Anim. Reprod., v.10, n.3, p.00-00, Jul./Sept. 2013

Doppler ultrasonography as a tool for ovarian management

J.H.M. Viana[1,2,5], E.K.N. Arashiro[1,2], L.G.B. Siqueira[1], A.M. Ghetti[3], V.S. Areas[4], C.R.B. Guimarães[2], M.P. Palhao[2], L.S.A. Camargo[1,2], C.A.C. Fernandes[2]

[1]Embrapa Dairy Cattle Research Center, Juiz de Fora, MG, Brazil
[2]University Jose do Rosario Vellano, Alfenas, MG, Brazil
[3]Fluminense Federal University, Niterói, RJ, Brazil
[4]Espirito Santo Federal University, Alegre, ES, Brazil

Abstract

Doppler ultrasonography allows the characterization and measurement of blood flow, and can be used to indirectly make inference regarding the functionality of different organs, including the ovaries. Several studies highlighted the importance of an adequate blood flow for follicle development and acquisition of ovulatory potential, and for progesterone secretion by the corpus luteum. Due to some particularities of the ovarian vascular anatomy, however, different strategies had to be developed to measure the blood flow detected by Doppler imaging. Some of these approaches were successful to characterize the patterns of blood flow throughout the estrous cycle, pregnancy, and early postpartum, but require post-acquisition image processing and do not allow real time decisions to be taken. The subjective evaluation of blood flow has been alternatively used in field conditions aiming to early detect non-pregnant animals, select embryo recipients, or predict artificial insemination and *in vitro* fertilization outcomes. In summary, color-Doppler imaging provides important information about the function of corpus luteum and follicles, supporting clinical diagnoses and management decisions. Nonetheless, the adoption of Doppler ultrasonography as a routine exam, instead of a limited use in an individual basis, requires further development of feasible blood flow evaluation procedures and the establishment of reference values.

Keywords: blood flow, cattle, reproduction, vascular dynamics.

Introduction

The development of image technologies significantly contributed to advances in the field of biological sciences. The possibility of a non-invasive, real time approach to assess tissues and organs was of great importance for the study of many physiological mechanisms and for the diagnosis of pathological conditions. For these purposes, several of technologies, from the X-ray to the modern computer tomography, have been used in human and veterinary medicine. The B-mode ultrasonography, for example, had a central role in the characterization of ovarian follicle dynamics in different domestic species and in the subsequent development of several protocols to control ovarian function for assisted reproductive technologies (ARTs) such as timed artificial insemination, superovulation, and oocyte pick-up (Adams *et al*., 2008). The versatility and the number of potential use have made the B-mode ultrasound a valuable and widespread adopted tool in animal reproduction sciences.

Over the past few decades, various new technologies for image diagnosis with potential use in reproductive medicine emerged, including the Doppler ultrasonography (King, 2006). The Doppler technology allows the identification of movement of cells or tissues and is particularly important in the characterization and measurement of blood flow. The vascular dynamics is very closely related to the function of different organs, including those from the reproductive tract, and consequently is an important evaluation parameter of reproductive function. Doppler ultrasonography is routinely used in human gynecology and obstetrics, frequently as the diagnostic gold standard, and also to predict *in vitro* fertilization outcomes (Chui *et al*., 1997; Borini *et al*., 2001). The use of Doppler ultrasonography in animal reproduction research is more recent but not less important, and several studies have demonstrated the relationship of blood flow and ovarian and uterine function throughout the estrous cycle and pregnancy (Acosta *et al*., 2002, 2003; Miyamoto *et al*., 2005; Honnens *et al*., 2008; Herzog *et al*., 2010). In spite of its potential usefulness for reproductive management (Miyamoto *et al*., 2006; Matsui and Miyamoto, 2009), and differently from the human medicine, the adoption of Doppler imaging technology in large animal practice is still limited.

Two main factors have contributed to this low use of Doppler technology in cattle: 1) the availability and cost of portable devices, and 2) the relative lack of knowledge by practitioners about its potentials as a tool to evaluate reproductive function. Along with the recent technological advances in electronics and data processing computers there is a trend for the reduction in both size and price of portable ultrasound devices (Herzog and Bollwein, 2007). The present article aims

[5]Corresponding author: henrique.viana@embrapa.br
Phone: +55(32)3311-7438
Received: May 5, 2013
Accepted: xxx

to review some of the particularities of the use of Doppler ultrasonography in the evaluation of ovarian function and its implications for cattle reproductive management, considering the basic mechanisms of the technique, the vascular physiology of the ovary, and the alternatives to evaluate its vascularization.

