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Research Article

Ultrasonographic Descriptions of Reproductive Tracts of Cattle, History of Veterinary Ultrasound and its Current Practice in Nepal

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Abstract: Reproductive ultrasonography is a diagnostic technique used in the study of structures of the reproductive tract and to detect their pathological state. It is one of the non-invasive techniques used for the detection of variation and abnormalities in ovarian, uterine and other reproductive structures. Since, the development of diagnostic ultrasound in early 1940s, it has widely been used in detecting tissue structure and abnormalities in both humans and animals. In 1956, ultrasonography was first used in cattle to detect back-fat thickness. In Nepalese veterinary practice, ultrasound was first introduced in 2007 AD. Since, 2007 to date, ultrasound is being used in both large and small animal practice in various researches, institutions and clinics of Nepal. This article reviews the ultrasonographic description of ovaries and uterus, their pathological conditions, the use of ultrasonography in pregnancy diagnosis, detection of early embryonic death, fetal mummification and maceration based on the various journal papers, proceedings, books, and online articles. This article also documents the history of ultrasonography in veterinary practice of Nepal based on personal interviews and presents the prospects of reproductive ultrasonography in the context of Nepal. Reproductive ultrasonography can provide early, accurate and more reliable diagnosis in comparison to transrectal palpation and hence gaining popularity in modern reproductive management of cattle. Cost-effective and portable ultrasound equipment is now commercially available and are earning popularity day by day.

Keywords: Ultrasonography, Nepal, History, Veterinary, Reproductive, Cattle.

1. INTRODUCTION

Ultrasonography is a cross-sectional imaging technique that uses high-frequency sound waves to see the internal structures and pathology of tissues (Hayward, 2012). The probe containing piezoelectric crystal generates the sound waves and receives its reflection, which is then displayed on the screen. Currently, ultrasonography is used in various fields of medicine. Among the various uses of ultrasonography, its application in reproductive management of cattle is gaining popularity day by day. Ultrasonography enables the practitioner to visualize the architecture of the ovaries, uterus, reproductive vasculature and surrounding structures (Dhakal, 2015). Since the introduction of transrectal palpation, Gray-scale ultrasonography has been the most important technological inventions in the field of large animal research and clinical reproduction (Ginther, 1986). Reproductive ultrasound has become so popular in modern practice and somehow has replaced transrectal palpation in pregnancy determination, and diagnoses associated with uterine and ovarian infections (Fricke & Lamb, 2002). One of the greatest advantages of ultrasonography is that it is easy to use, non-invasive and cheaper in comparison to other diagnostic tools (Y. Ribadu & Nakao, 2002). Pregnancy detection, fetal sexing, observation of twins, visualization of early embryonic death and estrus synchronization can be efficiently performed with ultrasound in cattle. Ultrasonography has upgraded the managemental practices of cattle.

Principles of Ultrasonography

Ultrasonography is the technique where sound waves are passed through tissue interphases and their echoes from various tissues are received back and are

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displayed on a screen. Generally, reproductive ultrasound uses the sound waves of around 3.5 MHz, 5 MHz and 7.5 MHz (Y. Ribadu & Nakao, 2002). The sound waves are generated by a piezoelectric crystal that has a unique property to convert electrical waves into sound waves and vice-versa (N. Ahmad, 1997). The reflection in the form of echo occurs due to the difference of acoustic impedance between the tissue interphases.

In reproductive management of animals, a-B-mode (brightness modality) scanners are generally used (Y. Ribadu & Nakao, 2002). B- Mode ultrasound displays an image of a cross-section of tissue. The amplitude or intensity of echoes defines the brightness of the image in B- mode ultrasound. A hyperechoic tissue or organ appears bright and anechoic object appears dark in the ultrasound image. A hypoechoic tissue or organ appears in between grey and black color. Ultrasound images are best observed in a dark room where the human eye can visualize more shades of grey (Y. Ribadu & Nakao, 2002). Talking about transducer, linear probes containing piezoelectric crystals in rows are generally used in transrectal ultrasonography. The linear probe displays a rectangular image. Sometimes, sectorial probes are also used which produce a pie-shaped image.

