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Scanning the Future: Veterinary Reproductive Ultrasonography

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ABSTRACT

Reproductive ultrasonography can be an extensively preferred diagnostic modality in farm and companion animals for maximizing reproductive health in future days. The shape, contour, size, and position of the structure being analyzed, as well as its echogenicity, which is determined by the amplitude of the echoes received, are being used to describe ultrasound pictures. Reproductive ultrasonography has introduced a new dimension to animal reproduction by allowing not only visualization of the reproductive tract but also early pregnancy diagnosis, surveillance of embryonic or fetus development, and detection of the estrous cycle phase. Confirmation of ovarian and uterine ailments such as cystic ovarian follicle, endometritis, hydrometra, mucometra, and pyometra can be easily diagnosed for the earliest treatment regimens. The technique is a simple, safe, non-invasive, cost-effective, and practical way to confirm reproductive status in farm and companion animals from 30 days of gestation onwards, and by trans-rectal approach, the earliest diagnosis of pregnancy and non-pregnancy in small ruminants and swine can be confirmed from day 20 onwards. As a result, it appears that veterinary reproductive ultrasonography is an excellent technique for managing fertility in farm and companion animals.

Introduction

Despite its numerous and relevant applications in improving the reproductive efficiency of farm and companion animals, very few veterinarians in India use the ultrasound technique for the handling of reproductive complications (Fricke, 2002). Furthermore, many ultrasonographers in veterinary establishments around the country exclusively utilize the ultrasound scanner to identify early pregnancy and for research purposes. The use of ultrasonography regularly can lead to more precise drug administration based on the diagnosis. In large ruminants, it can also minimize, or even eliminate, the typical range of error of manual rectal palpation of physiological and pathological ovaries and uterine conditions.

The application of real-time, B-mode diagnostic ultrasonography has been increasing as an imaging

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technique in small ruminant reproduction, as it becomes more apparent that its use can produce solutions to many unanswered questions, its concomitant disorders, and pregnancy diagnosis (Pujar et al., 2016). No embryonic deaths or abortions were seen as after continuous scanning during the pregnancy, the kids born were morphologically normal and viable (Padilla-Rivas et al., 2005). Ultrasonography has a widespread application in Veterinary Gynaecology for assessment of the stage of pregnancy and even records the number of fetuses. The trans-rectal approach provides an accurate diagnosis just on day 23 of gestation (Pujar et al., 2017; Mali et al., 2019), whereas the trans-abdominal approach is on day 35th of pregnancy (Suguna et al., 2008).

The use of reproductive ultrasonography has made significant strides in the diagnosis of canine infertility (Feliciano et al., 2013; Polisca et al., 2013). Years ago, the use of reproductive ultrasonography was limited to the diagnosis of pregnancy in canine reproduction. Over the years with advancements and the use of ultrasonography has extended from diagnosis to monitoring of pregnancy, prediction of dates of parturition, monitoring threatened or induced abortions, treatment of endometritis, pyometra, sub involution, stump granuloma, stump pyometra, and ovarian remnant syndrome (Freitas et al., 2016). The ultrasonography is not only a diagnostic tool but can be utilized for monitoring the effect of treatment in different reproductive problems. Hence, it seems clear that veterinary reproductive ultrasonography is an ideal tool for fertility management in farm and companion animals.

Ultrasound basic principle

It's crucial to remember that the image on the ultrasound screen depicts a fine segment of an organ that looks suspiciously like a weakly magnified histology cut. As a consequence, the probe acts like a knife, carving through organ tissue from top to bottom. B-mode, ultrasonography thus presents a flattened two-dimensional image of a finely-cut section of the target organ (Pierson and Ginther, 1984). As the probe moves across a tissue surface, ultrasound images are rapidly updated and superimposed one on the pinnacle of the other. The quick sequence of tissue segment views provides the appearance that the structures are moving in a cartoon-like manner. It is critical to have a clear understanding of the three-dimensional shape of the organ in space when interpreting sectional images of an instrument on the screen.