Doppler ultrasonography: basic principles, limitations, and alternatives for ovarian evaluation

The Doppler Effect was first described in 1842 by Christian Johan Doppler. It is a natural phenomenon characterized by the apparent change in the sound wave frequency when the source of the wave moves towards or away from the receptor. The difference between the frequency generated and received (Doppler shift) is proportional to the velocity of the movement. This effect also occurs with ultrasound waves, and the frequency difference caused by the movement of blood cells allows for the detection and measurement of blood flow (Ginther, 2007). The change in the frequency of reflected waves relative to emitted waves can be positive, when blood cells move towards de transducer; or negative, when cells move away from the transducer (Herzog and Bollwein, 2007).

The Doppler shift can be represented in the ultrasound screen as a colored image (color and power Doppler) or as a graph (spectral Doppler). In the color flow mode, the Doppler shift is shown as a spectrum of one or two colors, usually red and blue, which represents the direction of blood flow. In the power flow mode the color represents the intensity of the Doppler signal, regardless of direction. In the spectral flow mode, the Doppler shift is represented as a chart (speed by time). In an artery, for example, the spectral graph will typically have a wave form corresponding to the arterial pulse in each cardiac cycle (systole and diastole), while in veins the flow will be almost constant, without a pulse pattern (Herzog and Bollwein, 2007). The spectral flow mode allows a detailed analysis of flow distribution and waveform, and consequently the calculation of velocity and indices (pulsatility index [PI], resistance index [RI]), in one particular vessel. The color flow mode, on the other hand, provides less flow information, but allows an overview of the blood flow perfusion in a region.

Most of the objective measurements of blood flow performed by Doppler ultrasonography, including calculation of flow velocity and indices, are usually performed in large and straight arteries and veins. However, the evaluation of blood flow in the genital system, especially the ovary may be particularly challenging. Arterioles and venules detectable by ultrasound in follicles and corpora lutea represents only part of the vascular plexuses of these structures (Jiang *et al*., 2003), and besides having very small diameters they show slow blood flow, requiring the Doppler function to be set for a high sensitivity (usually by decreasing pulse repetition frequency [PRF]). This high sensitivity, however, requires a well-immobilized patient during the exam, what is frequently difficult to achieve in large animals. The interference of breathing and peristaltic movements may also impair correct caliper positioning during the exam and so spectral mode Doppler is hardly used in routine evaluations of cattle ovaries. Additionally, the Doppler shift is affected by the angle (insonation angle) between the Doppler beam and the flow direction; therefore calculation of blood flow velocity requires the knowledge of the vessel orientation (Ginther, 2007). Besides being small, most of the vessels in the follicular wall or around the corpus luteum are tortuous, and it is difficult to establish the insonation angle and, consequently, to have a correct estimation of flow velocity. Finally, the architecture of the ovarian artery, which is coiled around the ovarian vein, reduces the pulse pattern of the ovarian arterioles, affecting PI and RI.

To overcome these limitations, some alternative approaches have been used for the assessment of blood flow in follicles and corpora lutea. One of the most used is the measurement of the colored area in one or a few representative images of the area under study (Acosta *et al*., 2002; Ginther *et al*., 2007; Rauch *et al*., 2008). In this approach, the ovarian structure is scanned and the color-Doppler mode is used to identify the blood flow, and cross section images of the central area or of the areas with greatest Doppler signal are recorded and later measured using software for image analysis. Alternatively, power mode Doppler can be used and pixel analysis performed in the colored areas to have a semiquantitative assessment of luteal or follicle blood flow (Lüttgenau *et al*., 2011a, b). This methodology is relatively simple to perform and results are usually coherent with the expected physiological variation in blood flow (Herzog *et al*., 2010). Unfortunately, images post-processing prevents real-time decisions to be taken. Although a high repeatability was reported for blood flow evaluation based on single images (Lüttgenau *et al*., 2011b), bias may also occur due to the unevenly distribution of angiogenesis, especially in the follicle, where largest vessels are present mainly in the basal region (Jiang *et al*., 2003). During transrectal ultrasound exam of the ovaries, it is not always possible to adjust the angle of scanning in order to have in the same ("representative") picture the cross section of a follicle on its maximum diameter and on the area with the maximum number of colored pixels. This could explain, to some extent, the relatively low correlation between follicle blood flow (FBF) and follicle diameter observed during dominant follicle growth (Lüttgenau *et al*., 2011a; Arashiro *et al*., 2012), comparing to that observed between corpus luteum blood flow (CLBF) and corpus luteum (CL) size.

