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APPLICATION OF COLOUR DOPPLER ULTRASONOGRAPHY FOR OVARIAN BLOOD FLOW EXAMINATION IN COWS – A REVIEW

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ABSTRACT

The purpose of this review article is to summarize the data concerning the application of colour Doppler ultrasonography for examination of the ovaries in cows.

The main indication for performing a Doppler ultrasound of the ovaries is to determine the changes in the ovarian blood flow during the follicular and luteal phases of the estrus cycle. The available literature shows that measurement of follicular blood flow is suitable for identifying the future dominant follicle at an early stage of development and for predicting the viability of the follicles after deviation. Additionally, examining the number of follicles with detectable blood flow was applied to predict the superovulatory response. In this regard, measurement of follicular blood flow by color Doppler ultrasonography has used to detect follicular development and to predict the correct time of ovulation.

Colour Doppler ultrasonography is used to differentiate follicular and luteal cysts, which has crucial importance in choosing appropriate treatment. The better sensitivity of this diagnostic method (92.3%) than B-mode ultrasonography (61.5%) in determination of the follicular luteinization degree, shows that blood flow intensity is more accurate indicator for detection of active luteal tissue compared to wall thickness measurement.

During the entire estrus cycle, there is higher positive correlation between luteal blood flow (LBF) and the progesterone level than between corpus luteum cross-sectional area (corpus luteum size) and progesterone (P4). Because of this fact, LBF increase can be used to establish early luteal function. On the base of close relationship between LBF and P4 values during the early and late luteal phases, it is accepted that LBF determination may distinguish developing (functional) and regressing (nonfunctional) corpora lutea with the same size. In conclusion, the current literature analysis indicates that transrectal colour Doppler ultrasonography is useful technique for examination of the reproductive organs blood flow and opens a new window for ovarian function investigation in cows.

Key words: Colour Doppler, ultrasonography, ovarian blood flow, cows.

Introduction

In recent decades, ultrasound examination of the reproductive system has been the main method for determination of the reproductive performance in large ruminants (Ginther, 2014). Conventional B-mode ultrasonography allows for real-time imaging of the female reproductive organs by generating two-dimensional grayscale images. The application of this technology allows for further knowledge of the reproductive processes in cattle and supports the development of some reproductive biotechnologies. Ultrasound examination has helped to reveal the peculiarities of the folliculogenesis process and provides an idea of the so-called wave-like pattern of follicular growth in cows (Ginther *et al.*, 1989; Fortune *et al.*, 1991). The conducted scientific research has contributed to the routine use of ultrasound in veterinary medicine for the assessment of reproductive health, early pregnancy diagnosis, determination of sex and viability of the fetus, and others (Panarace *et al.*, 2006; Ginther, 2007).

The physiological angiogenesis plays an essential role in the female reproductive system, occurring cyclically in the ovary and uterus. Hemodynamic changes occurring during the ongoing processes in the ovaries that occur during follicular growth, ovulation, and development of the subsequent corpus luteum (CL) can be detected using Doppler ultrasonography (Collins *et al.*, 1991; Brannstrom *et al.*, 1998; Acosta *et al.*, 2002). This method is a diagnostic procedure that uses an ultrasound scanner to convert sound waves into an image of blood flow in body tissues and organs. It provides clinically relevant information about the vascularization of specific organs or structures within those organs and can be used to assess their functionality. Among the many possibilities for using this technology in bovine reproduction, is the determination of uterine blood flow during pregnancy (Bollwein *et al.*, 2002), assessment of follicular and luteal blood perfusion in the ovarian circulation in relation to the phase of the estrous cycle in cows (Acosta *et al.*, 2002; Acosta *et al.*, 2003; Acosta *et al.*, 2005; Ginther, 2007; Ginther *et al.*, 2007). Colour Doppler ultrasonography is a non-invasive technique for assessing ovarian vascular function, allowing visualization of blood flow in a specific area in the wall of preovulatory follicles (Brannstrom *et al.*, 1998) or CL (Miyazaki *et al.*, 1998; Acosta *et al.*, 2002). Transrectal colour Doppler is also used to study uterine blood flow (Ford and Chenault, 1981; Ginther, 2007).

Basic principles of Doppler ultrasound examination

Ultrasound research technologies in the field of reproduction include the production of images by ultrasound in gray scale, color ultrasound Doppler, and the production of spectral Doppler plots (ultrasonography) of changing blood velocities within the arterial and venous pulse. Many research findings and discoveries over the past 30 years have resulted from the application of these technologies (Bollwein *et al.*, 2000; Ginther, 2007; Ginther, 2014; Bollwein *et al.*, 2016; Pugliesi *et al.*, 2023).