Characteristics and resolutions of probes

The probe is the most delicate part of the ultrasound system. Probes with a frequency of 3 to 10 MHz are being used in the veterinary industry. Linear, curvilinear, and sectorial probes are the three different types of probes. For trans-rectal ultrasonography investigations of the ovaries and uterus, linear or curved probes are preferred in theriogenology. This linear probe has a predefined row of crystals that are selected electronically to generate a rectangular image. For tissues positioned close to the probe, the linear probe gives a good resolution. One or even more crystals in the curvilinear and sector probes form a beam in the shape of a pie slice due to their position. The curvilinear and sectorial probes have the advantage of not requiring a large contact surface and scanning a substantially larger surface. The sectorial probe is appropriate for ultrasound-guided transvaginal aspiration of bovine follicles in IVF Technology and for examining the fetus during trans-abdominal ultrasound imaging. Tissue penetration will be deeper with a lower frequency, but the resolution will be lesser (Rantanen and Ewing, 1981).

Terminology and Interpretation of B-Mode Ultrasound Images

The shape, contour, size, and position of the structure being analyzed, as well as its echogenicity, which is determined by the amplitude of the echoes received, are used to describe ultrasound images. The bulk of sound waves is reflected in the probe by an echogenic structure, which appears on the screen as white to various degrees of grey. An echogenic structure does not produce echoes; instead, the waves are transmitted to deeper tissues. Follicular fluid, which appears black on the screen, is an example of an echogenic structure. The terms hypoechogenic and hyperechogenic denote a decrease and an increase in relative echogenicity in comparison to surrounding tissue, respectively, whilst is echogenic denotes similar echogenicity to surrounding tissue.

Different Types of Ultrasonography

A-mode: The A-mode (amplitude mode) generates a one-dimensional display depicting echo amplitudes for various depths. The axes are amplitude and depth. The A-mode is widely used for the diagnosis of pregnancy in small animals, sheep, and horses.

B-mode: Most modern ultrasound for examining the reproductive tract of animals is B-mode (Brightness mode), real-time scanners in which the ultrasound imaging is a two-dimensional display proportional to the amplitude of the echoes. Real-time imaging is a moving display in which echoes are continuously collected and events such as fetus leg movements and the heartbeat can be monitored as they occur.

M-mode: The M-Mode (motion mode) imaging technique is a modification of the B-Mode and is used to assess moving structures such as the heart. The change in reflector depth over time is depicted as a simple line graph with depth and time as axes.

Doppler ultrasound systems: The Doppler ultrasound systems use the motion of blood toward, away, or at an angle to the transducer to construct dazzling multicolour images of flow patterns.

Scanning technique: In bubaline, equine, caprine, ovine, and porcine trans-rectal, as well as trans-abdominal scanning, is routinely performed to visualize physiological and pathological conditions of the reproductive system. During mid or late gestation, trans-abdominal ultrasonography is frequently performed with the animal standing to determine the status of the fetus, such as fetal movements, fetal heart beats, umbilicus blood flow, and placentomes integrity.

Trans-rectal scanning technique in bovine and bubaline

Mostly linear or curvilinear probe having a frequency in between 5 to 10 MHz is used for trans-rectal scanning. Preparing the animal for trans-rectal ultrasound is analogous to preparing the animal for rectal palpation. The transducer is dipped into the gel. Because the rectal wall is normally moist, the use of a coupling medium for trans-rectal exams may be superfluous. To avoid rectal tears, utilize the same care as with rectal palpation while inserting the greased hand and transducer into the rectum. Faecal material should be eliminated before examination since it can produce aberrations in the ultrasound image. It is critical to take special precautions to guarantee that the transducer was advanced within the lumen of the rectum and not into a blind pocket which can be done by extending the fingers beyond the transducer's tip during major forward motions.

