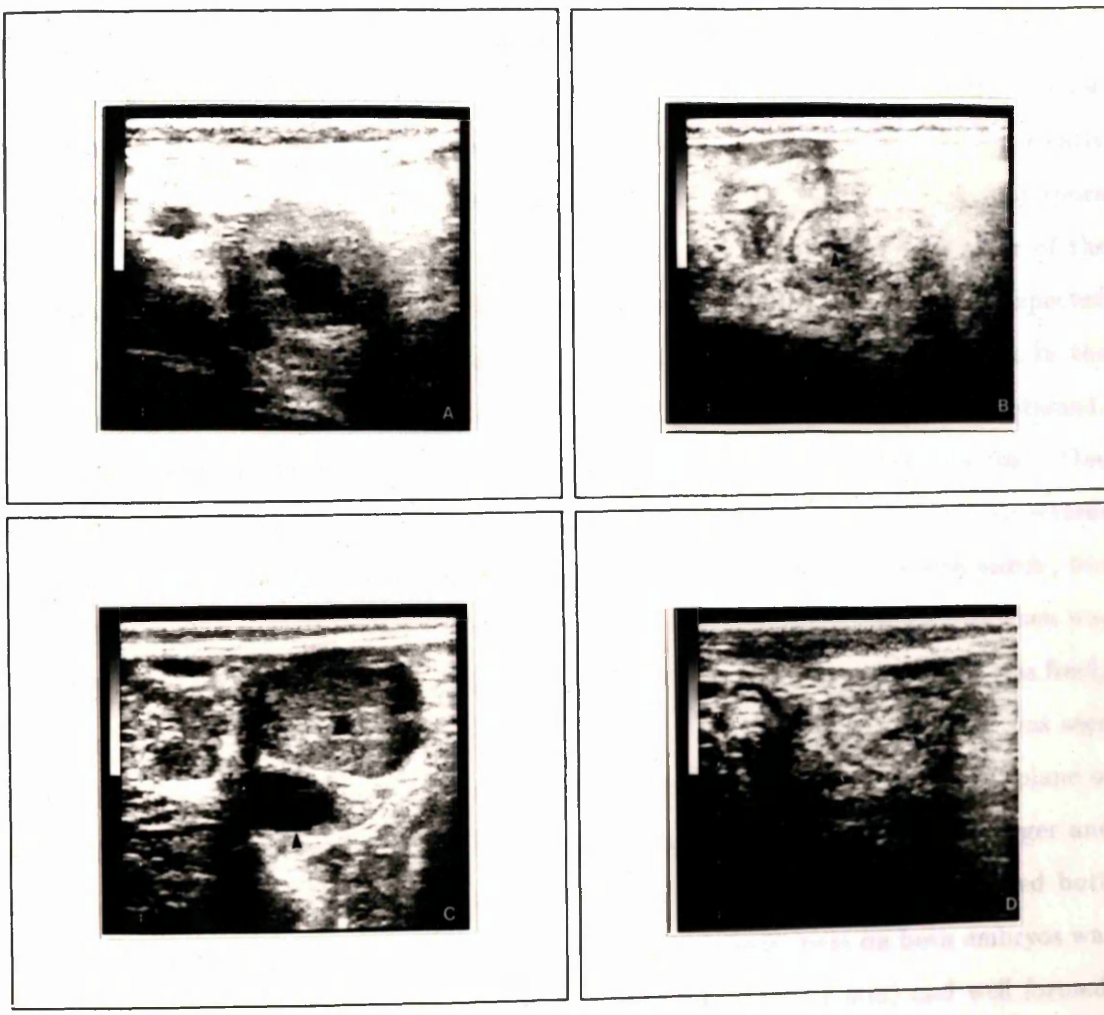



Figure 3.36: Cow 29 on Days 18 and 27 after breeding. **A** and **B**. Day 18. The ovary was not well imaged; the lacuna and the echotexture of the luteal gland are poorly defined (central picture). The lumen of the horn is distended (arrow), and the uterine muscle layers are difficult to be seen. **C** and **D**. Day 27. The ovary shows a poorly defined corpus luteum and a large follicle (arrow). The lacuna is very small. The uterine horn has a distended lumen, larger than seen at the first scanning and an echogenic structure can be seen within it (arrow).

when imaged for few seconds. The embryo could be seen in subsequent images, but the heart beat was no longer detected. The positive diagnosis was sustained based on the corpora lutea and apparently viable embryos.

On Day 29 after breeding, a third scan was performed. The two corpora lutea were about the same size as observed in the prior scan (21 x 20 mm), with normal echogenicity and a well defined border. The horns were seen with a large lumen, however visualization of the embryo was still difficult because of their size. Embryonic heart beat was clearly detected in both embryos. The diagnosis was maintained as positive, based on the corpora lutea and the embryonic heart beat. At this stage a slight difference in the size of the embryos began to be noticed. Though small for the gestational day, which may be expected with twins, the embryo on the ipsilateral horn seemed to be bigger than the one in the contralateral horn. The latter could be observed by magnification of the frame obtained.

Ultrasonographic visualization of the embryos on Day 36, was easily achieved. One embryo, located in the ipsilateral horn, was clearly seen and the embryonic structures easily distinguishable. The other embryo, on the contralateral horn, was also visible, but the embryonic structures were not easily discernible. Their position within the lumen was also different. In longitudinal plane, the embryo ipsilateral to the corpus luteum was freely positioned in the center of the nonechogenic lumen. The contralateral embryo was seen at the lower portion of the nonechogenic lumen of the uterine horn, in the same plane of view. The last scan was on Day 43 after last standing oestrus. The lumen was larger and hence visualization of the small embryo was difficult. The image obtained showed both horns at the same frame on the scan of the ultrasound. A heart beat on both embryos was detected and the corpora lutea were larger (22 x 26 mm, 25 x 22 mm) and well formed. The embryos were obviously distinguishable by their location within the horns and by their size, one measuring 25 mm and the other 20 mm, in the ipsilateral and contralateral horns respectively. The diagnosis was positive for twins pregnancy. See sequence of Figures 3.37 to 3.39).

## 3.3 Discussion

This study accomplished the objective of accessing the efficiency of B-mode ultrasound scanning for determining early pregnancy diagnosis in cattle under field conditions. For

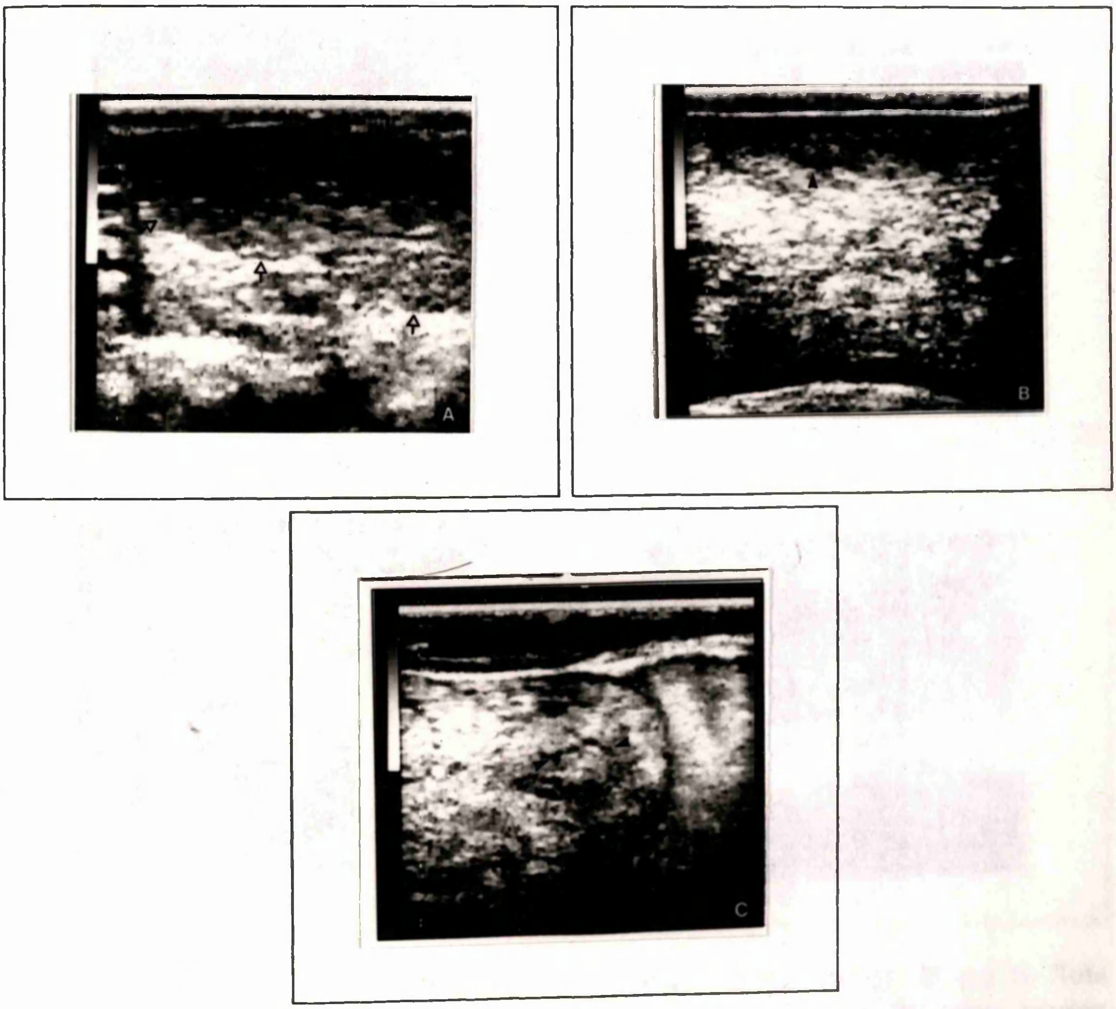



Figure 3.37: Twin pregnancy on Day 18 of gestation, Cow 06. **A**, **B** and **C**. The two corpora lutea have a well defined appearance (open arrows). Both uterine horns show a distended nonechogenic lumen (arrows).

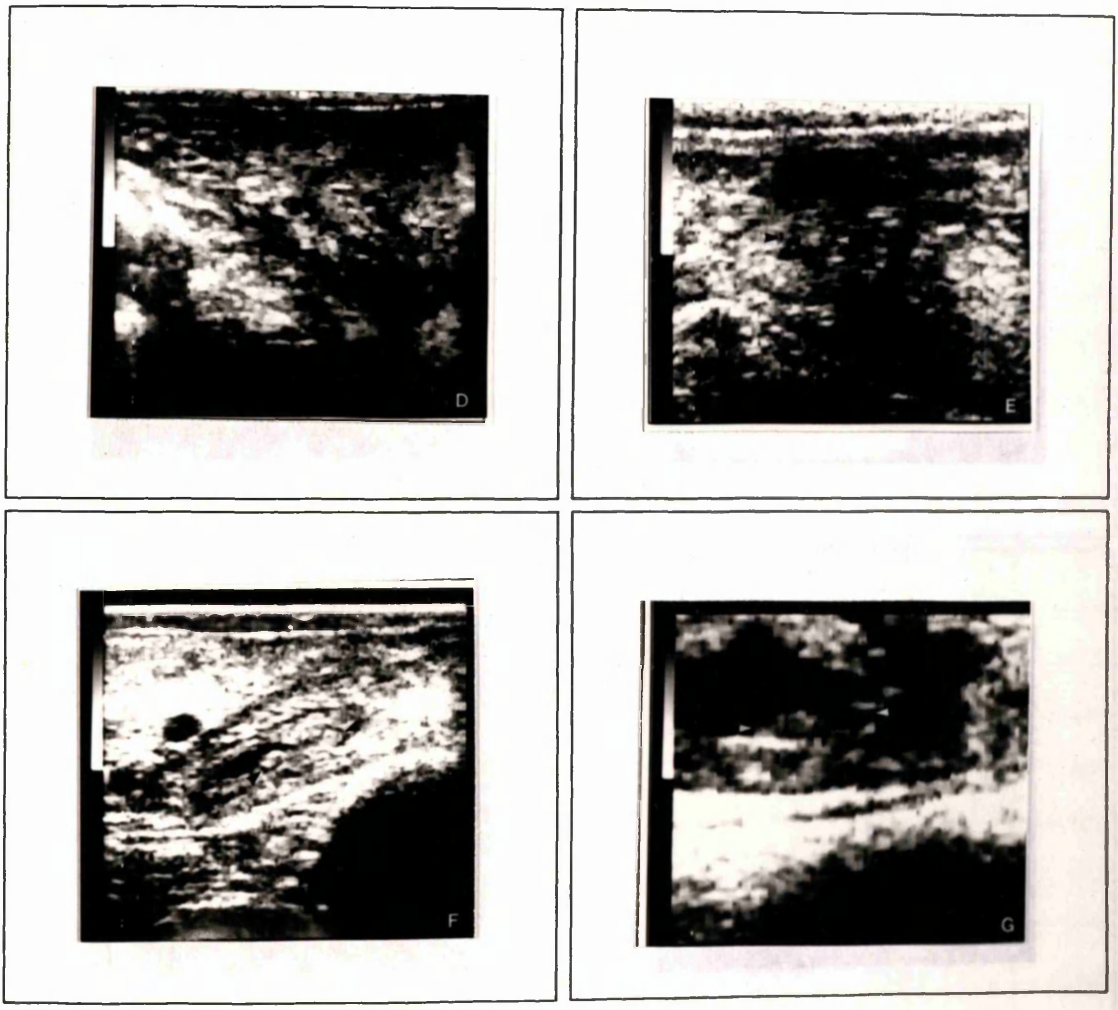



Figure 3.38: Twin pregnancy on Days 24 and 29 of gestation. Cow 06. **D** and **E**. Note the small lumen already present on Day 24. The horn ipsilateral to the ovary bearing the corpora lutea shows a larger lumen. The contralateral horn exhibits a smaller lumen. The embryos are small, but can be seen and heart beat was detected at the scanning (arrows). **F** and **G**. The embryos, on Day 29, can be seen (arrows). The embryo within the contralateral horn was visualized with a enlarged image. Heart beats were detected during the scanning.

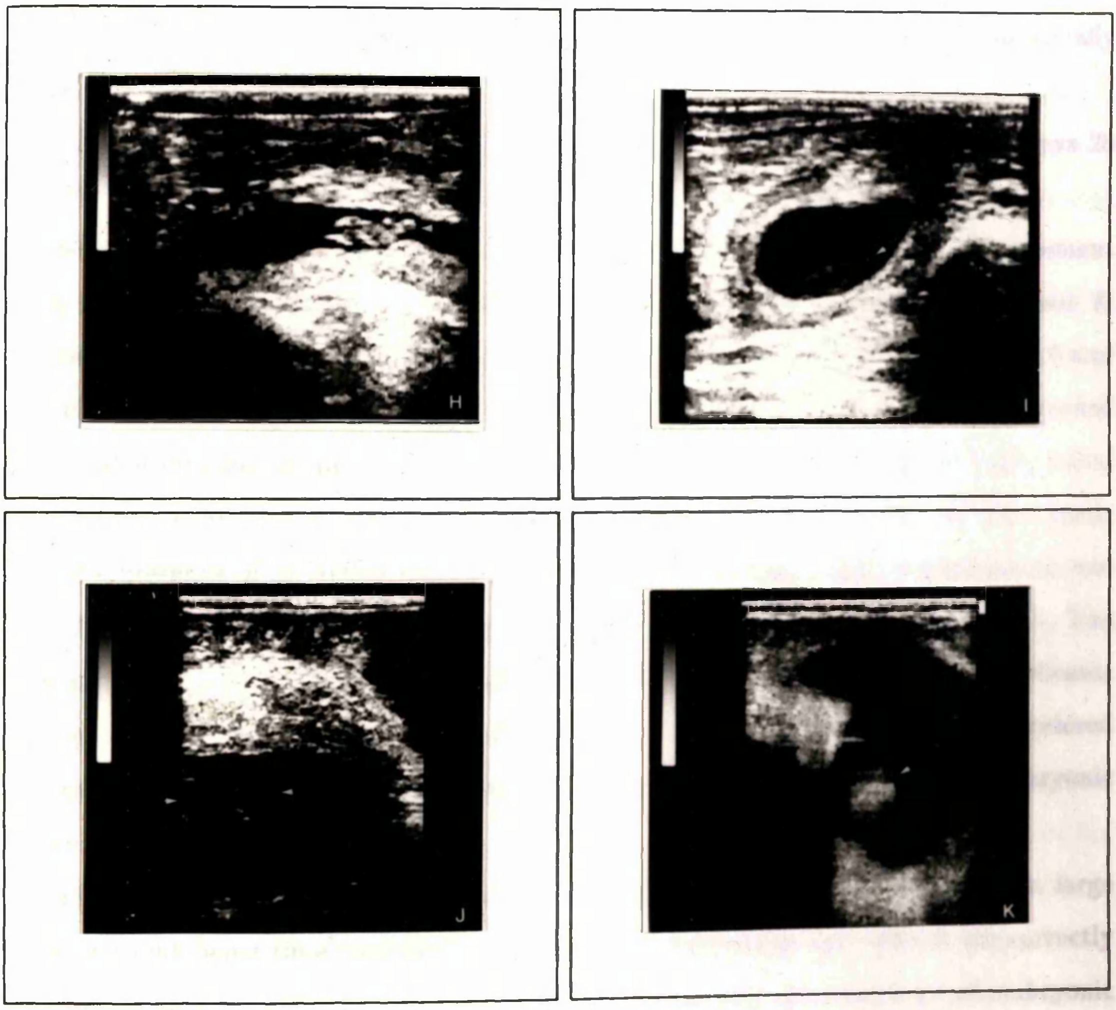


Figure 3.39: Twin pregnancy on Days 36 and 43. Cow 06. **H** and **I**. Twin foetuses on Day 36 of gestation. Note the difference in their size and position within the different horns (arrows). **J** and **K**. Day 43 of gestation. The embryos are clearly separated. The position they occupy within the horns remains different (arrows).

this purpose, a variety of factors that could affect the effectiveness of this method was identified and analysed, and the parameters of examination were defined to help to achieve a high degree of accuracy.

Using a high frequency transducer (7.5 MHz), cows were examined from Days 16 to 34 after breeding. As a matter of organization and analysis of the results, examinations were performed in three different gestational stages, Days 16 to 20, Days 21 to 27 and finally Days 28 to 34 after breeding.

The collected data showed that the later stage of scanning, the period from Days 28 to 34 of gestation, proved to be 100% accurate for identification of

both positive and negative diagnoses. See Table A.3. This result was in agreement with Fissore (Fissore, Edmondson, Pashen & Bondurant 1986) and Pierson (Pierson & Ginther 1984*a*), although these authors were using transducers with frequencies of 3.5 and 5.0 MHz. At this gestational stage, the criteria used for diagnosing pregnancy consisted of the identification of an uterine lumen, with a relatively large amount of fluid; foetal membranes, exhibiting a turgid appearance; a viable foetus with detectable heart beat; and the presence of an active corpus luteum, showing its characteristic echotexture and well defined outline, emerging from the ovary usually ipsilateral to the pregnant horn. The absence of an active corpus luteum and a viable foetus with heart beat, were an indicator of a negative diagnosis. The failure to detect even one of these criteria, was considered as negative. Non detection of heart beat at this age is commonly indicative of embryonic death.

This window of time would be highly suitable for diagnosing pregnancy in a large herd, without being time consuming; as long as the procedures and criteria are correctly applied. There is, also, the advantage that the probability of occurrence of embryonic loss, which would change the results, is greatly reduced (Kastelic, Northey & Ginther 1991), as the majority of embryonic deaths occur before day 18 of gestation (Curran et al. 1986*b*, Roberts, Schalue-Francis, Francis & Keisler 1990) and also up to Day 24.

The gestational stage ranging from Days 21 to 27, also proved to be a highly accurate time for diagnosing positive and negative pregnancy. The results showed a 100% accuracy. See Table A.3. Using 3.5 and 5.0 MHz transducers, there are several studies, performed at this time, that showed successful results (Hanzen & Delsaux 1987, Kastelic et al. 1988, Totey, Singh, Taneja & Talwar 1991, Kastelic, Northey & Ginther 1991). The scanning criteria used in this stage were similar to that in the later gestational period (28 to 34). The uterine lumen was, obviously, small compared to the later stage, but foetus and heart beat were readily visible.

On Day 21, the early embryo is occasionally not clearly visible and hence, detection of the heart is difficult. When the embryo can be visualized, but the heart beat is not detected, a detailed observation of the corpus luteum is essential. A positive diagnosis, in such case, can be supported by the presence of an evidently active corpus luteum. If the corpus luteum does not show a consistent well formed appearance, the diagnosis may certainly be negative. The failure to identify both the embryo and the heart beat, even with observation of an apparently well formed corpus luteum, would be reason for a negative diagnosis. There have been studies reporting that the luteal function may be prolonged after embryonic death (Humblot, Camous, Martal, Charlery, Jeanguyot, Thibier & Sasser 1988). A consecutive scanning would, certainly, provide an accurate diagnosis, with the absence of a viable embryo and the presence of an actively regressing corpus luteum.

In this experiment, uncertain diagnoses did not occur during this period. A negative diagnosis at Day 21 was given when both criteria, a small lumen in the horn and a corpus luteum in the ovary, could not be observed. The presence of a noticeable regressing corpus luteum was also indicative of negative pregnancy, even with the observation of a small lumen and an apparently viable embryo (heart beat). Also, the visualization of an apparently active luteal gland and non detection of a viable embryo was indicative of non pregnancy; since embryonic loss is a possibility at this stage. Kastelic (Kastelic, Bergfelt & Ginther 1991) reported, in his experiments, that embryonic death (failure to detect embryo proper) could be detected in heifers on Days 21 and 22, and the onset of luteal regression was detected on Days 19 and 22, respectively; and the second ovulation was observed on Day 23 for each animal. He also detected pregnancy (presence of heart beat) on Day 20 and confirmed on Day 21; and on Day 22 embryonic death was detected, with onset of luteal gland regression and second ovulation detected on Days 19 and 23, respectively.

At Day 21, one cow (C11) was diagnosed as a non pregnant animal, although carrying an embryo like structure within the lumen and an apparently active corpus luteum. Imaging the horn to obtain an appropriated visualization of the embryo was not achieved and the embryonic heart beat was not detected. This absence of a truly discernible embryo and its heart beat, was interpreted as a presumptive embryonic death, since the findings were adhering to the criteria used, and thus, a negative diagnosis was concluded. Echogenic structures resembling embryonic membranes (producing specular reflection) within a considerable large amount of uterine fluid, could be seen at the time of the second scanning, but no presence of an embryo proper, which confirmed the diagnosis. The persistence of the corpus luteum undergoing slow regressing was also observed. It seemed that there was further development of the uterine lumen, between the first and second examinations, but not probably the embryo, since it was not observed. The luteal gland seemed to start regressing, with change in its echotexture, after an increase in its size since the last observation. A presumptive embryonic death was concluded, as no prior scan was performed and nor heart beat detected. The embryonic death, in this cow, seemed to occur before the onset of luteal regression. Kastelic (Kastelic, Northey & Ginther 1991) reported that in cases that embryonic death preceded luteal regression, the volume of uterine contents seemed to be maintained until approximately the time that the cervical patency was detected. Presumably, embryonic death occurring 16 days after standing oestrus or later, may extend the lifespan of the corpus luteum.

This time slot (Day 21 to 27) would be appropriate for a relatively skillful ultrasonographer, scanning at a reduced rate of turn over, as there is necessity for close scrutiny during the examination, to identify the early embryo and heart beat. However, a careful scanning, in this time space, would be altogether applicable for pregnancy diagnosis, but confined to being used on a small herd number; being more suitable to the ultrasonographer and to the herdsman who is monitoring his breeding programme.

The examination from Days 16 to 20, presented a much more challenging proposition for routine pregnancy diagnosis. The corpus luteum is known to persist as an active unit until Day 16 post ovulation, even if conception has not taken place (Omran 1989). Thus, from Day 17 the regressing luteal gland may be noticeable in a detailed examination, consisting in changes in its echogenicity and flattening of its outline. This process lasts till approximately Day 19, when the new follicles are seen emerging from the ovarian stroma.

The presence of a discrete fluid filled lumen had to be supported by the presence of an active corpus luteum. On occasions, it was possible to identify an echogenic structure (early embryo) within the nonechogenic lumen of the horn. The observation of a presumed early embryo could be of great value for obtaining a confident diagnosis.

During the course of the study in this gestational stage, four embryonic losses were observed to have apparently occurred. As at the time of first scans, Days 16, 17 and 19, an embryonic heart beat is usually not perceptible by ultrasonography, the positive diagnosis could not be based on the visible beating heart of the early embryo; but it was based on the presence of a visible early embryo and apparently well formed luteal gland. Among these presumptive early embryonic losses, one cow (C11) was diagnosed as uncertain negative for a probable early embryonic death. The other three cows (C13, C31 and C26) were diagnosed as pregnant, based on the presence of an early embryo and observation of an active corpus luteum.

During the interval between fertilization and implantation of the embryo to the uterine wall, embryonic signalling is necessary for maternal recognition of pregnancy. This signal, there are likely to be more than one, seems to induce hormonal changes associated with pregnancy, such as uterine transformation necessary for the process of implantation and also mantainance of the luteal gland. The process of attachment of the bovine embryo to the uterine wall occurs during the 11 to 12 days after fertilization (Winters, Green & Comstock 1942). The failure to progress pregnancy, after fertilization has occurred, resulting from early embryonic death before the moment at which antiluteolytic signal is presented (Day 16), is of high incidence in the early stage of gestation. Research works in this area reported an estimated 51.7% of embryonic death from Days 16 to 34 (Hawk, Wiltbank, Kidder & Casida 1955) and an incidence of 25% of embryonic mortality before Day 25 post insemination (Laing 1949).

