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Reproductive processes in cows and the ultrasonography use

Los procesos reproductivos en vacas y el uso de la ultrasonografía



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Abstract

Transrectal ultrasonography is a technology used in cattle reproductive management as a means of gestation diagnosis and estrous cycle monitoring. Likewise, it has application in the management, diagnosis and treatment of both reproductive processes and in reproductive biotechnology programs. The objective of the work was to carry out a bibliographic review on the reproductive processes in cows and the ultrasonography use. A bibliographic review was carried out including publications from 2022 and previous years. Criteria that support the development achieved in the use of ultrasound were considered, based on the logical analysis of the consulted bibliography. The keywords used were ultrasonography, ultrasound, cattle, bovine reproduction, transrectal palpation, estrous cycle, follicular dynamics, ovarian function, Doppler, corpus luteum functionality, early pregnancy diagnosis, embryonic death. Transrectal ultrasonography has increased our knowledge and understanding of bovine reproductive physiology. Technological advances have allowed its use in the reproductive management of cattle to increase. Ultrasonography is a revolutionary advance in research that has influenced the efficiency of cow reproductive management.

Keywords: ultrasound, cattle, transrectal palpation, reproduction.

Resumen

La ultrasonografía transrectal es una tecnología utilizada en el manejo reproductivo de los bovinos como medio del diagnóstico de gestación y de seguimiento del ciclo estral. Asimismo, tiene aplicación en el manejo, diagnóstico y tratamiento tanto de procesos reproductivos como en programas de biotecnología reproductiva. El objetivo del trabajo fue realizar una revisión bibliográfica sobre los procesos reproductivos en vacas y el uso de la ultrasonografía. Se realizó una revisión bibliográfica incluyendo publicaciones del 2022 y años previos. Se consideraron criterios que sustentan el desarrollo alcanzado en el uso de ultrasonido, basados en el análisis lógico de la bibliografía consultada. Las palabras clave utilizadas fueron ultrasonografía, ecografía, bovinos, reproducción bovina, palpación transrectal, ciclo estral, dinámica folicular, función ovárica, Doppler, funcionalidad del cuerpo lúteo, diagnóstico precoz de la preñez, muerte embrionaria. La ultrasonografía transrectal ha aumentado nuestro conocimiento y comprensión de la



fisiología de la reproducción del bovino. Los avances tecnológicos han permitido que su empleo en el manejo reproductivo del ganado bovino se incremente. La ultrasonografía es un avance revolucionario en la investigación que ha influenciado la eficiencia en el manejo reproductivo de la vaca.

Palabras claves: ecografía, bovinos, palpación transrectal, reproducción.

INTRODUCTION

The decline in fertility observed in cattle in recent decades and the increasing requirements of farmers have made periodic reproductive monitoring essential to urgently identify and solve problems in reproductive efficiency. Traditionally, the main diagnostic methods for reproductive monitoring in cattle included rectal palpation, vaginal discharge inspection and vaginoscopy (Quintela *et al.*, 2012). Ultrasonography is a tool that has greatly contributed to facilitate the reproductive management of cattle, it has been shown that cows have two or three waves of follicular development during an estrous cycle (Ginther *et al.*, 1989), it has also allowed monitoring individual follicles during their development and determining follicular development patterns (Pierson & Ginther, 1984; Sirois & Fortune, 1988), it also allows the diagnosis of gestation (Kastelic *et al.*, 1988), as well as fetal sex determination (Curran *et al.*, 1989), diagnosis of reproductive organs and postpartum follow-up (Avellaneda *et al.*, 2014), pathologies such as ovarian cysts (Farin *et al.*, 1990), to which is added doppler ultrasonography for blood flow among other functions (Constantino-Rocha *et al.*, 2019; Holton *et al.*, 2022). B-mode and real-time transrectal ultrasonography was initially used as a means of diagnosing the estrous cycle, and currently has great application in management, diagnosis and treatment in both specialized reproductive processes and reproductive biotechnology programs (Kramer *et al.*, 2016). The contribution of ultrasonography in the study of cow reproductive processes is of great interest because it has great impact on reproductive efficiency. Some studies have shown that a *corpus luteum* (CL) is detectable by transrectal ultrasonography from two days after ovulation until day 21 of the subsequent cycle, while progesterone (P4) concentrations only rise from day six to day 16 of the cycle, so ultrasonography may be a better indicator of reproductive status than P4 measurement (McDougall & Rhodes, 1999). Since the 1990s, ultrasonography has become a diagnostic method used worldwide as a result of advances in its development: smaller size, high level of autonomy, high image quality and affordable prices. It improves the identification of the different stages of the estrous cycle and in the diagnosis of ovarian and uterine pathologies and pregnancy diagnosis, as well as its possible alterations (embryonic mortality, fetal malformations, among others) and helps to determine fetal sex from day 55 of bovine gestation (Quintela *et al.*, 2012). As a research and diagnostic tool it has been used in many discoveries, most of which would otherwise have escaped detection for years (Griffin & Ginther, 1992). There are some reviews on the subject of ultrasonography, but the focus on the cow and her reproductive processes has been little explored, as well as



the practical aspect in which its use is increasingly necessary. Therefore, the objective of the study was to perform a literature review on the reproductive processes in cows and the use of ultrasonography.

