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Clinical application of ultrasound in bovine reproduction: A review

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Abstract
The contributions of ultrasonography in the recent years have been increases tremendously in the field of animal reproduction. The ultrasound adds new perspectives to our general understanding of reproductive biology. The trans-rectal real time B- mode ultrasonography is a non- invasive research tool use in bovine reproduction. It helps in assessment of follicular dynamics during estrous cycle by the research scholar to the early pregnancy diagnosis by the bovine practitioners. The application of this technology includes the understanding of follicular dynamics, detection of early pregnancy, fetal viability, early diagnosis of reproductive tract abnormality, identification of fetal sex, diagnosis and monitoring of ovarian cysts during treatment as well as ultrasound guided reproductive methods. The involvement of ultrasonography with novel or pre-existing reproductive technology further increases the reproductive efficiency. Improvement of extension instruction projects to prepare veterinarian to involve ultrasound for routine assessments is a basic advance toward fast execution of this innovation into the dairy business. In this review, the writing relating to the utilization of ultrasonography in bovine reproduction is surveyed. The applications and restrictions of ultrasonography are featured.

Keywords: Corpus luteum, follicle, fetal sexing, ultrasonography

Introduction

The transrectal, real- time, B-mode ultrasonography represents a technological advancement that has revolutionized knowledge of veterinary reproductive biology. New exploration and information generated through ultrasonic imaging has clarified the nature of complex reproductive processes in cattle including ovarian follicular dynamics, corpus luteum function, and fetal development. Early integration of ultrasound technology to the dairy sector included novel techniques similar as trans-vaginal follicular aspiration and ultrasound guided ovum pick up leads to the growth of dairy sector (Jaśkowski *et al*. 2021) [5]. The linear array real time B-mode ultrasound scanner meets the requirement of veterinary application in animal reproduction. In general most ultrasound machines consist of central processing unit, transducer and a monitor upon which ultrasound image is visualize by the operator. The transducer or probe emit and receive the high frequency ultrasound waves. The transducer head consist of series of piezo-electric crystals arranged in the row. These crystals emit high frequency sound waves upon being energized through compression and rarefaction by electric current. The linear transducer presented the rectangular image while sector probe exhibit pie shaped image on the field of scan (Ribadu, 1999) [12]. Ultrasound waves are the sound waves inaudible to human being and operate at the frequency of 1 to 10 MHz (Woo, 2002) [17]. The B-mode is a brightness modality and is a two dimensional ultrasound image composed of bright dots representing the ultrasound echoes. The brightness of either dot is determined by the amplitude of returned echo signal. Bovine reproductive organs are generally scrutinized per-rectum using a linear-array transducer specifically manufactured for transrectal use (DesCôteaux *et al.* 2009) [2]. Linear- array transducers of 5.0 and 7.5 MHz frequences ranges are mostly used in cattle to perform reproductive ultrasound examinations. The penetration power of ultrasound waves and image resolution is inversely dependent upon each other. The lower frequency; higher the penetration power of sound wave but the image resolution will be poor (Fricke, 2002) [4]. Therefore, a 5.0-MHz transducer results in higher depth of penetration and lower image detail, whereas a 7.5-MHz transducer results in lower depth of penetration and better image detail. An ultrasound scanner equipped with a 5.0-MHz transducer is most useful for bovine field practitioners conducting routine reproductive examinations; small ovarian structures similar as small follicles are imaged with a 7.5-MHz transducer.

Technique

The ultrasonic assessment is done in a manner analogous to the trans-rectal palpation. The genitalia are trans-rectally palpated in the standard way; the transducer is progressed cranially along the rectal floor. The urinary bladder is the landmark structure in the pelvic cavity. It creates the regular picture of an empty organ containing anechoic liquid and extends cranio-ventrally to shape the body of the bladder. Ventrally to the bladder, the pelvic floor should be visible. In non pregnant animal, neck of the uterus (cervix) found dorsally to the body of bladder (DesCôteaux *et al.* 2009) [2]. The cervical designs that can be distinguished incorporate the cervical rings and a focal, hyperechoic line which addresses the cervical channel. Promptly cranial to the cervix, for the most part in the midline, seem the body and horns of the uterus. Every so often, the uterus can likewise be tracked down horizontal to the urinary bladder. On account of a linear probe, the transducer is currently turned from one side to another to deliver longitudinal pictures of the uterus.