Presumptive embryonic losses were noted in the present study, where the findings obtained were indicative of established pregnancy that failed to progress. Whether the death of the embryo preceeded or not the onset of regression of the luteal gland, was not confidently established. Kastelic (Kastelic, Bergfelt & Ginther 1991) showed in his experiment, that in some cases in which embryonic death preceeded luteal regression, the volume of the uterine fluid (conceptus) seemed to decrease gradually from the time of embryonic death to the day prior to detection of ovulation and the corpus luteum was observed to persist longer.

The findings of the current experiment showed that during the interval between the first and the second scanning, of these cows that underwent embryonic loss, there was maintenance of the luteal gland and further development of the conceptus. The later was based on the enlargement of the lumen of the horn, but which failed to continue growing at the expected rate between the two scans. These results may lead to a conclusion that the onset regression of the luteal gland proceeded the embryonic death.

In cattle, the majority of embryonic deaths occur before day 18 of gestation (Roberts et al. 1990). When it takes place at or after Day 16, the corpus luteum may persists for a period of time before total regression and thus, the incidence of false positive pregnancy diagnosis is observed. Inevitably, in this period of gestation, the early embryonic loss makes a 100% accuracy of diagnosis impossible.

Three uncertain diagnosis were obtained in this period of scans (Day 16 to 19). The uncertain diagnoses were considered to have a reliability higher than 50% for negative diagnosis, but not 100% accurate, which was why they were classified as uncertain negative diagnose. One of these cows (C11) was already discussed. A second uncertain negative diagnosis (C30) was achieved based on the collection of fluid within the uterine lumen, which was considered to be an amount too large for the gestational age, although the corpus luteum had apparently normal echogenicity and well defined border. The subsequent scans showed regression of the luteal gland and the presence of no embryo. A third animal uncertainly diagnosed as negative, exhibited a lumen with an amount of fluid compatible with the gestational age, though not clearly seen, and a luteal gland not confidently determined as an active gland. In the consecutive examinations, the cow showed an increase in the uterine lumen and even the presence of an echogenic structure within it. The negative diagnosis was confirmed by the absence of a viable embryo. The doubtful diagnoses, could be related to the physiologic intrauterine fluid collection that are commonly seen in the early gestational stage, being a reason for difficult distinction between them (Kastelic, Bergfelt & Ginther 1991); and also to infertility problems in the history of the animal (Chaffaux et al. 1986). Later, it was found that cow 30 had had a mucopurulent vulval discharge for about one month and had received treatment. The discharge was last observed four days before the standing oestrus, which led to the session of scans performed in this study.

All cows in this experiment, early diagnosed as pregnant that failed to continue pregnancy, as well as the uncertain diagnoses, showed long intervals to return to standing oestrus (between services). The intervals observed varied between 32 to 80 days. Olds (Olds 1969) reported that long interval between services (26 days or more) and missed oestrus can be as damaging as repeated services, for the animal production programe. Some of these cows not only had a long interval, but also had repeated oestrus. This occurrence is indicative of animals of low fertility, which consequently can make early pregnancy diagnosis difficult, due to early embryonic mortality.

The statistical results at this stage were not as accurate as in the other two stages of gestation (Days 21 to 27 and 28 to 34). This early stage showed 100% sensitivity, as there was no incorrect positive pregnancy diagnosed. The specificity was 67%, as the early embryonic losses led to failure to progress in pregnancy. The negative predictive value was 100% accurate, since no incorrect negative pregnancy was present. The positive predictive value was 85% correct, since the incidence of incorrect positive pregnancy was observed due to the embryonic losses. It was evident that a strict criteria must be applied, during this stage, in the interpretation of the findings, aiming to accurately diagnose.

During the course of the research, it was observed that the presence of large follicle(s) at the same ovary bearing the corpus luteum, was not necessarily indicative of a regressing luteal gland, if the corpus luteum had a well defined shape and echotexture. This conclusion was achieved by the presence of large or medium follicles, with a corpus luteum in the same ovary, in cows that were diagnosed pregnant and later calved. Another important point, is the size of the corpus luteum. Some cases showed a large corpus luteum at the first scan and an increase in its size at the time of the second examination, but showing a reduction in size in subsequent scans. Also developing follicles were observed in the same ovary bearing the luteal gland, throughout the scans. Thus, specific size of a luteal gland at different gestational stage is not possible to be defined, as it varies between animals. What is certainly possible is the definition of the echotexture of the gland, which has to be the same throughout the gestational period. The corpora lutea presenting lacuna, usually showed reduction in the size of the latter as pregnancy progressed.

There are a variety of factors which influence the accuracy of early ultrasound pregnancy diagnosis, among these, are the day of examination after breeding; the skill of the ultrasonographer; the previous knowledge of the breeding history of the animal under examination and the frequency of the transducer used to perform the scanning. The collected data showed that there is a considerable difference between the accuracy of the scanning performed between Days 16 to 19 and those performed at a later gestational stage, on Days 20 to 26. It was concluded that the difference in the accuracy of the scans carried out before and after Day 20 are somewhat related to early embryonic mortality.

It would appear that the figures could be construed in one of two ways: if the criteria for failure in the early pregnancy diagnoses is simply taken as a failure to identify pregnancy at later dates, the accuracy would be 88%. However, if confidence in the first scan, which an early embryo was clearly distinguishable in the images obtained and the luteal gland clearly active was maintained, the discrepancy could be put down to early embryonic loss and therefore the accuracy of this first scanning period would be increased to 100%.

The results of studies performed with transrectal ultrasound to determine the accuracy of this technique, in early stage of gestation in cattle, have shown a large variety of findings and some controversial opinions. Most of the previous studies were carried out using transducer of 3.0, 3.5 and 5.0 MHz and very few used the 7.5 MHz (Omran 1989, Kastelic, Bergfelt & Ginther 1991). The use of a 7.5 MHz transducer in this current experiment, enabled visualization of the early conceptus at the first day of scanning (Day 16), as also reported by Kastelic (Kastelic, Bergfelt & Ginther 1991), Pierson and Ginther (Pierson & Ginther 1984*a*). The embryo proper was first visualized on Days 16 to 19, which is not in agreement with some reports (Pierson & Ginther 1984*a*, Fissore et al. 1986, Hanzen & Delsaux 1987). The heart beat was first detected on Day 20 and in all cows from this day, this observation agrees with those already reported (Omran 1989, Kastelic et al. 1988, Curran et al. 1986*a*) (Tables A.1 and B.1, tb-2, tb-3,tb-4,tb-5 and tb-6).

Using a 7.5 MHz transducer, Omran (Omran 1989) studied animals during the oestrus cycle and diagnosed pregnancy from Day 10 to 20. His experiment proved that knowing the breeding history of the animal under examination, cows could be accurately diagnosed between Days 14 to 17 after service, and even not knowing the breeding history of the animal, a 100% accuracy could be achieved from Day 17 after service. Using the same frequency of transducer, Kastelic and others (Kastelic, Bergfelt & Ginther 1991), working with pregnancy diagnosis between Days 10 to 24, reported that the embryonic vesicle could be apparently seen in the uterine horn from Days 12 to 14. The presence of intrauterine fluid was visualized from Days 10 to 16, but not distinguished from free intrauterine fluid collection, and hence concluding that pregnancy diagnosis before Day 16 could be confounded; considering an accurate diagnosis before this day not more than an opinion.

It is apparent that the time at the first visualization of the conceptus varies markedly with the frequency of transducer used. This present experiment showed that this technique is relatively easy to apply, harmless and confidence is a matter of practice. Undoubtedly, to substantiate the findings of early embryonic mortality, confidence and experience in the use of the ultrasonographic technique is a prime consideration. It was evident that a strict criteria must be applied, during the early stages of gestation (Days 16 to 19), in the interpretation of the findings, aiming to accurate diagnose. Nevertheless it could be an exciting prospect, if this was a realistic and proven method from the point of view of monitoring herd breeding programmes. The use of the technique in the early gestational stage (Day 16 to 19), would be more for research purpose, as it is too time consuming and still to be substantiated. However, studies on much larger numbers, with the increasing quality of the scanning equipment and the improvement of the technique, may yet allow this to be a feasible proposition in herd situations.

As the results suggested, the earliest day for early ultrasonographic pregnancy diagnosis in cattle under farm conditions, capable of giving an optimal result with less cost for the cattle breeder, appeared to be Day 20 after breeding. At this stage, the embryo and embryonic heart beat can be easily visualized, which minimises the incidence of false positive diagnosis due to embryonic mortality, giving a maximum of confidence to the veterinarian or technician performing the examination.

# Chapter 4

# Sex Determination of the Foetus

Foetal sex determination is of important value in the animal breeding industry. Techniques for sex determination of the calf, before pregnancy is established, have been studied and used in the cattle industry, using bull semen, for the enhancement of the reproductive potential of herds (Herr & Reed 1991). There are also, several methods for sexing embryos and foetuses. These methods give highly accurate results at a very early stage of development of the embryo. These are methods that require specialized laboratory facilities and skilled technicians who are able to manipulate foetal fluids and the embryo proper in its early stage of development.

The reproductive organs of the bovine, arise in the early stage of development of the embryo. The primitive reproductive structures of both male and female gonads are present in all embryos. This period of development of the reproductive structures, during which both gonads are present in the embryo, is called the indifferent stage. Later, the gonads develop characteristics of one or other of the sexes, usually with degeneration of the inappropriate structures. This period of development of the sex specific characteristics is called the differentiated stage of the gonad (Noden & de Lahunta 1985).

The external bovine genitalia, are initially formed on the caudoventral surface of the abdominal wall, between the hind limbs. Inomata (Inomata et al. 1982) described that these primordial structures were seen as poorly defined elevations known as the genital tubercle, genital swellings and cloacal folds, which developed into the male or female gonad. This stage was known to occur in up to the 2 cm stage embryo (39 Day). The differentiated stage of the gonads occurred at about the stage of the 2 to 2.5 cm embryo (39 to 43 Day).

The genital tubercle was seen to become vertically elongated and the cloacal folds were separated into the urogenital folds and the anal folds. The genital swellings were still round, being seen as vaguely outlined elevations. From the 2.5 to the 3 cm stage (43 to 46 Day), the differentiation of the external genitalia was observed. In both male and female, the tip of the genital tubercle was directed toward the caudal region and the genital swellings, which were becoming clearly elevated, on either side of the genital tubercle. Also two pairs of nipples appeared close to the midline cranial to the genital tubercle. In the male, the anogenital raphe and the urogenital orifice appeared at sites caudal to the genital tubercle. The opening of the urogenital orifice formed communications with the urogenital sinus between both urogenital folds; whereas in the female, a lack of the urogenital orifice and anogenital raphe was observed. When gonadal sexual differentiation could be detected from a superficial view of the external genitalia, the gonads could be discerned as whether they were male or female. At the 3 cm stage, the genital tubercle began to migrate along the abdominal wall, keeping to the midline between the umbilical cord and the tail, moving toward the umbilicus in the male and toward the tail in the female foetuses (Figure 4.1.

Inomata found that, in the male, the genital tubercle become elongated to form the penis, the urogenital folds enclosed the penis to form the prepuce and the genital swellings become enlarged to develop into the scrotum. In the female, the genital swellings completely disappeared and the urogenital folds developed to enclose the genital tubercle, forming the labia

(Inomata et al. 1982).

Recently, research in the field of ultrasonography, applied to farm animals, has discovered another advantage in the application of ultrasound techniques. The transrectal ultrasound technique has allowed determination of the sex of the foetus in its early stage of development. Sex determination of bovine and equine foetuses is possible by visualization and location of the genital tubercle, in the early foetal stage of development or by visualization of the prepuce, scrotum and mammary gland in later stages of development. Relatively few works have been carried out in this field. The feasibility and accuracy of sex determination of the bovine foetus in the early stage of gonad differentiation is as yet questionable.

In a field study, sex of bovine foetuses, using a 3 and 5 MHz transducer by the tran-

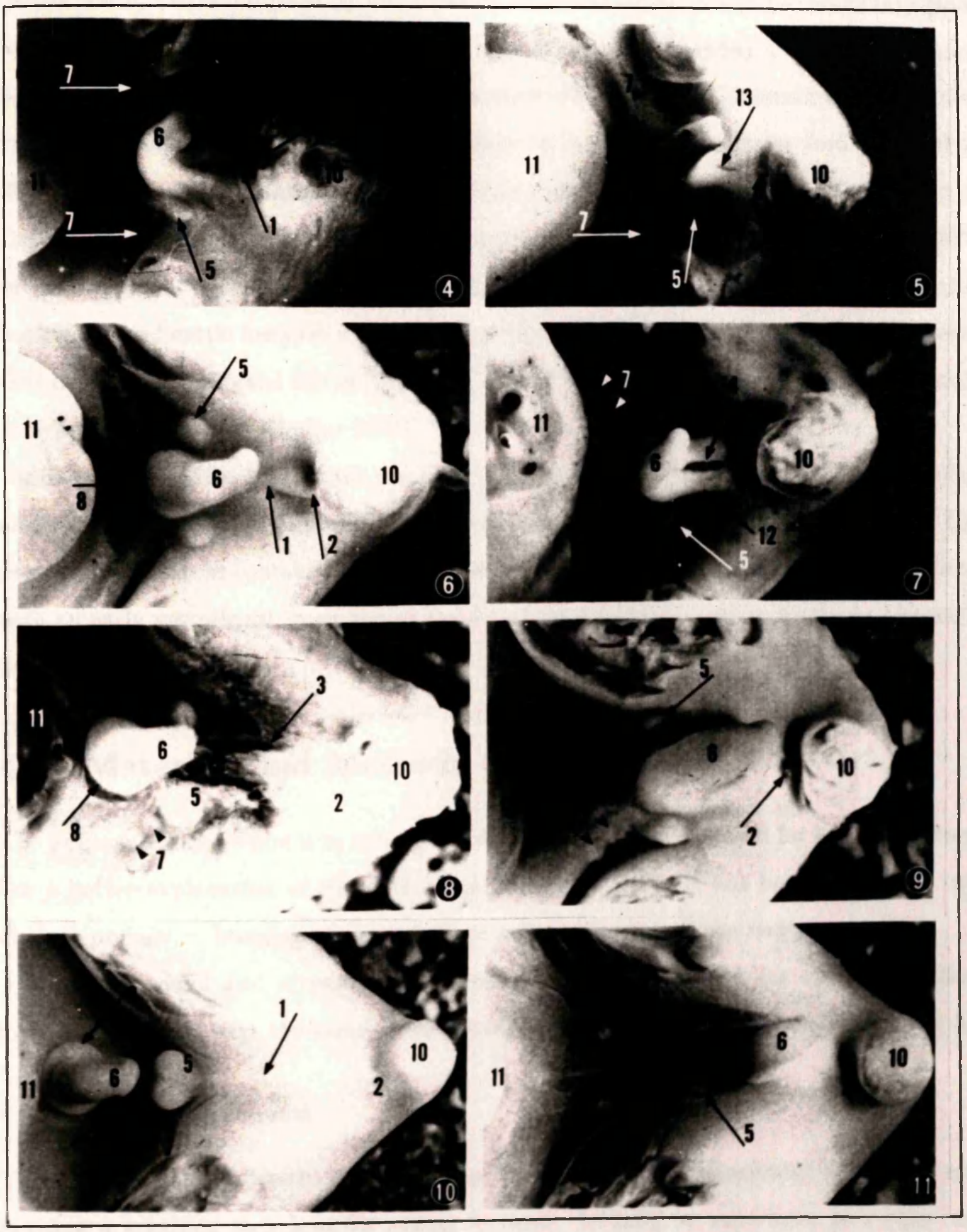



Figure 4.1: The gross development of the bovine external genitalia. Photos kindly given by Inomata et al (Inomata et al. 1982). Photos 4, 6, 8 and 10 are showing the male external genitalia of the foetus on Days 44, 49, 52 and 58, respectively. Photos 5, 7, 9, and 11 are showing the external genitalia of the female foetus on Days 45, 49, 53, and 57, respectively. **1.** Anogenital raphe. **2.** Anus. **3.** Artificial cleft. **4.** Cloacal membrane. **5.** Genital swelling. **6.** Genital tubercle. **7.**Nipple. **8.** Prepuce. **9.** Tail. **10.** Tail cut. **11.** Umbilical cord. **12.** Urogenital fold. **13.** Urogenital Groove. **14.** Urogenital orifice.

srectal route, was determined by visualization of scrotal swellings and the mammary gland between Days 100 and 120 of gestation (Muller & Wittkowski 1986). In early studies, using a 5 MHz transducer, Curran and others reported that the sex determination of equine foetuses was first possible in males from Days 58 to 97, and in females from Days 57 to 68 (Curran & Ginther 1989); and in the bovine the genital tubercle was first identified on Day 50, with reliable determination of the sex beginning on Day 55 (Curran et al. 1989). In later reports, Curran showed that, under farm conditions, the optimal age for sexing both horse and cattle foetuses was from Days 59 to 68 (Curran 1992), and also determined that between Days 55 and 64, the sex of equine and bovine foetuses could be appropriately determined (Curran & Ginther 1991).

Using a 7.5 MHz transrectal transducer for sex determination of the bovine foetus, under field conditions, cows were examined between Days 49 and 62 of gestation. The present study was undertaken to discover whether this could be an accurate technique in such an early gestational stage and to describe the techniques used to locate and identify the genital tubercle.

## 4.1 Materials and Methods

The purpose of this section is to attempt to describe the procedure used for sexing foetuses. For a better explanation of the skills acquired in this study, it has been divided in two distinct periods — learning of the technique and application of the technique.

The ultrasound and accessory equipment, and the preparation for the examination used in this study, were the same as employed for early pregnancy diagnosis (Chapter 3).

### 4.1.1 Training Period

This phase was given over to learning the process of using the ultrasound equipment and devising a scanning technique for sexing foetuses. Initially, a waterbath technique was used. An appropriate sink was filled with water and early pregnant uteri of slaughtered animals were immersed in it. Before immersion, the reproductive tract was separated from the neighbouring viscera, keeping just the cervix, uterine body, horns and ovaries. Subsequently, the specimens were washed, placed directly into the sink and held in such a way as to mimic their position in the live animal. The level of the water was sufficient to keep the uterus deep enough to be scanned. The scanner, transducer and video cassette recorder were mounted on a bench beside the sink, where they were convenient for the operator.

Even though the water served as a coupling medium between the transducer and the structure to be scanned, the former was placed within a plastic glove containing a thin film of ultrasound coupling gel. This precaution avoided any hazard regarding the insulating capacity of the transducer, as well as enhancing transmission of the sound waves. Thereafter, the transducer was introduced within the water in the sink, over the pregnant uterus. Firmly holding the transducer, keeping its face over the dorsal surface of the reproductive tract, the scanning took place. Positioned over the dorsal surface of the caudal end of the reproductive tract, the probe was moved cranially, enabling visualization of the cervix, uterine body and then uterine horns.

Reports in the literature of external examination of bovine foetuses ex utero (Inomata et al. 1982) indicate that, at the indifferent stage of development of the external genitalia, the genital tubercle is located in the midline between the tail and the attachment of the umbilicus to the abdomen, exactly between the hind limbs. The external morphological determination of the sex of the foetuses is first possible at the 2.5 to 3.0 cm stage, which is equivalent to Days 42 to 46, based on the published crown-rump length (Winters et al. 1942). Sex is discernable by an urogenital orifice and anogenital raphe in the male. For both male and female, the genital tubercle appears as a small elevation, and the genital swellings, cloacal folds and nipples are visible. During differentiation of the external genitalia, the genital tubercle begins to migrate, from its initial position, along the abdominal wall, towards the umbilicus or tail at the 3 to 4 cm stage (Days 45 to 51). In the male foetus, the genital tubercle migrates towards the umbilical attachment, distancing it from the anus. This anogenital distance is notably increased from the 3.6 to the 3.9 cm stages (Days 49 to 50). In the female foetus, the genital tubercle, migrates towards the tail, reducing, therefore, the anogenital distance. This migration in the male is complete by the 5 cm stage (Day 54). By this stage, the genital swellings already resemble the adult scrotum.

In this experiment, to perform the technique, determination of the sex was based on the identification and location of the genital tubercle relative to the location of the umbilical cord attachment and the tail.

On locating the foetus, the transducer was slowly moved around it. The first step to be followed was looking for the umbilicus, as it proved to be the best way of locating and identifying the genital tubercle. As the equipment was being used for the first time for this aim, the operator had no defined scanning technique, and obtaining specific planes of view was rather difficult. Compared with the scanning applied to the live animal, this technique was easier, as the foetal movements were absent and even manipulation of the foetus was possible. Furthermore, the reproductive tract was visible and the transducer was moved freely, not being limited by the rectal wall.

As the foetuses studied were from slaughtered cows, where gestational ages were unknown, prediction of foetal age was essential for recognizing the stages of genital development of the foetuses. For predicting foetal age, the crown-rump length measurement technique described in (White, Russel, Wright & Whyte 1985) was used. Thus, with the age determined, the appearance of the foetal external genitalia at different stages of development could be correctly observed.

The foetuses used (18 foetuses) were older then seventy days. At this stage, the genitalia of the foetuses already resembled the adult animal (Inomata et al. 1982, Winters et al. 1942, Curran et al. 1989). After identification and determination of the sex of the foetus within the uterus, the latter was opened to confirm the sex of the animal. On the first scanning session, the sex of two foetuses was wrongly determined, but on following scanning session there was improvement of the technique and no more mistakes were made.

Simultaneous to the water bath technique, scans in non pregnant cows were performed to improve the ultrasonographic scanning technique. A group of four cows was used for daily examinations. Changes in the appearance of the reproductive organs, throughout the reproductive cycle of the animals, were observed and identified.

Considerable skill was acquired during this training stage, which was subsequently applied to the further studies.

### 4.1.2 Experimental Period

This period corresponded with the use of transrectal ultrasound technique for sexing bovine foetuses in pregnant cows under farm conditions.

Most of the cows used for early pregnancy diagnosis that had conceived (Chapter 3) were used in this experiment for sexing their foetuses. For this purpose, the days of pregnancy ranged mainly from Days 50 to 54 post breeding. Some animals were scanned earlier or later (Days 48 to 62) than the gestational age determined for the experiment, with the intention of comparing the different stages. Usually a single examination was considered to be sufficient for sexing each animal for the purpose of this experiment as this would be the only commercially feasible time allowance under farm conditions.

The ultrasound examinations were normally confined to the horns. However, in the animals that were not used in the early pregnancy diagnosis experiment, and were thus being scanned for the first time, imaging of the ovaries was usually performed to confirm the reproductive status of the animal. Once within the rectum and having located the foetus, the sex differentiation scanning technique was established. Obtaining a clear static image was sometimes difficult, as the foetus had its own movement and rectal contractions in the cow under examination often presented problems.

There were some structures which ultrasonographically resembled the genital tubercle. In some cases, the tail vertebrae provided a confusing image in the perineal region, mainly when the tail was directed ventrally and cranially towards the umbilicus running between the hind legs, making it very difficult to distinguish the tail vertebrae from the genital tubercle in the early stages of gonad differentiation. Spinal vertebrae, pelvic limb bones and hooves were easily confused with the bilobar hyperechoic appearance of the genital tubercle (Figures 4.2). Often, a cross section of the umbilical cord, at the level of its attachment to the abdomen of the foetus, showed echogenic structures not related to the genital tubercle. This echogenic appearance of structures within the area of the umbilicus seemed to be the wall of the umbilical veins. Occasionally, in longitudinal plane, these structures were visualized as hyperechoic lines inserted between black lines. These anechoic regions appeared to be the lumen of the umbilical vessels, and the bright lines, the walls of the vessels (Figures 4.6, and 4.8.A) Therefore, thorough scanning was essential, and in cases of uncertainty, a second examination was necessary for determining the sex of the foetus with greater confidence. Localization and identification of foetal landmarks such as the head,a beating heart or a pulsating umbilical cord were helpful in locating the genital tubercle (Figure 4.3).