Occasionally Doppler's ultrasonography is used to visualize the vasculature of the reproductive system at various stages of developmental cycles. It is based on measurements of relative change in returned ultrasound wave frequency when compared with the transmitted frequency. Doppler ultrasound is often combined with a B- mode ultrasound.

Techniques of reproductive ultrasound

Before performing the ultrasonography, the proper restraining of the animal is required. Cattle are initially tied in a proper place to restrict the animal movement. The rectal contents are then removed and a transrectal probe is introduced into the rectum just above the reproductive structures. Sometimes, a preliminary exploration of the reproductive tract via rectal palpation is also performed to have an idea about the location of the reproductive organs (Y. Ribadu & Nakao, 2002). Initially, the face of the transducer is coupled with a suitable gel and covered with plastic before inserting it in the rectum. The face of the transducer is firmly pressed against the walls of the rectum and moved systematically to view various reproductive parts. Occasionally, curved probes are also used to detect the reproductive organs.

Ultrasonographic description of various reproductive organs of cattle

1. Ovary:

The bovine ovary is a structure that provides different ultrasonographic images depending upon the phase of its development. Basically, an ovary consists of a corpus luteum, follicles, corpora haemorrhagica, ovarian vessels, cysts and ovarian stroma (Pierson and Ginther, 1988). Among all the structures, ovarian follicles and corpus luteum are the most viewed structures.

1.1 Follicles

Follicles are relatively easy to identify as they contain anechoic fluid inside them and appear as a black structure in an ultrasound image (Fricke & Lamb, 2002). Ovarian follicles are fluid-filled structures that are surrounded by an outer layer of thecal cell and an inner layer of granulosa cell (Fricke & Lamb, 2002). Follicles generally appear dark with diameters <25mm and are surrounded by a fine wall (Hanzen, Pieterse, Szenci, & Drost, 2000). Due to the continuous presence of follicular waves during the estrus cycle, there is a constant presence of large follicle > 8mm in ovary except for few days of the cycle (DesCôteaux, L., Gnemmi, G., Colloton, 2010). In ultrasound, follicles greater than 10mm in diameter can be detected with much ease. Follicles from 10-15mm of diameter can be identified with an accuracy of 90 % and follicle greater than 15mm can be identified with an accuracy of 100% (Aslan *et al.*, 2000). Ovulation can be detected by acute loss in the structure of large follicle >13mm in diameter (Pierson & Ginther, 1984). According to Ginther *et al.*, 1989 follicles typically ovulate when they are about 13-17 mm in diameter.

1.2 Corpus luteum

Corpus luteum is the structure that is formed after ovulation. It is very essential in the identification of puberty, stage of estrus cycle and pregnancy. It is a real challenge to identify corpus luteum via ultrasound because their morphological form changes continuously depending upon their stage of development (Luc DesCôteaux *et al.*, 2010). The corpus luteum may either be compact or maybe with a cavity. During the first 10 days post-ovulation, around 30-50 % corpus luteum develops a cavity which slowly regresses and becomes compact (Kito, S., Okuda, K., Miyazawa, K., 1986). A well-defined border of corpus luteum is visible after 3-4 days post ovulation (Luc DesCôteaux, 2010). The luteal tissue is generally more echogenic at the early stages, which become less echogenic at the end-stage due to more vascularization (DesCôteaux, L., Gnemmi, G., Colloton, 2010).

2. UTERUS

2.1 Uterus at diestrus phase

During diestrus, the uterus is quite homogenous in terms of echogenicity. Due to the circulating level of progesterone, it prepares itself for the implantation of the embryo. The uterus loses its tone has less endometrial liquid and becomes thinner (DesCôteaux, L., Gnemmi, G., Colloton, 2010). The uterus is more highly coiled during diestrus. (Pierson & Ginther, 1988). The presence of a higher concentration of fluid during diestrus is considered pathological

(Colazo, Ambrose, & Kastelic, 2010). Diestrus is generally detected by the presence of corpus luteum in ultrasound.