While utilizing a sector probe, the administrator can turn the shaft through 90 degrees and subsequently change the checking plane from longitudinal to cross over comparable to the body plane. As such cross over segments of the uterus can be acquired. The transducer can be turned further along the side to see the ovaries. Care ought to be taken to dispense each indistinguishable ovary to the correct side. The goal of ultrasound at a frequency of 5.0 MHz is sufficiently high to distinguish vesicular structure with a breadth of 3 to 5 mm. At the lower frequencies of 3 to 3.5 MHz vesicles of 6 to 8 mm should be visible (Perry, 2016) [10]. To additional increment picture quality, 7.5 MHz ultrasonography can be utilized. Sound waves at this frequency give an insignificantly preferable goal over that got with 5 MHz. The infiltration profundity of sound waves at 7.5 MHz is, notwithstanding, simply 4 to 5 cm. Therefore the utilization of high frequency ultrasonography is confined to the assessment of constructions that are very near the transducer. The ultrasound waves of 5 MHz enter around 8 to 10 cm, consequently permitting the assessment of the ovaries and uterus during early pregnancy. Since ultrasound at 3.5 MHz enters 12 to 15 cm, or more profound, it tends to be conveniently applied in the later phases of pregnancy, or in cows with abnormally enlarged genitalia (Terzano, 2012) [15]. Thus higher the frequency of probe greater the resolution power while the lower frequency have better penetration ability.

Ovarian structures

Follicles: The follicles are portrayed by the anechoic round about region of the follicular lumen. The liquid substance normally gives no reflection. The mass of a follicle can seldom be recognized. The state of the follicle is as a rule round. Follicles with a width of smaller than 2 mm are too little to possibly be perceptible. After ovulation has occurred, the ovulation gloom or some other indication of ovulation - with the exception of the vanishing of the enormous hypoechoic follicular design cannot be distinguished. During early metestrus, the echographic picture of the ovary compares to that of an ovary with no critical practical designs. During follicular phase, the dominant follicle selected to ovulate enlarges and ovulation occurs when the follicles has reached the size of 12 to 20mm as represented in Fig. 1 (Evans, 2003) [3]. Most estrus follicles arrive at their greatest size 36 hrs before ovulation. Ovarian blood vessels might be mistaken for ovarian follicles. Broadened veins are seen during the luteal stage because of an expanded blood stream to the corpus luteum. They can be recognized by moving the transducer: veins can, in contrast to follicles, be followed during looking over a more drawn out distance and are for the most part seen on the boundary of the ovary, in the ovarian hilus, and around the corpus luteum. However, recent advances in the use of color Doppler ultrasound technique to show haemodynamic changes in the local circulation of the follicle helps in to distinquish follicles from ovarian blood vessels. Ovarian follicular development in cows happens in wave fashion. At the hour of ovulation another follicle is develops to form into a predominant follicle, to become further during metestrus, and to arrive at its greatest width during the early luteal stage. Not long after this diestrus follicle has arrived at its greatest width, the second follicular wave begins and another prevailing follicle creates. On account of a 2-stage follicular cycle this one will end in ovulation. Some dairy cattle show a 2 wave pattern, others a 3 wave of follicular development. In contrast four waves have been commonly recorded in *Bos indicus* cows (Bo *et al*. 2003) [1]. In a 3-stage follicular advancement the second predominant follicle additionally relapses and a third follicle forms into the estrus follicle. The cows with two waves per cycle tend to have shorter interoestrous interval (19.8 days) and large preovulatory follicles than those animals with three waves (22.5 days) (Lucy, 2007) [7].

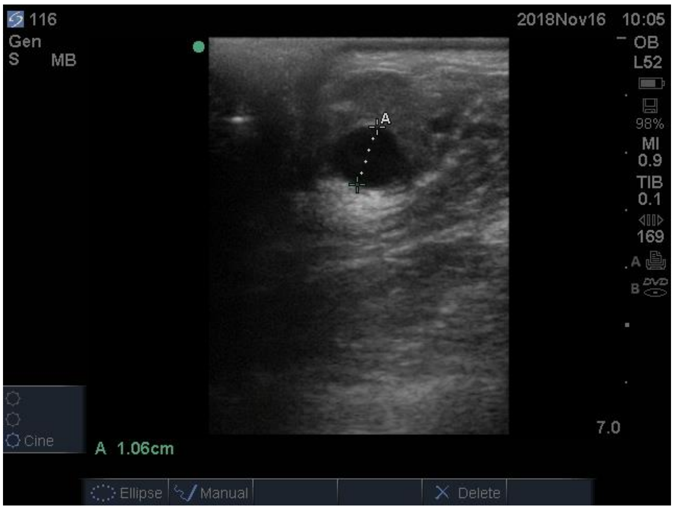

Fig 1: Ultrasonographic image of ovary shows follicle of diameter 1.06 cm.

