Bovine Reproductive Ultrasonography: A Review

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Abstract. The advent of ultrasonography over a decade ago has contributed tremendously in the field of bovine reproduction. Ultrasound imaging has shown that cattle exhibit 2 or 3 waves of follicular development during an estrous cycle. Ultrasonography allows monitoring of individual follicles as they grow and/or regress over time and patterns of follicular development can thus be determined with relative precision. Other important applications of ultrasonography in bovine reproduction includes; pregnancy diagnosis, fetal sex determination, folliculocenteses, diagnosis of abnormalities of the reproductive organs, monitoring of treatment of ovarian cysts, monitoring of postpartum genital resumption, ultrasound-guided centesis and male genital ultrasonography. Recent applications of ultrasonography in bovine reproduction includes doppler ultrasonography for genital blood flow and mammary ultrasonography. The use of ultrasonography is indispensable in most studies in which morphology (structure) and function are correlated. In this paper, the literature pertaining to the use of ultrasonography in bovine reproduction is reviewed. The applications and limitations of ultrasonography are highlighted. The non-invasive nature of ultrasonography makes it an excellent clinical and research tool in bovine reproduction.

Key words: Bovine, Reproduction, Ultrasonography.

The application of real-time B-mode ultrasonography to bovine reproduction has grown rapidly in the last decade. It is apparent that ultrasonography has provided answers to a number of hitherto unanswered questions with regard to bovine reproductive cycle and its concurrent disorders. One of the greatest advantages of ultrasonography is that it is totally non-invasive and so repeated examinations of an animal’s reproductive tract can be performed without impairing its breeding potential or having adverse effect on the conceptus. Real-time ultrasonic examination has allowed the monitoring of individual follicles on a daily basis. It is now clearly established that the growth of follicles in bovine ovaries occurs in a wave-like manner with two or three waves per estrous cycle.

Accurate early pregnancy detection can be achieved easily and instantly through the use of ultrasonography. Ultrasonographic fetometry allows the estimation of fetal age, assessment of progression of fetal growth and diagnosis of pregnancy disorders. Furthermore, fetal sex can be determined by ultrasonography.

The course of uterine involution (postpartum) can be monitored by ultrasonography. Characteristics of uterine disorders (endometritis, pyometra, hydrometra) have also been documented [1]. Ultrasonography has helped tremendously in the diagnosis and differentiating the types of ovarian cysts in cattle [2–5] and ovarian tumors [1]. The use of ultrasonography in monitoring the response to treatment of ovarian cysts in cattle has also been reported [3, 6, 7].

Ultrasonically guided amnio- and allantocente-
ses in pregnant cows [8] and ultrasound guided transvaginal folliculocentesis for *in-vitro* fertilization [9] and mammary ultrasonography [10, 11] are other important applications of ultrasonography. The use of ultrasonography to help predict observed oestrus in dairy cows after administration of prostaglandin has been reported recently [12]. Currently, there is scanty of information on the use of ultrasonography in bovine male reproduction. Ultrasonographic characteristics of bull testes and accessory sex glands have been reported [13–15]. The potential for the use of ultrasonography in bovine male reproduction is enormous.

Although some review articles on bovine and large animal ultrasonography have appeared previously [16, 17], the need to have an up-to-date information on the rapidly growing field of bovine reproductive ultrasonography prompted this review.

**Principles of Ultrasonography**

Ultrasound is defined as any sound frequency above the normal hearing range of the human ear; i.e. greater than 20,000 Hz [18]. Detailed reviews of basic principles of transrectal ultrasonography have been reported [18–21]. Briefly, ultrasonography utilizes high frequency sound waves to produce cross sectional images of the tissues and internal organs. The sound waves are produced by vibrations of specialized crystals (piezo-electrical crystals) housed in the ultrasound transducer. Vibrations of the crystals are produced by pulses of electric current. A proportion of sound waves reflected back to the transducer is converted to electric current and displayed as an echo on the ultrasound viewing screen. The transducer, therefore, acts as both the sender and receiver of echoes. The echoes are evident on the viewing screen as varying shades of gray (black to white). The absolute value of the acoustic impedance of any tissue is relatively unimportant, because it is the magnitude of the difference in acoustic impedance at tissue interface that determine the amount of reflection of the beam [18].

Most ultrasound scanners used in bovine reproduction currently are B-mode (brightness modality) real-time scanners. In B-mode ultrasonography, the image is a two-dimensional display of dots (pixels), the brightness of the dots is proportional to the amplitude of the reflected echoes returning to the transducer. Real-time refers to the ability to image movements (e.g. fetal heart beat or motion) as it occurs. Dynamics of some reproductive structures or events (i.e. ovulation) could be studied by video tape recordings of real-time ultrasound examinations.

