AN ULTRASONOGRAPHIC STUDY OF POST-PARTUM INVOLUTION OF THE BOVINE UTERUS

A Thesis submitted to the Faculty of Veterinary Medicine
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BY

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DEDICATION

I specially dedicate this work to my late father Induna Mwanamwambwa Situmbeko Liwakala. Your words will always be an inspiration; "*Mwana-Onge uku yete.....*".

DECLARATION

I Ilyamupu Situmbeko, do hereby declare that the work in this thesis is original, was carried out by me and has not been presented for an award of a degree in any other University.

SUMMARY

The reproductive performance of the postpartum cow has a great influence on the overall reproductive success of the herd and therefore on the production and profits of the entire dairy enterprise. Extended calving intervals and premature culling due to infertility are a source of financial loss for dairy producers world-wide. Following calving, successful re-breeding depends on the resumption of ovarian cyclicity and completion of uterine involution. While follicular activity has been well characterised, there are few detailed studies of uterine involution using ultrasonography. This study was carried out with the objective of monitoring normal uterine involution and identifying features indicative of uterine pathology using real time B-mode ultrasound scanning.

In preliminary experiments uteri recovered from abattoirs were examined and measured and scan planes were selected for serial monitoring of postpartum cows. Twenty seven cows were then examined five times (Examinations 1-5) during the periods of 2-9, 12-17, 23-29, 43-49 and 54-61 days postpartum. For 21 of the cows certain features were identified ultrasonographically. Volumes of uterine fluids with variable echogenicity were observed during examinations 1 and 2. The fluid was anaechoic during the early part of Examination 1, becoming echogenic in the later part of Examination 1 and remaining so until Examination 3. By Examination 3 no fluid was seen except that associated with oestrus. Caruncles were visualised as oval echogenic structures during Examination 1 and the diameter of the uterine horns was greater than 60 mm. The size of the previously gravid horn reduced markedly during the first four examinations. Mean diameter was 45.1 mm during Examination 2 and 29.7 mm by Examination 5. After Examination 3 further changes were reduction in the size of the horns

and the observation of features associated with the oestrous cycle. Regression of the cervix was slower than that of the horns. The cervical lumen was visualised during Examination 1 and not thereafter. The cervix remained greater than 60 mm in diameter until Examination 3 when a mean diameter was 52.0 mm was recorded. By Examination 5 the structure was 44.7 mm in diameter.

Six cows were classified as abnormal because of periparturient problems or endometritis. These cows showed delayed involution, characterised by the persistence of a visible cervical lumen beyond 12 days postpartum, caruncles visible after 17 days postpartum, a uterine horn diameter of greater than 60 mm or the presence of echogenic fluid after 23 days postpartum and a cervix of greater than 60 mm in diameter beyond 54 days postpartum.

In conclusion this study showed that it was possible to characterise the process of involution ultrasonographically, and to recognise certain features which were indicative of delayed involution or uterine pathology.

Chapter 1

Review of the Literature

1.1. The Reproductive Cycle of the Cow

1.1.1. Anatomy and Histology of the Bovine Reproductive Tract

Since the anatomy of the reproductive organs changes with such factors as age and the physiological state, the initial description will refer to a non-pregnant parous cow.

The ovaries are oval structures, located in the pelvic cavity and generally lying near the middle of the lateral margin of the pelvic inlet. They measure about 3.5 to 4.0 cm in length, 2.5 cm in width and about 1.5 cm in thickness. The ovaries are attached to the body wall by the broad ligament. The latter is important during rectal palpation as it may be used as a landmark for locating the ovaries. The surface of the ovary may show various projecting structures such as follicles, corpora lutea and cysts (Getty, 1975).

Histologically the ovaries consist of the cortex and the medulla. In the cortex are found follicles, corpora lutea, interstitial cells and stromal elements. The medulla is centrally located and contains large blood vessels, lymphatics and nerves. An epithelial layer of simple squamous or cuboidal cells form the germinal epithelium (Banks, 1993).

The uterus consists of the two horns, body and neck (cervix). Each horn is approximately 35 cm long (Getty, 1975). From the body the two horns run cranially and for about a third of their initial length are covered in a common serosal and muscular wrapping. This part of the horns looks like part of the body on the outside. For a short length from a point where the horns become free of this wrapping, the two horns are joined together dorsally and ventrally by the dorsal and ventral intercornual ligaments respectively. During rectal palpation the dorsal ligament may be hooked with a finger to draw the organ caudally. The horns tend to form a spiral by first curving ventrally, cranially and laterally and then caudally and

dorsally. The spiral position of the uterus is more prounced during dioestrus than during the periovulatory period (Pierson and Ginther, 1987). The body is short, measuring only about 3 cm (Dyce et al., 1987). Caudally the body give rise to the cervix (neck), a firm muscular structure whose lumen, the cervical canal, is spiral and tightly closed except during oestrus and at parturition.

Microscopically the uterus consists of three layers, namely, a mucous membrane (endometrium), an intermediate smooth muscle layer (myometrium) and an outer serous layer (perimetrium) (Frandson and Spurgeon, 1992). The epithelial covering of the endometrium consists of simple columnar cells (Banks, 1993). Caruncles are non-glandular regions of the endometrium. The lamina propria-submucosa in these regions is highly vascularised. The myometrium consists of a thick inner circular layer and a thin outer longitudinal layer of smooth muscle. Between the two layers is a vascular layer. The perimetrium or tunica serosa is the serous covering of the uterus and is continuous with the broad ligament which supports the reproductive organs.

The mucosa-submucosa of the cervix has circular and longitudinal folds with many secondary and tertiary folds. The epithelium is simple columnar with goblet cells which secrete mucus during oestrus and pregnancy. The inner circular layer of smooth muscle has abundant elastic fibers (Priedkalns, 1993).

The vagina and the vestibule make up the remaining part of the female genital tract, the two being demarcated from each other by the urethral opening. The vagina has flat longitudinal and circular folds. The epithelial layer is stratified squamous but in the cranial portion has a surface of columnar and globlet cells on the stratified squamous epithelium. The tunica muscularis is made up of two smooth muscle layers. The tunica adventitia or tunica serosa, cranially, consists of loose connective tissue (Priedkalns, 1993). The vestibule is the tubular portion between the vagina and the labia of the vulva. It is short and slopes ventrally to the opening between the labia of the vulva. At the caudal

extremity, between the labia, is the fossa containing the glans of the clitoris. The histology of the vestibular wall is similar to that of the caudal portion of the vagina but has more lymphatic nodules and vessels and blood vessels. It also has cavernous tissue (Banks, 1993).

The vulva makes up the external portion of the genitalia. It consists of thick wrinkled lips (labia) with a skin covering that has apocrine sweat and holocrine sebaceous glands. In the hypodermis are striated muscle fibres (Priedkalns, 1993).

1.1.2. Physiology of the oestrous cycle

Knowledge of the reproductive cycle is very important in the reproductive management of the cow. Manipulation of the oestrous cycle, such as for oestrus synchronisation or superovulation, is based on such knowledge, as is the understanding of pathological conditions such as cystic ovarian disease or anoestrus.

The cow is a polyoestrus, non-seasonal breeder with recurring cycles of 21 days on average (Arthur et al., 1996a). Though the oestrous cycle may be divided into four stages, the only behaviourally distinct stage is oestrus. This is the stage when the cow stands to be mounted by the bull. It lasts, on average, 18 hours with ovulation occurring 25-30 hours after the pre-ovulatory surge of luteinising hormone (LH). The other stages in the oestrous cycle are pro-oestrus, metoestrus and dioestrus. These are less well behaviourally defined.

During pro-oestrus there is increased follicular growth and one follicle grows to maturity. The maturing follicle actively secretes the steroid hormone oestrogen whose influence results in the animal coming into oestrus shortly before ovulation. During this period the corpus luteum from the previous cycle is regressing as a result of prostaglandin secretion from the uterus, and with it progesterone levels fall.

Metoestrus follows the end of oestrus. The mature follicle has ovulated and the remaining structure forms the corpus haemorrhagicum,

which becomes the new corpus luteum. Dioestrus is the stage when the corpus luteum is the dominant structure. The corpus luteum takes about 7 days to reach its mature size of 2-2.5 cm. The corpus luteum secretes progesterone which prepares the reproductive tract for pregnancy. Progesterone is secreted for at least two thirds of the oestrous cycle (Hunter, 1982b).

Following a successful mating or insemination pregnancy occurs, the duration of which is about 280 days. During this period cyclic activity is suspended. Cyclicity resumes after a period of physiological anoestrus post-calving. Once cyclicity has resumed and uterine involution is completed the animal is ready to support another pregnancy.

Follicular growth and atresia occurs even before puberty. At puberty correct hormonal balance is established and follicular growth culminates in ovulation. Follicular growth occurs in two or three waves, each characterised by the development of one dominant follicle and many smaller follicles (Sirois and Fortune, 1988). During the last wave the dominant follicle ovulates. The events of follicular development from antrum formation to ovulation have been well described by Arthur and others (1996b). Follicle-stimulating hormone (FSH) receptors are present on small antral follicles together with few or no luteinising hormone (LH) receptors. The latter are important for ovulation. As follicular growth progresses the granulosa cells of the follicle gain the ability to aromatise androgens to oestrogens. LH receptors develop in theca interna cells of most follicles and in the granulosa cells of those follicles with high levels of aromatisation. It is these follicles which are capable of ovulation under the right stimulation by gonadotrophic hormones. The other follicles become atretic.

After ovulation, the follicle wall collapses and the site is invaded by blood vessels and cells from the theca layer which surrounded the follicle. The corpus luteum then starts to form by hypertrophy and luteinisation of the granulosa and thecal cells. Two days after ovulation it is about 1.4 cm in diameter and soft, with a dull cream colour (Arthur et

al., 1996a). By the seventh to eighth day of dioestrus it has reached a maximum size of 2-2.5 cm and is yellow to orange in colour. The centre of the yellow body may be occupied by a cavity containing a yellow fluid (Kilo et al., 1986). The mature corpus luteum maintains this size until the onset of pro-oestrus, then regresses rapidly. Old corpora lutea of previous pregnancies persist as small irregular white projections on the surface of the ovary. The term corpus albicans is derived from their white colour (Arthur et al., 1996a).

Hormones involved in controlling the oestrous cycle are secreted by the hypothalamus, pituitary gland and the ovaries. The uterus also has a regulatory function. The hypothalamus controls the pituitary gland by secreting a hormone called gonadotrophin-releasing hormone (GnRH). This hormone stimulates synthesis and release of two hormones, follicle stimulating hormone (FSH) and luteinising hormone (LH) from the anterior pituitary. Increases in the release of gonadotrophin-releasing hormone cause increases in the release of the other two hormones. Follicle stimulating hormone promotes follicular growth. The follicles, in turn, produce oestrogen and as the plasma level of the latter increases FSH is inhibited by a negative feedback effect. Luteinising hormone is responsible for maturation and ovulation of the dominant follicle, and the formation and maintenance of the corpus luteum. The corpus luteum so formed produces progesterone which inhibits production of more LH (Arthur et al., 1996a).

Of the various steroidal hormones secreted by the bovine ovary the most important are oestrogens and progesterone (Peters and Ball, 1996). Oestradiol-17β is considered the principal biologically active oestrogen. The synthesis of oestrogens in the ovary by both the theca interna and granulosa cells is under the influence of LH and FSH. Oestradiol-17β peripheral blood levels are low for most of the oestrous cycle, only rising four days before oestrus to reach a peak on the day of or the day before standing oestrus; the peak triggering the LH surge. Oestradiol is responsible for behavioural signs of oestrus. It also, due to

high concentrations during the follicular phase of the cycle, induces the pre-ovulatory gonadotrophin surge by a positive feedback mechanism, increasing the frequency of hypothalamic GnRH secretion and the sensitivity of the anterior pituitary to GnRH. In the luteal phase of the cycle oestradiol levels are low and this, together with progesterone exert a negative feedback effect on LH secretion (Peters, 1985).

Inhibin is a non steroidal hormone produced by granulosa cells. It functions in blocking the action of FSH at the release and binding sites. It is thought to be produced by the dominant follicle during the pre-ovulatory period, thus preventing further development of follicles (Henderson and Franchimont, 1983).

Progesterone is produced by the cells of the corpus luteum. Peripheral plasma concentrations of progesterone reflect luteal development, maintenance and regression. The rise in progesterone concentrations is reported to begin after ovulation, increasing rapidly over the first four days of the cycle to reach peak levels around day 8. Concentrations remain stable until day 17, decreasing to basal levels before the next oestrus and ovulation in the event that the cow is not pregnant (Peters and Ball, 1995). High levels of progesterone exert a negative feedback effect on the release of gonadotrophins though, despite this, it is known that two or three waves of follicular growth occur (Sirois and Fortune, 1988). The other effect of progesterone is on the uterus where it inhibits uterine activity but stimulates glandular development. Towards the end of the non-pregnant cycle, when progesterone levels fall, the negative feedback effect is removed and development of the pre-ovulatory follicle occurs. With this, increased oestradiol secretion occurs and the LH surge is triggered to cause ovulation and the beginning of a new cycle.

Prostaglandin is the endogenous luteolytic agent. It is produced from the endometrium of the uterus, and secreted into the uterine vein, which by its close proximity with the ovarian artery is transferred into the latter by a counter-current mechanism. Prostaglandin is reported to be

released from about day 15 of the cycle in pulses for a few days and until progesterone levels reach base line (Kindahl et al., 1981).

It is now recognised that the ovary is an extra-hypothalamic source of oxytocin. Oxytocin is produced by granulosa and luteal cells (Stormshak et al., 1995). Following release, oxytocin binds to its receptors in the endometrium and triggers the release of prostaglandin $F_{2\alpha}$. Plasma oxytocin levels are basal around oestrus. Luteal oxytocin concentration reaches a peak about day 9 and pulsatile release begins about day 17, the time of luteal regression. The episodic pulses of oxytocin release are coupled to the release of prostaglandin (Wathes and Lamming, 1995).

1.1.3. Pregnancy and Parturition

The period of intrauterine development of the foetus is termed pregnancy or gestation. In the bovine this lasts about 280 days. Changes occur in the reproductive organs that facilitates this process. The end of the gestation period is marked by events leading to parturition

The uterus undergoes gradual increase in size as pregnancy progresses. This process includes proliferation, growth and stretching. The cervix is tightly closed by a highly viscous mucus plug during pregnancy which liquefies just before parturition. With impending parturition the vulva and vagina become highly vascularised and oedematous (Jainudeen and Hafez, 1993).

Parturition involves dilation of the cervix, expulsion of the foetus and expulsion of the foetal membranes, events which are termed the three stages of labour. The first stage is characterised by relaxation of the cervix under the influence of oestrogens and commencement of painful uterine contractions which result in discomfort and restlessness. In the second stage the allantochorion ruptures as the foetus passes through the cervix. Distension of the cervix by the foetus initiates Ferguson's reflex which causes an increase in the release of oxytocin from the

posterior pituitary. This, in turn, accentuates myometrial contractions and the foetus is eventually expelled. The third stage involves expulsion of the foetal membranes (Jainudeen and Hafez, 1993).

1.1.4. Endocrinology of pregnancy and parturition

Following conception the corpus luteum is retained as the corpus luteum of pregnancy. Progesterone is responsible for maintenance of pregnancy. The main source of progesterone in the cow is the corpus luteum but the placenta also produces small amounts of the hormone. Progesterone plasma levels rise in early pregnancy to reach a plateaux which is maintained until about 20-30 days before parturition. At this time plasma concentrations begin to decline. Oestrogen levels are low during gestation but begin to rise about 30 days before parturition and reach peak values 2-5 days before calving. Prolactin concentrations only rise within hours of parturition and decline by 30 hours after parturition (Arthur et al., 1996d).

Parturition is brought about by the interaction of hormonal, neural and mechanical factors. The events are triggered by the maturation of foetal hypothalamo-pituitary-adrenal (HPA) axis (Jainudeen and Hafez, 1993). Foetal plasma cortisol increase at the end of pregnancy due to a signal from the foetal hypothalamic-pituitary axis. This stimulates the placenta to convert progesterone to oestrogen. High maternal levels of oestrogen cause release of prostaglandin $F_{2\alpha}$ from the endometrium and consequently regression of the corpus luteum of pregnancy. Myometrial activity is under the influence of progesterone and oestrogen through their effects on the release of prostaglandin $F_{2\alpha}$. Oestrogens increase myometrial activity through stimulation of prostaglandin release. Oestrogens also causes the cervix to soften and dilate. Prostaglandin plasma levels rise as progesterone levels fall. Plasma oxytocin levels rise

at parturition due to Ferguson's reflex as described above but only for a short period and fall again by 6 hours after parturition (Jainudeen and Hafez, 1993).

1.2. The Postpartum Period

The postpartum period begins with parturition and ends with completion of uterine involution, resumption of cyclic ovarian activity and normal oestrous behaviour (Garcia and Larsson, 1982). It is an important component of the calving interval and offers a great challenge to both the farmer and the veterinarian. Prolonged calving intervals and early culling of cows due to reproductive failure cause substantial economic losses world-wide. The economic goal of a dairy enterprise is to optimise profits and since postpartum disorders have a negative effect on the productivity of the cow it is in the economic interest of the producer to control them. This will ensure good reproductive efficiency. The postpartum period is crucial because it is at this time that the return to normal sexual activity has to compete with the carry-over effects of the previous pregnancy and parturition together with the nutritional requirements of the high yielding cow. The dairy cow has not been specially bred for high reproductive efficiency but for high milk yield. The need for special attention to be paid to the dairy cow therefore arises if good reproductive efficiency is to be achieved and this is especially true after parturition since the next successful gestation depends on the return to normal of the uterine environment and the resumption of normal oestrous behaviour.

During pregnancy, there is distension and distortion of uterine tissue together with the heightened glandular development needed to support the conceptus. At term the gravid uterus is not only occupied by one or two foetuses, but also by enveloping membranes and placental fluids causing distension of the uterine wall and lumen (Hunter, 1980). Therefore immediately after parturition, the uterus is a large flabby sac, nearly 1m long, weighing approximately 9 kg (Gier and Marion, 1968) and containing up to 1.5ℓ of fluid (Michael, 1995). To restore the organ to its normal non-pregnant state the uterus has to undergo a period of

shrinkage, repair and glandular regression, a process called involution. Involution involves changes in the connective tissue, musculature and epithelial layers.

Involutionary changes are made up of three partly co-ordinated and overlapping processes, namely; reduction in size, loss of tissue and tissue repair (Rasbech, 1950; Gier and Marion, 1968). It is reported that the reduction in size progresses in a decreasing logarithmic scale (Gier and Marion, 1968). Morrow and others (1969b) reported that the reduction was slow during the first 4-10 days but becoming accelerated from 10-14 days postpartum. However Gier and Marion (1968) reported a uniform regression in the same period.

Hanzen (1982) found, by measuring myometrial electrical activity, that soon after parturition the contractions were propagated in the tubo-cervical direction. This is said to help in the expulsion of the foetal membranes and in minimising the size of the uterine cavity size and hence reducing the chance of infection entering via the cervix and the open birth canal (Ruesse, 1982). These uterine contractions continue for several days but decrease in regularity, amplitude and duration (Arthur et al., 1996c). During the first 24 hours, abdominal straining may be seen but by 3 to 5 days postpartum these contractions are barely noticeable.

Uterine contractions seem to be a result of the influence of oxytocin released by the suckling reflex, on the smooth muscle (Hunter, 1982a). Contractions result in atrophy and reduction in size of myofibrils from 750 to 400 microns on the first day to less than 200 microns over the next few days (Gier and Marion, 1968). Reports of examination by rectal palpation state that the uterus is definable by 4-7 days (Morrow et al., 1969b) but, in some cases, not until 8-10 days postpartum (Rasbech, 1950). By day 5 the uterus has contracted to almost half its gravid length. A further gross change is the loss of water from the uterine tissues as shown by a rapid decrease in the weight of the organ. Gier and Marion (1968) found that the weight of the uterus decreased from approximately 9.0 kg at parturition to 1.0 kg at day 30.

It is reported that the cervix constricts rapidly immediately after parturition. By 4 days it will only admit the insertion of two fingers. It is not distinguishable from the uterus in the first few days but as it becomes firm it can be palpated cranial to the pelvis by 4-7 days postpartum (Gier and Marion, 1968). The changes in the size of the cervix are due to elimination of tissue fluid and a reduction in muscle tissue. The diameter of the cervix is about 15 cm at 2 days postpartum, 9-11 cm at 10 days, 7-8 cm by 30 days and 5-6 cm by 60 days (Gier and Marion, 1968). A slight enlargement which is associated with final sloughing of caruncular masses (Gier and Marion, 1968) and lochial discharge (Morrow et al., 1969b) is detected at 10 days. The regression of the cervix is uniform but slower than the regression of the post-partum gravid horn (Moller, 1970; Garcia and Larsson, 1982). At parturition the gravid horn diameter is much larger than that of the cervix. However by 18 days postpartum the two are of almost equal diameter and remain so for 3-5 days before the horns shrinks below the size of the cervix (Morrow et al., 1969b).

Tissue loss is through the lochial discharge which is made up of mucus, detritus and blood (Rasbech, 1950). Though lochia is derived from the remains of foetal fluids, blood from ruptured umbilical vessels and shreds of foetal membranes, the main component is said to be sloughed caruncular surface tissue debris. Following parturition the caruncles are crumbled masses of septum and blood vessels covering an endometrial base. They measure 70 mm in length, 35 mm in width and 25 mm thick (Gier and Marion, 1968). The sequence of events in the regression of the caruncles is reported as degenerative change, peripheral ischaemia, necrosis and sloughing (Michael, 1995). By day 5 postpartum there is complete septal disorganisation through necrosis as a result of vasoconstriction and leukocytic infiltration (Gier and Marion, 1968). Within two days the caruncular blood vessels have constricted almost to the point of occlusion. By day 5 necrosis has progressed such that the septal material is being sloughed. As the caruncular mass is sloughed away, it leaves protruding remnants of blood vessels from

which blood oozes into the luminal fluids for at least 10 days. This is the main component of the lochial discharge seen in cattle for 10-12 days postpartum. Sloughing is completed by 15 days postpartum. Tissue losses after 19 days includes further constriction of blood vessels, regression of uterine glands, contraction of tissue with reduction in number and volume of cells (Gier and Marion, 1968).

The endometrium is highly oedematous just after calving but this oedema regresses slowly during the 4 or 5 days that follow (Gier and Marion, 1968). The lochial discharge is usually yellowish brown to reddish brown (Arthur et al., 1996c) and the total amount voided varies between individuals. Primipara void about 500 ml, with a few individuals showing no lochia at all (Rasbech, 1950) due to complete absorption of lochia (Arthur et al., 1996c). Pluripara void up to 2000 ml. The lochia becomes mixed with blood at about 10 days (Rasbech, 1950). After day 14 it becomes lymph-like and reduces in quantity until it disappears by day 18.

Endometrial repair begins immediately after parturition in those areas which were not seriously damaged during parturition and re-covers the entire intercaruncular luminal surface by 8 days. The raw surfaces of caruncles are covered mainly by growth of cells from the surrounding uterine glands and is completed after day 25. Meanwhile, the caruncles are reducing in size, such that by 40-60 days they consist of small protrusions 4-8 mm in diameter and 4-6 mm high (Gier and Marion, 1968).

The previously gravid horn never regresses to its pre-gravid size, but remains much larger even during the next pregnancy (Gier and Marion, 1968; Morrow et al., 1969b; Moller, 1970).

Review of the literature indicates that there is a wide range in the reported time taken for the completion of involution. Garcia (1982), in a survey of nine publications, found that the interval from parturition to clinically completed involution ranged from 18 to 50 days. Miettinen

(1990) noted that this variation could be a result of different definitions of involution and differences in the intervals between clinical examinations.

1.2.1. Methods used for monitoring the rate of uterine involution

Most methods used for measuring the rate of uterine involution are highly subjective. This has resulted in different and sometimes contradictory opinions.

Rectal palpation is the method which has been used most frequently (Garcia and Larsson, 1982; Bastidas et al., 1984). Using this method uterine size, tone, symmetry and position are used as indicators of uterine involution (Garcia and Larsson, 1982). Uterine involution is considered complete when the uterus has returned to its normal pelvic position, the horns are of nearly equal diameter and no uterine wall enlargement is detected (Fonseca et al., 1983; Heinonen et al., 1988; Miettinen, 1990; Tian and Noakes, 1991). Normal involutional changes occurring after day 30 postpartum are unlikely to be detected by rectal palpation as further reduction in size of the organ is minimal.

Morphological and histological studies of uterine slaughter house specimens (Gier and Marion, 1968) though more objective than rectal palpation, are of limited diagnostic application. Histological changes during uterine involution such as regeneration of uterine glands continue even when no changes in uterine size is detected by rectal palpation. When the latter method is used to monitor involution the period of time until completion is obviously longer than that of rectal palpation.

1.2.2. Factors influencing uterine involution

Uterine involution is affected by many factors, some of which accelerate the process while others slow it down as shown by the following studies (Marion et al., 1968; Morrow et al., 1969b; Kay, 1978; Dohoo, 1983). However, the subjective nature of the methods used in determining the completion of uterine involution and the experimental design may account for the differences encountered in these studies.

Parity and age

Parity and age are important when assessing uterine involution. Many authors have shown that uterine involution takes a few days longer in pluriparous compared to primiparous cows (Rasbech, 1950; Marion et al., 1968; Morrow et al., 1969b; Fonseca et al., 1983; Oltenacu et al., 1983; Etherington et al., 1985), though a few studies (Moller, 1970; Miettinen, 1990) found parity to have no significant effect on the rate of involution. Since even a normal calving may result in some scar tissue formation, the former group may be justified in concluding that the more calves a cow has borne, the greater the chance that the involution process will be extended. In one study involving 76 cows it was reported that the rate of uterine regression in primiparous cows was eight days longer than in pluriparous cows (Bastidas et al., 1984). However, no explanation for this observation was given.

Season

The effect of season is not clear. Morrow et al., (1969b) found season to have no significant effect on uterine involution. Bastidas and others (1984) found the rate of involution slower in cows which calved in the dry season compared to those calving during the rainy season. However, most reports indicate that involution is more rapid in summer (Marion et al., 1968). In fact, Lewis and others (1984) demonstrated that

heat stress during the last third of gestation or immediately after parturition reduced the period required for completion of uterine involution completion. Etherington and others (1985) attributed this to the fact that cows calving in warm months had a decreased incidence of retained placenta.

Suckling

It is reported that involution is faster in suckled animals than in non-suckled animals (Roberts, 1971a). However, Moller (1970) found that suckling had no significant effect on the speed of uterine involution when compared to animals which were milked. Arthur and others (1996c) believe that the differences in such reports may be a result of a breed influence on return to cyclicity.

Periparturient abnormalities

Periparturient abnormalities such as retained placenta, dystocia, hypocalcaemia, ketosis and metritis all delay uterine involution (Morrow et al., 1966; Piper et al., 1978; Sandals et al., 1979; Dohoo, 1983). Retained foetal membranes and uterine infection are discussed in detail in section 1.2.3.

Effect of prostaglandins

The postpartum uterus produces high concentrations of prostaglandins for about three weeks postpartum (Eley et al., 1981; Lindell et al., 1982; Bosu et al., 1984; Madej et al., 1984; Thompson et al., 1987; Slama et al., 1991). In normal animals the duration of prostaglandin $F_{2\alpha}$ release and the magnitude of elevation is reported to be important (Lindell et al., 1982). In these animals, cows with a long duration of postpartum prostaglandin $F_{2\alpha}$ release had a short involution period. Furthermore a continuously higher elevation seemed to promote faster involution.

Postpartum ovarian activity

Postpartum ovarian activity has been well reviewed by Garcia (1982). The onset of ovarian cyclic activity has been monitored by rectal palpation, visual oestrus detection, blood hormone profiles, milk progesterone and changes in cervical mucus (Mather and Melancon, 1981).