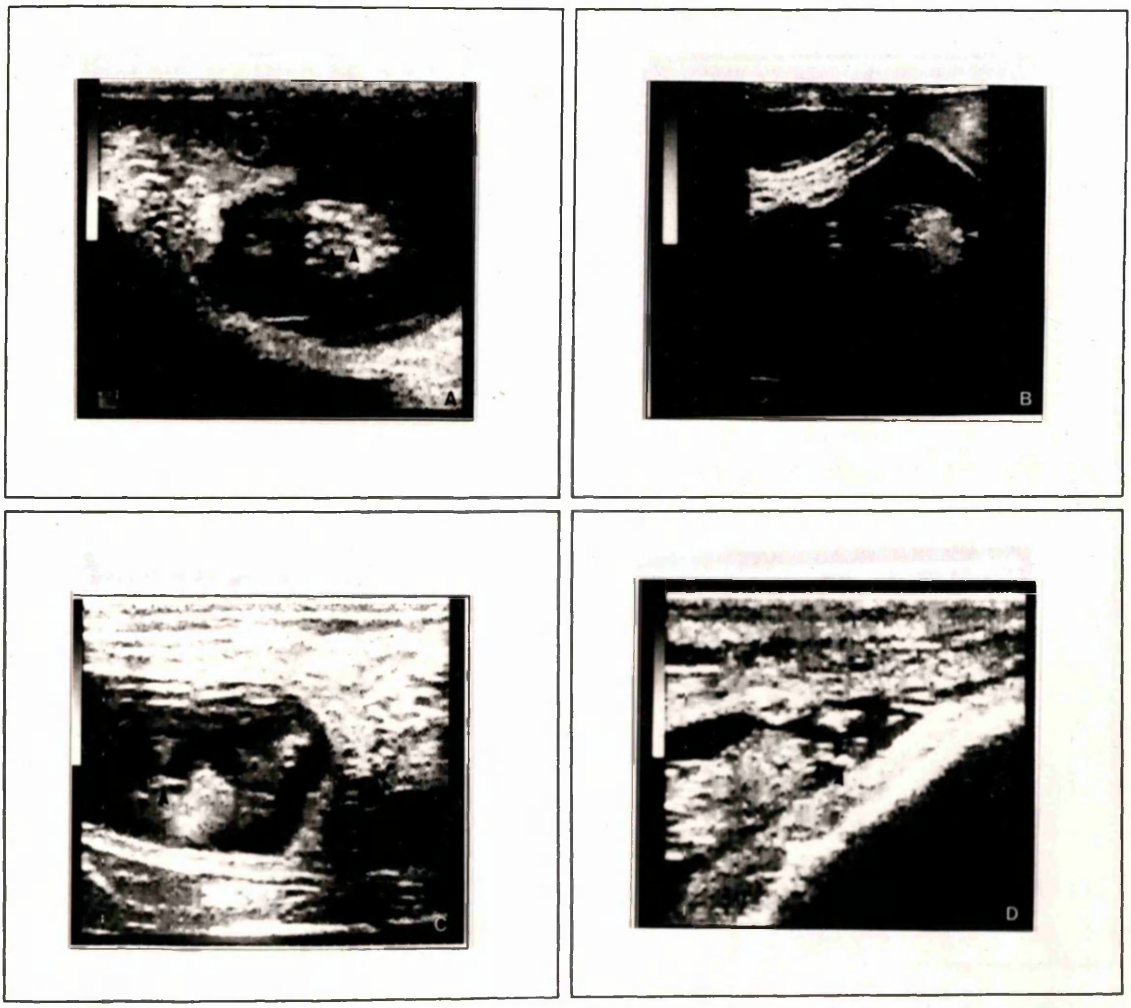


Figure 4.2: Structures presenting difficulties in sex determination. **A.** Foetus 54 day old (centre). This cross section, at the extreme caudal region of the foetus, shows a longitudinal view of the tail (arrows), running from the dorsum toward distal to the foetus. The hind limbs are on the left side of the image. **B.** Cross section of a Day 62 foetus. Note the bilobar appearance of the tail vertebrae in a cross section at the level of its insertion to the foetus body (arrow). **C.** This oblique sagittal plane, of a 51 day old foetus, shows the tail running ventrally, cranial to the foetus. It is seen as hyperechoic longitudinal line (arrow). The left hind leg of the foetus is directed upwards, enabling visualization of the umbilical region. **D.** Day 60 foetus. Dorsal plane at the level of the proximal region of the limbs. The cross section of the hind limbs presents similar appearance to the male genitalia, at this region (arrows).

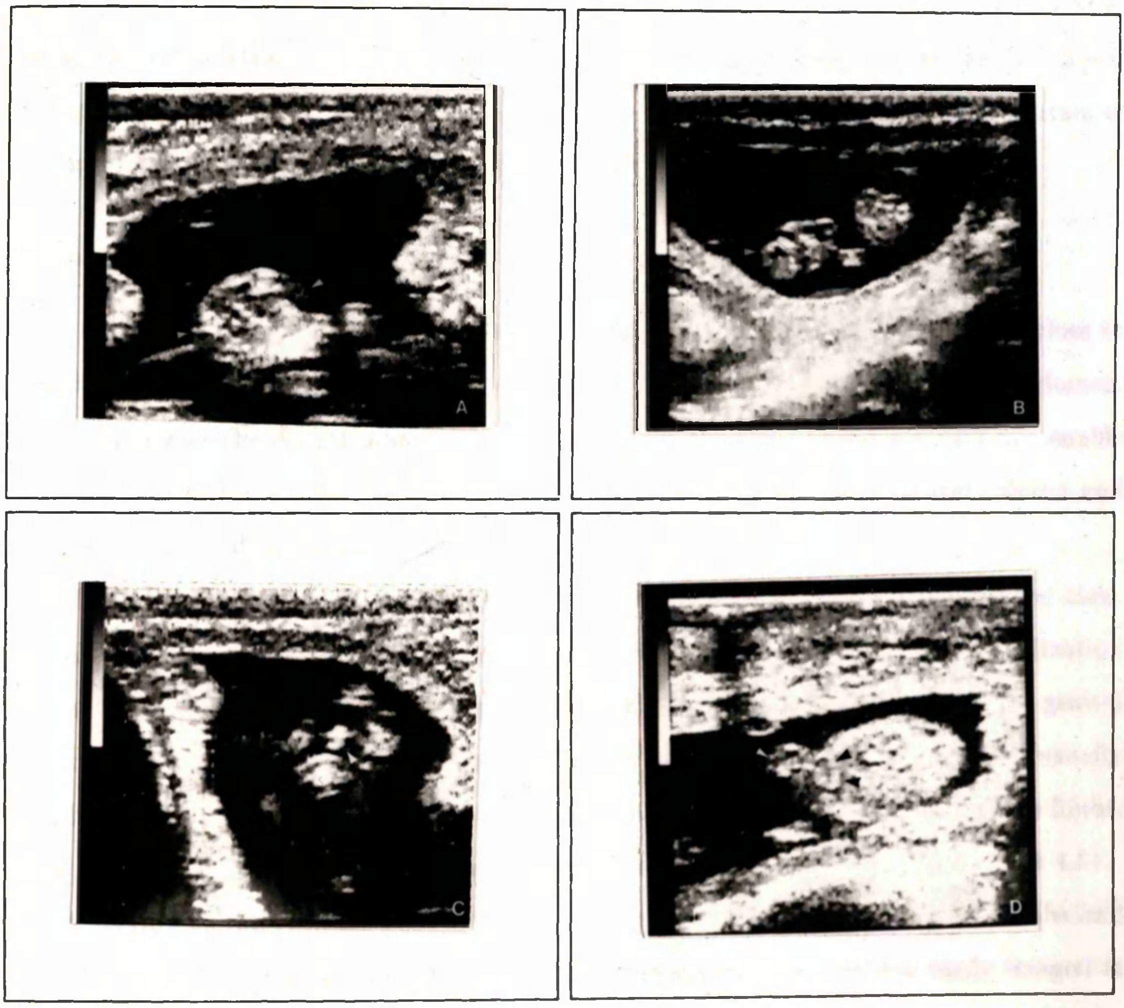



Figure 4.3: Foetal landmarks for ultrasonographic examinations. **A.** The head of a 51 day old foetus (arrows). Note the clearly defined structures of the foetus. Close to the foetus muzzle, the fore limbs are seen in cross section as hyperechoic structures. The abdominal region is partially seen. **B.** Day 48 foetus (central frame). The head, neck and trunk of the foetus are visible. The folded appearance of the foetus cerebrum is observed (arrow). **C.** The 51 day old foetus is seen with its caudal region upwards the top of the image. At the extreme caudal region, the tail is seen in cross section and cranial to it, the hind limbs are seen showing the same appearance as the tail. Between the hind limbs, the genital tubercle of the male foetus is distinguished (arrow). The umbilical cord is seen close to the genital tubercle, as a hyperechoic area. **D.** An oblique plane of a 60 day foetus. The heart is noticeable in the thorax of the foetus (black arrows). Note its muscle, giving it a characteristic appearance. the umbilical cord is the longitudinal region seen running close to the heart, at the top left side of the latter (white arrow).

### Scanning Planes

Many different scanning planes were achieved in the experiment. Some provided a clear image of the location of the genital tubercle. Recognising in which plane the foetus was being imaged, on the screen of the ultrasound scanner, was essential for identification of the gender, otherwise, mistakes were easily made.

### Dorsal Plane

This plane was achieved in three sections at different regions of the foetus body: close to the dorsum, at the midway level of the trunk and at the ventral portion of the abdomen.

Scanning in the dorsal plane of the foetus at the level of the dorsum, did not enable visualization of the gender. In this section of the dorsal plane, the vertebral column and also the ribs could be seen (Figure 4.4).

The section of this plane at the level of the proximal region of the limbs, allowed identification and location of the external genitalia. Rarely, this view provided visualization of the ventral portion of the rib cage, making difficult the identification of the genital tubercle at the abdomen, in the male foetus. This section of the dorsal plane usually exhibited the tail vertebrae in cross or longitudinal plane and cross section of the limbs. The ventral portion of the abdominal region was also present in this plane (Figure 4.5).

A section at the extreme ventral region of the foetus showed the umbilicus, limbs and usually the tail in cross section. For both sexes, the genital tubercle was easily imaged in longitudinal view. This was a very reliable plane and also commonly achieved during the examinations. Occasionally, the head could be visualized (Figure 4.6).

It was concluded that to image the relevant structures identifiable with the different genders within the foetal body, the dorsal plane section had to be situated more ventral than dorsal to the foetus body; otherwise, the hyperechoic structures used for deciding the gender of the genders could be confused with ribs, pelvic bones and even the umbilical region could probably not be imaged, preventing sex determination of the foetus.

The dorsal plane view could be obtained by holding the transducer lateral, cranial or caudal to the foetal body; allowing the sound beam to pass through the latter in a dorsal plane (Figure 4.7).

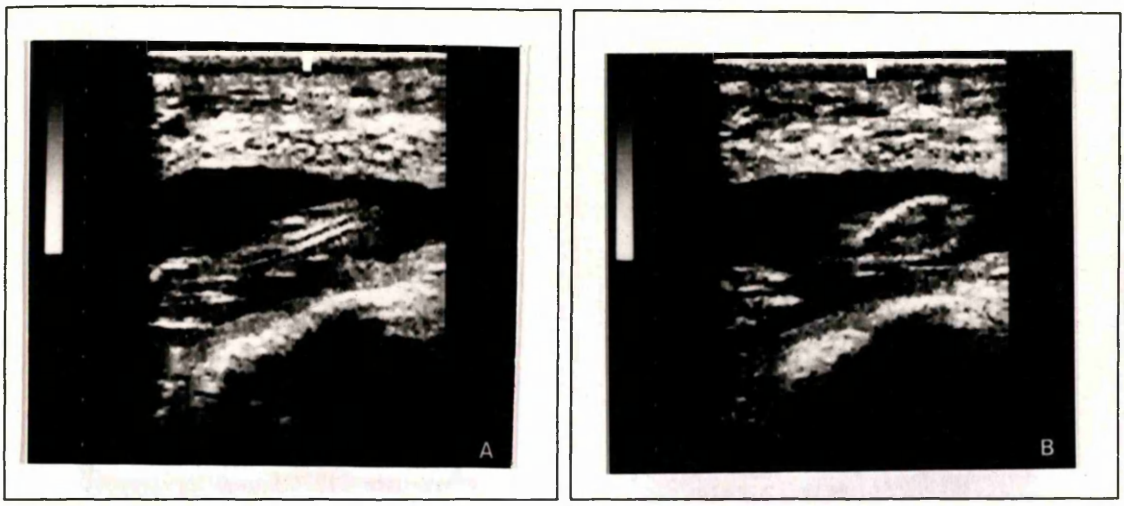



Figure 4.4: Dorsal plane close to the dorsum of the foetus. **A.** Day 60 foetus, visualized in dorsal plane at the level of the dorsum. The head of the foetus is directed to the left side of the image, and the cranial bones are perceptible. The column vertebrae are clearly seen, running from the cranial to the caudal region of the foetus. **B.** The same foetus, Day 60, showing the rib cage. Note that sex organs are not visible at this section.

### Sagittal Plane

A true sagittal or median plane of the foetus was rarely obtained. This plane was not consistent in early stages of differentiation of the gonad, since the migration of the genital tubercle could be at its initial stage and ultrasonogaphic visualization was difficult. In a later stage of migration of the organs differentiating the gender, the sex could be easily discerned by location of the external genitalia under the tail or close to the umbilicus, for female and male respectively.

The position of the hind limbs could occasionally prevent location of the genital tubercle in early stages of differentiation, and at times the hind limb bones could be confused with the tubercle. At stages in which the scrotum was already present, this could be missed when one of the hind legs was on the near plane of the image, leaving the scrotum in the background of the frame. When imaged in this plane, the umbilical cord was always seen in longitudinal view (Figure 4.8).

This plane was achieved by holding the transducer dorsal, ventral, cranial, or caudal to the foetal body, permitting the sound beam to pass through the foetus in sagittal plane (Figure 4.9).

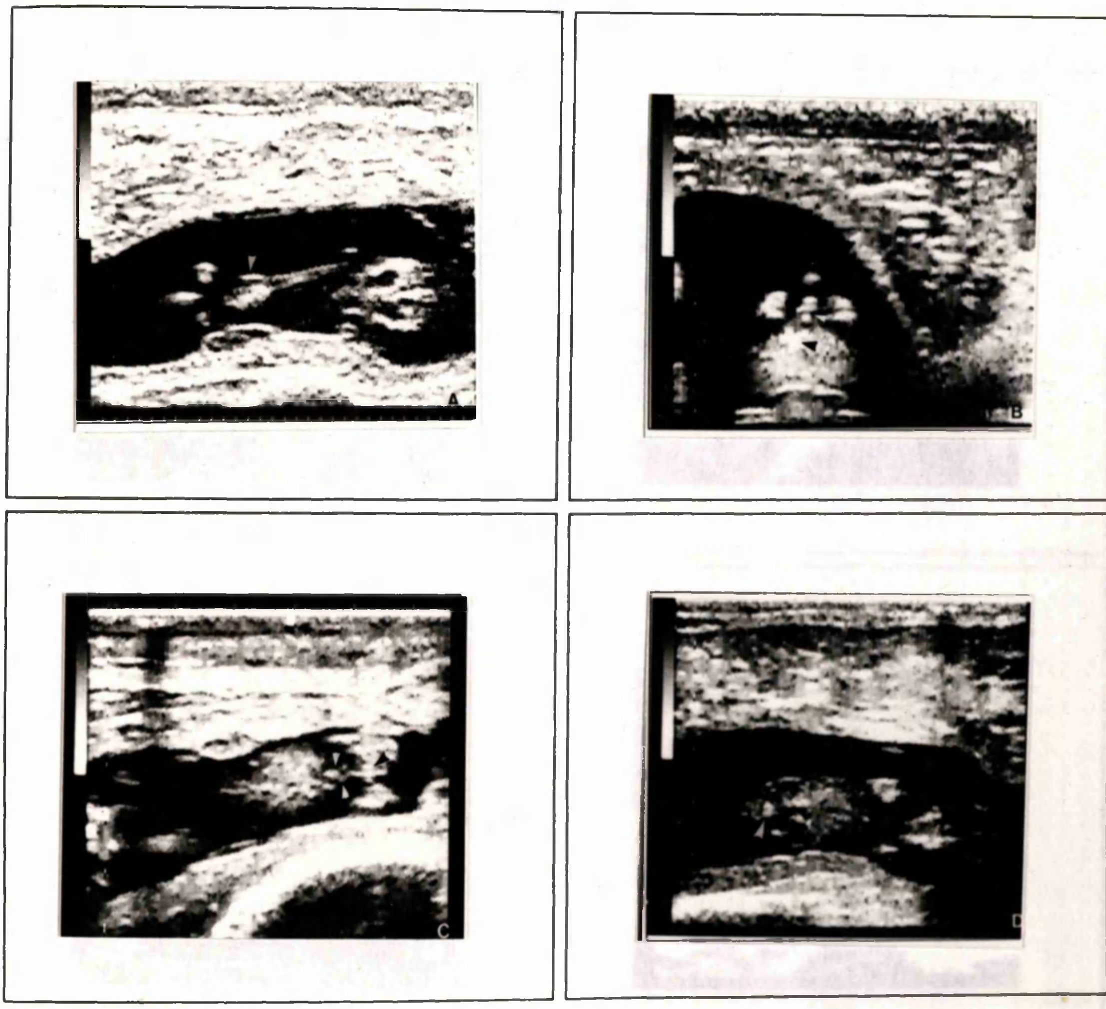



Figure 4.5: Dorsal plane at the midway level of the foetus trunk. **A.** Male foetus on Day 51 of gestation, with cranial portion to the right side of the picture. Note the defined lines of the skull. The ventral portion of the rib cage is present in this section of the dorsal plane. The umbilicus exhibits a hyperechoic appearance (arrow), and caudal to it the hind limbs are seen in cross section. The tail also has a hyperechoic appearance, seen as a line caudal to the hind limbs. **B.** The caudal region of the foetus, on Day 54 of gestation, is seen in this picture. The tail is the hyperechoic rounded structure appearing at the top of the image of the foetus. Ventral to it, the genital tubercle is seen (arrow). The hind limbs are located lateral to each side of the genital tubercle, in cross section view. The hyperechoic area seen close to the genital tubercle is the umbilicus (black arrow). **C.** Day 60 male foetus. The head of the foetus is situated on the left side of the image. At the caudal region, the limbs are seen in cross section. The genitalia of the foetus is clearly seen. The scrotum is between the limbs (black arrow) and the penis at this age is clearly seen (white arrows). **C.** A 54 day female foetus. The contour of the head is seen at the right side of the frame. The genital tubercle is visible under the tail (arrow).

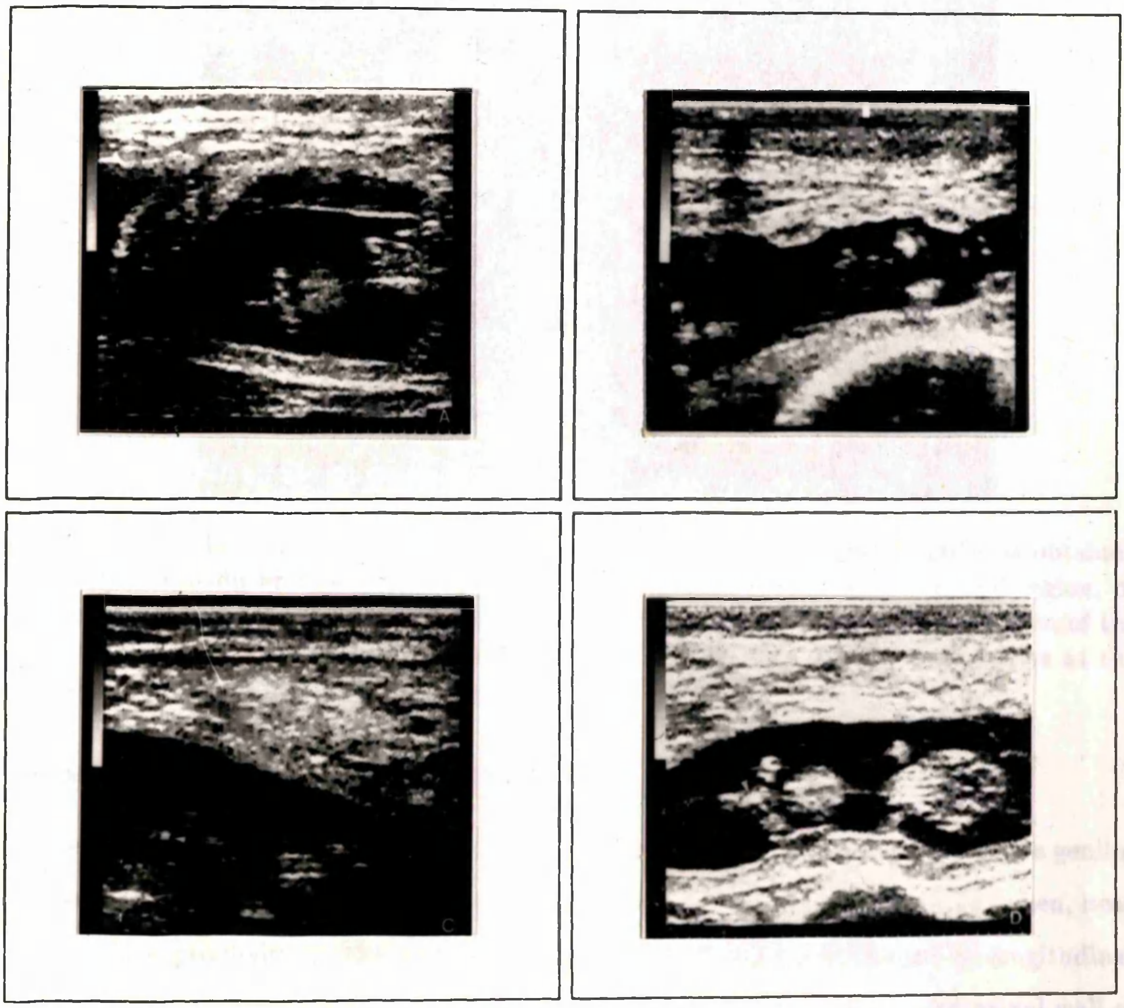


Figure 4.6: Dorsal plane at the extreme ventral region of the foetus. **A**. A 51 day male foetus. Note the genital tubercle located cranial to the hind limbs, migrating toward the umbilicus (arrows). **B**. Male foetus on Day 60. The genital tubercle has a bilobar appearance (arrow). The scrotum is not present in this image, but was seen between the hind limbs. The echogenic structure cranial to the genital tubercle is the umbilicus, with a trilobar appearance. The tail is seen as an hyperechoic structure caudal to the hind limbs, and both are seen in cross section. **C**. Female foetus on Day 54. The genital tubercle is in close proximity to the tail vertebrae (arrow). The limbs are seen in cross section and the head is observed on the right side of the picture. **D**. Day 51 female foetus. Limbs, umbilical region and head are clearly visible. The tip of the genital tubercle is forward caudally, close to the tail (white arrow). The genital swellings are visualized on both sides caudal to the genital tubercle (black arrows).

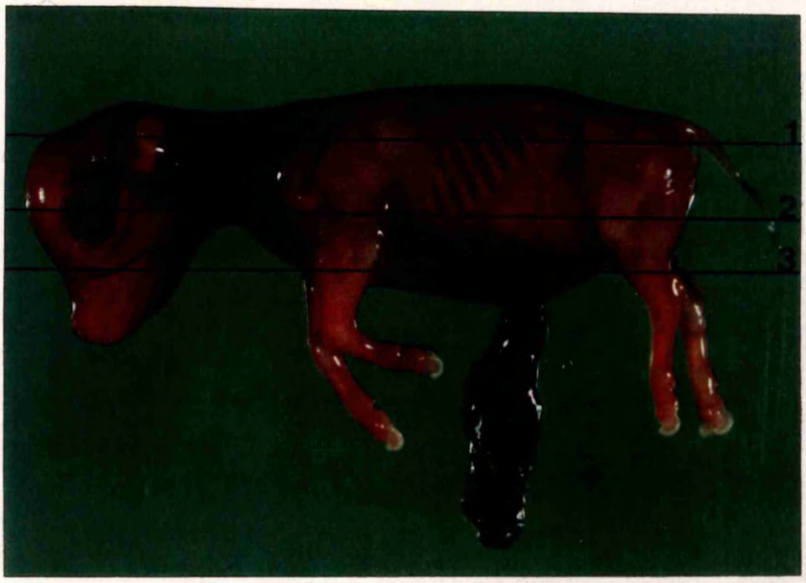



Figure 4.7: Illustration of the dorsal plane of the foetus. The dorsal plane is obtained with the transducer face positioned parallel to the foetal body at the lateral region, or perpendicular to the cranial or caudal region. **1.** Dorsal plane close to the dorsum of the foetus. **2.** Dorsal plane at the central region of the foetus body. **3.** Dorsal plane at the extreme ventral region of the foetus.

### Transverse or Cross Sectional Plane

A section of the foetus body in this plane, at the level of the umbilicus , showed the genital tubercle, seen as a hyperechoic round structure at the ventral wall of the abdomen, near or in close proximity to the umbilicus. The umbilical cord was imaged in longitudinal plane in this section, where it was running perpendicular to the ventral abdominal wall of the foetus. An oblique plane of the umbilical cord was seen in this section, when it was running parallel to the foetal abdomen (Figure 4.10).