Background

In Mexico, cattle ranching has been developed in extensive grazing systems, reproductive efficiency is important in herd profitability as well as natural resources in cow-calf production systems, it is influenced by herd management practices ([Lassala et al., 2020](#)). The cow should produce one calf per year, an annual pregnancy test, helps to eliminate inefficient cows, a herd in defined breeding season that identifies and eliminates non-pregnant cows, and incorporates heifers, allows to be efficient ([Gwynn et al., 2018](#)). Increasing the percentage of pregnant cows' results in more calves weaned, giving more profit per cow ([Burns et al., 2010](#)). Eliminating empty or subfertile cows and the use of replacement heifers improves herd fertility, the money saved can help improve facilities ([Martínez-Albarrán et al., 2021](#)). Pregnancy diagnosis is performed in 31 % of herds, 23 % discard non-pregnant cows, less than 10 % use estrous cycle synchronization methods, heifers are usually inseminated after two years of age (73 %) ([Lassala et al., 2020](#)). The most common methods to determine pregnancy are: transrectal palpation, transrectal ultrasonography ([Kramer et al., 2016](#)) and determination of hormone levels by blood tests ([Szelényi et al., 2015](#); [Engida et al., 2022](#)) and in advanced gestations trans-abdominal ultrasonography ([Hayawie-Lazim et al., 2016](#)). When all these methods are performed correctly, they are safe for the cow and the fetus, although there are several factors that naturally cause early pregnancy loss ([Ryan et al., 1993](#); [Inskeep et al., 2005](#)). "Ultrasound" is the sound whose vibration frequency is higher than the limit perceptible by the human ear (20000 Hz), the ultrasounds emitted by the probes of ultrasonography equipment have a frequency generally comprised between two and 10 million Hz ([Segura-Grau et al., 2014](#)), through the use of ultrasonic waves, equipment has been developed that has diverse applications and is used in human ([Rehman-Khan, 2022](#)) and animal medicine ([Guedes et al., 2022](#)), which have been called ultrasound scanners ([Díaz-Rodríguez et al., 2007](#)), the "ultrasound" used in medicine, allows the exploration of the body interior by means of ultrasound ([Gargus et al., 2020](#)). Economic pressure demands an increase in productivity and an increase in technologies for cattle management. Therefore, keys to fertility management have been identified: 1) data management, 2) genetic selection, 3) nutritional management, 4) disease control, 5) reproductive management, 6) synchronization, 7) rapid diagnosis of reproductive status ([Mee, 2007](#); [Terry et al., 2020](#); [Brito et al., 2021](#)). Future trends to improve fertility will be possible including 1) development of fertility phenotypes, 2) specific genomic fertility markers, 3) early and rapid pregnancy detection, 4) increased use of activity monitors, 5) improved breeding protocols, 6) automated online sensors for phenotypes, and 7) capturing and mining data



sources (Crowe *et al.*, 2018). Ultrasonography is indispensable in studies correlating bovine reproductive morphology (structure) and function (Ribadu & Nakao, 1999). For the study of effects of hormones and drugs commonly used in reproduction, on follicular vascularization, observed by Doppler ultrasonography (Pereira-de Moraes *et al.*, 2021). Fetal sex determination and embryonic mortality monitoring are less likely to be applied in herd management, however, they are valuable for reproductive physiology research (Beal *et al.*, 1992; Kamimura *et al.*, 1997; Chagas & Lopes, 2005). Ultrasonic signals were observed as a natural phenomenon. In 1779, Lazzaro Spallanzani discovered waves associated with the hunting of bats (Martínez-Rodríguez *et al.*, 2007). By plugging the bat's ear with wax he deduced that when flying it must produce some sound that was reflected on objects (Águila-Carbelo *et al.*, 2019). Pierre and Jacques Curie in 1880, discovered the phenomenon of piezoelectricity, present in crystals that are deformed by internal forces, when subjected to electrical energy, producing oscillations in the form of a wave above the range audible to humans, they experimented on quartz and tourmaline crystals (Martínez-Serrano, 1995; Zhou *et al.*, 2011). In 1883 Galton's whistle appeared, for the control of dogs by a sound inaudible to humans (Águila-Carbelo *et al.*, 2019). In 1842 Johann Christian Doppler observed the change in frequency emitted by a sound source. The effect, Doppler, occurs when a lens and observer move in relative motion (Martínez-Rodríguez *et al.*, 2007). Armand Hippolyte L. Fizeau, in 1848, generalized Doppler's work by applying his theory to light (Paolinelli, 2013). Richardson initiated in 1912 the use of ultrasound to detect submerged objects. Langevin and Chilowsky, produced in 1917 the first piezoelectric ultrasound generator (Águila-Carbelo *et al.*, 2019). During 1930 and 1940, ultrasound was applied for medical purposes in Germany, Austria, France and Switzerland, in neuralgia, myalgia, arthritis and osteoarthritis, (Edler & Lindström, 2004). Karl Dussik, in 1942 used it to explore brain anomalies. John Julian Wild studied, using the A-mode, the changes of breast tissue waves with the equipment at a frequency of 15 megacycles to detect tumors, began its use in the brain, and in cardiology to study oscillations of the mitral valve (Águila-Carbelo *et al.*, 2019). Douglas Howry in 1947, pioneered the visualization of carcinoma and soft tissues (Howry *et al.*, 1954). Ultrasonography in the reproductive management of cows has great relevance, since in order to be more efficient the reproductive diagnosis should be performed when reincorporating the calving cow as soon as possible to the reproductive management program to reduce the number of days open (La Torre, 2001). The use of this technology in the variation in the elapsed interval to estrus, after the application of PGF2 α , was understood to transrectal palpation, to monitor the growth patterns of individual follicles (Sartori & Barros, 2011).