During pregnancy ovarian activity is minimal. Follicles, though present, show a progressive decrease in size (Leslie, 1983). Schirar and Martinet (1982), quoting Casida (1968), indicated that the average diameter of the largest follicle was about 12 mm in the second month of pregnancy, about 9 mm at the fifth month and 4 mm in the eighth or ninth month. There is still little follicular activity at parturition. The prominent ovarian structure during gestation is the corpus luteum of pregnancy and with the right ovary being more active than the left this is more often on the right ovary (Morrow et al., 1969a). Although the corpus luteum regresses and ceases to function just before parturition it may still be palpable by day 20 postpartum (Morrow et al., 1966) although it ceased to function before parturition. It is believed that the regressing corpus luteum of pregnancy has no effect on the involution of the uterus or ovarian function (reviewed, Leslie, 1983).

Follicular growth is said to begin 4-10 days after calving (Morrow et al., 1966; Morrow et al., 1969a; Kesler et al., 1978; Kesler et al., 1979). Follicles which have reached the ovulatory size are present in the ovary by day 15 (Kesler et al., 1980). It is reported that the first postpartum ovulation occurs between days 10-25 (Morrow et al., 1966). The corpus luteum formed from this ovulation is said to be smaller than subsequent corpora lutea. It was reported that this small, immature corpus luteum is responsible for the short oestrous cycle which often follows (Marion and Gier, 1968; Morrow, 1971). Behavioural signs of oestrus are rarely exhibited prior to this first ovulation, and when they are displayed they

are usually weak (Moller, 1970; Morrow et al., 1969a). The fact that no signs are shown in most cases means that the first postpartum ovulation may not be accompanied by oestrus. The incidence of "silent" heat, however, decreases in subsequent cycles (Bulman and Lamming, 1978). Though ovulation occurs early postpartum, conception rates at this time are low.

The interaction between the postpartum uterus and resumption of cyclic activity in the postpartum period has been reviewed by Schirar and Martinet (1982). In the cow, hysterectomy after regression of the corpus luteum of pregnancy and before first ovulation had no effect on follicular development and the time of occurance of the first ovulation. However, the life span of the corpus luteum produced by the first ovulation did appear to be influenced by the involuting uterus through the release of prostaglandins. This release is said to be triggered by factors such as postpartum uterine inflammation and through the action of oxytocin, especially in suckling animals (reviewed Schirar and Martinet, 1982).

1.2.3. Uterine and ovarian pathology

Cystic ovaries

Cystic ovaries are characterised by one or more persistent fluid-filled structure larger than a mature follicle (i.e. > 25 mm in diameter) in one or both ovaries (Arthur et al., 1996b). Cysts are considered pathological when they cause aberrant reproductive function. In this case they become an important cause of infertility. Cysts develop as a result of ovulation failure. They are a common occurrence before the first postpartum ovulation (Morrow et al., 1966; Kesler et al., 1979) but will normally regress spontaneously at this stage (Morrow et al., 1966; Garcia and Larsson, 1982).

Retained placentae

Since expulsion of foetal membranes take place after birth of the calf, retained foetal membranes has been a difficult condition to define because the line of demarcation between physiological and pathological retention is not easy to draw. Many authors regard foetal membranes to be retained by 24 hours post-partum (Halpern et al., 1985; Curtis et al., 1985; Markusfeld, 1987; Heinonen and Heinonen, 1989; Mellado and Reyes, 1994) although van Werven and others (1992) demonstrated that a retention of as short a duration as six hours had adverse effects on the reproductive performance of older cows.

Retained foetal membranes are a common condition during the postpartum period in the dairy cow. The incidence of the condition varies from one location to the another and in a survey of 12 publications Laven and Peters (1996) found the incidence to vary from 2.0% in New Zealand to as high as 39% in Bangladesh.

Many factors such as breed, year, season, herd, length of gestation, parturition induction, dystocia, hypocalcaemia, multiple birth, age of cow, abortion, heredity, fatty liver, selenium/vitamin E , vitamin A, iodine, and general nutrition are thought to be involved in causing this condition. How they induce the condition is not well understood (reviewed Laven and Peters, 1996).

The most important way in which retained foetal membranes affect fertility is that the condition predisposes to endometritis and hence delayed uterine involution (Morrow et al., 1966; Curtis et al., 1985; Borsberry and Dobson, 1989; van Werven et al., 1992). In these complicated cases fertility, in terms of increased calving to conception interval and lowered pregnancy rate at first service, is reduced and calving intervals are longer (Heinonen and Heinonen 1989; Mellado and Reyes, 1994). Permanent sterility may then result due to pyometra, perimetritis, salphingitis and severe damage to the endometrium (Roberts, 1971b).

However, there are reports that even in uncomplicated cases of retained foetal membranes, fertility is lowered (Borsberry and Dobson, 1989; Heinonen and Heinonen 1989), though others have noted no such effect (Dahoo and Martin, 1984). Other economic losses due to the condition are a loss in milk sales and the cost of veterinary services.

Metritis and endometritis

Uterine infection is an important cause of infertility in the cow. Inflammatory conditions involving the uterus have been given various terms (endometritis, metritis, metritis complex, lochiometra), which has often led to confusion (Messier et al., 1984; Callahan and Horstman, 1987; van Werven et al., 1992; Lowder, 1993). A useful definition is that of Blood and Studdert, (1996) where metritis is the inflammation of the whole uterus while endometritis is the inflammation of the endometrium.

Metritis occurs within a few days of parturition. The toxin producing bacteria which colonise the uterus at this time commonly cause septicemia. A fetid reddish-brown serous vaginal discharge is observed. On gentle rectal palpation, the uterus is thin walled and fluid filled and there is loss of myometrial tone (Callahan and Horstman, 1987). The animal is febrile, inappetant and has a low milk yield (Dohmen and Loohuis, 1996). Metritis is a potentially fatal condition whose management is generally agreed to be systemic administration of antibiotics and fluid therapy (Bretzlaff, 1987). On the other hand, the recognition of endometritis is crucial due to its varied clinical signs.

While metritis may show systemic clinical signs, endometritis does not. This makes diagnosis difficult considering the fact that normal uterine involution itself is an inflammatory process. The clinical signs commonly observed in endometritis are, a vaginal discharge that may or may not be malodorous, thickened uterine horns and delayed uterine involution (Lowder, 1993).

During the early postpartum period there is sloughing of caruncular tissue in the normal process of uterine involution. The disrupted uterine

epithelium then comes into contact with fluid and tissue debris that can support bacterial growth (Bretzlaff, 1987). Parturition being a non-aseptic process, bacterial contamination of the uterus at, or after parturition (from faeces, bedding, or on hands, chains or instruments) is common. It is reported that 93% of cows acquire an infection within 15 days of parturition; a percentage that falls to 9% by days 46 to 60 (Elliott et al., 1968). Ability to overcome this infection depends on a number of factors including the level of contamination and the defences of the body. Thus, during the first 14 days after calving most cows will have this infection (Studer and Morrow, 1978; Miller et al., 1980) but spontaneous recovery occurs in the majority of cases. The bacterium most commonly isolated from a uterus with endometritis is the gram-positive *Actinomyces pyogenes (*formerly *Corynebacterium pyogenes)* (Miller et al., 1980; Bretzlaff, 1987) which is often found together with gram-negative anaerobes such as *Fusobacterium* species. *Bacteroides* and *Clostridium* species are some of the other anaerobes commonly recovered (Ruder, et al., 1981; Steffan et al., 1984).

Retained placenta, poor environmental hygiene, ketosis, hypocalcemia, poor nutrition, altered endocrine balances, twins, parity, prolonged gestation, high ambient temperature, still-birth, unsanitary obstetric equipment and procedure are all factors thought to predispose to endometritis (Harrison, 1984; Markusfeld, 1984; Weaver, 1985; Olson, et al., 1986; Bretzlaff, 1987; Callahan and Horstman, 1987).

Endometritis is diagnosed by various methods including rectal palpation, vaginal speculum examination, uterine culture and uterine biopsy (Studer and Morrow, 1978; Miller et al., 1980; Messier, et al., 1984; Gonzalez, et al., 1985). Vaginoscopy is considered a more accurate method for diagnosing subacute/chronic endometritis than rectal palpation (Miller et al., 1980), but the use of the two methods together is preferable.

Severe endometritis delays the process of uterine involution, prolongs the calving-to-first oestrus interval, increases the mean calving-

to-first service interval, number of services per conception and consequently prolongs the inter-calving interval (Lindell et al., 1982; Coleman et al., 1985; Borsberry and Dobson, 1989; Lowder, 1993). Furthermore, financial losses occur which are attributed to milk and meat withdrawal time if an antibiotic therapy regime is selected for treatment.

The selection of a treatment plan is the most controversial aspect of the condition. In fact whether treatment is required or not remains a question of open discussion (Miller et al., 1980; Bretzlaff, 1987). In one extensive study Miller et al., (1980) found that treatment of mild cases of endometritis was of little value as infertility, measured by first service conception rate, percentage of cows open after day 150 and mean days open, in the untreated and treated cows was not significantly different. In severe cases, cows were found to show low fertility despite the treatment. The benefit of treatment was questioned as the aim in treatment is not merely for clinical recovery but, most importantly, for restoration of normal fertility.

Pyometra

After ovulation and formation of a corpus luteum, endometritis can become chronic and normal oestrous cycles continue or develop into pyometra and resultant anoestrus. Pyometra is characterised by accumulation of purulent exudate in the uterus, persistence of the corpus luteum and anoestrus. On rectal palpation the uterine wall is thicker than normal, and the uterus is distended with fluctuating fluid (Gustafsson, 1980). Unless ovarian cyclicity resumes, spontaneous recovery in true pyometra is rare and the condition can go on for months without any improvement (Gustafsson, 1980).

1.3. Dairy Herd Fertility

1.3.1. The annual production cycle of the dairy cow

The annual production cycle of the dairy cow begins after the heifer has calved. The main aim then is that the cow should give optimal milk yield from the available feed and conceive in sufficient time to calve at the same time the following year (Esslemont, et al., 1985).

It is generally accepted that a calving interval of 13 months for first-calf heifers and 12 months for cows is optimal for milk production (Louca and Legates, 1968; Esslemont and Ellis, 1974; Britt, 1975). Following calving, the most crucial date in the production cycle is the date of conception which, in turn, controls the next lactation. Since gestation length (normally around 280 days) can not under normal circumstances be controlled, the animal needs to conceive by day 85 postpartum to maintain a 365 day calving interval. Breeding of cows normally starts well before 85 days postpartum with some farmers opting for a first service around day 56. However, usually the first service is around day 65. Widespread use of artificial insemination now involves the Stockman in the detection of oestrus. It is expected that oestrus should have been observed by day 42.

After calving the animal is in lactation for about 305 days, this period therefore includes part of the next gestation period. Cows are normally dried off about 2 months before term to enable replacement of body condition lost during the lactation, involution of mammary tissue and to allow for the proper growth of the foetus (Esslemont et al., 1985).

1.3.2. Herd fertility programmes

In recent years it has become common practice for dairy farmers to arrange a reproductive health (herd fertility) programme with the veterinary surgeon. This is in an effort to maximise reproductive efficiency and to minimise reproductive diseases and problems.

A herd fertility programme involves regularly scheduled farm visits by the same veterinary surgeon for routine work in accordance with farm records. Good management by the farmer and a good recording system are both vital for the programme to work. Finally, the data is analysed by the veterinary surgeon and progress assessment made (Radostits et al., 1994).

Radostits and others (1994) have suggested that before the start of a programme the objectives of the farmer should be considered. Following this, regular visits to the farm can be undertaken by the veterinary surgeon with a frequency dependent on herd size and calving pattern. Monthly visits may suffice for herds of less than 100 animals while large herds need more frequent visits, usually as often as once weekly for herds of 150 cows or more.

During these visits animals in the following categories should be examined; cows which had difficulties at calving, those which had retained foetal membranes, metritis or abnormal vulval discharge, those not seen in oestrus by day 40, cows showing nymphomania, cows presented for pregnancy diagnosis and cows showing oestrus after being diagnosed pregnant (Noakes, 1986).

It is suggested that an accurate and permanent record keeping system is used to identify each cow (Radostits et al., 1994). Castle and Watkins (1984) advise that freeze-branding or ear tags with numbers are possible identification methods for this purpose. However, they suggest that other methods such as neck chains or coloured and numbered plastic collars are equally useful. Freeze-branding is useful on dark

haired and fairly old animals. The freeze-branding of young animals will result in distortion of the number as the animal grows. Temporary markings for management purposes, such as identifying a cow with mastitis, are advisable. Such identification may take the form of a coloured sticky tape around the tail.

Having identified each individual animal a record can be constructed of calving date and calving history, lactation number or number of calves, post calving/pre-breeding checks, oestrus and insemination dates (with the sire identification), oestrus not observed, veterinary treatment and pregnancy diagnosis results, drying off date, culling or calving. The daily milk output record for a cow will help in breeding or culling decisions as well as a guide to feeding. Visual aids such as circular calendars provide a summary of records at a glance.

There are several dairy recording programmes on computer. In the UK, for instance, the Dairy Information System (DAISY) is widely used (Esslemont et al., 1991). There are similar recording systems in other countries such as the National Animal Health Monitoring System (NAHMS) in the United States and the Animal Productivity and Health Information Network (APHIN) in Canada (Radostits et al., 1994). Information ranging from action lists to an overview of health, fertility and production for the whole year can be retrieved from these programmes (Esslemont et al., 1991).

1.3.3. Herd fertility evaluation

Having obtained accurate records for all cows in the herd, the fertility of the herd can be evaluated, the purpose being either to correct problems or confirm satisfactory performance (Blood et al., 1978). Several reproductive parameters are used to measure and monitor performance. These are summarised in Table 1.1 modified from Esslemont and Peeler (1993).

For a number of years the *Calving Interval* has been used as a measure of dairy herd fertility (Fielden et al., 1980). This is the interval in days from one calving to the next calving in the individual cow. The interval should be 365 days or less. The use of the Calving Interval as a main indicator of fertility has received much criticism. It has been pointed out that this measure ignores important factors such as the policy of the farmer in relation to the interval to first possible service, the length of service season and culling decisions (Esslemont, 1992; Esslemont, 1995). To have a Calving Interval a cow must have calved twice, therefore first lactation animals are excluded, as are those failing to conceive. If corrective measures are to be taken promptly, the Calving Interval is of little value because of its historic nature, measuring events which occurred from 9-12 months previously.

The mean Calving Intervals of all cows in the herd at a specific time is the *Calving Index* which should be 365 days. Like the Calving Interval the Calving Index as its drawbacks and it may seriously underestimate fertility problems in the herd.

A more immediate measure of fertility is the *Calving-to-Conception Interval*. This measures the interval in days from calving to the first fertile insemination. With a gestation length of about 280 days, the mean Calving-to-Conception interval should be 85 days to maintain the 365 day Calving Index. Both the *Calving-to-first-Service Interval* (average number of days from calving until a cow is bred for the first time) and the *Pregnancy Rate* affect the Calving-to-Conception Interval. *Days to first service* for a herd is influenced by the onset of ovarian function postpartum, heat detection, and a management policy on when first breeding should occur postpartum (Rounsaville et al., 1979; Bailie, 1982).

To achieve an 85 day Calving-to-Conception Interval cows must be bred early postpartum (Louca and Legates, 1968; Bozworth et al., 1972; Lauderdale, 1974; Fielden et al., 1980). However the first allowable service should not be less than 45 days from calving. Some

cows may be bred successfully as early as 40 days postpartum. However, higher fertility levels are not reached until 60 days or later postpartum. Milk production may be reduced in cows bred too early postpartum and therefore good milkers should not be served until at least 50 days. First lactation heifers should also not be served until 50 days post calving because they usually have a longer Calving-to-First Oestrus Interval than older cows (King and McLeod, 1983). Since producers may not observe or detect all possible heats many start breeding their cows at the first oestrus after 45 days postpartum.

The *Pregnancy Rate* is the number of services resulting in a confirmed pregnancy and usually is expressed as a percentage of the total number of services. It can be calculated for first service or all services. A 60% rate for the first services or 55% for all services is the target.

Accurate oestrus detection is vital where artificial insemination is used extensively in the herd. Indeed, extended calving intervals may be a result of poor oestrus detection. Simple observation of behavioural patterns by the may not be adequate in achieving high detection rates, in which case oestrus detection aids may provide a useful adjunct. Tail paint, heat mount detectors or steers injected with testosterone (Williamson, 1980) are reported to be better than mere observation, though each is successful and preferred in a particular environment (Williamson et al., 1972; Esslemont and Bryant, 1976). Measurement of progesterone levels in blood or milk has also been shown to be quite accurate for oestrus detection (McCaughey and Cooper, 1980; Watson, 1996). There are several methods used to measure oestrus detection; the commonest method calculates the mean interval between services. The target heat detection rate is 80%.

Annual replacement of dairy cows usually runs at about 25% of the herd (Esslemont, 1992). Cows may be culled for mastitis, lameness or indeed, bad behaviour or simply need for cash, but about a third of

these cows are culled primarily for failure to conceive (Klingborg, 1987). The suggested target is that 95% of cows calving should conceive again.

Other measures of fertility such as the *Fertex Score* have been described (Esslemont, 1995). This is a financial score which calculates the total cost to a farmer of any extra day the cow remains open (extending the Calving Interval), the cost of extra culls (beyond the performance target) and the cost of extra inseminations per conception. This is based on a penalty/bonus principle. The Fertex Score is finally reported per hundred animals and carries either a minus (penalty) or a positive (bonus) sign. In the case of a bonus situation the cost becomes a gain. An example of this calculation is shown in Table 1.2 The performance targets for the herd were as follows:- mean Calving Interval of 360 days, culling due to reproductive failure target of 5% and the mean inseminations per conception of 2.0. Costs were calculated as £2.50 per day per cow beyond the target Calving Interval, £500 per each % cull above target, and an insemination cost of £20.

Table 1.2. shows the Fertex score for the herd (X) with a mean calving interval of 380 days, culling rate of 10% and mean number of inseminations per conception of 2.5. The closer the Fertex score is to zero the better the performance. The calculation for Herd X indicates a minus fertex score of £8500/100 cows, a situation where there is room for improvement.

Table 1.1: Performance targets for UK dairy herds (modified from Esslemont and Peeler, 1993)

Fertility Index	Target
Mean calving interval (days)	370
Mean calving to first oestrus (days)	45
Mean interval to 1st service (days)	67
Mean calving to conception (days)	89
Oestrus detection rate (%)	80
Conception to first service (%)	60
All service pregnancy rate (%)	55
Cows served of cows calved (%)	92
Proportion conceiving of calved (%)	86
Proportion conceiving of served (%)	93
Culling for failure to conceive	7
Total culling rate (%)	16
Number of services per conception	<1.7
Herd Fertex (per 100 cows)	+ 890

Table 1.2: Example of calculating the Fertex Score of Herd X

Parameter	Standard	penalty / bonus
Mean calving interval	360	£2.50/day/cow
Culling rate	5%	£500.00/1%/100 cows
Inseminations/Conception	2.0	£20.00

Herd X		Calculation	£/cow	£/100 cows
Calving Interval	380	-(380-360) 2.50	-50	-5000
Culling rate	10	-(10-5) 500	-25	-2500
Inseminations/ Conception -	2.5	-(2.5-2.0)	-10	+1000
Fertex Score			-85	-8500

1.4. Ultrasonography

Ultrasonic waves are high frequency sound waves beyond the human audible sound frequency range of 20-20,000 Hertz (Hz). For diagnostic purposes ultrasound waves of the frequency 1-10 MHz are used.

1.4.1. Historic background

Ultrasound takes its evolution from the discovery of the piezoelectric effect in 1880. The piezoelectric effect allows the conversion of the mechanical energy of echoes into electric current. Ultrasound was further developed for the detection of submarines during the second world war. Since then major advances have been made which have been of benefit to both human medicine and the livestock industry. In the human field ultrasound was originally used for obstetrical examination. More recently it has become used in many areas of human medicine. Ultrasound has also become a very important diagnostic and research tool in the field of veterinary medicine (King, 1971; Chaffaux et al., 1986; Pierson, 1987; Kahn, 1989; Curran and Ginther, 1991).

1.4.2 Generation and detection of ultrasound

Numerous publications give detailed descriptions of the principles of ultrasound and the types of equipment available (McDickens, 1981; Bartum and Crown, 1983; Ginther, 1995; Goddard, 1995). Diagnostic ultrasound is based on a pulse-echo principle. The ultrasound beam is emitted by a transducer (also called probe or scanhead) which contains the piezoelectric crystals. When high voltage current is applied to these crystals they become deformed and vibrate, thus sending high frequency

sound waves through the tissues. Upon reaching a boundary between tissues of different densities reflection occurs. The echoes produced travel back and are received by the transducer. The echo signal is transmitted to a scanner and is analysed according to the strength and the depth of reflection. This is done in a repetitive co-ordinated manner to build up an ultrasound image (Goddard, 1995). Thus the ultrasound machine is made up of a transducer for transmitting pulses and receiving echoes and a scanner for analysing the echoes.

1.4.3 Equipment

The Transducer (probe, scanhead)

Generally transducers are manufactured with a fixed resonant frequency, for example, 3.5 MHz, 5 MHz or 7.5 MHz. Tissue penetration and resolution depend on the frequency of the transducer used. The choice of which frequency to use depends on the location of the structures to be studied relative to the transducer position. The lower the frequency, the greater the depth of penetration but this advantage is counteracted by a loss of resolution. Broadly, a 7.5 MHz transducer is used for detailed ovarian and early pregnancy studies, a 5 MHz for pregnancy work after 40 days and a 3.5 MHz for late pregnancy examinations (Boyd, 1995).

Transducers are of two types, one being the linear array transducer and the other, the sector transducer. The former produces a rectangular shaped field (Barr, 1990) and has the advantage of a wide field of view close to the scanning surface. Ultrasonic examination of the ovaries generally utilises this transducer type (Pierson and Ginther, 1984). The disadvantage of this type of transducer is that it requires a relatively large area of patient contact making it unsuitable for use in small animals. The latter type of transducer produces a sector image which looks like a wedge of cake. Its advantage is that visualisation of some structures inaccessible to the linear array transducer is possible

e.g. viewing between the ribs. This type of transducer is commonly used in small animals.

The Ultrasound Scanner

This is the image display. The image on the scanner can be displayed in several modes (A-Mode, M-Mode, B-Mode) but of greatest importance in bovine reproduction is the B-Mode display (Brightness Mode; Bartum and Crown, 1983). In B-Mode the ultrasonic image is a two dimensional display of dots. The brightness of the dots is proportional to the echo strength. In real time (as opposed to static) the echoes are continuously recorded and the screen image is rapidly updated making it possible to observe the movement of structures. The Real-time B-Mode Scanner is commonly used in veterinary medicine.

1.4.4. Image interpretation and echogenicity

Images on the scanner are shown in various shades of gray ranging from black to white. Images are classified as anechoic (black), hypoechoic (shades of grey) or hyperechoic (white) (Ginther, 1995). Structures which do not reflect the ultrasound beam are said to be anechoic and appear black in the image. Examples of anechoic tissues are fluid filled structures like follicles in the ovaries, the urinary bladder and foetal fluids. Structures which reflect ultrasound are termed echogenic. These can either be low reflectors (hypoechoic) which appears in shades of grey or high reflectors (hyperechoic) and appear white. Generally soft tissues are hypoechoic while bone and high density structures like the cervix are hyperechoic.

1.4.5. Artifacts or image distortions

Artifacts observed during reproductive ultrasonography have been discussed in detail by Ginther (1995). Correct interpretation of an ultrasound image requires knowledge of artifactual (image) distortions which may be mistaken for normal or pathological structures or changes. Tissue artifacts commonly seen in Bovine reproduction using a two dimensional Real-time scanner are as follows:-

Acoustic shadowing. This occurs when the ultrasound beam is blocked or deviated. When the beam meets a highly reflective structure such as bone it is blocked and the structures distal to this can only be seen by altering the angle of the incident beam. (Figure 1.1A)

Reverbration (Re-echo). This is seen when sound from a highly reflective surface is reflected back into the tissues by the surface of the transducer. The beam continues to bounce back and forth between structure and transducer and results in a series of lines that are equidistant on the screen. An example is when the ultrasound beam impinges upon the bladder wall, gas in the bowels or bone. It is also seen at the top of the screen if the initial transmission from the probe is poor. In this case air, a highly reflective interface, is interposed between the transducer and the patient. The ultrasound beam bounces back and forth between the transducer and the air, forming spurious echoes (Figure 1.1B).

Acoustic enhancement (through transmission). When ultrasound passes through a reflector-free structure, for example fluid-filled structures, attenuation of the beam by echo production does not occur. When the beam emerges on the other side of the structure the pulse is greater than in the tissues on each side of the structure. The beam will then appear as a column of relatively brighter echoes beneath the fluid-filled structure. Ovarian follicles, cysts and embryonic vesicles are some of the structures which commonly give rise to enhancement (Figure 1.C).

The area of increased echogenicity is not a structure but should be taken as a pointer to a proximal fluid filled structure.

Reflection. During specular reflection the ultrasound beam strikes a smooth interface which is wider than the beam width and perpendicular to the transducer. For an echo to be detected by the transducer the pulse must hit the specular reflector at approximately a right angle. The echo will result in an edge thickness artifact. Of the pulse that hits the specular reflector at a right angle, only a small portion is reflected. The major portion goes through as a transmitted pulse. If the wall on the opposite side of an encapsulated, fluid filled structure is smooth enough it will again act as a specular reflector and again the edge thickening artifact occurs. The smooth surface of the external uterus, endometrial folds, embryonic vesicles, and uterine cysts are all examples of specular reflectors. Frequently, the uterine lumen may be seen as a bright echogenic line when the uterus is viewed longitudinally (Figure 1.1D).

Refraction. This occurs when the ultrasound beam traverses tissues of different acoustic impedance. The sound waves transmitted to the second medium change direction. A reflector encountered after refraction will appear slightly shifted from its real position (Figure 1.1E).

1.5. Ultrasound in Bovine Reproduction

In 1980 Palmer and Driancourt produced the first report of transrectal imaging in the mare. Ultrasonography has since assumed a wide application in female bovine reproduction. Its use for pregnancy diagnosis led to its application in the detection of early embryonic death (Pierson and Ginther, 1984; Chaffaux et al., 1986; Curran et al., 1986a), foetal imaging (Kahn, 1989; Kahn, 1990) and foetal sexing (Curran and Ginther, 1991) in the bovine.

Ultrasound has been of great assistance in understanding ovarian follicular growth and regression. It was used to establish that follicular growth during the bovine oestrous cycle occurred in wave-like patterns

(Pierson, 1987; Sirois and Fortune, 1988). Follicular dynamics have also been studied during pregnancy (Guilbault et al., 1986; Pierson and Ginther, 1986) and the postpartum period (Rajamahendran and Taylor, 1990, Savio et al., 1990a). Observation of ovulation and the development and structure of the corpus luteum has been documented (Sprecher et al., 1989; Pieterse et al., 1990).

1.5.1 Ultrasonography of the oestrous cycle

The reproductive tract of the cow undergoes various changes associated with different stages of the oestrous cycle. These changes were routinely monitored by transrectal palpation (Hancock, 1962; Edwards, 1965; Settergren, 1980). Early reports of ovarian dynamics were based on this technique and on studies of slaughter house specimens or marking of the structures with Indian ink (Dufor et al., 1972). The introduction of ultrasound into veterinary science has provided a non-invasive investigative method which has been able to supplement previous studies.