2.2 Uterus at Periestrus phase

Periestrus phase includes proestrus, estrus and the beginning of the metestrus. During periestrus, the uterus is more heterogeneous in terms of echogenicity (DesCôteaux, L., Gnemmi, G., Colloton, 2010). During this period there is a high level of circulating estrogen, the uterus is more swollen, its tone increases and there is the presence of fluid inside the uterus. The ultrasound image looks much darker and the horn is less coiled in comparison to the diestrus (Y. Ribadu & Nakao, 2002). The presence of rosette-shaped mucus in the uterine lumen is very typical during this period (Palgrave, 2012).

3. Pregnancy

Pregnancy diagnosis is a very important application of ultrasonography. Early pregnancy diagnosis after insemination helps in better management of the pregnant animals and early submission of non-pregnant animals for subsequent breeding (Thompson *et al.,* 1995). The sensitivity of ultrasonography to detect pregnancy on the 24th day, 26th day and 28th day is 93.05, 100 and 100 (Moharrami *et al.,* 2013). From day 29th one can have a diagnosis of pregnancy with 100 % accuracy (Romano *et al.,* 2006). Pregnancy detection via transrectal ultrasound was found superior to transvaginal ultrasound (Altun & Gürbulak, 2011). Ultrasound also can measure the length and volume of the fetus (Kastelic *et al.,* 1988). In buffaloes, pregnancy has been detected even on the 19th day with ultrasonography (Pawshe *et al.,* 1994). Although an early diagnosis of pregnancy is possible as early as the 9th day (Boyd *et al.,* 1988). For 100 % accuracy days after 25 are appropriate (Lamb & Fricke, 2014). The heartbeat in the embryo can be detected from the time it is visible in Ultrasound, generally after 26th day (Pierson, R.A. and Ginther, 1984).

4. Twin pregnancy

Twin pregnancy is not very common. Ultrasonography allows us the detection of the twins with high accuracy rates. The rate of twinning is found in increasing rate (Day *et al.,* 1997). Early detection of twin pregnancy can help a farmer to provide better management to the animal. Abortions, dystocia, post-partum metritis, ketosis, and fatty liver are very common in bovine carrying twins (Van Saun, 2001).

There Are Two Types of Twins

a. Monozygous

Ultrasound image shows monozygous twins very close to each other and generally with single corpus luteum (DesCôteaux *et al.,* 2010).

b. Dizygous

Dizygous twins are either in the same horn on in different horn. Ultrasound image generally reveals two corpus luteum during dizygous twin formation (DeCôteaux *et al.,* 2010).

5. Early embryonic and fetal death

Death of the embryo from fertilization to the end of organogenesis is called Early Embryonic Death and after organogenesis is called fetal death (Ayalon, 1978). The majority of the embryonic death occurs before 25th day which goes unnoticed. The time between 25 and 42 is important for the attachment or embryonic membrane to the uterine epithelium. Generally, ultrasound allows the detection of fetal death where there is a loss of normal fetal architecture and the amniotic fluid is cloudy (DesCôteaux, 2010). In the case of mummification, there is the presence of hyperechoic tissues like bones with shadows and the ultrasound image is poor.

Abnormalities and Pathological conditions of the reproductive tract of cattle

Cystic ovary in cattle is characterized by the abnormal presence of large anovulatory follicles which persist for a long period without the presence of corpus luteum and generally hampering the normal estrus cycle (DesCôteaux *et al.,* 2010). The cysts are larger than 25 mm in diameter and persist for more than 10 days (Seguin, 1980).