Significance and Potential of Transrectal Ultrasonography

Transrectal ultrasonography has been available for reproductive management decisions since the mid-1980s, and provides a wide range of morphologic information without invading or altering tissues. Examinations can be performed repeatedly over many days, or a dynamic event such as ovulation can be monitored in its entirety by continuous observation for 30 minutes or more. The inclusion of ultrasonic examinations in experimental protocols provides the opportunity to associate changing morphology with hormonal and other functional changes (Griffin & Ginther, 1992; Ginther, 2014). Real-time B-mode ultrasound provided the opportunity to improve methods of ovarian function assessment and pregnancy diagnosis in cattle, determination of fetal sex in early gestation from day 55 to 85 and verification of embryo viability by fetal heartbeat monitoring are unique methods involving ultrasound scanning. These techniques and the method for evaluating artificial insemination can be used to improve reproductive management in cattle (Beal *et al.*, 1992; DesCôteaux *et al.*, 2009). Within these reproductive technologies, the importance of ultrasonography is to extend the advantage of early reproductive diagnosis that, in some production systems, lies in the separation of groups of pregnant and empty females to implement reproductive strategies that increase overall fertility. Ultrasonography is a technique of accuracy in the early diagnosis of pregnancy in cows, both in natural mating programs (Alonso-Alanusa *et al.*, 2012), as well as fixed-time artificial insemination (Beal *et al.*, 1989) over that obtained by rectal palpation (Gwynn *et al.*, 2018). Clinical researchers have taken advantage of technological developments such as solid-state circuitry, real-time imaging, color and power Doppler, transrectal and transvaginal ultrasonography, and 3D imaging to improve research and diagnosis in very diverse areas (Campbell, 2013).

B-MODE ULTRASONOGRAPHY

Follicular dynamics in the cow

The wave-like follicular growth theory was originally proposed by Rajakoski in 1960, who based on visual and histological observations, proposed that the growth of ovarian follicles develops in the form of waves and that during an estrous cycle two follicular waves propagate, this theory was questioned for more than 20 years, during which multiple studies were conducted with contradictory results on the nature of follicular growth ((Rajakoski, 1960; Adams *et al.*, 2008; Henao-Restrepo, 2010; Bó *et al.*, 2020). This theory was confirmed in cattle thanks to the use of ultrasonography in the 1980s (Pierson & Ginther, 1984). Since the 70s Rosalyn Sussman Yallow and Solomon A. Berson worked with the radioimmunoassay (Zárate & Manuel, 2011), this technique allows to quantify and differentiate the characteristic values of reproductive hormones in serum, during the