Ultrasound scanners are equipped with transducers of varying frequencies. The most commonly used frequencies in bovine reproduction are 3.5, 5.0 and 7.5 MHz. The higher the frequency of the transmitted sound waves, the better the image resolution, but the shallower the depth of penetration [2, 18]. There are two types of scanners; linear array and sector. Linear array transducers have piezo-electric crystals arranged in rows and as such the image produced by linear array transducer appears rectangular. Sector transducers, on the other hand, have only a few such crystals and the image produced is pie-shaped corresponding to the field of scan. Mechanical sector scanners offer multi-frequency capability in a variety of scan head design with 3.0, 5.0 and 7.5 MHz crystals in a single scan head. Because of the versatility of these machines, they are also more expensive than linear-array systems [22]. Recent ultrasound scanners have a wider frequency range and accept a full line of single and dual frequency probes. Digital image port for computer image storage is also available. Battery-powered portable ultrasound scanners are also currently available.

Doppler ultrasonography which detects turbulence within blood vessels and direction of flow, is also a useful diagnostic tool in bovine reproduction. The Doppler phenomenon is the change in sound frequency of a moving object as perceived by a stationary observer. Doppler ultrasound machines detect frequency change and, therefore, movement which is converted to an audible signal [23].

The major considerations in selecting an ultrasound scanner are price, resolution quality, portability, serviceability and technical support [24]. Other factors include memory capabilities, remote control, transducer and cable design (I or T), single or dual frequency probe and above all, the use for which the machine would be put. For routine bovine reproductive ultrasonography (early pregnancy diagnosis, pathology of the ovaries and uterus, fetal sexing etc) a 5 MHz linear recta
transducer seem to be the most versatile and effective. However, a 7.5 MHz linear transducer is recommended for follicular dynamics studies. For transvaginal oocyte recoveries, a convex-linear transducer gives better results.

**Techniques**

For transrectal or transcutaneous ultrasound scanning in cattle, no sedation is indicated as the procedure is totally non-invasive and well tolerated. Adequate restraint is however required and the scanner should be placed at a sensible distance from the cow/bull on the side opposite the operator’s rectalling arm. All precautions that apply to palpation per rectum are applicable to transrectal scanning.

All feces from the rectum should be evacuated prior to introduction of the transducer. It is often advantageous to carry out a preliminary exploration of the topography of the reproductive tract before commencing the ultrasonographic examination. The transducer face is lubricated with a suitable coupling medium and is usually covered by a lubricated plastic sleeve before insertion in a cupped, lubricated hand through the anal opening. It is then progressed cranially along the rectal floor to overlie the reproductive tract. The transducer face must be pressed firmly against the rectal mucosa in order to effect ultrasound transmission through the rectal wall into abdominal viscera. The probe is moved across the reproductive tract in a thorough and systemic manner [23, 25].

**Basal requirements**

To make an accurate diagnosis via an ultrasonographic examination, ambient lighting is imperative. A darkroom is ideal for viewing the monitor and helps the human eye recognize as many shades of gray as possible. When examinations are carried out in lighted conditions, some type of hood must be draped over the monitor to facilitate effective gray-shade delineation [24].

Interposition of any contaminating feces will prevent ultrasound transmission and produce poor imaging and artefactual interference. The ultrasound screen and the human eye should be at similar level for accurate interpretation of ultrasound images.

**Interpretation of ultrasound images**

Interpretation of ultrasonographs of the reproductive tract require a thorough understanding of the composition of the images and an awareness of the possible artifacts which can occur and lead to a misdiagnosis. As sound waves pass through the tissues and surrounding areas they may be modified in a number of ways. Sound waves passing through body structures will encounter tissue interfaces and the returning echoes will be of varying strengths and so produce a variety of images.

The ultrasonic characteristic of a tissue depends on its ability to reflect sound waves. Liquids do not reflect sound waves (i.e. are nonechogenic or anechoic) and are represented on the viewing screen as black. The ultrasonic images of liquid-containing portions of structures such as ovarian follicles, embryonic vesicles appear black. Dense tissues (e.g. bone) reflect a large proportion of the transmitted sound waves (i.e. echogenic) and is represented on the viewing screen as light gray or white. Various tissues and contents of the reproductive tract appear on the screen in varying shades of gray depending upon their echogenicity [18].

Some common artifacts include; distant enhancement (occurs when the incident sound beam strikes the far wall of a fluid-filled structure; e.g follicle, embryonic vesicle), refraction artifacts, specular reflection, reverberation artifacts, acoustic shadowing [23]. A detailed review of imaging artifacts in diagnostic ultrasound has been published [26].