Ultrasonography of the ovaries

Ultrasonographic ovarian images are primarily composed of follicles and corpora lutea. Follicles appear as anechoic areas surrounded by a thin hyperechoic wall, ranging in size from 2 mm to 2 cm. Luteal tissue is less echogenic than the surrounding ovarian parenchyma, has an irregular shape and a thin hypoechoic border zone (Pierson and Ginther, 1984; Pierson, 1987). Follicles develop at regular intervals of several days in a wave-like pattern. Usually a minimum of two waves is observed and each wave is characterised by the development of one large dominant follicle and a variable number of smaller follicles (Sirois and Fortune, 1988). This follicular growth begins on day two of the cycle. The dominant follicle of the last wave proceeds to ovulation. The average diameter of the ovulatory follicle at standing oestrus is 15 mm, ovulation

usually occurs when the follicle reaches 2 cm in size. The occurrence of ovulation is indicated by the disappearance of this large follicle and the subsequent formation of a corpus haemorrhagicum (Pierson and Ginther, 1984). The site of ovulation may be visualised and the apposed walls of the collapsed follicle can occasionally be distinguished from the surrounding stroma. The corpus haemorrhagium is identified as an area with a central, less echoic core surrounded by a relatively hyperechoic peripheral edge (Figure 1.2.D) The border of the corpus luteum is identifiable by day three and the mature corpus luteum grows to a size of 2.5 cm. In about 43% cases the corpus luteum has a lacunae which may be as large as 22 mm (Pierson and Ginther, 1984). The regressing corpus luteum reduces in size, becomes hyperechoic and is eventually indistinguishable from ovarian stroma.

Ultrasonography of the uterus

On transrectal clinical examinations the uterus is described as contracted and firm during oestrus. The muscularis is physiologically contractile so that when the uterus is palpated per rectum this muscular irritability, coupled with the marked vascularity conveys a highly characteristic tonic turgidity to the palpating fingers. The horns feel erect and coiled (Arthur et al., 1996a). This turgidity is associated with increased vascular development and oedema in the uterus as shown by post-mortem study reports (Hansel and Asdell, 1951; Hackett and Hafs, 1969). It is present a day before and a day after oestrus but is at its maximum during oestrus. The dioestrus uterus is flabby, highly coiled and tortuous (Garverick et al., 1971; Bartol et al., 1981).

Ultrasonographically, two distinct appearances of the non- pregnant uterus are seen relating to the periovulatory and dioestrus periods (Fissore and others, 1986). Increases in intrauterine and intravaginal fluids are detected just before ovulation till 3 days after ovulation at which time volumes of fluid start to decrease to reach base-line levels by day 6 or 7 (Pierson and Ginther, 1987). As the uterus becomes oedematous,

towards ovulation, it starts to uncoil. The ultrasonographic picture of the periovulatory uterus shows a heterogeneous echo texture. The endometrial folds are seen clearly (Fissore and others, 1986). The uterus shows thickening and the lumen contains anechoic fluid (Figure 1.3A). As it becomes turgid, few cross sections of the uterine horns are seen in the ultrasonographic image. Following ovulation the uterus starts to coil up until approximately day 6 or 7. It then remains in this state until days 16 or 17 (Pierson and Ginther, 1987). Uterine thickness decreases. During the dioestrus period, the uterus displays a homogeneous echo texture and more cross sections of the horns are seen in the image (Figure 1.3B).

1.5.2. Ultrasonography and pregnancy diagnosis

One of the earliest applications of ultrasound in the livestock industry was pregnancy diagnosis. The first indication of pregnancy, ultrasonographically, is the observation of the embryonic vesicle between 10 and 14 days post-insemination (Pierson and Ginther, 1984; Curran et al., 1986a). This appears as a discrete, nonechogenic area in the horn ipsilateral to the ovary bearing the corpus luteum. By day 28 post service, it is possible to visualise the embryo within the embryonic vesicle and the heart beat is clearly visible (Reeves et al., 1984; Curran et al., 1986b). Placentomes are seen from around day 34. A detailed description of the ultrasonographic appearance of bovine conceptus, from the time of the embryonic vesicle is detected till day 60, has been given by Curran and others in two separate papers (1986a & 1986b). An example of examination of the pregnant uterus is shown in Figures 1.3C and 1.3.D.

Chaffaux and others (1986) found ultrasound to be 100% reliable for diagnosing non-pregnant cows from day 40 post-insemination. Others found the accuracy of ultrasound for identifying pregnant cows to be 33% by day 16 and increasing to 100% by day 20 (Boyd et al., 1990).

Rajamahendran and others (1994) in a survey of ten publications reported variations in reliability from 33% at 16 days to 100% at 45 days. The type of transducer, number of examinations, age and parity of the animal, day of examination post-insemination, the reporting criteria and the experience of the operator have all been cited as sources of this variation. Considering these factors it has been suggested that the most realistic date for early pregnancy diagnosis in the cow using ultrasound is day 30 (Rajamahendran et al., 1994).

There are reports of the use of ultrasound in foetal sexing (Muller and Wittkowski, 1986; Curran and Ginther, 1991; Curran, 1992). These are based on visualisation of the genital tubercle, the embryonic structure which differentiates into the penis in males and clitoris in females (Noden and De Lahunta, 1985). It begins to develop between the hind limbs of the undifferentiated foetus, gradually migrating towards the umbilicus in males and beneath the tail in females. With a high resolution transducer and an experienced ultrasonographer it is possible to determine the sex of the foetus as early as day 56 post service fairly accurately.

1.5.3. Ultrasonography of uterine and ovarian pathology

Traditionally ovarian cysts have been diagnosed on the basis of clinical signs, rectal palpation and measurement of either serum or milk progesterone levels. Pathological ovarian cystic structures are classified as either follicular or luteal cysts. Clinical signs are of continuous oestrus, short oestrous cycles or anoestrus (Booth, 1988). Anoestrus, though commonly observed in luteal cystic conditions, can occur with follicular cysts, thus making diagnosis difficult on the basis of clinical signs alone. By rectal palpation, follicular cysts are thin-walled and soft, while luteal cysts are firm, rubbery and thick-walled (Farian et al., 1992). However, it can be difficult to differentiate a follicular cyst from a luteal cyst on rectal palpation alone (McLeod and Williams 1991; Farian et al., 1992).

Measurement of progesterone is the standard method for confirming cyst type (Booth, 1988; Farian et al., 1990; Ribadu et al., 1994b; Jeffcoate and Ayliffe, 1995). Luteal cysts are associated with relatively high progesterone levels in milk (>2.0 ng/ml) and blood (>0.5 ng/ml) compared to basal levels with follicular cysts (Booth 1988; Farian et al., 1990; Farian et al., 1992). However, reports are emerging showing that cows with cysts secrete variable amounts of progesterone (Blowey, 1992). Also cysts may occur at the same time with a corpus luteum. Darwash (1997), in a more recent study, found that approximately 37% of UK dairy cows show some form of abnormal ovarian hormone pattern. Thus the interpretation of progesterone analyses may not be straightforward.

Ultrasound has been used successfully for diagnosing cystic ovarian disease and monitoring progress of the disease following treatment (Ribadu et al., 1994a; Ribadu et al., 1994b; Jeffcoate and Ayliffe, 1995). Ultrasonographically, a luteal cyst shows a rim of luteinised tissue measuring about 2-4 mm in thickness (Figure 1.4B), sometimes accompanied by trabeculae which extend into the antrum of the cyst (Omran et al., 1988; Farian et al., 1990). Follicular cysts have an uninterrupted anechoic antrum; the inner wall is thin and relatively smooth (Figure 1.4A) (Farian et al., 1990). Ultrasound has been shown effective in detecting luteal cysts than follicular cysts in the cow (Farian et al., 1990). However the combination of ultrasonography, rectal palpation, clinical signs and progesterone immunoassays can help in reaching a definitive diagnosis.

Ultrasonographic studies of uterine pathology have been reported (Fissore et al., 1986). These conditions include endometritis, pyometra, and mummified or macerated foetuses. In general, inflammatory conditions are characterised by a distended lumen filled to a varying degree with partially echogenic "snowy" patches (Fissore et al., 1986).

1.5.4. Ultrasonography of the postpartum cow

Most studies of the early postpartum period have been restricted to ovarian activity (Rajamahendran and Taylor, 1990; Savio et al., 1990a; Savio et al., 1990b). Ultrasonographic studies of uterine involution are quite limited (Okano and Tomizuka, 1987; Kamimura et al., 1993). The purpose of this study was to shed more light on the subject to help our understanding of the process.

1.6. Objectives of the Study

The objectives of this study were three fold:

1. To gain relevant experience with the use of ultrasound in Bovine female reproduction.
2. To establish base measurement parameters for heifers and parous cows and to compare ultrasonographic pictures of normal and pathologic uterine specimens.
3. To use the information gained above in serial monitoring of post-partum uterine involutional changes and recognise changes indicating uterine pathology.

Figure 1.1. Artifacts commonly seen during ultrasonic reproductive examination of the bovine.

A. Acoustic shadowing. Cow 74. The ultrasound beam is reflected by the pelvic bone and no structures are visible distal to this. u = uterine horns; b = urinary bladder; p = pelvic bone

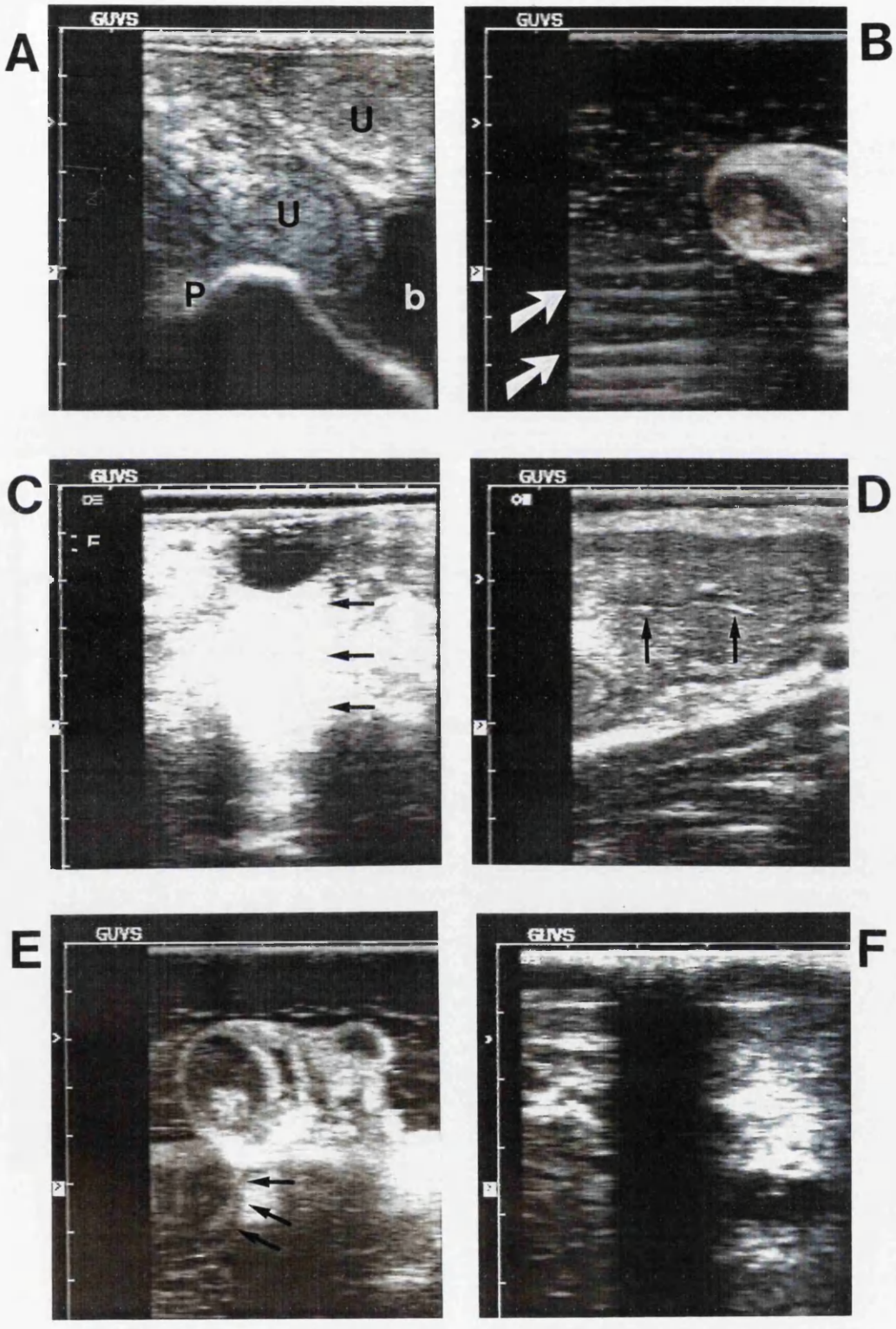
B. Reverberation. Specimen of an ovary examined in a water-bath. A series of lines are visible caused by the beam hitting the highly reflective surface of the water-bath (arrows).

C. Acoustic enhancement. Cow 90. Large follicle on left ovary. A bright column occurs distal to the follicle because the beam is poorly attenuated on passage through the follicle, relative to the tissue on either side of the follicle (arrows).

D. Specular reflection (arrows) visible as a bright echogenic line at the lumen of the uterus. Cow 97, longitudinal section of the uterus.

E. Refraction, causing a partial mirror image (arrows) of an ovary examined in a water-bath.

F. Black column caused by faecal material on the face of the transducer, blocking the transmission of the ultrasound beam.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U
P
b
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E
GUYS
F
GUYS

Figure 1.2. Ultrasonographic examination of the bovine ovary

A. Ultrasound image of a small inactive ovary. Cow 8 at 33 days postpartum. Note that only one small (5mm) follicle is visible (arrow).

B. Ultrasound image of an ovary showing developing follicles. Five small and medium follicles are visible (F). Cow 20, 42 days postpartum.

C. Ultrasound image of an ovary with a large pre-ovulatory follicle, 1.8 cm in diameter. Cow 71 at 60 days postpartum.

D. Ultrasound image of an ovary showing a corpus haemorrhagicum 24 hours after ovulation.

E. Ultrasound image of an ovary containing an immature corpus luteum. Note lacuna. Cow 98, 46 days postpartum.

F. Ultrasound image of an ovary showing a corpus luteum of pregnancy. The structure measured 2.5 cm in diameter. Cow 80.

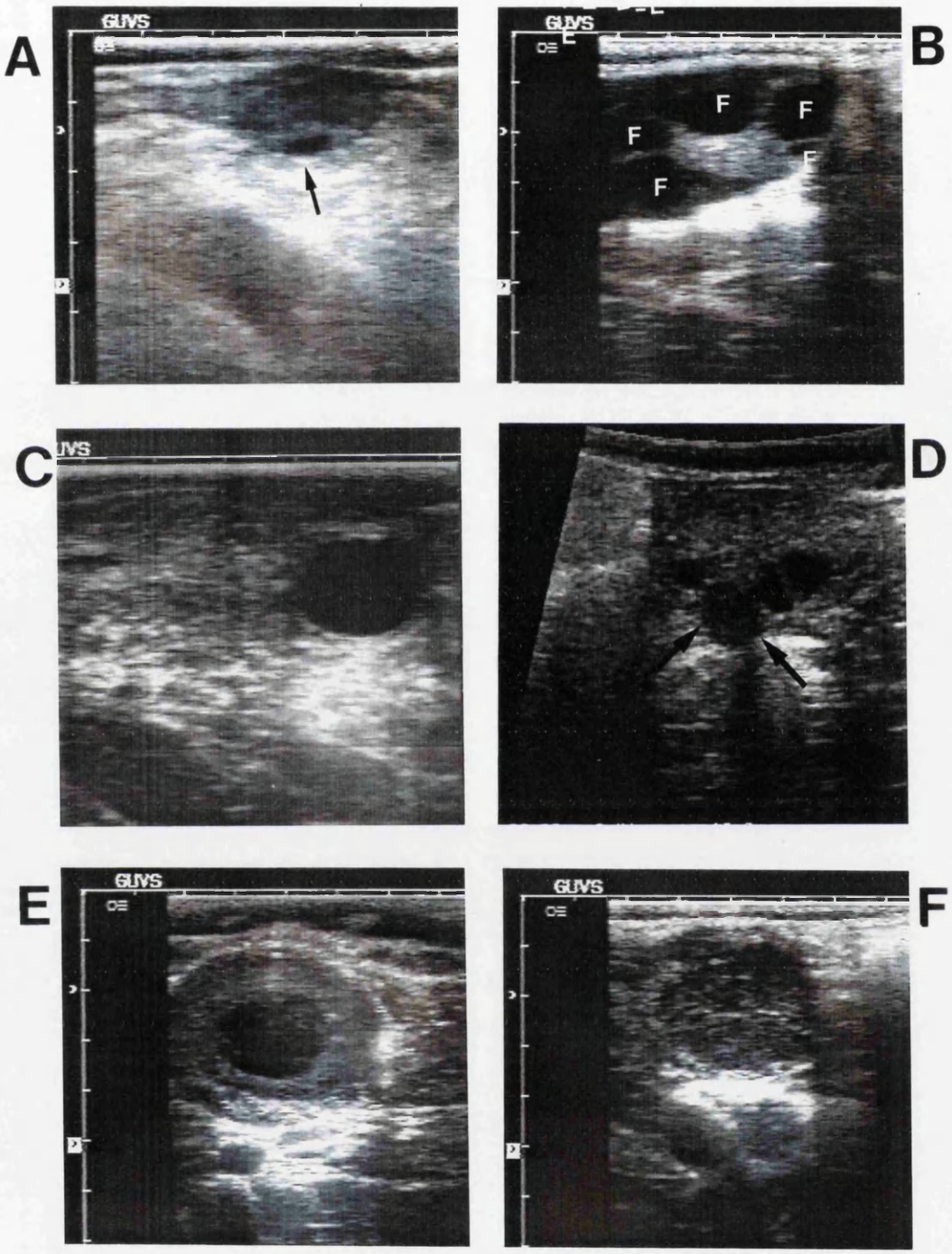
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Figure 1.3. Ultrasonographic examination of the bovine uterus.

A. Uterus during oestrus. There is anechoic fluid in the lumen and the uterus has an oedematous appearance with marked endometrial folds. Cow 90 at 29 days postpartum.

B. Ultrasound image of uterine horns during dioestrus. Cow 96 at 45 days postpartum.

C. Ultrasound image of a gravid uterus. The foetus and amniotic membrane (white arrow) are visible. Cow 80.

D. Ultrasound image of a gravid uterus. Note visible placentomes (P). Cow 80.

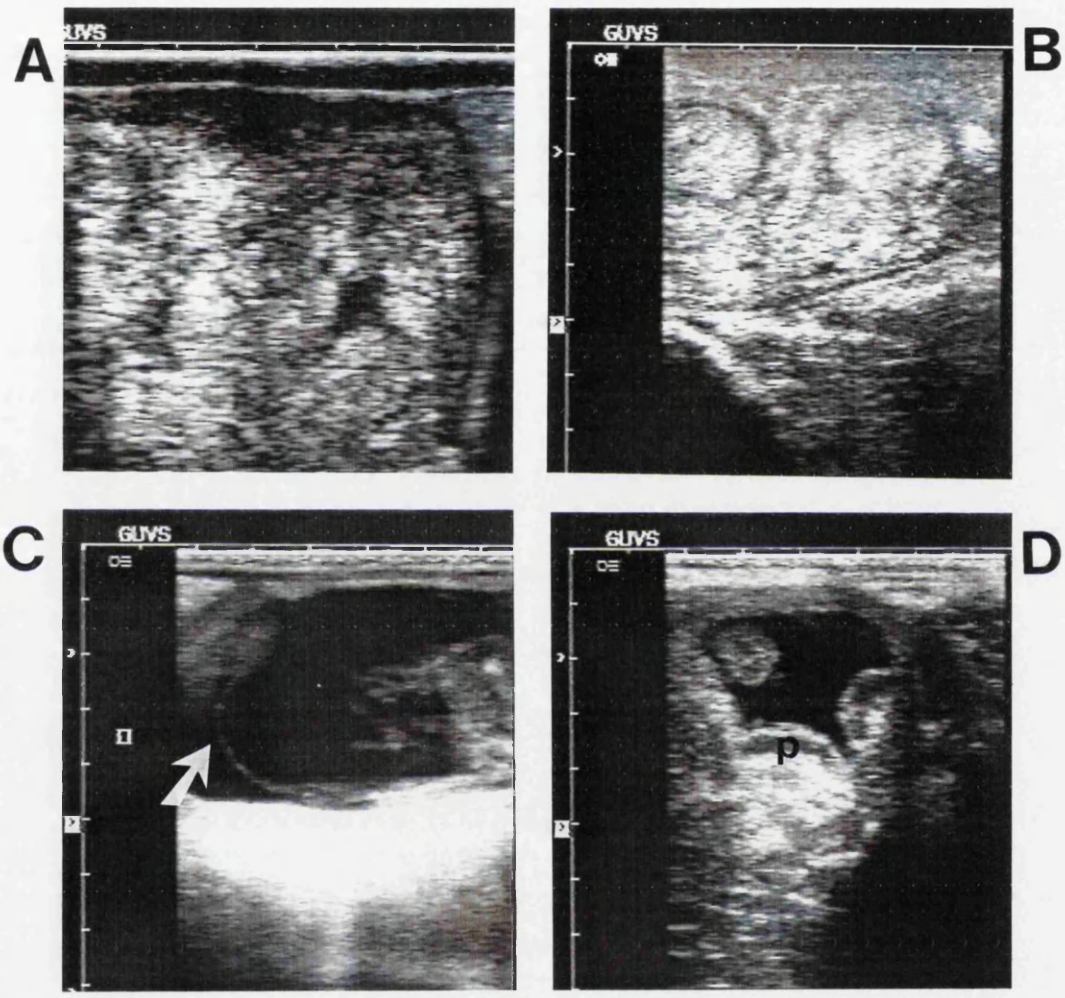
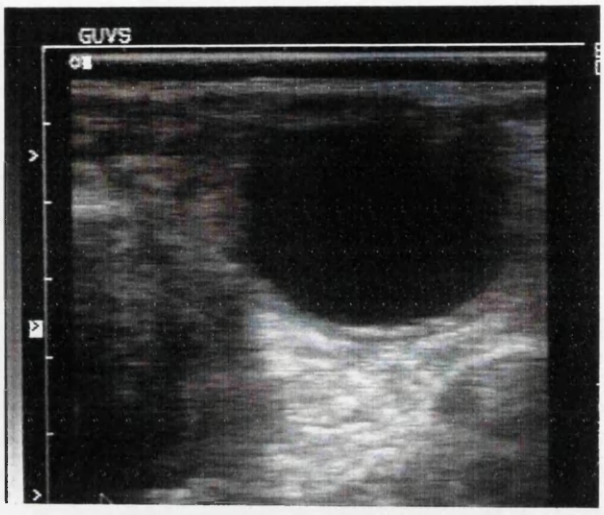

A
B
C
D
GUVS
p

Figure 1.4. Ovarian pathology.

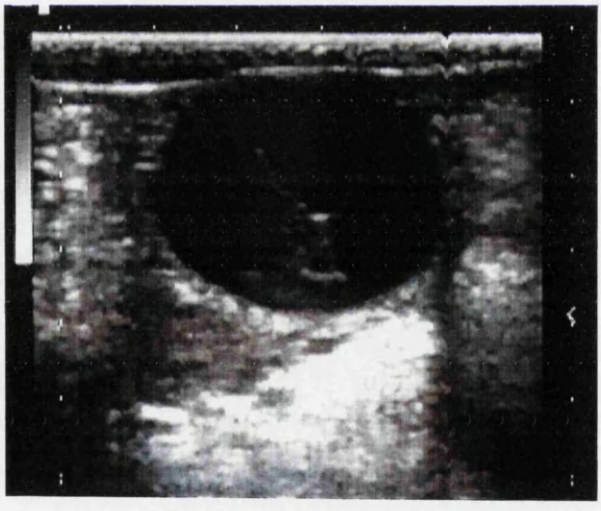
A. Ultrasound image of a follicular cyst. Cow 74, 35 days postpartum. The cyst is solitary and has a fairly smooth rim surrounding anaechoic fluid.

B. Ultrasound image of a luteal cyst. Note the rim of luteal tissue and the trabecula in the cavity of the cyst.

A



B



Chapter 2

Materials and Methods

2.1. Ultrasound Equipment

2.2. Experimental Protocol

2.2.1. Abattoir specimens

2.2.2. Scanning of Water-Bath Specimens

2.2.3. Experimental Animals

2.2.4. Scanning of Experimental Animals

2.2.5. Herd Fertility Records

2.3. Statistical Analysis

2.1. Ultrasound Equipment

A portable, real-time B-mode, two dimensional ultrasound scanner was used throughout this study (Concept/MCV, Dynamic Imaging, Livingston, Scotland). Imaging was carried out using a 7.5 MHz linear array transducer with a 6 cm crystal face (Figure 2.1.A). Electronic callipers were used to measure structures after freezing the image with the freeze-frame facility. Three degrees of magnification were available to allow examination of detail. The scanner was also equipped with video output enabling clinical examinations to be recorded for later review. Images were recorded on Super VHS tape using a super VHS video cassette recorder (Panasonic AG 7330; Figure 2.1.B). Video tapes were reviewed in the laboratory using an Interspec scanner (Ambler, USA) and selected images were printed on Sony black and white paper (UPC 3020) using a Sony Video Printer (UP-300P). Structures were also measured from video tapes using this equipment after an initial calibration.

2.2. Experimental Protocol

2.2.1. Abattoir Specimens

The abattoir specimens used to establish base-line uterine measurement parameters for nuliparous heifers were collected from Paisley abattoir. These tracts were recovered from beef heifers under 30 months of age which were slaughtered for human consumption during the 1996 Bovine Spongiform Encephalopathy outbreak in Great Britain. A total of 25 specimens were collected.

Specimens from parous Holstein-Friesian cows were collected from Larkhall abattoir. These cows were slaughtered for disposal during the same outbreak. The specimens in this latter group were categorised

as normal, as having mild endometritis or as having severe endometritis in a manner similar to that described by Miller et al., (1980) and Studer and Morrow (1981). Specimens were classified according to the following criteria as follows:-

Class I. Normal uterus (Figure 2.2.A). A smooth and rounded uterine outer surface. No discharge observed at the external cervical orifice, with "dry" mucosa or clear oestrous mucus.

Class II. Mild endometritis (Figure 2.2.B). Increased discharge, mucus cloudy or containing small clumps of pus but without odour. Uterus may have longitudinal grooves.

Class III. Severe endometritis (Figure 2.2.C). Palpable fluid in the uterine lumen. Purulent discharge ranging from thick, creamy to cheesy or thin and red on massaging the horns. Fetid discharge. Palpable caruncles.

These specimens were severed at the urethral orifice then taken to the laboratory for scanning. A total of 25 specimens were collected for each group.

2.2.2. Scanning of water-bath specimens

Scanning was carried out with the dorsal surface of the tract nearest to the transducer to imitate transrectal examination in the live animal. The specimens were scanned in a water bath with one hand holding the uterus immersed and the other hand guiding the transducer from 3 cm above the area of interest. In an initial study selected uteri were scanned and then frozen for a minimum of 48 hours. The frozen uteri were sectioned with an electric band-saw along the scan planes

shown in Figure 2.3. For scan plane 1 an image of a transverse section was taken at the level of mid cervix. For scan plane 2, a longitudinal scan was made of the cervix. For scan planes 3 and 4, transverse and longitudinal scans were made of the uterine body, respectively. Planes 5 and 6 were transverse and longitudinal sections of the horn at the level of intercornual ligaments. Representative photographs are shown in Chapter 3, Figures 3.1-3.3. When measurements were made, each of the uterine horns was measured in the transverse plane at the level of the intercornual ligaments. The cervix was measured mid-cervix. Horizontal and vertical diameters were recorded.

2.2.3. Experimental Animals

Animals used in this study were Holstein-Friesian cows calving between the months of September 1996 and February 1997 on Glasgow University Cochno Farm. Cows were housed in a cubicle house, with access to a silage face. They were milked twice daily and concentrates were fed in the milking parlour. A total of 27 animals with parity ranging from one to eleven were included in the study (Table 2.1). Oestrus detection was carried out by the stockman by observing the animals twice daily. Heat mount detectors were also used in aiding oestrus detection.

2.2.4. Scanning of Experimental Animals

Serial examination of the cows was carried from parturition until around 65 days postpartum or until service. Each cow was examined during the time periods shown in Table 2.2. These examination dates were selected to ensure an examination during the first and second week postpartum, the period of greatest physical reduction in uterine size, and as close to 26, 45 and 56 days postpartum. The latter dates correspond to typical examinations carried out during routine herd fertility visits.