Another approach routinely used by both researchers and practitioners is the subjective evaluation of Doppler images (Ghetti *et al*., 2012). In this methodology the technician ranks blood flow based on a general overview of the Doppler signal in all areas of the structure, based on a mental reconstruction of the three-dimensional image of the vascularization. When there is few Doppler signal, as occurs in small follicles, blood flow can also be simply classified as “present” or “absent” (Pancarci *et al*., 2012). The subjective approach is a fast and straightforward way to evaluate blood flow, in which diagnostic and decisions can be taken during the exam and, consequently, is suitable to most of the field reproductive management routines. The subjective approach, as any other methodology based on visual ranking instead of objective measurement, is more difficult to standardize and requires previous experience of the evaluator (Siqueira *et al*., 2013). Moreover, it will be more efficient when the amount of detectable blood flow is larger, as occurs when we evaluate blood flow in the CL, in comparison to evaluation in follicles; or when the expected differences are greater, as occurs in day-20 CL between pregnant and nonpregnant cows. Previous studies, however, reported a good correlation of blood flow between objective and subjective methods, for both follicles and CL (R = 0.72 and R = 0.78, respectively; P < 0.0001), in a single evaluation (Areas *et al*., 2012; Ghetti *et al*., 2012). Figure 1 illustrates the subjective and objective approaches to evaluate the CL.

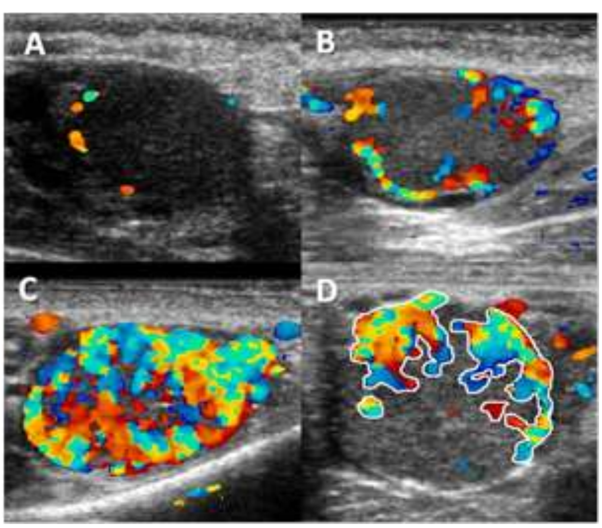

Figure 1. Corpus luteum blood flow evaluation with color flow Doppler imaging. A-C: Corpus luteum subjectively scored as presenting low (A), intermediate (B) or high (C) vascularization. D: Corpus luteum with Doppler signal (colored) area delimited (in white) for further objective measurement with image assisted computer analysis.

Three-dimensional (3D) modeling was alternatively used to objective evaluation of blood flow volume in corpora lutea (Jokubkiene *et al*., 2006) and follicles (Lozano *et al*., 2007) in human and, more recently, in cattle (Arashiro *et al*., 2012). This new approach allowed an objective measurement of blood flow, using a set of images that covers the whole structure under analysis. Besides, it is possible to reconstruct the vascular architecture of follicles and CL, what was previously done only with invasive methodologies such as vascular polymer injection and subsequent corrosion of organic tissues (Jiang *et al*., 2003). The 3D modeling approach also requires post-acquisition image processing and therefore is time-consuming, but may open new possibilities for ovarian vascular dynamics studies. Figure 2 shows 3D models of a CL on day 12 of the estrous cycle with a typical distribution of blood flow (from the periphery to the center; [2B]), and a CL with an unusual vascular architecture (from the center to the periphery; [2D]). Although this uncommon blood flow pattern, the cow bearing this CL was later diagnosed as pregnant (personal data).

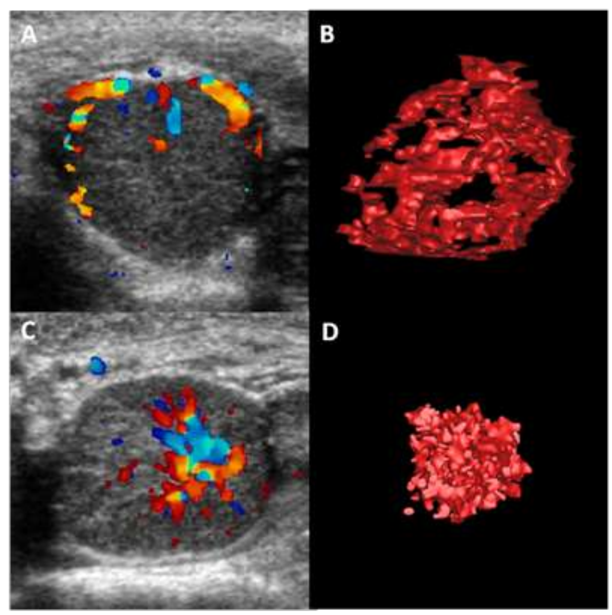

Figure 2. Three-dimensional modeling of corpus luteum vascularization. A-D: Color flow Doppler imaging (A, C) and respective 3D models (B, D) of a corpus luteum with a typical distribution of blood flow (from the periphery to the center; A, B), and of a corpus luteum with an unusual vascular architecture (from the center to the periphery; C, D).

