Clinical and research applications of real-time ultrasonography in bovine reproduction: A review

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Abstract

Transrectal real-time ultrasonography has proved to be a rapid and reliable technique for studying reproductive functions in cattle. Through ultrasonography it is now established that follicular growth occurs in wave-like patterns during each estrous cycle. It has been shown that follicular growth and regression continue during early pregnancy, as well as in the postpartum anestrous period. Ultrasound has also helped us to understand the influence of dominant follicles on medium and small follicles. Among the numerous demonstrated applications of ultrasonography, early pregnancy diagnosis, fetal sexing, and postpartum reproductive management appear to be promising areas for immediate application. The new information that has been generated through ultrasound has thrown light on hitherto poorly understood areas of ovarian follicular dynamics, corpus luteum function, pregnancy establishment, and embryonic development in cattle, thereby opening newer areas for research. Still there is great potential for the continued application of this technology to further our understanding of the reproductive processes and to maximize reproductive efficiency of the bovine species. The significant contributions of real-time ultrasonography to the study of bovine reproduction in general and its practical applications in particular are discussed in this paper. The need for taking up technology assessment studies and for the introduction of low-cost portable equipment are stressed. Literature search for this review was done by scanning Current Contents Series 1991–92, AGRICOLA 1980–92, and MEDLINE 1990–92.

Résumé

Revue de l'utilisation de l'imagerie avec les ultrasons en reproduction bovine dans les domaines de diagnostic médical et de recherche

L'échographie effectuée par voie transrectale est une méthode fiable et rapide pour suivre le cycle reproducteur chez les bovins. L'imagerie avec les ultrasons a permis de visualiser la croissance folliculaire, laquelle se présente sous forme d'ondes à chaque cycle d'oestrus. De plus, il est démontré que la croissance et la régression folliculaires se poursuivent jusqu'au début de la gestation et durant la période d'anestrus post-partum. L'échographie a aussi contribuée à déterminer le rôle des follicules dominants sur les follicules de moindre taille. Le diagnostic précoce de gestation, la détermination du sexe du foetus et la régie de la reproduction en période post-partum représentent des domaines prometteurs d'utilisation immédiate de l'échographie. Les informations générées par l'imagerie avec les ultrasons ont permis d'élucider plusieurs questions dans les domaines peu connus de la dynamique ovarienne folliculaire, de la fonction du corps jaune, de l'implantation et du développement embryonnaire chez la vache, et ainsi d'élargir les domaines de recherche. L'utilisation de cette technologie permettra d'améliorer nos connaissances sur le processus de la reproduction et de maximiser les performances en reproduction chez les bovins. Dans ce communiqué, les auteurs discutent de l'importante contribution de l'échographie en reproduction bovine en général et de son utilisation pratique. De plus, ils soulignent la nécessité d'effectuer des études technologiques d'évaluation et de la mise en marché d'un équipement portatif à prix populaire. La revue de la littérature a été effectuée en consultant Current Contents, séries 1991–1992, AGRICOLA 1980–1992 et MEDLINE 1990–1992.

(Traduit par Dr Thérèse Lanthier)

Can Vet J 1994; 35: 563–572

Introduction

Real-time ultrasonography has gained tremendous popularity in recent years as a diagnostic as well as a research tool in veterinary and animal science. Reports on the diverse applications of ultrasonography, continued improvements to imaging quality, availability of portable ultrasound scanners, and reduction in equipment costs have led to its general acceptance by the veterinary profession. As a diagnostic aid, ultrasonography is well suited for bovine practice, particularly for the examination of reproductive organs. The technique is noninvasive, relatively simple and effective, safe to both the subject and the operator, portable, and ultrarapid, since the ultrasonic image facilitates immediate interpretation and diagnosis in most circumstances. Even though the first report on the application of ultrasound for pregnancy detection in sheep was made over 25 years ago (1), the high cost of the equipment has contributed to the

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reluctance of the veterinary profession to adopt it, in contrast to its widespread acceptance as a clinical tool in human medicine. Interest in ultrasonography among both veterinarians and animal scientists began to grow in the early eighties, following reports (2–4) on the usefulness of the technique in studying the reproductive organs of the cow. Since that time, a dramatic growth in the application of real-time ultrasonography has been witnessed. Over the past decade, several applications of ultrasonography in bovine reproduction have been described (5–34, Table 1).

Reviews on the use of ultrasound in diagnostic veterinary medicine and its research applications in cattle and other farm animals have appeared recently (35–41). However, with the widening scope of ultrasonography, an enormous amount of information is being generated rapidly. It is impossible for practitioners to keep abreast of all published information. Periodic "progress reports" in the form of review articles thus serve to fill in the information gaps. This review aims to recapitulate the significant contributions made by real-time ultrasonography in the past decade and update the more recent developments in diagnostic ultrasonography with particular reference to bovine reproduction. We hope that such a focused review will be useful, specifically to those associated with bovine reproduction.

Table 1. Applications of real-time ultrasonography in bovine reproduction

Application	Reference
I. Female Reproductive System	
• follicular dynamics during	
a) estrous cycle	5,6
b) pregnancy	7,8
c) postpartum period	9,10
d) superovulation	11,12
• ovulation process	13,14
• corpus luteum morphology and growth	15,16
• normal uterine status	3,17
• pregnancy diagnosis and detection of early embryonic death	18,19
• fetal imaging	20,21
• fetal sex	22
• uterine involution	23
• cystic ovarian conditions	24,25
• changes during induced embryo mortality	26,27
• pathological conditions of the reproductive system	17,28,29
II. Male Reproductive System	
• testicular tissue	30,31
• status of accessory sex glands	32
III. Other applications	
• collection of oocytes from in situ ovarian follicles	33
• artificial insemination training	34

Materials and methods

The literature search for this review was performed by computer-aided scanning of Current Contents Series 1991 and 1992 (Institute for Scientific Information, Philadelphia, Pennsylvania, USA), MEDLINE 1990 to 1992 (Excerpta Medica, New York, New York, USA), and CD-ROM AGRICOLA 1980 to July 1992 (National Agricultural Library, Beltsville, Maryland, USA). Recent issues (July 1992 onwards) of popular animal and veterinary science journals available at the University of British Columbia libraries, and research papers available on personal files of the authors were also consulted for relevant literature. Ultrasound, Ultrasonography, Ultrasonic, Bovine, Cattle, Reproduction, Follicle, Cyst, Cystic, Uterus, Ovary, Fetus, Fetometry, Imaging, Sexing, Pregnancy diagnosis, and Corpus luteum were the key words used for literature search. We scrutinized all the literature that we had access to, and excluded or included papers based on the following criteria. All papers reporting a new application of ultrasound were included. Reports supporting or contesting previous findings were included or excluded at our discretion, depending on their importance and relevance to context.

