Bovine Reproductive 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 dis- orders. One of the greatest advantages of ultrasonography is that it is totally non-invasive and so repeated examinations of an animal's re- productive 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 preg- nancy 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. Ultrasonically guided amnio- and allantocente-ses in pregnant cows 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 repro- duction. Ultrasonographic characteristics of bull testes and accessory sex glands have been report- ed

Principles of Ultrasonography

Ultrasound is defined as any sound frequency above the the normal hearing range of the human ear; i.e. greater than 20,000 Hz .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 (pi- ezo-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).

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 (pix-els), 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 ex- aminations.

Ultrasound scanners are equipped with trans- ducers 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]. 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 ma- chines detect frequency change and, therefore, movement which is converted to an audible signal [23].

The major considerations in selecting an ultra- sound scanner are price, resolution quality, portability, serviceability and technical support [24]. For routine bovine reproductive ultrasonography (ear- ly pregnancy diagnosis, pathology of the ovaries and uterus, fetal sexing etc) a 5 MHz linear rectal 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 trans- ducer 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.

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 .

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 ultra- sound 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 pass- ing 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 repro- ductive tract appear on the screen in varying shadesof gray depending upon their echogenicity .

Ultrasonography of Normal Ovarian Structures

Follicles

Follicles typically appear as anechoic regions within the ovarian stroma. However, it is not usually possible to distinguish the follicular wall from the surrounding stroma (apart from large pre-ovulatory follicles). Follicles do not always appear round due to transferred pressure from the transducer on the surrounding ovarian tissue.

Ovulation/ Active ovary

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. The components of the active ovary, including follicles, corpora lutea and ovarian stroma, have various echogenicities and therefore will appear as varying shades of grey on ultrasound examination

Corpora luteam

Corpora lutea may be seen on the ovaries of most cows as true anoestrus is rare and the corpus luteum (CL) is present for two thirds of the oestrous cycle. Luteal tissue appears as distinctly echogenic areas within the ovarian stroma. A central lacuna (fluid-filled cavity) may be seen within a normal CL and should not be confused with the presence of a luteal cyst. Compared to a luteal cyst, a normal CL with a central lacuna is less than 25 mm in diameter and the lacuna occupies less than one third of the entire CL. The CL may usually be identified on ultrasound examination 4 days after ovulation occurs. If fertilisation of the ovum does not occur and pregnancy is not established, the CL reaches peak size 16 days post-ovulation and then begins to regress. Therefore, repeated examination of the ovaries can provide useful information regarding stage of the cycle through observation of changes to the CL. Additionally, persistence of the CL may assist in the determination of early pregnancy diagnosis. The embryonic vesicle can usually be found in the uterine horn ipsilateral to the ovary containing the CL

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Ultrasonography of the Uterus during the Estrous Cycle

The ultrasono- graphic appearance of the uterus was influenced by the stage of the estrous cycle. Uterine echotex- ture was characteristically dark during the follicular phase (estrus) reflecting an extensive degree of ede- ma of the endometrium. The uterine horns were maximally curled during luteal dominance but were less curled during follicular dominance. The uterus has different echogenic appearances depending on the stage of the oestrous cycle. Viewing the uterine horn in cross section, where the uterus is circular in appearance may enable the endometrium, myometrium and uterine lumen and its contents to be identified more easily. When the cow is in oestrus, the endometrium becomes oedematous and therefore the endometrial folds become more prominent. The lumen also has a varying appearance depending on intraluminal fluid accumulation at different stages of the cycle. In the periovulatory period, the uterine lumen appears anechoic due to mucus accumulation. It is important to differentiate between the appearance of a large amount of mucus in the uterus and early pregnancy. This can be done through examination of the ovaries for the presence of follicles and corpora lutea in addition to the presence/absence of a foetus, foetal membranes and placentomes (cotyledon/caruncle unit)

Ultrasonographic Detection of Pregnancy

Early and accurate identification of the non-pregnant cow is essential to improve the overall reproductive efficiency of the

herd. Although the experienced operatormay be capable of detecting a pregnancyas early as day 17 post-breeding/ artificial insemination (AI), the length of examination time is increased as theentire uterus must be carefully evaluated to confidently diagnose a non-pregnant. state. Additionally, diagnosis of pregnancy at this stage should be considered with caution due to typically high rates of early embryonic loss. Most operators can diagnose pregnancy under farm conditions quickly, easily and accurately by day 30 through the use of transrectal ultrasonography. Therefore, it is generally advisable to perform ultrasound examinations for pregnancy diagnosis around day 30 post-breeding/AI. A positive diagnosis of pregnancy may be made without visualisation of the embryo on ultrasound examination. This is done through identification of allantoic fluid, foetal membranes and placentomes.

