aSPRA Asociación Peruana de Reproducción Animal

www.spermova.pe

SPERMOVA

Spermova 2017; 7(2): 85-92

Artículo de revisión - Review article DOI. 10.18548/aspe/0005.15

CURRENT STATUS, EMERGING TECHNOLOGIES, AND TRENDS IN VETERINARY ULTRASONOGRAPHY APPLIED TO CATTLE REPRODUCTION

Estado actual, tecnologías emergentes y tendencias de la ultrasonografia veterinaria aplicada a la reproducción del ganado

J.H.M. Viana[1]*, L.G.B. Siqueira[2]

1 Embrapa Recursos Genéticos e Biotecnologia, Brasília, DF, 70770-917 Brazil
2 Embrapa Gado de Leite, Juiz de Fora, MG, 36038-330 Brazil

* Corresponding author
Address: Embrapa Cenargen, PqEB, Av. W5 N, Brasilia, DF, 70770-917 Brazil. Phone: +55-61-34484700, Fax: +55-61-3340-3624

E-mail: henrique.viana@embrapa.br

ABSTRACT

The B-mode and color Doppler ultrasonography provide a safe, non-invasive, real-time approach to assess reproductive tissues and organs in female cattle, and thus have a number of potential uses in reproductive management. On the ovary, antral follicle population can be counted, and follicular growth, regression and ovulation can be monitored during sequential exams. The corpus luteum can be identified, and evaluated according to size, echotexture, and vascularization. Ultrasonography allows an early diagnosis of pregnancy based in the detection of the embryonic vesicle from day 24 of gestation. Alternatively, non-pregnancy can be indirectly inferred according to corpus luteum vascularization 20 days after insemination. Embryo and fetus morphology and vital signs can monitored throughout gestation, and fetal sex can be determined by the visualization of the relative position of the genital tubercle. In addition, a range of pathologic conditions of the ovary and uterus can be diagnosed based on sonographic images. The use of ultrasonography was also key for the development of many assisted reproductive technologies, such as timed artificial insemination, modern superovulation protocols, procedures for the recovery of cumulus-oocyte complexes aiming in vitro embryo production, and is a valuable tool for the selection and management of embryo recipients. In the future, emerging technologies such as computer-assisted image analysis, 3D ultrasonography, and 3D modeling have the potential to improve the accuracy of sonographic diagnosis, or even create new potential uses for ultrasonography in animal reproduction.

Keywords: 3D ultrasound, bovine, color Doppler, pregnancy diagnosis, reproductive biotechnologies

RESUMEN

La ecografía de modo B y dopler color proporciona un enfoque seguro, no invasivo y en tiempo real para evaluar tejidos y órganos reproductivos en vacas, por lo tanto, tiene una serie de usos potenciales en el manejo reproductivo. En el ovario, se puede realizar el conteo de la población de folículos antrales, y realizar el seguimiento del crecimiento folicular, la regresión y la ovulación durante monitoreo secuencial. El cuerpo lúteo puede ser identificado y evaluarse según el tamaño, ecotextura y vascularización. La ecografía permite un diagnóstico precoz de la preñez basado en la detección de la vesícula embrionaria desde el día 24 de la gestación. Alternativamente, la ño preñez puede inferirse indirectamente de acuerdo con la vascularización del cuerpo lúteo a los 20 días después de la inseminación. La morfología y los signos vitales del embrión y del feto pueden monitorearse durante toda la gestación, y el sexo del feto puede determinarse mediante la visualización de la posición relativa del tubérculo genital. Además, se puede diagnosticar una variedad de condiciones patológicas del ovario y el útero según las imágenes ecográficas. El uso de ultrasonografía también fue clave para el desarrollo de muchas tecnologías de reproducción asistida, como la inseminación artificial a tiempo fijo, los protocolos modernos de superovulación, procedimientos para la recuperación de complejos-ovocito-cumulus que apuntan a la producción de embriones in vitro, y es una herramienta valiosa para la selección y manejo de las receptoras de embriones. En el futuro, las tecnologías emergentes como el análisis de imágenes asistidas por computadora, la ecografía 3D y el modelado 3D tienen el potencial de mejorar la precisión del diagnóstico ecográfico o incluso crear nuevos usos potenciales para la ecografía en la reproducción animal.

Palabras clave: Ultrasonografía 3D, bovino, Doppler color, Diagnóstico de preñez, biotecnología reproductiva.

INTRODUCTION

The use of B-mode ultrasonography was a major advance in large animal theriogenology. Ultrasonography provided valuable information for the study of reproductive physiology and pathological conditions, and is therefore considered one of the most important background for the scientific advances that occurred in the field of animal reproduction in the past few decades (Ginther, 2014). Moreover, the development of portable and less expensive devices gave support to a widespread adoption of ultrasonography by veterinarians, which currently use imaging diagnostic routinely in cattle reproductive management. The ultrasound exam provides a safe, non-invasive, real-time approach to assess reproductive tissues and organs, allowing fast diagnoses and decision making, with a number of potential uses in field service.

As the development of ultrasonography progresses, improvements in the technology results in the expansion of new potential uses for this tool. The introduction of high-resolution ultrasonography associated with computer-assisted image analysis, for example, has dramatically increased image quality and accuracy of the diagnosis. Conversely, color Doppler ultrasound provides information about the functional status of organs and tissues and, thus, allows inferences that were not possible using a conventional gray-scale B-mode ultrasound. More recently, progress in 3D ultrasonography offered the possibility of a direct quantification of the volume of a given tissue and the analysis of organ architecture, similar to what is currently done by computed tomography (CT) and magnetic resonance imaging (MRI).

In this review, we present an overview of the current status, emerging technologies and future perspectives for the use of ultrasonography in bovine reproductive management, associated or not with reproductive biotechnologies, focused in females.