Equipment for ultrasound scanning

Sector and linear-array, real-time [B-mode (brightness modality)], ultrasound devices are commonly available for veterinary applications. In general, linear-array transducers are used with linear-array imagers, and sector transducers are used with sector scanners. Scanners that can be used with both sector and linear-array transducers are also available. A linear-array transducer has several piezo electric crystals (which emit high frequency sound waves on being energized) arranged in a row, while the sector transducer has only a few such crystals. The other major difference between the two systems is that the image produced by the linear-array transducer appears rectangular on the screen, whereas the sector transducer produces a pie-shaped image corresponding to the field of scan. Readers interested in detailed information on the principles and types of ultrasound equipment available are directed to the relevant literature (42–46). The most common approach for scanning bovine reproductive organs is per rectum, using a transrectal transducer and a linear-array scanner. Transvaginal scanning is performed, usually with sector transducers, for certain special applications. Transducers usually come in 3.0, 3.5, 5.0, and 7.5 MHz frequency ranges. The tissue penetration of sound waves and the image resolution depend on the frequency of the transducer used. Accordingly, a 3.0 MHz transducer will give greater tissue penetration but minimum detail, whereas a 7.5 MHz transducer will give minimum tissue penetration yet maximum resolution. A transducer of 5.0 MHz is a general purpose one, providing reasonably detailed images of ovaries and uterus.

Continued improvements to the existing models of ultrasound scanners are essential. Presently, most of the clinical ultrasound equipment used for diagnostic purposes in cattle is manufactured primarily for the medical profession and equipped with modified transducers for veterinary use. Easily portable but sturdily built scanners, capable of withstanding tough field conditions, particularly dust, moisture, sunlight, extremes of temperature, and voltage fluctuations, need to be designed for veterinary use. Szenci (47) described the practicality of using a battery (direct current) operated portable ultrasound scanner rather than the widely used alternating current machines. The total weight of the equipment was only 6 kg, including the battery, a built-in video recorder, and transducer. The machine was capable of providing four hours of continuous operation without recharging, and it could be recharged from an automobile cigarette lighter outlet. If transducers with advanced features such as the ability to alter frequencies between 3.5, 5.0, and 7.5 MHz could be built, it would be of great advantage because of the wide range of applications that they would provide. An important factor that presently restricts the use of ultrasonography is the cost of the equipment; for example, the present cost of a basic model with a general purpose transducer is about $25,000. If cheaper machines become available, more and more practitioners will be interested in this technology.

Applications of ultrasound to ovarian physiology

Follicular turnover during estrous cycles

Ovarian follicular growth and regression have long been subjects of speculation and controversy, resulting in conflicting hypotheses (48). Now, through the use of ultrasound, it has become firmly established that follicular growth during the bovine estrous cycle occurs in wave-like patterns. Even though there are reports indicating the appearance of two, three, and, sometimes, even four waves during each cycle , a two-wave pattern in cows and a three-wave pattern in heifers appear to be the norm (5,6,49). A follicular wave involves the synchronous growth of a group of follicles from which one attains dominance over the others to become the dominant follicle. Each dominant follicle has a growing phase and a static phase, each lasting about 5–6 d. The first-wave dominant follicle is anovulatory. It remains dominant for 4–5 d, and generally by day 11 or 12 of the cycle it loses its dominance and begins to regress. In the meantime, the second wave of follicles has been recruited and selection of the second-wave dominant follicle has taken place. In a two-wave cycle, this follicle goes on to ovulate, while in a three-wave cycle, the second dominant follicle also regresses making way for yet another cohort of follicles, the third wave.

Follicular turnover during pregnancy

Ultrasonographic studies performed during early pregnancy (7,8,50,51) indicate that waves of follicular growth continue even during pregnancy. A wave-like pattern of follicular growth and atresia continues until at least day 60 in pregnant cows, just as it does in normally cycling cows. One study (51) revealed that pregnant cows have more follicles detectable by ultrasound than do nonpregnant cows; however, there appeared to be no difference in the size of the dominant follicles between the two groups. Earlier, it had been reported (50) that pregnant heifers have smaller dominant follicles than do nonpregnant heifers. The continuous secretion of progesterone during pregnancy is thought to be responsible for follicular turnover. Follicular turnover during mid and late gestation has not been investigated.

Follicular turnover during the postpartum period

Ovarian activity in postpartum dairy and beef cows has been characterized (9,10,52,53), and distinct differences are evident between the two. Studies on dairy cows by Rajamahendran and Taylor (9) and Savio *et al* (10) resulted in consistent observations that a) in more than 80% of cows, the first ovulation was unaccompanied by overt signs of estrus, and b) the length of the second cycle (luteal phase following first ovulation) was about 23 d. The length of the first cycle was of either short, 17 ± 7 d (9), or normal, 22 ± 9 d (10), duration. Even though a predominantly two-wave follicular growth pattern was observed in these studies, one, two, and three-wave patterns occurred, depending on the initial day of dominant follicle detection. If the dominant follicle was detected after 20 d postpartum, the cycle duration was consistently short (10).

Studies on beef suckler cows (52,53) indicated that patterns of follicular growth differ between first and second cycles. Similar to the observations made for dairy cows, it was recorded in both studies that over 80% of the cows did not show any estrus behavior before the first ovulation. However, the beef cows, unlike dairy cows, had a short luteal phase (<10 d) following the first postpartum ovulation.

Further studies are obviously needed in this very important area to add to the available information. The postpartum period in the dairy animal is a crucial window which could possibly be manipulated with the help of ultrasound to improve reproductive efficiency by closely monitoring the animals. Detection of silent estrus and timely insemination of postpartum cows will play an effective role in reducing the number of open days, thereby improving reproductive efficiency of the herd (see comments at the end of this article).

Follicular development during superovulation

Ovarian responses to superovulation have been studied in heifers (11,12), cows (54), and buffaloes (55). Even though it is possible to categorize animals as good, average, and poor responders, based on ultrasonic imaging of ovaries, there seems to be no significant advantage in using ultrasound over rectal palpation, particularly for assessment of ovarian response. In a recent report (56), the usefulness of ultrasound in monitoring superovulation was critically evaluated, and it was concluded that neither is it possible to obtain an accurate estimate of follicles or luteal structures, nor is it possible to follow the development of individual follicles by ultrasound scanning. Thus, the limitations seem to outnumber the advantages of using ultrasound for monitoring ovarian responses in superovulated cattle. Therefore, for the embryo transfer practitioner, the use of ultrasound for the above purpose may not be justified. However, the use of ultrasound should not be neglected by researchers studying variations in superovulation responses, as it may provide very useful information. If daily ultrasound monitoring is feasible, the technique could help when having to make such crucial decisions as whether to continue with follicle stimulating hormone (FSH) treatment, if the ovarian response is poor after the first few FSH injections.

Investigations on the influence of a dominant follicle on superovulation responses have been initiated (57–60). No conclusive evidence has been presented in these studies to suggest a positive or negative effect of the dominant follicle on superovulation. However, there seems to be a negative influence exerted by the dominant follicle that is present at the time of superovulation on embryo yield.