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 [or the scrotum and mammary glands . 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 .

Ultrasonographic Fetometry

Although a cow’s calving date can be predicted with reasonable precision from a knowledge of ser- vice date (whether AI or supervised natural mating), it is however difficult when cows are run together with a bull, mating is frequently not ob- served 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.

The organs evaluated included eyeball, brain case, stomach, trunk, ribs, metacarpal diaphysis, os ilium and os ischii, scrotum and umbilical cord. For determination of the size of organs, the transducer was ma-nipulated so that the largest sonographic section of the structure was obtained on the screen. Re- gression and correlation coefficients of fetal structures were calculated depending on days of gestation. It was concluded that intrauterine 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]. the mean difference between the actual and predicted calving dates as 0.9 � 9.0 S.D (days).

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 com- pletion of uterine involution.
Ultrasonographic evaluation of the diameter and shape of the postpartum uterus, echotexture and layers Uter- ine involution was completed at approximately 40 days based on ultrasonic assessment of uterine horn diameters.

Ultrasonographic Diagnosis of Ovarian and Uterine Abnormalities

Ovarian cysts

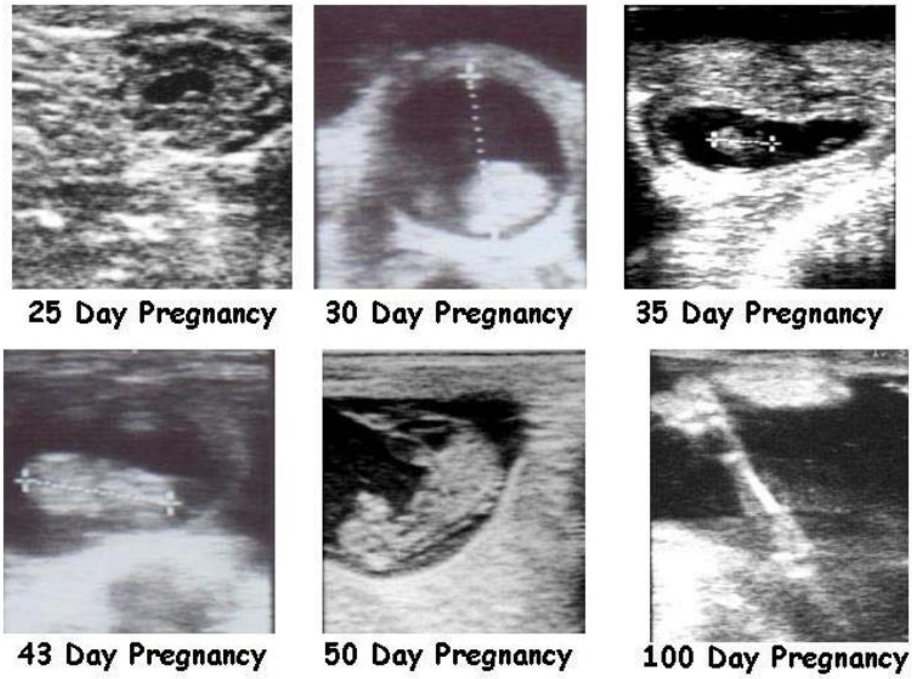
Ovarian cysts is an important cause of infertility in cattle. It is characterized by the presence of one or more follicular structures larger than 25 mm in diameter for 10 or more days in the absence of a corpus luteum [101]. Ovarian cysts are either folli- cular (thin-walled) or luteal (thick-walled). 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 accu- rately 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. 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 cor- pora 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 present- ing 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.

Ul- trasonography is an effective tool for monitoring the response of cystic ovaries to therapy. Edmond- son et al. [3] first reported the use of ultrasound to monitor the response of cystic ovaries following treatment. In a cow treated with gonadotropic re- leasing hormone, the cyst wall increased in thickness from 2 mm to 6 mm over a two week period. 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 . Uterine abnormalities recognised during ultrasonography included endometritis, pyometra, fetal maceration and fetal mummifica- tion. 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 suspended in nonechogenic fetal fluids with a thickened uterine wall. Fetal mummification, on the other hand, appeared as poorly defined mass (fetal mum- my) with complete absence of uterine fluids.
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.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 superovula- tion and embryo recovery or the occurrence of estrus[129]. Ooe *et al.* [130] showed that ultra-sound-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].



'igure 4. Ultrasound images of the bovine fetus at various stages of development.