Corpora lutea: The corpus luteum have a well defined border and a mottled echogenic appearance that is less echogenic than ovarian stroma. The corpus luteum shows up as a grey color and oval design with much separated from the other structures on the ovary. Corpora lutea with pits, supposed cystic corpora lutea, can likewise be analyzed by ultrasonography (Fig. 2). The corpus luteum develops by hypertrophy and luteinization of the granulosa and thecal cells that lined the follicle (Noakes, 2009) [8]. The development of corpus luteum is extremely rapid in a way that it reaches the 10 mm in diameter within 2 days. The corpus luteum attains it maximum size by the 7 to 8 day of the cycle. The greatest diameter of corpus luteum varies in a range of 20- 25 mm. However, quickly following ovulation, the creating luteal tissue cannot yet be perceived. The youthful corpus luteum just turns out to be sonographically recognizable 2 to 4 days post-ovulation. Corpora lutea have a mean width of 14 mm and a mean length of somewhere in the range of 18 and 21 mm when they previously become discernible on the third day after ovulation (Ribadu and Nakao, 1999) [12]. After luteolysis has started the biggest distance across of the corpus luteum quickly tumbles to be 23 mm.

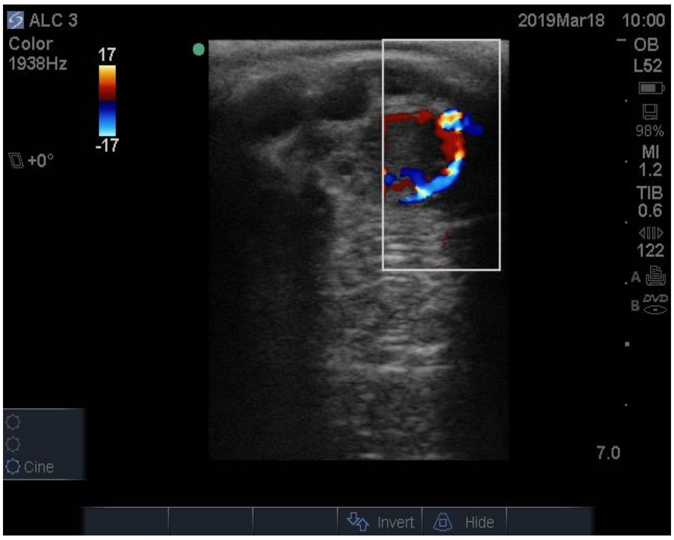

Fig 2: Doppler ultrasonographic image of corpus luteum. The blue and red color shows the flow of blood towards and away from the probe

At 5 MHz frequency, corpora lutea are dependably perceivable from their initial advancement to the furthest limit of diestrus. The dim sizes of the corpora lutea of various ages fluctuate very little and can't be utilized for symptomatic reason. Simultaneously, corpora lutea of pregnancy can't be recognized based on their echogenicity from those of the cycle. The pits inside corpora lutea are typically oval, infrequently round, and almost in every case halfway situated inside the organ. The biggest measurement of the cavities normally changes from simple millimeters to 1.5 cm. The echogenicity of the pits is like that of follicles. Slight reflections can sometimes be seen inside the cavity's liquid. The luteinized divider is utilized to separate cystic corpora lutea from ovarian follicles. As during the estrous cycle, cystic corpora lutea can likewise be found during the principal long stretches of pregnancy. The ripeness of creatures with a cystic corpus luteum is equivalent to that of cows with the strong kind of the corpus luteum. With the guide of ultrasonography the quantity of corpora lutea on an ovary can be resolved precisely. The pit in cystic corpora lutea arrived at its biggest width between days 8 and 10 of the cycle. A while later they diminished in frequency and size with time. Until day 10, 33% to a portion of all corpora lutea in typical cycles contain a sonographically noticeable cavity. Spell *et al*. 2001 [13] determined that the concentration of progesterone in day 7 of estrous cycle was not associated with luteal diameter. The area and volume of corpus lutea were correlated with the concentration of progesterone. Echogenicity of the corpus luteum in buffalo is like that of cows, yet it is more modest (10 to 14 mm), more compacter, and the rate of event of focal depression is less incessant. Although ultrasound is more accurate than rectal palpation in determining the ovarian follicles yet it is difficult to differentiate between developing and regressing corpora lutea.