B-mode ultrasound uses ultrasound waves that are emitted from the probe and directed at organs and tissues, then reflected back to the transducer at different frequencies depending on the density of the tissue and processed by the ultrasound module into a grayscale image (Pierson *et al.*, 1988). A good knowledge of the anatomy of the blood supply to the genitals is required for Doppler ultrasound examination. The ovarian artery is a branch of the aorta and divides into a uterine and ovarian branch about 6 cm from the respective ovary (Ginther and Del Campo, 1974). The ovarian branch divides in the mesoovarium of the ovary into two or three branches, and each branch subdivides into two or three smaller branches. As a result, four to nine arteries enter the hilus of the ovary. The arterial branch that supplies the corpus luteum in cattle increases in diameter by two or three times by the time the CL reaches maximum development (Lamond and Drost, 1974). It is assumed that the same arterial branch previously supplied the preovulatory follicle.

Doppler ultrasound allows the assessment of blood flow and blood perfusion, which can be used to assess the functionality of reproductive structures and organs. These blood parameters are examined by Doppler ultrasonography. In the context of vascularization, the method identifies changes in the frequency of ultrasound waves that are sent from the transducer (source) and reflected by red blood cells (target), which are constantly changing their position relative to the transducer. When red blood cells move towards the transducer, the frequency of the reflected ultrasound waves is greater than the frequency of the transmitted waves, resulting in a positive Doppler shift. When the frequency of the reflected waves is lower than that of the transmitted waves, such as when red blood cells move away from the transducer, a negative Doppler shift occurs (Ginther and Utt, 2004). There are three main modes of Doppler ultrasound: spectral, power, and colour. Each mode can be used to assess different parameters. In spectral Doppler, the frequency shift of the ultrasound waves is plotted as a graph to visualize blood flow over a period of time. This mode is useful in assessing the waveform and distribution of blood flow and can be used to calculate: blood flow velocity, pulsatility index, and resistance index (Ginther and Utt, 2004).

Doppler ultrasonography of uterine blood flow

The spectral Doppler mode is commonly used to assess blood flow in larger blood vessels such as the uterine artery (Fig. 1), but due to limitations, such as difficulty in establishing the correct angle between the transducer and the blood flow and the significant potential for inaccurate results, it is hardly used to measure blood flow in smaller vessels such as the ovarian vasculature (Viana *et al.*, 2013).

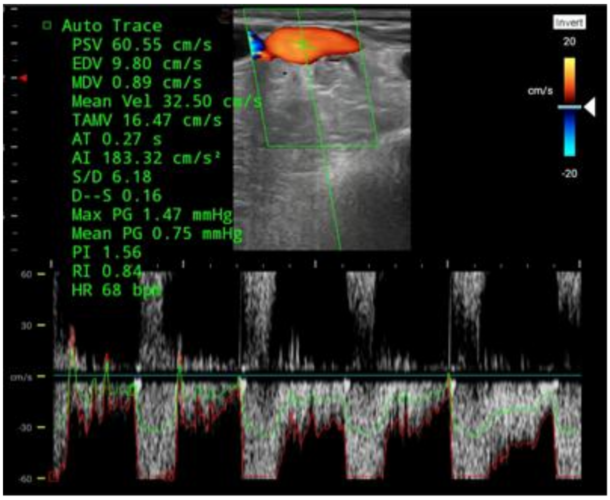

Figure 1: Spectral Doppler ultrasound of the uterine artery in a cow with measurements of the Doppler indices

Power Doppler mode measures the movement of blood cells through a given blood vessel over a certain period of time, known as blood flow intensity, and is better for imaging structures with very little blood flow, such as the wall area (basement membrane) of dominant follicles (Bollwein *et al.*, 2016). Doppler shift can be visualized as colors, usually ranging from yellow to red, superimposed on a B-mode ultrasound image, where the color intensity represents the intensity of blood flow. These colors indicate the vascularization of a structure but do not provide information about the direction of blood flow. In color Doppler, Doppler shift can also be visualized as color signals superimposed on a conventional B-mode image. However, higher frequencies or positive changes are indicated by colors ranging from yellow to red, and lower relative frequency changes or negative changes are indicated by colors ranging from green to blue. The colour gradients described above are the most conventionally used: however, they can be modified according to ultrasound settings and operator preference. Using information about the color signal, the percentage of a given area that has blood circulation can be objectively estimated using specific software (Ginther and Utt, 2004; Herzog *et al.*, 2010).