B-mode and color Doppler ultrasonography in reproductive management

In cattle, the possibility of approaching the female genital tract throughout the estrous cycle and early pregnancy by rectal palpation permits the use of high frequency ultrasound transducers (5.0 to 8.0 MHz) so that detailed images can be obtained by scanning ovaries, uterus, fetus, and placenta. On the ovary, antral follicular growth, regression and ovulation can be monitored in a real-time fashion during sequential exams. In the early years of ultrasonography, the resolution of the available equipment allowed the detection of follicles ≥4 to 5 mm (Pierson and Ginther, 1984a), but currently the technological advances reduced this limit of detection to approximately 1 mm in diameter (Jaiswal et al., 2004), using transducer of the same frequency (7.5 MHz).

Sonographic monitoring follicles resulted in the comprehensive characterization of follicular dynamics in different species (Ginther et al., 2001a). In cattle, usually two or three waves of follicle growth occur during an estrous cycle (Sirois and Fortune, 1988). Each follicular wave is characterized by the emergence of a cohort of small follicles, from which one will undergo a process of selection (referred to as deviation) and become dominant, suppressing further growth of subordinated follicles within a wave. A dominant follicle will develop and may ovulate under if progesterone (P4) concentrations are low and a preovulatory LH surge occurs. Conversely, in conditions of high P4 concentrations such as mid-cycle (diestrus) or pregnancy, the dominant follicle will undergo atresia followed by the emergence of a new follicular wave (Ginther, 2016). Due to the dynamics of follicular waves during an estrous cycle, or in the prepubertal period, or early pregnancy, it is not possible to characterize the functional status (i.e., growth or atresia) of a given follicle based on a single ultrasound exam. Nevertheless, studies on the patterns of follicular dynamics for each breed of cattle have determined the average follicular diameter associated to deviation, acquisition of ovulatory capacity, and ovulation (Ginther et al., 2001b; Sartori et al., 2001; Sartori and Barros 2011). In addition, cystic ovarian disease may also be diagnosed based on the size of a dominant follicle (Hamilton et al., 1995; Peter, 2004). All these reference values aid in the interpretation of sonographic findings in gynecological exams of cows and heifers during the reproductive management routine.

Another important application of ultrasound scanning is the quantitative assessment of the ovarian reserve, i.e., follicular population can be counted or estimated based on real-time imaging. The number of follicles present on the ovaries in a given moment is affected by the stage of follicular growth dynamics and fluctuates as a follicular wave emerges or a dominant follicle is selected (Pierson and Ginther 1984a). Although oscillations in antral follicle count (AFC) are expected, there is a moderate to high repeatability of AFC within individuals between waves and estrous cycles (Singh et al., 2004; Morotti et al., 2017). Follicle population is negatively affected by undernutrition, including during fetal life (Evans et al., 2012) when primordial germ cells are forming the ovarian reserve. There is evidence that Bos taurus cows with low AFC have decreased pregnancy rates after artificial insemination (Mossa et al., 2012), but similar results were not observed in Bos indicus (Santos et al., 2016).

Within an ovarian follicle, a complex vascular network develops surrounding the theca layer (Jiang et al., 2003). Most of these vessels are capillary or small diameter veins and so can hardly be detected in small follicles using standard color Doppler flow imaging. As follicles grow, color Doppler signals become more evident and detectable; leading to the observation that vascularization in preovulatory follicles was associated with subsequent fertility (Siddiqui et al., 2009a). Positive Doppler signal, i.e., the presence of blood flow, can also be used as a selection criterion to decide whether to inseminate a female, since follicles without signal are probably undergoing atresia (Acosta et al., 2005) and would therefore be less fertile. In most cases, however, preovulatory follicles will present some degree of vascularization (Ghetti et al., 2012) and the possibility of using these differences to predict fertility is still controversial.

Ovarian ultrasonography allows the visualization of luteal tissue and, thus, development and regression of the corpus luteum (CL) can be monitored throughout its lifespan (Pierson and Ginther, 1984a). The presence of a CL is key to characterize cyclic ovarian activity and is crucial for pregnancy establishment and maintenance in cattle; therefore, a correct identification of the CL is critical in reproductive management. Ultrasonography has a higher sensitivity and specificity in CL detection if compared with rectal palpation (Sprecher et al., 1989). Moreover, because the CL is a highly vascularized gland, color Doppler ultrasonography can add valuable information about CL functional status (Matsui and Miyamoto, 2009). There is an association between CL size, vascularization, and P4 throughout the estrous cycle (Herzog et al., 2010). However, during spontaneous or PGF2a-induced luteolysis, loss of function (drop in P4 production) precedes morphological regression (Viana et al., 1999; Siqueira et al., 2009) and this temporal difference may hamper conclusions of CL sonographic exams close to the end of the estrous cycle.

The uterus has an intermediary echogenicity, but can still be discriminated from surrounding tissues in the sonographic image because of the acoustic interface (Pierson and Ginther, 1987). In cattle, uterine ligaments permit some mobility of the uterine horns, which can appear during ultrasound imaging either as longitudinal or transversal cross-sections. In the non-pregnant uterus, uterine lumen is visualized as a hyperechoic thin line and endometrium thickness varies according to P4 and estradiol stimuli during different phases of the cycle (Souza et al., 2011). In pregnant cattle, ultrasound pregnancy diagnostic is only possible after the phase of embryo elongation (days 19 to 20), after the embryonic vesicle progressively increases in volume (Pierson and Ginther, 1984b). For the best accuracy and reliability, however, the exam should be performed after day 24 of gestation (Pieterse et al., 1990).

Nevertheless, pregnancy diagnosis can be performed much earlier by ultrasonography than by rectal palpation and with a higher accuracy (Pierson and Ginther, 1984b). If non-pregnant cattle are identified early after mating, insemination, or embryo transfer, re-synchronization or breeding re-scheduling occurs earlier, which ultimately reduces inter-insemination intervals and, subsequently, the average calving interval of the herd. In this regard, color Doppler ultrasonography can be used for the early detection of non-pregnant cattle, based on the evaluation of CL blood flow as early as Day 20 of the cycle (Siqueira et al., 2013). Because a functional CL is mandatory for pregnancy establishment, the lack of proper blood flow within the CL on day 20 after breeding/AI suggest functional luteolysis and indicates non-pregnancy status (Matsui and Miyamoto, 2009). This approach has a moderate positive predictive value, due to synchronization failures or possible early embryonic losses after maternal recognition of pregnancy (after Day 20), but has an extremely high negative predictive value, i.e., is very accurate to detect animals that did not became pregnant after breeding/AI (Siqueira et al., 2013). Thus, this is a valuable tool to improve reproductive performance, particularly when overall pregnancy rate is low, as commonly observed in high-producing dairy herds.