Pregnant uterus

Until day 25 of pregnancy, the cross sectional breadth of the allantochorionic and amniotic vesicle is still little to such an extent that a liquid undeveloped vesicle can be identified by utilizing higher frequency ultrasonography of 5 MHz or more (Taverne and Noakes, 2018) [14]. From around day 25 how much liquid in the allanto-chorionic vesicle increments quickly so the early stage vesicle's cross over width likewise turns out to be impressively more noteworthy. The liquid will lie in the horn ipsilateral to the corpus luteum. Between days 21 and 25 of pregnancy much fluid inside the undeveloped vesicle has normally expanded so much that it becomes more straightforward to picture by ultrasonography. The biggest fluid accumulation is most often seen interestingly distal to the bend of the uterine horn. Here the incipient organism and its encompassing amnion become perceivable interestingly. During this earliest period of the sonographic pregnancy analysis, specific consideration should be paid to affirm that the noticed liquid gathering is intrauterine (Fig.3). A positive pregnancy conclusion must be made with assurance once a developing conceptus has been distinguished. Before day 25 of pregnancy it can frequently be hard to track down the incipient organism itself. Ultrasonography have the following advantages firstly non-pregnant cows can be found earlier (28–32 days after breeding), and diagnostic information on the status of the ovaries and uterus can be obtained (Wang *et al*., 2020). Secondly, the viability of the embryo or fetus can be assessed, eg, by visualization of a fetal heart beat (Day 22) (Zalesky, 1993) [18]. Thirdly, twins are more readily detected and promptly action should be taken. Fourth, sex of the fetus can be determined and age of the conceptus can be estimated more accurately. The farmers can be shown the conceptus, which could be helping in diagnosis the cases of embryonic losses in the herd.

Fig 3: USG image of 45 days of pregnancy

Fetal Sexing

To develop the production in the cattle farms many cattle operation are developing the strategies to use fetal sexing as marketing or purchasing tool. Male and female fetuses can be differentiated by recognizing the relative position of genital tubercle and development of genital swellings at approximately day 50 of gestation (Jost, 1971). The ultrasound probe should be placed to produce a frontal cross-sectional, or sagittal image of the ventral body surface of the fetus. The optimum timing for fetal sexing in large breed of cattle is between day 55 to 70 of gestation while ideal time period for small breed between day 55 to 80 of gestation (Lamb and Fricke, 2005) [6].

The umbilicus can be used as landmark in determining the location of genital tubercle or presence of scrotum. In the male, genital tubercle is located just adjacent to and caudal to the umbilicus, whereas the genital tubercle in the female located just ventral to the tail. The accurate diagnosis of sex in each pregnant cows veterinarian should access the three locations firstly adjacent to the umbilicus, where the umbilicus enter the abdomen for the genital tubercle. Secondly, the area between the hind limbs for scrotum and thirdly, ventral to tail possible for female genital tubercle (Quintela *et al*. 2011) [11]. In embryo transfer technique, determination of sex after successful transfer of embryo to recipients allows marketing of male and female embryo before the parturition (Pellegrino *et al*., 2016) [9]. As the veterinarian become proficient at fetal sexing, commercial farm industries will utilize this technique to increase the marketability and efficiency of the farm.

Conclusion

As an exploration tool, trans-rectal ultrasound has revolutionized our understanding of reproductive biology. Although there are numerous implicit operations of ultrasound for use in reproductive operation of dairy cattle, combining ultrasound for early gestation opinion with timed AI along with early discovery of twin fetus will probably affect in the widest uses of this technology. Development of integrated reproductive operation systems that combine ultrasound with new and being reproductive technologies will further enhance the practical operations of ultrasonography. Further exploration on the efficacy of protocols that integrate this technology with timed AI protocols for synchronization of ovulation, discrimination operation strategies for cows carrying twin fetuses, and thorough profitable analyses on the use of ultrasound for reproductive operation of dairy cattle must be conducted before wide integration of ultrasound occurs in the dairy assiduity.

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