different reproductive stages of cows ([Herrera et al., 1993](#)). It is applicable for the diagnosis of pregnant cows, with a quantitative result 24 days after insemination ([Sasser & Ruder, 1987](#)). Although this analytical tool is highly specific, sensitive and low cost per sample, it has the disadvantage of requiring highly specialized equipment and personnel, licenses for handling and disposal of radioactive products ([Ehrhardt et al., 2000](#)). The radioimmunoassay allowed the development of profiles of hormones such as progesterone in cow's milk samples collected twice a week, which made possible the selection of cows with abnormal progesterone patterns, and this allowed the study of some treatments of subfertile cows ([Lamming & Bulman, 1976](#)). The study of follicular dynamics began in the middle of the 20th century, but progress has been particularly rapid in the last three decades through the use of tools that have allowed serial and non-invasive examination being able to observe in real time the changes in the ovaries thanks to ultrasonography even during gestation ([Kastelic et al., 1990](#)). Studies to date support the concept that the ovarian pair acts as a single unit and influences follicular development primarily through systemic endocrine pathways involving ovarian and uterine products, gonadotropins and their receptors. Dominant and subordinate follicles go through growth, static and regressive phases that have distinct characteristics that are the basis for diagnosing follicular status ([Adams et al., 2008](#)). The monitoring of follicular dynamics by means of ultrasonography in cattle allowed observing that there can be two or three follicular waves in a cycle ([Ginther et al., 1989](#)), this has allowed more precise studies on hormones related to reproduction in cattle such as the association between follicle stimulating hormone (FSH) peaks and the appearance of follicular waves in cattle, which has been studied by techniques such as the ablation of the dominant follicle ([Adams et al., 1991](#)). Treatment of cows that have not been detected in estrus, but have a detectable corpus luteum, has focused on the use of PGF2 α . In cows with a detectable corpus luteum by ultrasonography, treatment with PGF2 α resulted in 55 % of animals being detected in estrus within six days of treatment ([Smith et al., 1998](#)). Ultrasonography assisted in the study of changes in follicular and luteal structures, as well as the measurement of estradiol and progesterone concentrations in cows, in lactating Hereford x Angus breeds during postpartum anestrus, monitoring follicular size, ovulation and corpus luteum formation and regression, the interval from calving to first ovulation was 82 days. Follicular growth patterns were different before the first and second postpartum ovulation. The first postpartum estrous cycle began with ovulation of a follicle similar in size to the follicle of the second ([Perry et al., 1991](#)). These studies are of great importance and are carried out by means of ultrasonography, in addition to the fact that differences in follicular dynamics and their changes due to various factors can be studied ([Ayala et al., 2019](#)).



Estrous cycle and ovulation determination

The use of ultrasound confirmed that follicles grow, regress and are replaced by other large follicles continuously throughout the cycle. Also that estradiol concentrations increase in preovulatory follicles until the preovulatory LH peak and then decrease rapidly. Furthermore that gonadotropin controls follicular steroidogenesis, and that high estradiol concentrations suppress progesterone production before the LH peak (Hansel & Convey, 1983). Ultrasonography is a tool with high sensitivity and specificity to detect ovarian follicles, corpora lutea or cystic structures, useful to determine the stage of a cow's estrous cycle or diagnose ovarian pathologies such as subclinical endometritis and ovarian cysts (Quintela *et al.*, 2012). The opportunity to monitor the estrous cycle and the ovaries can be performed daily by ultrasonography, also the gestation diagnosis can be performed from 32 days after artificial insemination (Kastelic *et al.*, 1988), with this it is possible to observe the effect of hormonal treatments such as gonadotropin-releasing hormone (GnRH) and Prostaglandin F_{2α} on the ovaries (Bonacker *et al.*, 2020), for which should be monitored from day -7 to day +4 and even +7 to determine follicular and luteal activities, the response of the dominant (largest) follicle in cyclic and non-cyclic cows is of great interest (Thompson *et al.*, 1999). Poor estrous detection remains a limitation to achieving high reproductive performance in herds due to decreased estrous expression in high-producing cows. One strategy to increase serum progesterone concentrations prior to artificial insemination (AI) during estrus is to administer GnRH or human chorionic gonadotropin (hCG) from day five to seven of the estrous cycle to induce ovulation of a dominant first wave follicle and form a corpus luteum, these studies have been made possible by monitoring the estrous cycle using ultrasonography (Cunha *et al.*, 2021).

Development of superovulation protocols and treatments

Ultrasonography helped greatly in the development of superovulation protocols and treatments. The objective of treatments to induce superovulation in embryo transfer programs is to obtain the maximum number of transferable embryos with a high probability of producing gestations, which is possible to observe in real time by means of ultrasonography (Torres-Simental *et al.*, 2021). The conventional protocol for superovulation was originally based on a greater superovulatory response when treatments were initiated eight to 12 days after estrus, these early studies did not evaluate the specific follicular status of the animals at the initiation of treatments, because ultrasound monitoring in cattle was in the early stages of development and was not available in many laboratories (Bó & Mapletoft, 2014). Through information generated through ultrasonography, it is now known that eight to 12 days after estrus (equivalent to seven to 11 days after ovulation) would be the approximate time of appearance of the second follicular wave, and a cluster of growing follicles is present at that time. However,



the day of onset of the second follicular wave has been shown to differ between two-wave cycles and three-wave cycles (one to two days earlier in three-wave cycles) and between individual animals (Ginther *et al.*, 1989). In this regard, it has been clearly demonstrated that superovulatory response was optimized when treatments were initiated at the time of follicular wave onset, initiating gonadotropin treatments only one day before or after surge onset significantly reduced superovulatory response (Nasser *et al.*, 1993; Bergfelt *et al.*, 1997; Cirit *et al.*, 2019). Superovulation in cows is influenced in a multifactorial manner so this must be considered when developing a protocol, the use of ultrasonography is necessary when following up these treatments (Mikkola *et al.*, 2020).