During ultrasound scanning of the pregnant uterus, embryo length and development can be evaluated and vital signs such as heartbeat can be clearly identified after Day 25 of gestation (Curran et al., 1986; Breukelman et al., 2004). By Doppler ultrasonography, blood flow can be detected in the placentomes, umbilical cord, and within fetal main vessels and heart (Herzog and Bollwein, 2007). The Doppler spectral mode is also used to calculate circulatory parameters such as heart rate, blood flow velocity, pulsatility and resistance indexes (PI and RI, respectively) in different fetal blood vessels. Blood flow in the uterus increases as pregnancy progresses (Herzog et al., 2011a) and is moderately correlated to fetal size and birth weight (Herzog et al., 2011b).

During a normal gestation, fetal sex can be determined using B-mode ultrasonography by visualization of the genital tubercle (GT) and its position on the fetal body (Curran and Ginther 1991). At early gestation (from days ~40 to ~55), GT is observed between the hind limbs. From Day 55 onwards, GT gradually moves towards the insertion of the umbilicus cord in males or towards the tail in females. These distinct positions may be detected after day 55 of pregnancy in cattle and allow sex diagnosis with high accuracy.

Finally, a wide range of pregnancy abnormalities can be detected by ultrasonography, including fetal malformations, underdevelopment, abnormal placentation, and early embryonic death. Embryo loss is visualized by distinct characteristics of sonographic features such as lack of embryo heartbeat, embryo disintegration, fragmentation of the amniotic vesicle, and reduction of uterine fluid content. The outcome of fetal death in late pregnancy may be fetal mummification or maceration and, in these cases, hyperechoic bone structures will be clearly visible on the sonographic image. Sonographic diagnosis of uterine infections relies on the visualization of accumulated hypoechoic contents within the uterine lumen (Meira et al., 2012). The echogenic appearance of such contents will vary according to the infection severity and amount of cell debris and pus in the uterine mucus.

Ultrasonography and the development of assisted reproductive biotechnologies

The use of ultrasonography has boosted the remarkable development of assisted reproductive technologies (ARTs) observed in the past three decades. The characterization of ovarian follicular and luteal dynamics throughout the estrous cycle was the basis for the subsequent development of numerous protocols to control ovarian function for the application of ARTs (Adams et al., 2008). Ultrasonography allowed sequential, daily (or even hourly) evaluation of size, shape, echotexture, and vascularization of follicles and corpora lutea, both under physiological circumstances and/or after treatment with different exogenous hormones (P4, FSH, LH, eCG, etc) and, therefore, gave support to the development of novel protocols for artificial insemination (AI) and embryo production.

Additionally, ultrasonography has currently multiple uses to support ARTs, from the selection of animals suitable to be used as donors or recipients to the continuous evaluation of pregnancy outcome. In Brazil, for example, the use of AI in cattle had a two-fold increase in the past 20 years and a significant part of this transformation is credited to the use of timed artificial insemination (TAI), which currently represents more than 70% of all inseminations (Sartori et al., 2016). Along with the increase in the use of AI, the emergence of companies in the field of in vitro embryo production (IVEP) in 1998, led to an exponential increase in the number of embryo production (Viana et al., 2017). It is noteworthy that the vast majority of embryos transferred are produced using immature cumulus-oocyte complexes recovered by transvaginal ultrasound-guided follicle aspiration (also known as ovum pick-up, OPU).

Protocols for TAI were developed to overcome two of the main limitations for a widespread use of AI in cattle: 1) the low efficiency of visual estrus detection and 2) the increasing costs and reduced availability of workforce in countryside areas

(Senger, 1994). There is a range of TAI protocols that differ in duration, type and dose of hormones, but they all have the same goal: to ensure that a fully-grown, functional dominant follicle is present and able to ovulate a viable oocyte within a given time-frame (Wiltbank and Pursley, 2014). To achieve this goal, protocols were designed to synchronize follicular wave emergence (Sá Filho et al., 2011), stimulate follicle growth (Sales et al., 2016), and induce ovulation (Ferreira et al., 2015). All TAI steps are possible because of previous studies on follicular dynamics using ultrasonography (Adams et al., 2008; Ginther et al., 2014). Moreover, ultrasound imaging of the ovaries has been used also to evaluate the efficacy of TAI protocols and predict subsequent pregnancy rates (Sá Filho et al., 2010). Alternatively, sonographic evaluation of follicle development at the end of the hormonal protocol can be used to categorize animals according to follicle size and perform TAI in blocks, at different timepoints, which has been demonstrated to improve pregnancy rates (Pfeifer et al., 2015).

Superovulation (SOV) protocols used to produce in vivo embryos were developed empirically, before the use of ultrasonography to study bovine ovarian physiology. Nonetheless, the evolving knowledge about follicular dynamics fulfilled important gaps in the understanding of the SOV process, e.g., determining the reason for better results when protocols started around mid-cycle (turnover of follicular waves, Knopf et al., 1989) or why treatments should last about four days (growth rate of the dominant follicle, Ginther et al., 2001b; García Guerra et al., 2015). Moreover, this knowledge was fundamental for the development of modern SOV protocols, which include synchronization of follicular wave emergence by the removal of the dominant follicle, exogenous control of circulating P4, and ovulation induction (Martins et al., 2012; Bo and Mapletoft, 2014). Ultrasound imaging is also a useful tool to select potential embryo donors based on follicle population (Singh et al., 2004), as well as to identify donors likely to be non-responsive to conventional FSH treatments, and to evaluate overall ovarian SOV response, particularly in those animals with a high number of corpora lutea in each ovary (Minare et al., 2013).