Trans-rectal scanning technique in caprine and ovine

The aim of ultrasonographic evaluation will define the scanning technology to be used (trans-rectal or trans-abdominal). Although trans-abdominal ultrasonography is easy to execute, a thorough examination of the genital system necessitates the use of high-resolution probes employed in a trans-rectal technique. As a result, the trans-rectal approach is the procedure of choice for non-pregnant and early pregnant females undergoing ultrasound examinations. A linear or curvilinear probe can be made rigid by using AI sheaths or PVC pipes and easily used for trans-rectal scanning in small ruminants (Mali et al., 2019). Trans-rectal examination in small ruminants and swine mostly carried out in standing position by restraining manually. To improve the image, faeces can be removed from the rectum, and a hydrosoluble gel must be introduced into the rectum to avoid injury to the mucosa and improve sound wave transmission. The probe (6 to 8 MHz) is placed into the rectum, orientating the transducer perpendicularly to the ventral abdominal wall. The urinary bladder is detected first; it can be seen as an echogenic structure. The uterine body will be dorsal to the bladder, and each uterine horn will be on its respective side. The position of the uterus varies depending on the quantity of fluid in the bladder, the size of the uterus, and the female's age and number of parities. When the bladder is reached, the probe must be rotated laterally clockwise and counter clockwise to view the uterine horns and both ovaries completely. By trans-rectal approach, the earliest diagnosis of pregnancy and non-pregnancy can be confirmed from day 20 onwards in small ruminants and swine.

Trans-abdominal scanning technique in farm animals

During mid or late gestation, trans-abdominal ultrasonography is frequently performed with the animal standing to determine the state of the fetus, such as fetal movements, fetal heartbeats, umbilicus blood flow, and placentomes integrity. The convex transducer having a frequency between 3 to 5 MHz should be placed in the inguinal region just cranial to the udder of the large and small ruminants whereas in swine, just dorsal to the last teat. To eliminate the presence of air and increase contact between the transducer and skin, the hair in the inguinal area of some breeds may be trimmed, and the probe may be liberally smeared with contact gel. By trans-abdominal approach in large ruminants, is mostly used during mid or late gestation because the fetus moved in the abdominal area during this period and it is preferable to use trans-rectal scanning to monitor the fetus and other aspects of pregnancy.

Trans-abdominal scanning technique in companion animals

In canines, 3 to 6 MHz transducer preferably convex sector which allow a larger area to be covered is ideal for evaluation of normal uterus whereas high-frequency transducers of 7.5-10 MHz are ideal in felines. Canines and felines are usually examined in dorsal, lateral recumbence, or standing position. Most animal owners may object to removing the ventral abdominal hair coat, which is a typical technique for obtaining the clearest picture. The use of alcohol or other wetting agents before applying acoustic gel to an unclipped hair coat may improve image quality by lowering the amount of hair between the transducer and the skin. A full urine bladder improves the visibility of the uterus. A distended bladder facilitates scanning by acting as an auditory window through which the uterine body can be seen between the urine bladders ventrally and the colon dorsally.

Evaluation of status of reproductive tract in large and small ruminants

A. Uterus: The uterus and uterine horns are the first structures to be assessed during trans-rectal ultrasonography. The non-gravid, fully involuted uterus is a muscular structure that generates an echogenic image whose echogenicity is dependent on uterine tone and luminal contents. As a result, echogenicity varies during the luteal and follicular stages of the estrus cycle (Griffin and Ginther, 1992). The endometrial wall thickness in bovine and bubaline can be measured for confirmation of animals is having subclinical endometritis or not. Generally, endometrial wall thickness is up to 8 mm in normal cyclic animals, if it increases more than 8 mm and the texture of the endometrium becomes hyper-echoic then it can be easily diagnosed as subclinical endometritis. In small ruminants, endometrial wall thickness is up to 3-5 mm in normal cyclic animals and if it increases more than 5 mm and the texture of endometrium becomes hyper-echoic then it can be easily diagnosed as endometritis. Pathological conditions of the uterus such as pyometra, Mucometra, and Hydrometra can be easily diagnosed by Ultrasonography techniques only which are remains undiagnosed in most of these small ruminants.