Computer-assisted ultrasound image analysis was first reported for the evaluation of CL and follicle echotexture (Singh *et al*., 2003), and is a requirement for most studies on the objective blood flow evaluation in both 2D and 3D Doppler imaging. Different computer programs were used in ovarian Doppler evaluation, including open-source (Image-J, Acosta *et al*., 2002) and commercial versions of general purpose (Adobe Photoshop, Acosta *et al*., 2002; FixFoto, Lüttgenau *et al*., 2011b; Mimics, Arashiro *et al*., 2012) or custom image analysis software (PixelFlux, Rauch *et al*., 2008; VOCAL, Jokubkiene *et al*., 2006). Software choice must consider the goals and complexity of each study, the Doppler ultrasound device available, as well as the type and resolution of the image files generated.

Vascularization and ovarian function

The development of the ovarian follicle is closely related to the development of a vascular network in the theca layer, which provides nutrients, hormones and oxygen to support follicle growth and steroidogenesis. There are no blood vessels in the granulosa layer and metabolites reach the intrafollicular environment by diffusion. The vascular plexus of medium and large follicles are supplied by individual spiral arteries but drained by several veins (Jiang *et al*., 2003). There are many evidences demonstrating the relationship between vascularization and follicular function. Angiogenesis and ischaemia are related to the fate of follicles, although it is not clear whether they are cause or consequence of follicle development or atresia (Jiang *et al*., 2003). Blood flow affects the composition of follicular fluid (Rodgers and Irving-Rodgers, 2010) and is positively correlated to intrafollicular estradiol concentrations and estradiol:progesterone ratios (Pancarci *et al*., 2012). Follicular blood flow increases during follicle deviation (Arashiro *et al*., 2012) and reaches a peak in the preovulatory follicle after the onset of the LH surge or 0.5 h after GnRH injection (Acosta *et al*., 2003), supporting the concept that a close association between blood flow and the morphological and functional changes precedes ovulation. Consequently, FBF affects oocyte quality and its developmental potential (Sutton *et al*., 2003). Follicle vascularization is also associated to responsiveness to exogenous stimulation and to further embryo implantation rates (Lozano *et al*., 2007), and has been used in human medicine to predict *in vitro* fertilization outcomes (Chui *et al*., 1997).

The CL is highly vascularized and shows the greatest blood flow per unit of tissue in the body (Acosta *et al*., 2002). Luteogenesis is characterized by intensive angiogenesis, and CLBF increases 3 to 4-fold in the first 96 h after ovulation (Acosta *et al*., 2003). Most of steroidogenic cells are adjacent to one or more capillaries (Reynolds *et al*., 2000) and luteal blood flow perfusion is so intense that affects acoustic impedance of the organ, resulting in the hypoechoic echotexture characteristic of the CL (Singh *et al*., 1997). Neovascularization during CL development is important to support the rapid growth of luteal tissue and is essential for both the provision of substrate for progesterone biosynthesis and for the delivery of steroids to the general circulation (Miyazaki *et al*., 1998). Plasma progesterone concentrations are positively correlated to CLBF throughout the estrous cycle, reflecting the development and regression of luteal tissue (Herzog and Bollwein, 2007). Progesterone secretion is the gold standard for functional evaluation of the CL, particularly during luteolysis, when changes in luteal tissue volume are slower and more progressive than in luteal function (Niswender *et al*., 1994). Coherently, the correlations between CLBF or progesterone and CL size are not as strong as between CLBF and plasma progesterone, demonstrating that Doppler ultrasonography improves the accuracy of diagnoses by adding physiological information from the organ (Herzog *et al*., 2010). Interestingly, both natural and PGF2α-induced luteolysis are characterized by an initial acute increase in blood flow, followed by a significant drop 24 to 48 h later (Ginther *et al*., 2007). This initial rise is associated with a transient vasodilation in peripheral vessels of the CL, driven by an increase in endothelial nitric oxide synthase stimulated by the PGF2α (Shirasuna *et al*., 2008), and must be taken into account as a factor affecting single evaluations of the CLBF performed during the expected period of luteolysis, specially when the goal is to early identify non-pregnant animals (Siqueira *et al*., 2013).