Uterine blood flow has a characteristic pattern in the cow with the highest mean blood flow velocity values during proestrus and diestrus (Bollwein *et al.*, 2000). During diestrus, blood flow velocity remains at a relatively constant low level (Bollwein *et al.*, 2002). Changes in uterine blood flow velocity associated with the estrus cycle correlate with plasma concentrations of estrogens and progesterone, although the correlations are only moderate (Bollwein *et al.*, 2016). This indicates that not only steroid sexual hormones, but also other factors are involved in the regulation of uterine blood flow.

Doppler ultrasonography of follicular blood flow

Changes in follicular blood perfusion have been studied during follicular growth (Acosta *et al.*, 2005). Sufficient blood supply is of utmost importance for follicles to reach the dominance phase, as good vascularization is established in dominant and preovulatory follicles, but not in atretic follicles (Acosta *et al.*, 2005; Miyamoto *et al.*, 2006). Follicular blood perfusion is also positively correlated with steroid hormone concentrations in follicular fluid (Pancarci *et al.*, 2012; de Tarso *et al.*, 2017). Positive correlations between follicular blood supply and follicular estradiol/progesterone ratio have also been reported in a study of dairy cows, using color Doppler ultrasonography (Pancarci *et al.*, 2012). In the same study, the percentage of estradiol active follicles was greater when follicular blood flow was detected and positive associations were observed between estradiol concentrations and follicle size (Fig. 2).

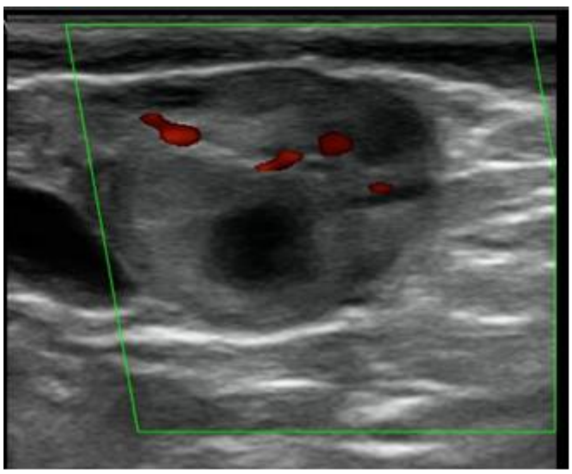

Figure 2: Colour Doppler ultrasonography of the ovary in a cow with small follicles.

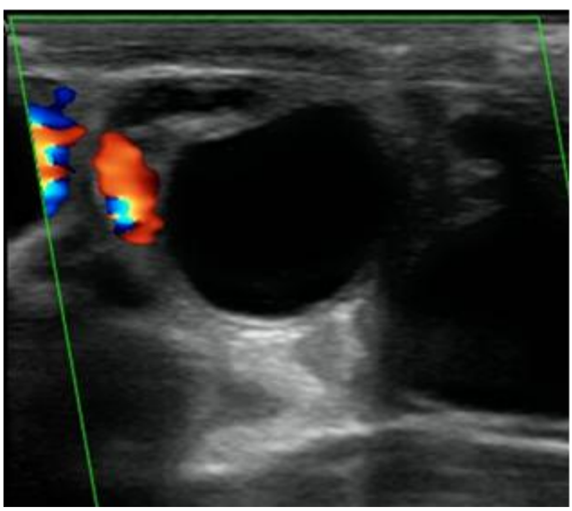

Figure 3: Colour Doppler sonographic image of the preovulatory follicle in a cow.

Blood perfusion of the preovulatory follicle also positively correlates with the diameter and vascularization of the subsequent corpus luteum and its ability to secrete progesterone (de Tarso *et al.*, 2017). These results suggest that the vascularization of follicles can be used as a tool to demonstrate their functionality (Fig. 3). Therefore, since larger dominant follicles have greater vascularization and a better ability to secrete estradiol (Pancarci *et al.*, 2012; Perry *et al.*, 2014), it can be concluded that they have greater functionality than smaller follicles, especially since larger follicles have higher rates of ovulation and subsequent CL and have resulted in higher pregnancy rates compared to smaller follicles (Perry *et al.*, 2007; Pugliesi *et al.*, 2016).