There Are Two Types of Ovarian Cyst

a. Follicular cyst:

They generally have a thin wall which is generally less than 3mm in diameter (Kahn, 2010) and the structure appear as anechoic and dark (Y. Ribadu & Nakao, 2002). The follicular cyst may be multiple and are present on one or both the ovaries.

b. Luteal cyst:

They have a thick wall greater than 3mm in diameter which appears grey with the central anechoic dark region. (Y. Ribadu & Nakao, 2002). Incidence of cystic CLs ranges from 25.2% to 78.8% during diestrus and decreases during estrus (Kahn, 2010).

2. Pyometra:

Pyometra is the accumulation of pus inside the uterus or uterine horn. They generally occur via bacterial invasion into the uterus. In ultrasonography they appear as a homogenous black anechoic region or there may be the presence of some echogenic debris appearing white in black fluid and uterine lumen are distended (DesCôteaux *et al.,* 2010). When the uterine content is very thick then it has similar echogenicity like that of the uterine wall (Aba *et al.,* 1995). During pyometra, there is no evidence of the placentome, fetus and fetal membrane (Zambrano-Varón J., 2015).

3. Endometritis

Endometritis is the inflammation of the endometrium. In ultrasound image the condition is characterized by thickening of the endometrial wall, irregular echogenicity of the endometrium and accumulation of the small amount of intraluminal fluid which may be anechoic or there may be the presence of echogenic material at various degrees (DesCôteaux, L., Gnemmi, G., Colloton, 2010).

4. Mummification:

During mummification, the ultrasound image appears as a poorly defined mass with a complete absence of uterine fluid (Y. Ribadu & Nakao, 2002) Ultrasound alone cannot confirm the condition.

5. Maceration

In ultrasonography during maceration the uterine wall is thickened, the bones are observed as an echogenic area suspended in a non-echogenic uterine fluid.

Besides, above pathological conditions, ultrasound also can be used for the diagnosis of birth abnormalities like hypoplasia of ovary, uterus, and vagina (DesCôteaux *et al.*, 2010). It is also beneficial in identification conditions like hydrometra, mucometra, and reproductive tumors.

History and current practice of veterinary ultrasound in Nepal

The global use of diagnostic ultrasound started in 1942 when Dr. Karl Theodore Dussik of Austria first used it in the detection of brain tumors of a human patient (Newman & Rozycki, 1998). With the beginning of the use of ultrasonography in human disease diagnosis, it led an idea to veterinarians to use it for the study of animal tissues. In the year 1956 ultrasound was used to detect backfat thickness in cattle by Douglass Howry at the University of Colorado medical center (Temple *et al.*, 1956). In the same year, an A-mode ultrasound was used to detect backfat thickness in pig (Dumont, 1957). In Nepal, there is no documentation of the use of Ultrasound before 2007. In 2007 the first ultrasound machine (Honda HV-1500, Honda electronics, Japan) was introduced in Nepal by Dr. Bhuminand Devkota (Devkota, 2019). Since then various researches in animals were conducted by the use of transrectal ultrasonography (Bhuminand *et al.*, 2009; 2014).

Currently, reproductive transrectal ultrasound is in practice in the National cattle research program in Chitwan (Acharya, 2019) and the National Buffalo research program in Sunsari (Devkota, 2019). Nepalese army also uses transrectal ultrasound to study the reproductive status of the horse (Dhakal, 2019). For the diagnostic purpose in 2019, Hamro Veterinary Sewa Sahakari of Jhapa district bought a transrectal ultrasound machine (Versatile vet ultrasound farm scan-L600) in about 4 lakh NC (Sapkota, 2019). Diagnostic ultrasound in other hands in small animal practice is being used in Animal Medical Center, Kathmandu (Chand, 2018), Agriculture and Forestry University, Chitwan (Pokhrel, 2019) and in various private animal clinics of Nepal. In paklihawa campus, ultrasound was first used to detect the presence of chylothorax due to heart worm in dog (Singh *et al.*, 2018).