**Ultrasonography of Normal Ovarian Structures**

**Follicles**

The ultrasonographic anatomy of the ovaries of the cow has been described in detail [3, 27–29]. Antral follicles of various sizes appear as non-echogenic structures which could be distinguished from blood vessels in cross-section by the elongated appearance of the latter [29, 30].

There was a linear relationship between follicle diameter measured by in vivo ultrasonography and follicle diameter determined after slaughter [31]. Correlation coefficients of 0.7–0.9 for various sizes of follicular structures were recorded between in vivo ultrasonography and postmortem slicing of excised ovaries [31–35]. These studies showed that transrectal ultrasonography is a reliable method for identifying and measuring follicles in bovine ovaries. In a recent study on ultrasound echotex-
nature of follicles and functional and endocrine status in 32 heifers [36], it was shown that the echotexture characteristics of the ultrasound images of the follicle antrum and wall (after ovariectomy) were correlated with the functional and endocrine status of a follicle. Mean pixel (picture element) values (grey scale: black=0; white=255) for the follicle wall and stroma increased progressively from the growing to regressing phases of the dominant follicle of the first follicular wave. Pixel heterogeneity of the antrum and wall were negatively correlated with estradiol and estradiol:progesterone ratio in follicular fluid.

Ovarian ultrasonography has shown that the development of bovine follicles occurs in a distinct, striking regular pattern [37]. Each wave consists of the contemporaneous emergence of a group (cohort) of follicles 5 mm or more in diameter. Within several days, one follicle has grown larger than the cohort and is considered dominant [38–41]. Ultrasonography has revealed the existence of two [32, 42, 43] or three [40, 41] follicular waves per estrous cycle in heifers.

Each dominant follicle has a growing phase and a static phase; each lasting for about 5–6 days. The dominant follicle of the first wave is anovulatory. It remains dominant for 4–5 days, and generally by day 11 or 12 of the estrous cycle, it loses its dominance and begins to regress which lasts for 5–7 days. In the meantime, the second wave of follicles has been recruited and selection of the second wave dominant follicle has occurred, this dominant follicle goes on to ovulate. In a three wave cycle, however, this second dominant follicle regresses, making way for yet another group of follicles, with the third dominant follicle ovulating [43, 44]. Analysis of follicular dynamics has shown that dominant non-ovulatory follicles remain as the largest follicle for several days after the start of the succeeding wave, which implied that a dominant follicle retains morphological dominance (largest follicle on a pair of ovaries) longer than it retains functional dominance (ability to suppress growth of other follicles) [45].

Ultrasonography has been used to study ovarian response to superovulation in cattle [46–49]. The influence of a dominant follicle on the superovulatory response has been reported in heifers and cows [50–53]. The presence of a dominant follicle seem to have a negative influence on superovulatory response. Thus, assessment of follicular status prior to initiation of a superovulatory treatment may be an important consideration. Guilbault et al. [54] have indicated that decreased superovulatory response and embryo recovery in FSH-primed heifers may be due to the presence of higher numbers of follicles 7–10 mm and >10 mm prior to the initiation of the superovulatory treatment which limited recruitment of follicles 2–3 mm during the superovulatory treatment.

Follicular dynamics in heifers and cows during early pregnancy were investigated [55–57]. A wave-like pattern of growth and regression of large follicles was observed through the first 60–70 days of pregnancy in all the studies. It was concluded that the follicular dynamics during the first 60–70 days of pregnancy were similar to those during non pregnancy. Bergfelt et al. [58] have reported that the continued periodic emergence of anovulatory follicular waves in pregnant heifers was associated with continued production of progesterone as evidenced by the continued periodic emergence of waves in non-bred progesterone-treated heifers.

**Ovulation**

Determination of ovulation by ultrasound examination was reported by Larsson [59]. The ovaries of 8 heifers were examined by ultrasonography every 4th hour during and after estrus. Ovulation was depicted by the absence of a preovulatory follicle that was present at a previous examination and subsequently confirmed by the development of corpus luteum at the same spot. The usefulness of ultrasonography performed at 2-hourly intervals for detecting the onset of ovulation was also demonstrated [60].

**Corpora lutea**

The ultrasonic characteristics of corpora lutea (CL) has been described [5, 28, 29, 61]. Generally, a CL is identified ultrasonically from 3 days after ovulation. A developing CL appears on the ultrasound image as a poorly defined, irregular, greyish-black structure with echogenic spots all within the ovary; a mid-cycle CL is a well defined granular, greyish echoic structure with a demarcation line visible between it and the ovarian stroma; in a regressing CL the demarcation line is faint, owing to the slight difference in echogenicity between the tissues [33]. Corpora lutea with
cavities appear as a centrally located nonechogenic area surrounded by greyish echogenic luteal structure. The size and shape of the cavities varies according to the cyclic stages of the CL [2, 62, 63]. The presence or size of central fluid-filled cavity affected neither the plasma progesterone secretion nor subsequent reproductive performance [34]. These structures represent a normal variation of CL in bovine.