B. Ovary: The ovaries appear elliptic, round, or almond shape with a hyper-echoic outline. In the bovine and bubaline diameter of the ovaries is around 15 × 35 mm whereas in small ruminants, 12 × 22 mm depending on the animal's reproductive stage. The ability of technicians to identify ovarian structures is dependent on their knowledge and experience. In general, follicles have an anechoic spherical structure because they contain fluid, whereas the corpus luteum has a somewhat hypo-echoic texture since it is a transitory tissue part of the ovary. The ovary in anoestrous females is small, with follicles ranging in size from 2 to 4 mm (Pierson and Ginther, 1984). Follicles are detected as black entities with a smooth spherical contour due to fluid in the antrum. The ovulatory size of Graafian follicle (GF) in bovine and bubaline is ranging from 11 to 18 mm (Fricke, 2002) whereas 8 to 10 mm in small ruminants (Sharma and Sood, 2019). The size of GF varies based on the animal's parity and breed. Pathological conditions in the ovaries such as cystic ovarian follicle (COF) both of luteal cyst or follicular cyst origin can be easily diagnosed and confirmed by ultrasonography (Souza et al., 2013). In bovine and bubaline, the COF of luteal cyst origin is typically single with a diameter of more than 20 mm and a thickness of more than 3 mm, but in follicular cysts, the COF is either single or multiple with a diameter of more than 20 mm and a thickness of less than 3 mm (Fig. 1). Although goats have the same incidence as cattle, there is little information on the diagnosis and pathogenesis of follicular cysts. Diagnosis of the cystic condition is difficult in goats due to the limitation in performing rectal palpation and unavailability of ultrasound equipment under field conditions. Ultrasonography is considered the gold standard in goats for the accurate identification of ovarian cysts (Medan et al., 2004). A follicular cyst in goats is described as a fluid-filled, thin-walled ovarian structure that is larger than 10 mm in diameter and will last for more than 10 days (Souza et al., 2013).

Evaluation of status of reproductive tract in canine and feline

A. Uterus: In dogs and cats, the uterus is positioned between the urinary bladder ventrally and the descending colon dorsally. The non-gravid uterus can sometimes

Fig. 1: In cattle, 1. Graafian follicle, 2. Cystic ovarian follicle, 3. Both ovaries

be imaged. It is identified as a solid, homogenous relative hypoechoic structure. Even when a non-gravid uterine body is, it is sometimes difficult to identify the uterine horns, which become lost in small bowel echoes and mesenteric fat. The uterus can be distinguished from the small bowel by the absence of peristalsis and luminal gas, which frequently produces a poor acoustic shadow. Ultrasound is often used to diagnose pyometra, especially if vaginal discharge is minimal or absent. It is possible to diagnose pyometra before clinical signs. An enlarged uterus and uterine horns are among the ultrasonographic findings. The enlargement is usually symmetrical, but segmental or focal alterations might occur. The luminal contents are often homogeneous and anechoic, with very significant distal amplification, or echogenic. In endometritis, the uterine lumen shows minimal fluid accumulation but the endometrium is hyperechoic. The uterus can be visualized dorsal to the bladder. The endometrium is thickened and the uterine lumen contains anechoic or hypo-echoic material. Postpartum uterine involution can be monitored with ultrasound. Sub involution can be diagnosed by ultrasound where anechoic fluid accumulation is seen with hyperechoic areas of placental zones.