Major Uses of Reproductive Ultrasound in Cattle

There are multiple uses of ultrasonography in cattle. It is used to understand the situations during various stages of the estrus cycle. It is also used to study the status of corpus luteum and ovarian follicles. Similarly, it is used in early pregnancy diagnosis and to detect early embryonic death. Fetal sex determination can be performed via ultrasound. It is a very useful tool to study the uterine involution process. It accurately helps in the identification of cystic ovary. It can be used during estrus synchronization programs. It is a very essential diagnostic tool in the identification of the pathological condition of the reproductive tracts. With the use of transrectal ultrasound, proper management of the cattle according to its condition can be done. It allows better diagnosis of reproductive tract than rectal palpation and is being used with constant modification and advancement.

Prospects of Reproductive ultrasound in the context of Nepal

The use of Reproductive ultrasonography in Nepal is not so popular to date. Although, Reproductive ultrasound was introduced in 2007 (Devkota, 2019), it is yet to gain popularity in the context of Nepal. It may be due to the lack of well-trained practitioners and poor economic status of farmers. In Nepal, most of the diagnosis of reproductive abnormality in cattle is based on rectal palpation. In such a situation, ultrasonography can provide a new approach to deal with reproductive problems. Ultrasound can differentiate the layers of soft tissue and also differentiates it well from fluid with clear distinction. As Ultrasound is a non-invasive and innocuous method, it has a wide safety margin in comparison to other radiological methods. As ultrasound is comparatively cheap and easy to operate it's gaining popularity in veterinary practice for the diagnosis of the various reproductive structures.

In the context of Nepal, the practice of using transrectal ultrasound can help farmers and veterinarians to identify the condition of the reproductive tract. In large bovines, the method is relatively easy to perform under field conditions under limited resources (Shah, 2011). Ultrasound is very helpful in differentiating the various layers of soft tissue and also differentiates it well from fluid with clear distinction. It can be an effective diagnostic tool for the detection of abnormality and pathological state of the bovine reproductive tract.

CONCLUSION

Ultrasonography is indeed a very efficient diagnostic tool in the context of Nepal. It has a wide safety margin as it is a non-invasive and innocuous method in comparison to other diagnostic tools. Being cost-effective with portable machines, it can be operated in field conditions and can be used in all part of the country. Ultrasonography is relatively more accurate than transrectal palpation. The use of transrectal ultrasound is gaining popularity in Nepal with constant modification and advancement. With proper training and a certain level of skills, the practitioner can now offer services to the farmer for better reproductive management and enhanced productivity.