Table 2. Accurancy/predictive value of ultrasound in pregnancy diagnosis

Animal	n	Transducer frequency (MHz)	Days post-AI	Accuracy of positive diagnosis or PPV[a] (%)	Comments	Reference
Cows and heifers	113	3.5	45	100	Accuracy of positive diagnosis varied from 93–100% from 30 d onwards. Accuracy of negative diagnosis varied from 68–100%	19
Cows	100	3.5/5.0	27–29	*97	NPV[b] = 92%	47
Cows and heifers	320	3.0	25–30	94	Ultrasonic observations confirmed by rectal palpation on day 60. Embryo visible by day 30	70
Cows	80	3.5	42	100	Accuracy of +ve diagnosis was 100% in cows aged <2 years even by d 28, but 0% in cows aged >8 years	71
Heifers	36	5.0	20–22	100	Observed only 50% accuracy for +ve diagnosis between d 10 and 18	72
Cows	85	5.0	26–29 30–33	*89 *90	NPV = 100% NPV = 94%	73
Cows	39	7.5	16 20	33 100		74
Cows	148	5.0	21–25 26–33	*68 *90	NPV = 64% NPV = 97%	75
Cows and heifers	200	5.0	23–31	*70	Cows and heifers combined. PPV for cows=65%; heifers=87%	76
Cows	143	5.0	25–35	*100	PPV was 100% beyond day 27 and the NPV was 100% beyond day 28	77

[a] PPV = Positive predictive value (Figures with asterisk indicate PPV)
[b] NPV = Negative predictive value
AI = Artificial insemination
Heifer = Bred nulliparous female

Assessment of ovulation and corpus luteum dynamics

Ovulation can be unmistakably detected by ultrasonography, since it is characterized by the abrupt disappearance of the large ovulatory follicle (13,14). Even though the process of ovulation in cattle has apparently not been studied by continuous ultrasonic monitoring, various reports indicate the usefulness of ultrasonography performed at 2 h (61), 3 h (55), and 4 h (13) intervals for detecting ovulation and determining the temporal relationships among estrus, ovulation, progesterone and luteinizing hormone (LH) levels, milk yield, and body temperature. One study (61) showed that the rise in vaginal temperature is a reliable measure of the times of ovulation and LH surge. The time interval to ovulation tends to be dependent upon parity, with pluriparous cows ovulating earlier than their biparous counterparts.

The ultrasonic appearance of the corpus luteum (CL) has previously been described (4,51,62–64). Kastelic *et al* (64) reported that up to 73% of CL were detectable by day 0. However, the findings of Pieterse *et al* (16) contradict the above observation in that only a 43% positive predictive value and 33% sensitivity were obtainable with ultrasound for detecting young (days 1–4 postovulation) CL. Such a huge variation between the two reports in the reliability of ultrasound for CL detection is bound to raise questions about the credibility of this technique. Even though both studies used 5 MHz transducers and linear array scanners, differences in their scanning methods and equipment may have contributed to the variations. In the one study (64), a transrectal approach was adopted, while a transvaginal approach was used in the other (16). Further, the sensitivity of the ultrasound scanners used could have been different, as they were from different manufacturers (Tokyo Keiki LS-200H in the former study versus Pie Medical-400 in the latter). Generally, with a good quality scanner and general purpose (5 MHz) transducer, coupled with adequate experience, there should be no difficulty in determining the CL status of cattle from day 3 onwards. The use of ultrasound for accurate determination of CL status could also be of particular advantage in certain exotic bovine species, such as the water buffalo, in which accurate detection of a CL by transrectal palpation often proves difficult, even during the mid-luteal phase (65). Development of accessory CL, following human chorionic gonadotrophin (hCG)-induced ovulation of the first-wave dominant follicle, has been monitored (66) by ultrasound.

Ultrasonography in pregnancy diagnosis

The first reported use of real-time ultrasonography for pregnancy diagnosis in cattle was in 1982 by Chaffaux *et al* (2). They used a 3.5 MHz transducer for transrectal ultrasonography and observed irregularly shaped nonechogenic structures in the lumen of the uterus from day 28 postinsemination. The embryo proper was identified within this vesicle from day 35 onwards. Pierson and Ginther (67) later reported that it is possible to recognize the presence of an embryo within the uterus between days 12 and 14 following insemination. In their study, eight heifers were bred at estrus and transrectal ultrasound scanning was done daily from day 0 to day 50 postbreeding. Curran *et al* (68,69) characterized the ultrasonic anatomy of the developing bovine conceptus from days 10 to 60. An embryonic vesicle was first detectable at a mean 11.7 ± 0.4 d and the embryo proper was visible by 20.3 ± 0.3 d postbreeding. A 5.0 MHz transducer was used and the most significant finding of the study was that it is possible to detect embryonic loss in heifers as early as day 20 postbreeding. The successful application of ultrasound to early pregnancy diagnosis in cattle under farm conditions was soon reported (70). A 3.0 MHz transducer was used to scan 320 cows and heifers. Pregnancy was detectable as early as day 25 postartificial insemination (AI), and the embryo was visible by day 30. The ultrasonic observations were later confirmed on day 60 by rectal palpation. Accuracy of positive diagnosis was 94%, when animals were examined by ultrasound at about 41 days postbreeding.

Several reports on the application of real-time ultrasonography for pregnancy diagnosis in cattle are available, but inconsistencies exist among them in terms of the reliability of the results for early diagnosis. Since it would be quite exhaustive to discuss the wide variations among the reports, we have summarized the information from some of the important references (19,47,70–77) in Table 2, so that readers may make their own judgement.

The level of accuracy in pregnancy diagnosis achievable through ultrasound appears to vary widely and may depend on a variety of factors. The type of ultrasound equipment used (sector or linear), frequency of transducer selected, scanning frequency (whether the animal was scanned once or several times), age and parity of the animal selected, stage at which examined (number of days postinsemination), the reporting criteria chosen, and the experience of the operator all seem to contribute to the variability. Expression of diagnostic accuracy in terms of the positive or negative predictive value is perhaps the most accepted criterion for reporting. The positive and negative predictive values may be expressed as (a/a+b) x 100 or (c/c+d) x 100, respectively, where "a" is the number of correct positive diagnoses, "b" is the number of incorrect positive diagnoses, "c" is the number of number of correct negative diagnoses, and "d" is the number of incorrect negative diagnoses made (see Table 2 for examples).

It is evident that a 5 MHz or a 7.5 MHz transducer tends to provide more reliable results than does a 3.0 MHz or a 3.5 MHz transducer for early pregnancy diagnosis in cattle. However, the reliable period for pregnancy diagnosis with a positive predictive value of over 95% varies between days 20 and 42 postbreeding. Based on these results, the most realistic early date for reliable pregnancy diagnosis by ultrasound under field conditions may be day 30 postbreeding. Even if early pregnancy is confirmed through ultrasound, it is still advisable to watch the animal closely for estrus and to reconfirm pregnancy around day 60, either ultrasonically or through a carefully conducted transrectal palpation, to rule out chances of embryonic loss after the first ultrasonic confirmation of pregnancy. Even though reports suggest the possibility of confirming pregnancies using ultrasound between days 16 and 21 (68,69,78,79), one should realize that these studies were performed under a research setting, often with repeated and frequent examinations. Since it is extremely difficult to satisfy research requirements under field conditions, attempts to confirm pregnancies earlier than day 30 is not advisable. If the diagnosis can wait, it is probably advisable to delay the examination by an extra five days to minimize the risk of possible trauma to the early fetus, particularly if the operator is not well experienced in ultrasonography.