B. Ovary: Ovarian ultrasonography is still limited by the fact that normal, quiescent ovaries in medium to small size dogs can be difficult to visualize. A 7.5 MHz probe is recommended for exploring canine ovaries, while a 5.0 MHz probe can be used in larger dogs. Comparatively, ovaries are small, oval to bean-shaped. Ovaries are more visible during proestrus because of follicular development. It is difficult to monitor ovulation by ultrasound in canines as the pre-ovulatory follicle undergoes luteinisation (England et al., 2009). Ultrasound cannot accurately differentiate between different forms of ovarian cysts in dogs. Cystic ovaries on USG appear irregular with numerous anechoic sacs containing fluid. Larger follicular cysts possess a characteristic thin wall, an anechoic centre, and acoustic amplification at the distal end. The ovary's outer surface may be uneven. An elevation in ovarian size is also possible. Ovarian tumors can be identified ultrasonographically as a mass lesion in one or both ovaries. Exclusion of splenic, renal, or lymph node masses is frequently used to make an ovarian mass lesion diagnosis. Ovarian tumors appear as irregular mixed masses within the ovarian parenchyma. They are usually cystic with multiple small anechoic compartments. There may be anechoic areas separated by echogenic septae.

Pregnancy diagnosis in bovine and bubaline

Early identification of non-pregnant dairy animals after breeding enhances reproductive efficiency and pregnancy rate in cows and buffaloes by shortening the gap between AI services and increasing the number of AI services. Ultrasound is a speedy way for detecting pregnancy, and expert palpators quickly adjust to ultrasound technology by recognizing embryonic vesicle, embryo proper with heartbeats, amniotic membrane, and corpus luteum verum on day 30 post-breeding (Fig. 2; Ribadu and Nakao, 1999). Also, embryonic or fetal aging can be done by measuring the crown-rump length and head diameter (Transverse) of the embryo from day 30. Approximately at day 30, crown-rump length (CRL) is 1cm in length and it grows daily by 1 mm up to day 40, and later on, growth is comparatively more. In cattle and buffaloes, gestational aging up to day 60 is important later on there is no practical application. The concept behind 30 days pregnancy diagnosis in large ruminants is that estrus interval of 21 days if animals did not show post-AI estrus after 21 days can be scanned by ultrasonography on day 30 of gestation. If the animal is pregnant on day 30 well and good but if the animal is non-pregnant then based on ovarian and uterine status, estrus induction can be carried out by either hormonal or non-hormonal regimens. So, by employing the concept

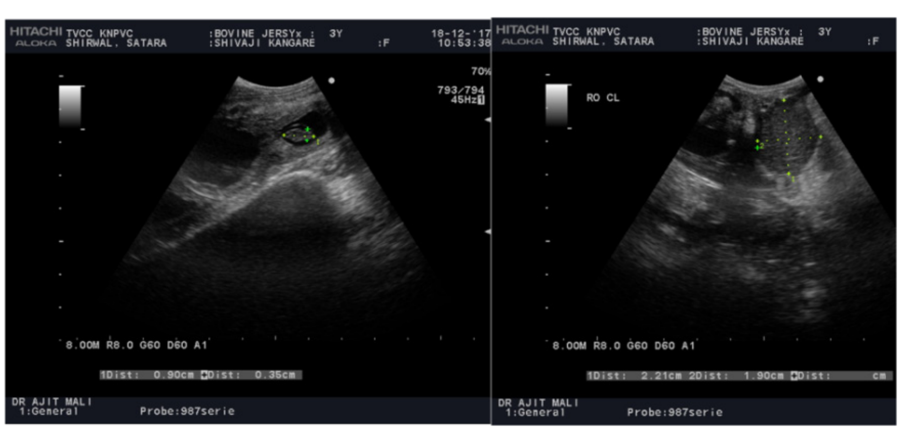

Fig. 2: Pregnancy diagnosis in cattle on day 32 (embryo with crown-rump length and HD measurement and CL Verum)

of 30 days pregnancy diagnosis, we can obtain the earliest detection of pregnant or non-pregnant status with any pathological condition of the ovary or uterus, as well as less and more accurate drugs for estrus induction, which reduces the cost of treatment as well as the time interval.