Implantation process and early embryonic death in cattle

One of the advantages of ultrasonography is the diagnosis and follow-up of gestation in cows, although embryonic mortality has a substantial impact on cow fertility. Most embryonic losses occur during the first few days after fertilization and during the implantation process. Primary attention has often been given to infectious agents, but non-infectious causes probably account for 70 % or more of cases (Vanroose *et al.*, 2000). The incidence of embryonic losses is usually higher than perinatal losses, early embryonic death occurs before fetal calcification, and complete embryo resorption, mummification, maceration, or abortion is often observed. Gestational age, cause of death and the source of progesterone for gestational maintenance are the factors that affect the outcome of embryonic or fetal death (Givens & Marley, 2008). Dead embryos, between the time of implantation and calcification, are also resorbed (Sha, 2019). Successful delivery follows conceptus survival during embryonic and fetal development. Generally, product losses occur during the first 42 to 50 days after mating or insemination (Inskeep *et al.*, 2005). There are a variety of readily adoptable management factors that can directly increase embryonic survival or ameliorate the consequences of low embryonic survival rates (Parmar *et al.*, 2016). Gestation loss may be due to trauma caused at gestation diagnosis, either by rectal palpation or ultrasound. In cattle, early gestation diagnosis is usually performed between 35 and 50 days of gestation, however, Vaillancourt *et al.* (1979) found no evidence of increased embryonic loss, Baxter & Ward (1997) reported that ultrasound examination has no detrimental effects on the fetus, rectal palpation is also a safe procedure when performed correctly (Vanroose *et al.*, 2000).



Diagnosis of pregnancy

Fetal morphometry by ultrasonography is useful for evaluation of fetal development, estimation of gestational age, and prediction of calving if the mating date is unknown (Fitzgerald *et al.*, 2015). While gestational age determination is feasible during early pregnancy in cattle and becomes more difficult in late gestation due to the size of the fetus and its position in the maternal abdomen (Buczinski, 2009). Determination of placentoma size, fetal thoracic, abdominal and umbilical diameter, and heart width by ultrasound are the reliable variables for predicting gestational age (Nagel *et al.*, 2020). In studies by Chaudhary & Purohit, (2012) early gestational loss can be detected by ultrasonography up to day 55 of gestation, although the highest incidence occurs around day 35 of gestation. Examination and monitoring of gestation is very important as there are crucial periods for gestation loss during the first trimester of gestation in cows (López-Gatius, 2012), even though it can occur at other stages and for a variety of causes, producing a negative impact on the reproductive and economic performance of herds (Wiltbank *et al.*, 2016; Franco *et al.*, 2020). The first period of losses occurs during the first week after insemination with lack of fertilization and early embryo death, particularly under specific environmental and hormonal conditions, between 20 and 50 % of high-producing lactating dairy cows experience gestation loss due to causes such as heat stress, inflammatory diseases and loss of body condition (Fernández-Novo *et al.*, 2020). A second fundamental period, is from days eight to 27 after mating, contemplates embryo elongation and the period of "maternal recognition of pregnancy" with average losses of 30 % but with a variation of 25 to 41 %, maintenance of CL by interferon-tau and changes in prostaglandin secretion, as well as failures in trophoblast elongation (Hirayama *et al.*, 2014). The third critical period is during the second month of pregnancy, between days 28 to 60, with losses of 12 %. Delays or defects in the development of chorioallantoic placentomas or the embryo result in CL regression or embryo death (Pinedo *et al.*, 2020). A fourth period is during the third month with 2 %, strategies to reduce these losses require a multifactorial approach, in addition to the diagnostic support of ultrasonography (Wiltbank *et al.*, 2016). Bovine abortion, also a limiting factor, can occur sporadically or endemically or as an outbreak and can be of infectious and non-infectious origin. The main infectious agents with or without tropism for fetal membranes and/or fetuses are Brucellosis, Leptospirosis, Bovine Viral Diarrhea (BVD), Aspergillosis, Neosporosis. Although ultrasound does not directly diagnose these diseases, gestational follow-up helps in the diagnosis based on history or history of abortion (Rivera *et al.*, 2018), as well as symptoms related to reproductive organ problems such as metritis, endometritis and pyometra can also be observed (Vallejo-Timaran *et al.*, 2020). It is important to continue researching on the problem of bovine abortion, for which ultrasonography is a very useful tool (Rivera, 2001). It is common that gestation diagnosis in experiments is performed by transrectal ultrasonography between 30 and 47 days after insemination (Oosthuizen *et al.*, 2020).



Detection of non-pregnant cows is of utmost importance to reduce days open, as one of the most critical problems is to achieve efficient estrus detection. In addition, valuable information about embryo viability, the presence of twins, normal fetal development or fetal sex can be obtained from the ultrasonography image (Quintela *et al.*, 2012; Kanazawa *et al.*, 2016). This is of great relevance since the goal of having reproductive efficiency has a direct impact on the viability of livestock, which implies the earliest possible diagnosis of any problems, making ultrasonography a very useful tool (Kelley *et al.*, 2017; Abdelnaby *et al.*, 2018).