Fetal imaging by ultrasound

Ultrasound has been used to monitor and document morphological changes in the bovine fetus at different stages of gestation. Conventionally, studies of embryonic development in cattle have depended on aborted fetuses, or those obtained at necropsy or slaughter. One of the early reports on the use of transrectal real-time ultrasonography to study early embryonic development in cattle was by Pierson and Ginther (67). They reported that the embryonic vesicle gradually increased in length until day 26 when it started encroaching into the opposite horn. By day 32, the embryonic vesicle fully occupied both horns. The heart beat was visualized between days 26 and 29. Another study from the same laboratory (69) reported the detection of ultrasonographically identifiable characteristics of the bovine conceptus during days 20 to 60 for gross fetal morphology, fetal heart beat, allantois, spinal cord, limb buds, amnion, optic vesicle, optic lens, split hooves, ribs, and fetal movement. Other workers (78) also found the technique useful to monitor embryonic growth and suggested that such close monitoring of the embryo would help to investigate early embryonic death in cattle.

Kahn (20,21) used transrectal ultrasonography with sector transducers (3.5 MHz and 5.0 MHz) to characterize changes in the size of fetal organs and body parts and to register the relative frequency of different intrauterine positions between 30 d and 10 mo of gestation. Until the fourth month, anterior and posterior presentations occurred with almost equal frequency, with a predominance of anterior presentations during months 5–7. Most large organs of the head, thorax, abdomen, and pelvis were visible with ultrasound during the first four months. Accessibility to the latter three body regions was restricted later on. However, in about 80% of the cases examined, the head was accessible throughout all stages of pregnancy. Fluid filled structures (eyes, braincase, heart, and stomach) were most easily recognizable because of the nonechogenic nature of their contents. Visualization of the entire fetus was difficult in advanced stages of pregnancy on account of the limited field of view and depth penetration of the sound waves. For close range visualization of the fetus, the 5 MHz transducer was used, while the 3.5 MHz transducer was preferred for viewing internal organs. The study demonstrated that gross anatomical structures of the bovine fetus can be observed in utero by transrectal ultrasound.

In a similar study performed on *Bos indicus* cows (79), the embryonic vesicle was first observable between days 18 and 20, fetal heart beat was detectable by day 22.6, and fetal movements were seen by day 50.7 ± 1.0. Growth of the embryo proper increased steadily till day 39, with a rapid increase in growth rate thereafter. The overall growth of the *Bos indicus* embryo was slower than that of the *Bos taurus* embryo.

In summary, sonographic imaging is a promising versatile technique to monitor fetal growth and well-being. The results available clearly demonstrate that transrectal ultrasound scanning is a precise technique for the determination of age and intrauterine development of the bovine fetus, and has several distinct advantages over conventional methods.

Fetal sex determination by ultrasound

One of the early reports of visualization of male and female characteristics of bovine fetuses by real-time ultrasound was by Muller and Wittkowski (80). Eighty-two cows were studied between 57 and 120 d gestation using both 3.0 MHz and 5.0 MHz transducers. The scrotal swelling in male and mammary glands in female fetuses were the references for sex determination with an accuracy of 94% between days 70 and 120. Kahn (21) reported that accurate sex determination can be performed after day 60 and that the gender of a male fetus was less difficult to determine than that of a female, based on the presence or absence of a scrotum.

Reports of fetal gender determination based on the genital tubercle are also available (22,81,82). In both sexes, the genital tubercle (forerunner of penis and clitoris) was easily recognizable by ultrasound as a prominent bilobular structure. However, it was not a useful ultrasonic indicator of sex until it reached the vicinity of the umbilical cord in males and the tail in females. On days 48 and 49, the genital tubercle was located between the hind limbs. In male fetuses it then moved cranially, reaching a point just caudal to the umbilical cord by day 56, on average. In female fetuses, it reached a point near the tail around day 54 (82). Curran and Ginther (22) reported up to 100% accuracy in sex determination by ultrasonography between days 50 and 100. However, considerable experience was found to be essential for the accurate determination of sex. The average time required to determine fetal sex varied from 2–15 min per cow (22,83).

In summary, prenatal sex determination is possible in cattle by transrectal real-time ultrasonography under both research and farm conditions. It appears to be a rapid and reliable technique. Accuracy of sex determination by an experienced operator is nearly 100% between days 60 and 70 of gestation. Diagnoses were inaccurate or impossible before 60 d of gestation and became less reliable as the age of the fetus advanced beyond 100 d. Predetermining the sex of fetuses carried by pregnant recipient animals will be of great advantage to commercial embryo transfer companies in planning marketing strategies. Diagnosis of fetal sex will also help dairy farmers in deciding whether or not to retain pregnant cows already earmarked for culling, depending on the sex of the fetus. Sexing of twins is also advantageous as it allows selective termination of unwanted pregnancies, such as those involving a male and a female fetus, which would otherwise bear a freemartin.

Ultrasonography in the diagnosis of ovarian and uterine abnormalities

The ultrasonographic appearance of a cystic CL was first described by Reeves *et al* (3). The usefulness of ultrasonography in diagnosing cystic ovarian conditions of the cow were soon reported by others (24,25,29,84) and provided practical diagnostic guidelines for differentiating follicular and luteal cysts. Follicular cysts revealed large (25–55 mm) nonechogenic areas with very thin walls. Luteal cysts on the contrary appeared as nonechogenic areas surrounded by echogenic tissue of varying thickness (2–5 mm). In cystic cows, treated with gonadotropin-releasing hormone, the wall of the cyst increased in thickness from 2 mm to 6 mm over a two-week period (29). Rajamahendran and Walton (85) and Peter *et al* (86) used ultrasonography to monitor the dynamics of follicular cyst formation following steroid administration in dairy cows. Prater *et al* (87) described the usefulness of ultrasound in detecting ovarian neoplasia.

Ultrasonography has also been found to be immensely useful in diagnosing uterine pathological conditions. Detailed studies (17,28) of common uterine pathological conditions, such as endometritis, pyometra, mucometra, and mummified and macerated fetuses, indicate that images of inflammatory conditions of the uterus are generally characterized by a distended lumen, filled to varying degrees with partially echogenic "snowy" patches. In conditions where fetal remnants are present, images allow visualization of the fragments. For instance, in the macerated fetal condition, the fetal bones were identifiable as echogenic particles in the uterine lumen suspended in the fetal fluids, and the uterine walls were thickened. In mummified fetuses, the uterine fluids were absent and the fetal mummy appeared as a poorly defined echogenic mass.

Studies have investigated the fate of the bovine conceptus and CL after embryonic death had been induced during early gestation using luteolytic substances, the intrauterine administration of colchicine, an antimitotic agent, or pure cultures of *Actinomyces pyogenes* (26,27). Through sequential ultrasound evaluations, changes in embryonic viability, cervical patency, uterine fluid volumes, and CL status were recorded. Both studies concluded that the CL of pregnancy was maintained when embryonic death resulted from the administration of colchicine or from bacterial invasion. In contrast, when abortion was induced with a luteolytic agent, the CL regressed within 24 to 72 h. Additionally, significant observations were that embryonic loss following luteolysis was characterized by rapid loss of the conceptus with minimal degeneration. The elimination of the conceptus and its breakdown products were primarily by expulsion through the cervix rather than by resorption (26).

Ultrasonography has thus been found to be a useful clinical tool for monitoring the response of cystic ovaries to therapy, studying induced cyst formation, studying conceptus loss following induced abortions, and diagnosing several kinds of ovarian and uterine pathological conditions.