A cross section of the umbilical cord was the most reliable way to achieve a diagnosis. Locating the umbilicus was indispensable for the use of this technique. After finding the umbilical cord, the transducer was slowly moved in parallel along its length, which was thus imaged in longitudinal plane. On reaching the level of the attachment of the umbilical cord to the abdomen (umbilicus), the transducer was slowly rotated to obtain a cross section of the umbilicus. The cross section of the umbilical cord was achieved by placing the transducer at right angles to it, parallel to the abdomen wall. With positive male diagnosis, a bilobar hyperechoic structure appeared near or in close proximity to the

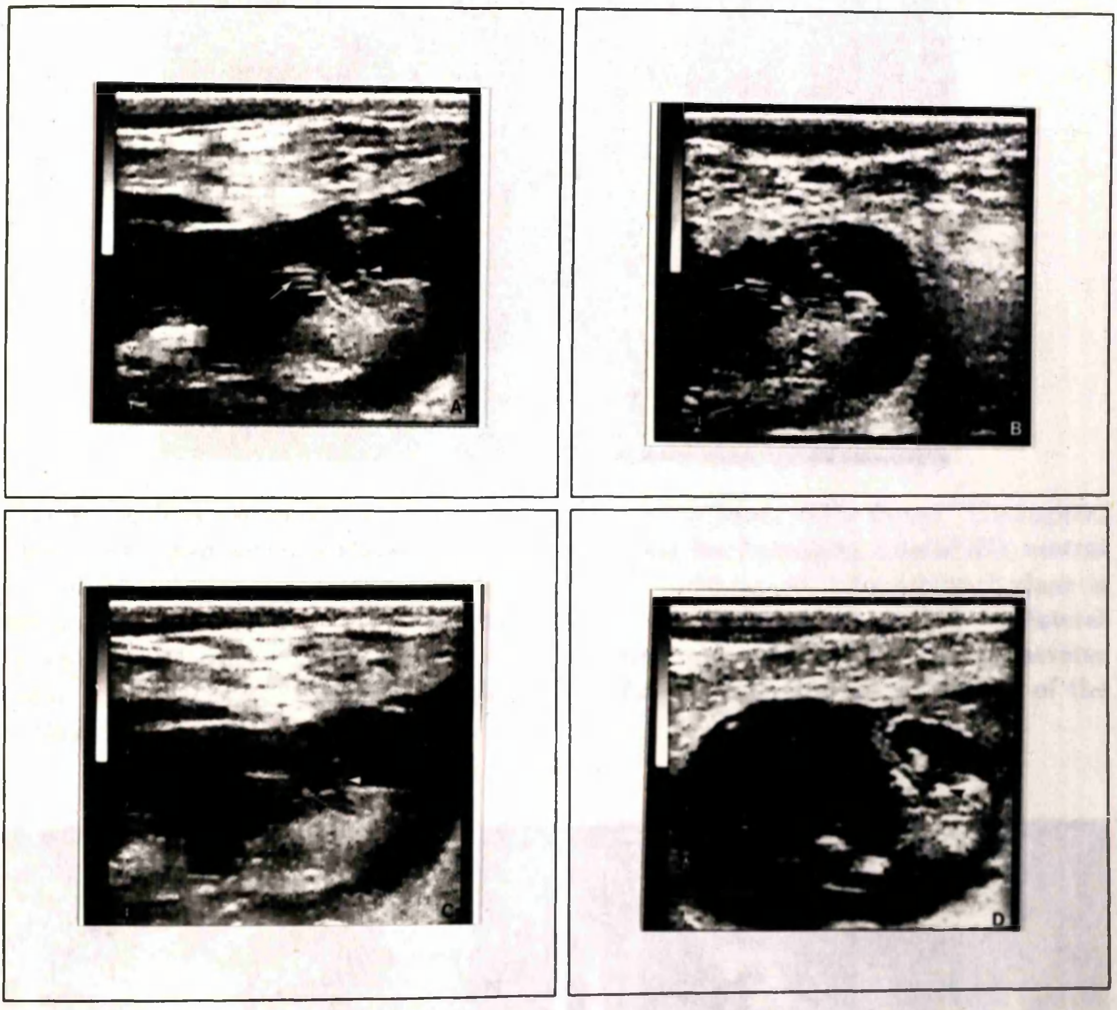



Figure 4.8: Sagittal plane of the foetus body. **A**, **B** and **C**. Cow 18 at Day 57 of gestation of a male foetus. Note the genital tubercle in close proximity to the umbilical cord and seen as a small hyperechoic structure protruding from the abdomen (black arrow head). The scrotum is also seen in some views, caudal to the genital tubercle (white arrow head). The umbilical cord is in longitudinal plane and shows the hyperechoic lines representing the umbilical vessels wall (white arrow). Occasionally, the hind limbs do not allow visualization of the scrotum. **D**. Male foetus on Day 54 of gestation. The genital tubercle is covered by the right hind limb and the umbilical cord that is running from the same region, in this view. The genital swellings are discretely seen near the hind limbs (arrow).

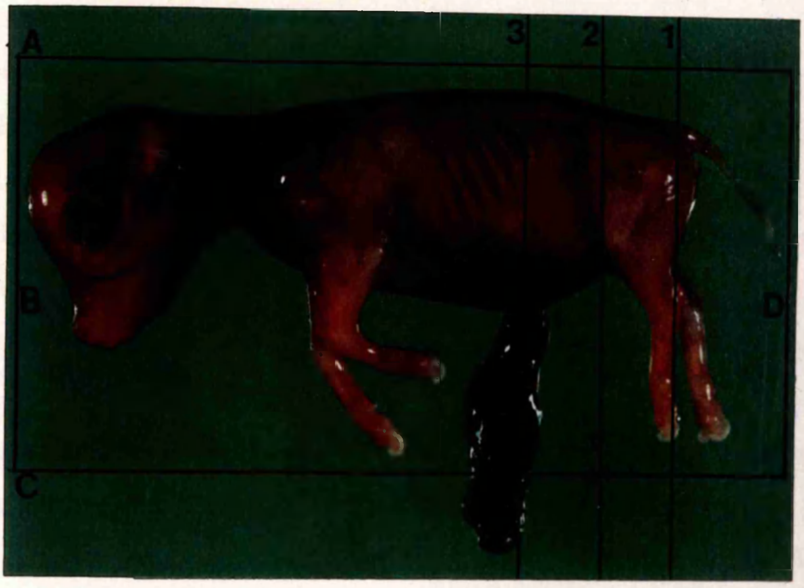



Figure 4.9: Illustration of the sagittal and the transverse planes of the foetus. The sagittal plane is obtained with the transducer positioned facing the dorsal (A), cranial (B), ventral (C) or caudal (D) region of the foetal body. The transverse or cross sectional plane is obtained by placing the transducer perpendicular to the foetal body at the dorsal, lateral or ventral regions. **1.** Transverse plane at the extreme caudal region. **2.** The transverse plane at the caudal region of the umbilicus. **3.** The transverse plane at the level of the umbilicus.

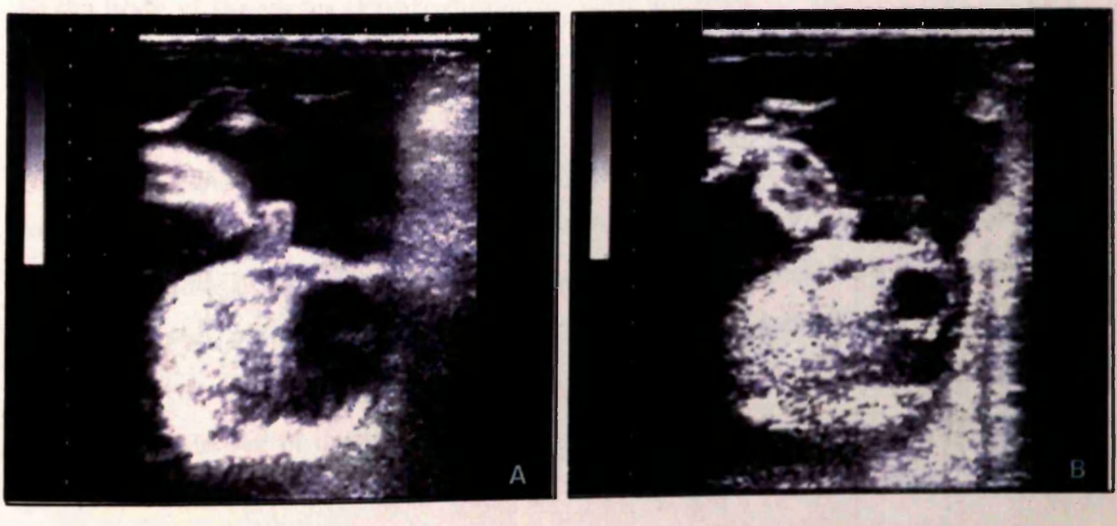



Figure 4.10: Transverse plane of the foetal body at the level of the umbilicus. **A.** Transverse plane of the abdomen showing the umbilicus and the prepuce in longitudinal plane. Foetus male at Day 100 of gestation, obtained in water bath scanning. **B.** The same foetus showing the umbilical cord in oblique view.

umbilicus, depending on the gestational age. Whereas for a positive female diagnosis, this structure was absent on scanning this region (Figure 4.11).

This plane with a section between the umbilicus and the hind limbs, provided visualization of the scrotum in the longitudinal plane, when this was present. If running parallel caudal to the foetus, the umbilicus could occasionally be seen in cross section. It also could be present in longitudinal plane. The hind limbs could, in some images, be visualized. In older female foetuses, the mammary gland could be observed (Figure 4.12).

A section of the dorsal plane at the extreme caudal region was also observed. This view could show the entire hind limbs or only parts of it. The scrotum, when present, was seen in longitudinal plane. In this view, a cross section of the vertebrae column could be visualized directly opposite to the scrotum. The mammary gland could be seen in older female foetuses (Figure 4.13). The tail could be seen in cross section at the level of its insertion with the foetal body, resembling the ultrasonographic appearance of the genital tubercle. The tail could also be imaged in longitudinal plane, covering the perineal region, which was the location of the organs for deciding the gender in the female foetus (Figure 4.2 A and C). The umbilicus was usually not present in this image, but occasionally could be seen in both cross section and longitudinal plane.

The transverse plane was achieved by placing the transducer dorsal, ventral, or lateral to the body of the foetus (Figure 4.9).

### Oblique Plane

This plane was frequently seen, usually as a deviation of one of the planes previously explained. It was obtained by holding the transducer angled towards the sides of the foetus. Section in this plane could be obtained at the extreme caudal region, ventral abdominal wall, perineal region, abdomen and through the inguinal region and others.

There were some sections in the oblique plane that could be a cause of errors in determining the sex of the foetus. Identification of these sections was essential to achieve an accurate sex determination.

- **Oblique section, close to the transverse plane, at the extreme caudal region of the foetus.** This section was achieved by holding the transducer at an oblique angle to the foetal body, as a deviation of the transverse plane at the

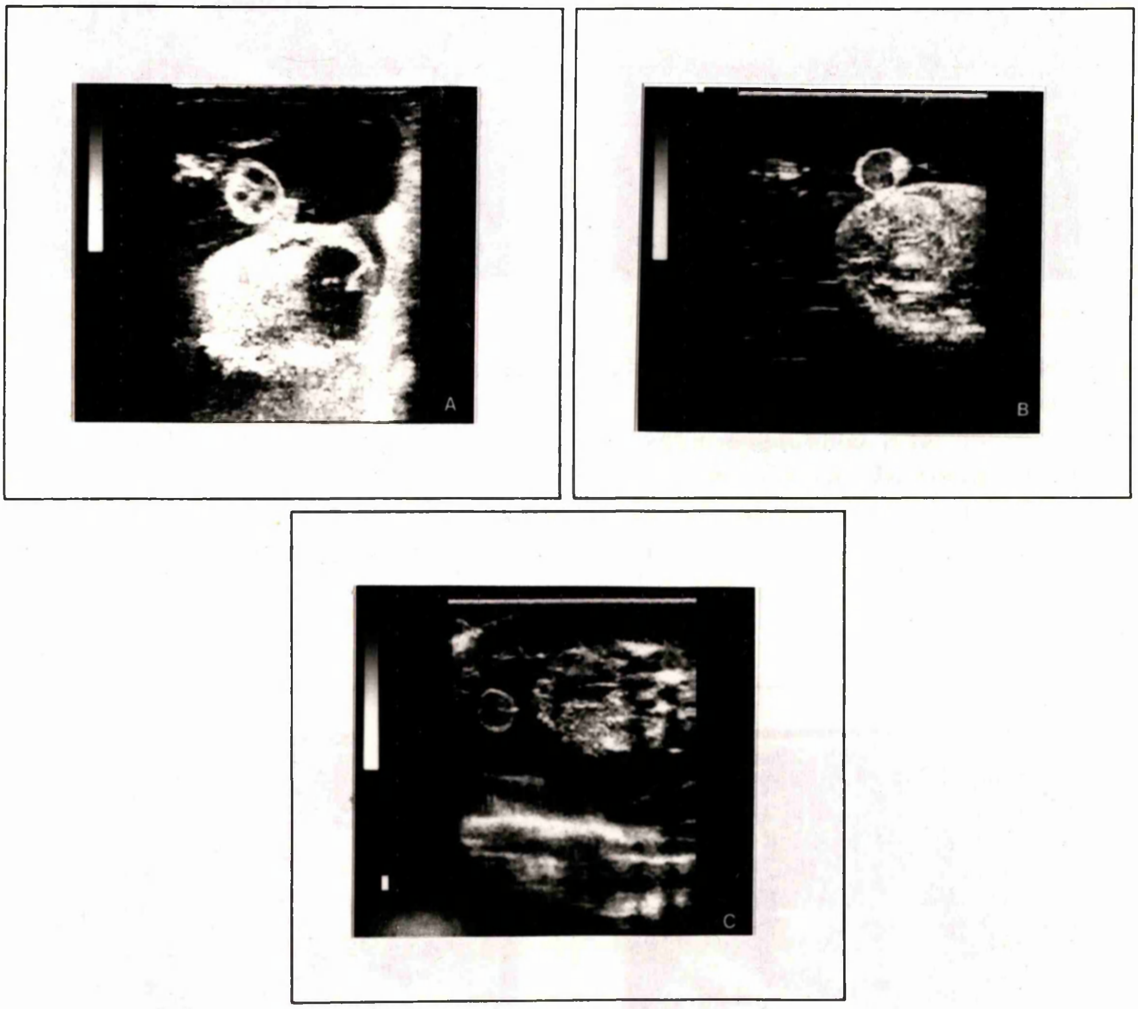



Figure 4.11: Cross section of the umbilical cord of the foetus. **A** and **B.** Showing the umbilical cord in cross section plane. Note the genital tubercle in close proximity to the former, appearing as an hyperechoic rounded structure (arrows). The foetuses are male at Days 100 and 74 of gestation, respectively. Images obtained in water bath technique. **C.** The same plane of view, showing the umbilical region in a 73 day old female. Note the absence of the genital tubercle close to the umbilical cord. Scan in water bath technique.

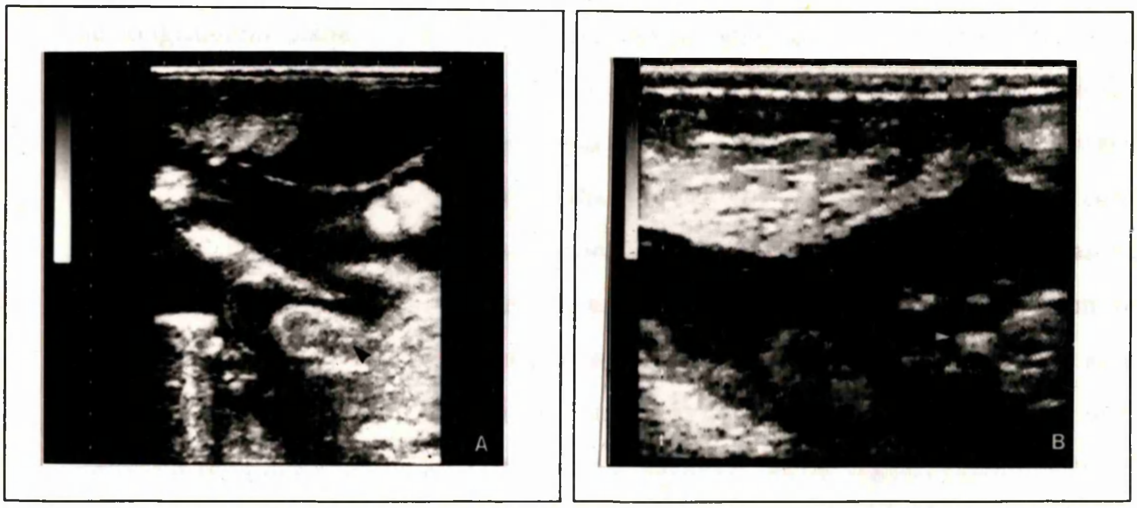



Figure 4.12: Transverse plane caudal to the umbilicus of the foetus. **A.** Day 100 male, scanned in water bath. The cross section plane was achieved at the region between the scrotum and the hind limbs. The scrotum is seen in longitudinal plane (arrow). The umbilical cord is also visible in longitudinal view near the scrotum. **B.** Foetus on Day 62 of gestation. Cross section caudal to the umbilicus. The scrotum (arrow) and hind limbs are seen in longitudinal plane.

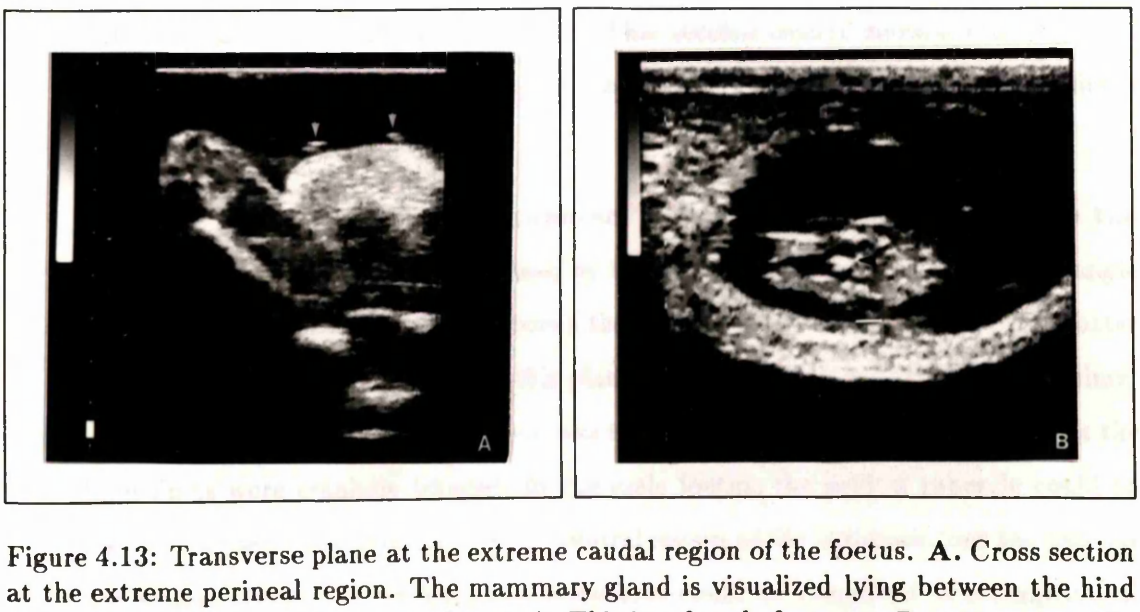



Figure 4.13: Transverse plane at the extreme caudal region of the foetus. **A.** Cross section at the extreme perineal region. The mammary gland is visualized lying between the hind limbs. Note the teats of the organ (arrows). This is a female foetus on Day 82, scanned in water bath. **B.** A male foetus at Day 54 of gestation. Note the genital swellings between the hind limbs, as an hyperechoic structure protruding from the abdomen (arrow). The hind limbs are seen in longitudinal plane.

extreme caudal region of the foetus. The image obtained showed the hind limbs in longitudinal plane. When the section was situated at the level of the tail, this could be seen in longitudinal plane, running distal to the body. Sometimes the tail did not enable visualization of the foetal genitalia. The scrotum was seen between the hind legs, in the background of the frame. In this case, the umbilical cord was not visualized and the scrotum appeared protruding from the abdomen, as an echogenic bilobar structure. When the section was at the caudal region, but more cranial to the tail, this was not easily seen. In this case, the tail appeared as a small hyperechoic area opposite to the scrotum or genital tubercle, which were seen between the hind limbs. This section of the extreme caudal region, extending more cranially, could image the umbilicus. Portions of the head of the foetus could also be present, when this was bent, with the head toward the ventral region of the body. In this presentation of the foetus, in early stages of migration of the gonad, the genital tubercle was seen as a small hyperechoic structure close to a cross section of the umbilical cord, and the forming scrotum could be also observed, when present at the stage of scan. Occasionally, in male foetuses that had the genitalia already resembling that of the adult, both scrotum and prepuce could be imaged close to the longitudinal view of the umbilical cord. This section clearly showed that the hind limbs were imaged caudally, which was confirmed by the position in which the limbs were presented (Figure 4.14).

- **Oblique section, close to the transverse plane, at the region caudal to the umbilicus.** This section was obtained by holding the transducer at an oblique angle to the foetal body, at the region between the umbilicus and the hind limbs. The latter were usually completely imaged in this plane. Part of the ventral region of the animal could also be seen, but the umbilicus was not visible. It was clearly noticed that the hind limbs were cranially imaged. In the male foetus, the genital tubercle could be seen as a hyperechoic structure at the ventral region of the abdomen, but the tail was not visible. When the scrotum was present, it could be visualized in a longitudinal plane dependent from the abdomen, between the hind limbs, or in cross section at the same region. Both the genital tubercle and scrotum were usually present in the same image, however, the former was difficult to discern. In the female foetus,

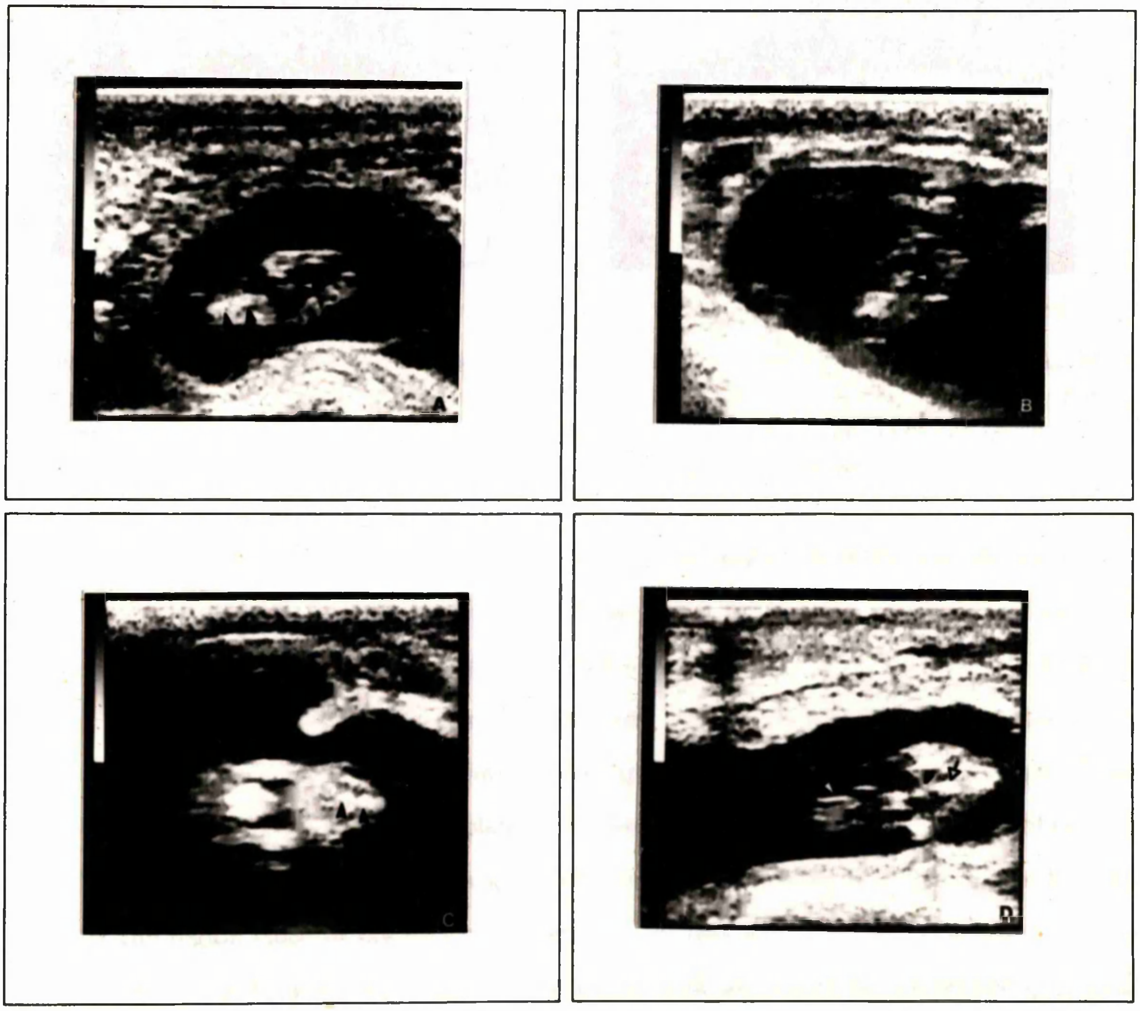



Figure 4.14: Oblique section at the extreme caudal region of the foetus. **A.** Male foetus on Day 54 of gestation. The genital swellings are seen between the hind limbs (white arrow). The tail is visualized in longitudinal plane, running distal to the foetal body (black arrows). **B.** The same plane in a foetus 51 day old. Note the tail vertebrae in cross section (black arrow) opposite to the genital tubercle (white arrow). **C.** The genital tubercle and swellings are seen (arrows). The tail vertebrae are situated caudal to the genital swellings and appears in cross section. The foetus is 54 day old. **D.** The umbilical cord (white arrow), prepuce (black arrow) and the scrotum (open arrow) are seen in this image. The tail is caudal to the scrotum. The foetus is 60 days old.

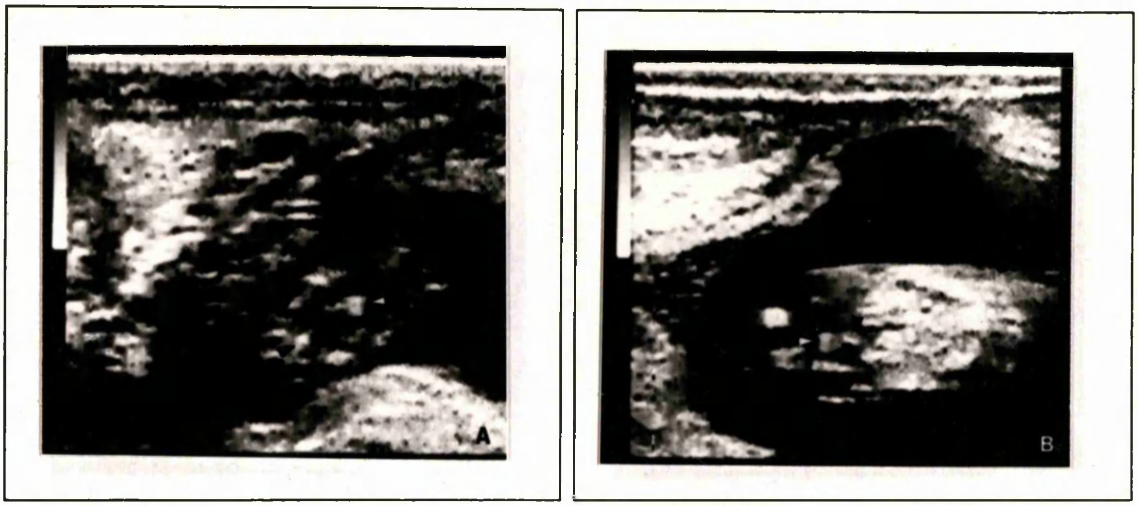



Figure 4.15: Oblique transverse plane caudal to the umbilicus, in male foetus. **A.** Male foetus at Days 54 of gestation. The scrotum is seen between the hind legs (white arrow). The genital tubercle is cranial to the scrotum (black arrow). **B.** Day 62 foetus. The genital tubercle (black arrow) is cranial to the scrotum (white arrow).

the genital tubercle resembled the scrotum in the male, however, the tail vertebrae was present near it, between the hind limbs. The tail vertebrae was viewed in cross section, appearing as an echogenic structure caudal to the genital tubercle, between the hind limbs. The presence of the tail vertebrae in the female and its absence in male was, in fact, a relevant point for distinguishing both sexes. The presence of the tail vertebrae in female was explained by the level in which the section was obtained. That is, to image the female genital tubercle, the oblique section had to be located at the region close to the tail, more perineal. In this case, the tail vertebrae could be imaged as well, as at that region, they are in close proximity to the female genitalia. The absence of this structure in the male foetuses, was also due to the section of the plane. To image the prepuce and scrotum, the section had to reach a larger portion of the ventral abdominal region and extend caudally. To image the tail, the section had to be more dorsal to the foetal body and, so, the genital tubercle would not be present in this specific oblique plane. The opposite was observed when imaging the male genitalia, the tail was not seen, because the section was more ventral to the foetal body (Figures 4.15 and 4.16).