Color flow Doppler imaging in the reproductive management

Color flow Doppler imaging can be used to assess the amount and pattern of blood flow within the ovary, and indirectly indicates its functionality. This is particularly useful to characterize the presence of luteal tissue, as in early developing CL, tumors, or in luteinized cysts (Rauch *et al*., 2008), and to make indirect inference regarding CL function, i.e., progesterone secretion. Visualization of CLBF may therefore be used either in studies of ovarian physiology or for reproductive management decisions. A CL that does not present enough Doppler signals (colored pixels), an evidence of lack of blood supply, may be considered as not functional; the same CL, however, may present a normal size (diameter or area). From this point of view, gray-scale ultrasonography is limited to differentiate from a functional *vs*. a nonfunctional CL, if they have similar size. This type of assessment of functionality is only possible by using Doppler imaging. Indeed, blood flow has been suggested to be more appropriate than size for CL function evaluation (Herzog *et al*., 2010), because CL vascularization plays a key role in regulating luteal function (Miyamoto *et al*., 2005). Evaluations of CLBF could, consequently, be useful to detect pregnancy failures or to predict pregnancy rates after embryo transfer or timed-AI.

In cattle and other domestic mammals, a functional CL is required for pregnancy establishment, because progesterone supports preimplantation embryonic development, allowing maternal recognition of pregnancy (Mann and Lamming, 1999). Failure in conception or the presence of a non-viable embryo results in absence of maternal recognition of pregnancy, which will consequently lead to CL regression (reviewed by Niswender *et al*., 2000). Luteolysis is primarily associated to a progressive decrease in the blood flow to the CL in response to prostaglandin F2α and luteal oxytocin. Although the CL´s loss of function (progesterone secretion) responds rapidly to the decrease in blood flow, its morphological regression (reduction in size) only becomes evident hours later (Niswender *et al*., 1994). This asynchrony between functional and morphological changes limits the usefulness of CL evaluation by conventional B-mode ultrasonography, based on the evaluation of CL size (diameter or area), to determine luteal function around the time of luteolysis (Kastelic *et al*., 1990). The use of color-Doppler ultrasound can overcome these limitations because it allows real-time CLBF assessment, an indirect measure of CL functionality, especially by the end of the estrous cycle (Miyamoto *et al*., 2006; Herzog *et al*., 2010). In the reproductive management routine, an important strategy to improve reproductive efficiency is to detect failure in conception as early as possible. It has been suggested that color-Doppler imaging could be used to early detect nonpregnant animals, by evaluating CLBF (Matsui and Miyamoto, 2009; Siqueira *et al*., 2013).

Although gray-scale ultrasonography has been successfully used for transrectal early pregnancy diagnosis in cattle (Pierson and Ginther, 1984), this approach presents low reliability and accuracy when performed earlier than 26 days post AI (Pieterse *et al*., 1990) because clear visualization of the embryo/vesicle in the uterine lumen at this stage is difficult and time consuming (Quintela *et al*., 2012). Therefore, considering that a functional CL is mandatory for pregnancy establishment, the lack of CLBF on specific days of the estrous cycle may be used as a direct indicator of nonpregnancy. In fact, previous studies have already suggested that a decrease in CLBF around 19 to 21 days post AI is an indicator of nonpregnancy (Matsui and Miyamoto, 2009) and also that color Doppler flow imaging could be useful for an accurate early pregnancy diagnosis in cattle (Matsui and Miyamoto, 2009; Quintela *et al*., 2012). Briefly, the idea is to detect, by color-Doppler imaging, normal-sized, non-functional corpora lutea, which indicates failure in conception. Results of these types of approaches are still controversial, and some authors recommend CLBF for early pregnancy diagnosis (Matsui and Miyamoto, 2009; Quintela *et al*., 2012) but others reported low accuracy (Utt *et al*., 2009) and reliability (Herzog *et al*., 2011) of this diagnostic method. Perhaps, the explanation for this apparent disagreement lays on the fact that CLBF evaluation has been performed at different days of the estrous cycle in those studies. A recent report showed high accuracy and reliability of pregnancy diagnoses performed on day 20 post AI, using subjective evaluation of CLBF as an unique criterion (Siqueira *et al*., 2013). The subjective evaluation of CLBF is a feasible approach, and its adoption in the reproductive routine may allow for the early resynchronization of nonpregnant animals and consequent reduction in days open and calving intervals.