Kähn and Ludlow [64] compared palpation per rectum and transrectal ultrasonography (with a 5 MHz transducer) with ovarian dissection for identifying mid-cycle CL and obtained a sensitivity of 85% for both methods. Complete agreement was reported between in-vivo ultrasonography and post-mortem slicing of ovaries for identification of CL and the presence of CL with cavities [61]. Pieterse et al. [33] reported that the sensitivity and predictive value of palpation for detecting mid-cycle CL were 83.3% and 73.2% and those of transvaginal ultrasonography (with a 5 MHz transducer) were 80.6% and 85.3%, respectively. In another study, Ribadu et al. [5] compared the evaluation of mid-cycle CL by palpation per rectum, ultrasonography (with 7.5 MHz transducer) and plasma progesterone concentration and obtained a sensitivity, specificity and positive predictive value of palpation as 85%, 95.7% and 89.7%, respectively. Ultrasonography had a sensitivity of 95%, a specificity of 100% and a positive predictive value of 100%.

Quirk et al. [65] stated that decreases in the size of the CL in heifers coincided with decreases in the plasma concentrations of progesterone. Sprech et al. [66] obtained a correlation coefficient of 0.68 between milk progesterone measured by radioimmunoassay and luteal diameter measured by ultrasonography. Song-Chang et al. [67] also reported a significant correlation between CL size (as determined by ultrasonography) and milk progesterone concentration in 16 dairy cows. Kastelic et al. [68] concluded that an ultrasonographic assessment of CL was a viable alternative to measurement of plasma progesterone concentration for the evaluation of luteal function. It should be noted however, that even though a high correlation exists between the CL diameter measured by ultrasound and plasma progesterone concentration during most days of the estrous cycle; the correlation was absent during the last few days of the estrous cycle when sharp decline of progesterone concentration was not accompanied by a commensurate decrease in CL size as visualized by ultrasonography [5, 69]. A similar trend was observed by Kamimura et al. [34] who reported that the estimated volume of corpus luteum (by ultrasonography) and plasma progesterone concentration in early postpartum cows increased with similar profiles during the luteal growth but decreased more slowly than the fall in plasma progesterone level during luteal regression.

### Ultrasonography of the Uterus during the Estrous Cycle

The ultrasonic anatomy of the uterine horn has been described [29, 32]. The ultrasound image of the uterus showed a distinctly echogenic structure with different layers of the uterine horn reflected by differing echotextures. The echotexture of the endometrium was characterized by the presence of non-specular reflections with dark and bright signals seen within the ultrasound image of the endometrium.

The changes in the morphology of the uterus during the estrous cycle has been characterized ultrasonically in 22 Holstein heifers monitored during 58 interovulatory intervals [70]. The ultrasonographic appearance of the uterus was influenced by the stage of the estrous cycle. Uterine echotexture was characteristically dark during the follicular phase (estrus) reflecting an extensive degree of edema of the endometrium. The uterine horns were maximally curled during luteal dominance but were less curled during follicular dominance.

### Ultrasonographic Detection of Pregnancy

The early and accurate diagnosis of pregnancy in cattle is essential for the maintenance of high levels of reproductive efficiency. Chaffaux et al. [71] first reported the use of real-time ultrasonography to diagnose pregnancy in cattle. Using a 3.5 MHz transducer, they observed irregularly shaped nonechogenic structures in the lumen of the uterus from day 28 post-insemination. Later, several authors [72–81] reported on the use of ultrasonography to diagnose pregnancy in cattle.

Pierson and Ginther [72] using a 5 MHz transducer, reported the presence of discrete
nonechogenic areas within the uterus at days 12 and 14. The changes in the ultrasonographic appearance of the bovine conceptus from time of first detection of the embryo proper (day 20) to day 60 of pregnancy was reported [75]. The embryo was seen initially as a small echogenic line on day 20. By days 22 to 30, the embryo had a prominent C-shape. Boyd et al. [82], using a 7.5 MHz transducer in 22 Friesian dairy cows reported that the conceptus was tentatively observed at day 13 within the vesicle. The vesicle suddenly enlarged at day 19 and the heart beat was detected in the embryo at day 22.