- **Oblique section, close to the transverse plane, at the level of the umbilicus.** This was a plane used for diagnosing male foetuses. The transducer was placed

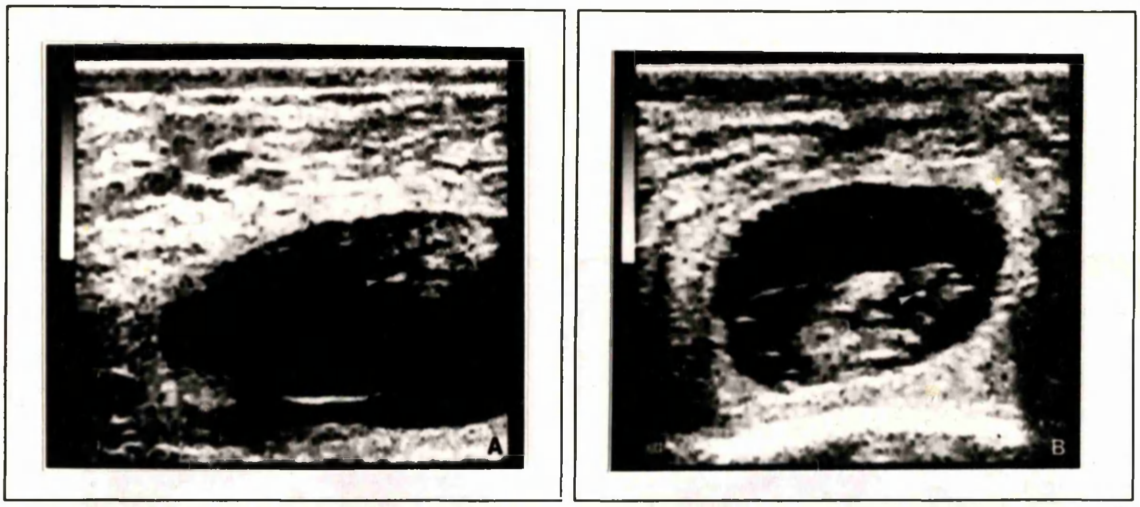



Figure 4.16: Oblique transverse plane caudal to the umbilicus in female foetus. **A** and **B.** Female foetuses at Day 51 and 55 of gestation, respectively. In this plane the genital tubercle is visualized between the hind limbs (black arrows), and caudal to it, the tail vertebrae can be seen (white arrows).

at an oblique angle to the lateral region of the foetus, at the level of the umbilicus. The hind limbs were not seen, but hyperechoic areas situated at the sides of the abdominal region were indicative of pelvic bones. In some frames, the vertebrae column could be visualized opposite to the umbilical cord. The hyperechoic genital tubercle was imaged in longitudinal section, close to the umbilical cord, which was also seen in longitudinal plane (Figure 4.17).

- **Oblique section, close to the dorsal plane, at the level of the proximal region of the hind limbs.** This plane resembled the oblique section, close to the transverse plane, at the level of the umbilicus and also at the region caudal to the umbilicus in males. This plane was solely used in female foetuses. Compared with the oblique section close to the transverse plane at the level of the umbilicus, this oblique dorsal plane at the proximal region of the hind limbs showed no longitudinal view of the umbilical cord. The proximal region of the hind limbs could be distinguished and, occasionally, the hooves could be seen in this plane, appearing beside or caudal to the tail vertebrae. The main difference was observed by the presence of other echogenic structures close to the genital tubercle. These echogenic structures represented the tail vertebrae. Compared with the oblique section close to the dorsal plane at the region caudal to the umbilicus, in male, the tail vertebrae, observed in this plane,

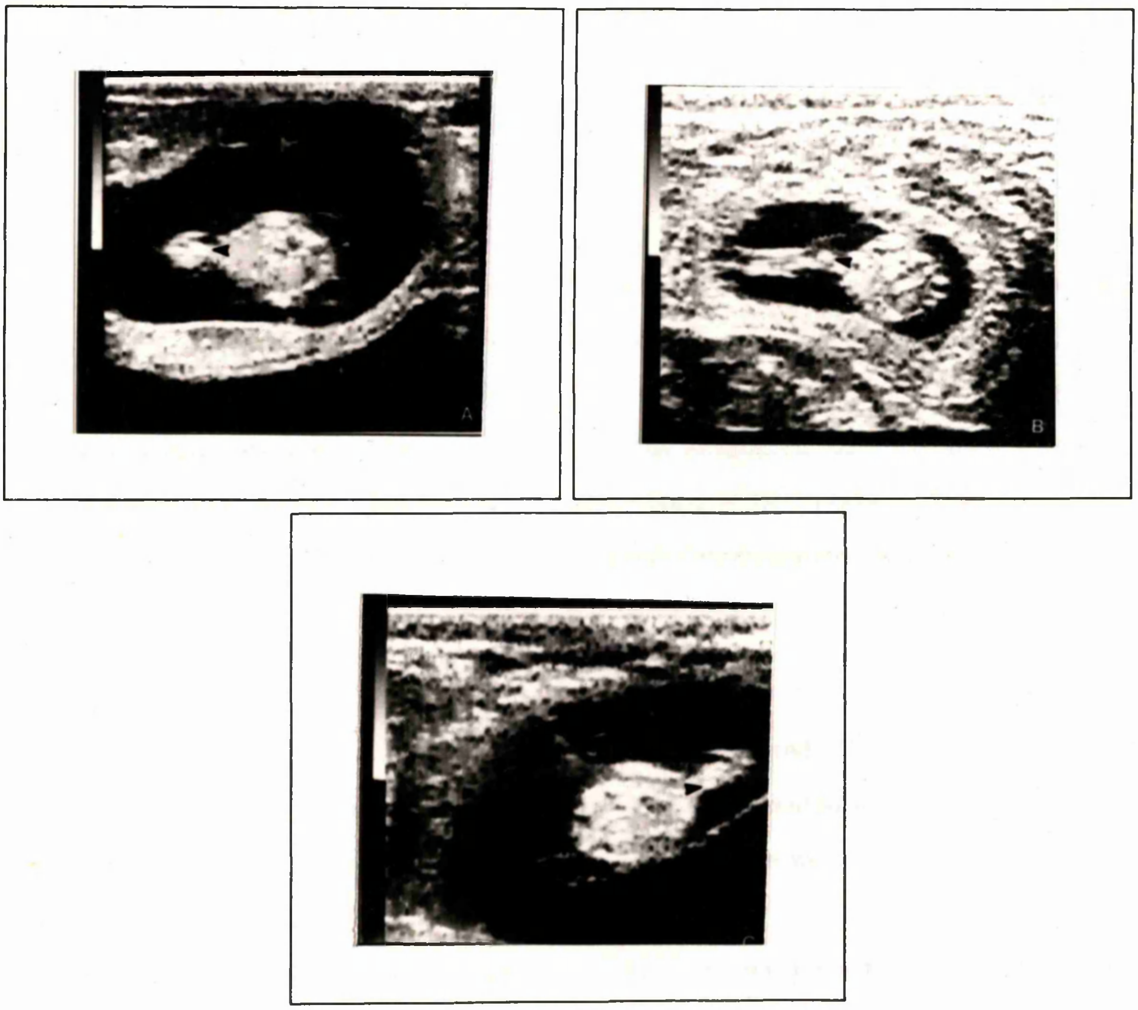


Figure 4.17: Oblique transverse plane at the umbilical region of the foetus. **A**, **B** and **C**. Foetuses at Days 54, 51 and 51, respectively. Note the hyperechoic appearance of the male genital tubercle (arrows) in close proximity to the umbilical cord, which is seen in longitudinal plane. The hyperechoic structures seen in both lateral side of the foetal trunk are portions of the hind limb bones.

confirmed the difference between the sexes, in both planes. The hind limbs were not entirely imaged in this view (Figure 4.18).

- **Oblique section, close to the transverse plane, at the level of the extreme perineal region through the umbilical cord.** The oblique section was through the umbilical cord, at the level of the perineal region. This plane was commonly obtained and provided a reliable location for detecting of the gender of both sexes. The umbilicus was usually seen in longitudinal section, running toward the caudal region of the foetus. The hind limbs could be seen in cross section and the tail in longitudinal or cross section. The tail running cranially between the hind limbs, was visualized in longitudinal plane and could cover the genital tubercle in the early stages of the gender migration or even give an erroneous idea of the location of the organs of determination of the gender, as the former was seen as an elongated structure, running toward the umbilicus. In this case, the anogenital distance could appear as reduced, which could give rise to a wrong location of the organs of determination of the gender and therefore create an error in sex determination (Figure 4.19).

## 4.2 Results

The animals used for the experiment were not specially selected. Which meant that they were of differing age and size. Some of the smaller cows presented greater problems with scanning, due to the the narrowness of the rectum; and some were of poor temperament, making prolonged scanning difficult.

If during the actual scanning session, the structures used for sex differentiation were not confidently identified, the recorded images were later reviewed to reach a diagnosis. After reviewing the scanning, the sex of the foetus was then determined. During the experiment, this procedure was required to be carried out twice. Two scanned cows were pregnant with twins and, in both cases, the sex of two foetuses, one from each cow, could not be determined at the time of the original scan.

At the end of the experiment, all recorded images of the scans performed, were assessed for a detailed analysis and determination of the planes of viewing the foetuses. During the review of the scans, some discrepancies between the first diagnoses and the current analysis of the image arose. These discordant results were closely related to the plane of

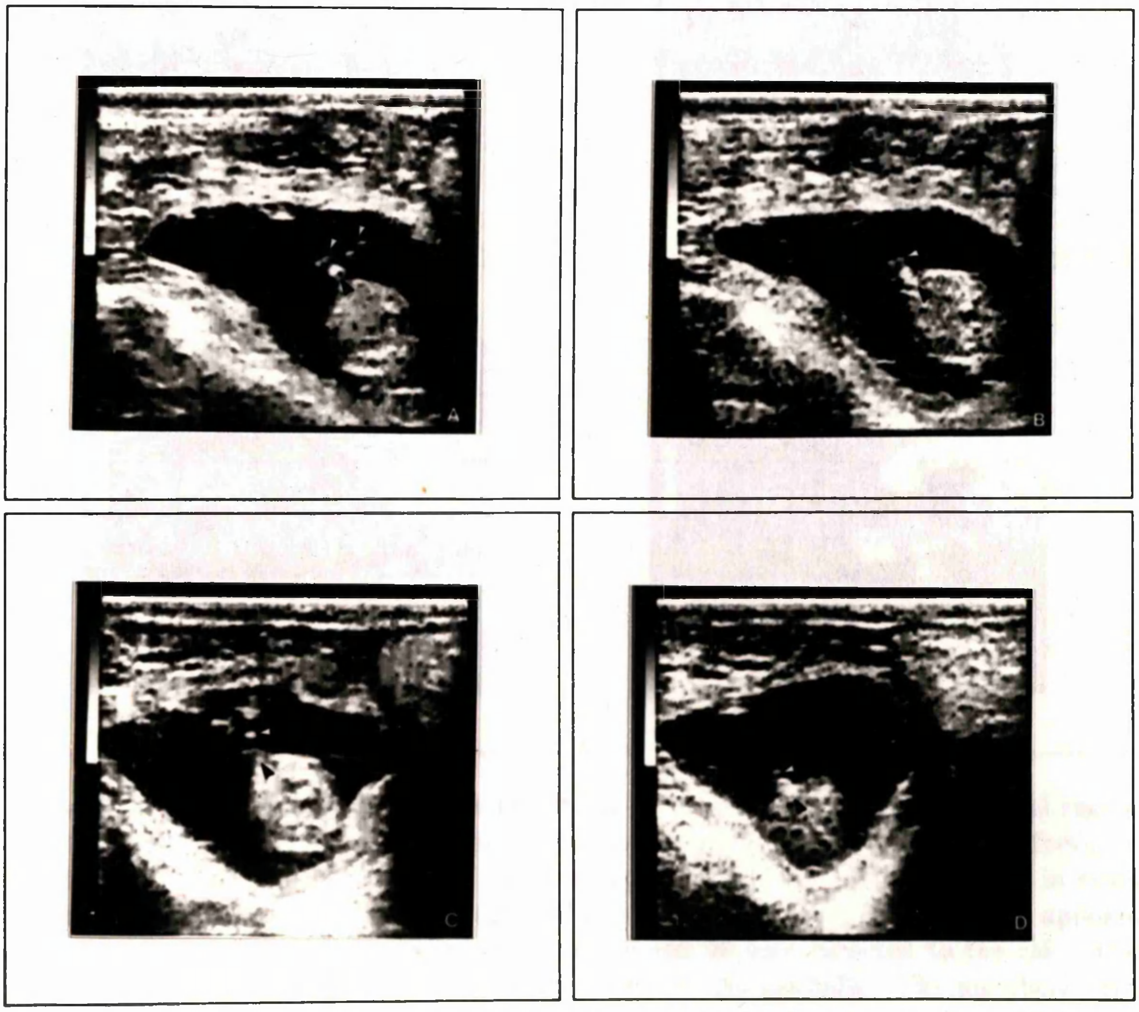



Figure 4.18: Oblique dorsal plane, at the level of the proximal region of the hind limbs. **A, B, C** and **D**. Cow 19 on Day 55 of gestation. The genital tubercle of the female foetus is visible at the extreme caudal region of the trunk (black arrows). The tail vertebrae can be seen as echogenic structures close to the genital tubercle (white arrows). The hooves and the proximal region of the hind limbs can be observed in some images.

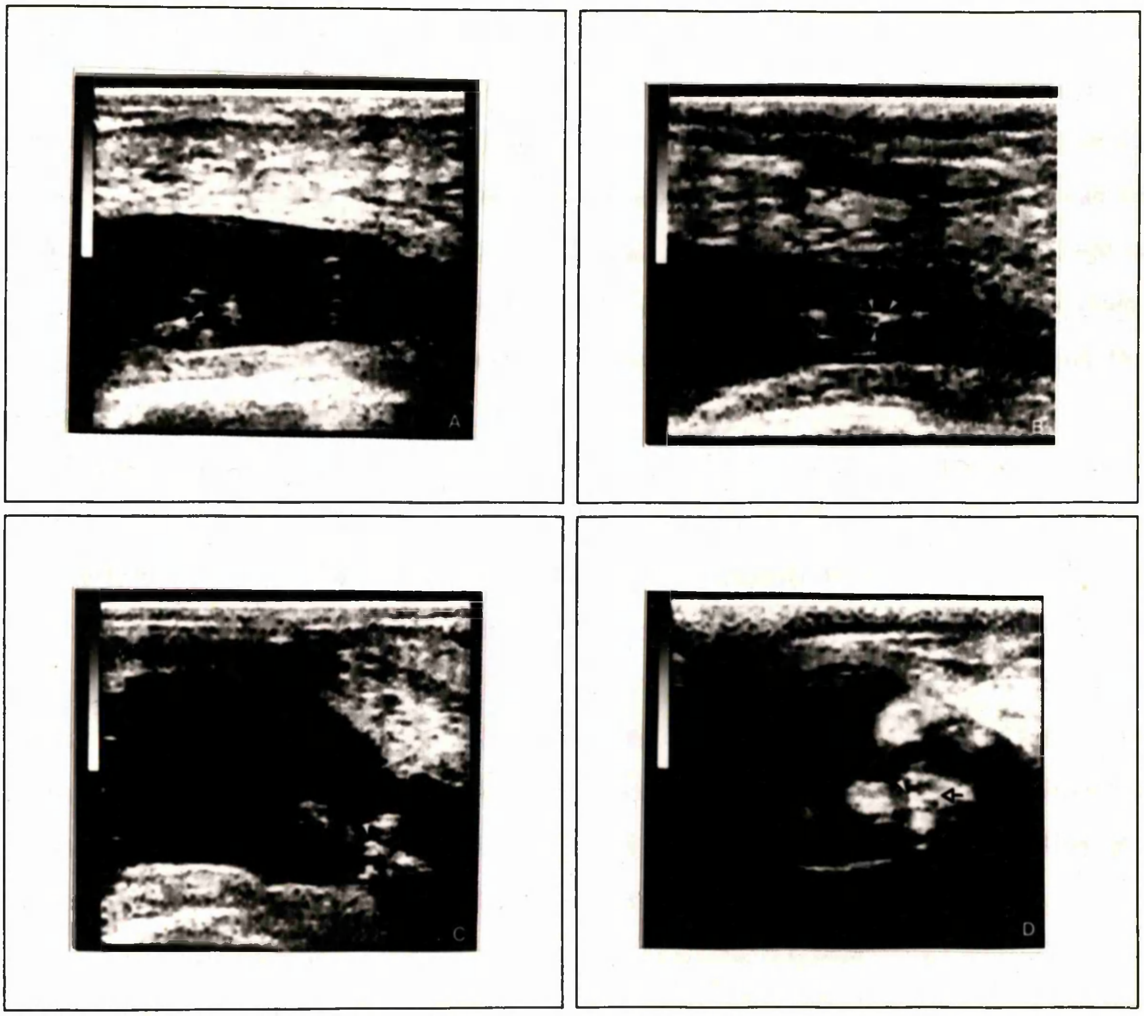


Figure 4.19: Oblique section, close to the dorsal plane, at the level of the perineal region through the umbilicus. **A.** Female foetus, on Day 54. The caudal region of the foetus is to the left side of the image. At the extreme caudal region, the tail can be seen in cross section. Close to the tail, the female genital tubercle can be noticed (arrow). It appears as a bright structure, where the genital tubercle can be seen directed to the tail. The hind limbs are seen in cross section, cranio lateral to the genitalia. The umbilical cord is also imaged in cross section and is seen cranial to the hind limbs. **B.** Cow 20, female foetus on Day 52. The cranial region of the foetus is on the left side of the frame. The umbilicus is cranial to the hind limbs, which are seen in cross section. The genital tubercle is near the tail. It appears as a hyperechoic structure slightly caudal to the hind limbs (arrow head) .Caudal to the genital tubercle, on its both sides, the genital swellings can be seen (arrows). **C.** Day 54 old male foetus. The umbilical cord is running forward the caudal region. Close to it, the genital tubercle is clearly seen (arrow). The tail is visible caudal to the genital tubercle and appears as a hyperechoic line at the extreme caudal region of the foetus. **D.** Male foetus on Day 54 of gestation. The umbilical cord is running toward the genital tubercle. Note the foetal hind limbs extended cranially, thus, it gives a wrong location of the organ of gender. Detailed observation enables one to see the hoof of the right hind limb at the same level of the genital tubercle. The genital tubercle (white arrow) is near the umbilical cord. Caudal to the genital tubercle, the genital swellings can be seen as an single hyperechoic structure (open arrow).

view of the foetus. The experience acquired during the course of the experiment, certainly helped to identify and understand the planes and sections of the foetuses during the review, producing conclusions which were not perceptible at the time of the original scans during the course of the experiment. The results of reviewing the recorded scans, which can be referred to as the second phase results, were compatible with the calving results, except in one specific case (C11). The results of the first sex determination will be initially discussed, but the results of the second examination will be also described and commented on the Section 4.2.3.

The earliest day used for sex determination of the foetuses was Day 48 after breeding. The examination performed at this stage did not give confidence in determining the sex of the foetus. At this stage, the genital tubercle was located between the hind legs, in the midline between the tail and the umbilical cord. The view obtained of the foetus was an oblique section close to the dorsal plane. The tail of the foetus seemed to be bent towards the ventral region, running between the hind limbs. Differentiation of the external genitalia by ultrasonographic visualization of these structures, did not show the existence of any morphological difference between the male and female gender at this age. The anatomical localization of the genital tubercle was also not perceptible.

The ultimate confirmation of the sex of each foetus was achieved at the time of calving. The average time spent to achieve determination of the sex of the foetuses was 2 minutes and 45 seconds, ranging from 30 seconds to 8 minute, approximately. A total of 29 examinations were performed in 22 animals, and 24 foetus were sexed. Two cows were pregnant with twins (9%). Two abortions were observed (9%). One of the twinning pregnancy had only one calf. At the time of confirmation of the sex, two foetus had no confirmation of the sex. At birth, 15 (63%) calves were male, 7 (29%) were female and 2 (8%) unknown. These two calves in which sex was not confirmed, were due to one abortion with no foetus recovered, and one twin not born. There was

another abortion, but the foetus was recovered and the sex determined. The results of the actual scanning showed 15 males and 9 females; amongst these, 16 (73%) were correctly diagnosed and 6 (27%) were wrongly diagnosed. The second phase results determined 16 males and 8 females; amongst which 21 (95%) were correct and 1 (5%) was wrong (see Table A.4).

There were many structures which could be ultrasonographically confused with the genital tubercle, due to a similar bilobar hyperechoic appearance. Location of foetal landmarks such as umbilical cord, head and heart was essential for orientation purposes.

Abortions during the course of the studies occurred, but not at the time of the period of the scans. The reasons for the abortions were unknown.

### 4.2.1 Twin Pregnancy

During the course of the experiment, two cows were diagnosed as carrying twin foetuses. One cow (C06) was first used in the prior study, for early pregnancy diagnosis, where the twin pregnancy was observed at the time of first scanning (Day 18). The other cow (C27) was first examined in this experiment, for foetal sexing. Both animals were examined for sexing their foetuses at stages later than Day 50. The pregnancy had normal development and a heart beat was detected in all foetuses at the sex determination examinations.

#### Cow 06

This cow was used in the experiment for diagnosing early pregnancy. The twin pregnancy was diagnosed at the time of the first scanning (Day 18), when two corpora lutea were visualized in one ovary and the conceptuses could be imaged, one in each horn. Subsequent scans, on Days 24, 29, 36 and 43, demonstrated viable embryos, with heart beat and normal development observed.

The embryos were noticed to exhibit differences in size and position of implantation within the horn, as pregnancy progressed. The embryo presented within the horn ipsilateral to the ovary bearing the two corpora lutea was notably bigger than the embryo within the contralateral horn, 25 mm and 20 mm respectively (see Chapter 3).

The first attempt at sexing the foetuses was on Day 56. Both foetuses were imaged on the ultrasound screen, and their heart beat detected. The difference in size and position within the uterine horns remained evident. Determination of the sex of the larger foetus was confidently achieved, and it was concluded to be a male foetus. The sex of the smaller foetus was not confidently diagnosed, its position and constant movement made visualization of the gender not clearly perceptable. The foetus was diagnosed as a male.

At the end of the examination, the recorded scanning was reviewed. Visualization of both foetuses was clearly obtained and therefore the gender could be located and the sex determined — both male (Figure 4.20).

The cow gave birth to just one live calf. Observation of the uterus of the cow did not show the presence of another foetus. The calf was healthy, but considered to be small for the size of its mother and also compared to the calves previously produced by the cow.

### Cow 27

This animal was not used for diagnosing pregnancy in early stages of gestation. It was first brought to be scanned on Day 49 after service. As the animal was not part of the first experiment, and thus no prior observation of its ovaries was done, at the time of the scan the ovaries were first examined. Images of the ovaries revealed two corpora lutea, one in each ovary. The presence of two luteal glands was suggestive of a twin pregnancy. The transducer was directed to search for the foetuses in the uterine horns.

Scanning of the uterine horns showed the presence of two very small foetuses. The small size of the foetuses was perhaps not unexpected as a result of the twining. Determination of the sex at this day (Day 49) was not possible. Identification of the foetal body structures was not clearly obtained, and thus determination of the sex at such an early stage was impracticable.

The animal was rescanned on Day 53. The image of the foetuses was easily missed, as the cow was restless and stood poorly for examination. Locating the foetuses within the large amount of uterine fluid, was difficult due to their size. Identification and location of the genital tubercle was therefore a problem but a few images were obtained allowing visualization of the echogenic appearance of the genital tubercle. The sex was determined for one male and one female, but not with confidence. After the scan, the recorded images were reviewed and the sex confidently confirmed as one male and one female foetus (Figures 4.21 and 4.22). The animal calved and the determination of the sex of the foetuses was corrected — male and female twins.

### 4.2.2 Abortions

Two late abortions occurred after the end of the ultrasonographic examination. The foetus of one cow was recovered, but the other foetus could not be recovered, and thus, the sex of this foetus was not confirmed. The day the abortion took place was also not known.