Ultrasound in oocyte aspiration and in other applications

Rapid developments are taking place in bovine in vitro fertilization (IVF) leading to increased success in production of IVF embryos. Bovine IVF largely depends on oocytes aspirated from ovaries collected at slaughter, although laparotomy and laparoscopy are surgical approaches to procure follicular oocytes from the live cow. While repeated laparotomy for follicular aspiration is not a feasible proposition for obvious reasons (surgical trauma, postsurgical adhesions, and ethical considerations), laparoscopic follicular aspiration has been demonstrated to be a feasible and repeatable technique (88), yielding an up to 88% recovery rate. However, the long term effects of laparoscopy on the reproductive function and general health of animals subjected to the procedure are of serious concern, due to the invasive nature of the technique.

Over the years, alternative methods of obtaining follicular oocytes have been investigated. A technique for the aspiration of bovine oocytes during transvaginal ultrasound scanning of ovaries was first described by Pieterse *et al* (33), using a 5.0 MHz sector transducer. Both normally cycling and superovulated cows were used for transvaginal oocyte aspirations, and a 27.4% oocyte recovery was attained. Ovaries of superovulated cows were found to be easier to handle and aspirate, while puncturing small follicles was often difficult and risky, with increased chances of injury to the cow's rectum or even the operator's hand.

In a follow up study (89), repeated follicular aspiration was performed on 21 cows up to three times during each estrous cycle, at approximately six-day intervals over a three-month period. On every occasion, all follicles more than 3 mm in size were punctured and aspirated. The mean total number of follicles punctured per cycle was 12.6 ± 0.3, with the maximum number of follicles for puncture being available on day 3 or 4. The overall oocyte recovery rate was 55%, demonstrating substantial improvement in the efficiency. The IVF rate of transvaginally collected oocytes was significantly higher than that of oocytes aspirated from ovaries obtained at slaughter. The repeated interventions had no apparent deleterious effect on the estrous cycles of the animals studied (90). The findings indicated that transvaginal, ultrasound-guided, follicular aspiration is less traumatic and less invasive than laparoscopy, and that it is possible to repeat the procedure without follicular stimulation and without affecting the cyclicity of the animal. Repeated oocyte collections for IVF from the same cow was possible over several months, and more than 30 transferable embryos per cow could be produced in one year. These findings were further substantiated (91) by demonstrating that without any hormonal pretreatment, immature oocytes collected nonsurgically from cows during normal estrous cycles can be used successfully for in vitro production of viable embryos. This report proposed the transvaginal, ultrasound-guided, follicular aspiration technique in combination with IVF as an attractive and potential alternative to superovulation for bovine embryo production.

Apart from oocyte aspiration, the transvaginal, ultrasound-guided, puncture technique may also be useful for several other applications; for example, aspiration of fetal fluids for sex determination, biochemical analysis, and hormonal estimation; sampling of uterine contents for diagnostic purposes; subsampling of follicular fluid and fluid in the central cavity of CL; injection of substances into the ovaries, follicles, uterus, et cetera; and perhaps even selective elimination of embryos during the early stage of unwanted twin pregnancies. Successful collection of bovine fetal fluid through a transvaginal, ultrasound-guided, puncture technique has already been reported (92). The earliest successful aspiration of amniotic fluid was on day 44 of pregnancy. Even though this study demonstrated the possibility of repeated sampling of amniotic and allantoic fluids up to five times at weekly intervals, the risk of intrauterine fetal death increased with repeated punctures.

The usefulness of real-time ultrasonography in AI training has been reported (34). Ultrasound was used to evaluate the efficiency of AI trainees in depositing semen at the desired site by using a brass bead and string attached to the AI sheath, which was deposited at the site of AI. The location of the bead was immediately confirmed by ultrasound scanning. This method of training is reportedly quick, easy, and effective.

Ultrasonography in male reproduction

Breeding soundness assessments of dairy bulls are currently based on semen parameters, scrotal circumference, and testicular palpation. Specialized examination procedures, such as testicular biopsy, thermography, or tonometry, may involve risks to the reproductive potential of the bulls. Because of this, there has been interest in the application of ultrasonography to assess the normal anatomical appearance of bull testes (30) and accessory sex glands (32). Studies (93,94) have also investigated the possible correlation between ultrasonically determined testicular parameters and breeding soundness score parameters. The testicular diameter measured accurately by ultrasound correlated well with testicular circumference, weight, and volume (94). However, a routine testicular ultrasound examination contributes no significant additional information that could be of value in breeding soundness evaluations (93). Sidibe *et al* (31) also found ultrasonography not useful for the objective evaluation of artificially induced testicular degeneration in the bull. Since ultrasound waves have no adverse effects on testicular development, sperm production, and semen quality (95), scope for increased application of ultrasonography in male reproduction still remains. Despite the discouraging results obtained (31,93), real-time ultrasonography may have significant advantages over other techniques in the diagnosis of testicular and epididymal abnormalities. The most promising application, however, seems to be in the diagnosis of pathological conditions of the accessory sex glands, since digital palpation has severe limitations.

Comment

In less than a decade, the quantum of new information generated through the use of diagnostic ultrasound in the field of bovine reproduction is enormous. The advent of ultrasound technology, with the important capability of following the sequential growth and demise of follicles has improved our understanding of folliculogenesis and CL dynamics during different reproductive states. This has contributed richly towards a better understanding of the complex subject of animal reproduction as a whole. The reputation of ultrasound for safety (96) and ease of operation only strengthens its potential for future applications. Even though the technology has been in vogue for over 10 yr, no study has so far systematically analyzed the cost-effectiveness of ultrasonography in routine herd management. Will the routine use of ultrasound improve the economic performance of such herds? As of today, there is no definite answer to this question. A preliminary evaluation along these lines was conducted in the authors' laboratory (97), where ultrasound imaging is being used for routine check-up of all cows (herd size ≃ 40 milch animals) between 30 and 40 d postpartum to evaluate their reproductive status. Based on the findings, appropriate managemental measures are taken immediately. This procedure helps in early detection of silent estrus, anestrus, and cystic ovarian conditions, and has proved useful in reducing days to first service, days open, and calving interval. It seems apparent that continuing the practice will further help in improving the efficiency of the herd. It would be worth taking up such investigations on large herds, so that critical evaluations could be made of the economic benefits that ultrasound promises to offer.

The feasibility of transcervical, ultrasound-guided, intrafallopian placement of gametes, zygotes and embryos has been demonstrated in human reproduction (98). Such novel approaches may have practical value in bovine reproduction as well. Particularly in the transfer of valuable, in vitro produced, zygotes to the recipient fallopian tube to overcome in vitro developmental blocks, thereby increasing the chances of successful implantation.