Comparison of transrectal palpation, transrectal ultrasonography and blood tests

When performing reproductive tract diagnosis ultrasonography has several advantages compared to transrectal palpation, although this is still the most common method for determining pregnancy in cattle, both methods must be performed competently (Annandale *et al.*, 2019), for which training must generally be using private cattle, this can be difficult because producers may believe that transrectal palpation by inexperienced students increases the risk of pregnancy loss, so experiments have been designed with support for both techniques, obtaining results with no difference in pregnancy loss between cattle palpated by students and by clinical ultrasound, which supports the safety of using privately owned animals for student bovine palpation and training in pregnancy diagnosis without affecting early pregnancy loss (Bond *et al.*, 2019). McDougall & Rhodes (1999) in their study on the detection of a *corpus luteum* in anestrus cows, where they compared the results of hand palpation, transrectal ultrasonography and plasma progesterone concentration by radioimmunoassay with each other, finding that ultrasonography may provide a better diagnostic tool for examining cows that were not detected in estrus compared to hand palpation or measurement of progesterone concentration. It was also shown that cows that were not detected in estrus but had a corpus luteum have significantly lower reproductive performance than herdmates that showed estrus. On the other hand Gwynn *et al.* (2018) mentions that with ultrasonography unlike blood tests, evaluation of fetal viability, detection of twins, determination of fetal age and sex can be performed, results are immediately available and there is opportunity to discuss with the veterinarian other animal health problems on the farm, fostering the relationship of the veterinarian with the farmer.

Estrus synchronization programs

Synchronization programs were improved by the understanding of the waveform follicular growth pattern (Pierson & Ginther, 1984), and its real-time monitoring with the ultrasonography use, research on exogenous control of *corpus luteum* (CL) life span, as well as the use of GnRH agonists to induce ovulation were observed in detail by ultrasonography (Macmillan & Thatcher, 1991), and in general the prediction and



synchronization of estrus continues to be studied in search of alternatives for various livestock production systems (Pluta *et al.*, 2021). The OvSynch protocol (PGF2 α and GnRH) was developed and fully validated for use in cows (Pursley *et al.*, 1995), along with its variations, this protocol remains the most widely used estrus synchronization protocol in cattle (Burke *et al.*, 1996; Pursley *et al.*, 1997a; Pursley *et al.*, 1997b; Bisinotto *et al.*, 2014; Tippenhauer *et al.*, 2021), even under heat stress conditions (De la Sota *et al.*, 1997; Aréchiga *et al.*, 1998). Treatment of cows that have not been detected in estrus, but have a detectable corpus luteum, has mainly focused on the use of PGF2 α (Martins *et al.*, 2021; Wilke *et al.*, 2021). In beef cattle, one of the characteristics of anovulatory cows is reduced LH pulsatility, which impairs follicle growth and impairs the achievement or maintenance of follicular dominance. However, most anovulatory cows are characterized by moderately sized follicles that respond to induced ovulation. Studies using PGF2 α for purposes other than luteolysis have shown that it stimulates LH release (Lopes *et al.*, 2020). Progesterone-based synchronization treatments should be used with caution because of variability in postpartum cow cyclicity. Non-cycling animals that do not respond to treatments may continue to cycle normally. Conception rates of cows after second service vary from 67 and 58 % for cyclic or noncyclic cows, respectively (Beal *et al.*, 1984). There are three key features of successful synchronization of synchronization programs. 1) It is necessary to induce the appearance of a new follicular wave and control follicle development to ensure that a growing follicle of adequate size for ovulation is available at the end of the protocol. 2) The duration of the protocol, the period of exposure to progesterone, which generally varies from five to nine days, should be defined. 3) At the end of the protocol, circulating progesterone concentrations should be minimal with synchronous induction of ovulation to coincide with insemination (Prata *et al.*, 2020; Madureira *et al.*, 2020). In synchronization protocols for AI, exogenous progesterone is used to suppress the LH pulse rate and delay estrus and ovulation, this leads to a decrease in circulating progesterone concentrations and stimulates follicle maturation and ovulation. First developed in the 1970s, intravaginal devices deliver exogenous progesterone in a sustained manner, the controlled-release intravaginal device (CIDR) and the P4-releasing intravaginal device (PRID-Delta) are the only devices licensed for use in cattle in Canada (Zwiefelhofer *et al.*, 2021). Ultrasonography laid the foundation for the development of OvSynch, which was the first estrus synchronization protocol that achieved sufficient synchronization of ovulation in the herd to allow the use of fixed-time artificial insemination (Pursley *et al.*, 1995). By means of transrectal ultrasonography it has been possible to confirm anestrus, the absence of CL and the absence of signs of estrus, and to determine the presence of follicles ≥ 10 mm on day 0, to increase the effectiveness in OvSynch programs (Ahuja *et al.*, 2005). Ultrasonography has been used in studies to determine the pregnancy rate after treatment with GnRH + PGF2 α for ovulation induction in *Bos taurus* x *Bos indicus* cows in anestrus and low body condition during the summer months in a tropical environment, this study compared fixed-time