Cows showing incomplete uterine involution on the fifth examination were examined at weekly intervals until involution was considered to be complete. The parity, calving date and calving history were all recorded. An example of the recording sheet used during these examinations is presented in Appendix 1.

For transrectal examination animals were restrained in a cattle crush with a head-lock. A manual rectal examination was carried out and the size and position of the reproductive tract was recorded. The probe was inserted in the rectum and rotated 90° to give an image of a transverse section of the cervix and the uterine horns at the intercornual ligaments and the cervix. The horizontal and vertical diameters of the cervix and horns at the level of the intercornual ligaments were recorded. In addition the endometrial area and thickness were measured.

2.2.5. Herd Fertility Records

All cow breeding information was recorded by the stockman on the farm and stored in the computerised dairy fertility programme "DairyChamp (University of Michigan) by staff within Glasgow University Farm Animal and Production Division. At the end of the study herd fertility records were obtained for all animals. An example of a record sheet is presented in Appendix 2.

2.3. Statistical Analysis

Student's t test was used to test differences between means.

Table 2.1. Lactation numbers of study animals.

Lactation number	Number of animals
1	4
2	3
3	7
4	5
5	1
6	2
7	2
8	1
11	2
Total	27

Table 2.2. Examination protocol

Examination number	Days postpartum
1	2-9
2	12-17
3	23-29
4	43-49
5	54-61

Figure 2.1. Ultrasound equipment used in the study.

A. Ultrasound scanner Concept/MCV equipped with a 7.5MHz linear array transducer.

B. Panasonic Super VHS Video Cassette Recorder

A

B

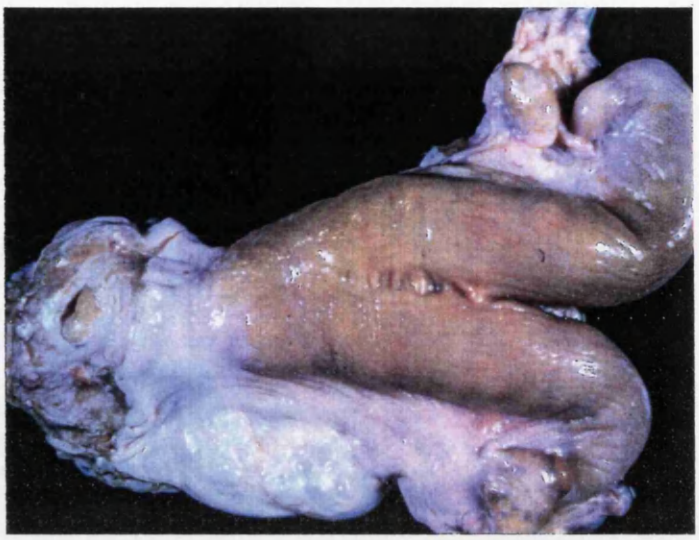
Figure 2.2. Specimens of uteri from parous cows.

A. Specimen classified as normal. Note that horns of approximately equal diameter.

B. Specimen classified as showing mild endometritis.

C. Specimen classified as showing severe endometritis. Disparity between horns is obvious. Note the presence of a mucopulurent discharge.

A



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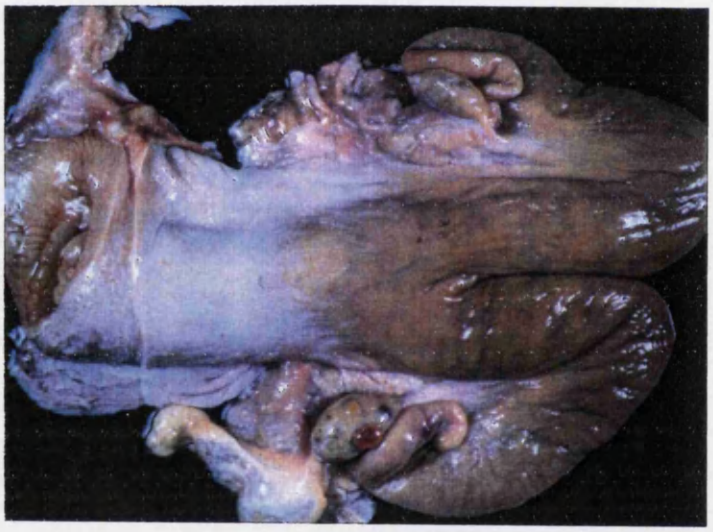


C

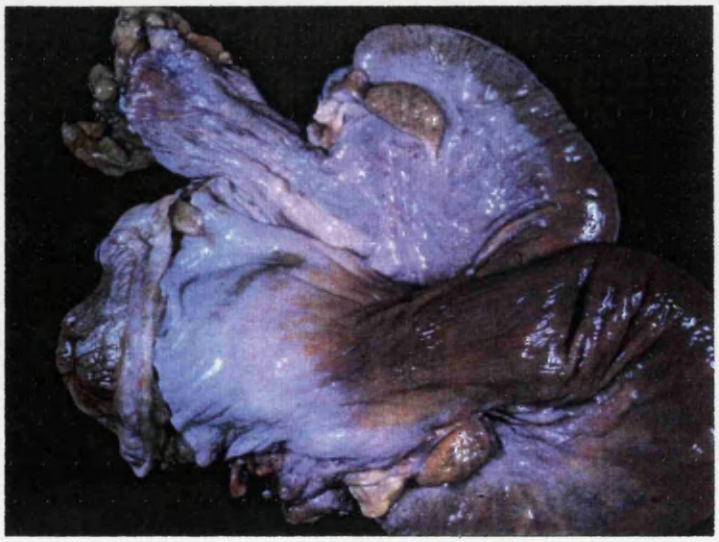


Figure 2.3. Scanning planes examined in a preliminary study.

1. Longitudinal section of the cervix at mid-cervix.

2. Transverse section of the cervix.

3. Longitudinal section of the uterine body midway along the length of the body.

4. Transverse section of the uterine body.

5. Longitudinal section of the uterine horns at the level of the intercornual ligaments.

6. Transverse section of the uterine horns at the level of the intercornual ligaments.

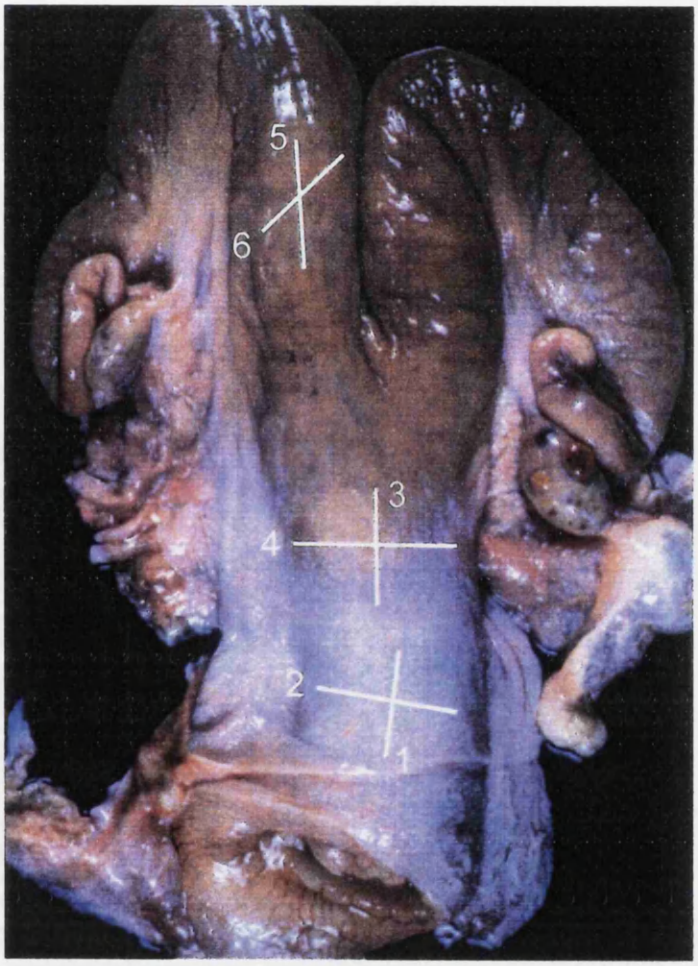

5
6
3
4
2
1

Figure 2.4. Ultrasonographic uterine measurements.

a. Schematic diagram of a cross section of uterine horn. A, endometrium; B, inner circular layer of myometrium; C, vascular layer; D. outer longitudinal layer of myometrium; E, perimetrium.

b. Schematic diagram marking the horizontal and vertical diameters of the uterine horn.

c. Schematic diagram showing endometrial area of uterine horn.

d. Schematic diagram showing endometrial thickness of uterine horn.

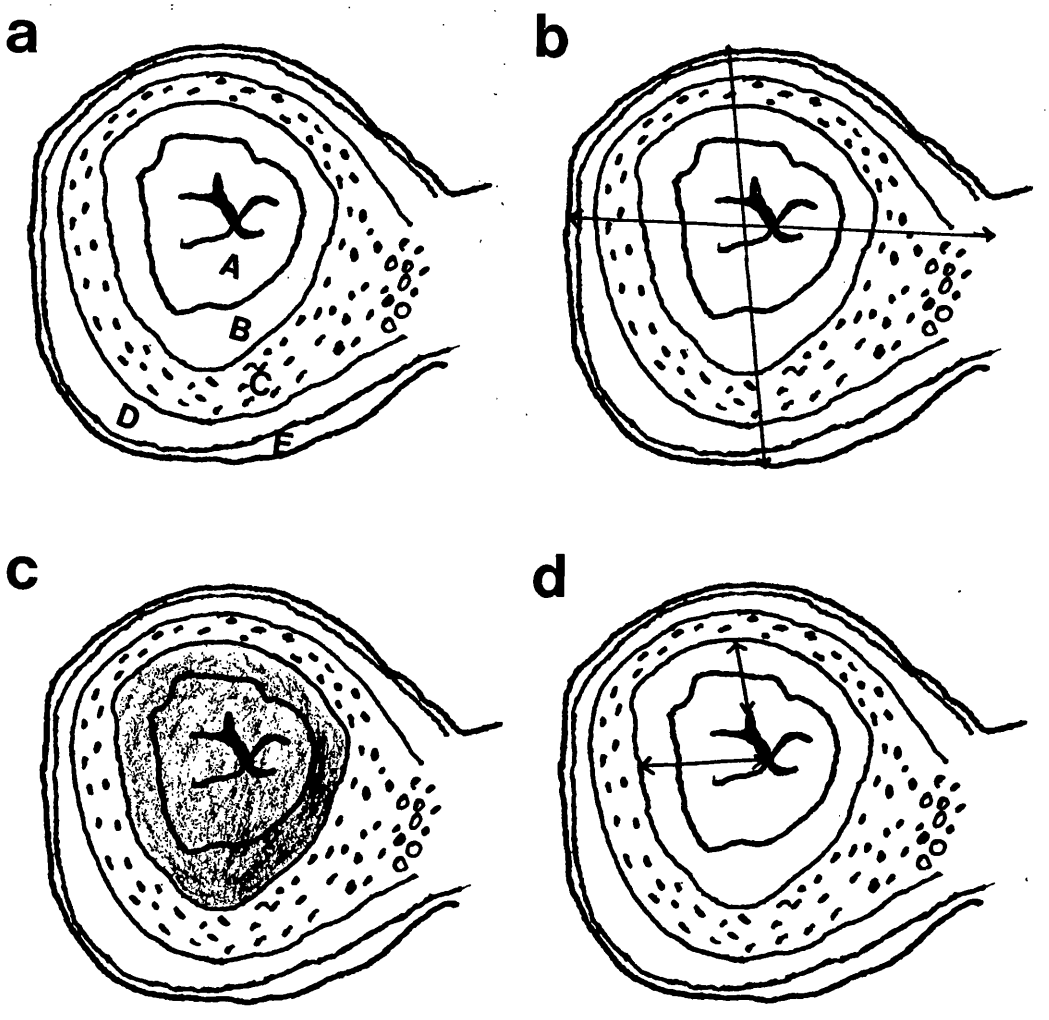

a
A
B
C
D
E
b
c
d

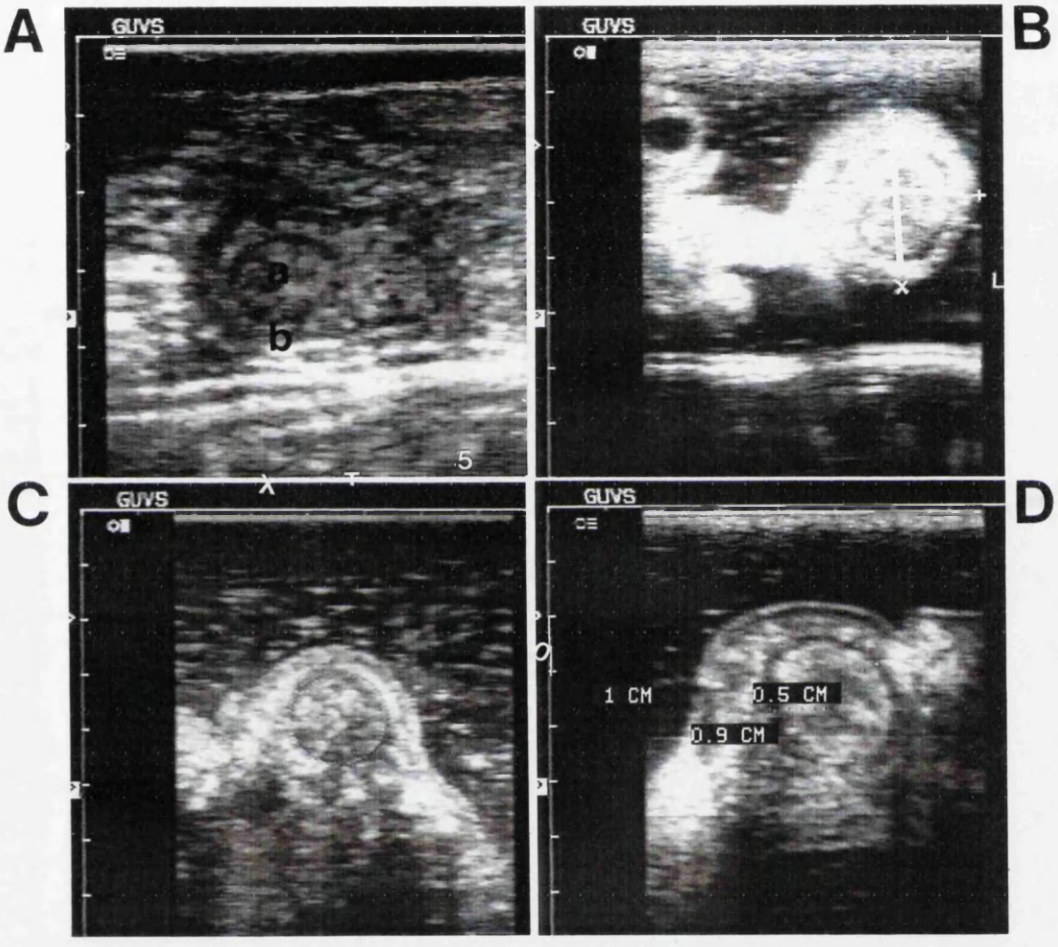
Figure 2.5. Ultrasonographic uterine measurements.

A. Ultrasonographic picture showing the various layers of a uterine horn indicated schematically in Figure 2.4a. a, endometrium; b, myometrium. The hypoechoic ring between a and b represents the vascular layer.

B. Ultrasonographic picture showing markings of the horizontal and vertical diameters of the uterine horn.

C. Ultrasonographic picture showing endometrial area of uterine horn.

D. Ultrasonographic picture showing endometrial thickness of uterine horn.


A
GUVS
a
b
5
B
GUVS
L
C
GUVS
D
GUVS
1 CM
0.5 CM
0.9 CM

Chapter 3

Results

Section 3.1. Selection of Ultrasound Scan Planes

3.1.1. Introduction

3.1.2. Comparison of ultrasound images and sectioned specimens

3.1.3. Application to live animals

Section 3.2. Ultrasonic Examination of Bovine Uteri in a Water-bath

3.2.1. Introduction

3.2.2. Measurement of uteri recovered from heifers

3.2.3. Measurement of uteri recovered from parous cows

3.2.4. Measurement of uteri recovered from parous cows with endometritis

Section 3.3. Serial Ultrasonography of the ReproductiveTract of Post-partum Dairy Cows

3.3. 1. Introduction

3.3. 2. Monitoring of normal cows

3.3. 3. Monitoring of cows with post-partum abnormalities

Section 3.1

Selection of Ultrasound Scan Planes

3.1.1. Introduction

Prior to scanning either uterine specimens or live animals preliminary experiments were carried out to determine which scan planes were the most appropriate for use during the serial monitoring of post-partum cows. This was approached two ways. Firstly, comparisons were made between the images produced by scanning uterine specimens in a water-bath and the anatomical structures identified after sectioning the same specimens on a bandsaw. Secondly, the same scan planes were used to examine uteri of non-pregnant cows. Cervix, uterine body and uterine horns were examined in both transverse and longitudinal sections. The scan planes used are illustrated in Figure 2.3.

3.1.2. Comparison of ultrasound images and sectioned specimens

Ultrasonic images of cervix, uterine body and uterine horns, obtained by scanning specimens in a water-bath, are presented in Figures 3.1.-3.3., together with corresponding pictures obtained by sectioning the specimens with a band-saw. Cut at mid-cervix in the transverse plane the cervix was tightly closed and the cervical canal was indistinct (Figure 3.1.A). The mucosa of the cervical plicae was highly folded. Ultrasonographically, the cervix appeared as an almost circular, highly echogenic structure characterised by acoustic shadowing distally. The lumen was not visible (Figure 3.1.C).

On sectioning the cervix in the longitudinal plane the intravaginal and intra-uterine portions of the cervix were visible and the cervical canal appeared highly convoluted (Figure 3.1.B). On scanning in this plane the bounderies between the cervix and vagina, caudally, and the uterine body, cranially, were indistinct. Dorsal and ventral boundaries of the cervix could be visualised. The cervical canal was visible, represented by a centrally located hyperechoic line (Figure 3.1.D).

The uterine body, cut in transverse section midway between the cervix and uterine horns, is shown in Figure 3.2.A. The lumen and endometrial folds were clearly visible. This feature was not distinct on ultrasonic scanning (Figure 3.2.C). Sectioned in the longitudinal plane, it was noted that the uterine body was short and again the lumen was clearly visible (Figure 3.2.B). On ultrasonic scanning of the same structure the lumen of the uterus was hypoechoic and highlighted by bright areas of specular reflection (Figure 3.2.D).

The uterine horns of band-saw specimens were of about equal size, with lumen and endometrial folds visible in both transverse and longitudinal sections (Figures 3.3.A & B). A highly vascular layer was seen surrounding the uterus. In transverse section ultrasonographically, the horns were circular and less echogenic than the cervix. The lumen was visible; the position marked by a hypoechoic irregular line (Figure 3.3.C). In transverse section a circular hypoechoic area was visible between the endometrium and myometrium. There was no distinct demarcation between the myometrium and the outer layer of perimetrium, ultrasonographically. Endometrial folds were more distinct in the longitudinal plane and gave rise to a folded appearance (Figure 3.3.D).

Specimens with endometritis

Uteri showing different degrees of endometritis were subjected to the same procedure of scanning, freezing and sectioning on the band-saw. Figures 3.4 and 3.5 show specimens of uterine horn scanned in the transverse plane, with mild and severe endometritis, respectively. There was an obvious disparity in the size of the right and left horn and an accumulation of abnormal fluid in the uterine lumen of both types of specimens, visible after sectioning. In severe cases of endometritis caruncles were very obvious. Ultrasound scans usually showed luminal distension, with the lumen containing variable volumes of fluid. The fluid differed from the anaechoic fluid seen during oestrus (Figure 1.3.A) ranging from partially echogenic (Figure 3.5.C and D) to hyperechoic (Figure 3.5.B and E), depending on the severity of the condition. The volume of fluid varied with small volumes seen in mild cases (Figure 3.4) and large volumes in severe cases (Figure 3.5). Endometrial folds were evident on scanning cases with severe endometritis.

3.1.3. Application to live animals

The scan planes used for the examination of uterine specimens in a water-bath were applied in non-pregnant live cows. The ultrasonographic images obtained are shown in Figure 3.6. A transverse section of the cervix showed a homogenous structure with acoustic shadowing distally (Figure 3.6.A). In longitudinal section the cervical canal was barely visible as a centrally located hyperechoic line (Figure 3.6.B). The uterine body appeared in transverse section as a circular structure with a an anechoic area in the centre representing the uterine lumen (Figure 3.6.C). In comparison to the uterine horns the layers of the uterine body were indistinct. In longitudinal section a hypoechoic line in the centre of the structure represented the uterine lumen (Figure 3.6.D). The horns

appeared circular in transverse section and the myometrium was demarcated from the endometrium by a hypoechoic ring (Figure 3.6.E).

Based on the information accumulated in these two experiments it was decided to use two scan planes for subsequent studies. The transverse section of the cervix was selected (Figure 3.6.A) because it was possible to repeatably identify the area of mid-cervix by palpation and because the borders of the structure were easily identified in the ultrasound image. The transverse section of the uterine horns at the level of the intercornual ligaments was selected (Figure 3.6.E) because the ligaments supplied an identifiable landmark for repeated measurements and because the myometrial and endometrial layers of the uterus were distinct in the ultrasound image.

Figure 3.1. Comparison of uterine specimens scanned in the water-bath and examined after sectioning with a band-saw (cervix).

A. Transverse section of the cervix (at mid-cervix). Note the highly folded mucosa. The cervix is tightly closed and the central lumen of the cervical canal is indistinct. a. cervical canal. b. cervical folds or plicae. c. broad ligament.

B. Longitudinal section of the cervix. Note the highly convoluted cervical canal. a. lumen of body of uterus. b. cervical canal. c. external cervical orifice. d. vagina. e. intravaginal portion of cervix

C. Ultrasound image of the specimen shown in A. In transverse section the cervix appeared almost circular and was highly echogenic (black arrow). The image was obscured by acoustic shadowing distally (white arrow).

D. Ultrasound image of specimen shown in B. In longitudinal section the uterine and vaginal boundaries of the cervix were indistinct. Mucosal folds were difficult to discern. Dorsal and ventral boundaries of the cervix were imaged (arrows). The cervical canal is represented by a centrally located hyperechoic line (small arrows).

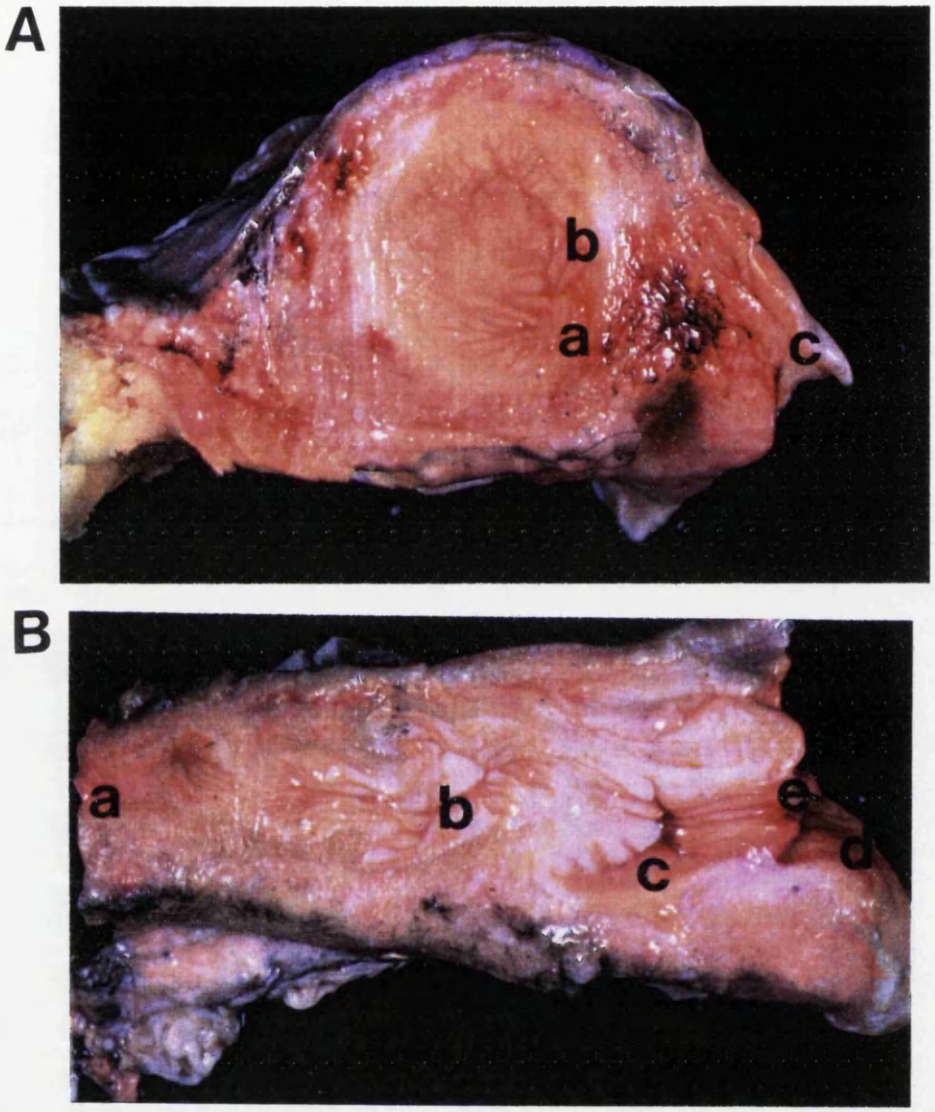

A
b
a
c
B
a
b
c
e
d
C
D

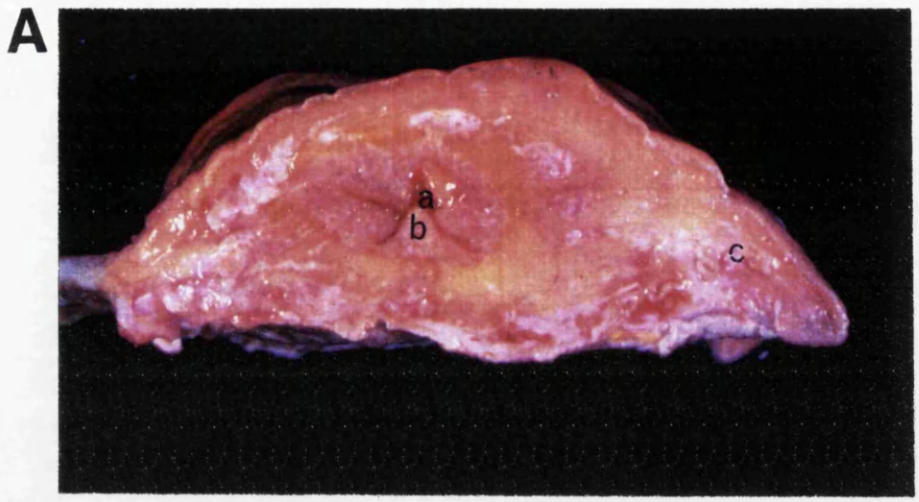
Figure 3.2. Comparison of uterine specimens scanned in the water-bath and examined after sectioning with a band-saw (uterine body).