Colour Doppler analysis of follicular blood flow contributes to elucidating the role of follicular vascularization on fertility; however, it currently has limited use for measuring follicular perfusion for reproductive health management in large ruminants. Transrectal colour Doppler ultrasound imaging and colour Doppler ultrasound diagnostics have become useful technologies for noninvasively examining the vascularization of the internal genital organs in large ruminants (Acosta *et al.*, 2005; Herzog and Bollwein, 2007; Ginther, 2007; Ginther, 2014). In ultrasound imaging, the degree and direction of blood flow in tissues are displayed in color on a scale between yellow and red on the one hand and between blue and green on the other. In ultrasound examination, placement of the probe across the relevant artery produces a graphical image showing the arterial pulsation and includes the resistance index (RI), which is calculated as the ratio of the difference between the peak systolic frequency (PSF) shift and the end diastolic frequency (EDF) shift to the peak systolic frequency shift (Bollwein *et al.*, 2016). Reduced RI indicates increased vascular perfusion of tissues supplied by the artery downstream of the RI assessment. As an example of research findings in follicular function using colour Doppler technologies, the percentage of preovulatory follicles with blood flow signals is greater in cattle (Siddiqui *et al.*, 2009) and mares (Silva *et al.*, 2006) that subsequently become pregnant.

Colour Doppler ultrasonography can be used to differentiate between follicular and luteal cysts, which is of important practical importance in choosing the appropriate treatment. The sensitivity of diagnosing luteinized follicles using B-mode (61.5%) and color Doppler ultrasonography (92.3%) indicates that blood flow more accurately reflects active luteal tissue than wall thickness (Rauch *et al.*, 2008).

In cows, the preovulatory follicle has been studied by colour Doppler (Acosta *et al.*, 2005; Siddiqui *et al.*, 2009), but limited attention has been paid to smaller follicles (e.g., <10 mm). In an initial study, there was no significant difference between future dominant and subordinate follicles in the number of follicles with detectable Doppler blood flow signals in their wall before diameter deviation (Acosta *et al.*, 2005). After deviation, the number of subordinate follicles with detectable blood flow decreased.

Doppler ultrasonography of luteal blood flow

The corpus luteum (CL) is a transient endocrine structure that secretes primarily progesterone (P4), which is essential for the establishment of pregnancy in cows (Mann and Lamming, 1995). The development of the CL after ovulation is often divided into three main phases: early luteal phase or luteogenesis; mid-luteal or static phase; and regression or luteolysis (Niswender *et al.*, 2000). Maternal recognition of pregnancy is associated with the maintenance of a fully functional CL secreting adequate amounts of P4 to support early embryonic development (Lonergan, 2011).

The color flow mode generates real-time, easy-to-interpret images of blood flow that depict the vascularization of the CL. The use of colour Doppler devices for examination is a good diagnostic tool for comparative analysis, taking into account changes over time or individual differences between animals, or even relative cut-off values.

Therefore, after visual assessment, the CL still needs to be quantified. In this regard, it should be borne in mind that the CL is surrounded by capillaries and small vessels (Macchiarelli *et al.*, 1998), which makes the correct assessment of its vascularization extremely careful and precise.

Extensive angiogenesis and distribution of luteal cells within the CL are visualized sonographically as a hypoechoic imaging pattern, and changes in this pattern may reflect different luteal phases and function (Tom *et al.*, 1998). In this regard, previous studies have investigated correlations between plasma P4 and quantitative CL echotexture defined as echogenicity modeling (Siqueira *et al.*, 2009; Siqueira *et al.*, 2019). Overall, it appears that pixel brightness (mean pixel value) within the luteal tissue has limited value for inferences regarding CL function (plasma P4 secretion). In contrast, variation in pixel brightness in luteal tissue, i.e. pixel heterogeneity, appears to be a much better indicator of luteal function and has been found to have a relatively high correlation with plasma P4 during luteolysis (Siqueira *et al.*, 2009). Analysis of circulating progesterone concentrations is the most reliable way to assess CL function, as its primary function is to secrete progesterone during diestrus and pregnancy. However, the time and expense associated with determining progesterone concentrations are major drawbacks to its routine use in reproductive management.

Due to the rapidity and reliability of conventional B-mode ultrasonography, this technique is used to detect the presence of the corpus luteum and determine its size. This allows an assessment of its function, as CL diameter (Spell *et al.*, 2001; Velho *et al.*, 2022) and CL zone/area (Berger *et al.*, 2017; Pugliesi *et al.*, 2019) are related to circulating progesterone concentrations. However, this correlation is reduced during luteolysis because the rate of decrease in circulating progesterone concentrations is greater than the decrease in CL size (Kastelic *et al.*, 1990; Assey *et al.*, 1993).