Tentative early pregnancy diagnosis (before detection of an embryo proper) was based on the finding of discrete, nonechogenic structure or line within the uterine lumen but must be subsequently confirmed by the progressive elongation of the nonechogenic area and eventual detection of embryo proper. Definitive diagnosis was unreliable before day 18 using a 5 MHz transducer [83] and before day 16 using a 7.5 MHz transducer [84]. A wide variation in the levels of accuracy for pregnancy diagnosis (70–100%) has been reported by several authors [76, 77, 83, 85–88]. The level of accuracy depends on factors like; the type of ultrasound equipment used (sector or linear), transducer frequency (3.5, 5.0 or 7.5 MHz), stage of gestation and the experience of the operator. Generally, a 5 MHz or 7.5 MHz transducer tends to provide more reliable results than does a 3.0 MHz transducer for early pregnancy diagnosis. However, the reliable period of for pregnancy diagnosis with a positive predictive value of over 95% varies between days 20 and 42 postbreeding [17].

The use of ultrasonography for pregnancy diagnosis has no detrimental effect on either the dam or the fetus. In a recent study, Baxter and Ward [80] showed that the use of ultrasound scanners for early pregnancy diagnosis (at 30 to 40 days of gestation) did not increase the rate of fetal loss. The advantages of using ultrasonography for pregnancy diagnosis are that the presence of an embryo can be detected earlier than by palpation per rectum and that direct physical manipulation of the gravid reproductive tract is unnecessary with ultrasonography. Thus, the risk of inducing embryonic mortality is greatly reduced [89].

Fetal Sex Determination by Ultrasonography

Fetal sex determination has several implications in the animal breeding industry. The gender of fetuses can be detected by visualization of the location of the genital tubercle [90] or the scrotum and mammary glands [91]. The most appropriate time of ultrasonographic sex determination is 55 to 60 days of gestation and the technique can be accurate even under farm conditions [92].

In another study, Viana and Marx [93] used ultrasonography to determine the sex of bovine fetuses between 60th -90th day of pregnancy in 716 embryo recipient cows and heifers. Verification tests on calves born revealed a correct diagnosis of 96.3%. It was concluded that this technique offers a new tool for the management of cattle industry. It should be emphasized however, that ultrasonic identification of the genital tubercle or the scrotum and mammary glands for sexing purposes requires considerable experience.

Ultrasonographic Fetometry

Although a cow's calving date can be predicted with reasonable precision from a knowledge of service date (whether AI or supervised natural mating), it is however difficult when cows are run together with a bull, mating is frequently not observed and the dates of service may be unknown. Accurate estimate of calving dates could be a good management tool as cows can be rationed more precisely in late pregnancy to achieve the appropriate level of body condition at calving [94]. Management at calving could also be eased if cows could be grouped according to their expected calving dates.

Estimation of fetal age, monitoring of fetal growth across time and diagnosis of pregnancy disorders can be performed by ultrasonographic fetometry. Growth curves of fetal structures based on ultrasonographic fetometry have been reported [95, 96]. Kähn [96] described in detail the ultrasonic fetometry of fetuses in 19 pregnant heifers. A total of 485 examinations were carried out between 2 to 10 months of pregnancy. The organs evaluated included eyeball, brain case, stomach, trunk, ribs, metacarpal diaphysis, os ilium and os ischii, scrotum and umbilical cord. For determina-
tion of the size of organs, the transducer was manipulated so that the largest sonographic section of the structure was obtained on the screen. Regression and correlation coefficients of fetal structures were calculated depending on days of gestation. It was concluded that intra-uterine development of the bovine fetus and its gestational age may be judged from the size of its organs and parts of the body.

Ultrasonographic fetometry has been shown to provide a precise estimation of gestational age and prediction of calving dates [94]. In a study of 300 pregnant beef cows between 35 and 125 days of gestation, Wright et al. [94] reported the mean difference between the actual and predicted calving dates as $0.9 \pm 9.0$ S.D (days). It was concluded that the accuracy and precision of the prediction of calving date were sufficient to be of benefit in the management of cows in late pregnancy and at calving.

**Ultrasonography of Postpartum Uterus**

Serial ultrasonographic assessment of bovine postpartum uterine involution has shown that total uterine diameter and endometrial thickness decreased over time to reach a static size at completion of uterine involution.

Ultrasonographic evaluation of the diameter and shape of the postpartum uterus, echotexture and layers of the uterine wall and intraluminal fluid accumulation has been reported in cows [97]. Uterine involution was completed at approximately 40 days based on ultrasonic assessment of uterine horn diameters. Kamimura et al. [34] also monitored the progress of uterine involution by twice-weekly ultrasonography in 40 Holstein cows and reported completion of uterine involution by 41.5 days postpartum.

Uterine involution in 15 Charolais cows were monitored by Santos et al. [98] using an ultrasound scanner at 3 day intervals from 8 to 40 days after calving. For cows with a normal parturition, uterine involution was completed an average of 28.12 ± 55 days after calving vs 32.75 ± 1.13 days for cows with dystocia. There were highly significant differences between cows with dystocia and those calving normally in the time taken for involution of the uterine horns. Taking the above studies together, uterine involution seem to be completed by day 28–40 postpartum based on ultrasonographic assessment. The difference in the mean day of completion of uterine involution may be attributed to differences in sample sizes, parity and level of management.