artificial insemination that was more effective than artificial insemination after estrus detection, both for achieving pregnancies in anestrus cows in poor body condition during the warmer months (Ahuja *et al.*, 2005). The use of ultrasonography greatly helps by being one of the main drivers of a successful synchronization regimen at the farm level, in conjunction with body condition and days postpartum at the start of treatment, as well as an ally of artificial insemination, which remains the most cost-effective way to increase genetic progress in cattle herds (Randi *et al.*, 2021).

Incorporating the Doppler Effect in ultrasonography

The wavelength of light varies with the relative motion between the source and the observer. Color Doppler ultrasound uses this effect to show moving structures in a range of color. The basic principle lies in the observation of how the frequency of an ultrasonic beam alters when it encounters a moving object (Fernández-Sánchez, 2012). In the field of cattle reproduction, it has been of great help in identifying the hemodynamic changes that take place in the genital tract during its different stages. Using this information, the different applications of this tool at the field level have been investigated (Ginther, 2014; Yáñez *et al.*, 2022). This technology has contributed to further research on the reproductive processes of the cow, as for example in a study conducted with Holstein cows, it was found that cows with fewer antral follicles have a larger diameter preovulatory follicle, a preovulatory follicle with greater blood flow, a larger CL and with greater area of blood perfusion, which have higher serum levels of progesterone, than cows with a greater number of antral follicles (Bonato *et al.*, 2022).

Doppler ultrasonography in superovulation and embryo transfer

The management of technologies related to ovarian function in cattle has been driven to several advances thanks to the use of Doppler ultrasonography (Viana *et al.*, 2018), in embryo transfer its main use is to be more efficient in the choice of recipients, based on the quality of their *corpus luteum*, although it can also serve us to monitor the process of superovulation, embryo transfer and implantation (Pugliesi *et al.*, 2018). Singh *et al.* (1998) analyzed the ultrasonographic image of the corpora lutea of several heifers and related it to their histomorphological characteristics, luteal content and plasma progesterone levels. The authors conclude that recipients with higher quality corpora lutea will be more favorable candidates for embryo implantation. In recent years, transrectal Doppler ultrasonography has proven to be a useful tool that can determine uterine blood flow during the estrous cycle, gestation and puerperium (Honnens *et al.*, 2008). Visual assessment of corpus luteum blood flow 14 days after embryo transfer is effective for the detection of non-pregnant recipients. Day seven ovarian assessment information and validation of visual scores for corpus luteum blood flow improved prediction accuracy. This methodology increases flexibility in the use of recipients, allowing resynchronization of



about 79 % of non-pregnant animals between nine and 14 days earlier ([Guimarães et al., 2015](#)).

Study of the functionality of the CL its number and apparent quality with Doppler ultrasonography

Regarding the measurement of blood flow in the corpus luteum by color Doppler ultrasound, there are works that relate blood flow in the CL to the response to the administration of prostaglandins and others study it for resynchronization purposes 21 days after fixed-time insemination ([Palhão et al., 2020](#)). It can also be used to identify non-pregnant animals based on the decrease in corpus luteum blood flow associated with luteolysis. In the study by [Utt et al. \(2009\)](#) the accuracy of non-pregnancy diagnosis was not dependent on the ultrasound experience of the operator, implying that less learning time may be required for this method. However, due to embryo loss between days 17 to 33 and the number of cows identified as pregnant that later lost the embryo, the use of this method alone for early pregnancy diagnosis in an embryo transfer program is not recommended ([Siqueira et al., 2013](#)). In cattle, there are two types of *corpus luteum*: homogeneous and cavitory. Although they are considered equal in their hormonal activity, the function of the cavitory *corpus luteum* is questioned. Consequently, females with cavitory *corpus luteum* are considered less valuable for assisted reproductive techniques. Recent studies have concluded that the cavitory *corpus luteum* may give the embryo better chances of survival at the time of gestation recognition and, consequently, may have a positive effect ([Jaśkowski et al., 2022](#)).