Pregnancy diagnosis in caprine and ovine

Early pregnancy diagnosis in animals improves reproductive efficiency and pregnancy rate in small ruminants. On day 25 post-breeding, experienced palpators adjust quickly to ultrasound technology by detecting embryonic vesicle, embryo proper with heartbeats, amniotic membrane, umbilicus, and corpus luteum verum. From day 25, embryonic or fetal aging can be performed by measuring the crown-rump length and head diameter (transverse) of the embryo (Fig. 3). Twins or triplets can be easily confirmed at day 25 of gestation for further pregnancy or parturition care by measuring the number of embryos and corpus luteum (s) present on both ovaries. After 50 days of pregnancy to later stages of gestation, only trans-abdominal scanning is recommended to better results without any complications to pregnancy in small ruminants (Suguna et al. 2008). During the middle to late pregnancy, the whole fetus is unable locates so crown-rump length is difficult to measure so fetal aging can be done based on measuring Bi-parietal diameter and placentomes only. In most small ruminants, breeding history is unknown it's better to start with trans-abdominal scanning first if any pregnancy-related structures are not observed then only we can proceed for trans-rectal scanning in small ruminants for further confirmation of reproductive status.

Pregnancy diagnosis in canine and feline

Uterine enlargement is seen as pregnancy detection of a gestational sac. The gestational sacs were first observed on day twenty after breeding in the dog. Clinically, it is preferable to ask the client to wait until day twenty-five or more after the final day of breeding for ultrasonographic confirmation of pregnancy, as gestational sacs with viable embryos can be recognized with high certainty at that time. At post-breeding day 25, the embryo is semi-circular, gestational sacs are of an ampullary shape and the embryo can be seen with the heartbeat. From day 28 elongation of the gestational sac starts. Between days 30-35 limb buds are seen. From 35 to 37 days; the fetuses are very active and cardiac activity is visible (Mantis, 2008). The gestational sacs became more oblong in the longitudinal direction. Fetuses are highly active and cardiac activity is easily identifiable. From 35 -40 days fetal organs such as the bladder and stomach are imaged. The shape of limbs is also visible (Fig. 4). The fetal sac showed comparatively less fluid than fetal mass than in previous stages. From 40 days onwards it is very easy to calculate biparietal diameter (BPD) as ossification is very prominent. It is difficult to measure CRL as fetuses grow bigger. Bony structures (ribs, vertebrae, and skull) are visible. From day 51 to 60 days it is not possible to take lumen or CRL measurements, because the fetuses fill the lumen and are too large to fit on the screen. Fetal vertebrae, ribs, liver, intestines, and heart are well visualized in all fetuses. Fetuses are active, with cardiac activity readily visible. Infertility due to Foetal resorption can be diagnosed by ultrasonography. In these cases, few or all sacs were reduced in size, and thickening of the sac wall was seen. It is possible to estimate litter size in early