Ultrasonographic evaluation of uterine involution in 11 Swedish cows with retained fetal membranes [99] revealed accumulation of snowy fluid and thickening of the endometrium and uterine walls in all cows. In a recent study, Torres et al. [100] monitored uterine involution in 33 dairy cows and reported that uterine involution was completed at 4 weeks postpartum in cows with normal puerperium (n=12) but was delayed in 23.8% of cows with abnormal puerperium (n=21).

These studies have demonstrated that ultrasonography is a useful tool in monitoring the progress and completion of uterine involution.

**Ultrasonographic Diagnosis of Ovarian and Uterine Abnormalities**

**Ovarian cysts**

Ovarian cysts is an important cause of infertility in cattle. It is characterized by the presence of one or more follicular structures larger than 25 mm in diameter for 10 or more days in the absence of a corpus luteum [101]. Ovarian cysts are either follicular (thin-walled) or luteal (thick-walled). The difficulties of differentiating accurately between follicular and luteal cysts by palpation per rectum is well documented [102–106]. It is pertinent to distinguish follicular cysts from luteal cysts accurately as the approach to treatment may differ depending on the diagnosis. Ultrasonography provides a method of accurately differentiating the types of cysts. The ultrasound appearance of follicular and luteal cysts have been documented [1, 3–5, 107–110]. By ultrasonography, a follicular cyst appeared as a uniformly nonechogenic ovarian structure >25 mm in diameter with a wall <3 mm thick. Luteal cysts, on the other hand, appeared as nonechogenic structure >25 mm in diameter with grey patches within the antrum or along the inner cyst wall and a wall thickness >3 mm. It is necessary to distinguish luteal cysts from cystic corpus luteum as the latter is not pathological. For clear differentiation between the two, various criteria must be considered which include; overall size, shape of the cavity, thickness of the luteinized wall.
and echogenicity of the interior area. Cystic corpora lutea are usually no larger than 3 cm in diameter and the wall is about 5–10 mm thick. Corpora lutea are rarely spherical usually presenting an oval shape on the screen. The image of the cavities depend on direction of the ultrasound beam and is either circular or oval. The fluid-filled cavities of CL only seldom present reflections more often being homogeneous and near-black, whereas reflections are frequently observed in luteal cysts [1, 5].

Ultrasonography has proved to be useful in monitoring the dynamics of experimental ovarian cyst formation following the administration of steroids in heifers and cows [111–114]. Cyst turnover in spontaneous occurring ovarian cysts in cows [115] was also monitored.

The ultrasound appearance of ovarian tumor have been reported [1, 116]. A compact, highly echogenic image interrupted by nonechogenic blood vessels was observed in a cow with granulosa cell tumor [1].

Ovarian cysts after treatment

Prior to the introduction of ultrasonography, assessment of luteinization of follicular cysts following therapy was performed by palpation per rectum. However, incorrect assessment of luteinization of follicular cysts by palpation per rectum after treatment has been a problem [117, 118]. Ultrasonography is an effective tool for monitoring the response of cystic ovaries to therapy. Edmondson et al. [3] first reported the use of ultrasound to monitor the response of cystic ovaries following treatment. In a cow treated with gonadotropic releasing hormone, the cyst wall increased in thickness from 2 mm to 6 mm over a two week period.

In a study on the use of ultrasound and progesterone profile to monitor the response of ovarian follicular cysts in 5 cows treated with gonadotropic releasing hormone, Ribadu et al. [6] have observed ultrasound changes after treatment which included; clouding of the uniformly nonechogenic antrum of cysts, luteinization of cysts wall, reduction in cyst size (resolution) and/or development of 1–4 corpora lutea on the ovary bearing the cyst or on the contralateral ovary. Multiple ovulation occasionally occur following GnRH therapy of ovarian follicular cysts.

Treatment of cows with ovarian cysts (follicular or luteal) with progesterone releasing intravaginal device (PRID) or gonadotropic releasing hormone (GnRH) did not seem to have any immediate effect on either follicular or luteal cysts, but consistently resulted in estrus and ovulation; with subsequent CL formation as monitored by ultrasonography. Treatment of luteal cysts with prostaglandin caused marked decreases in cyst size with rough echotexture of the luteinized rim of the cyst within 2–4 days and the formation of new CL within 1 week after treatment [7]. Ohnami et al. [119] also monitored the response of ovarian cysts in 9 cows treated with GnRH analogue. Ultrasonography detected doughnut-like luteal structures after treatment which would not have been detected by palpation per rectum alone. Subsequent hormone measurement confirmed the luteal structures were fully functional.