The perspective of a clinical use of in vitro fertilization emerged mainly after the birth of Louise Brown in 1978 (Steptoe and Edwards, 1978). At that time, oocyte recovery used to be performed by laparoscopy, an invasive method that might cause sequels and is of limited use, particularly in farm animals. In both human medicine and animal practice, OPU procedures were vastly adopted later (Feichtinger and Kemeter, 1986; Pieterse et al., 1988) and became the technique of choice for the recovery of cumulus-oocyte complexes for IVEP. Currently, OPU is part of more than 94% of all transfers of embryos produced in vitro (Perry 2016).

As ultrasonography is currently a key step in the commercial IVEP workflow, characteristics of the equipment such as image quality and probe design are of great importance (Hashimoto et al., 1999; Bols et al., 2004). Similarly, to what occurs in the production of embryo in vivo (by SOV), potential oocyte donors can be selected based on the ovarian antral follicle count (Boni et al., 1997). Different studies demonstrated that donors with greater COC yield are those that produces more embryos and, consequently, a greater number of pregnancies (Pontes et al., 2010; Monteiro et al., 2017). Interestingly, pregnancy rate does not seem to be affected by ovarian follicle population (Feres et al., 2016).

To increase the number and quality of follicles at the time of OPU in Bos taurus breeds and thus improve IVEP results, hormonal protocols were developed to synchronize follicular wave emergence (Ramos et al., 2010) and stimulate follicle growth (de Roover et al., 2008; Sendag et al., 2008). Another potential use of ultrasonography in IVEP programs is the evaluation of follicular blood flow. In humans, vascularization has been used to predict follicle status and oocyte quality in patients undergoing ART cycles (Sutton et al., 2003; Lozano et al., 2007). The same approach could be used in bovine oocyte donors (Siddiqui et al., 2009b). In fact, some studies have highlighted the importance of vascularization upon follicle viability, growth, ability to become dominant, and ovulate (Acosta et al., 2003; 2005; Pancarci et al., 2012). In commercial embryo production laboratories, however, oocytes are usually not tracked individually during the entire process and, therefore, embryos cannot be linked to the status of the follicle of origin.

In embryo transfer programs, either for embryos produced in vivo or in vitro, synchronized recipients are routinely selected based on the presence and quality of the CL on days 7 or 8 after estrus (natural or induced). Because there is a positive relationship between luteal tissue volume and P4 production (Stormshak et al., 1963; apud Wiltbank, 1994), the evaluation of CL size could potentially give a clue about plasma P4 concentrations and, indirectly, the likelihood of pregnancy (Siqueira et al., 2009). In this regard, morphological evaluation of CL by ultrasonography represents a significant progress, in comparison to rectal palpation, with gains both in objectivity and accuracy (Sprecher et al., 1989).

More recently, the use of color Doppler imaging allowed functional evaluation of the CL, based on the differences in blood flow. Size of the CL, vascularization, and P4 secretion are positively correlated throughout the estrous cycle (Herzog et al., 2010) and, therefore, color Doppler could provide an additional criterion to select embryo recipients. So far, the predictive value of color Doppler ultrasound for the selection of eligible embryo recipients is still controversial (Lüttgenau et al., 2011; Guimarães et al., 2015). This apparent contradiction is probably caused by the complex interplay among CL function, P4 metabolism, endometrial timing, embryo stage, and development potential (Lonergan, 2011). Nevertheless, there is a consensus that CL function affects the likelihood of pregnancy and CL morphological characteristics are still the main criteria for embryo recipient selection. In this regard, the use of ultrasonography in embryo transfer programs definitely increases the accuracy in the measurement of CL size (Siqueira et al., 2009) and is indispensable to evaluate additional parameters such as echotexture and vascularization of the CL, as well as to measure endometrial thickness.

Emerging technologies: computer-assisted image analysis and 3D ultrasonography

Ultrasonographic images are composed of thousands of picture elements, called pixels, which represents in a scale of 256 shades of gray (0 = black; 255 = white) the intensity of the echo (i.e., echogenicity) generated in one specific area of the tissue by reflection of the ultrasound waves (Pierson and Adams, 1995; Singh et al., 1997, 1998; Tom et al., 1998). Thus, the sonographic image reflects variations in tissue density. The human eye, however, is not able to differentiate so many shades of gray and, consequently, part of this information is lost during visual evaluation of ultrasound images. Moreover, individual differences in visual perception of pixel brightness may result in inconsistency during exams. To overcome this limitation, computer algorithms can be used to transform images in a numeric matrix, which can be subsequently analyzed for mean pixel value and pixel heterogeneity (Singh et al., 1998, 2003). Thus, this computer-assisted image analysis allows quantitative and objective assessment of sonographic image attributes, overcoming the subjectivity and low sensibility of visual analysis.

In animal reproduction, computer-assisted image analysis has been used for the evaluation of CL, follicle, and uterine echotexture (Singh et al., 2003; Siqueira et al., 2009; Scully et al., 2015). Studies show that CL echotexture, for example, is associated with luteal tissue area (Singh et al., 1997), and reflect luteal function throughout the estrous cycle (Singh et al., 1997; Tom et al., 1998; Siqueira et al., 2009). In the past few decades, there was a significant advance in hardware and software capacity for data processing, analysis, and interpretation. Therefore, the perspective for the future is an increasing integration between ultrasonographic exams and image technologies. The outcome of image analysis, however, depend upon image quality (resolution and lack of artifacts). In this regard, further development of ultrasound technology may reduce inconsistencies currently observed among studies and allow the use of echotexture, for example, as a criterion to select embryo recipients with better CL quality.

Computer-assisted analysis is also a requirement for the objective measurement of color Doppler signal area or volume. Different software can be used for that, including open-source (Image-J, Acosta et al., 2003) and commercial versions of general purpose (FixFoto, Lüttgenau et al., 2011; Mimics, Arashiro et al., 2013) or custom-developed image analysis software (PixelFlux, Herzog et al., 2010).