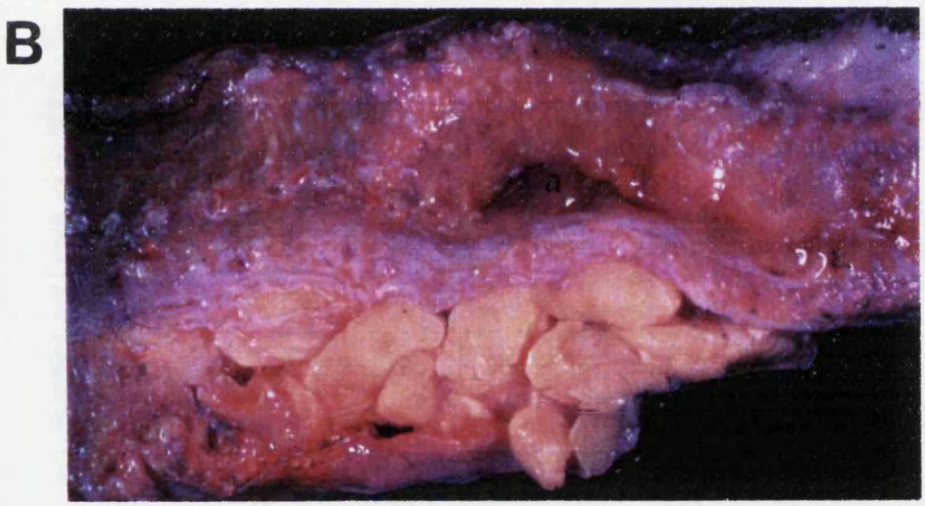
A. Transverse section of the uterine body (at mid uterine body). a. lumen of uterine body. b. endometrial folds. c. broad ligament

B. Longitudinal section of uterine body. Note the visible lumen (a).

C. Ultrasound image of a transverse section of the same specimen as in A above. Note that the lumen of the uterine body is represented by a centrally located hypoechoic area (arrows).

D. Ultrasound image of a longitudinal section of the same specimen as in B above. Note that the lumen of the uterine body is represented by a centrally located hypoechoic area highlighted by areas of specular reflection (arrows).


A
a
b
c


B
a

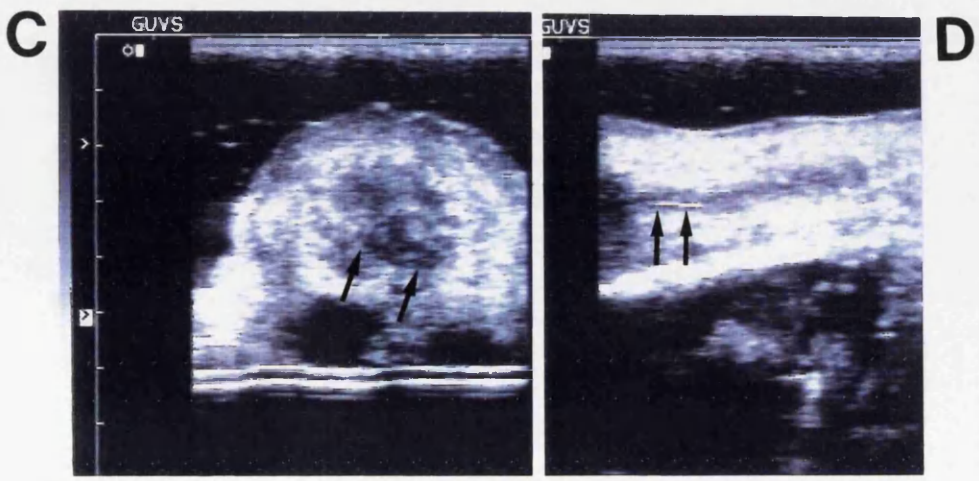

C
GUVS
GUVS
D

Figure 3.3. Comparison of uterine specimens scanned in the water-bath and examined after sectioning with a band-saw (uterine horns).

A. Transverse section of uterine horns at the level of the intercornual ligament. The small lumen can be seen. a. endometrial folds. b. vascular layer. c. left ovary

B. Longitudinal section of uterine horn. a. uterine lumen. b. vascular layer.

C. Ultrasound image of a transverse section of the uterine horns as shown in 3.A. Note folded mucosa (large arrow) and ring of vascular layer (small arrows).

D. Ultrasound image of a longitudinal section of uterine horn as shown in 3.B. Note folded endometrium (arrows).

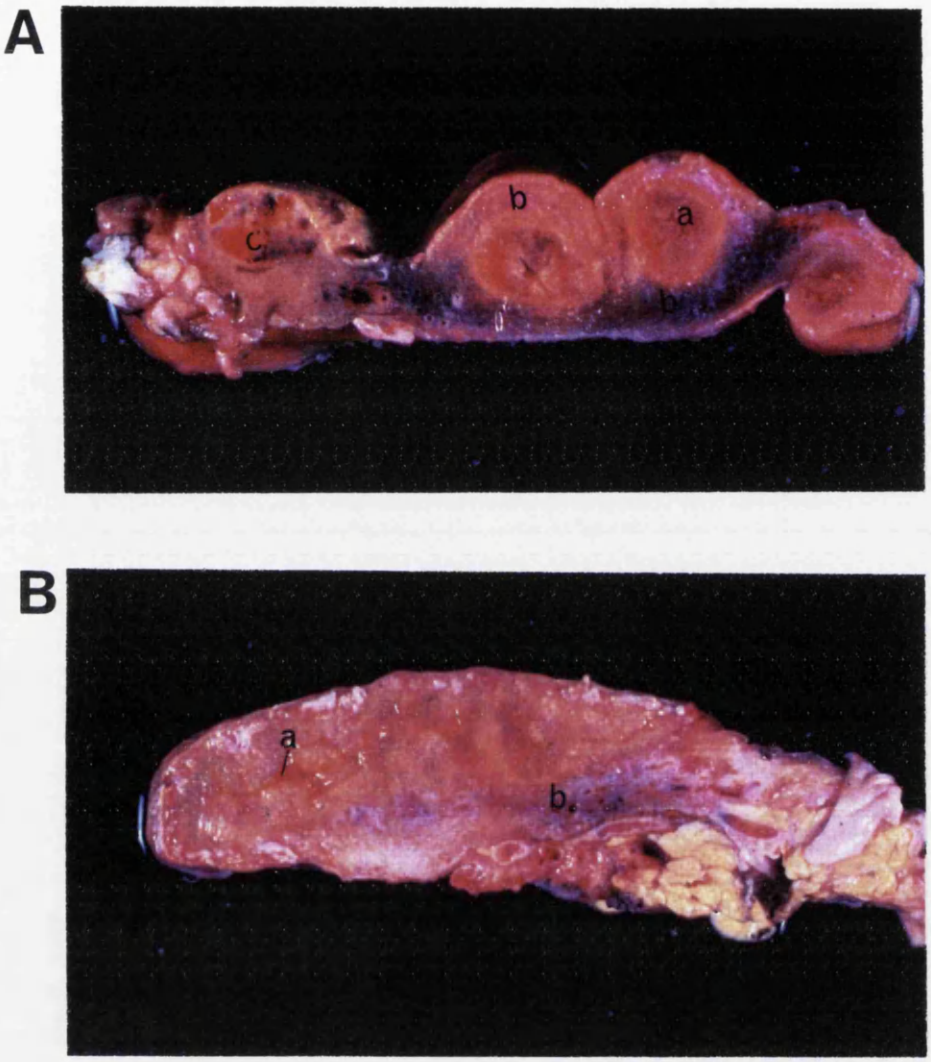

A
b
a
c
B
a
b
C
GUVS
D

Figure 3.4. Comparison of uterine specimens scanned in the water-bath and examined after sectioning with a band-saw (uterine horns-mild endometritis).

A. Transverse section of uterine horns. Note disparity in horn size and abnormal fluid in uterine lumen.

B-C. Ultrasonographic images representative of mild endometritis. Note hyperechoic fluid in the uterine lumen (B, arrow) and loss of tissue detail in (C).

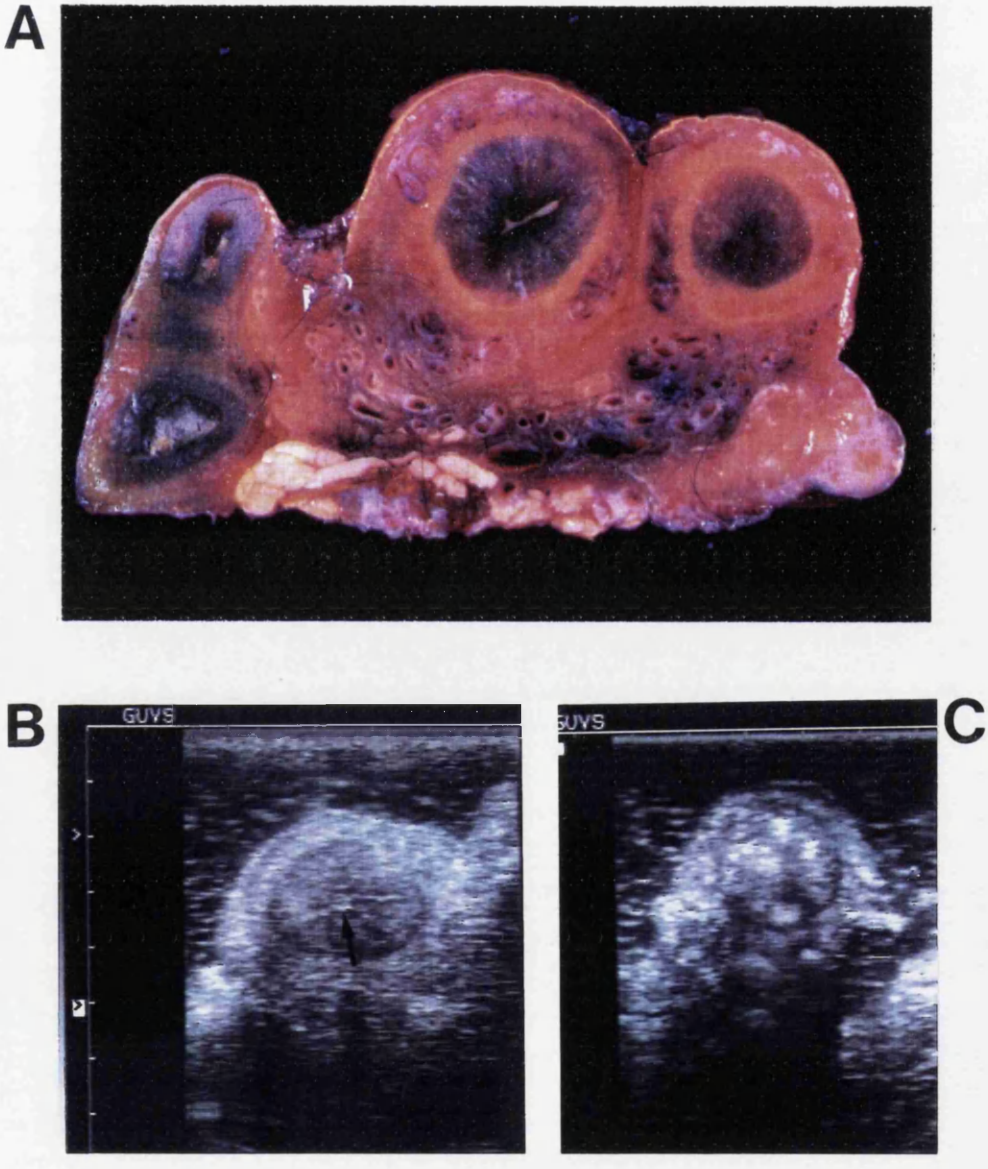

A
B
GUYS
GUYS
C

Figure 3.5. Comparison of uterine specimens scanned in the water-bath and examined after sectioning with a band-saw (uterine horns-severe endometritis).

A. Transverse section of uterine horns. Note disparity in horn size, large amount of abnormal fluid in uterine lumen and caruncles.

B-E. Ultrasonographic pictures representative of severe endometritis. Note hyperechoic fluid in uterine lumen (B, arrow), partially echogenic fluid in (C) and (D, arrows) and complete loss of tissue detail in (E).

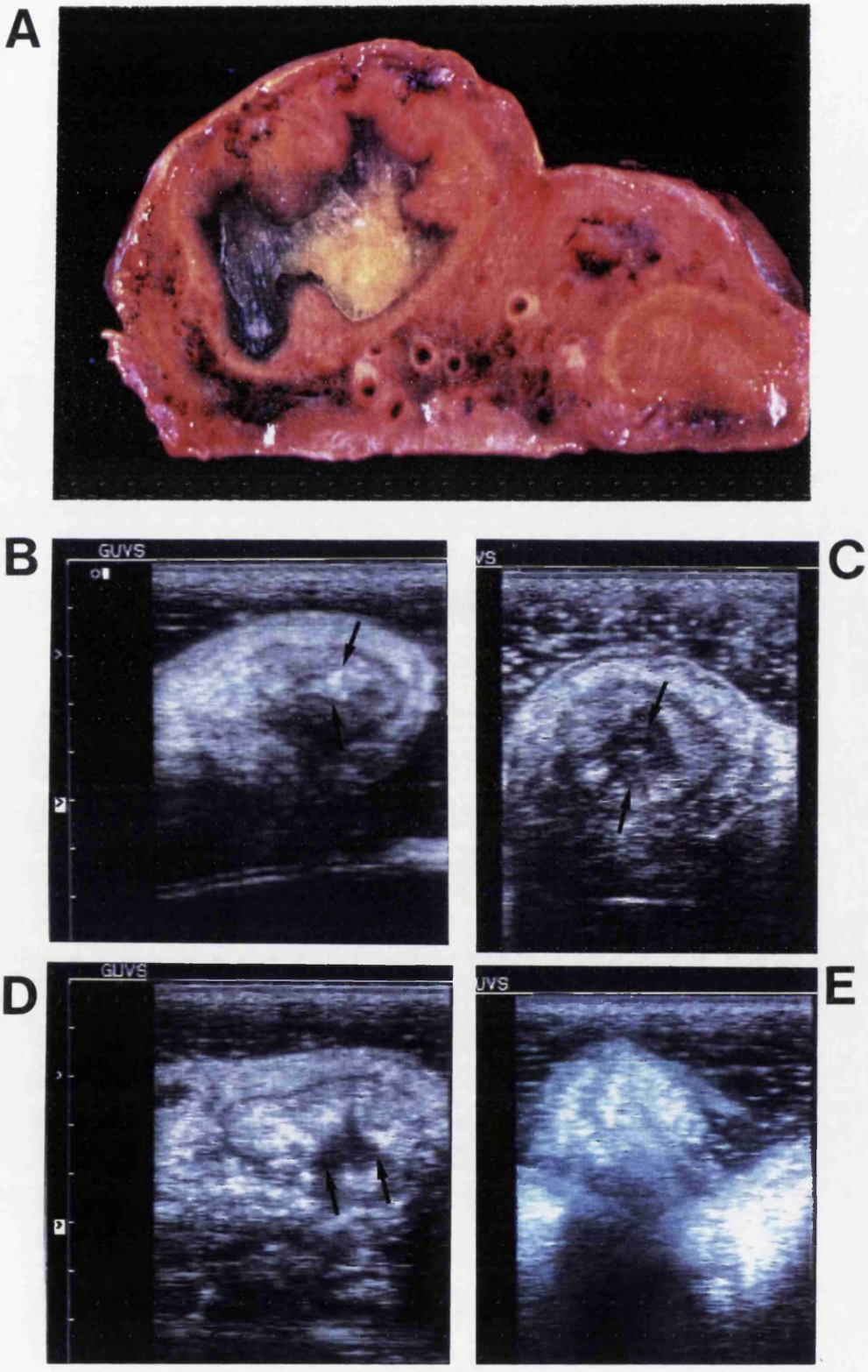

A
B
GUVS
C
D
GUVS
E
GUVS

Figure 3.6. Ultrasonographic images obtained on examination of live animals using the scan planes shown in Figure 2.3.

A. Ultrasonic image of a transverse section of mid-cervix. Note that cervical folds are visible.

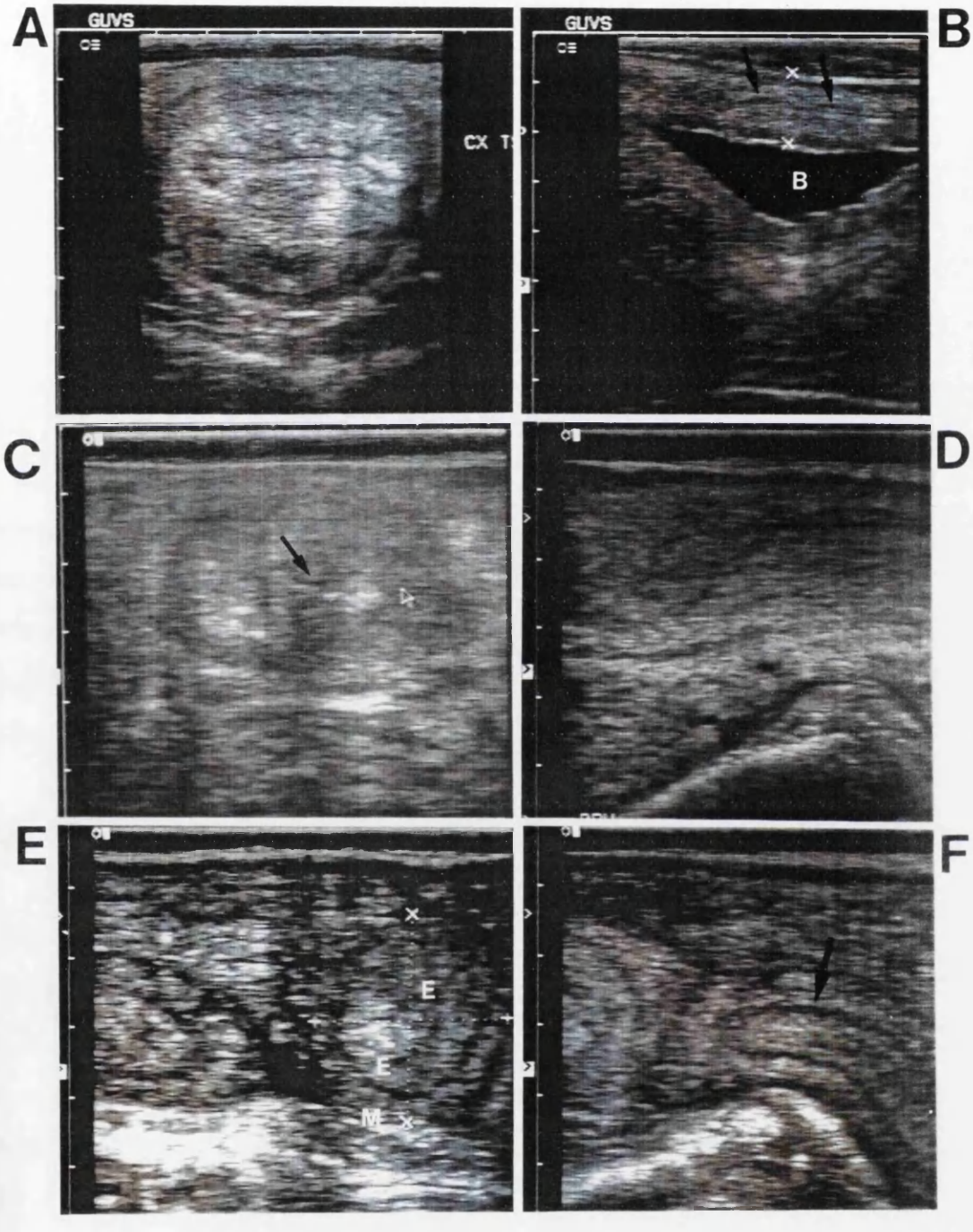
B. Ultrasonic image of a longitudinal section of the cervix. The cervical canal is barely visible (arrows). The dorsal and ventral borders of the cervix are marked (white x). The cervix overlies bladder (B).

C. Ultrasonic image of a transverse section of the uterine body at mid-body. The lumen is visible as an anechoic area (black arrow). The uterine layers are indistinct in comparison to a transverse section of uterine horns.

D. Ultrasonic image of a longitudinal section of the uterine body. The uterine lumen is represented by a hypoechoic line.

E. Ultrasonic image of a transverse section of the uterine horns at the intercornual ligament. Note the encircling myometrium (M) demarcated from the endometrium (E) by a hypoechoic ring. The endometrium surrounds a somewhat anechoic lumen.

F. Ultrasonic image of a longitudinal section of the uterine horns. Note demarcation between endometrium and myometrium (arrow).


A
GUVS
CX T
B
GUVS
B
C
D
E
E
F

Section 3.2

Ultrasonic Examination of Bovine Uteri in a Water-bath

3.2.1. Introduction

In order to establish a database of measurements of uterine cervix and horns a preliminary study was carried out using uteri collected from the abattoir and measured in a water-bath. Specimens were collected from 25 heifers, 25 parous, cull cows and 50 parous cows with endometritis. The horizontal and vertical diameter of the cervix, right and left horns were recorded for each specimen (mm). Each measurement was made at mid-cervix and at the level of the intercornual ligaments. In addition, endometrial area (mm^2) and endometrial thickness were recorded for all parous cows.

3.2.2. Measurement of uteri recovered from heifers

Measurements of the cervix and uterine horns of 25 heifer uteri are presented in Table 3.1. The mean horizontal diameter of the cervix of these heifer specimens was 46.8 mm (range 36.3-59.4 mm) and the mean vertical diameter was 36.5 mm (range 28.3-46.2 mm). The mean horizontal and vertical diameters of the right horn measured 27.1 mm (range 20.0-46.2 mm) and 25.7 mm (range 19.3-30.8 mm), respectively. The mean horizontal and vertical diameters of the left horn were 26.1 mm (range 22.3-30.9 mm) and 25.0 mm (range 21.6-30.2 mm), respectively. There was no significant difference between the dimensions of the two horns ($p > 0.05$). The area and the thickness of the endometrium were not measured in heifer uterine specimens.

Table 3.1: Horizontal and vertical diameters of the cervix and uterine horns of heifer uteri imaged ultrasonically in a water-bath (mm).

Sample	CERVIX (mm)		RIGHT HORN (mm)		LEFT HORN (mm)	
	Horizontal	Vertical	Horizontal	Vertical	Horizontal	Vertical
1	57.4	34.5	31.7	28.7	27.0	29.0
2	41.6	30.9	31.9	29.1	28.1	26.5
3	41.1	32.1	24.6	22.3	25.0	26.6
4	59.4	40.8	26.0	24.5	28.6	22.5
5	56.1	45.5	28.5	21.2	22.3	22.3
6	46.4	36.0	23.7	26.2	25.2	25.7
7	36.3	28.3	26.2	29.1	27.9	26.4
8	50.7	46.1	29.8	30.9	39.0	25.3
9	44.7	34.8	29.7	25.8	28.1	30.2
10	55.8	40.0	26.0	20.8	24.4	23.1
11	54.5	40.7	30.2	30.1	25.1	24.8
12	59.4	46.2	28.6	22.5	28.4	24.4
13	54.5	40.5	29.0	25.6	31.2	26.7
14	43.7	32.9	31.3	23.5	30.4	25.0
15	37.1	33.7	23.5	25.0	24.4	24.8
16	39.3	34.8	30.4	28.8	31.5	24.2
17	42.2	34.2	25.2	26.2	26.1	24.7
18	47.6	39	32.6	29.8	27.5	24.3
19	44.9	34.2	25.0	26.8	22.9	26.3
20	41.8	34.4	20.0	22.0	25.2	21.8
21	44.2	37.8	25.2	25.4	23.1	24.0
22	46.6	35.9	27.5	25.2	24.4	26.7
23	39.0	33.0	21.2	19.3	23.3	21.6
24	40.2	30.9	22.9	24.7	24.8	22.9
25	45.3	35.7	26.8	29.8	26.2	24.4
Mean	46.8	36.5	27.1	25.7	26.1	25.0
Range	36.3-59.4	28.3-46.2	20.0-32.6	19.3-30.9	22.3-39.0	21.6-30.2

3.2.3. Measurement of uteri recovered from parous cows

Measurements made of the cervix and uterine horns of 25 uteri from parous cows are presented in Table 3.2a. The mean horizontal diameter of the cervix was 48.7 mm (range 39.3-57.3 mm) and the vertical diameter was 44.5 mm (35.7-56.5 mm). These dimensions were generally greater than for heifer specimens.

The mean horizontal and vertical diameters of the right uterine horn were 33.7 mm (range 27.7-37.9 mm) and 32.3 mm (range 26.3-36.7 mm), respectively. The mean horizontal and vertical diameters of the left horn were 31.9 mm (range 25.4-36.4 mm) and 31.3 mm (range 26.3-35.6 mm), respectively. The mean horizontal diameter of the right horn was significantly greater than the left horn mean horizontal diameter ($p < 0.05$).

Measurements of the endometrial area and thickness are presented in Table 3.2.b. The mean endometrial area was 357 mm^2 (range 210-609 mm^2) and 313 mm^2 (range 169-531 mm^2) for the right and left horns, respectively. The mean endometrial thickness was 9 mm (range 07-10 mm) and 8 mm (range 06-12 mm) for the right and left horns, respectively. There was no significant difference in mean area or mean endometrial thickness between the right and left uterine horn ($p > 0.05$).

3.2.4. Measurement of uteri recovered from cows with endometritis

Measurements of the cervix and uterine horns of 50 uteri classified as having either mild or severe endometritis are presented in Tables 3.3.a,b and 3.4.a,b. It was assumed that the larger of the two horns was the previously gravid horn and the data was collated as 'previously gravid uterine horn' (PGUH) and 'previously non-gravid uterine horn' (PNGUH).

Table 3.2a: Horizontal and vertical diameters of the cervix and uterine horns of uteri from parous cows imaged ultrasonically in a water-bath (mm).

Sample	CERVIX (mm)		RIGHT HORN (mm)		LEFT HORN (mm)	
	Horizontal	Vertical	Horizontal	Vertical	Horizontal	Vertical
1	51.5	49.5	35.0	36.5	30.9	28.1
2	39.3	37.4	33.8	29.4	28.3	26.6
3	45.8	38.4	35.1	36.7	28.2	27.5
4	40.9	52.7	31.9	34.4	30.5	29.8
5	45.8	36.1	34.4	31.9	34.4	33.2
6	52.8	43.4	34.4	30.9	34.4	29.2
7	50.4	43.4	32.1	28.6	32.1	32.0
8	48.2	40.3	27.7	26.3	29.8	30.2
9	55.0	44.5	32.1	31.7	29.8	26.3
10	45.9	44.5	36.0	36.2	34.4	32.7
11	57.3	56.5	32.1	31.7	34.4	35.1
12	45.9	46.4	34.4	36.3	32.1	32.5
13	55.0	51.0	32.1	29.4	32.2	34.7
14	46.3	35.7	34.1	29.3	25.4	34.1
15	49.0	44.9	38.4	31.4	32.0	30.7
16	50.0	45.1	32.1	29.0	31.0	30.8
17	47.8	37.6	34.1	29.8	35.5	28.4
18	47.0	47.2	35.3	33.9	35.0	33.4
19	43.2	41.5	34.2	32.6	33.2	30.7
20	50.4	44.9	33.5	31.8	36.4	34.9
21	49.9	47.6	37.9	34.8	31.0	30.8
22	51.2	46.6	31.3	31.9	29.6	29.4
23	50.1	43.8	37.2	33.4	33.8	32.9
24	53.2	48.6	33.2	35.5	34.4	35.6
25	46.2	45.7	32.7	35.1	28.3	33.5
Mean	48.7	44.5	33.7	32.3	31.9	31.3
Range	39.3-57.3	35.7-56.5	27.7-37.9	26.3-36.7	25.4-36.4	26.3-35.6

Table 3.2b. Endometrial area (mm^2) and thickness (mm) of the uterine horns specimens from parous cows imaged ultrasonographically in a water-bath.

Sample Number	Endometrial Area (mm^2) Right horn	Left horn	Endometrial Thickness (cm) Right horn	Left horn
1	333	264	08	08
2	375	222	10	07
3	317	270	08	06
4	267	169	07	06
5	382	451	10	10
6	249	200	08	08
7	230	276	08	06
8	210	237	08	08
9	225	187	08	07
10	609	206	10	08
11	283	440	10	11
12	440	402	10	12
13	452	480	10	08
14	242	231	08	08
15	410	224	10	08
16	257	304	08	09
17	346	422	10	10
18	323	389	10	10
19	440	304	10	10
20	431	531	08	08
21	486	270	09	10
22	393	332	08	10
23	373	322	10	08
24	353	351	08	07
25	497	330	09	08
Mean (SEM)	357 (20)	313 (20)	09 (0.2)	08 (0.3)
Range	210-609	169-531	07-10	06-12

SEM = Standard error of the mean

For 80% of the specimens with mild endometritis the dimensions of the cervix could not be measured accurately as the dimensions were greater than the transducer crystal face. These specimens were allocated a measurement of >60 mm (Table 3.3.a). For all the uteri with severe endometritis both the horizontal and vertical diameters of the cervix exceded 60 mm (Table 3.4.a).