Jou et al. [120] monitored the ovarian response in cows with follicular cysts treated with GnRH followed by prostaglandin 10–12 days later (n=15) and saline followed by prostaglandin 10–12 days later (control; n=17) and reported no significant difference in the time from treatment to detection of the first CL or cyst disappearance in the 2 groups. Although the success rates following GnRH therapy of ovarian cysts varied amongst the various studies quoted above, the visualization by ultrasonography of cyst luteinization and, or the presence of CL in cows after treatment showed that in responding cases evidence of therapeutic success could be confirmed quickly [6]. Ultrasonography is thus, a useful tool for monitoring the response of ovarian cysts to treatment.

Uterine abnormalities

The usefulness of ultrasonography in diagnosing pathological conditions of the uterus has been reported [1, 121]. Uterine abnormalities recognised during ultrasonography included endometritis, pyometra, fetal maceration and fetal mummification. Ultrasonographic appearance of inflammatory conditions of the uterus were characterized by distended lumen filled to varying degrees with partially echogenic, diffuse, flaky reflections. The degree of echogenicity depended on the consistency of the fluid. When the uterine content was very thick and full of leucocytes and tissue debris; the echogenicity resembled that of uterine wall [1]. In fetal maceration, the fetal bones were identified as echogenic structures in the uterine lumen suspend-
ed in nonechogenic fetal fluids with a thickened uterine wall. Fetal mummification, on the other hand, appeared as poorly defined mass (fetal mummy) with complete absence of uterine fluids.

These studies have indicated that, ultrasonography is an effective tool for diagnosing and studying uterine abnormalities in cows.

Although the use of ultrasonography in diagnosis of hydrometra in sheep and goats have been fairly documented [122–125], there is scanty information on its application in bovine. Ultrasonography may be helpful in diagnosis of hydrometra and mucometra in cattle.

**Ultrasonographic Folliculocentesis**

Ultrasound-guided transvaginal oocyte aspiration is helpful in obtaining ova from clinically infertile, but valuable cows for *in vitro* fertilization. In this way, the genetic potential of such donor cows could be propagated.

Pieterse *et al.* [9] described a technique for the repeated collection of bovine oocytes using transvaginal ultrasound guided aspiration during the normal estrous cycle and after stimulation with PMSG in 10 cows. A total of 36 transvaginal procedures were performed during which 54 oocytes were recovered from 197 follicles.

Moyo and Dobson [126] using a 7.5 MHz linear array ultrasound transducer collected oocytes from 5 cows superovulated with ovine follicle stimulating hormone (oFSH). From 124 follicles punctured, oocyte recovery rate of 48% was recorded. In another study [127], an oocyte recovery rate of 50–70% has been reported in cows.

In a study on the development of oocytes derived from the first dominant follicles of beef cows, Otoi *et al.* [128] monitored follicle development in 26 Japanese black beef cows by daily ultrasonography from day of estrus (day 0) until the first dominant follicle was aspirated. It was observed that the percentage of oocytes from first dominant follicles that developed to blastocysts tended to decrease with increasing follicular diameter and estrous cycle days. Also, recovery of oocytes by transvaginal ultrasound-guided follicle aspiration once or twice a week for up to 12 weeks in heifers did not affect subsequent response to superovulation and embryo recovery or the occurrence of estrus [129]. Ooe *et al.* [130] showed that ultrasound-guided transvaginal oocyte aspiration can be performed repeatedly and safely at different phases of the estrous cycle and stages of gestation in pregnant cows.

In addition to collection of oocytes for *in vitro* fertilization, ultrasound guided folliculocentesis also allows the collection of follicular fluid for hormonal studies [131].

**Ultrasound Guided Amnio- and Allantocentesis**

Amniocentesis is the collection of fetal amniotic fluid. The interactions between the fetus and the dam may be studied through the technique of amnio and allantocentesis. Specific objectives of amniocentesis includes; correlation of changes in biochemical constituents with gestational age, early determination of fetal sex by cytological analysis and as a source of fetal cells for the determination of prenatal transgenesis.

Transvaginal ultrasound guided amnio and allantocentesis has been reported in 7 Dutch Friesian cows [8]. The pregnant uterus was rectally positioned against the puncture needle which was mounted on the intravaginally inserted ultrasound transducer (sector). It was shown that amniotic and allantoic fluid could be obtained in most ultrasound guided attempts and one fetus was carried to term even after repeated (weekly) amnio and allantocenteses. The risks of fetal deaths resulting from repeated punctures of the pregnant uterus (due to excessive manipulation and bacterial contamination) in a large percentage of animals limits the application of this procedure in routine pregnancy evaluation. However, collection of fetal amniotic fluid using ultrasound-guided transvaginal technique seem to be promising. In a recent study, Garcia and Salaheddine [132] have described the technical aspects of a safe and reliable ultrasound-guided transvaginal method of amniotic fluid collection from heifers (n=93) with day 79 to 90 bovine conceptuses using a very fine aspiration needle. Collection of amniotic fluid samples was successful in 88 out of 93 (94.6%) attempts, and no complications of pregnancy losses were recorded as consequence of the procedure.
Mammary Ultrasonography

Ultrasonography has important applications in the bovine mammary glands. Ultrasound studies have characterized not only the normal ultrasonographic appearance of the mammary glands and teats but also some pathologic lesions of the mammary glands.