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REFERENCES

1. Aba, M.A., Forsberg, M., Kindhal, H., Sumar, J., & Edqvist, L. (1995). Endocrine changes after mating in pregnant and non-pregnant llamas and alpacas. *Acta Vet Scand*, *36*, 489–498.
2. Acharya, M. (2019). Use of ultrasound in cattle in Nepal. (S. Jyoti, Interviewer)
3. Altun, Ö., & Gürbulak, K. (2011). A Comparison of Diagnosis of Early Pregnancy in Dairy Cows Via Transrectal and Transvaginal Ultrasound Scanning. *J Fac Vet Med Univ Erciyes*, *8*(1), 17–21.
4. Aslan, S., Findik, M., Erünal-Maral, N., Kalender, H., & Celebi, M. S. E. (2000). Comparison of various examination methods used in ovarian diagnostics in cattle. *Dtsch Tierarztl Wochenschr*, *107*(6), 227–230.
5. Ayalon, N. (1978). A review of embryonic mortality in cattle. *J. Reprod, Fert.*, *54*, 483–493.
6. Bhuminand, D., Bohara, T.P., Kumar, S., Motozumi, M., & Yoh-Ichi, M. (2009). Proceedings of the British Society of Animal Science. *Ovarian Status, Uterine Pathology and Physical Condition of Anoestrus and Culled Buffaloes in Chitwan, Nepal*, 15–15. https://doi.org/10.1017/S1752756200028544
7. Boyd, J.S., Omran, S.N., & Ayliffe, T. R. (1988). Use of a high frequency transducer with real time B-mode ultrasound scanning to identify early pregnancy in cows. *Vet. Rec.*, *123*, 8–11.
8. Chand, B. (2018). Use of diagnostic ultrasound in Nepal. (S. Jyoti, Interviewer)
9. Colazo, M. G., Ambrose, D. J., & Kastelic, J. P. (2010). Practical uses for transrectal ultrasonography in reproductive management of cattle. *World Buiatrics Congress*, (March 2014), 1–22. Retrieved from https://www.researchgate.net/publication/260597639
10. Day, J.D., Weaver, L.D., & Franti, C. (1997). Association of twin pregnancy diagnosis and parturition with days open, days pregnant at diagnosis, parity, and milk production in dairy cattle. *Bov Pract*, *2*, 25–28.
11. Devkota, B. (2019, 7 28). Introduction of transrectal ultrasound in Nepal. (S. Jyoti, Interviewer)
12. Devkota, B., Bohara, T.P., & Yamagishi, N. (2012). Seasonal Variation of Anestrus Conditions in Buffaloes (Bubalus bubalis) in Southern Nepal. Asian Journal of Animal and Veterinary Advances, 7(9), 910–914.
13. DesCôteaux, L., Gnemmi, G., & Colloton, J. (2010). *Ruminant and Camelid Reproductive Ultrasonography*.
14. Dhakal, I. (2015). Practical application of transrectal ultrasonography for reproductive management of cattle and buffaloes. *The Blue Cross.*
15. Dhakal, I. (2019). Use of transrectal ultrasound in the horse. (S. Jyoti, Interviewer)
16. Dumont, B. L. (1957). Nouvelles methods pour l'estimation de la qualite des carcasses sur les porcs vivants. *Joint Food and Agriculture Organization of the United Nations/European Association for Animal Production Meeting on Pig Progeny Testing*.
17. Fricke, P., & Lamb, C. (2002). Practical Applications of Ultrasound for Reproductive Management of Beef and Dairy Cattle. *The Applied Reproductive Strategies in Beef Cattle Workshop*, 228–246. Retrieved from http://beefrepro.unl.edu/proceedings/2002manhattan/2002ManhattanARSBC.pdf
18. Hanzen, C. H., Pieterse, M., Szenci, O., & Drost, M. (2000). Relative Accuracy of the Identification of Ovarian Structures in the Cow by Ultrasonography and Palpation Per Rectum. *Veterinary Journal*, *159*(2), 161–170. https://doi.org/10.1053/tvjl.1999.0398
19. Hayward, N. (2012). BSAVA Manual of Canine and Feline Ultrasonography. In British Small Animal Veterinary Association (Vol. 53).
20. Kahn, C. M. (2010). Cystic Ovary Disease (10th edition ed.). Whitehouse Station, NJ: The Merck Veterinary Manual.
21. Kastelic, J. P., Curran, S., Pierson, R. A., & Ginther, O. J. (1988). Ultrasonic evaluation of the bovine conceptus. Theriogenology, 29(1), 39-54.
22. Kito S., Okuda K., & Miyazawa K., S. K. (1986). Study on the appearance of the cavity in the corpus luteum of cows by using ultrasonic scanning. *Theriogenology*, *25*, 325–333.
23. Lamb, G. C., & Fricke, P. M. (2014). Ultrasound – Early Pregnancy Diagnosis and Fetal Sexing. *Applied Reproductive Strategies in Beef Cattle*, (October), 219–229.