In cases of mild endometritis the PGUH measured 41.2 mm (range 29.8-49.8 mm) and 39.5 (range 31.5-48.8 mm) in the horizontal and vertical diameter, respectively. A mean endometrial area of 610 mm^2 (range 310-972 mm^2) and thickness of 11 mm (range 09-13 mm) was recorded (Table 3.3.b). The mean horizontal and vertical diameters of the PNGUH were 34.4 mm (range 20.8-43.4 mm) and 32.6 mm (range 25.4-40.5 mm) respectively. A mean endometrial area of 425 mm^2 (range 175-678 mm^2) and thickness 10 mm (range 07-12 mm) was recorded.

Measurements for specimens showing severe endometritis were generally greater than those with mild endometritis. With severe endometritis the mean diameters of the PGUH measured measured 49.1 mm (range 34.4-60.0 mm) and 49.0 mm (range 37.0-63.6 mm) in the horizontal and vertical diameter, respectively (Table 3.4.a). A mean endometrial area of 997 mm^2 (range 547-1807 mm^2) and thickness of 13 mm (range 08-16 mm) was recorded (Table 3.4.b). The PNGUH measured 41.4 mm (range 30.6-49.4 mm) and 39.6 mm (range 26.9-50.3 mm) in the horizontal and vertical planes, respectively (Table 3.4.a). A mean endometrial area of 704 mm^2 (range 227-1235 mm^2) and thickness of 11 mm (range 08-16) was recorded (Table 3.4.b). The mean diameters of the uterine horns, endometrial area and thickness were all greater in cases of mild or severe endometritis than in normal parous cow specimens. These differences were statistically significant ($p<0.05$).

Table 3.3a: Horizontal and vertical diameters of the cervix and uterine horns of uteri from parous cows with mild endometritis imaged ultrasonically in a water-bath (mm).

Sample	CERVIX (mm)		PGUH (mm)		PNGUH (mm)	
	Horizontal	Vertical	Horizontal	Vertical	Horizontal	Vertical
1	44.1	42.6	49.8	44.7	42.1	40.5
2	48.1	50.9	48.5	48.8	20.8	25.4
3	*	*	47.0	45.4	31.9	31.7
4	*	47.4	40.7	37.6	43.4	34.1
5	*	46	38.0	38.6	34.8	29.4
6	*	36.7	42.4	40.1	34.4	30.6
7	45.9	47.0	31.7	31.5	29.8	26.2
8	*	*	43.8	36.5	32.1	34.0
9	57.8	49.9	44.0	33.4	43.0	30.7
10	*	49.1	29.8	33.3	30.5	26.7
11	*	*	40.03	41.3	33.0	29.2
12	*	*	45.9	42.8	35.0	36.0
13	*	*	43.6	46.4	36.7	30.5
14	*	*	39.9	36.9	34.9	36.7
15	*	51.6	39.0	41.8	39.2	34.4
16	*	52.4	40.7	40.7	31.3	33.1
17	*	47.3	33.3	32.0	27.4	33.6
18	52.8	51.2	39.1	33.6	31.9	31.1
19	*	43.7	42.7	36.4	33.6	32.1
20	*	48.3	40.2	40.9	37.7	36.0
21	*	*	42.6	40.0	38.2	36.7
22	*	48.2	39.9	40.5	33.3	35.0
23	*	*	43.2	42.1	35.1	36.8
24	*	*	43.0	38.6	34.4	33.9
25	*	*	41.2	43.7	34.9	30.6
Mean	-	-	41.2	39.5	34.4	32.6
Range	44.1->60	36.7->60	29.8-49.8	31.5-48.8	20.8-43.4	25.4-40.5

*Greater than 60mm.
PGUH- Previously gravid uterine horn.
PNGUH- Previously non gravid uterine horn.

Table 3.3b. Endometrial area (mm^2) and thickness (mm) of the uterine horns of specimens from parous cows with mild endometritis imaged ultrasonographically in a water-bath.

Sample	Endometrial Area (mm^2)		Endometrial Thickness (mm)	
	PGUH	PNGUH	PGUH	PNGUH
1	619	480	10	11
2	972	175	12	08
3	735	427	10	09
4	310	335	09	10
5	456	335	10	07
6	690	400	10	07
7	373	330	10	10
8	480	380	10	10
9	421	411	11	11
10	511	349	12	11
11	786	477	09	10
12	747	566	12	10
13	614	448	12	10
14	578	491	12	12
15	543	678	09	12
16	707	511	12	12
17	449	391	10	08
18	650	479	11	10
19	496	423	11	10
20	715	389	12	09
21	700	270	13	10
22	668	474	11	10
23	726	573	12	12
24	578	403	11	10
25	703	441	12	11
Mean	610	425	11	10
Range	310-972	175-678	09-13	07-12

PGUH- Previously gravid uterine horn.
PNGUH- Previously non gravid uterine horn.

Table 3.4a. Horizontal and vertical diameters of the cervix and uterine horns of uteri from parous cows with severe endometritis imaged ultrasonically in a water-bath (mm).

Sample	CERVIX (mm)		PGUH (mm)		PNGUH (mm)	
	Horizontal	Vertical	Horizontal	Vertical	Horizontal	Vertical
1	*	*	57.5	51.0	44.9	45.2
2	*	*	52.3	53.1	41.2	40.9
3	*	*	55.4	49.2	49.4	39.2
4	*	*	60.0	63.6	37.7	38.0
5	*	*	51.4	43.4	36.3	35.0
6	*	*	34.4	37.0	30.6	26.9
7	*	*	41.6	43.5	45.9	35.7
8	*	*	54.9	56.0	48.2	40.1
9	*	*	44.7	42.2	47.0	39.2
10	*	*	43.7	44.0	32.5	37.4
11	*	*	49.4	45.1	39.6	40.2
12	*	*	48.5	49.0	45.1	42.0
13	*	*	52.2	52.0	40.5	41.7
14	*	*	57.1	54.6	49.1	47.2
15	*	*	48.1	49.6	39.2	34.4
16	*	*	40.1	42.3	36.1	38.0
17	*	*	56.8	49.8	48.7	50.3
18	*	*	45.6	48.2	33.0	35.1
19	*	*	51.6	55.5	43.0	44.2
20	*	*	45.1	47.2	37.1	37.0
21	*	*	48.8	49.6	39.0	41.2
22	*	*	52.6	52.0	46.1	43.0
23	*	*	42.1	46.3	38.2	36.6
24	*	*	41.6	47.2	40.0	38.2
25	*	*	52.1	53.8	46.6	42.1
Mean	>60	>60	49.1	49.0	41.4	39.6
Range	-	-	34.4-60	37.0-63.6	30.6-49.4	26.9-50.3

*Greater than 60mm.
PGUH- Previously gravid uterine horn.
PNGUH- Previously non gravid uterine horn.

Table 3.4b. Endometrial area (mm^2) and thickness (mm) of the uterine horns of specimens from parous cows with severe endometritis imaged ultrasonographically in a water-bath.

	Endometrial Area (mm^2)		Endometrial Thickness (mm)	
Sample	Right horn	Left horn	Right horn	Left horn
1	1081	1040	14	16
2	1678	1235	14	14
3	1576	227	15	12
4	1807	453	20	14
5	547	402	11	09
6	611	423	09	08
7	1018	920	13	11
8	1519	633	20	11
9	634	662	12	12
10	712	482	14	10
11	818	621	12	10
12	966	727	12	12
13	1000	816	13	12
14	1103	902	13	13
15	867	729	12	12
16	912	818	12	10
17	1062	762	14	12
18	807	720	12	10
19	906	733	12	10
20	802	618	12	10
21	933	762	13	10
22	911	716	12	12
23	781	633	12	10
24	906	816	11	14
25	961	749	14	10
Mean	997	704	13	11
Range	547-1807	227-1235	09-20	08-16

Section 3.3

Serial Ultrasonography of the Reproductive Tract of Postpartum Dairy Cows

3.3.1. Introduction

Reproductive fertility records for the 27 animals examined during this study are summarised in Table 3.5. Of the 27 animals included, 6 animals were classified as abnormal. These animals suffered an abnormal periparturient reproductive event as follows; two animals retained their foetal membranes and one subsequently developed endometritis. One cow gave birth to a dead calf and later developed endometritis. One cow gave birth to twin calves. One cow required a caesarean operation following dystocia. One cow had metritis. The abnormalities are detailed in Table 3.7. Of the 21 animals in the normal group, a management decision was made to cull two of the cows before the end of the study because of poor udder conformation and mastitis. Fertility details for these animals are not included in Table 3.5.

3.3.2. Monitoring of normal cows

Cows were examined 5 times as follows, with days postpartum in brackets ; Examination 1 (2-9 days postpartum), Examination 2 (12-17 days post partum), Examination 3 (23-29 days postpartum), Examination 4 (43-49 days postpartum) and Examination 5 (54-61 days postpartum).

All ultrasound scans were preceded by manual rectal palpation. A summary of manual examinations is presented in Table 3.6. It was noted that the uterus in all cows was intra-abdominal during Examination 1. Two cows were examined at 2 days postpartum (Cows 84 and 97), at which time the uterus was very flaccid and the borders of the uterus could not be defined by rectal palpation. These animals were examined again at day 7 and at day 9 postpartum, respectively. The remainder of the cows were examined from day 4 postpartum when the uterus could be defined by rectal palpation. The cervix was always palpable.

The uterus gradually returned to the intra-pelvic position over successive examinations. By the fourth examination the uterus was intra-pelvic in all animals. It was possible to palpate ovaries during the first examination. The right ovary was palpable in 47% of the cows while the left ovary was palpable in 58% of the animals. By the third examination both ovaries were palpable in all animals. Resumption of cyclicity was not observed until the second examination at which time 21% (4 animals) were cycling. By the third examination 95% (18 animals) of the animals were cycling. One animal (Cow 33) remained acyclic until examination five.

There was an inverse relationship between the horizontal diameter of the cervix and time postpartum (Figure 3.10A). During Examination 1 the mean horizontal diameter of the cervix was greater than 60 mm (Figures 3.10A, 3.11). Ultrasonographically, a fluid filled lumen could be imaged. This feature persisted for up to 9 days postpartum (Figure 3.7.A, C) but was not observed in normal cows beyond this examination. The

mean diameter of the cervix was still greater than 60 mm during the second examination in the majority of the animals. During subsequent examinations the mean diameter decreased, reducing to a mean value of 44.7 mm (range 34.2-49.9 mm) by Examination 5 (Figures 3.10A, 3.11).

For most cows the diameter of both the previously gravid and previously non-gravid horns exceeded 60 mm during Examination 1 (Figures 3.10B and 3.10C). Ultrasonographically, the uterine lumen could be visualised filled with variable volumes of fluid. The luminal fluid was typically anechoic during the first 5-7 days postpartum, becoming partially echogenic thereafter (Figure 3.7.B,E). By Examination 2 the uterine lumen had reduced in size and typically contained a small volume of highly echogenic material (Figure 3.7.F). Caruncles were evident during Examination 1 (Figure 3.7.B). Typically, the caruncles reduced in size until, by the second examination, they were not visible. The horizontal diameter of the previously gravid horn reduced markedly during the first four examinations (Figures 3.10B). This reduction was highly significant ($p<0.01$). The reduction in horn diameter between Examination 4 and Examination 5 was not statistically significant. The mean diameter of the previously gravid uterine horn was 45.1 mm (range 28.0 - 60 mm), 35.1 mm (range 22.3-37.1 mm), 31.5 mm (range 22.3-37.1 mm) and 29.7 mm (range 20.2-37 mm) by Examinations 2, 3, 4 and 5, respectively. Reduction in the horizontal diameter of the previously non-gravid uterine horn was highly significant ($p<0.01$) between Examinations 2 and 3 (Figure 3.10C). Thereafter, the reduction in size was not significant. The horizontal diameter of this horn was 41.6 mm (range 28.1-60 mm), 32.7 mm (range 24.6-50 mm), 30.1 mm (range 19.8-38 mm) and 28 m (range 18.6-34.6 mm) by Examinations 2, 3, 4 and 5, respectively. Disparity between the previously gravid and previously non gravid horns was statistically significant ($p<0.05$) at Examination 2 (figure 3.11) but not thereafter. The diameter of the previously gravid uterine horn (mean diameter of 29.7 mm) remained slightly larger than the

previously non-gravid uterine horn (mean diameter of 28.0 mm), however, this difference was not significant.

Examples of ultrasonic imaging during Examination 3 are shown in Fgure 3.8.A-D. This period was characterised by resumption of cyclicity. Fluid visualised in the uterine lumen was anechoic, a characteristic of the pro-oestrus/oestrus period. Endometrial folds were marked in oestrus animals. These features were accompanied by the presence of a large pre-ovulatory follicle on one ovary (Fgure 3.8.B). No luminal fluid was detectable during the luteal phase of the cycle (Fgure 3.8.A). Further reduction in the diameter of both uterine horns was noted until the fourth examination when the two horns could, typically, be visualised in the same field Figure 3.9.B and D. Little change in the diameters of the horns or other features was detected ultrasonographically thereafter.

Changes in the endometrial area is represented graphically in Figure 3.12. It was not possible to measure the area of the endometrium during Examination 1 due to the large size of the uterine lumen. The endometrial area of the previously gravid horn reduced with each successive postpartum examination. During Examination 2 the mean endometrial area was 623 mm^2 (range 200-950 mm^2) and by Examination 5 it was 259 mm^2 (range 210-870 mm^2). The changes in endometrial area between successive examinations was not statistically significant. Reduction in endometrial area of the previously non gravid horn was similar to that of the previously gravid uterine horn. The mean endometrial area of this horn was 539 mm^2 (range 210-1120 mm^2) during Examination 2 and 287 mm^2 (range 120-480 mm^2) by Examination 5. The reduction in the endometrial area of this horn between Examinations 2 and 3 was statistically significant. At each successive examination beyond Examination 2 the area of the endometrium was greater in the previously gravid horn, though this difference was not statistically significant.

Changes in the endometrial thickness of the uterine horns with successive examinations postpartum are shown on Figure 3.13. The

thickness of the endometrium decreased during successive examinations. The endometrium in the previously gravid uterine horn measured, on average, 11 mm (range 8-16 mm) during Examination 2. By Examination 5 the endometrial thickness had reduced to 8 mm (range 6-10 mm). No statistical difference was noted between two successive examinations except between examination 4 and 5. The endometrium of the previously non gravid horn was thinner than that of the previously gravid horn. This difference was statistically significant at all examinations ($p < 0.05$). The endometrium measured 9 mm (range 6-14 mm) during Examination 2 and was 7 mm (range 6-10 mm) by Examination 5. The reduction in endometrial thickness of the previously non gravid horn was not statistically significant between successive examinations.

Examination of the ovaries was not the principal aim of this study. However, certain observations relating to postpartum resumption of cyclicity were noted and are shown on Figure 3.14.A-D. An anovulatory follicle was observed on the ovary of cow 14 at 30 days postpartum. This structure had the ultrasound appearance of a small luteal cyst. The structure was 2.5 cm with a rim of luteal tissue and a small fluid-filled lacuna crossed by trabeculae. Follicular cysts were also encountered during the study. A follicular cyst was detected on the ovary of cow 74 35 days post-partum and appeared in the ultrasound image as an anechoic, fluid filled structure greater in size than an ovulatory follicle. The example shown measured 38.6 mm by 36.1 mm in diameter.

Table 3.5. Reproductive fertility parameters for animals in the study (arrow shows where cows with abnormalities begin).

Cow number	Lactation number	Calving date	Calving to first heat	Calving to second heat	Calving to first service	Calving to conception	Services per conception	Remarks
03	3	8.1.97	14	28	50	68	2	pregnant
11	2	10.12.96	68		68	108	2	pregnant
27	1	27.10.96	27	53	27	91	3	pregnant
31	3	5.2.97	14	34	82	-	-	culled
33	1	4.12.96	62	132	62	132	2	pregnant
40	3	30.1.97	19	77	77	112	2	pregnant
41	4	17.1.97	38	49	49	139	3	pregnant
43	1	18.2.97	26	46	73	73	1	pregnant
45	6	2.2.97	24	42	-	-	-	culled
55	4	13.1.97	17	34	62	110	2	pregnant
57	4	11.1.97	16	83	83	128	2	pregnant
73	4	9.2.97	24	28	54	80	2	pregnant
74	3	17.10.96	52	86	52	86	2	pregnant
76	4	2.2.97	54	98	54	98	2	pregnant
84	7	1.1.97	21	50	50	90	2	pregnant
90	2	26.10.96	59	84	59	84	2	pregnant
97	1	18.10.96	36	64	36	106	3	pregnant
98	3	3.2.97	37	54	54	54	1	pregnant
118	7	25.1.97	49	62	62	62	1	pregnant
→ 05	11	18.2.97	17	35	-	-	-	culled
08	2	20.10.96	37	-	-	-	-	culled
20	6	17.9.96	62		62	-	-	culled
37	8	31.1.97	27	32	56	119	2	pregnant
60	3	29.11.96	78	106	78	-	-	culled
96	3	21.2.97	19	101	55	-	3	culled

Table 3.6. Manual rectal examinations of reproductive organs of normal cows prior to transrectal ultrasonic scanning.

	Uterus Position	Number of Animals	Ovarian status	Number of Animals
Examination 1	Intra-abdominal	19/19	Right ovary palpable	9/19
Range 2-9	Intra-pelvic	none	Left ovary palpable	11/19
Days p.p.	All palpable	none	Cycling	none
Examination 2	Intra-abdominal	18/19	Right ovary palpable	15/19
Range 12-17	Intra-pelvic	1/19	Left ovary palpable	17/19
Days p.p.	All palpable	9/19	Cycling	4/19
Examination 3	Intra-abdominal	2/19	Right ovary palpable	19/19
Range 23-29	Intra-pelvic	17/19	Left ovary palpable	19/19
Days p.p.	All palpable	16/19	Cycling	18/19
Examination 4	Intra-abdominal	none	Right ovary palpable	19/19
Range 43-49	Intra-pelvic	19/19	Left ovary palpable	19/19
Days p.p.	All palpable	19/19	Cycling	18/19
Examination 5	Intra-abdominal	none	Right ovary palpable	19/19
Range 54-61	Intra-pelvic	19/19	Left ovary palpable	19/19
Days p.p.	All palpable	19/19	Cycling	19/19

Figure 3.7. Ultrasonographic findings in normal cows during Examinations 1 (2-9 days postpartum) and 2 (12-17 days postpartum).

A. Transverse section of the cervix 2 days postpartum (Cow 97). Note the visible lumen (white arrow). The horizontal diameter of the cervix is > 60 mm.

B. Transverse section through a uterine horn 7 days postpartum (Cow 98). Note presence of caruncles (c) and anaechoic fluid.

C. Transverse section of the cervix 9 days postpartum (Cow 97). Note that the lumen is still visible (white arrow). Note also the heterogenic appearance of the cervix.

D. Transverse section through uterine horn 9 days postpartum (Cow 97). Note that the horizontal diameter of the horn is still > 60 mm. Caruncles are visible (c) and uterine tissue appears oedematous.

E. Transverse section through uterine horn 9 days postpartum (Cow 97). Note that the uterine lumen contains echogenic fluid (f). This appearance may persist into Examination 2.

F. Transverse section of uterine horn at 13 days postpartum (Cow 97). Note hyperechoic fluid in the lumen (arrows).

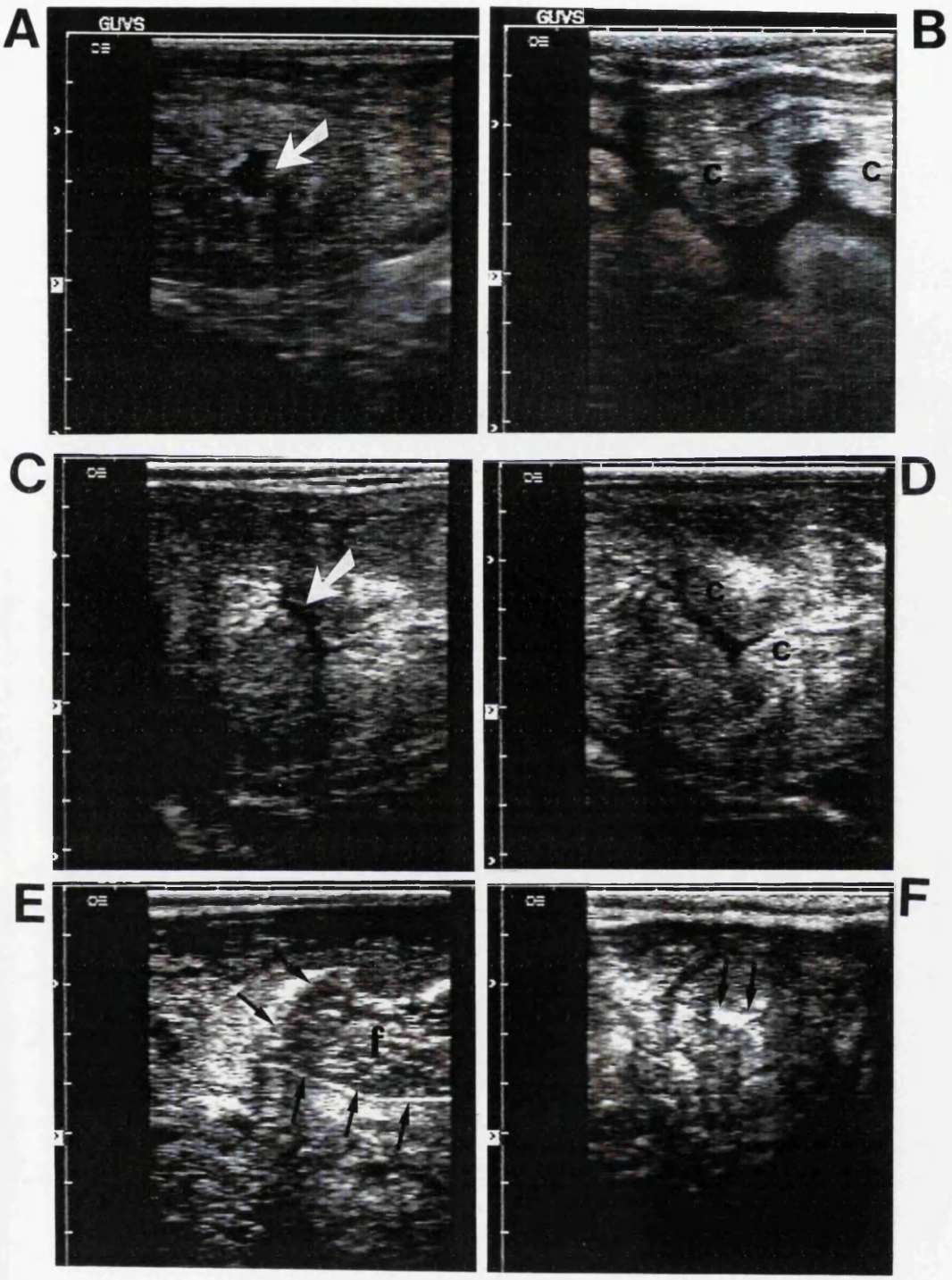

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Figure 3.8. Ultrasonographic findings in normal cows during Examination 3 (23-29 days postpartum).

A. Transverse section of uterine horn 27 days postpartum (Cow 27). Note absence of any visible fluid.

B. A section of an ovary showing a large (1.8 cm) pre-ovulatory follicle (Fo) at 29 days postpartum (Cow 29).

C and D. Transverse section of uterine horn approaching first oestrus in Cow 29, above, at 29 days postpartum. Note oedematous swelling of the endometrium and anoechoic fluid in the uterine lumen (arrows).

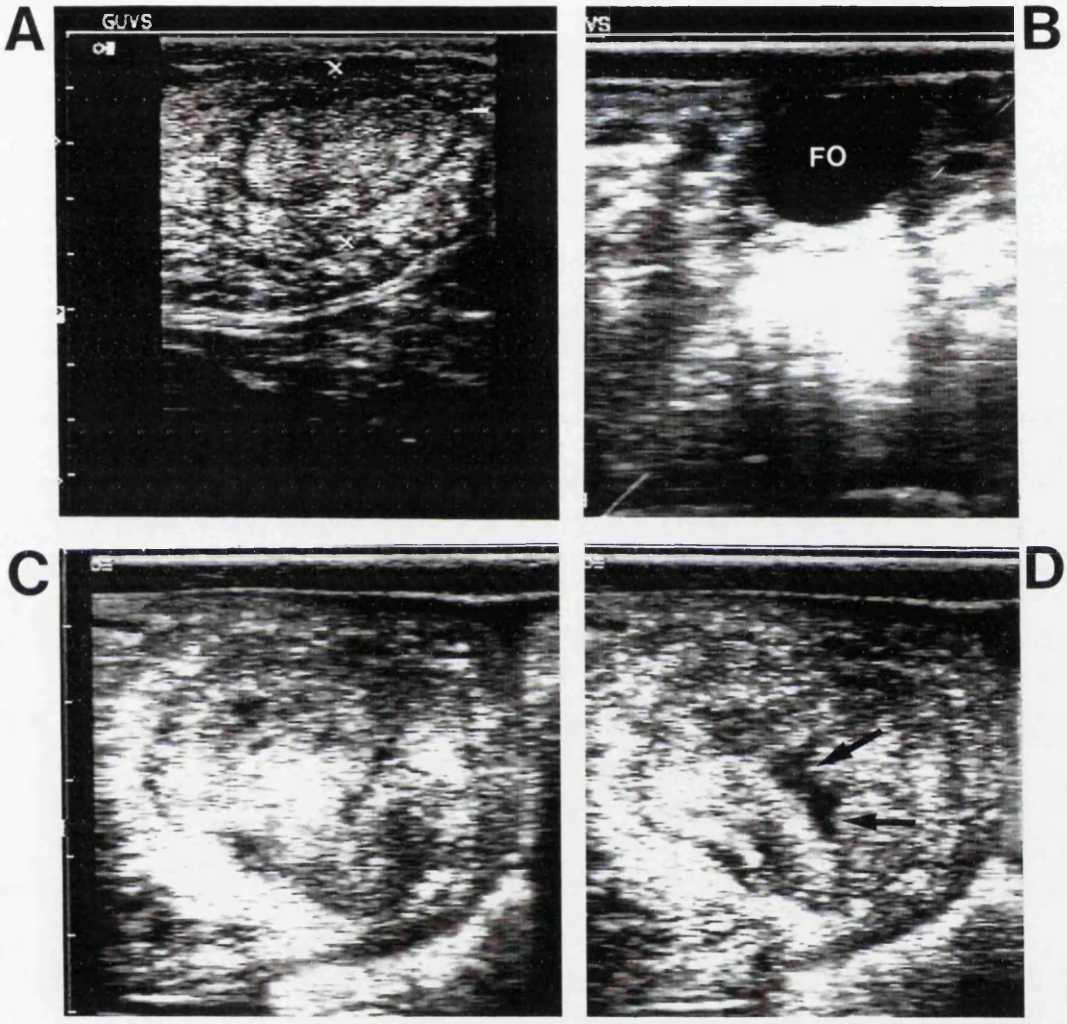
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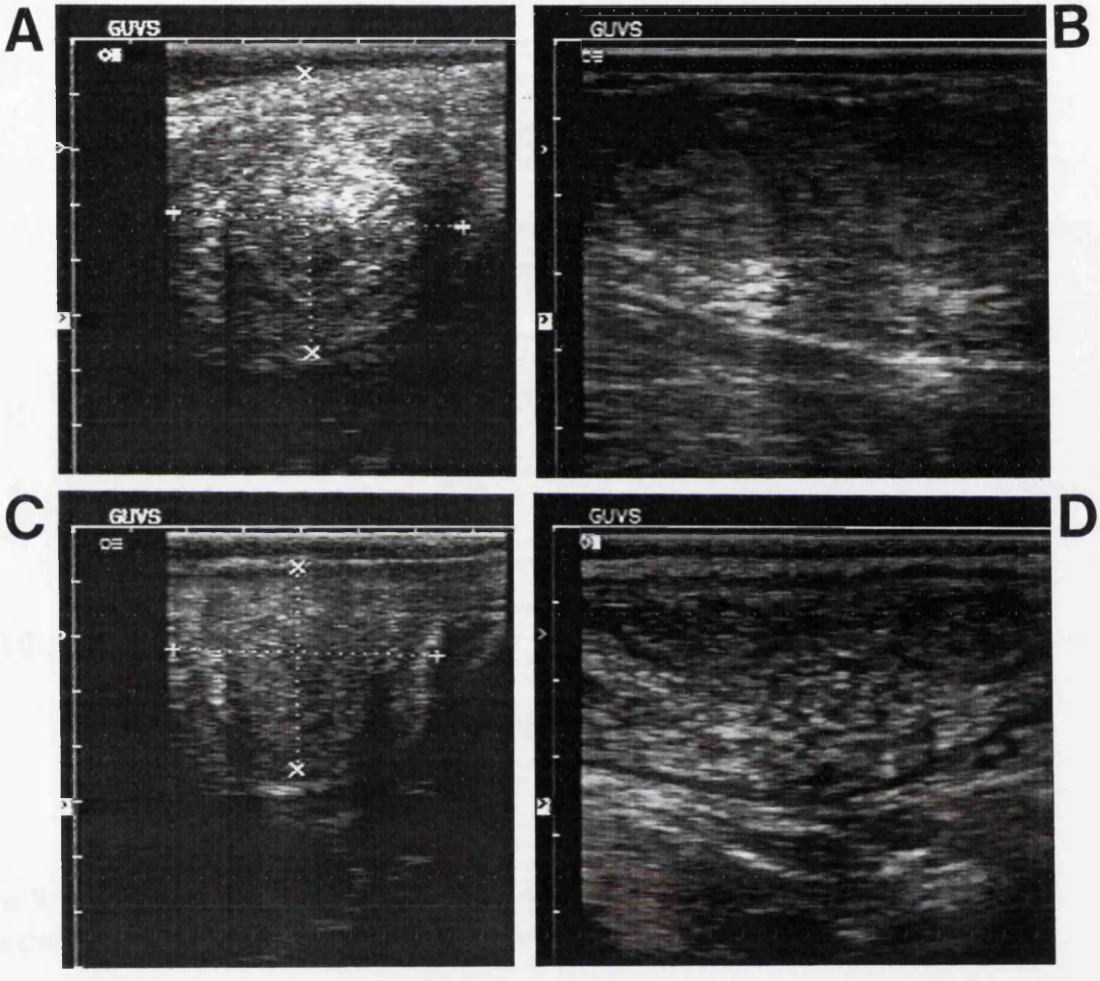
Figure 3.9. Ultrasonographic findings in normal cows during Examinations 4 (43-49 days postpartum) and 5 (54-61 days postpartum).