Three-dimensional (3D) ultrasonography is a technological evolution with potential applications in animal reproduction. Ultrasound consoles equipped with the 3D imaging function have built-in software that reconstruct the 3D architecture of examined organs and tissues and display a real-time 3D image (although data processing may require a small-time lapse between acquisition and 3D reconstruct). Alternatively, 3D modeling algorithms can be used to reconstruct 3D structures from a set of frames obtained during B-mode ultrasound scanning of an organ (Arashiro et al., 2013). The 3D modeling approach requires post-acquisition image processing and therefore is time-consuming, but on the other hand offers the possibility of manually differentiate layers within a tissue and, thus, does not depend on clear differences in echogenicity (as occurs, for example, between the fetus and the surrounding amniotic fluid) to create the 3D model.

In humans, 3D images are used for instance to identify fetal malformations that are better visualized in a geometrical surface, rather than in a slice, as for cleft lip (Tonni et al., 2016). In animal sciences, 3D ultrasonography has been used to create 3D models of fetus in domestic (Hildebrandt et al., 2009) and wild species (Drews et al., 2008), aiming to improve fetus biometry and study fetal pose and behavior within the uterus. Another possible use of 3D images is to calculate the volume of a given structure (Scully et al., 2014), which is particularly useful when the object under study has an irregular shape that is poorly explained by uni- or bi-dimensional measures (i.e., diameter, area, etc.). For that, 3D modeling is an alternative to objectively measure vascularization of follicles and corpora lutea. Due to the unevenly distribution of vessels surrounding these structures, assessment of vascularization based on the area of color Doppler signals in a single image is obviously prone to bias (Arashiro et al., 2013). The 3D reconstruction of the vascular architecture allows the calculation of the volume of vascularization and its association to follicle growth (Arashiro et al., 2013), as well as the construction of virtual and physical 3D models for study (Viana et al., 2013). Recently, studies of ovarian physiology demonstrated that the spatial relationship between follicles and corpora lutea within the ovary affect follicle size and growth rate, as well as CL vascularization and lifespan, probably due to angiocoupling between adjacent structures (Ginther et al., 2016). These evidences highlight the importance of ovarian architecture, a factor that has been neglected in most studies of follicular dynamics and luteal function, and that will require further use of 3D ultrasound to be completely understood.

REFERENCES

- Acosta TJ, Hayashi KG, Ohtani M, Miyamoto A. Local changes in blood flow within the preovulatory follicle wall and early corpus luteum in cows. Reproduction, 2003. 125:759-767.
- Acosta TJ, Hayashi KG, Matsui M, Miyamoto M. Changes in follicular vascularity during the first follicular wave in lacting cows. J Reprod Dev, 2005. 51: 273-280.
- Adams GP, Jaiswal R, Singh J, Malhi P. Progress in understanding ovarian follicular dynamics in cattle. Theriogenology, 2008. 69:72-80.
- Arashiro EKN, Palhao MP, Santos JRL, Fontes R, Siqueira LGB, Henry MRJM, Viana JHM. Three-dimensional modeling of color Doppler images: a new approach to study follicular vascularization in cattle. Anim Reprod, 2013. 10:662-669.
- Bó GA, Mapletoft RJ. Historical perspectives and recent research on superovulation in cattle. Theriogenology, 2014. 81:38-48. doi: 10.1016/j.theriogenology. 2013.09.020.
- Bols PE, Leroy JL, Vanholder T, Van Soom A. A comparison of a mechanical sector and a linear array transducer for ultrasound-guided transvaginal oocyte retrieval (OPU) in the cow. Theriogenology, 2004. 62:906-914.
- Boni R, Roelofsen MWM, Pieterse MC, Kogut J, Kruip TAM Follicular dynamics, repeatability and predictability of follicular recruitment in cows undergoing repeated follicular puncture. Theriogenology,1997. 48:277-289.
- Breukelman SP, Reinders JM, Jonker FH, de Ruigh L, Kaal LM, van Wagtendonk-de Leeuw AM, Vos PL, Dieleman SJ, Beckers JF, Perényi Z, Taverne MA. Fetometry and fetal heart rates between Day 35 and 108 in bovine pregnancies resulting from transfer of either MOET, IVP-co-culture or IVP-SOF embryos. Theriogenology, 2004. 61:867-882.
- Curran S, Pierson RA, Ginther OJ. Ultrasonographic appearance of the bovine conceptus from days 20 through 60. J Am Vet Med Assoc,1986. 189:1295-1302.
- Curran S, Ginther OJ. Ultrasonic determination of fetal gender in horses and cattle under farm conditions. Theriogenology, 1991. 36:809-814.
- De Roover R, Feugang JM, Bols PE, Genicot G, Hanzen CH. Effects of ovum pick-up frequency and FSH stimulation: a retrospective study on seven years of beef cattle in vitro embryo production. Reprod Domest Anim, 2008. 43:239-245. doi: 10.1111/j.1439-0531.2007.00873.x.
- Drews B, Hermes R, Göritz F, Gray C, Kurz J, Lueders I, Hildebrandt TB. Early embryo development in the elephant assessed by serial ultrasound examinations. Theriogenology, 2008. 69:1120-1128. doi: 10.1016/j.theriogenology.2008.01.026.
- Evans AC, Mossa F, Walsh SW, Scheetz D, Jimenez-Krassel F, Ireland JL, Smith GW, Ireland JJ. Effects of maternal environment during gestation on ovarian folliculogenesis and consequences for fertility in bovine offspring. Reprod

Domest Anim, 2012. 47:31-37. doi: 10.1111/j.1439-0531.2012.02052.x.