In embryo transfer programs, synchronized recipients are routinely selected based on the presence and quality of the CL on days 7 or 8 of the estrous cycle. The relationship between CL quality, assessed by size or echotexture, and pregnancy rates have been evaluated after the transfer of embryos produced *in vivo* or *in vitro* (Siqueira *et al*., 2009). Although there is a general consensus that CL function affects the likelihood of pregnancy, the relatively poor association between CL morphological characteristics and progesterone production limits the predictive value of B-mode ultrasonography for recipient selection. Progesterone secretion and CL blood flow are positively correlated throughout the estrous cycle (Herzog *et al*., 2010) and therefore color-Doppler ultrasonography could be a valuable tool to select embryo recipients. Our preliminary results (Guimarães *et al*.; Universidade José do Rosário Vellano, Alfenas, MG; unpublished data) however, showed no differences in CLBF between embryo recipients later diagnosed as pregnant or nonpregnant. This apparent contradiction may be caused by the complex interplay among embryo quality, embryo developmental stage, and endometrial timing by progesterone (Lonergan, 2011). Although the role of progesterone in the establishment and maintenance of pregnancy is well known (Mann and Lamming, 1999), the minimum threshold for the establishment of pregnancy is controversial (Spell *et al*., 2001) and consequently it is difficult to determine a cutoff value. Moreover, in spite of characterizing the association of CLBF and progesterone during the estrous cycle (Herzog *et al*., 2010), the same group observed that single Doppler evaluations during mid-luteal phase (on day 9 of the estrous cycle) failed to correlate CLBF and plasma P4 concentration (Lüttgenau *et al*., 2011b). Thus, it is likely that Doppler evaluation, as occurs with CL size and even with progesterone concentration, will provide additional information to interpret further pregnancy rates after embryo transfer, but may be of limited use as a tool to select recipients.

Follicular blood flow evaluation has been used to predict follicle status and oocyte quality in human medicine (Sutton *et al*., 2003; Lozano *et al*., 2007). In cattle, there are some studies highlighting the importance of vascularization on follicle viability, growth, ability to become dominant and ovulate (Acosta *et al*., 2003; 2005; Pancarci *et al*., 2012), but few reports associating vascularity of preovulatory follicles and subsequent fertility (Siddiqui *et al*., 2009a). Although evidences of a direct relationship between blood flow and the fate of the follicle were previously reported (Acosta *et al*., 2005), the use of FBF in a reproductive routine still requires the establishment of feasible evaluation procedures and respective reference values or criteria. For example, FBF can be detected from small (4-6 mm) to preovulatory follicles and this information can be used to predict oocyte quality and *in vitro* embryo production (Siddiqui *et al*., 2009b; Pancarci *et al*., 2012). In the commercial embryo production laboratories, however, oocytes are not tracked individually throughout the process and therefore embryos cannot be linked to the status of the follicle of origin. A single exam of FBF can be performed in preovulatory follicles, for example as selection criteria to decide for insemination. Follicles presenting no Doppler signal are probably undergoing atresia (Acosta *et al*., 2005) and will be related to conception failures. 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 controversial. Actually, there is a significant variation in other predictors of follicle status, such as in intrafollicular concentrations of estradiol, even among growing follicles of the same diameter and presenting estradiol:progesterone ratios higher than 1 (Viana *et al*., 2013). The physiological relevance of this variation is unclear, and consequently the same is possible for minor differences in FBF.

In summary, color Doppler imaging provides important information about the function of CL and follicles, supporting clinical diagnoses and management decisions. For example, the technology can be used for the characterization of luteal tissue or for the early detection of luteolysis and prediction of pregnancy failures. Nonetheless, the adoption of Doppler ultrasonography as a routine exam, instead of a limited use in an individual basis, still requires further development of feasible blood flow evaluation procedures and the establishment of reference values for a broad range of uses.

Acknowledgments

This study was supported by grants from FAPEMIG (Projects CVZ APQ 02863/09 and PPM 0067/11) and CAPES.

References

Acosta TJ, Yoshizawa N, Ohtani M, Miyamoto A. 2002. Local changes in blood flow within the early and midcycle corpus luteum after prostaglandin F(2 alpha) injection in the cow. *Biol Reprod*, 66:651-658.

Acosta TJ, Hayashi KG, Ohtani M, Miyamoto A. 2003. Local changes in blood flow within the preovulatory follicle wall and early corpus luteum in cows. *Reproduction*, 125:759-767.

Acosta TJ, Hayashi KG, Matsui M, Miyamoto M. 2005. Changes in follicular vascularity during the first follicular wave in lacting cows. *J Reprod Dev*, 51: 273-280.

Adams GP, Jaiswal R, Singh J, Malhi P. 2008. Progress in understanding ovarian follicular dynamics in cattle. *Theriogenology*, 69:72-80.

Arashiro EKN, Palhao MP, Santos JRL, Fontes R, Henry M, Viana JHM. 2012. Three dimentional modeling of color Doppler ultrasound images: a new approach to the evaluation of follicular vascularization in cattle. *Reprod Domest Anim*, 47:489. (abstract).

Areas VS, Siqueira LGB, Ghetti AM, Souza ED, Arashiro EKN, Camargo LSA, Viana JHM. 2012. Evaluation of corpora lutea vascularization by color Doppler during the period of maternal recognition of pregnancy: preliminary data. *Anim Reprod*, 9:673. (abstract).

Borini A, Maccolini A, Tallarini A, Bonu MA, Sciajno R, Flamigni C. 2001. Perifollicular vascularity and its relationship with oocyte maturity and IVF outcome. *Ann NY Acad Sci*, 943:64-67.