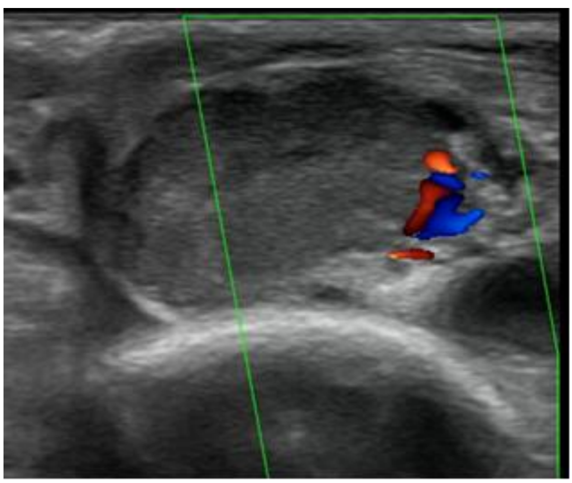

Figure 4: Colour Doppler ultrasound view of active CL in a cow

Over the entire estrus cycle, the correlation between luteal blood flow and progesterone is higher than the correlation between corpus luteum cross-sectional area (corpus luteum size) and progesterone. Because the increase in blood supply to the corpus luteum in cows is closely related to increased plasma concentrations of progesterone, luteal blood flow can be used to establish early luteal function (Bollwein *et al.*, 2016).

The reduction in luteal volume is associated with lower concentrations of circulating progesterone (Vasconcelos *et al.*, 2001). Given the close relationship between luteal blood flow and progesterone during the early and late luteal phases, luteal blood flow can be used to distinguish developing (functional) and regressing (nonfunctional) corpora lutea of the same size (Herzog *et al.*, 2010; Herzog *et al.*, 2011).

Characteristic changes are observed in luteal blood flow during the estrous cycle, which are strongly correlated with progesterone levels. Furthermore, color Doppler imaging has shown that there is no decrease, but rather an increase, in luteal blood flow at the onset of luteolysis in cows (Herzog and Bollwein, 1998). The corpus luteum is a highly vascularized structure during diestrus (Fig. 4), but undergoes a significant reduction in blood perfusion and luteal tissue volume during luteolysis (Ginther *et al.*, 2007).

Several studies have used color Doppler to characterize morphological and functional changes in the CL during this period (Miyamoto *et al.*, 2006; Herzog *et al.*, 2010; Rocha *et al.*, 2019). It has been shown that a decrease in luteal blood perfusion occurs before structural regression (Niswender *et al.*, 2000; Herzog *et al.*, 2010) and the correlation between CL blood perfusion and circulating progesterone concentrations is greater during luteolysis than the correlation between CL area and progesterone outside this period (Rocha *et al.*, 2019). For these reasons, the use of colour Doppler to assess the functional status of the CL during luteolysis has increased significantly in research activity over the past few years and has provided valuable information about cyclic changes in CL function.

Corpus luteal blood flow (CLBF) changes dramatically in pregnant and non-pregnant cows from day 16 onwards (Siqueira *et al.*, 2019), and the subjective score can be used effectively to predict non-pregnant status (Siqueira *et al.*, 2013; Pugliesi *et al.*, 2014). On the other hand, studies such as those to determine the relationship between CLBF production and progesterone require distinguishing between subtle changes in CLBF (Herzog *et al.*, 2010; Pugliesi *et al.*, 2019). Siqueira *et al.* (2013.) concluded that cows with no color signals in the central area of the CL in their ovaries were non-pregnant, while Pugliesi *et al.* (2014) considered cows non-pregnant when the percentage of their luteal area with blood perfusion signals was ≤ 25% or when their CL area was < 2 cm^2. It should be noted that both studies reported high sensitivity (probability of the test giving a positive result when the cows were actually pregnant) and negative predictive value (probability of the cow not being pregnant when diagnosed as non-pregnant).

Conclusion

In conclusion, the current literature review indicates that transrectal colour Doppler ultrasonography is useful technique for examination of the reproductive organs blood flow. The ovarian blood flood intensity depends from the estrous cycle phases. Presence of close relationship between some blood flow indices of different ovarian structures and the hormonal production can be successfully used as an indicator for their functional activity. All abovementioned information opens a new window for investigation of physiological and pathological changes of the ovarian function in cows.

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