- Feichtinger W, Kemeter P. Transvaginal sector scan sonography for needle guided transvaginal follicle aspiration and other applications in gynecologic routine and research. Fertil Steril, 1986. 45:722-725.
- Feres LF, Camargo LSA, Palhao MP, Brandao FZ, Viana JHM. Effect of production efficiency in hte likelihood of pregnancy of in vitro derived bovine embryos. Reprod Fertil Develop, 2015. 27:210.
- Ferreira RM, Ayres H, Gimenes LU, Torres FP, Lima FA, Veras MB, Guida TG, Sala RV, Baruselli PS. Inducing ovulation with oestradiol cypionate allows flexibility in the timing of insemination and removes the need for gonadotrophin-releasing hormone in timed AI protocols for dairy cows. Reprod Fertil Dev, 2017. 29:468-475. doi: 10.1071/RD15270.
- García Guerra A, Tribulo A, Yapura J, Adams GP, Singh J Mapletoft RJ. Lengthened superstimulatory treatment in cattle: Evidence for rescue of follicles within a wave rather than continuous recruitment of new follicles. Theriogenology, 2015. 84:467-476. doi: 10.1016/j.theriogenology.2015.03.037.
- Ghetti AM, Siqueira LGB, Areas VS, Arashiro EKN, Fernandes CAC, Palhao MP, Viana JHM. Comparison of objective and subjective methods for evaluating the vascular pattern of the preovulatory follicle using color Doppler. Anim Reprod, 2012. 9:657.
- Ginther OJ, Beg MA, Bergfelt DR, Donadeu FX, Kot K. Follicle selection in monovular species. Biol Reprod, 2001a. 65:638-647.
- Ginther OJ, Bergfelt DR, Beg MA, Kot K. Follicle Selection in Cattle: Relationships among Growth Rate, Diameter Ranking, and Capacity for Dominance. Biol Reprod, 2001b. 65:345-350.
- Ginther OJ. How ultrasound technologies have expanded and revolutionized research in reproduction in large animals. Theriogenology, 2014. 81:112-125. doi: 10.1016/j.theriogenology.2013.09.007.
- Ginther OJ. The theory of follicle selection in cattle. Domest Anim Endocrinol, 2016. 57:85-99. doi: 10.1016/j.domaniend.2016.06.002.
- Ginther OJ, Siddiqui MA, Baldrighi JM. Functional angiocoupling between follicles and adjacent corpus luteum in heifers. Theriogenology, 2016. 86:465-471. doi: 10.1016/j.theriogenology.2016.03.002.
- Guimarães CRB, Oliveira ME, Rossi JR, Fernandes CAC, Viana JHM, Palhao MP. Corpus Luteum blood flow evaluation on d21 to improve the management of embryo recipient herds. Theriogenology, 2015. 84:237-241.
- Hamilton SA, Garverick HA, Keisler DH, Xu ZZ, Loos K, Youngquist RS, Salfen BE. Characterization of ovarian follicular cysts and associated endocrine profiles in dairy cows. Biol Reprod, 1995. 53:890–898.
- Hashimoto S, Takakura R, Minami N, Yamada M. Ultrasound-guided follicle aspiration: Effect of the frequency of a linear transvaginal probe on the collection of bovine oocytes. Theriogenology, 1999. 52:131-138.
- Herzog K, Bollwein H. Application of Doppler ultrasonography in cattle reproduction. Reprod Domest Anim, 2007. 42:51-58.
- Herzog K, Brockhan-Lüdemann M, Kasle M, Beindorff N, Paul V, Niemann H, Bollwein H. Luteal blood flow is a more appropriate indicator for luteal function during the bovine estrous cycle than luteal size. Theriogenology, 2010. 73:691-697.
- Herzog K, Voss C, Kastelic JP, Beindorff N, Paul V, Niemann H, Bollwein H. Luteal blood flow increases during the first three weeks of pregnancy in lactating dairy cows. Theriogenology, 2011a. 75:549-554. doi: 10.1016/j.theriogenology.2010.09.024.
- Herzog K, Koerte J, Flachowsky G, Bollwein, H. 2011b. Variability of uterine blood flow in lactating cows during the second half of gestation. Theriogenology 75:1688-1694. doi: 10.1016/j.theriogenology.2010.12.033.
- Hildebrandt TB, Drews B, Kurz J, Hermes R, Yang S, Göritz F. Pregnancy monitoring in dogs and cats using 3D and 4D ultrasonography. Reprod Domest Anim, 2009. 44:125-128. doi: 10.1111/j.1439-0531.2009.01429.x.
- Jaiswal RS, Singh J, Adams GP. Developmental pattern of small antral follicles in the bovine ovary. Biol Reprod, 2004. 71:1244-1251.
- Jiang JY, Macchiarelli G, Tsang BK, Sato E. Capillary angiogenesis and degeneration in bovine ovarian antral follicles. Reproduction, 2003. 125:211-223.
- Knopf L, Kastelic JP, Schallenberger E, Ginther OJ. Ovarian follicular dynamics in heifers: test of two-wave hypothesis by ultrasonically monitoring individual follicles. Domest Anim Endocrinol, 1989. 6:111-119.
- Lonergan P. Influence of progesterone on oocyte quality and embryo development in cows. Theriogenology, 2011. 76:1594-1601.
- Lozano DH, Frydman N, Levaillant JM, Fay S, Frydman R, Fanchin R. The 3D vascular status of the follicle after HCG administration is qualitatively rather than quantitatively associated with its reproductive competence. Hum Reprod, 2007. 22:1095-1099.
- Lüttgenau J, Ulbrich SE,Beindorff N, Honnens A, Herzog K, Bollwein H. Plasma progesterone concentrations in the mid-luteal phase are dependent on luteal size, but independent of luteal blood flow and gene expression in lactating dairy cows. Anim Reprod Sci, 2011. 125:20-29.
- Martins CM, Rodrigues CA, Vieira LM, Mapletoft RJ, Bó GA, Sá Filho MF, Baruselli PS. The effect of timing of the induction of ovulation on embryo production in superstimulated lactating Holstein cows undergoing fixed-time artificial insemination. Theriogenology, 2012. 78:974-980. doi: 10.1016/j.theriogenology.2012.05.007.
- Matsui M, Miyamoto A. Evaluation of ovarian blood flow by color Doppler ultrasound: practical use for reproductive management in the cow. Vet J, 2009. 181:232-240. doi: 10.1016/j.tvjl.2008.02.027.
- Meira, E.B. Jr.; Henriques, L.C.; Sá, L.R.; Gregory, L. 2012. Comparison of ultrasonography and histopathology for the diagnosis of endometritis in Holstein-Friesian cows. J Dairy Sci 95:6969-6973. doi: 10.3168/jds.2011-4950.
- Minare TP, Arashiro EKN, Wohlres-Viana S, Siqueira LGB, Palhão MP, Cordeiro CEC, Fernandes CAC, Viana JHM. Characterization of individual differences in the efficiency of superovulation in the Gyr breed. Anim Reprod, 2013. 10:546-546.
- Monteiro FM, Batista EOS, Vieira LM, Bayeux BM, Accorsi M, Campanholi SP, Dias EAR, Souza AH Baruselli PS. Beef donor cows with high number of retrieved COC produce more in vitro embryos compared with cows with low number of COC after repeated ovum pick-up sessions. Theriogenology, 2017. 90:54-58. doi: 10.1016/j.theriogenology.2016.11.002.
- Morotti F, Santos GM, Júnior CK, Silva-Santos KC, Roso VM, Seneda MM. Correlation between phenotype, genotype and antral follicle population in beef heifers.