24. Moharrami, Y., Khodabandehlo, V., Ayubi, M. R., & Mosaferi, S. (2013). Accuracy rate of early pregnancy diagnosis in Holstein heifers by transrectal ultrasonography using 7. 5MHz linear array transducer. *European Journal of Experimental Biology*, *3*(3), 678–680.
25. Ahmad, N. (1997). Basic Principles of Diagnostic Ultrasound. *Pakistan Vet. J .*, *17*(3), 231–261. https://doi.org/10.1007/978-1-4684-3486-6_21
26. Newman, P. G., & Rozycki, G. S. (1998). The history of ultrasound. *Surgical Clinics of North America*, *78*(2), 179–195. https://doi.org/10.1016/S0039-6109(05)70308-X
27. Palgrave, K. (2012). *Bovine Reproduction: Clinical ultrasound booklet with Easi-Scan*.
28. Pawshe, C.H., Appa Rao, K.B.C., Totey, S. M. (1994). Ultrasonographic Imaging to Monitor Early Pregnancy and Embryonic Development In The Buffalo (Bubalus bubalis). *Theriogenology*, *41*, 697–709.
29. Pierson, R.A., & Ginther, O. J. (1986). Ovarian follicular populations during early pregnancy in heifers. *Theriogenology*, *26*, 649–659
30. Pierson, R.A., & Ginther, O. J. (1988). Ultrasonic imaging of the ovaries and uterus in cattle. *Theriogenology*, *29*, 21–37.
31. Pierson, R.A., & Ginther, O. J. (1984). Ultrasonography for Detection of Pregnancy and Study of Embryonic Development in Heifers. *Theriogenology*, *22*(2), 225–233.
32. Pierson, R. A., & Ginther, O. J. (1984). Ultrasonography of the bovine ovary. *Theriogenology*, *21*(3), 495–504. https://doi.org/10.1016/0093-691X(84)90411-4
33. Pokhrel, S. (2019). Use of diagnostic ultrasound in Nepal. (S. Jyoti, Interviewer)
34. Romano, J. E., Thompson, J. A., Forrest, D. W., Westhusin, M. E., Tomaszweski, M. A., & Kraemer, D. C. (2006). *Early pregnancy diagnosis by transrectal ultrasonography in dairy cattle*. *66*, 1034–1041. https://doi.org/10.1016/j.theriogenology.2006.02.044
35. Sapkota, D. (2019). Introduction of transrectal ultrasound in Nepal. (P. Gautam, Interviewer)
36. Seguin, B. (1980). *Current Therapy in Theriogenology* (D. Morrow, ed.). Philadelphia: W.B Saunders CO.
37. Shah, S. P. (2011). Fundamentals. In S. P. Shah, D. D. Price, G. Bukhman, S. Shah, & E. Wroe, Manual of Ultrasound for Resource Limited Settings (first edition ed., pp. 19-28). Boston MA , USA: Partners in Health, Program in infectious Disease and Social Change, Havard Medical School.
38. Singh, D.K., Adhikari, A., Rimal, S., & Singh, K. (2018). Chylothorax due to heart worm. The Blue Cross, 15, 83–86.
39. Temple, R.S., Stonaker, H.H., Howry, D., Posakony, G., & Hazaleus, M. H. (1956). Ultrasonic and conductivity methods for estimating fat thickness in live cattle. *Am Soc Anim Prod West Sec Proc*, 477–481.
40. Thompson, J.A., Mainrh, W.E., Etherington, W.G., Momont, H.W., Mark, L.K. (1995). Evaluation of the benefits of the timing of pregnancy testing by transrectal palpation in dairy cattle. *J. American Vet. Med. Asso.*, *207*, 1462–1465.
41. Van Saun, R.J. (2001). Comparison of pre - and postpartum performance of Holstein dairy cows having either a single or twin pregnancy. *Proceedings of the Annual Convention of the American Association of Bovine Practitioners*, 204.
42. Ribadu, A.Y., & Nakao, T. (2002). Bovine Reproductive Ultrasonography: A Review. *Journal of Reproduction and Development*, *45*(1), 13–28. https://doi.org/10.1262/jrd.45.13
43. Zambrano-Varón, J. (2015). Reproductive Application of Ultrasound in the Female Buffalo (Bubalus bubalis). In G. N. Purohit, *Bubaline Theriogenology* (p. A5709.1215). Ithaca NY: International Veterinary Information Service.