A. Transverse section of the cervix at 44 days postpartum (Cow 118). The horizontal and vertical diameters of the cervix are 50.3 and 50.2 mm, respectively. Note the lumen of the cervical canal is not visible.

B. Transverse section of uterine horns at 42 days postpartum (Cow 74). Note that the two horns are small enough to appear in one field view.

C. Transverse section of the cervix at 55 days postpartum (Cow 118). The horizontal and vertical diameters of the cervix are now 45.9 and 36.7 mm, respectively.

D. Transverse section of uterine horns at 52 days postpartum (Cow 74). No major change is noted when compared to Examination 4 in B, above.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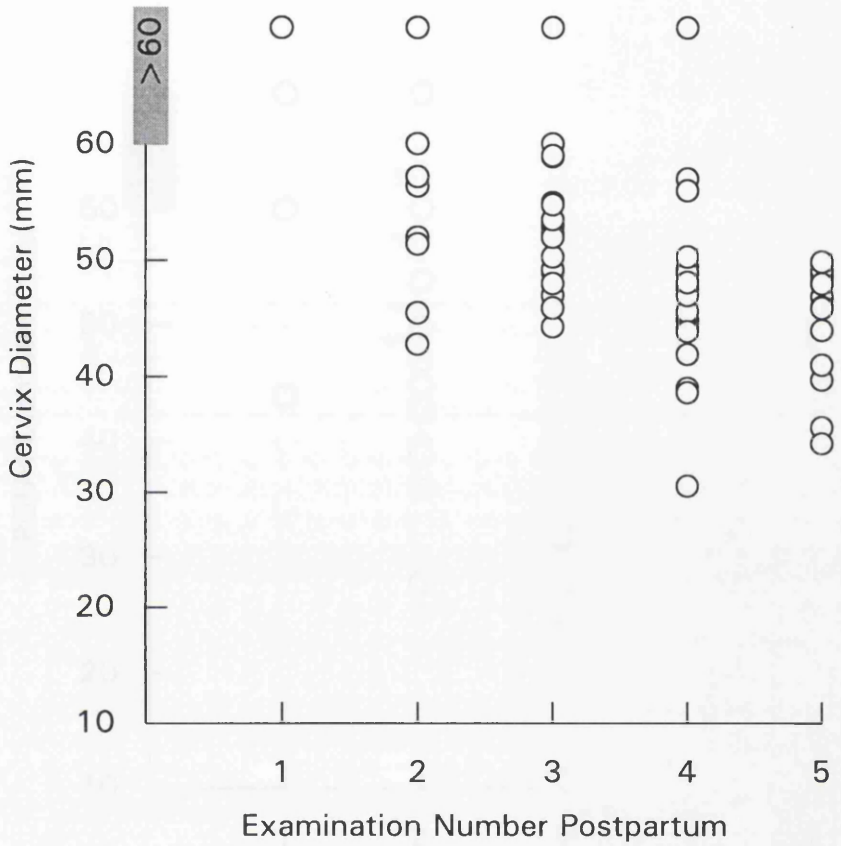


Figure 3.10A. Scatter plot showing changes in horizontal diameter of the cervix during successive examinations in normal cows.

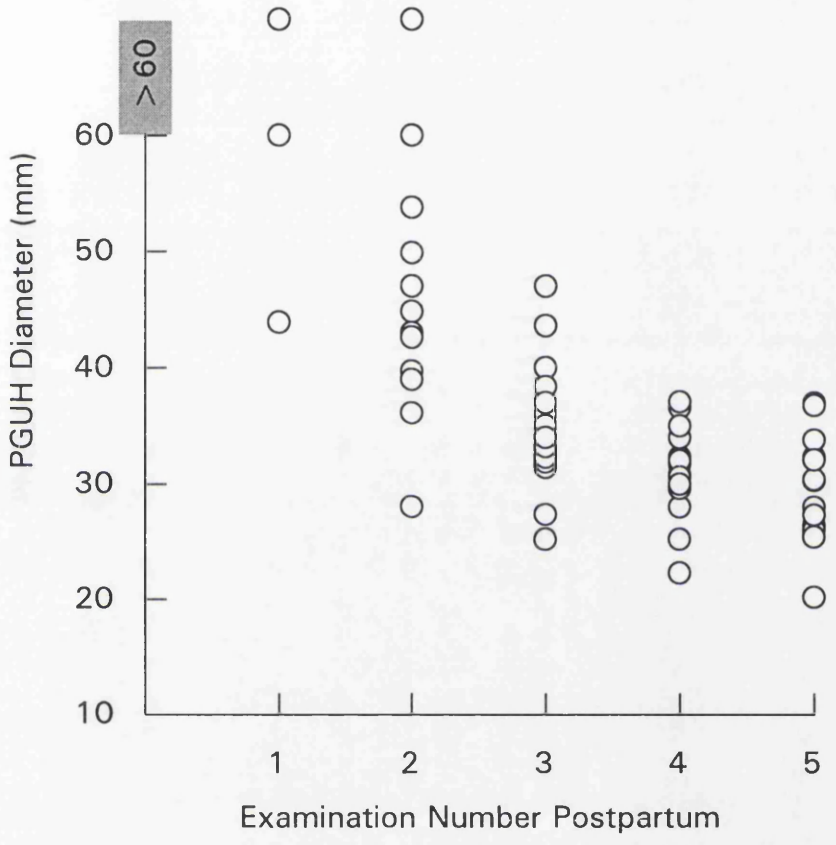


Figure 3.10B. Scatter plot showing changes in uterine horn diameter of the previously gravid uterine horn (PGUH) during successive examinations in normal cows.

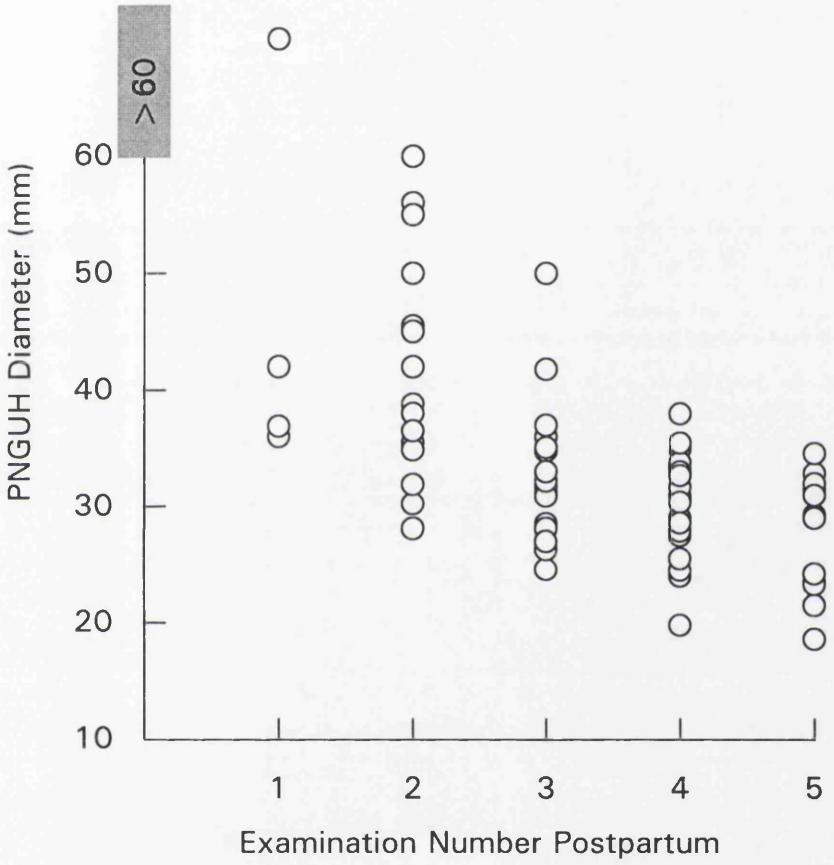


Figure 3.10C. Scatter plot showing changes in uterine horn diameter of the previously non-gravid uterine horn (PNGUH) during successive examinations in normal cows.

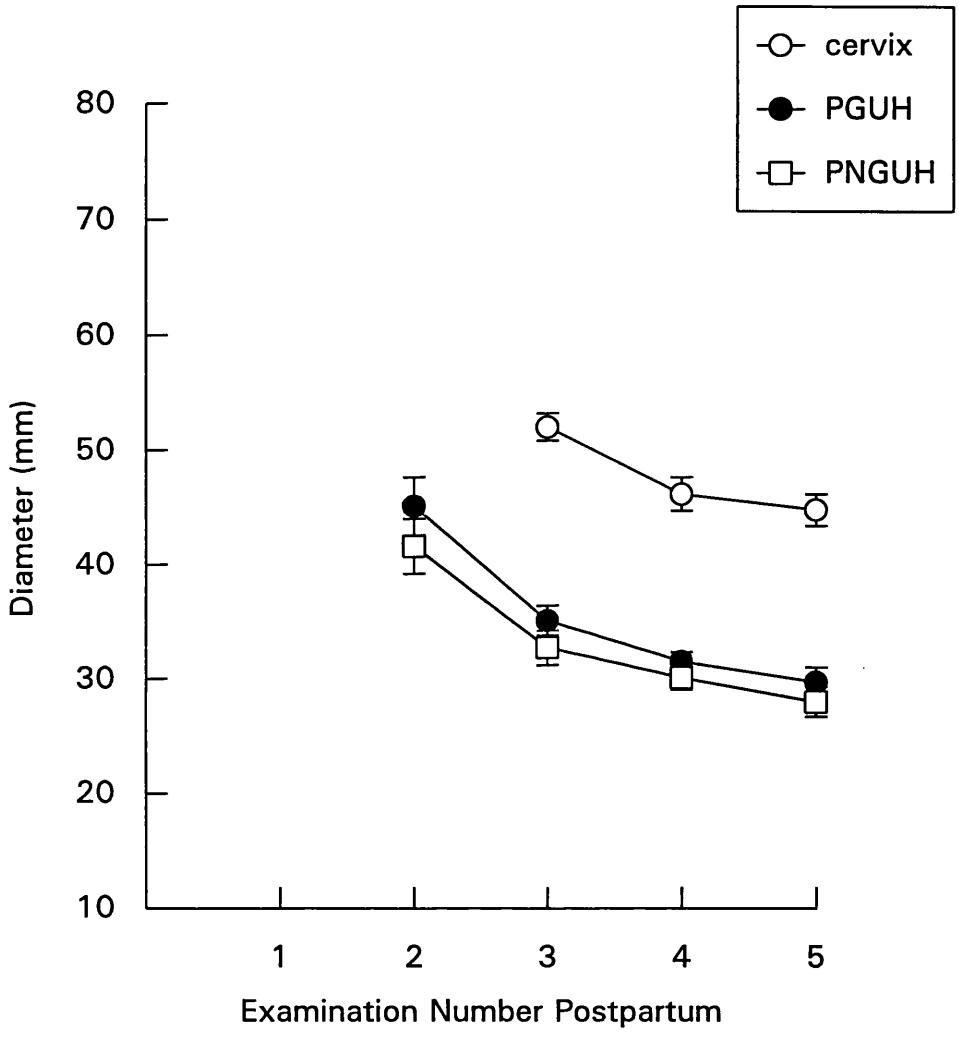


Figure 3.11. Changes in diameters of the cervix, previously gravid uterine horn (PGUH) and previously non-gravid uterine horn during successive examinations in normal cows (Mean ± SEM).

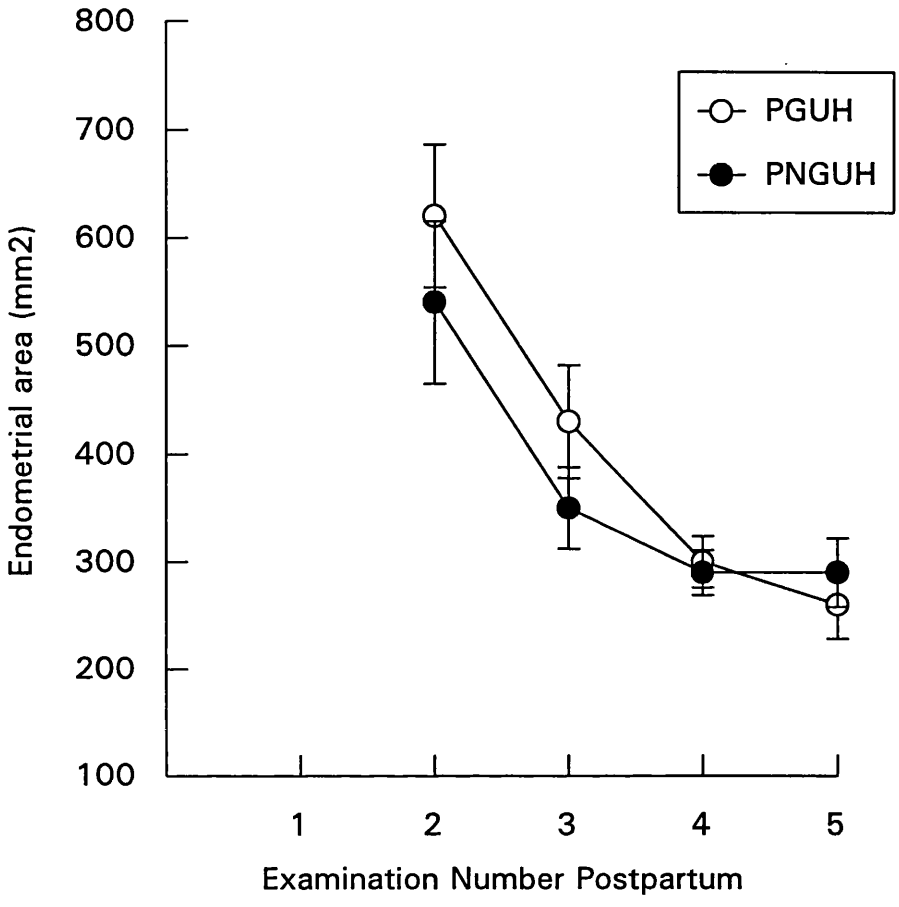


Figure 3.12. Changes in endometrial area of previously gravid uterine horn (PGUH) and previously non-gravid uterine horn during successive examinations in normal cows (Mean ± SEM).

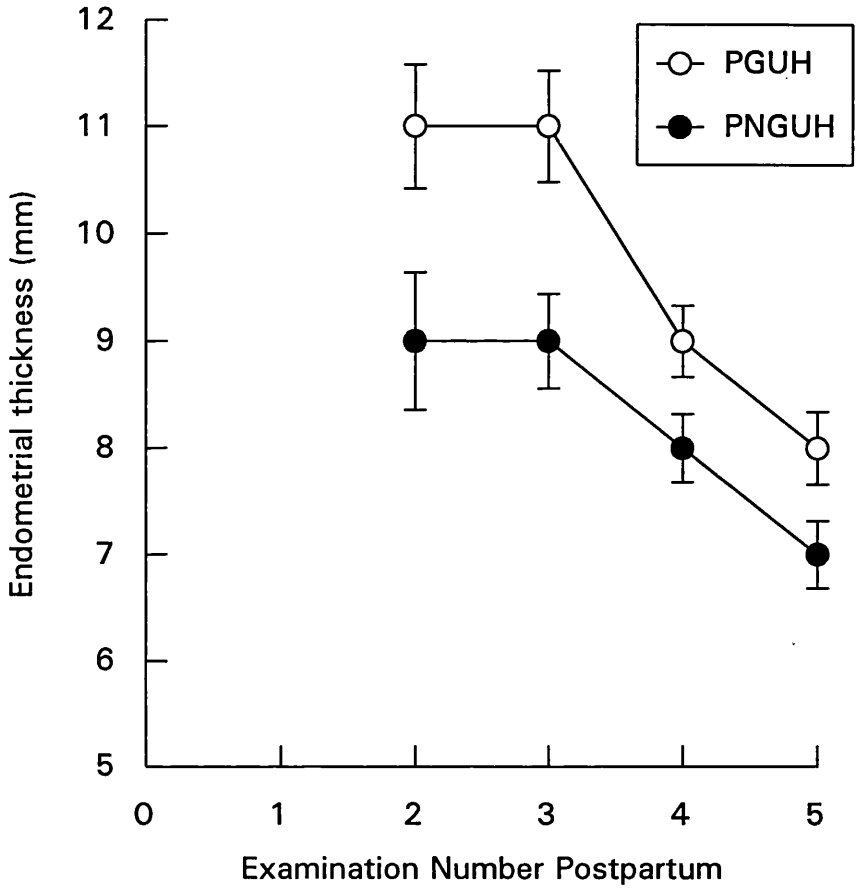


Figure 3.13. Changes in endometrial thickness of previously gravid uterine horn (PGUH) and previously non-gravid uterine horn during successive examinations in normal cows (Mean ± SEM).

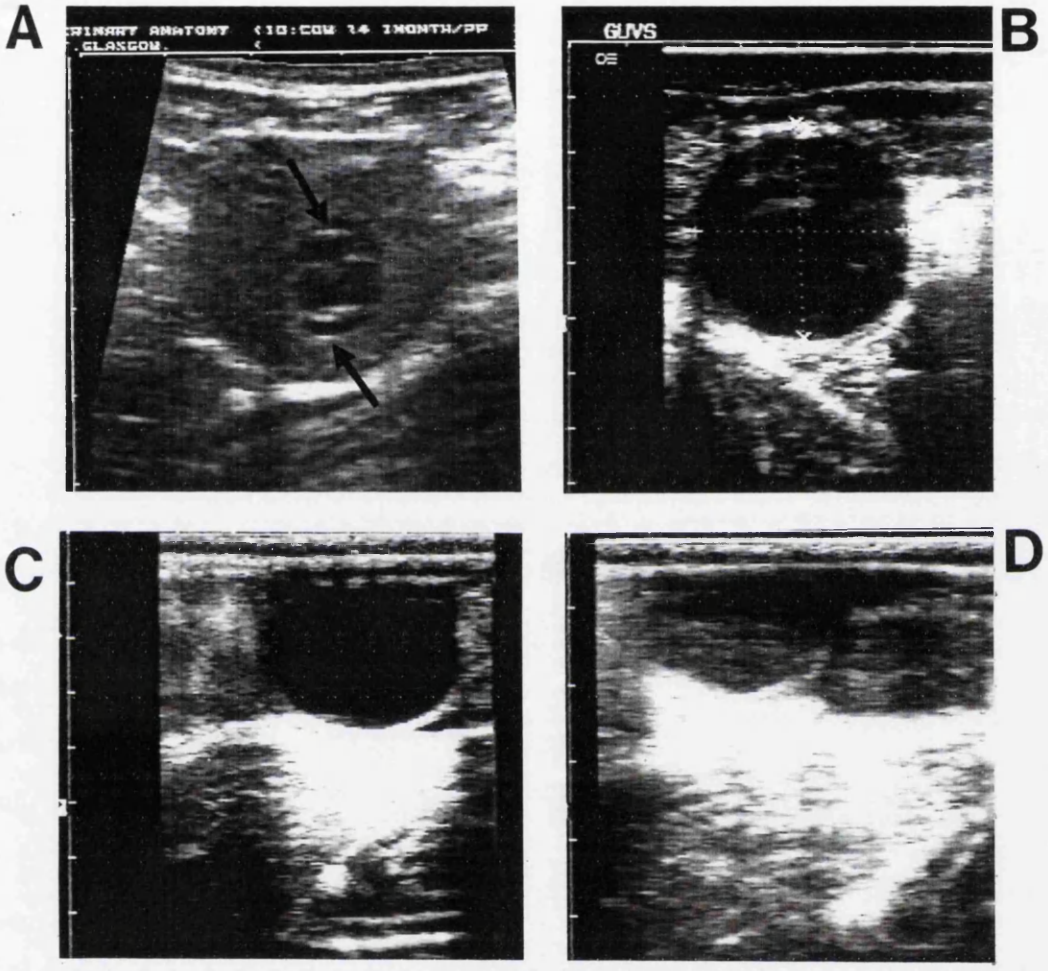
Figure 3.14. Incidental ovarian findings during the postpartum period.

A. Anovulatory follicle. 30 days postpartum (Cow 14). Note rim of luteal tissue and small lacuna (arrows).

B. Ultrasound image of an ovary with a follicular cyst 35 days postpartum (Cow 74). Note the smooth outline of the cystic cavity which measured 38.6 mm by 36.1 mm in horizontal and vertical diameters.

C. Ultrasound image of the same ovary with the follicular cyst 17 days later, 52 days postpartum. The cyst has regressed following treatment. and now measures 33.6 by 27.3 mm.

D. Section of the other ovary at the same examination showing two corpora lutea, thus indicating that a double ovulation has occured.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3.3.3. Monitoring of cows with post-partum abnormalities

Six cows were classified as abnormal because of periparturient problems. These abnormalities are listed in Table 3.7. Findings upon rectal examination prior to ultrasound scanning are summarised in Table 3.8. Cow 8, upon which the caesarian operation was performed was not examined rectally until Examination 3, to facilitate the healing process. For this reason it was excluded from the summary information indicating ovarian palpability before ultrasonic scanning. It was, however, included in the data for uterine position as the uterus was still intra-abdominal on Examination 3. The whole uterus could not be examined in any of the abnormal cows during Examinations 1 and 2 as it was intra-abdominal in each case. By Examination 3 the uterus was intra-pelvic in 33% of the animals. At Examination 5 the uterus was still intra-abdominal in two animals, Cow 8 which had the caesarean operation and cow 20 which had a still birth followed by endometritis, although the organ could be retracted into the pelvis for examination. In these two cases the uterus had returned to the pelvic position by days 73 and 75 postpartum, respectively. Both the right and left ovary were palpable in 20% of the cases during Examination 1, 40% during Examination 2 and in all animals by Examination 3. Cyclicity was observed in 20% of the animals by Examination 2, 80% by Examination 3 and in all the animals by Examination 4.

Figure 3.15 and 3.16 are ultrasound images of the abnormalities encountered in these animals. Where foetal membranes were retained threads of membrane remnants could be seen projecting into the uterine lumen at day 4 postpartum and had an echogenic appearance contrasting with the anechoic fluid (Cow 37, Figure 3.16.A). While in normal cows the uterine fluid was anechoic at this stage postpartum the uterine lumen contained no visible material. Figures 3.15.A. and B show the appearance of endometritis at 26 days postpartum. The uterine

lumen is distended with partially echogenic fluid and the endometrium is greatly enlarged. This contrasts with the normal cows which would either have no intrauterine fluid or the anechoic fluid and endometrial folding associated with oestrus. Figure 3.15.D shows a longitudinal section of the cervix of cow 8, 27 days after the caesarean operation. The cervical lumen is distended, a feature not seen in normal cows beyond 9 days postpartum. Figure 3.16 illustrates delayed reduction in uterine horn diameter. Only after Examination 5 could the the two horns be visualised in the same field, a feature observed at Examination 3 or 4 in the normal group. The same figure also shows a transverse section of the cervix after Examination five. Following the disappearance of visualisation of the cervical lumen there were no identifiable structural changes in the cervix other than those relating to size.

The herd fertility parameters of the abnormal cows are shown in Table 3.5. No attempt was made to rebreed two animals in this group. Those two animals were cow 5, an eleventh calver with retained foetal membranes and cow 8, upon which the caesarean operation was performed. It was decided to cull these two animals.

Table 3.7. Periparturient disorders of the abnormal cow group.

Cow Number	Lactation Number	Abnormality
05	11	Retained foetal membranes.
08	2	Caesarean after dystocia.
20	6	Still birth followed by endometritis.
37	8	Retained foetal membranes followed by endometritis.
60	3	Metritis.
96	3	Twin calving.

Table 3.8. Manual rectal examinations of reproductive organs of abnormal cows prior to transrectal ultrasonic scanning

	Uterine Position	Number of Animals	Ovarian Status	Number of Animals
Examination 1	Intra-abdominal	6/6	Right ovary palpable	1/5
Range 2-9	Intra-pelvic	none	Left ovary palpable	1/5
Days p.p.	All palpable	none	Cycling	none
Examination 2	Intra-abdominal	6/6	Right ovary palpable	2/5
Range 12-17	Intra-pelvic	none	Left ovary palpable	none
Days p.p.	All palpable	none	Cycling	2/5
Examination 3	Intra-abdominal	4/6	Right ovary palpable	5/5
Range 23-27	Intra-pelvic	2/6	Left ovary palpable	5/5
Days p.p.	All palpable	3/6	Cycling	4/5
Examination 4	Intra-abdominal	2/6	Right ovary palpable	5/5
Range 43-49	Intra-pelvic	4/6	Left ovary palpable	5/5
Days p.p.	All palpable	5/6	Cycling	5/5
Examination 5	Intra-abdominal	2/6	Right ovary palpable	5/5
Range 54-61	Intra-pelvic	4/6	Left ovary palpable	5/5
Days p.p.	All palpable	6/6	Cycling	5/5

Figure 3.15. Postpartum ultrasonographic findings in abnormal cows during Examinations 1-3.

A. Transverse section of uterine horn in a cow with retained foetal membranes at 4 days postpartum (Cow 37). Note threads of membranes in the uterine lumen.