There is wide scope for the use of ultrasound as a tool to increase our understanding of bovine reproduction and to manipulate the reproductive processes to maximize the reproductive efficiency of this species. We are confident that this modern technology will soon be a popular tool for early pregnancy diagnosis, prenatal sexing, and the reproductive health management of cattle. CVJ

References

1. Lindahl IL. Detection of pregnancy in sheep by means of ultrasound. Nature 1966; 212: 642–643.
2. Chaffaux S, Valon F, Martinez J. Evoluation de l'image echographique du produit de conception chez la vache. Bull Acad Vet Fr 1982; 55: 213–221.
3. Reeves JJ, Rantanen NW, Hauser M. Transrectal real-time ultrasound scanning of the cow reproductive tract. Theriogenology 1984; 21: 485–494.
4. Pierson RA, Ginther OJ. Ultrasonography of the bovine ovary. Theriogenology 1984; 21: 495–504.
5. Pierson RA, Ginther OJ. Follicular populations during the estrous cycle in heifers. I. Influence of day. Anim Reprod Sci 1987; 14: 165–176.
6. Sirois J, Fortune JE. Ovarian follicular dynamics during the estrous cycle in heifers monitored by real-time ultrasonography. Biol Reprod 1988; 39: 308–317.
7. Guilbault LA, Dufour JJ, Thatcher WW, Drost M, Haibel GK. Ovarian follicular development during early pregnancy in cattle. J Reprod Fertil 1986; 78: 127–135.
8. Pierson RA, Ginther OJ. Ovarian follicular populations during early pregnancy in heifers. Theriogenology 1986; 26: 649–659.
9. Rajamahendran R, Taylor C. Characterization of ovarian activity in postpartum dairy cows using ultrasound imaging and progesterone profiles. Anim Reprod Sci 1990; 22: 171–180.
10. Savio JD, Boland MP, Hynes N, Roche JF. Resumption of follicular activity in the early post partum period of dairy cows. J Reprod Fertil 1990; 88: 569–576.
11. Grasso F, Guilbault LA, Roy GL, Lussier JG. Ultrasonic determination of ovarian follicular development in superovulated heifers pretreated with FSH-P at the beginning of the estrous cycle. Theriogenology 1989; 31: 1209–1220.
12. Pierson RA, Ginther OJ. Follicular populations during the estrous cycle in heifers III: Time of selection of the ovulatory follicle. Anim Reprod Sci 1988; 16: 81–95.
13. Larsson B. Determination of ovulation by ultrasound examination and its relation to LH-peak in heifers. J Vet Med [A] 1987; 34: 749–754.
14. Rajamahendran R, Robinson J, Desbottes S. The use of ultrasonography to determine the onset of ovulation in dairy cows. Proc Annu Meet West Sec Am Soc Anim Sci 1988; 39: 309–311.
15. Sprecher DJ, Nebel RL, Whitman SS. The predictive value, sensitivity and specificity of palpation per rectum and transrectal ultrasonography for the determination of bovine luteal status. Theriogenology 1989; 31: 1165–1172.
16. Pieterse MC, Taverne MAM, Kruip AM, Willemse AH. Detection of corpora lutea and follicles in cows: a comparison of transvaginal ultrasonography and rectal palpation. Vet Rec 1990; 126: 552–554.
17. Fissore RA, Edmondson AJ, Pashen RL, Bondurant RH. The use of ultrasonography for the study of the bovine reproductive tract. II. Non pregnant, pregnant and pathological conditions of the uterus. Anim Reprod Sci 1986; 12: 167–177.
18. Taverne MAM, Szenci O, Szetag J, Piros A. Pregnancy diagnosis in cows with linear array real time ultrasound scanning: a preliminary note. Vet Q 1985; 7: 264–270.
19. Chaffaux S, Reddy GNS, Valon F, Thibier M. Transrectal real-time ultrasound scanning for diagnosing pregnancy and for monitoring embryonic mortality in dairy cattle. Anim Reprod Sci 1986; 10: 193–200.
20. Kahn W. Sonographic fetometry in the bovine. Theriogenology 1989; 31: 1105–1121.
21. Kahn W. Sonographic imaging of the bovine fetus. Theriogenology 1990; 33: 385–396.
22. Curran S, Ginther OJ. Ultrasonic determination of fetal gender in horses and cattle under farm conditions. Theriogenology 1991; 36: 809–814.
23. Okano A, Tomizuka T. Ultrasonic observation of postpartum uterine involution in the cow. Theriogenology 1987; 27: 369–377.
24. Farin PW, Youngquist RS, Parfet JR, Gaverick HA. Diagnosis of luteal and follicular ovarian cysts in dairy cows by sector scan ultrasonography. Theriogenology 1990; 34: 633–642.
25. Carroll DJ, Pierson RA, Hauser ER, Grummer RR, Combs DK. Variability of ovarian structures and plasma progesterone profiles in dairy cows with ovarian cysts. Theriogenology 1990; 34: 349–370.
26. Kastelic JP, Ginther OJ. Fate of conceptus and corpus luteum after induced embryonic loss in heifers. J Am Vet Med Assoc 1989; 194: 922–928.
27. Semambo DKN, Boyd JS, Taylor DJ, Ayliffe TR, Omran, SN. Ultrasonographic study of early embryonic loss induced by *Actinomyces pyogenes* in cattle. Vet Rec 1992; 131: 7–12.
28. Kahn W, Leidl W. Ultrasonic characteristics of pathological conditions of the bovine uterus and ovaries. Curr Top Vet Med Anim Sci 1989; 51: 53–65.
29. Edmondson AJ, Fissore RA, Pashen RL, Bondurant RH. The use of ultrasonography for the study of the bovine reproductive tract. I. Normal and pathological ovarian structures. Anim Reprod Sci 1986; 12: 157–165.
30. Pechman RD, Eilts BE. B-mode ultrasonography of the bull testicle. Theriogenology 1987; 27: 431–441.