Blood perfusion of the uterus by Doppler ultrasonography

Ovarian artery imaging can be observed with Doppler mode ultrasonography, although imaging of the lumen of the ovarian arteries may not be completely anechogenic ([Ginther, 2007](#)). Measurement of middle uterine artery blood flow may be useful in determining gestational age, for which Doppler ultrasound has been used to measure maternal and fetal blood vessels, allowing recognition and evaluation of high-risk gestations ([Bollwein et al., 2000](#)). A goal in commercial dairy herds is to determine as soon as possible whether an animal had an abortion and, although perfusion examination of CL and decreased uterine artery blood flow at day 34 (when pregnancy diagnosis is performed) does not provide a method to identify a risk of embryonic loss at this time, probably after day 34 of gestation it is more certain ([Kelley et al., 2017](#)). Until recently, studies of changes in uterine circulation during gestation in cows required the use of invasive methods, such as implantation of electromagnetic flow probes and the steady-state diffusion method ([Bollwein et al., 2002](#)). However, as time progresses, during all reproductive stages, characteristic changes in uterine blood flow have been observed using ultrasonography. For example, within the first three weeks after insemination, prior to embryo visibility by B-mode ultrasonography, differences in uterine and luteal blood supply were detected in



early pregnant cows compared to cyclic cows (Bollwein *et al.*, 2016), differences have also been observed in cows subjected to the Ovsynch protocol (Sharawy *et al.*, 2022). On the other hand, echotextural differences in placentomas have been observed in late gestation in cows, with significant changes in placental and uterine artery blood flow. These changes may be related to fetal membrane maturation, especially in the days close to calving (Demir *et al.*, 2022). During postpartum anestrus in cows, one of the organs that undergoes the most marked changes, found by Doppler ultrasonography, is the uterus (Abdelnaby *et al.*, 2018). In addition, it has been observed that cows with puerperal disorders show a late decrease in uterine blood flow in the first weeks after calving compared to healthy cows (Bollwein *et al.*, 2016). Currently, Doppler ultrasonography is playing an important role in artificial reproductive monitoring, such is the case of the finding, where exogenous estradiol benzoate was found to be responsible for increasing uterine blood flow in cows at four weeks postpartum (Rawy *et al.*, 2018), and in those that underwent a synchronization protocol, using a progesterone, GnRH and Prostaglandin F2 α device (Moonmanee *et al.*, 2018). Finally, ultrasonography has also been helpful in assessing uterine blood flow when supplementing heifers with the hormone melatonin (Brockus *et al.*, 2016) and cows and heifers with vitamin B3 (Gard *et al.*, 2021) during gestation in response to risk of gestation termination and level of toxicity, respectively.

Hyperprecocious gestation diagnosis with Doppler ultrasonography and resynchronization

Luteal morphology and blood flow in Japanese black cattle are effective for pregnancy diagnosis with ultrasonography. The corpus luteum tissue area is an accurate predictor for pregnancy diagnosis, with 100% sensitivity and specificity on day 21 of insemination. This method increases the chances for early reproduction in non-pregnant cattle (Kanazawa *et al.*, 2022) and the determination of heartbeat by color Doppler ultrasound as a sign of fetal viability (Fernández-Sánchez, 2012). The evaluation of luteal blood flow at days seven and 14 using Doppler ultrasound is a new reliable predictor of pregnancy. Therefore, this method can be useful for selection of recipient cows prior to embryo transfer and early pregnancy diagnosis under field conditions (Kanazawa *et al.*, 2016). Regarding resynchronization possibilities, protocols starting 14 days after the first insemination up to 24 days. The use of resynchronization programs with a short interval between inseminations leads to a higher proportion of pregnant cows within the first 30 days, which is associated with better reproductive performance, lower risk of discard and higher profitability (Guimarães-da Silva *et al.*, 2022). To perform this method, it is necessary to evaluate the functionality of the *corpus luteum* by color Doppler ultrasonography, since this method detects with high accuracy luteolysis 20 to 22 days after AI in cows that did not achieve pregnancy (Carvalho *et al.*, 2021; Cupper *et al.*, 2021; Stevenson *et al.*, 2021). The establishment of ovulation resynchronization programs accelerates genetic gain by increasing the number of calves born and reduces the number



of bulls needed on the farm. In addition, resynchronization programs can result in higher pregnancy rates at the end of the breeding season than insemination followed by natural mating in herds (Guimarães-da Silva *et al.*, 2022). Currently, complementary alternatives to synchronization programs have been studied, such as GnRH analog in the early luteal phase to improve fertility in repeat cows (>3 inseminations), administered five to seven days after insemination observing embryo survival (López-Gatius *et al.*, 2020). Doppler ultrasound equipment is commercially available, but the cost of the equipment is currently high. However, these ultrasound techniques have already been incorporated into research studies, and as this technology becomes more available and accessible, it is expected that they will be integrated into routine practice in the reproductive management of beef cattle (Herickhoff *et al.*, 2018).

CONCLUSIONS

Transrectal ultrasonography has increased our knowledge and understanding of bovine reproductive physiology. Diagnoses can be made earlier and more accurately than with transrectal palpation. Technological advances have led to the availability of affordable ultrasound equipment that is highly functional, portable, and inexpensive, which has greatly increased its use in the reproductive management of cattle. Ultrasonography is a revolutionary advance in research that has influenced the efficiency of cow reproductive management.

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