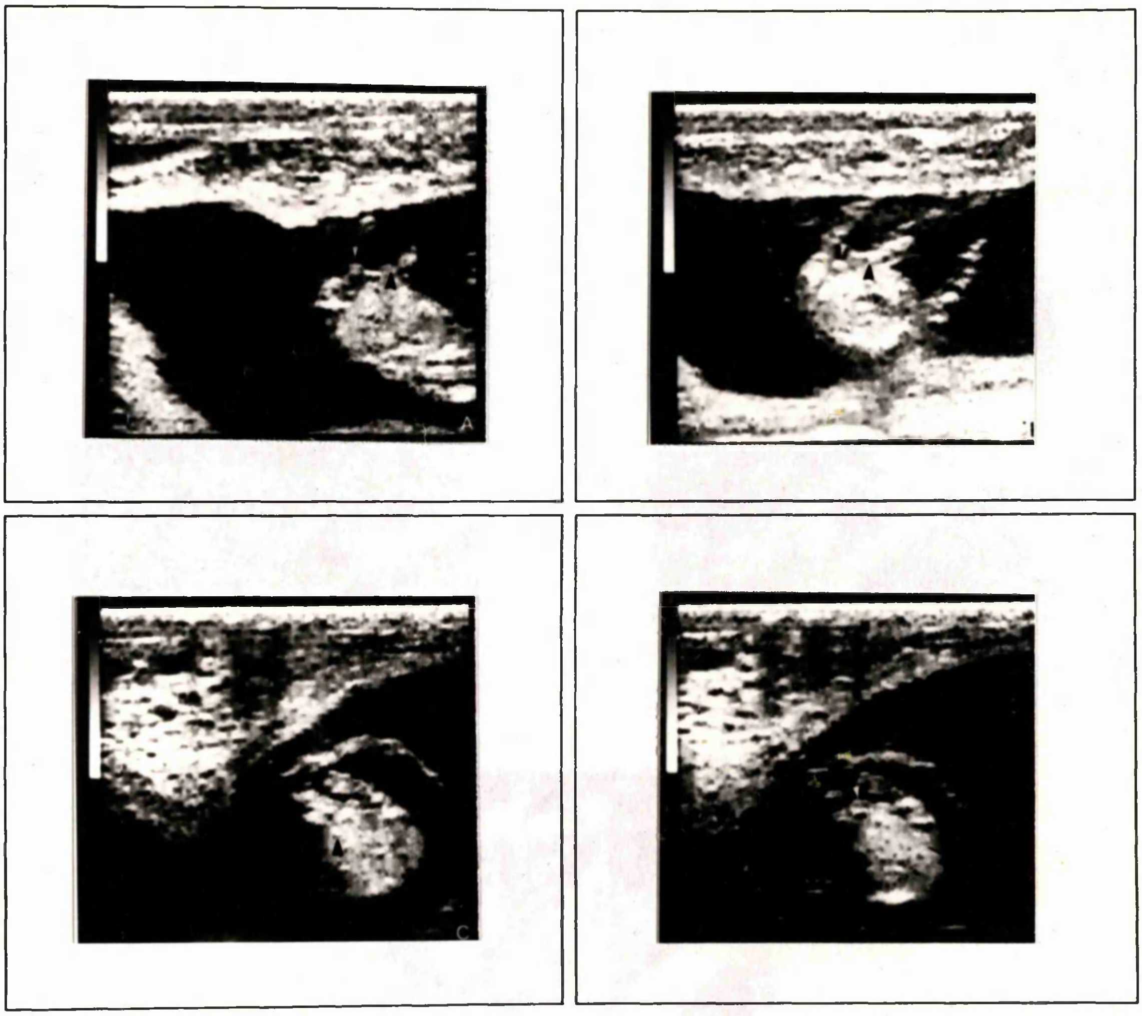


Figure 4.20: Twin pregnancy at Day 56, Cow 06, male foetuses. **A** and **B.** The foetus in the horn ipsilateral to the ovary bearing the two corpora lutea. Note the position of implantation of the foetus within the lumen. The head is not seen in this image shown, but it is at the right side of the view. The male gender is discernible between the hind limbs, at the caudal ventral region of the foetus body. The first image shows the scrotum (white arrow) between the hind limbs, which are not clearly seen in this image, but were seen in other views. The second frame shows the scrotum as a round echogenic structure between the hind limbs (white arrow). The prepuce (black arrow) is not clearly seen, due to the right hind limb bones and the umbilicus seen in the same region. **C** and **D.** The male foetus is in the contralateral horn. The difference in size and implantation is notable. In both images, the foetus presents the cranial region directed down, in the view. The first image shows the umbilical cord running to the abdomen. The genital tubercle is seen as a hyperechoic structure (black arrow) in close proximity to the umbilical cord. The scrotum is partially visualized lateral to the umbilical cord. The right hind limb is observed parallel to the foetal body, lateral to the scrotum. The second image, shows the scrotum as a dependent structure from the abdomen, between the hind limbs (white arrow).

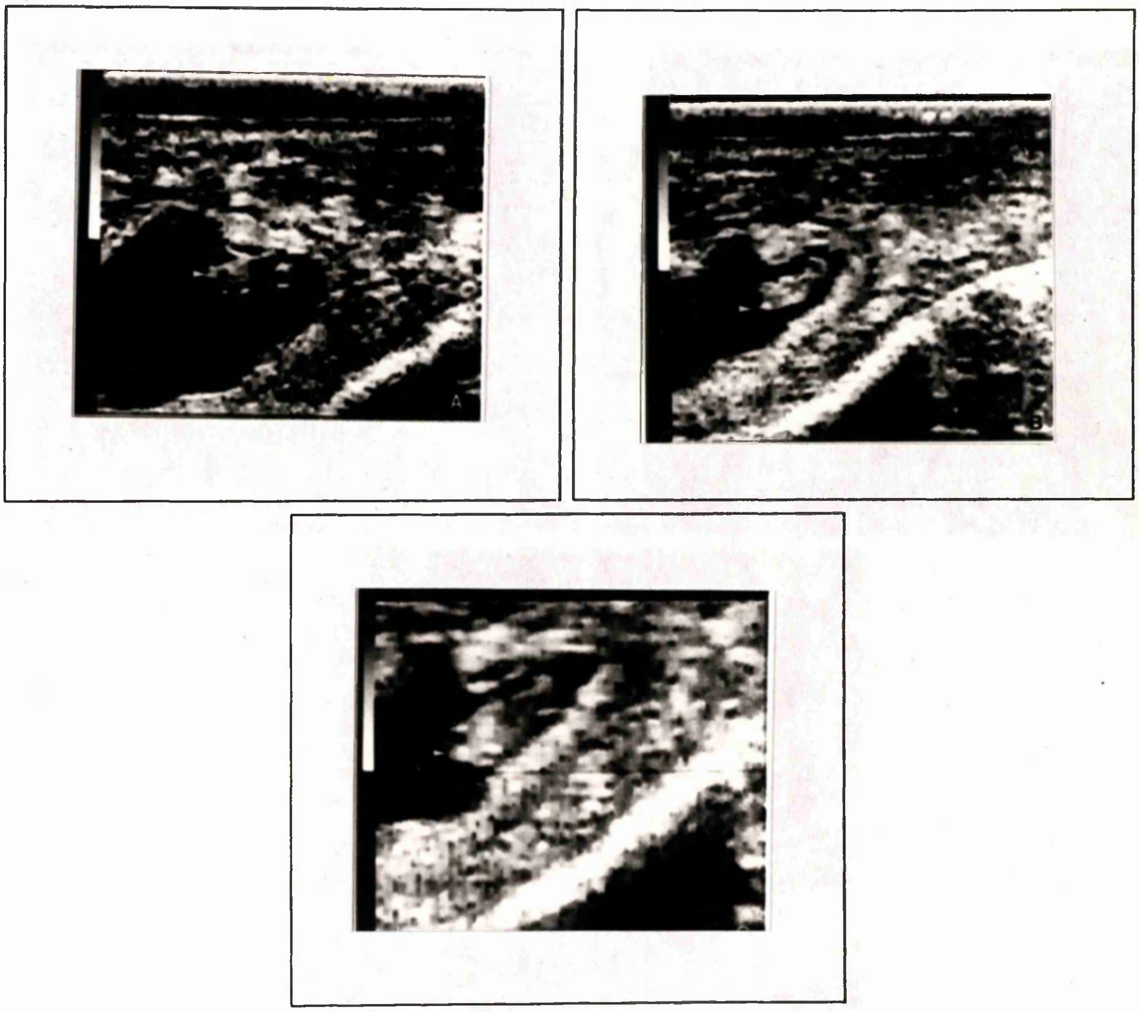


Figure 4.21: Twin pregnancy at Day 53 of gestation, Cow 27, female foetus. **A, B** and **C.** The foetus is very small, but its sex can be seen. Its head is directed up and to the right side of the image. A hyperechoic structure, the genital tubercle, can be seen at the extreme caudal region of the foetus. The umbilical region can be seen relatively defined at the abdomen of the foetus. The last image shows the umbilical cord region and no presence of echogenic structure close to it. The female genital tubercle is well defined in the last and first picture (white arrows).

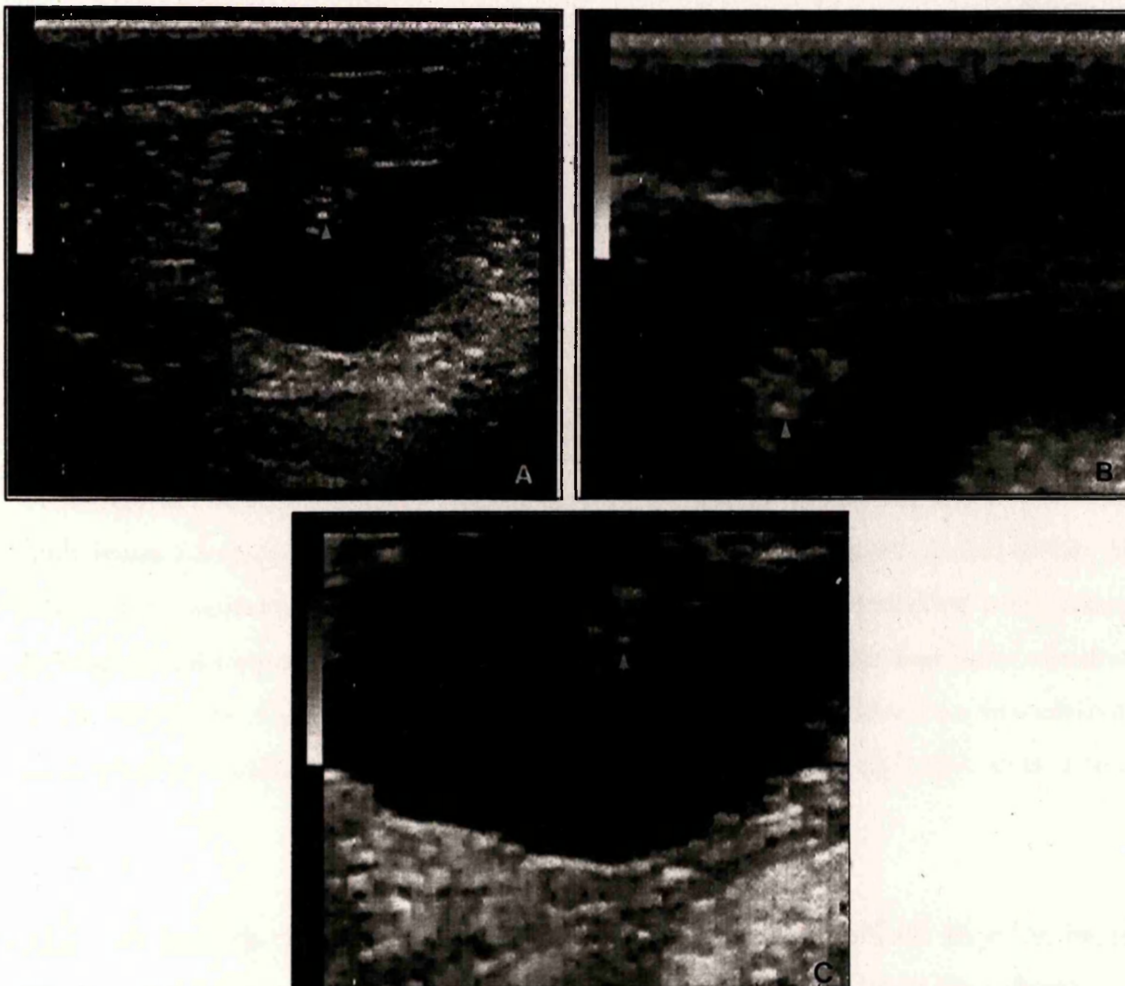



Figure 4.22: Twin pregnancy at Day 53 of gestation. **A**, **B** and **C**. The foetus is small, but the sex can still be determined. The foetus is facing to the right side of the image. The head is well defined. The umbilical region is also easily seen and the presence of a hyperechoic structure close to it is visible. This structure is the male genital tubercle, which is seen caudal to the umbilicus (arrows).

## Cow 18

This cow was not used for early pregnancy diagnosis. The animal was first scanned on Day 48 after breeding, attempting to see if ultrasonographic determination of the foetal sex was possible at this stage. The next scan was performed at a later stage, Day 57, to see whether scanning at a later stage would provide an easier imaging of the foetus and identification of the external genitalia.

Sex determination on Day 48 was not feasible, as already reported. Identification of the genital tubercle was achieved, but accurate location of the structure was not discernible at this stage (Figure 4.23) At the second examination, on Day 57, the external genitalia could be identified and located for sex determination. At this stage, the external genitalia already resembled that of the adult animal. The foetus was determined as male (Figure 4.8).

The foetus was imaged in oblique section close to the sagittal plane. Both prepuce and scrotum were visualized as small echogenic structures protruding from the abdomen of the foetus. Determination of the sex was not easily achieved in this plane. The hind limb bones resembled the scrotum and prepuce. With the movement of the foetus, the hind limbs frequently obscured visualization of the scrotum. The umbilical cord, imaged in longitudinal plane, was also, several times, seen beneath one of the hind limbs visualized on the first plane of the image. Once a detailed observation of the structures was achieved, a confident determination of the foetus sex was made, which was concluded to be a male.

## Cow 20

Also used for early pregnancy diagnosis, this cow was first scanned on Days 17, 24 and 31. The early embryo was imaged at the first scan (Day 17) and heart beat detected on the following examinations (see Chapter 3).

The cow was last scanned on Day 52, for sex determination of the foetus. Identification and location of the genital tubercle was achieved with an oblique section of the extreme caudal region of the foetus, sagittal plane of the caudal region and transverse plane of the foetus at the umbilical region. The location of the genital tubercle was suggestive of a female foetus. The tail was directed ventral toward the umbilicus, but the echogenic appearance of the organs differentiating the gender was visible. Other images clearly showed the umbilical cord running to the abdominal region and the echogenic structure

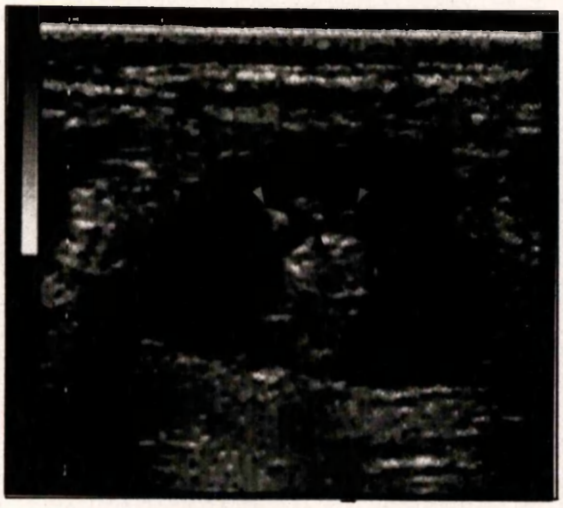


Figure 4.23: Cow 18, on Day 48 after breeding. The caudal region of the foetus appears on the top of the image. The genital tubercle is visualized, however its location is not possible to be determined (black arrow). It appears to be in its initial position, between the hind limbs (white arrows).

and appearance of the genital tubercle was seen close to the umbilicus, at the level of its attachment to the abdomen of the foetus. The sex was determined, as a female foetus, by visualization of the genital tubercle near the tail (Figures 4.19.B and 4.24). The abortion was observed 128 days after last scanning, that is, the cow aborted on Day 180 of gestation. The cause of the abortion was unknown, but the sex of the foetus was confirmed to be female.

### 4.2.3 Erroneous Diagnoses

Failure to correctly diagnosis the sex of some foetuses was observed during the experiment. This failure to determine the sex of some foetuses, was mainly observed during the first scans performed in the experiment and were closely related to the planes of view obtained. The planes which led to the inaccurate diagnosis were sections of the oblique plane close to the transverse and dorsal planes. With the experience acquired with the subsequent scans, as the study progressed, the incidence of mistakes decreased. Explanations and comparisons between the first and second results will be described.

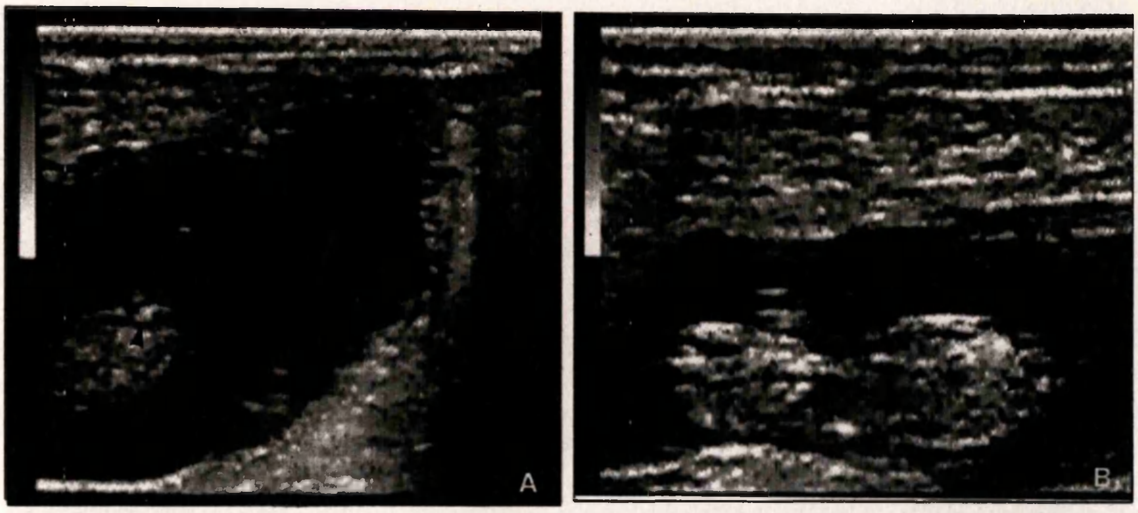



Figure 4.24: Cow 20, on Day 52 of gestation. **A.** The caudal region of the foetus is on the top and the head is not visible. The female genital tubercle can be seen as a hyperechoic structure at the perineal region of the foetus (arrow). **B.** The foetus is in dorsal plane. Note the genital tubercle on the extreme caudal region, close to the tail (arrow).

### Cow 05

Scanned on Day 51 after breeding, the foetus was imaged in an oblique section close to the dorsal plane. The first achieved images showed the ventral perineal region of the foetus. The umbilical region seemed to exhibit an hyperechoic structure caudal to the umbilicus. The hind limbs could be seen in cross section and the tail appeared as a discrete echogenic line caudal to the pelvic limbs. Another view showed the supposed genital tubercle located in position more cranial than the hind limbs and the tail could not be visualized. The umbilical region was seemingly near the genital tubercle, but not in close proximity to it. These findings appeared to be suggestive of a male foetus.

At the time of the second analysis, by reviewing the recorded scan, the findings showed a discrepancy in the results. The image exhibited a discernable genital tubercle located near the tail, more caudal than the hind limbs. Thus, the diagnosis was determined positive for a female foetus (Figures 4.25) .

### Cow 14

This animal was scanned on Day 54 after breeding. The images achieved were oblique sections close to the transverse plane, at the region caudal to the umbilicus. At this stage, the genital swellings have already fused to resemble that of the adult. In the plane of scan

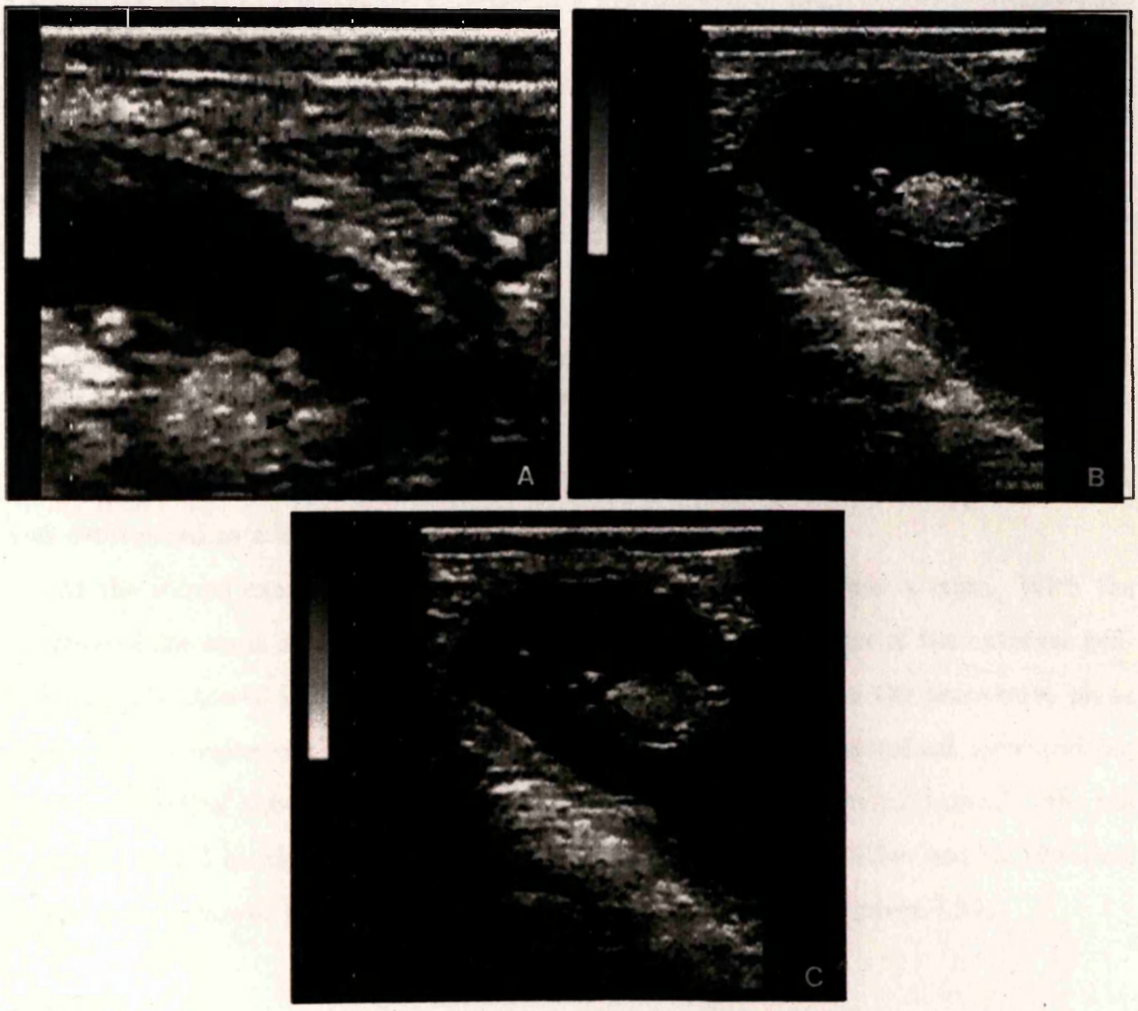



Figure 4.25: Cow 05 on Day 51 of gestation. **A.** The foetus is lying cranially to the left side of the picture. Slightly caudal to the hind limbs, the female genital tubercle (arrow) is observed close to the tail. **B.** The foetus is positioned cranially to the right side of the image. At the caudal region of the foetus, the genital tubercle (arrow) is seen close to the tail. The migration of the genital tubercle is noticed by its location. **C.** The hyperechoic appearance of the umbilicus at this plane resembled the male genital tubercle (arrow).

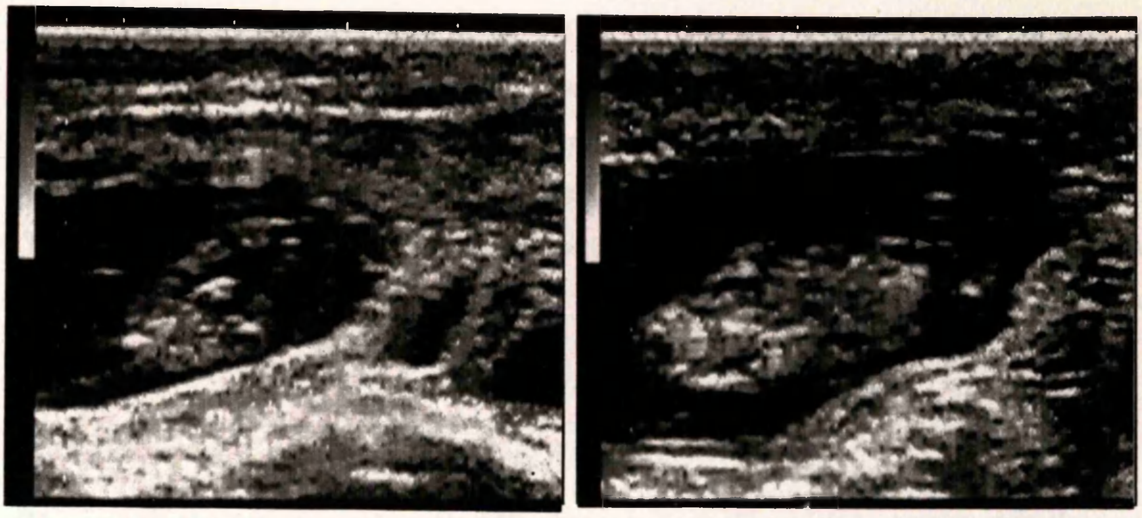


Figure 4.26: Cow 14 on Day 54 of gestation. Both images show the forming scrotum, between the hind limbs (arrow). The foetus is presenting in oblique transversal and oblique dorsal planes, respectively. The hind limbs are seen in both images.

obtained, the scrotum appeared to be the vulva, located between the hind legs. The sex was determined as a female foetus.