31. Sidibe M, Franco LA, Fredriksson G, Madej A, Malmgren L. Effects on testosterone, LH and cortisol concentrations, and on testicular ultrasonic appearance, of induced testicular degeneration in bulls. Proc 12th Int Congr Anim Reprod and AI 1992; 1: 162–164.
32. Weber JA, Hilt CJ, Woods GL. Ultrasonic appearance of bull accessory sex glands. Theriogenology 1988; 29: 1347–1353.
33. Pieterse MC, Kappen KA, Kruip ThAM, Taverne MAM. Aspiration of bovine oocytes during transvaginal ultrasound scanning of the ovaries. Theriogenology 1988; 30: 751–762.
34. Beal WE, Edwards RB, Kearnan JM. Use of B-mode, linear array ultrasonography for evaluating the technique of bovine artificial insemination. J Dairy Sci 1989; 72: 2198–2202.
35. Pierson RA, Ginther OJ. Ultrasonic imaging of the ovaries and uterus in cattle. Theriogenology 1988; 29: 21–37.
36. Perry RC, Beal WE, Corah LR. Reproductive applications of ultrasound in cattle. 1. Monitoring ovarian structures. Agri-Pract 1990; 11 (4, Jul/Aug): 28–32.
37. Boland MP, Murphy MG, Roche JF. The use of ultrasound to monitor ovarian function in farm animals. AgBiotech News Information 1990; 2: 841–845.
38. Sirois J, Fortune JE. Monitoring ovarian follicular development in cattle by ultrasound imaging: A 1990 update. Embryo Transfer News Letter 1990; 8 (4, Dec): 9–16.
39. Griffin PG, Ginther OJ. Research applications of ultrasonic imaging in reproductive biology. J Anim Sci 1992; 70: 953–972.
40. Perry RC, Beal WE, Corah LR. Reproductive applications of ultrasound in cattle 2. Monitoring uterine characteristics and pregnancy. Agri-Pract 1990; 11 (6, Nov/Dec): 31–35.
41. Peter AT, Jakovljevic S, Pierson RA. Use of real-time ultrasonography in bovine and equine reproduction. Compend Contin Educ Pract Vet 1992; 14: 1116–1125.
42. McDicken WN. Diagnostic Ultrasonics, Principles and Use of Instruments. 2nd ed. New York: John Wiley, 1981.
43. Bartum RJ, Crow HC. Real Time Ultrasound. Philadelphia: WB Saunders, 1983.
44. Powis RL. Ultrasound science for the veterinarian. In: Rantanen NW, ed. The Veterinary Clinics of North America: Equine Practice. Philadelphia: WB Saunders, 1986: 3–28.
45. Pierson RA, Kastelic JP, Ginther OJ. Basic principles and techniques for transrectal ultrasonography in cattle and horses. Theriogenology 1988; 29: 3–20.
46. Ligtvoet CM, Bom N, Gussenhoven WJ. Technical principles of ultrasound. In: Taverne MAM, Willemse AH, eds. Diagnostic Ultrasound and Animal Reproduction. Dordrecht: Kluwer, 1989: 1–9.
47. Szenci O. Early bovine, porcine and equine pregnancy diagnosis with a battery operated portable ultrasonic scanner. Proc 12th Int Congr Anim Reprod and AI 1992; 1: 168–170.
48. Spicer LJ, Echternkamp SE. Ovarian follicular growth, function and turnover in cattle: a review. J Anim Sci 1986; 62: 428–451.
49. Knopf L, Kastelic JP, Schallenberger E, Ginther OJ. Ovarian follicular dynamics in heifers: Test of two wave hypothesis by ultrasonically monitoring individual follicles. Domest Anim Endocrinol 1989; 6: 111–119.
50. Ginther OJ, Knopf L, Kastelic JP. Ovarian follicular dynamics in heifers during early pregnancy. Biol Reprod 1989; 41: 247–254.
51. Taylor C, Rajamahendran R. Follicular dynamics and corpus luteum growth and function in pregnant versus non pregnant dairy cows. J Dairy Sci 1991; 74: 115–123.
52. Perry RC, Corah LR, Kiracofe GH, Stevenson JS, Beal WE. Endocrine changes and ultrasonography of ovaries in suckled beef cows during resumption of postpartum estrous cycles. J Anim Sci 1991; 69: 2548–2555.
53. Murphy MG, Boland MP, Roche JF. Pattern of follicular growth and resumption of ovarian activity in postpartum beef suckler cows. J Reprod Fertil 1990; 90: 523–533.
54. Savio JD, Bongers H, Drost M, Lucy MC, Thatcher WW. Follicular dynamics and superovulatory response in Holstein cows treated with FSH-P in different endocrine states. Theriogenology 1991; 35: 915–929.
55. Manik RS, Madan ML, Ambrose JD, Singla SK, Chauhan MS. Real time ultrasound scanning for study of follicular population on the day of estrus and ovulation in buffaloes. Proc. 12th Int Congr Anim Reprod and AI 1992; 1: 234–236.
56. Robertson L, Cattoni JC, Shand RI, Jeffcoate IA. A critical evaluation of ultrasonic monitoring of superovulation in cattle. Br Vet J 1993; 149: 477–484.
57. Guilbault LA, Grasso F, Lussier JG, Rouillier P, Matton P. Decreased superovulatory responses in heifers superovulated in the presence of dominant follicle. J Reprod Fertil 1991; 91: 81–89.
58. Huhtinen M, Rainio V, Aalto J, Bredbacka P, Maki-Tanila A. Increased ovarian responses in the absence of a dominant follicle in superovulated cows. Theriogenology 1992; 37: 457–463.
59. Gray BW, Cartee RE, Stringfellow DA, Riddell MG, Riddell KP, Wright JC. The effects of FSH priming and dominant follicular regression on the superovulatory response of cattle. Theriogenology 1992; 37: 631–639.
60. Calder M, Rajamahendran R. Follicular growth, ovulation and embryo recovery in dairy cows given FSH at the beginning or middle of the estrous cycle. Theriogenology 1992; 38: 1163–1174.
61. Rajamahendran R, Robinson J, Desbottes S, Walton JSW. Temporal relationships among estrus, body temperature, milk yield, progesterone and luteinizing hormone levels, and ovulation in dairy cows. Theriogenology 1989; 31: 1173–1182.
62. Omran SN, Ayliffe TR, Boyd JS. Preliminary observations of bovine ovarian structures using B-mode real time ultrasound. Vet Rec 1988; 122: 465–466.
63. Pieterse MC. Ultrasonic characteristics of physiological structures of bovine ovaries. Curr Top Vet Med Anim Sci 1989; 51: 37–51.
64. Kastelic JR, Pierson RA, Ginther OJ. Ultrasonic morphology of corpora lutea and central cavitites during the estrous cycle and early pregnancy in heifers. Theriogenology 1990; 34: 487–498.
65. Ambrose JD, Manik RS, Singla SK, Madan ML. A simplified laparoscopy technique for repeated ovarian observation in the water buffalo (Bubalus bubalis). Theriogenology 1993; 40: 487–496.
66. Rajamahendran R, Sianangama PC. Effect of hCG on dominant follicles in cows: Accessory corpus luteum formation, progesterone production and pregnancy rates. J Reprod Fertil 1992; 95: 577–584.
67. Pierson RA, Ginther OJ. Ultrasonography for detection of pregnancy and study of embryonic development in heifers. Theriogenology 1984; 22: 225–233.
68. Curran S, Pierson RA, Ginther OJ. Ultrasonic appearance of the bovine conceptus from days 10 through 20. J Am Vet Med Assoc 1986; 189: 1289–1294.
69. Curran S, Pierson RA, Ginther OJ. Ultrasonic appearance of the bovine conceptus from 20 through 60 days. J Am Vet Med Assoc 1986; 189: 1295–1302.
70. Hanzen C, Delsaux B. Use of transrectal B-mode ultrasound imaging in bovine pregnancy diagnosis. Vet Rec 1987; 121: 200–202.
71. Hughes EA, Davies DAR. Practical uses of ultrasound in early pregnancy diagnosis in cattle. Vet Rec 1989; 124: 456–458.
72. Kastelic JP, Curran S, Ginther OJ. Accuracy of ultrasonography for pregnancy diagnosis on days 10 to 22 in heifers. Theriogenology 1989; 31: 813–820.
73. Willemse AH, Taverne MAM. Early pregnancy diagnosis in cattle by means of transrectal real time ultrasound scanning of the uterus. Curr Top Vet Med Anim Sci 1989; 51: 67–72.
74. Boyd JS, Omran SN, Ayliffe TR. Evaluation of real time B-mode ultrasound scanning for detecting early pregnancy in cows. Vet Rec 1990; 127: 350–352.
75. Pieterse MC, Szenci O, Willemse AH, Bajcsy CSA, Dieleman SJ, Taverne MAM. Early pregnancy diagnosis in cattle by means of linear array real time ultrasound scanning of the uterus and a qualitative and quantitative milk progesterone test. Theriogenology 1990; 33: 697–707.
76. Badtram GA, Gaines JD, Thomas CB, Bosu WTK. Factors influencing the accuracy of early pregnancy detection in cattle by real time ultrasound scanning of the uterus. Theriogenology 1991; 35: 1153–1167.
77. Ivkov V, Veselinovic S, Mickovski G, Medic D, Popovski K, Veselinovic S. Use of ultrasound in dairy cattle reproduction. Proc 12th Int Congr Anim Reprod and AI 1992; 1: 141–143.
78. Boyd JS, Omran SN, Ayliffe TR. Use of a high-frequency transducer with real time B-mode ultrasound scanning to identify early pregnancy in cows. Vet Rec 1988; 123: 8–11.
79. Totey SM, Singh G, Taneja M, Talwar GP. Ultrasonography for detection of early pregnancy following embryo transfer in unknown breeds of *Bos indicus* cows. Theriogenology 1991; 35: 487–497.
80. Muller E, Wittkowski G. Visualization of male and female characteristics of bovine fetuses by real time ultrasonics. Theriogenology 1986; 25: 571–574.
81. Curran S. Fetal sex determination in cattle and horses by ultrasonography. Theriogenology 1992; 37: 17–21.