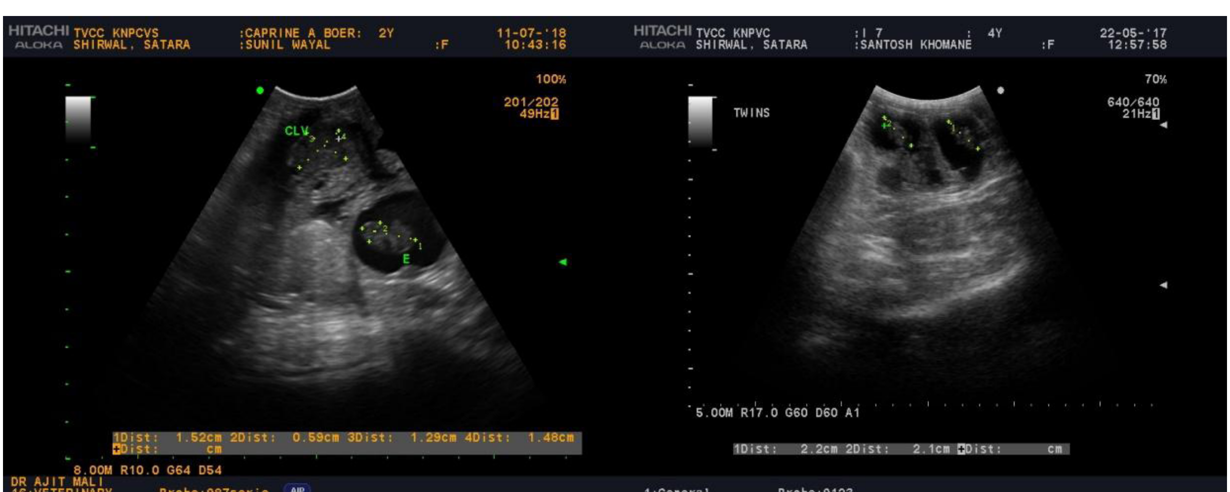

Fig. 3: Pregnancy diagnosis in caprine at Day 30 (left panel) and determination of twin pregnancies (right panel)

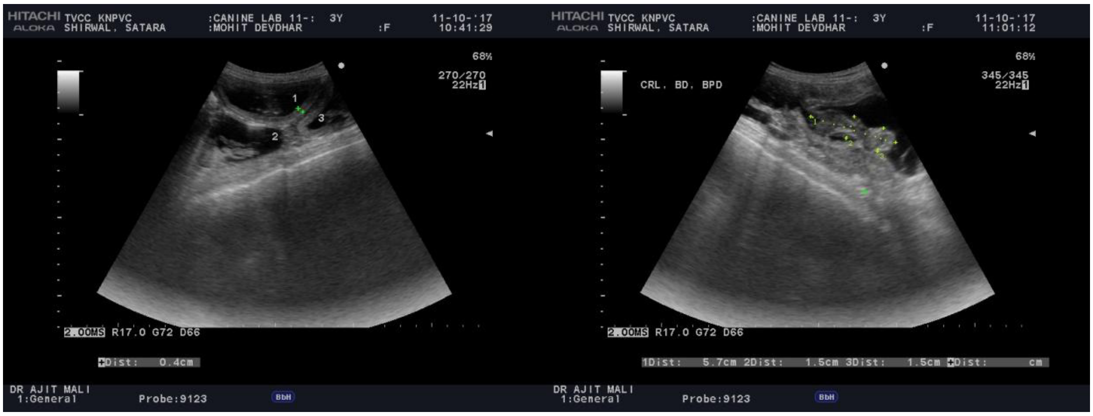

Fig. 4: Pregnancy diagnosis in canine at day 38 (left side) and measurement of crown-rump length, body diameter, and biparietal diameter (right side)

pregnancy in the smaller litter. As gestation advances, it becomes more difficult to estimate litter size.

Conclusion

Ultrasonography technique is a very easy, safe, economical, and practical way to confirm reproductive status either pregnant or non-pregnant from 30 days of gestation in farm and companion animals. In small ruminants and companion animals, it determines single or twins pregnancy as well as tentative litter size with gestational age in animals whose history of breeding or AI is unknown. The ultrasonography is used to diagnose various ovarian as well as uterine disorders on the spot of examination. Ultrasonography has introduced a new dimension to animal reproduction by allowing not only visualization of the reproductive tract but also early pregnancy diagnosis, surveillance of embryonic or fetal development, and detection of the estrous cycle phase. Confirmation of ovarian and uterine ailments such as cystic ovarian follicle, endometritis, hydrometra, mucometra, and pyometra can be easily diagnosed for the earliest treatment regimens. Ultrasonography is employed as both a diagnostic and applied research tool. Ultrasound is difficult to surpass in the realm of veterinary imaging because it is safe, has a wide range of uses, and is cost-effective. In the clinical discipline of animal reproduction, ultrasonography has emerged as the main diagnostic imaging modality of the twenty-first century. The scanner will never be able to replace the vet, but it can be a simple, obvious tool that assists him in diagnosing and reducing the range of error.

Competing Interest

There is no conflict of interest among the authors.

References

England GC, Russo M, Freeman SL. Follicular dynamics, ovulation, and conception rates in bitches. Reprod Domest Anim. 2009;44 Suppl 2:53-8. doi: 10.1111/j.1439-0531.2009.01416.x.