B. Transverse section of uterine horn in a cow with endometritis. Cow 71 26 days postpartum. Note distended lumen with partial echogenic fluid (arrows). The endometrium is grossly enlarged.

C. Longitudinal section of uterine horn of a cow with endometritis at 26 days postpartum (Cow 20). Note the distended lumen containing echogenic fluid (arrows).

D. Longitudinal section of the cervix of Cow 8, 27 days after the caesarean operation. Note centrally located distended lumen (white arrows). Upper and lower boundaries of the cervix are marked by white crosses.

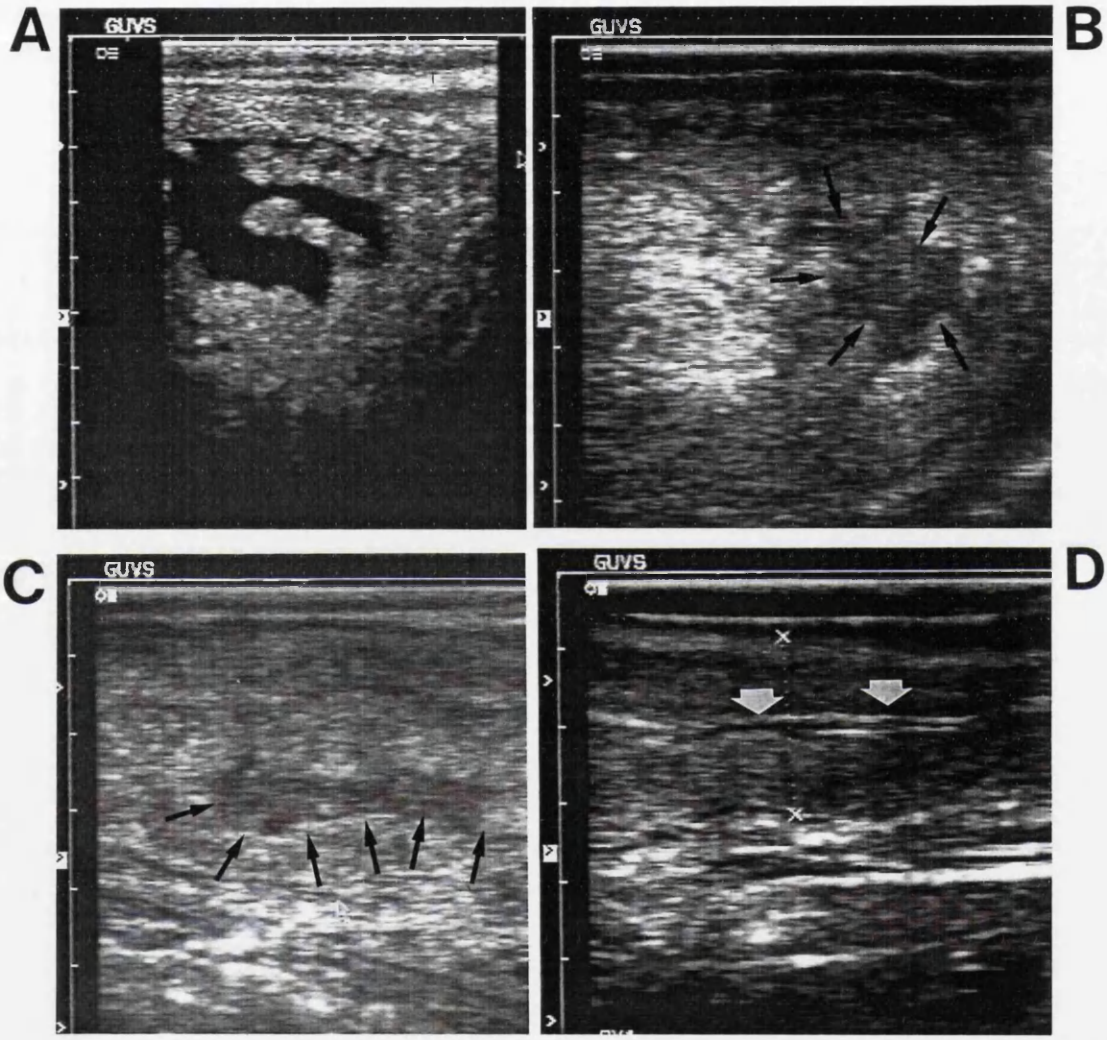
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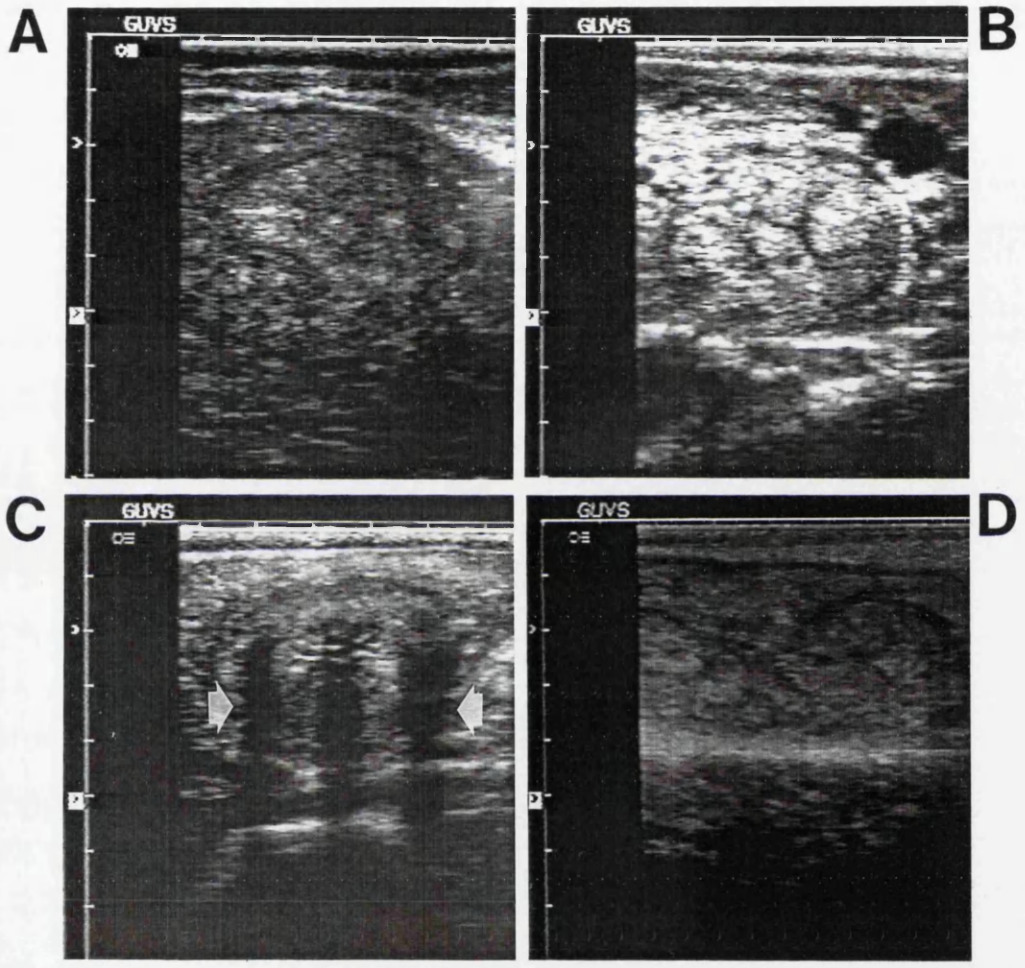
Figure 3.16. Postpartum ultrasonographic findings in abnormal cows during Examinations 4 and 5.

A. Ultrasound image of a transverse section of uterine horn. Cow 20, 44 days postpartum. Note that endometritis was detected in this cow 26 days postpartum. No abnormal fluid is now visible but the horn is still too large be visualised in a field.

B. Ultrasound image of a transverse section of the uterine horn of the same cow as in A, 54 days postpartum. The entire horn is now visible. Note also the oedematous appearance of the uterus with marked endometrial folding, indicating approaching oestrous (2 days before oestrus).

C. Ultrasound image of a transverse section of the cervix of Cow 20, 68 days postpartum. Note the acoustic shadowing (arrows) caused by hyperechoic connective tissue within the cervix.

D. Ultrasound image of a transverse section of uterine horns of Cow 20 at 75 days postpartum. The two horns are visible within one field of view.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Chapter 4

Discussion

Ultrasonography of the cow reproductive tract during the postpartum period has mainly focused on ovarian activity (Rajamahendran and Taylor, 1990; Savio et al., 1990a; Savio et al., 1990b). The resumption of ovarian activity after the physiological postpartum anoestrus period is essential. However, if a dairy cow is to calve again within 365 days it is also essential that involution of the uterus is completed without delay. There are few published studies examining involution of the uterus using ultrasound (Okano and Tomizuka, 1987; Kamimura et al., 1993). Therefore, this study was carried out to characterise the events of this process, ultrasonographically.

Throughout this study a 7.5 MHz linear array transducer was used. This high frequency transducer had the disadvantage of low depth of penetration into the tissues. This was a drawback, particularly during Examination 1 when the uterus was large and located deep in the abdominal cavity. Using a 3.5 or 5.0 MHz transducer would have helped to overcome this problem. However, low frequency transducers have the disadvantage of low resolution, resulting in loss of detail in the ultrasonic images. From the ultrasonic images shown in Section 3.1. it is clear that certain structures, for example, the boundaries and lumen of the cervix and uterine body, were difficult to visualise even using the high resolution 7.5 MHz. Since visualising detail of the cervix was an important component of Examination 1, the requirement for high resolution took precedence over depth of penetration.

The other limitation of the 7.5 MHz was the length of the crystal face, which was 60mm. Since a rectangular image is formed by a linear array transducer, the maximum width of the image produced was 60mm. This meant that dimensions greater than 60mm could not be measured precisely. A problem was encountered during measurement of the cervix of abattoir specimens with mild or severe endometritis, the cervix of live cows during Examinations 1 to 4, and the uterus of live cows during Examination 1. Wider measurements could have been made by using the

dual screen mode of the scanner, which allows the simultaneous display of two images. However, measurements obtained from such images would be subject to error as assembling the two parts of the image by visual judgement was not easy. It was decided that the procedure for using a dual screen mode was inaccurate and time consuming and unlikely to be practical under field conditions where a quick procedure is often desirable. Structures which were greater than 60mm in diameter were simply recorded as such. With collation of the data it became clear that this was actually a useful measurement since a uterine horn measurement greater than 60 mm, at and beyond day 23 postpartum, was always associated with some form of abnormality.

Initially six scanning planes were tested on uteri. The images obtained using these planes to scan abattoir specimens and live cows are shown in Figures 3.1-3.4. Two factors were considered in selecting the most appropriate scan planes for the study, one being clarity of image and the second, the repeatability of the measurement. It was evident that images produced by scanning the cervix longitudinally were indistinct and therefore of little value. Scanning of the uterine body in either longitudinal or transverse section was unsatisfactory because of lack of a distinct landmark to ensure that measurements were taken at the same location during successive examinations. For the same reason longitudinal examination of the uterine horns was unsatisfactory. Therefore, two scan planes were selected, a transverse section of the cervix at mid cervix, after location of this area by rectal palpation and transverse section of the uterine horns at the level of the intercornual ligament, also identified by rectal palpation. These scans were repeatable and rapid and used throughout the scanning of live animals.

The water-bath studies of the uterus provided a good training in scanning the normal and pathological reproductive tract of the female bovine. Comparisons could easily be made between the gross specimens and the ultrasound images. In gross specimens a layer of blood vessels surround the endometrium. Ultrasonographically, images of

the horns in cross section showed a circular hypoechoic area which divided the uterine horn into an outer and inner ring. The inner portion was taken to be the endometrium with its lumen, while the outer ring was termed the myometrium. Hypoechogenicity, however, is characteristic of fluids and it is likely that the hypoechoic circular structure is actually the vascular layer. Since the vascular layer is located between the inner circular and outer longitudinal layers of the tunica muscularis (see Figure 2.4) the area referred to as the endometrium may have included the inner circular layer of smooth muscle.

No significant difference was noted between the two horns of the abattoir specimens of heifer uteri, reflecting the fact that in nuliparous animals the horns are of equal diameter. The reproductive organs of parous cows were generally larger than those of heifers. Within this group the right horn was larger than the left horn. It is well recognised that the right ovary is more active than the left ovary (Morrow, et al., 1968) and consequently the right horn carries more pregnancies. The previously gravid horn never regresses to its pre-gravid size after each pregnancy. It was noted the dimensions of the cervix and uterine horns of all the parous cow specimens were less than 60 mm, whereas the same dimensions in live cows were greater than 60 mm during the early post-partum examinations. The likely explanation for this observation is that the abattoir specimens were recovered from cows culled probably many months after their last calving.

In some previous studies the criteria for classifying reproductive tracts according to severity of endometritis included dimensional differences between the uterine horns (Miller et al., 1980; Studer and Morrow, 1981). However, such classifications were based on an examination carried out between 21 and 35 days post-partum. In this study the reproductive history of the animals from which specimens were collected was not known. It was therefore difficult to classify specimens according to horn dimensions, since these were known to vary considerably in the early post-partum period. Rather than base the

classification on dimensions it was decided to put more emphasis on the uterine discharge.

The ultrasonographic appearance of endometritis in the live cow has been described as being characterised by a distended lumen filled to a varying degree with partially echogenic "snowy" patches (Fissore et al., 1986). The nature of the uterine luminal fluids seen in the water-bath specimens with endometritis in this study were of varied echogenicity. This seemed to be a reflection of the cellular composition of the fluids. The more purulent the fluid was, the more highly echogenic it appeared in the ultrasound image. In cases where the fluid echogenicity was similar to the echogenicity of the endometrium it was difficult to accurately measure endometrial thickness. This could have resulted in an overestimate of the endometrial thickness of some specimens. In some cases reflection from highly echogenic fluid tended to mask reflection from the surrounding area to such an extend that the image produced lacked tissue detail. Under such circumstances it was difficult to make accurate measurements. However, this problem was not encountered in live animals. The dimensions of the cervix of many of the abattoir specimens with endometritis exceded 60 mm, a finding not observed in normal live cows at and beyond 54 days post-partum. This finding lends support to the thesis that this measurement is a quick means of identifying an abnormality. However, without calving dates no firm conclusions can be drawn from this data.

Reports of extensive studies of endometritis using ultrasound are lacking in the literature. It would be interesting to determine if the same variation in echogenicity of luminal fluids could be recognised in live animals. Distinguishing one form from the other may not be very important in aiding treatment as uterine infections involve several different micro-organisms. However the recognition of all cases of endometritis is vital if treatment is to be instituted quickly.

The results of the serial monitoring of live animals indicated the uterus could not be scanned fully in the majority of animals during the

first few days postpartum, due to its large size and intra-abdominal position. This limitation has been noted by Kamimura and others (1993) who could not scan some animals during the first 10 days postpartum. In this study accurate measurements of the uterus could not be made during Examination 1, 2-9 days postpartum, and these dimensions were recorded as > 60 mm. Nevertheless, morphological observation of structures could be made. Typically the lumen of the uterus was filled with fluid with one of ultrasonographic appearances. In animals examined during the first 3-6 days of Examination 1, the fluid was anechoic. In animals examined towards the end of Examination 1 the fluid was partially echogenic. This change in echogenicity coincides with the period of caruncular sloughing reported by Gier and Marion (1968) in a study involving gross and histological examination. These researchers reported that necrosis of caruncular tissue advances to a point where septal material of caruncular tissue sloughed in chunks from 5 days postpartum. It therefore seem reasonable to conclude that this necrotic cellular mass imparts the echogenicity observed towards 9 days postpartum in this study. The volume of fluid was variable but fluid was present in all the animals. During the same period caruncles were clearly visualised protruding into the uterine lumen as oval echogenic structures.

During the period of 12-17 days postpartum (Examination 2) the uterus had reduced in size and accurate measurement of the uterine horns became possible. The mean diameter of the previously gravid uterine horn was 45.1mm (range 28.0-60.0). The uterus had a heterogeneous echotexture and images lacked tissue detail. This is a feature of oedema as noted by Pierson and Ginther (1987), who described the same condition during oestrus. The oedema seen at this time in the postpartum period is likely to be part of the involution process rather than due to oestrus. Postpartum oedema was also reported by Gier and Marion (1968) in an earlier study. In this current study the myometrium and the endometrium were separated by a hypoechoic ring lying between the two layers. Only a few animals still showed large

amounts of flocculant fluid in the uterine horns. Some animals showed no evidence of fluid at all, while others had hyperechoic material in the uterine lumen. The striking resemblance between the hyperechoic luminal material in these live animals and that seen in abattoir specimens with mild endometritis leads one to conclude that the hyperechoic material is pus-like material associated with mild endometritis. This seem reasonable as it is documented that 93% of cows acquire an infection within 15 days postpartum but recover such that by 46-60 days postpartum only 9% of animals are still infected (Elliott et al., 1968). Since it is reported that animals with mild endometritis tend to recover without treatment (Studer and Morrow, 1978; Miller et al., 1980), no action need be taken following observation of this hyperechoic material in the uterus.

During the period of 23-29 days postpartum (Examination 3), further reduction the size of the uterus was noted. The mean uterine horn diameter of the previously gravid horn was 35.1 mm (range 25.2-47.0). The uterus took on a distinct homogenous appearance and oedema was not observed. Anaechoic fluid was found in one cow which was in oestrus at the time of examination. Echogenic fluid was not observed during this period. This is in agreement with the report by Arthur and others (1996c) that lochia disappears by 18 days postpartum.

Features observed ultrasonographically from Examination 3 to the last examination (Examination 5) were mainly related to the oestrous cycle. The uterus showed two characteristic appearances. During dioestrus the uterus had a fairly homogenous appearance while prior to oestrus and for about three days after the uterus had a heterogeneous appearance with anechoic fluid in the uterine lumen and oedema in the uterine tissue. This is consistent with reports made by other researchers (Okano and Tomizuka, 1987; Kamimura et al., 1993). The fluid observed during oestrus was unevenly distributed in and between the two horns. Further reduction in uterine size was minimal between Examination 3 and Examination 5. The mean horizontal diameter of the previously gravid

uterine horn was 31.5 mm (range 22-37) and 29.7 mm (range 20.2-37.0) by Examinations 4 and 5, respectively. Reduction in diameter size between examinations 4 and 5 was not significant indicating completion of uterine involution. The previously non gravid horn was generally smaller than the previously gravid horn. Reduction in diameter size was significant only between examinations 2 and 3. This might show that involution of the previously gravid uterine horn is completed earlier than that of the previously gravid horn.

The endometrial area decreased with number of days postpartum (Figure 3.11). No significant change in endometrial area of the previously gravid uterine horn was noted from examination 2 onwards. For the previously non gravid uterine horn the change in endometrial area was significant between examinations 2 and 3. These results may reflect the inaccuracy of this measurement as it was affected by the presence of intraluminal fluid. In cases where there was fluid in the horn the calculation of area included the fluid. During examination 1 this was the lochial discharge and beyond Examination 2 was the effect of oestrus fluids. These fluids are usually unevenly distributed between the horns.

The greatest change in endometrial thickness of the previously gravid uterine horn occurred between Examination 1 and Examination 2. The reduction in uterine endometrial thickness between Examinations 2 and 3 was not statistically significant. The greatest change in endometrial thickness coincided with the period of caruncular sloughing, a process said to be completed by 15 days postpartum (Gier and Marion, 1968). Thereafter the endometrial surface becomes relatively smooth. A significant reduction in endometrial thickness was noted between Examination 4 and Examination 5. No good reason can be given for this observation.

Based on lack of further reduction in the size of the uterine horns it can be suggested that uterine involution was completed by Examination 4 (43-49 days postpartum). Other researchers who have monitored the

process ultrasonographically have reported completion in 40 days (Okano and Tomizuka, 1987) and 41.5 days (Kamimura et al., 1993).

Reduction in size of the cervix occurred more slowly than involution of the horns which is consistent with other reports (Gier and Marion, 1968; Moller, 1970). It has been reported that immediately after parturition the diameter of the previously gravid horn is greater than that of the cervix but the dimensions of the two become equal by 18 days postpartum and remains equal over the next 3-5 days before the horn shrinks below the size of the cervix (Morrow et al., 1969b). In this study, however, the two appeared to become equal in size earlier (during Examination 1, prior to 9 days) than had been reported. At Examination 2 (12-17 days postpartum) the previously gravid horn was already smaller than the cervix. The dimension of the cervix in this study were greater than 60 mm until Examination 3 (23-29 days postpartum) when it was 52.0 mm (range 60.0-44.3) in diameter. Throughout Examination 1 (2-9 days postpartum) the cervix had a fluid-filled open lumen. During Examination 2 the lumen of the cervix was not visible. Changes during the latter examinations involved reduction in size until its mean diameter was 44.8 mm (range 34.2-49.9) by examination five.

In cows with periparturient disorders involution was generally delayed. It was possible to visualise remnants of foetal membranes in cows which had retained their membranes. Membranes appeared as echogenic threads in the uterine lumen. The two cows with retained foetal membranes were old, with one in its eighth lactation and the other in its eleventh lactation. This is consistent with documented evidence that age is a predisposing factor to retained foetal membranes (reviewed Laven and Peters, 1996). One of these cows subsequently developed endometritis and the uterine lumen was distended with flocculant fluid after Examination 2. Interestingly, the cow which developed acute metritis immediately postpartum did not subsequently show endometritis. Presumably the infection responded to the aggressive antibiotic and anti-inflammatory treatment which was given within days of calving. Due to

the small sample size in the abnormal group no statistical comparison was attempted between this group and the normal cows in relation to differences in uterine involution and fertility parameters.

As stated earlier it was not the principal objective of this study to monitor ovarian function. However, by comparison of the herd records and ultrasonographic records in this study evidence of silent or missed heats was noted. In some cows large follicles (> 10 mm) and even corpora lutea were observed during one or more examinations before observed oestrus was recorded by the Dairyman. In these cases oestrus was not recorded until 50 days postpartum. However, non observed oestrus is recognised to be a very common problem in dairy cows with an incidence as high as 50% reported (Arthur et al., 1996b). Silent oestrus has a lower reported incidence of around 7% (Bulman and Lamming, 1978) and is said to be associated with the first heat postpartum (Morrow et al., 1969a). One heifer, however, seemed to show true delayed return to cyclicity. Herd records showed no observed oestrus until day 62 postpartum. Ultrasonography of the ovaries during the five examination periods only showed the presence of small follicles (< 5 mm) and no corpus luteum was detected. This animal had no periparturient abnormality.

An anovulatory follicle was observed on the ovary of one cow. Ultrasonographically the structure had a rim of luteal tissue like a luteal cyst except it was smaller in size (<2.5 mm). Ovulation failure during the postpartum period has been reported (Watson and Harwood, 1984). An anovulatory follicle is said to regress normally like a corpus luteum and does not affect the next oestrous cycle. This cow was culled so no further information was available. Another ovarian observation was the occurrence of ovarian cysts in two animals. These were luteal and follicular cysts and regressed following treatment.

When the reproductive performance parameters of the animals in this study were compared to performance targets for dairy herds in the UK, as recommended by Esslemont and Peeler (1993), a good mean

calving to first oestrus interval (36 days) was noted. However the animals had a longer calving to conception interval (97 days) than the recommended 89 days. Services per conception was also higher (2.1) than recommended (<1.7). Nonetheless these parameters were calculated for only a third of the herd and may not reflect the situation for the whole herd.

Conclusions

In conclusion, this study has shown that the process of uterine involution can be monitored ultrasonographically. An examination procedure involving visualisation and measurement of mid-cervix and uterine horns at the level of the intercornual ligament was found to be rapid and repeatable and could be applied by veterinary surgeons during routine fertility examinations. Further, the study suggests that certain features can be used to identify uterine pathology.

1. The persistence of a visible lumen in the cervix at and beyond 12 days post-partum.
2. Visible caruncles at and beyond 12 days postpartum.
3. A uterine horn diameter of greater than 60 mm at and beyond 23 days postpartum.
4. The presence of hyperechoic or echogenic fluid in the uterine lumen beyond day 23 postpartum.
5. Cervix horizontal diameter of more than 60 mm at and beyond day 54.

It is suggested that routine fertility examinations carried out around 26 days postpartum should be targeted at evaluating uterine involution, using the information listed above. At this stage postpartum endometritis can be detected and appropriate action taken. The routine fertility examination carried out around 45 days post-partum should additionally be targeted at establishing ovarian cyclicity. By this time the majority of the animals should be cycling. Since these fertility examinations are already used by veterinary practitioners it is hoped that the findings of this study can be utilised in practice. However, due to the limited number of animals in this study, further work is still needed to consolidate these results.

Appendix 1. Cow examination recording sheet.

Cow No.	Calving Date	Age (no.calves)	Calving comments	Others				
Exam No.	Exam Date	Days Post-Calf	Uterus			Ovaries		
			intrapelv/abdom	*all palpable y/n*	*retractable y/n*	*RO palpable y/n*	*LO palpable y/n*	*Cycling y/n*
			Ultrasound			Ultrasound		
			Cervix	*Right Horn*	*Left Horn*	*RO*	*LO*	
Exam No.	Exam Date	Days Post-Calf	Uterus			Ovaries		
			intrapelv/abdom	*all palpable y/n*	*retractable y/n*	*RO palpable y/n*	*LO palpable y/n*	*Cycling y/n*
			Ultrasound				Ultrasound	
			Cervix	*Right Horn*	*Left Horn*	*RO*	*LO*	
Exam No.	Exam Date	Days Post-Calf	Uterus			Ovaries		
			intrapelv/abdom	*all palpable y/n*	*retractable y/n*	*RO palpable y/n*	*LO palpable y/n*	*Cycling y/n*
			Ultrasound			Ultrasound		
			Cervix	*Right Horn*	*Left Horn*	*RO*	*LO*	

Appendix 2. Dairy-Champ animal herd fertility record print-out

COW CARD
FARM: COCHNO

Animal ID	: 097	Birth Date	: 2JUN94
Animal type	: COW	Bangs tag	:
Repro status	: CON PREGNANT	Breed assoc	:
Lact status	: LACTATING	Sire	: VALENTINO
Current lact	: 1	Dam	: 085
Current DIM	: 284	Location	:
Current DCC	: 178 Due date :13NOV97	Stall	: 0
Breeding code:		Group	:

Lact number	: 1	Dry Date	:	Calve-1st heat	: 36
Calving date	: 18OCT96	Services	: 3	Calve-1st serv	: 36
Calf ID	: 00262	Concep date	: 1FEB97	Calve-conception	: 106
Calf sex	: FEMALE	Concep type	: SERVED	Days milked	: 284
Calf sire	:	Concep sire	: HER	Days dry	:

18 OCT 96 CALVED; B:1; D:0; R:1; S:0; ; 0
18 OCT 96 CHANGE ID; 027
18 OCT 96 CALF ID; 00262; FEMALE;
20 OCT 96 CHANGE ID; 097
12 NOV 96 RECTAL; ; ; ; R:CL2; ;
23 NOV 96 AI; ETAZON JIM; ; ; ; ; ; 1.00; ; 0; 1.00
18 DEC 96 RECTAL; VET REQUEST; ; ; R:CL2; ;
18 DEC 96 RECHECK ON; 30 DEC 96; ;
21 DEC 96 AI; ETAZON JIM; ; ; ; ; ; 1.00; ; 0; 1.00
22 JAN 97 RECTAL; ; NORMAL; L:CL2; R:NO STRUCTURE; ;
22 JAN 97 RECHECK ON; 29 JAN 97; ;
28 JAN 97 OPEN; NORMAL; L:CL2; ; ;
28 JAN 97 TREATMENT; VETERINARIAN; IM (INTRAMUSCULAR); ESTRUMATE; KAMAR DVC
1 FEB 97 SERVED; HER; ;
4 FEB 97 RECHECK ON; 5 FEB 97; REPRO PROBLEM; OPEN + PG -7D
12 FEB 97 RECTAL; ; ; ; R:CL2; ;
25 FEB 97 PREGNANT; CONCEPTUS; ;
11 MAR 97 PREGNANT; ; ;
30 APR 97 PREGNANT; ; ;

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