82. Curran S, Kastelic JP, Ginther OJ. Determining sex of the bovine fetus by ultrasonic assessment of the relative location of the genital tubercle. Anim Reprod Sci 1989; 19: 217–227.
83. Wideman D, Dorn CG, Kraemer DC. Sex detection of the bovine fetus using linear array real-time ultrasonography. Theriogenology 1989; 31:272 (Abstract).
84. Sprecher DJ, Nebel RL. B-mode ultrasonic morphology of bovine follicular and luteal ovarian cysts. Agri-Pract 1988; 9 (2, Jan/Feb): 5–8.
85. Rajamahendran R, Walton JS. Effect of treatment with estradiol valerate on endocrine changes and ovarian follicular populations in dairy cows. Theriogenology 1990; 33: 441–452.
86. Peter AT, Asem EK, Padmakumar U. Dynamics of follicular growth in steroid induced cystic ovaries as determined by ultrasonography. Proc 12th Int Congr Anim Reprod AI 1992; 1: 257–259.
87. Prater PE, Shires M, Coley RB. Diagnostic aids for ovarian neoplasia: serologic interpretation and ultrasonography. Vet Med 1988; 83: 1273–1276.
88. Lambert RD, Bernard C, Rioux JE, Beland R, D'Amours D, Montreuil A. Endoscopy in cattle by the paralumbar route: Technique for ovarian examination and follicular aspiration. Theriogenology 1983; 20: 149–161.
89. Pieterse MC, Vos PLAM, Kruip ThAM, *et al.* Trans-vaginal ultrasound guided follicular aspiration of bovine oocytes. Theriogenology 1991; 35: 19–24.
90. Pieterse MC, Vos PLAM, Kruip TAM, Willemse AH, Taverne, MAM. Characteristics of bovine estrous cycles during repeated transvaginal, ultrasound guided puncturing of follicles for ovum pick-up. Theriogenology 1991; 35:401–413.
91. Kruip TAM, Pieterse MC, Beneden TH van, Vos PLAM, Wurth YA, Taverne MAM. A new method for bovine embryo production: a potential alternative to superovulation. Vet Rec 1991; 128: 208–210.
92. Vos PLAM, Pieterse MC, Weyden GC van der, Taverne MAM. Bovine fetal fluid collection: transvaginal, ultrasound-guided puncture technique. Vet Rec 1990; 127: 502–504.
93. Eilts BE, Pechman RD. B-Mode ultrasound observations of bull testes during breeding soundness examinations. Theriogenology 1988; 30: 1169–1175.
94. Cartee RE, Gray BW, Powe TA, Hudson RS, Whitesides J. Preliminary implications of B-mode ultrasonography of the testicles of beef bulls with normal breeding soundness examinations. Theriogenology 1989; 31: 1149–1157.
95. Coulter GH, Bailey DRC. Effects of ultrasonography on the bovine testes and semen quality. Theriogenology 1988; 30: 743–749.
96. Miller DL. Update on safety of diagnostic ultrasonography. J Clin Ultrasound 1991; 19: 531–540.
97. Taylor C, Rajamahendran R. The use of real-time ultrasonography in postpartum management of dairy cattle. J Dairy Sci 1991; 74 (Suppl) 1: 193 (Abstract).
98. Bustillo M, Schulman JD. Transcervical ultrasound guided intrafallopian placement of gametes, zygotes and embryos. J In Vitro Fertil Embryo Transf 1989; 6: 321–324.

Answers to Quiz Corner/Les réponses du Test Éclair

1. c — Some infected cats will die from FeLV infection; however, many more will recover and become immune.
   c — Quelques chats infectés par le FeLV mourront; cependant, beaucoup plus se rétabliront et seront immunisés.

2. c — Hypertonic enemas should not be administered to cats or small dogs, especially if the animal is obstipated.
   c — Les lavements hypertoniques ne devraient pas être administrés aux chats ou à de petits chiens, surtout s'ils souffrent de constipation opiniâtre.

3. d

4. b — These horses should not be allowed to eat anything before definitive treatment at a referral center. Treatment usually involves general anesthesia and softening of the feces. The other options would be appropriate.
   b — Ces chevaux ne devraient rien manger avant le traitement définitif au centre de référence. Le traitement implique habituellement une anesthésie générale et le ramollissement des fèces. Les autres options seraient appropriées.

5. a — Tetracyclines are specific treatment for chlamydial diseases of sheep.
   a — Le traitement aux tétracyclines est spécifique pour les infections chlamydiales des moutons.

6. e — Electric heating pads may cause thermal burns and are particularly dangerous for the unconscious or immobile patient. They have no place in clinical practice and their use only invites a lawsuit.
   e — Les coussins chauffants électriques peuvent causer des brûlures et ils sont particulièrement dangereux pour le patient inconscient ou immobilisé. Ils n'ont pas leur place dans une pratique vétérinaire et leur utilisation ne peut que vous attirer des poursuites judiciaires.

7. a

8. b — Calving interval is the most encompassing measure of reproductive performance. However, it is the slowest to change and, therefore, is not a good indicator of short-term performance to use for decision making.
   b — L'intervalle vêlage-vêlage est la mesure la plus globale de la performance de reproduction. Cependant, la modification de cet indice se fait très lentement et, par conséquent, cet indice n'est pas un bon indicateur de la performance à court terme à utiliser pour la prise de décision.

9. b — A tear of the medial meniscus occurs in 40% to 60% of cases involving rupture of the cranial cruciate ligament.
   b — Une déchirure du ménisque médial se produit dans 40 % à 60 % des cas de rupture du ligament croisé cranial.

10. e — The protein content of milk replacer should be at least 20%. The National Research Council recommends 22%. However, 20% is adequate provided that all of the protein ingredients are from milk sources.
    e — Le contenu protéique du lait de remplacement devrait être d'au moins 20 %. Le National Research Council recommande 22 %. Cependant, 20 % est un contenu adéquat en autant que tous les ingrédients protéiques proviennent de source lactée.