Theriogenology, 2017. 91:21-26. doi: 10.1016/j.theriogenology.2016.12.025.

- Mossa F, Walsh SW, Butler ST, Berry DP, Carter F, Lonergan P, Smith GW, Ireland JJ, Evans AC. Low numbers of ovarian follicles ≥3 mm in diameter are associated with low fertility in dairy cows. J Dairy Sci, 2012. 95:2355-2361. doi: 10.3168/jds.2011-4325.
- Pancarci SM, Ari UÇ, Atakisi O, Güngör O, Ciğremiș Y, Bollwein H. Nitric oxide concentrations, estradiol-17β progesterone ratio in follicular fluid, and COC quality with respect to perifollicular blood flow in cows. Anim Reprod Sci, 2012. 130:9-15.
- Perry G. 2015 statistics of embryo collection and transfer in domestic farm animals. Embryo Transfer News, 2016. 34:10-24.
- Peter AT. An update on cystic ovarian degeneration in cattle. Reprod Domest Anim, 2004. 39: 1–7.
- Pfeifer LF, Castro NA, Melo VT, Neves PM, Cestaro JP, Schneider A. Timed artificial insemination in blocks: A new alternative to improve fertility in lactating beef cows. Anim Reprod Sci, 2015. 163:89-96. doi: 10.1016/j.anireprosci.2015.10.002.
- Pierson RA, Adams GP. Computer-assisted image analysis, diagnostic ultrasonography and ovulation induction: strange bedfellows. Theriogenology, 1995. 43:105-112.
- Pierson RA, Ginther OJ. Ultrasonography of the bovine ovary. Theriogenology, 1984a. 21:495-504.
- Pierson RA, Ginther OJ. Ultrasonography for detection of pregnancy and study of embryonic development in heifers. Theriogenology,1984b. 22:225-33. (b)
- Pierson RA, Ginther OJ. Ultrasonographic appearance of the bovine uterus during the estrous cycle. J Am Vet Med Assoc, 1987. 190:995-1001.
- Pieterse MC, Kappen KA, Kruip TAM, Taverne MAM. Aspiration of bovine oocytes during transvaginal ultrasound scanning of the ovaries. Theriogenology, 1988. 30:751-762.
- Pieterse MC, Szenci O, Willemse AH, Bajcsy CSA, Dieleman SJ, Taverne MAM. Early pregnancy diagnosis in cattle by means of Linear-Array Real-Time ultrasound scanning of the uterus and a quantitative and qualitative milk progesterone test. Theriogenology, 1990. 33:697-707
- Pontes JH, Silva KC, Basso AC, Rigo AG, Ferreira CR, Santos GM, Sanches BV, Porcionato JP, Vieira PH, Faifer FS, Sterza FA, Schenk JL, Seneda MM. Large-scale in vitro embryo production and pregnancy rates from Bos taurus, Bos indicus, and indicus-taurus dairy cows using sexed sperm. Theriogenology, 2010. 74:1349-1355.
- Ramos AF, Rumpf R, Câmara JU, Mollo MR, Pivato I, Marques Jr AP, Sartori R. Effect of follicular wave synchronization on in vitro embryo production in heifers. Anim Reprod Sci, 2010. 117:201-207. doi: 10.1016/j.anireprosci.2009.04.009.
- Sá Filho MF, Crespilho AM, Santos JE, Perry GA, Baruselli PS. Ovarian follicle diameter at timed insemination and estrous response influence likelihood of ovulation and pregnancy after estrous synchronization with progesterone or progestin-based protocols in suckled Bos indicus cows. Anim Reprod Sci, 2010. 120:23-30. doi: 10.1016/j.anireprosci.2010.03.007.
- Sá Filho MF, Baldrighi JM, Sales JN, Crepaldi GA, Carvalho JB, Bó GA, Baruselli PS. Induction of ovarian follicular wave emergence and ovulation in progestin-based timed artificial insemination protocols for Bos indicus cattle. Anim Reprod Sci, 2011. 129:132-139. doi: 10.1016/j.anireprosci.2011.12.005.
- Sales JN, Bottino MP, Silva LA, Girotto RW, Massoneto JP, Souza JC, Baruselli PS. Effects of eCG are more pronounced in primiparous than multiparous Bos indicus cows submitted to a timed artificial insemination protocol. Theriogenology, 2016. 86:2290-2295. doi: 10.1016/j.theriogenology.2016.07.023.
- Santos GMGD, Silva-Santos KC, Barreiros TRR, Morotti F, Sanches BV, de Moraes FLZ, Blaschi W, Seneda, M.M. 2016. High numbers of antral follicles are positively associated with in vitro embryo production but not the conception rate for FTAI in Nelore cattle. Anim Reprod Sci 165:17-21. doi: 10.1016/j.anireprosci.2015.11.024.
- Sartori R, Fricke PM, Ferreira, J.C.; Ginther, O.J.; Wiltbank, M.C. 2001. Follicular deviation and acquisition of ovulatory capacity in bovine follicles. Biol Reprod 65:1403-1409.
- Sartori R, Barros CM. Reproductive cycles in Bos indicus cattle. Anim Reprod Sci, 2011. 124:244-250. doi: 10.1016/j.anireprosci.2011.02.006.
- Sartori R, Prata AB, Figueiredo ACS, Sanches BV, Pontes GCS, Viana JHM, Pontes JH, Vasconcelos JLM, Pereira MHC, Dode MAN, Monteiro Jr PLJ, Baruselli PS. Update and overview on assisted reproductive technologies (ARTs) in Brazil. Anim Reprod, 2016. 13:300-312.
- Scully S, Evans AC, Duffy P, Crowe MA. Characterization of follicle and CL development in beef heifers using high resolution three-dimensional ultrasonography. Theriogenology, 2014. 81:407-418. doi: 10.1016/j.theriogenology.2013.10.012.
- Scully, S.; Evans, A.C.; Carter, F.; Duffy, P.; Lonergan, P.; Crowe, M.A. 2015. Ultrasound monitoring of blood flow and echotexture of the corpus luteum and uterus during early pregnancy of beef heifers. Theriogenology 83:449-58. doi: 10.1016/j.theriogenology.2014.10.009.
- Sendag S, Cetin Y, Alan M, Hadeler KG, Niemann H. Effects of eCG and FSH on ovarian response, recovery rate and number and quality of oocytes obtained by ovum pick-up in Holstein cows. Anim Reprod Sci, 2008. 106:208-214. doi: 10.1016/j.anireprosci.2008.01.007.
- Senger PL. The estrus detection problem: new concepts, technologies, and possibilities. J Dairy Sci, 1994. 77:2745-2753.
- Siddiqui MAR, Almamun M, Ginther OJ. Blood flow in the wall of the preovulatory follicle and its relationship to pregnancy establishment in heifers. Anim Reprod Sci, 2009a. 113:287-92.
- Siddiqui MAR, Gastal EL, Gastal MO, Almamun M, Beg MA, Ginther OJ. Relationship of vascular perfusion of the wall of the preovulatory follicle to in vitro fertilization and embryo development in heifers. Reproduction, 2009b. 137:689-697.
- Singh J, Pierson RA, AdamsGP. Ultrasound image attributes of the bovine corpus luteum: structural and functional correlates. J Reprod Fertil, 1997. 109:35-44.
- Singh J, Pierson RA, Adams GP. Ultrasound image attributes of bovine ovarian follicles and endocrine and functional correlates. J Reprod Fertil, 1998. 112:19-29.
- Singh J, Adams GP, Pierson RA. Promise of new imaging technologies for assessing ovarian function. Anim Reprod Sci, 2003. 78:371-99.
- Singh J, Domínguez M, Jaiswal R, Adams GP. A simple ultrasound test to predict the superstimulatory response in cattle. Theriogenology, 2004. 62:227-243.
- Siqueira LG, Areas VS, Ghetti AM, Fonseca JF, Palhao MP, Fernandes CA, Viana JH. Color Doppler flow imaging for the early detection of nonpregnant cattle at 20 days after timed artificial insemination. J Dairy Sci, 2013. 96:6461-6472. doi: 10.3168/jds.2013-6814.