Chui DKC, Pugh ND, Walker SM, Gregory L, Shaw RW. 1997. Follicular vascularity - the predictive value of transvaginal power Doppler ultrasonography in an in vitro fertilization programme: a preliminary study. *Hum Reprod*, 12:191-196.

Ghetti AM, Siqueira LGB, Areas VS, Arashiro EKN, Fernandes CAC, Palhao MP, Viana JHM. 2012. Comparison of objective and subjective methods for evaluating the vascular pattern of the preovulatory follicle using color Doppler. *Anim Reprod*, 9:657. (abstract).

Ginther OJ (Ed.). 2007. *Ultrasonic Imaging and Animal Reproduction: Color-Doppler Ultrasonography*. Cross Plains, WI: Equiservices Publishing.

Ginther OJ, Silva LA, Araujo RR, Beg MA. 2007. Temporal associations among pulses of 13, 14-dihydro-15-keto-PGF2a, luteal blood flow, and luteolysis in cattle. *Biol Reprod*, 76:506-513.

Herzog K, Bollwein H. 2007. Application of Doppler ultrasonography in cattle reproduction. *Reprod Domest Anim*, 42:51-58.

Herzog K, Brockhan-Lüdemann M, Kasle M, Beindorff N, Paul V, Niemann H, Bollwein H. 2010. Luteal blood flow is a more appropriate indicator for luteal function during the bovine estrous cycle than luteal size. *Theriogenology*, 73:691-697.

Herzog H, Voss C, Kastelic JP, Beindorff N, Paul V, Niemann H, Bollwein H. 2011. Luteal blood flow increases during the first three weeks of pregnancy in lactating dairy cows. *Theriogenology*, 75:549-554.

Honnens A, Voss C, Herzog K, Niemann H, Rath D, Bollwein H. 2008. Uterine blood flow during the first 3 weeks of pregnancy in dairy cows. *Theriogenology*, 70:1048-1056.

Jiang JY, Macchiarelli G, Tsang BK, Sato E. 2003. Capillary angiogenesis and degeneration in bovine ovarian antral follicles. *Reproduction*, 125:211-223.

Jokubkiene L, Sladkevicius P, Rovas L, Valentin L. 2006. Assessment of changes in volume and vascularity of the ovaries during the normal menstrual cycle using three-dimensional power Doppler ultrasound. *Hum Reprod*, 21:2661-2668.

Kastelic JP, Bergfelt DR, Ginther OJ. 1990. Relationship between ultrasonic assessment of the