Feliciano MAR, Nepomuceno AC, Cardilli DJ, Nassar CL, Oliveira MEF, Kirnew MD, Almeida VT, Vicente WRR. B-mode ultrasound and Doppler mode for early-stage pregnancy diagnosis in Shi-Tzu bitches. Acta Scientiae Veterinariae, 2013; 41(1): 1-6.

Freitas LA, Mota GL, Silva HV, Carvalho CF, Silva LD. Can maternal-fetal hemodynamics influence prenatal development in dogs? Anim Reprod Sci. 2016;172:83-93. doi: 10.1016/j.anireprosci.2016.07.005.

Fricke PM. Scanning the future--ultrasonography as a reproductive management tool for dairy cattle. J Dairy Sci. 2002;85(8):1918-26. doi: 10.3168/jds.S0022-0302(02)74268-9.

Griffin PG, Ginther OJ. Research applications of ultrasonic imaging in reproductive biology. J Anim Sci. 1992;70(3):953-72. doi: 10.2527/1992.703953x.

Mali AB, Amle MB, Markandeya NM, Kumawat BL. Comparative efficacy of trans-rectal and trans-abdominal ultrasonography for early diagnosis of pregnancy and embryonic ageing in goats. Indian J Small Ruminants 2019; 25(2):171-175.

Mantis P. Ultrasonography of the urinary and genital system of the dog and cat. Iranian J Vet Surg 2008;63–71.

Medan MS, Watanabe G, Sasaki K, Taya K. Transrectal ultrasonic diagnosis of ovarian follicular cysts in goats and treatment with GnRH. Domest Anim Endocrinol. 2004;27(2):115-24. doi: 10.1016/j.domaniend.2004.03.006.

Padilla-Rivas GR, Sohnrey B, Holtz W. Early pregnancy detection by real-time ultrasonography in Boer goats. Small Rumin Res. 2005; 58:87-92. doi: 10.1016/j.smallrumres.2004.09.004.

Pierson RA, Ginther OJ. Ultrasonography of the bovine ovary. Theriogenology. 1984;21(3):495-504. doi: 10.1016/0093-691x(84)90411-4.

Polisca A, Zelli R, Troisi A, Orlandi R, Brecchia G, Boiti C. Power and pulsed Doppler evaluation of ovarian hemodynamic changes during diestrus in pregnant and nonpregnant bitches. Theriogenology. 2013;79(2):219-24. doi: 10.1016/j.theriogenology.2012.08.005.

Pujar NA Puro, Markandeya NM, Mali AB, . Kumawat BL. Studies on efficacy of select-Synch and Ovsynch protocols for induction and synchronization of estrus in Osmanabadi goats. Int J Sci Environ Technol. 2016; 5(6):4069-4073.

Pujar NA Puro, Markandeya NM, Mali AB, Kumawat BL. Comparative evaluation of therapeutic approaches for fecundity improvement in Osmanabadi goats. Intas Polivet 2017; 18 (1):100-102.

Rantanen NW, Ewing RL. Principles of ultrasound application in animals. Vet Radiol, 1981; 22(5):196–203.

Ribadu AY, Nakao T. Bovine reproductive ultrasonography: a review. J Reprod Dev. 1999; 45(1):13–28.

Sharma A, Sood P. Follicular dynamics in goats. The Haryana Veterinarian 2019; 58 (SI):1-7.

Souza J, Maia A, Brandão F, Vilela C, Oba E. Hormonal treatment of dairy goats affected by hydrometra associated or not with ovarian follicular cyst. Small Rumin Res. 2013; 111: 104-109.

Suguna K, Mehrotra S, Agarwal SK, Hoque M, Singh SK, Shanker U, Sarath T. Early pregnancy diagnosis and embryonic and fetal development using real-time B mode ultrasound in goats. J Small Rumi Res. 2008; 80: 80-86.