- Siqueira LG, Torres CA, Amorim LS, Souza ED, Camargo LS, Fernandes CA, Viana JH. Interrelationships among morphology, echotexture, and function of the bovine corpus luteum during the estrous cycle. Anim Reprod Sci, 2009. 115:18-28. doi: 10.1016/j.anireprosci.2008.11.009.
- Sirois J, Fortune JE. Ovarian follicular dynamics during the estrous cycle in heifers monitored by real-time ultrasonography. Biol Reprod, 1988. 39:308-317.
- Souza AH, Silva EP, Cunha AP, Gümen A, Ayres H, Brusveen DJ, Guenther JN, Wiltbank MC. Ultrasonographic evaluation of endometrial thickness near timed AI as a predictor of fertility in high-producing dairy cows. Theriogenology, 2011. 75:722-733. doi: 10.1016/j.theriogenology.2010.10.013.
- Sprecher DJ Nebel RL, Whitman SS. The predictive value, sensitivity and specificity of palpation per rectum and transrectal ultrasonography for the determination of bovine luteal status. Theriogenology, 1989. 31:1165-1172.
- Steptoe PC, Edwards RG. Birth after the reimplantation of a human embryo. Lancet, 1978. 2:366.
- Sutton ML, Gilchrist RB, Thompson JG. Effects of in vivo and in vitro environments on the metabolism of the cumulus-oocyte complex and its influence on oocyte development capacity. Hum Reprod, 2003. 9:35-48.
- Tom JW, Pierson RA, Adams GP. Quantitative echotexture analysis of bovine corpora lutea. Theriogenology, 1998. 49:1345-52.
- Tonni G, Rosignoli L, Palmisano M, Sepulveda W. Early detection of cleft lip by three-dimensional transvaginal ultrasound in niche mode in a fetus with trisomy 18 diagnosed by celocentesis. Cleft Palate Craniofac J, 2016. 53:745-748.
- Viana JHM, Ferreira AM, Sa WF, Camargo LSA. Morphologic and functional characteristics of corpora lutea during estrous cycle in Gir cattle. Arq Bras Med Vet Zootec, 1999. 51:251-256.
- Viana JHM, Arashiro EKN, Siqueira LGB, Ghetti AM, Areas VS, Guimarães CRB, Palhao MP, Camargo LSA, Fernandes CAC. Doppler ultrasonography as a tool for ovarian management. Anim Reprod, 2013. 10:215-222.
- Viana JHM, Figueiredo ACS, Siqueira LGB. Brazilian embryo industry in context: pitfalls, lessons, and expectations for the future. Anim Reprod, 2017. 14:476-481.
- Wiltbank MC. Cell types and hormonal mechanisms associated with mid cycle corpus luteum function. J Anim Sci, 1994. 72:1873–1883.
- Wiltbank MC, Pursley JR. The cow as an induced ovulator: timed AI after synchronization of ovulation. Theriogenology, 2014. 81:170-185. doi: 10.1016/j.theriogenology.2013.09.017.