Cartee et al. [10] examined the udders and teats of 4 cows (*in-vitro* in a water bath) and 3 live cows and found no difference in the ultrasonographic appearance of the mammary glands. Teats appeared as hypoechoic structures with anechoic lumens while the papillary ducts appeared as a thin anechoic area within folds of the inner teat layer. Ultrasonography detected the presence of a hypoechoic mass in a cow that was examined because of obstructed milk flow from a quarter.

In a further study [11], ultrasonic tomography was applied to evaluate the teat morphology and its use in preventing bovine mastitis.

Ultrasoundography of the Male Reproductive System

The establishment of normal ultrasonic parameters for testicular dimensions and characterization of normal ultrasonic appearance are necessary to allow studies on degenerative and pathological conditions of the bovine testicle. Transcutaneous ultrasonic imaging has been used to characterize the ultrasonic morphology of the testicles of the bull [13, 14]. The normal bull testicle was reported as being homogeneous and moderately echogenic. The head and tail of the epididymis were easily identified on all testes, but the epididymal body and ductus (vas) deferens were difficult to identify. Ultrasonic measurement of testicles was correlated with testicular circumference, weight and volume but not with scrotal circumference [133]. Ultrasonic scanning of the bovine testicle was found to have no detrimental effect on reproductive capacity (semen characteristics, testicular dimensions and consistency) after exposure to a 3-min scan with a 5.0 MHz transducer [134].

Recently, Bailey et al. [135] compared the reliability of caliper and ultrasonographic measurements of testicular length and width *in-vivo* in 10 bulls. The data demonstrated the reliability of using caliper and ultrasonographic imaging for measurements of testicular length and width. However, calipers were easier to use and demonstrated a higher degree of accuracy for measurement of testicular length. It was concluded that ultrasonography offers no added advantage over that of calipers for measuring the length of the *in-vivo* testicles except when only a width measurement is desired.

The ultrasonic anatomy of the accessory sex glands of the bull has been described [15]. Transrectal ultrasonography was used to measure the vesicular glands, ampullae and bulbourethral glands. Ultrasonic measurements were found to be repeatable in individual bulls over time and it was concluded that the anatomic relationships and organ dimensions were accurately imaged by ultrasonography.

Ultrasonography revealed testicular lesions in 95 bulls following *in vitro* injection of testes with crystalline, metallic or aqueous solutions [136]. Mickelson et al. [137] described the characteristic ultrasonographic changes in a bull with seminal vesiculitis. The changes observed included; increased glandular size, loss of normal lobular structure, increased glandular volume and the presence of bright fibrous tissue within the hyperechoic glandular parenchyma. Anderson et al. [138] used doppler ultrasonography and positive contrast cavernosography to evaluate a persistent penile hematoma in a bull. A wide scope exists for the use of ultrasonography in male reproduction.

Conclusions

The use of ultrasonography has allowed accurate monitoring of ovarian follicular development in heifers and postpartum cows on a daily basis in a non-invasive manner. This has contributed immensely to the understanding of follicular dynamics during the estrous cycle, early gestation and the postpartum period. In the areas of pregnancy diagnosis, fetal sex determination, folliculocenteses, amnio and allantocentesis, reproductive tract pathology, monitoring of normal and abnormal postpartum interval, diagnosis and evaluation of treatment of ovarian cysts, mammary ultrasonography, male reproduction; ultrasonography has proved to be a useful clinical and research tool. Ultrasonography is quite helpful for
individuals who are inexperienced in palpation per rectum as palpation skills are acquired quickly while at the same time making accurate diagnosis via ultrasonography.

One of the greatest constraints limiting the widespread use of ultrasonography in bovine reproduction is the high cost of the ultrasound machine. It is hoped that with the availability of several types of scanners (from different manufacturers) and improvements in the quality of scanners, the price may be affordable to a large number of veterinarians and research scientists and this will enhance its application in clinical as well as research studies of bovine reproduction.

It must be emphasized that the use of ultrasonography requires some skills. Such skills are usually obtained during university training or through attendance at continuous professional development (CPD) courses organized for such purposes. Furthermore, experience can be gained through the use of the ultrasound scanner repeatedly.

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