corpus luteum and plasma progesterone concentration in heifers. *Theriogenology* 33:1269-1278.
King AM. 2006. Development, advances and applications of diagnostic ultrasound in animals. *Vet J*, 171:408-420.
Lonergan P. 2011. Influence of progesterone on oocyte quality and embryo development in cows. *Theriogenology*, 76:1594-1601.
Lozano DH, Frydman N, Levaillant JM, Fay S, Frydman R, Fanchin R. 2007. The 3D vascular status of the follicle after HCG administration is qualitatively rather than quantitatively associated with its reproductive competence. *Hum Reprod*, 22:1095-1099.
Lüttgenau J, Beindorff N, Ulbrich SE, Kastelic JP, Bollwein H. 2011a. Low plasma progesterone concentrations are accompanied by reduced luteal blood flow and increased size of the dominant follicle in dairy cows. *Theriogenology*, 76:12-22.
Lüttgenau J, Ulbrich SE, Beindorff N, Honnens A, Herzog K, Bollwein H. 2011b. 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*, 125:20-29.
Mann GE, Lamming GE. 1999. Influence of progesterone during early pregnancy in cattle. *Reprod Domest Anim*, 34:269-274.
Matsui M, Miyamoto A. 2009. Evaluation of ovarian blood flow by colour Doppler ultrasound: practical use for reproductive management in the cow. *Vet J*, 181:232-240.
Miyamoto A, Shirasuna K, Wijayagunawardane MPB, Watanabe S, Hayashi M, Yamamoto D, Matsui M, Acosta TJ. 2005. Blood flow: a key regulatory component of corpus luteum function in the cow. *Domest Anim Endocrinol*, 29:329-339.
Miyamoto A, Shirasuna K, Hayashi K, Kamada D, Kawashima C, Kaneko E, Acosta TJ, Matsui M. 2006. A potential use of color ultrasound as a tool for reproductive management: new observations using color ultrasound scanning that were not possible with imaging only in black and white. *J Reprod Dev*, 52:153-160.
Miyazaki T, Tanaka M, Miyakoshi K, Minegishi K, Kasai K, Yoshimura Y. 1998. Power and colour Doppler ultrasonography for the evaluation of the vasculature of the human corpus luteum. *Hum Reprod*, 13:2836-2841.
Niswender GD, Juengel JL, Mcguire WJ, Belfiore CJ, Wiltbank MC. 1994. Luteal function: the estrous cycle and early pregnancy. *Biol Reprod*, 50:239-247.
Niswender GD, Juengel JL, Silva PJ, Rollyson MK, Mcintush EW. 2000. Mechanisms controlling the function and life span of the corpus luteum. *Physiol Rev*, 80:1-29.
Pancarci SM, Ari UÇ, Atakisi O, Güngör O, Ciğremiş Y, Bollwein H. 2012. Nitric oxide concentrations, estradiol-17β progesterone ratio in follicular fluid, and COC quality with respect to perifollicular blood flow in cows. *Anim Reprod Sci*, 130:9-15.
Pierson RA, Ginther OJ. 1984. Ultrasonography for the detection of pregnancy and study embryonic development in heifers. *Theriogenology*, 22:225-233.
Pieterse MC, Szenci O, Willemse AH, Bajcsy CSA, Dieleman SJ, Taverne MAM. 1990. Early pregnancy diagnosis in cattle by means of linear-array real-time ultrasound scanning of the uterus and a qualitative and quantitative milk progesterone test. *Theriogenology*, 33:697-707.
Quintela LA, Barrio M, Peña AI, Becerra JJ, Cainzos J, Herradón PG, Díaz C. 2012. Use of ultrasound in the reproductive management of dairy cattle. *Reprod Domest Anim*, 47:34-44.
Rauch A, Krüger L, Miyamoto A, Bollwein H. 2008. Colour Doppler sonography of cystic ovarian follicles in cows. *J Reprod Dev*, 54:447-453.
Reynolds LP, Grazul-Bilska AT, Redmer DA. 2000. Angiogenesis in the corpus luteum. *Endocrine*, 12:1-9.
Rodgers RJ, Irving-Rodgers HF. 2010. Formation of the ovarian follicular antrum and follicular fluid. *Biol Reprod*, 82:1021-1029.
Shirasuna K, Watanabe S, Asahi T, Wijayagunawardane MP, Sasahara K, Jiang C, Matsui M, Sasaki M, Shimizu T, Davis JS, Miyamoto A. 2008. Prostaglandin F2alpha increases endothelial nitric oxide synthase in the periphery of the bovine corpus luteum: the possible regulation of blood flow at an early stage of luteolysis. *Reproduction*, 135:527-539.
Siddiqui MAR, Almamun M, Ginther OJ. 2009a. Blood flow in the wall of the preovulatory follicle and its relationship to pregnancy establishment in heifers. *Anim Reprod Sci*, 113:287-92.
Siddiqui MAR, Gastal EL, Gastal MO, Almamun M, Beg MA, Ginther OJ. 2009b. Relationship of vascular perfusion of the wall of the preovulatory follicle to in vitro fertilization and embryo development in heifers. *Reproduction*, 137:689-697.
Singh J, Pierson RA, Adams GP. 1997. Ultrasound image attributes of the bovine corpus luteum: structural and functional correlates. *J Reprod Fertil*, 109:35-44.
Singh J, Adams GP, Pierson RA. 2003. Promise of new imaging technologies for assessing ovarian function. *Anim Reprod Sci*, 78:371-399.
Siqueira LG, Torres CA, Souza ED, Monteiro PL Jr, Arashiro EK, Camargo LS, Fernandes CA, Viana JH. 2009. Pregnancy rates and corpus luteum-related factors affecting pregnancy establishment in bovine recipients synchronized for fixed-time embryo transfer. *Theriogenology*, 72:949-958.
Siqueira LGB, Areas VS, Ghetti AM, Fonseca JF, Palhao MP, Fernandes CAC, Viana JHM. 2013. Color Doppler flow imaging for the early detection of nonpregnant cattle at 20 days after timed artificial insemination. *J Dairy Sci*. (in press).
Spell AR, Beal WE, Corah LR, Lamb GC. 2001. Evaluating recipient and embryo factors that affect

pregnancy rates of embryo transfer in beef cattle. *Theriogenology*, 56:287-297.

Sutton ML, Gilchrist RB, Thompson JG. 2003. 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*, 9:35-48.

Utt MD, Johnson III GL, Beal WE. 2009. The evaluation of corpus luteum blood flow using color-flow Doppler ultrasound for early pregnancy diagnosis in bovine embryo recipients. *Theriogenology*, 71:707-715.

Viana JH, Dorea MD, Siqueira LG, Arashiro EK, Camargo LS, Fernandes CA, Palhão MP. 2013. Occurrence and characteristics of residual follicles formed after transvaginal ultrasound-guided follicle aspiration in cattle. *Theriogenology*, 79:267-273.