At the second examination, it was concluded that the foetus was a male. With the practice of the scans and planes obtained; identification and location of the external genitalia clearly showed it to be a male. The oblique section close to the transverse plane at the caudal region of the umbilicus exhibited the scrotum in longitudinal view and not the female genital tubercle. In case of visualization of the female genital tubercle, the tail vertebrae would be also imaged near to the female genitalia. The other section obtained in this plane, showed the male gender cranial to the hind limbs (Figures 4.26).

### Cow 16

Scanned on Day 51, the oblique sections observed were close to the transverse plane at the extreme caudal region, the tail vertebrae could be seen and, between the hind limbs the genital tubercle was observed. At the time of the scan, the tail vertebrae were not noticed by the ultrasonographer, who immediately concluded that the region viewed on the scanner screen was a ventral portion of the abdomen, showing the genital tubercle between the hind legs.

Reviewing the images, it was not difficult to realize the mistake made at the first diagnosis. The caudal region of the foetus showed a cross section at the tail vertebrae at the region of its connection with foetal body, directly opposite in position to the genital tubercle. The genital tubercle was seen as a small hyperechoic portion situated near the umbilical cord, which could be partially seen. The section at the level of the umbilical cord exhibited a discrete hyperechoic structure at the level of the umbilical attachment. This structure was identified as the male genital tubercle. During the original scan, this structure was not distinguished. The review of the images determined the sex of the foetus as a male (Figure 4.17.C).

### Cow 19

The scanning was performed on Day 55 after breeding. The oblique section was close to the dorsal plane at the proximal region of the hind limbs. This plane allowed visualization of the abdomen which appeared with its caudal region presenting a pointed image. The echogenic genital tubercle appeared to be protruding from the abdomen of the foetus, which led to the conclusion that it should be of the male gender. Detailed observation of the recorded images, proved it not to be a male foetus. The level of the plane of the section did not show the lower region of the abdomen, where the male genital tubercle and scrotum, and the umbilicus would be seen. On review, the genital tubercle was seen protruding caudally from the foetal body, being that of the female gender and the echogenic structure appearing beneath it turned out to be the vertebrae (Figure 4.18)

### Cow 02

The cow was scanned on Day 62 after breeding. Visualized in oblique section, close to the transverse plane at the region caudal to the umbilicus, the foetus exhibited the ventral portion of its abdomen and the distal portion of the hind legs (hooves). A dependent structure apparently seen between the hind limbs induced the diagnosis of the sex of the foetus as a female.

In the later study of the recorded image, it was noticed that the ventral portion imaged corresponded to the region between the scrotum and the umbilicus. The protruding structure pending from the abdominal region was the scrotum of the foetus and partial visualization of the hind limbs could be seen deep to the male genitalia (Figure 4.15.C).

**Cow 11**

This cow was examined on Day 52 after breeding. The images obtained were very clear, however paradoxal. The oblique section was close to the transverse plane at the region caudal to the umbilicus. The dorsal plane of the extreme ventral region was also achieved in the scanning. The analysis of the recorded images, at the end of the experiment, was consistent with the result of the scanning, in spite of the location of some hyperechoic structures resembling the genital tubercle. The sex was determined as female.

The transverse plane clearly showed a bilobar hyperechoic structure at the extreme perineal region of the foetus. This structure was situated between the hind limbs, which appeared in cross section. Close to the bilobar hyperechoic structure, caudal to it, an echogenic structure could be seen, which seemed to be a cross section view of the tail. The umbilical cord was not clearly visible, but a rounded more echogenic area could be visualized at the ventral abdominal wall, which appeared to be the abdominal region of the umbilical attachment. An oblique section close to the dorsal plane showed an hyperechoic structure directed toward the tail, near to the latter. The dorsal planes at the extreme ventral region exhibited a cross section of the umbilicus and limbs. Between the hind limbs, an hyperechoic structure was observed and seemed to be directed toward the tail. Two small rounded structures were also present on either side cranial to the supposed genital tubercle. These structures were apparently similar to the genital swellings. The tail appeared to be the structure visualized in cross section close to the supposed tubercle. The cross section of the umbilicus surprisingly exhibited a hyperechoic structure in close proximity to it. As most of the findings suggested a female, the sex was so determined. However the result after calving showed a male foetus (Figures 4.27).

## 4.3 Discussion

The current experiment was carried out to evaluate the accuracy of B-mode ultrasound imaging to determine the sex of bovine foetuses under farm conditions. Aiming to achieve a reliable result, techniques and parameters of scanning were defined.

Ultrasonographic identification and location of the foetal external genitalia, in the early gestational stage, allowed determination of its sex. The genital tubercle is the embryonic structure, present in both male and female, that differentiates into the penis and

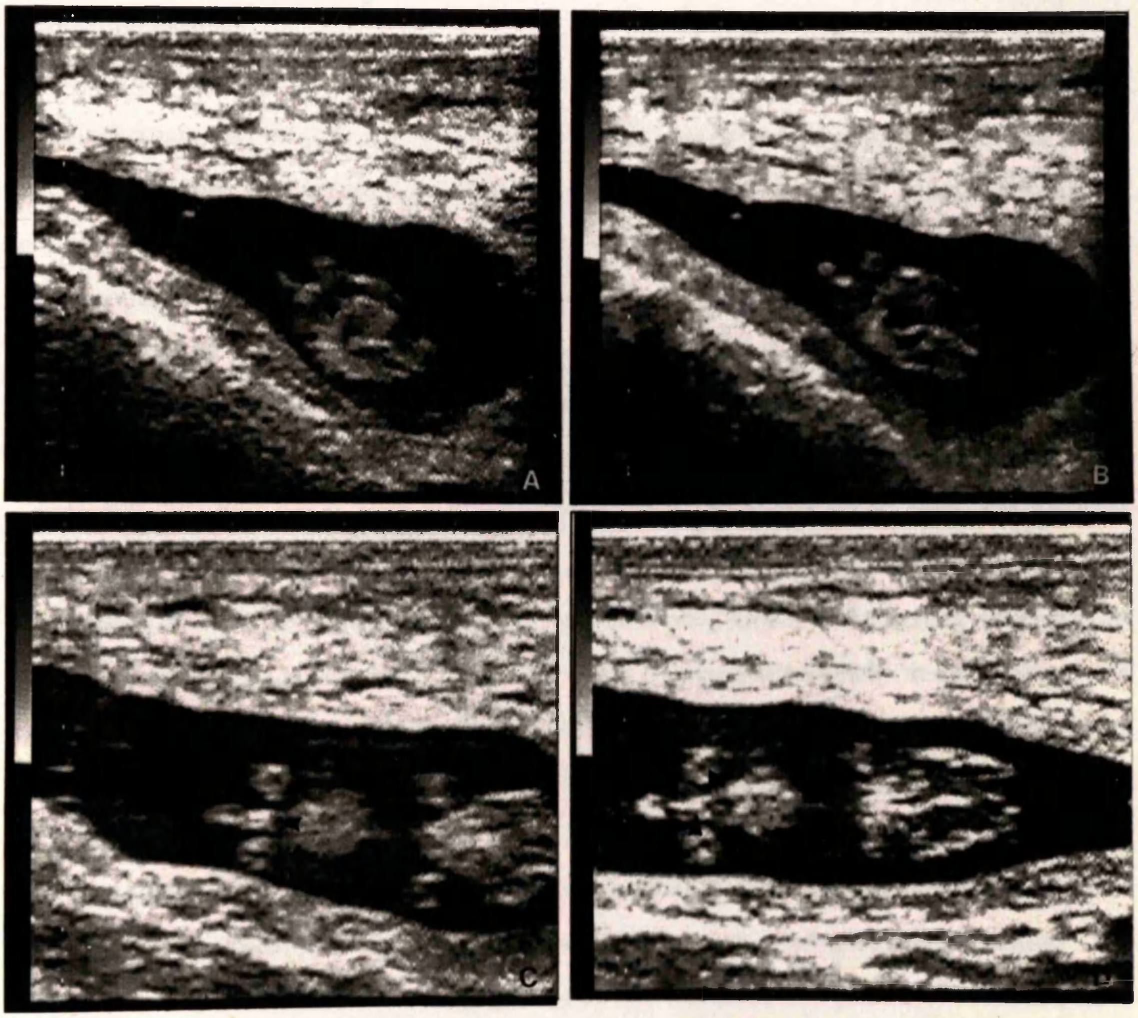


Figure 4.27: Cow 11 on Day 52 of gestation. **A** and **B**. Note the perineal region, the bilobar hyperechoic structure (arrow). The tail vertebrae is caudal to this structure. The hind limbs can be seen lateral to the hyperechoic structure, which resembles the female genital tubercle. **C**. The hyperechoic structure is directed forward the tail. The tail vertebrae is visualized caudal to the genital tubercle. **D**. Hyperechoic structures can be seen between the hind limbs and at the umbilical region (arrows). Caudal to the umbilicus the hyperechoic structure is observed and seems to be directed toward the tail.

clitoris in male and female foetuses, respectively. During the process of differentiation, the genital tubercle migrated from its initial position between the hind limbs, towards the umbilicus in males and towards the tail in females (Inomata et al. 1982). The genital tubercle is ultrasonographically seen as an hyperechoic structure usually presenting a bilobar appearance.

Using a 7.5 MHz transducer, pregnant cows were examined from Days 48 to 62 after breeding, aiming to identify and locate the genital tubercle for determination of the foetal sex. The number of animals expected to be used in this experiment was unfortunately reduced due to unforeseen circumstances. The cows used for early pregnancy diagnosis were supposed to be part of the sample of animals used in this current study. However the incidence of early embryonic mortality and repeated oestrus, as a consequence of low fertility animals, led to later pregnancies and hence contributed for the decrease in the number of cows to be scanned for sexing. Moreover the later abortions also led to a reduction in the sample used. It is also noticeable that the scans were not entirely concentrated in the days defined for sexing in this experiment Days 50 to 54. Although the sample was not of large proportion it appeared to prove a wide variety of planes of view of the foetuses made during the scans, which it is hoped will help in orientating and locating the genital tubercle for determination of the sex of the foetus. It also showed the ultrasonographic appearance of the genital tubercle on different days of the early stages of its differentiation.

Determination of the location of the genital tubercle was dependent on the plane of scanning. The transducer frequency used was also a factor of great importance. The failure to correctly diagnosis the cows proved to be related to the planes of view obtained during the scanning. The cross sectional view of the umbilical cord appeared to be the most reliable way for diagnosis. However, a cross section of the umbilical cord at the base of its attachment to the abdominal region, occasionally exhibited hyperechoic areas, which may be mistaken with the genital tubercle. These areas of hyperechogenicity may be referred to the wall of the umbilical veins or also some internal structure of the foetal body close to the region of the umbilical attachment. The sagittal plane was rarely achieved and seemed to produce inconstant results in the early stages of migration of the organ of gender. Due to the small size of the external genitalia at the early stages of development, it could be easily missed by the presence and movement of the hind limbs seen in this view.

The dorsal plane provided the best images obtained in the experiment and gave an easy location of the organ of gender. The foetus could be imaged in its entire length in this plane, showing the umbilical and perineal regions, enabling identification and location of the genital tubercle. The umbilical cord was seen in a cross sectional plane, in the sections made in this plane at the extreme ventral region, which was immensely helpful in locating and sexing the foetuses.

The cross sectional plane of the foetus at the level of the umbilical region or caudal to it proved to be a consistent useful plane of view for sexing. In the male, the genital tubercle could be seen close to the umbilicus and also the longitudinal presentation of the scrotum, when this was already formed. A section at the extreme caudal region also gave a good view for identification of the male when the scrotum of the male foetus was already developed, which could be seen dependent from the abdomen, between the hind legs.

The oblique plane was the most commonly achieved plane. Usually obtained as a deviation of the dorsal and transverse planes, the oblique plane presented difficulties to properly locating the genital tubercle. Always partially showing the foetus and also seemingly to show the foetus as if it was reduced, several sections of display gave rise a failure to correctly diagnose the sex of the foetus. Usually in this plane, the tail appeared running towards the umbilicus, and could cover the external genitalia if this was located close to the tail or even give a wrong location of the gender, if this was migrating towards the umbilicus. In a foetus from Day 55 to 56, for example, the genital swellings, that already resemble the adult scrotum, can be confused with the organ differentiating the female gender. It happened when the umbilical region was not clearly imaged and thus the genital tubercle was not seen. In this case, the presence of the hyperechoic structure between the hind limbs or even close to the tail, when the latter is bent towards the umbilicus, might have influenced the diagnosis of a female foetus.

It was evident that previous knowledge of the morphological appearance of the genital tubercle and also its relative position during the various stages of its differentiation was essential. In cases of oblique sections, the knowledge of the relative location of the genitalia, according to the age of the foetus scanned proved to be helpful. The erroneous diagnoses were a consequence of poor knowledge of the planes and of the echogenic structures that resembled the hyperechogenic appearance of the genital tubercle. With the experience acquired during the course of the research, the ultrasonographer became acquainted with the planes and the various foetal structures observed during the scans. Subsequently, at the time of the analysis of the scans, the mistakes were recognized and corrected. One specific case of sex determination was doubtful. This cow (C11) showed contradictory images. At the stage the animal was scanned, the male genitalia was still migrating toward the umbilical region and was thus visualized near the umbilicus, but not in close proximity to it. The genital swellings are also seen on both sides caudal to the genital tubercle (Inomata et al. 1982). However, some images obtained showed the genital tubercle in close proximity to the umbilicus and the presumptive genital swellings between the hind legs, distant of the genital tubercle. It appeared that the discrepancy in the achieved images could be partially explained if the hyperechoic supposed genital tubercle, that is seen close to the cross section of the tail vertebrae, could be part of the tail running toward the umbilicus. In this case, the tail was visualized in longitudinal plane at this region, resembling the genital tubercle of the female. However the distance between the genital tubercle and the supposed genital swellings would not match with the gross morphological findings of the external genitalia of the bovine foetuses at this age as reported by Inomata (Inomata et al. 1982).

Determination of the bovine foetal sex by identification and location of the genital tubercle, certainly gives an earlier result than by visualization of the mammary gland and the scrotum, as reported in (Muller & Wittkowski 1986). In this thesis, identification of the genital tubercle was achieved at the day of first scanning, Day 48, which is earlier than reported by Curran et al (Curran et al. 1989). Curran also showed that the reliability of the technique began on Day 55, indicating that the optimal day for sexing foetuses was from Day 55 to 64 (Curran & Ginther 1991). The results achieved in this thesis showed that determination of the foetal sex is possible with accuracy from Day 51 to 54.

It was concluded that determination of the plane of view had to be carefully carried out before the location of the genital tubercle was evaluated. It was convenient to first look for foetal landmarks for orientation purposes and also detailed observation of other embryonic structures, with an ultrasonographic appearance similar to that of the genital tubercle. Therefore, if the ultrasonographer attempted to hurry the examination, misinterpretations could be made.

Constant movement made by the cow under examination was reason for difficulty in performing the technique for sexing the foetus. It is also worth noting that the diagnosis became less consistent as foetal age increased, since the foetus became too large to be imaged within the range of the high frequency transducer.

The results achieved in this research were relatively successful. The incorrect diagnoses observed, at the beginning of the experiment, were later proved to be the consequence of the inexperience of the ultrasonographer. When the ultrasonographer was acquainted with the planes of view and the relative location of the genitalia related to these planes, the accuracy of the diagnosis was high (Table A.4).

The use of ultrasonography to access the identification and location of the genital tubercle, and its early stage of differentiation (Day 50 to 54), proved to be an accurate and non-invasive technique for determining the sex of the bovine foetus under farm conditions. It appeared that one or two examinations, in any case of doubt at the first attempt to sex, during the gestational stage between Days 50 and 54 was able to give precision in correctly determining the sex of the foetus. Although the technique is relatively easy to learn and apply, it requires considerable experience and skill to achieve high level of accuracy.

Ultrasonography for sexing bovine foetus is a highly efficient and feasible technique under farm conditions. It is a promising method that can allow the cattle breeder to plan in advance the future use of the calves which are yet to be born.

# Chapter 5

# Conclusions and Future Work

This chapter presents the achieved results and the alternatives for future work. The difficulties experienced during the experiment, and the knowledge necessary for successful application of the techniques are also described.

## 5.1 Assessment

### Gestational Diagnosis

The use of ultrasound imaging for evaluating ovarian structures and diagnosing early pregnancy in the bovine has been studied. There are several reports of the use of transrectal B-mode real time ultrasound examination for the study of ovarian structures (Pierson & Ginther 1984*b*, Fissore et al. 1986, Edmondson, Fissore, Pashen & Bondurant 1986, Pierson & Ginther 1988, Omran et al. 1988), gestational diagnosis and embryonic development (Pierson & Ginther 1984*a*, White et al. 1985, Chaffaux et al. 1986, Curran et al. 1986*a*, Curran et al. 1986*b*, Hanzen & Delsaux 1987, Kastelic et al. 1988). The gestational diagnosis studies were performed using transducers of frequencies varying from 3.0 to 5.0 MHz. Most of the examinations were accomplished on different days of the bovine oestrus cycle throughout early pregnancy. Some reports stated that pregnancy detection was possible by visualization of the early vesicle (conceptus) between Days 11 to 19; with first detection of embryonic heart beat between Days 19 to 33 after breeding.

Recently, using a 7.5 MHz transrectal transducer, research worker have reported the visualization of the early vesicles between Days 9 and 12 and first detection of embryonic heart beat between Days 19 and 20. Also high accuracy results could be achieved approximately on Day 17. Although using the same frequency transducer, there were some contradictory opinions, between these studies, about the accuracy of this approach in such an early stage of gestation (Omran 1989, Kastelic, Bergfelt & Ginther 1991).

The criteria used in those referred studies, for diagnosing pregnancy in the early stage of gestation (up to Day 19), were based on the presence of an active luteal gland and an amount of fluid within a horn, usually ipsilateral to the ovary bearing the corpus luteum, with the uterine horn exhibiting a smooth endometrial layer. The findings were later confirmed by visualization of an early embryo and embryonic heart beat from Day 19 onwards. Visualization and the development of the conceptus were followed from the very early stages throughout the later days of gestation, as already mentioned.

### Sex Determination

Sex determination of the bovine foetus has been poorly reported. The few experiments accomplished in this field, used transducer frequencies ranging from 3.0 to 5.0 MHz. Initially, searching for visualization of scrotal swellings and the mammary gland, ultrasound scans were performed between Days 100 and 110 of gestation (Muller & Wittkowski 1986). More recently, it has been reported that the bovine genital tubercle was first identified on Day 50, but a reliable determination of the sex was only possible from Day 55 (Curran et al. 1989). Later, it was shown that, under farm conditions, the optimal days for sexing foetuses was between Days 59 to 68 (Curran 1992).

## 5.2 Achievements

### Gestational Diagnosis

The use of a 7.5 MHz transrectal transducer under farm conditions, to diagnose pregnancy in the cow with a single examination, from Days 16 to 20, was evaluated. Other periods of gestational stage were also assessed to determine accuracy.

The approach used was similar to the ones already used for this purpose. The differences between this and the other techniques, may lie in the sequence of the observation of the reproductive structures. The procedure used in this research, starts by examination of an ovary followed by the ipsilateral horn, thereafter visualization of the contralateral ovary and horn; finally examining the uterine body, cervix and vagina.

Others parameters were considered, aiming to enhance the criteria used. These parameters consisted of a detailed observation of a discernible early embryo within the non echogenic intrauterine collection of fluid and an analysis of the amount of this fluid. This amount of fluid had to be compatible with the amount expected for the gestational age at the time of the scanning, in a positive diagnosis.

Ultrasound detection of pregnant and non pregnant cows was 100% accurate from Day 20. Embryonic heart beats were detected in all embryos from Day 20 onwards. Embryonic body structures and the associated membranes were clearly visualized on following days. In the early gestational stage, Day 16 to 19, the early embryo was clearly visualized within the vesicle, in 50% of the pregnant cows. Accuracy was considered to be high at this stage (Table A.2); however the incidence of early embryonic loss reduced the accuracy of the technique (88%). All results obtained during the experiment can be assessed in the Appendix A and Appendix B.

The results indicated the earliest day for diagnosing pregnancy was Day 20. At this stage, the embryonic heart beat and a discernible embryo are clearly imaged and, thus, highly accurate diagnoses can be given.

### Sex Determination

For this purpose, a scanning technique was defined. The planes of view of the foetus were analysed and described. The location of the gender and determination of the sex were found to be strictly tied to the plane of view. The criteria used allowed determination of the foetal sex with a high level of accuracy. At the results first obtained, the accuracy was 73% and at the second analysis the accuracy increased to 95.5% (Table A.4). All results obtained during the experiment can be assessed in the Appendix A and Appendix B.

The genital tubercle was identified at the time of first examination, Day 48. Its location could not be defined until Day 49 and appeared to be dependent on the plane of view. The dorsal plane at the extreme portion of the abdominal region proved to be the most reliable way of sexing. The umbilical cord could be seen in a cross section plane in this section. The oblique plane, provided, in some sections, erroneous location of the genital tubercle. In this plane, the image of the genital swellings, in their first stage of fusion to form the scrotum, could be confused with the female genital tubercle.

The results showed that identification of the genital tubercle can be possible before Day 50 of gestation. However, its location for determination of the sex is not feasible before Day 50 of gestation. It was also concluded that examination became difficult as foetal age increased, due to the large size of the foetus, when using a high frequency transducer. The results achieved indicated that sex of the bovine foetus can be accurately determined after 49 to 54 days of gestation. A standard technique for routine examination was defined, providing explanation of the possible planes of view found during the scans and indicating their advantages.

## 5.3 Encountered Difficulties

In general, the difficulties faced during the course of this research were almost the same for both experiments — gestational and sex diagnoses.

The constant movement and rectal straining of the animal under examination, was one of the main difficulties encountered in the technique. In these cases, structures were difficult to be analysed, since holding the image and slowly moving the transducer to observe the entire structure was not always possible for any length of time.

The plane of view of the structure or region scanned, also provided a great barrier for identifying, evaluating and locating the structures' components. Depending on the plane, structures could be missed or confused with similar ones.

For early pregnancy diagnosis, the embryonic mortality was a serious obstacle, resulting in a decrease of diagnostic accuracy. In fact, it was the main cause of the non feasibility of the technique before Day 20.

For sexing foetuses, structures resembling the organs of gender, in its early stage of differentiation, made location of the latter difficult. A thorough examination and an analysis of the location of these structures, at the specific age, helped to clarify the doubt.

## 5.4 Required Background

A knowledge of ultrasonographic principles and appearance of the structures are the first requisites to the use of ultrasound equipment. Knowledge of the gross anatomy and morphology of the organs is also required.

The experience of the ultrasonographer is of great importance. Familiarity with the approach used, allows the ultrasonographer to properly identify and analyse the structure examined, hence reducing the incidence of erroneous diagnoses.

## 5.5 Future Work

Future work in diagnosing and sexing bovine foetus could see improvements. The use of the techniques in a larger number of animals, with the use of equipment with higher resolution, could enhance the consistency of the method and even reduce the earliest day for diagnosis. With the knowledge acquired with the techniques defined, a skillful ultrasonographer would be able to achieve highly accurate results.

## 5.6 Final Words

According to the literature, this research work is the first, in the area of ultrasonography, to determine the sex of the bovine foetus using a 7.5 MHz transrectal transducer. Certainly, the successful results were deeply influenced by the higher resolution of the transducer used. The difficulties encountered were mostly related to the limited experience of the ultrasonographer. Nevertheless, a systematic technique for routine diagnosis was defined, presenting a variety of planes and sections of view that are commonly obtained during scanning and that may be helpful to easier identify and locate the gender of the foetus. The study related to the early gestational diagnosis was not the first with the use of a high frequency transducer, but showed some topics previously not reported.

Doubtless, with the constant improvement of the technology, the appearance of higher resolution ultrasonographic equipment will enable a reduction of this time window for foetal sex determination and early diagnosis of reproductive events and occurrences in the reproductive tract of the bovine. In the meantime, all results achieved in this field are certainly welcome to improve techniques and help to clarify doubts.

We hope that this research work has given a small contribution to the field of reproduction management of the cow; with its application in routine examinations of the reproductive tract of cows under farm conditions.

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# Appendix A

# Statistics

The statistic data obtained from the experiments are given in Tables A.1, A.2, A.3 and A.4. The abbreviations used in the tables are also given.

## A.1 Abbreviations

**CFO** - Conceptus First Observed

**CND** - Correct Negative Diagnosis

**CPD** - Correct Positive Diagnosis

**EEFO** - Early Embryo First Observed

**ELO** - Echogenic Loss Observed

**IND** - Incorrect Negative Diagnosis

**IPD** - Incorrect Positive Diagnosis

**LSO** - Last Standing Oestrus

**ND** - Negative Diagnosis

**NofA** - Number of Animals

**NofSc** - Number of Scannings

**PD** - Positive Diagnosis

**UND** - Uncertain Negative Diagnosis

**UPD** - Uncertain Positive Diagnosis

| LSO | PD | UPD | CPD | IPD | ND | UND | CND | IND | NofA | Scans | ELO | CFO | EEFO |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 16 | 1 | 0 | 0 | 1 | 0 | 1 | 1 | 0 | 2 | 2 | 0 | 0 | 1 |
| 17 | 4 | 0 | 3 | 1 | 0 | 1 | 1 | 0 | 4 | 5 | 0 | 3 | 2 |
| 18 | 4 | 0 | 4 | 0 | 1 | 1 | 2 | 0 | 6 | 6 | 0 | 3 | 2 |
| 19 | 9 | 0 | 8 | 1 | 2 | 0 | 2 | 0 | 11 | 11 | 0 | 5 | 4 |
| 20 | 2 | 0 | 2 | 0 | 0 | 0 | 0 | 0 | 2 | 2 | 0 | 0 | 0 |
| 21 | 0 | 0 | 0 | 0 | 1 | 0 | 1 | 0 | 1 | 1 | 1 | 0 | 0 |
| 23 | 3 | 0 | 3 | 0 | 1 | 0 | 1 | 0 | 4 | 4 | 0 | 0 | 0 |
| 24 | 6 | 0 | 6 | 0 | 3 | 0 | 3 | 0 | 8 | 9 | 2 | 0 | 0 |
| 25 | 0 | 0 | 0 | 0 | 2 | 0 | 2 | 0 | 2 | 2 | 0 | 0 | 0 |
| 26 | 6 | 0 | 6 | 0 | 1 | 0 | 1 | 0 | 7 | 7 | 1 | 0 | 0 |
| 27 | 2 | 0 | 2 | 0 | 1 | 0 | 1 | 0 | 3 | 3 | 0 | 0 | 0 |
| 28 | 0 | 0 | 0 | 0 | 3 | 0 | 3 | 0 | 3 | 3 | 1 | 0 | 0 |
| 29 | 3 | 0 | 3 | 0 | 0 | 0 | 0 | 0 | 3 | 3 | 0 | 0 | 0 |
| 30 | 7 | 0 | 7 | 0 | 0 | 0 | 0 | 0 | 7 | 7 | 0 | 0 | 0 |
| 31 | 5 | 0 | 5 | 0 | 2 | 0 | 2 | 0 | 7 | 7 | 0 | 0 | 0 |
| 32 | 0 | 0 | 0 | 0 | 1 | 0 | 1 | 0 | 1 | 1 | 0 | 0 | 0 |
| 33 | 2 | 0 | 2 | 0 | 1 | 0 | 1 | 0 | 3 | 3 | 0 | 0 | 0 |
| 34 | 2 | 0 | 2 | 0 | 0 | 0 | 0 | 0 | 2 | 2 | 0 | 0 | 0 |
| 35 | 0 | 0 | 0 | 0 | 1 | 0 | 1 | 0 | 1 | 1 | 0 | 0 | 0 |
| 36 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 0 |
| 38 | 0 | 0 | 0 | 0 | 1 | 0 | 1 | 0 | 1 | 1 | 0 | 0 | 0 |
| Total | 57 | 0 | 54 | 3 | 21 | 2 | 24 | 0 | 79 | 81 | 5 | 11 | 9 |

Table A.1: Early Ultrasound Diagnosis in Bovine – Collected Data.

| Last Standing Oestrus Day | 16 - 19 | 20 - 25 | 26 - 31 | 32 - 36 |
|---|---|---|---|---|
| Positive Pregnancy Diagnosis | 18 | 11 | 23 | 5 |
| Correct Positive Pregnancy Diagnosis | 15 | 11 | 23 | 5 |
| Incorrect Positive Pregnancy Diagnosis | 3 | 0 | 0 | 0 |
| Negative Pregnancy Diagnosis | 6 | 7 | 7 | 3 |
| Correct Negative Pregnancy Diagnosis | 6 | 7 | 7 | 3 |
| Incorrect Negative Pregnancy Diagnosis | 0 | 0 | 0 | 0 |
| Number of Animals Examined | 20 | 15 | 26 | 8 |
| Number of Scanning | 24 | 18 | 30 | 8 |
| Sensitivity | 100% | 100% | 100% | 100% |
| Specificity | 67% | 100% | 100% | 100% |
| Positive Predictive Value | 83% | 100% | 100% | 100% |
| Negative Predictive Value | 100% | 100% | 100% | 100% |
| Total Accuracy | 88% | 100% | 100% | 100% |

Table A.2: Statistics Data of Ultrasound Pregnancy Diagnosis.

| Last Standing Oestrus Day | 16 - 20 | 21 - 27 | 28 - 34 |
|---|---|---|---|
| Positive Pregnancy Diagnosis | 20 | 17 | 19 |
| Correct Positive Pregnancy Diagnosis | 17 | 17 | 19 |
| Incorrect Positive Pregnancy Diagnosis | 3 | 0 | 0 |
| Negative Pregnancy Diagnosis | 6 | 9 | 7 |
| Correct Negative Pregnancy Diagnosis | 6 | 9 | 7 |
| Incorrect Negative Pregnancy Diagnosis | 0 | 0 | 0 |
| Sensitivity | 100% | 100% | 100% |
| Specificity | 67% | 100% | 100% |
| Positive Predictive Value | 85% | 100% | 100% |
| Negative Predictive Value | 100% | 100% | 100% |
| Total Accuracy | 88% | 100% | 100% |

Table A.3: Accuracy of Pregnancy Diagnosis in Cows.

| | **First Phase Results** | | **Second Phase Results** | |
|---|---|---|---|---|
| | **Male** | **Female** | **Male** | **Female** |
| Diagnosed | 15 | 9 | 16 | 8 |
| Correct diagnosis | 11 | 5 | 14 | 7 |
| Incorrect diagnosis | 2 | 4 | 0 | 1 |
| Results not confirmed | 2 | 0 | 2 | 0 |

Table A.4: Sex Determination Results.

# Appendix B

# Ultrasonographic Examinations

This appendix presents mainly the data collected during the course of the experiments. They are summarized in form of tables. Additionally, the precise definitions of terms used in the thesis and abbreviations used in the tables are given. All dimension measurements are given in millimeters (mm).

## B.1 Definitions

- **Cow.** Also referred to as an animal.

- **Conceptus.** Also called early conceptus, was referred to as the components of the new generated life — embryo and embryonic membranes and fluids. This term was mainly used in early gestational stage — from fertilization until Day 19 of gestation. Ultrasonographically observed as a non-echogenic irregularly shaped amount of fluid within the smooth endometrial layer of the uterine horn. Seen in longitudinal plane, usually showed a small dilatation which was called vesicle, also seen in cross section as a rounded area.

- **Early Embryo.** Described as a faint ultrasonographic echogenic structure, usually resembling a comma or spot shape, visualized within the nonechogenic vesicle or conceptus. Used to name embryos up to Day 19 after fertilization. Ultrasonographically perceptable movements of the embryo were not observed, except for the rythmic heart beating that may be detected on Day 19 early embryo.

- **Embryo.** The ultrasonographic echogenic structure observed within the nonechogenic fluid filled uterine horn from Day 20 to 45 after fertilization. The embryonic heart beat was already perceptible in this stage, and as gestation progressed, some structures of the embryo body were clearly identified as well as body movement.

- **Foetus.** Was named the embryo older than 45 days, in which body structures and movements were easily perceptable and identified.

- **Lumen.** Known as the normal ultrasonographic nonechogenic region present within the uterus, between the endometrium layer. Here was defined as the discrete nonechogenic fluid collection present within the folded uterine horn in cyclic cows and which could also be present in early stages of gestation, commonly observed within the smooth endometrium of the contralateral horn of the pregnant cows until approximately Day 17 after fertilization.

- **Contralateral Horn.** Was defined as the uterine horn opposite to the ovary bearing the corpus luteum, that is, the horn usually not carrying the conceptus.

- **Ipsilateral Horn.** Was defined as the horn ipsilateral to the ovary bearing the corpus luteum, that is, the horn usually carrying the conceptus.

- **No Lumen.** Was the absence of an ultrasonographic perceptable lumen. Usually observed in non pregnant cows about the period of first examination.

- **Distended.** Was referred to as the lumen of the horn exhibitting a discrete ultrasonographic nonechogenic amount of fluid, usually related to conception. Used to define the size expected for the pregnant horn in early stage of gestation, from Day 16 to 20 after fertilization. In the contralateral horn the distended lumen was usually observed from Day 18 to 20 after fertilization.

- **Small.** Was referred to the size of the uterine lumen with ultrasonographic nonechogenic amount of fluid usually expected for pregnant cows from Day 21 to 23 of gestation.

- **Large.** Was referred to the size of the uterine lumen with ultrasonographic nonechogenic amount of fluid usually expected to be seen in a gestation older than 23 days.

## B.2 Abbreviations

**CIN** - Cow Identification Number

**CLs** - Corpora Lutea

**CL** - Corpus Luteum

**C** - Cow

**EE** - Early Embryo

**E** - Embryo

**F** - Female

**HB** - Heart Beat

**LFs** - Large Follicles

**LF** - Large Follicle

**LH** - Left Horn

**LO** - Left Ovary

**LSODay** - Last Standing Oestrus Day

**MFs** - Medium Follicles

**MF** - Medium Follicle

**M** - Male

**PD** - Pregnancy Diagnosis

**RH** - Right Horn

**RO** - Right Ovary

**S, (s), s** - Small

**SFs** - Small Follicles

**SF** - Small Follicle

**colap** - Colapsed

**cyst** - Cystic

**ech str** - Echogenic structure

**flat** - Flatenning

**lac** - Lacuna

**mul** - Multiple, several

**red** - Reducing

**reg** - Regressing

**sva** - Served again

**tw** - Twins

| CIN | Date | LSO Day | RO | RH | LO | LH | PD |
|---|---|---|---|---|---|---|---|
| C01 | 11/Sep/92 | 31 | 2 LFs (20) | Distended | MFs, SFs | Lumen | - |
| C02 | 11/Sep/92 | 31 | CL(24x31), 2 MFs | Embryo (21), Heart Beat | 1 LF, SFs | Large | + |
| C02 | 12/Oct/92 | 62 | CL | Foetus, Female | No observation | Large | + |
| C03 | 11/Sep/92 | 29 | CL, MFs, SFs | Embryo, Heart Beat | MFs, SFs | Large | + |
| C03 | 12/Oct/92 | 60 | CL(26x30), SFs | Foetus, Male | No observation | Large | + |
| C04 | 11/Sep/92 | 29 | CL(24x35), 1 MF, SFs | Embryo, Heart Beat | SFs | Large | + |
| C04 | 12/Oct/92 | 60 | CL(23x31) | Foetus, Out of reach | No observation | Large | + |
| C05 | 11/Sep/92 | 19 | 2 LFs, SFs | Lumen | Inert | No Lumen | - |
| C05 | 29/Sep/92 | 17 | CL(23x27), 1 LF, MFs | Distended, Early Embryo | SFs | Lumen | +sva |
| C05 | 6/Oct/92 | 24 | CL(27x25), MFs | Embryo, Heart Beat | MFs | Large | + |
| C05 | 12/Oct/92 | 30 | CL(27x28), 1 LF, MFs | Embryo, Heart Beat | 2 MFs | Large | + |
| C05 | 2/Nov/92 | 51 | No observation | Foetus, Male | No observation | Large | + |
| C06 | 11/Sep/92 | 18 | 2 CLs(20x20,20x15), SFs | Distended, Conceptus | 1 LF, SFs | Vesicle, Conceptus | +tw? |
| C06 | 17/Sep/92 | 24 | 2 CLs(20x20), MFs, SFs | Embryo, Small, HB | 1 LF, 1 MF, SFs | Embryo, Small, HB | +tw |
| C06 | 22/Sep/92 | 29 | 2 CLs(21x20), MFs | Embryo (bigger), HB | 2 MFs, SFs | Embryo (smaller), HB | +tw |
| C06 | 29/Sep/92 | 36 | 2 CLs(21x20), MFs | Embryo (bigger), HB | MFs | Embryo (smaller), HB | +tw |
| C06 | 6/Oct/92 | 43 | 2 CLs(22x26,25x22), SFs | Embryo (bigger), HB | 2 LFs | Embryo (smaller), HB | +tw |
| C06 | 19/Oct/92 | 56 | 2 CLs(19x22,23x20), SFs | Foetus, Male | No observation | Foetus, Sex? | +tw |
| C07 | 17/Sep/92 | 19 | 1 LF, MFs | Distended | CL, MFs | Distended, Conceptus | + |
| C07 | 22/Sep/92 | 24 | 1 LF(15x18), MFs | Large | CL(25x34) | Embryo, Heart Beat | + |
| C07 | 29/Sep/92 | 31 | 1 LF(13x14), MFs | Large | CL(29x25) | Embryo, Heart Beat | + |
| C07 | 19/Oct/92 | 51 | SFs(mul) | Large | CL | Foetus, Male | + |

Table B.1: Ultrasonographic Determination of the Reproductive Status in Dairy Cows.

| CIN | Date | LSO Day | RO | RH | LO | LH | PD |
|---|---|---|---|---|---|---|---|
| C08 | 17/Sep/92 | 19 | CL(lac,reg), SFs(mul) | Lumen | 1 LF(cyst,34x35), 1 MF, SFs | Lumen | - |
| C08 | 22/Sep/92 | 24 | CL(regressed), SFs(mul) | Lumen, NoE, NoHB | 1 LF(cyst,29x31), SFs(mul) | Distended | - |
| C09 | 22/Sep/92 | 20 | CL(29x32), MFs | Embryo, Heart Beat | 1 LF(09x12), SFs | Distended | + |
| C09 | 29/Sep/92 | 27 | CL(20x26), MFs | Embryo, Heart Beat | 1 LFs, 1 MF, SFs | Large | + |
| C09 | 6/Oct/92 | 34 | CL, 1 MF | Embryo, Heart Beat | 1 LF, 1 MF | Large | + |
| C09 | 12/Oct/92 | 40 | CL(20x21), 1 MF | Embryo, Heart Beat | 1 LF(13x13), SFs | Large | + |
| C09 | 23/Oct/92 | 51 | CL(28x29) | Foetus, Male? | No observation | Large | + |
| C09 | 26/Oct/92 | 54 | CL(28x27) | Foetus, Male | No observation | Large | + |
| C10 | 22/Sep/92 | 20 | CL(27x31), 1 MF, SFs | Embryo, Heart Beat | MFs, SFs | Distended | + |
| C10 | 29/Sep/92 | 27 | CL(23x30), SFs | Embryo, Heart Beat | 2 MFs, SFs | Large | + |
| C10 | 6/Oct/92 | 34 | CL(28x27), 1 MF, SFs | Embryo, Heart Beat | 2 MFs, SFs | Large | + |
| C10 | 23/Oct/92 | 51 | CL(25x24), MFs | Foetus, Female | SFs | Large | + |
| C10 | 26/Oct/92 | 54 | CL(24x26) | Foetus, Female | No observation | Large | + |
| C11 | 19/Oct/92 | 21 | CL(lac,24x28), 2 MFs | Distended (echo str) | SFs | Lumen | - |
| C11 | 23/Oct/92 | 25 | CL(lac,29x29), 1 LF, MFs | Large, NoE, NoHB | MFs, SFs | Distended | - |
| C11 | 26/Oct/92 | 28 | CL(lac,reg), MFs, SFs | Distended | 1 LF, MFs, SFs | No Lumen | - |
| C11 | 2/Nov/92 | 35 | CL(lac flat,reg,25x27), LF(20x15) | Distended | SFs | No Lumen | - |
| C11 | 9/Nov/92 | 42 | CL(lac flat,27x30), MFs | No Lumen | MFs | No Lumen | -sva |
| C11 | 30/Nov/92 | 17 | CL(lac,reg?,22x20), MFs, SFs | Vesicle (ech str) | SFs | Lumen | -? |
| C11 | 7/Dec/92 | 24 | CL(reg), MFs, SFs(mul) | Small (ech str), NoHB | No observation | Lumen | - |

Table B.2: Ultrasonographic Determination of the Reproductive Status in Dairy Cows.

| CIN | Date | LSO Day | RO | RH | LO | LH | PD |
|---|---|---|---|---|---|---|---|
| C12 | 19/Oct/92 | 19 | MFs, SFs | Distended | CL(lac,28x29), 1 MF | Distended, EE | + |
| C12 | 26/Oct/92 | 26 | 1 LF, MFs, SFs | Large | CL(27x31) | Embryo, HB | + |
| C12 | 2/Nov/92 | 33 | 2 MFs, SFs | Large | CL(26x26) | Embryo, HB | + |
| C12 | 23/Nov/92 | 54 | No observation | Large | No observation | Foetus, Male | + |
| C13 | 19/Oct/92 | 17 | SFs | Lumen | CL(lac,30x28), 1 LF | Distended, Conceptus | + |
| C13 | 26/Oct/92 | 24 | MFs, SFs | Lumen | CL(lac red,31x32), 1 MF | Distended (small for gest) | - |
| C13 | 2/Nov/92 | 31 | 1 LF | No Lumen | CL(lac red,reg,24x20), 1 MF | Distended (red) | - |
| C13 | 9/Nov/92 | 38 | MFs, SFs | Lumen | CL(reg,13x14), 2 MFs | Lumen | - |
| C14 | 26/Oct/92 | 19 | CL(25x27) | Distended, Early Embryo | 1 LF, 1 MF | Distended | + |
| C14 | 2/Oct/92 | 26 | CL(26x22), 1 LF, MFs | Embryo, Heart Beat | 2 LFs | Large | + |
| C14 | 9/Nov/92 | 33 | CL(25x30), MFs | Embryo, Heart Beat | 1 LF | Large | + |
| C14 | 30/Nov/92 | 54 | No observation | Foetus, Female | No observation | Large | + |
| C15 | 4/Nov/92 | 19 | 1 LF, 1 MF, SFs | Distended | CL(29x29) | Distended, Conceptus | + |
| C15 | 9/Nov/92 | 24 | 1 LF, MFs | Large | CL(31x27) | Embryo, Heart Beat | + |
| C15 | 16/Nov/92 | 31 | 1 LF, MFs | Large | CL(23x32) | Embryo, Heart Beat | + |
| C16 | 4/Nov/92 | 18 | MFs, SFs | Distended | CL(26x29), 1 LF | Distended, Conceptus | + |
| C16 | 9/Nov/92 | 23 | MFs | Small | CL(24x29) | Embryo, Heart Beat | + |
| C16 | 16/Nov/92 | 30 | 2 LFs, MFs | Large | CL(29x24) | Embryo, Heart Beat | + |
| C16 | 7/Dec/92 | 51 | No observation | Large | No observation | Foetus, Female | + |

Table B.3: Ultrasonographic Determination of the Reproductive Status in Dairy Cows.

| CIN | Date | LSO Day | RO | RH | LO | LH | PD |
|---|---|---|---|---|---|---|---|
| C17 | 25/Jan/93 | 52 | CL | Foetus, Male | No observation | Large | + |
| C18 | 1/Feb/93 | 48 | CL | Foetus, Female? | No observation | Large | + |
| C18 | 10/Feb/93 | 57 | CL | Foetus, Male | No observation | Large | + |
| C19 | 10/Feb/93 | 55 | CL | Foetus, Male | No observation | Large | + |
| C20 | 25/Jan/93 | 17 | CL(25x27) | Distended, Early Embryo | 2 LFs, 1 MF | Lumen | + |
| C20 | 1/Feb/93 | 24 | CL(23x25), SFs | Embryo, Heart Beat | 2 LFs, 1 MF | Large | + |
| C20 | 8/Feb/93 | 31 | CL(25x25), 1 LF, SFs | Embryo, Heart Beat | 1 LF, SFs | Large | + |
| C20 | 1/Mar/93 | 52 | CL | Foetus, Female | No observation | Large | + |
| C21 | 8/Feb/93 | 19 | CL(23x25), 1 MF, SFs(mul) | Distended, Conceptus | 2 LFs, SFs | Distended | + |
| C21 | 15/Feb/93 | 26 | Could not find | Embryo, Heart Beat | 1 LF, MFs, SFs | Large | + |
| C21 | 19/Feb/93 | 30 | CL(25x20), 1 MF, SFs (mul) | Embryo, Heart Beat | MFs, SFs | Large | + |
| C21 | 12/Mar/93 | 51 | CL | Foetus, Female | SFs | Large | + |
| C22 | 8/Feb/93 | 19 | CL(lacuna,31x30) | Distended, Conceptus | 1 LF, SFs | Distended | + |
| C22 | 15/Feb/93 | 26 | CL(lacuna,28x30), SFs | Embryo, Heart Beat | 2 LFs, SFs | Large | + |
| C22 | 19/Feb/93 | 30 | CL(lacuna,28x30) | Embryo, Heart Beat | 2 MFs, SFs | Large | + |
| C22 | 12/Mar/93 | 51 | CL | Foetus, Male | SFs | Large | + |
| C23 | 8/Feb/93 | 19 | 2 LFs, SFs | Distended | CL(23x28), 1 LF, SF | Distended, Conceptus | + |
| C23 | 15/Feb/93 | 26 | 1 LF, SFs | Large | CL(24x26), 1 MF, SFs | Embryo, Heart Beat | + |
| C23 | 19/Feb/93 | 30 | 1 MF, SFs | Large | CL(24x29), 2 SFs | Embryo, Heart Beat | + |
| C23 | 12/Mar/93 | 51 | No observation | Large | CL | Foetus, Male | + |

Table B.4: Ultrasonographic Determination of the Reproductive Status in Dairy Cows.

| CIN | Date | LSO Day | RO | RH | LO | LH | PD |
|---|---|---|---|---|---|---|---|
| C24 | 10/Feb/93 | 18 | CL(23x24), 1 LF, SFs | Distended, EE | 2 MFs, 1 SF | Distended | + |
| C24 | 15/Feb/93 | 23 | CL(24x21), SFs | Embryo, HB | 1 MF, SFs | Small | + |
| C24 | 22/Feb/93 | 30 | CL(25x24), 1 LF(15x15), SFs | Embryo, Heart Beat | SFs | Large | + |
| C24 | 15/Mar/93 | 51 | CL | Foetus, Female | No observation | Large | + |
| C24 | 17/Mar/93 | 53 | CL | Foetus, Female | SFs | Large | + |
| C25 | 10/Feb/93 | 18 | SFs | Distended | CL(s lac,24x32), 1 MF, SF | Distended, EE | + |
| C25 | 15/Feb/93 | 23 | 1 MF, SFs | Small | CL(lac colap,30x25), 1 MF | Embryo, HB | + |
| C25 | 22/Feb/93 | 30 | 1 MF, 1 SF | Large | CL(26x25), SFs | Embryo, HB | + |
| C25 | 15/Mar/93 | 51 | No observation | Large | CL | Foetus, Male | + |
| C25 | 17/Mar/93 | 53 | SFs | Large | CL | Foetus, Female | + |
| C11 | 15/Feb/93 | 17 | CL(23x22), 2 MFs | Distended, Conceptus | 1 MF, SFs | Lumen | + |
| C11 | 22/Feb/93 | 24 | CL(24x31), 1 MF, SFs | Embryo, Heart Beat | 1 MF, 1 SF | Small | + |
| C11 | 1/Mar/93 | 31 | CL(28x31), 1 MF, SFs(mul) | Embryo, Heart Beat | 2 MFs, SFs | Large | + |
| C11 | 22/Mar/93 | 52 | CL | Foetus, Female | MFs, SFs | Large | + |
| C26 | 22/Feb/93 | 19 | CL(enorm lac,44x42), 1 MF, SF | Distended, EE | 1 MF, 1 SF | Distended | + |
| C26 | 1/Mar/93 | 26 | CL(lac,reg,36x38), 2 MF, SF | Distended, NoE, NoHB | 1 MF, SFs(mul) | Distended | - |
| C26 | 8/Mar/93 | 33 | CL(lac red,32x28), 1 MF, SF | Lumen, NoE, NoHB | MFs, SFs(mul) | Lumen | - |
| C27 | 29/Mar/93 | 49 | CL(20x23), MFs, SFs | Embryo (s), Heart Beat | CL(25x29), 1 MF, 2 SFs | Embryo (s), HB | + tw |
| C27 | 2/Apr/93 | 53 | CL(16x22), 1 LF, 3 LFs | Foetus (s), Sex? | CL(27x25), SFs | Foetus (s), Male | + tw |
| C28 | 1/Feb/93 | 19 | CL(23x23), SFs | Distended, Early Embryo | 1 LF, SFs(mul) | Distended | + |
| C28 | 8/Mar/93 | 26 | CL(21x24) | Embryo, Heart Beat | 1 LF, SFs | Large | +died |

Table B.5: Ultrasonographic Determination of the Reproductive Status in Dairy Cows.

| CIN | Date | LSO Day | RO | RH | LO | LH | PD |
|---|---|---|---|---|---|---|---|
| C29 | 8/Mar/93 | 18 | CL(lac,24x20), 1 MF, 1 SF | Distended | SFs | Lumen | -? |
| C29 | 15/Mar/93 | 25 | CL(s lac,29x36), 1 LF, SFs | Small, NoE, NoHB | MFs, SFs | Lumen | - |
| C29 | 17/Mar/93 | 27 | CL(lac,reg,28x24), 2 LFs, SFs | Large, NoE, NoHB | 1 MF, SFs | Lumen | - |
| C30 | 15/Mar/93 | 16 | CL(29x29), 2 MFs, SFs | Distended (large for gest) | MFs, SFs | No Lumen | -? |
| C30 | 17/Mar/93 | 18 | CL(28x31), 1 LF, 1 MF, SF(mul) | Distended (large for gest) | MFs, SFs(mul) | Lumen | - |
| C30 | 22/Mar/93 | 23 | CL(s lac,28x22), 1 LF, 1MF, SF | Distended, NoE, NoHB | 1 MF, SFs | Lumen | - |
| C30 | 29/Mar/93 | 28 | CL(lac), MFs, SFs(mul) | Distended, NoE, NoHB | MFs, SFs | Lumen | - |
| C31 | 17/Mar/93 | 16 | 2 LFs, SFs | Lumen | CL(19x19), 3 MFs | Distended, EE | + |
| C31 | 26/Mar/93 | 25 | No scan. Rectal bleeding. | | | | |
| C31 | 29/Mar/93 | 28 | 2 LFs, SFs | Lumen | CL(flat lac,27x26), SFs | Distended, NoE, NoHB | - |
| C31 | 2/Apr/93 | 32 | 2 LFs, 1 SF | Lumen | CL(lac,24x30) | Distended | - |
| C32 | 16/Nov/92 | 54 | CL | Foetus, Male | No observation | Large | + |

Table B.6: Ultrasonographic Determination of the Reproductive Status in Dairy Cows.