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REVIEW ARTICLE

Targeted Application of Color Doppler Ultrasonography in Dairy Cows Management

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Abstract

The management of reproduction in dairy herds has profited tremendously from technical improvements, greater use of ovulation synchronization procedures, and fixed-time automated monitoring devices. The purpose of this review article is to highlight the importance of noninvasive color Doppler ultrasound imaging based on the target reproductive management of dairy cows. Noninvasive measurement of uterine and vaginal blood flow in dairy cows is variable during the estrous cycle, pregnancy, and puerperium. Transrectal uterine and vaginal arterial blood flow changes can be used to determine changes in female genital functions during the estrous cycle, pregnancy, and puerperium, according to the findings of our study. During these periods, there were noticeable changes in uterine and vaginal blood flow. During pregnancy, uterine blood flow is positively correlated with the gestational period. Meanwhile, vaginal blood flow increased significantly during the late gestation period. Regarding the OvSynch program, the vaginal blood flow showed a positive increase when the serum estrogen level was increased, while at the time of prostaglandin shots, the vaginal and uterine arterial blood flow were low. In conclusion, the genital blood flow of either uterine or vaginal blood is important for the prediction of pregnancy per artificial insemination in dairy cows.

Keywords: Color Doppler, Targeted reproductive management, Uterine artery, Vaginal artery

1. Introduction

In modern dairy farming, reproductive efficiency is crucial for maximizing productivity and profitability. Timely and accurate assessment of reproductive health is essential for optimizing breeding programs, minimizing infertility issues, and ensuring the sustainability of dairy operations [1]. In recent years, advanced imaging technologies, such as color Doppler ultrasonography (CDU), have emerged as valuable tools for diagnosing and monitoring reproductive management in dairy cows [2].

Traditionally, reproductive management in dairy cattle relies on manual palpation and visual assessment techniques, which are often subjective and limited in their ability to detect subtle abnormalities [3]. However, the advent of CDU has revolutionized the way veterinarians and producers approach reproductive health monitoring in dairy herds. CDU enables noninvasive visualization of reproductive organs and blood flow dynamics,

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providing valuable insights into ovarian function, uterine health, and pregnancy status [4].

The targeted application of CDU in dairy cow management encompasses various aspects of reproductive health, including estrus detection, follicular monitoring, pregnancy diagnosis, and the assessment of uterine and ovarian abnormalities [5]. By precisely assessing follicular and luteal dynamics, CDU facilitates accurate timing of artificial insemination (AI) and embryo transfer, thereby improving conception rates and reducing calving intervals [6].

Estrus synchronization is a crucial reproductive management technique in the dairy cattle industry, in which artificial insemination is used to breed the majority of animals. Estrus synchronization decreases the cost of estrus detection and eliminates the probability of error. The fundamental principle of estrus synchronization is the manipulation of the duration of the luteal phase of the estrus cycle [1−3].

Although several synchronization schemes using one or more hormones have been developed and used in commercial farms, the main idea of estrus synchronization is to alter the duration of the estrus cycle [4,5]. Prostaglandin F2 α (PGF2α) or its analogs can be used to shorten or extend the luteal phase. Exogenous progestogens can also be used to lengthen the phase. In addition, some programs have used estrogens and GnRH to change follicular wave development and decrease the interval between estrus and ovulation to boost conception rates [6,7].

Although estrus synchronization lowers the chance of conception during synchronized estrus, almost all reproductive performance may be enhanced by increasing the effectiveness and precision of estrus detection [7,8]. The main obstacle in estrus synchronization research has been establishing precise synchronization while minimizing the negative impact on the rate of conception during synchronized estrus. The current approach of ovulation synchronization enables planned breeding without detecting estrus [9]. Dairy farmers calculate the cost of medications and the time required to administer the protocol when deciding whether to utilize estrus synchronization. It can be more challenging to detect estrus in a large herd of synchronized cattle because it is impossible to distinguish which animals are actually in true estrus as many animals are in estrus simultaneously [9,10]. The solution to this problem is to create synchronized procedures that enable insemination to occur at certain times. For an estrus synchronization plan to be successful, herd managers need to be well organized and have a basic understanding of the program. The collaboration and communication between herd management, vets, and AI technologists must be effective [11−13].

Synchronization of ovulation procedures includes the use of a combination of two or more hormonal therapies to manage follicular wave dynamics, corpus luteum (CL) regression, and ovulation [11]. The use of GnRH and PGF2α has proven to be very successful in synchronizing estrus in cattle and buffaloes for timed insemination [14]. Ovulation synchronization (OvSynch), combination synchronization (Co-synch), select synchronization (Select-synch), and hybrid synchronization (HybridSynch) are four systems for synchronizing estrus with GnRH-PGF2α combinations [15].

Pursley *et al.* (1995) pioneered the OvSynch protocol, which has completely altered the dairy industry. In the OvSynch protocol, the first injection of GnRH was administered on day 0, followed by PGF2a after 7 days, and a second injection of GnRH 48 h later [11]. Pregnancy rates varied when cows were timed inseminated at 0, 8, 16, 24, or 32 h after the second GnRH injection in the OvSynch protocol. The highest pregnancy rate (45%) was attained when insemination was performed 16 h after the second GnRH injection [16].

According to Pursley *et al.* [11], the first GnRH injection modifies follicular growth by causing the dominant follicle, the largest follicle in the ovaries, to ovulate and create a new or extra CL. As a result, estrus typically does not start until the PGF2a injection lyses both the natural and secondary CL (which was created from the follicle that was made to ovulate by the initial GnRH injection).

Fixed-time artificial insemination (FTAI) protocols eliminate the need for estrus detection; therefore, prefixed timing of artificial insemination (AI) is key to improving reproductive efficiency in animals with poor estrus expression [17]. By accurately stimulating ovulation, OvSynch technology helps breeders boost reproductive efficiency and successfully permits artificial insemination.

However, OvSynch was unable to produce perfect synchrony of ovulation in all treated cows, and the pregnancy rate per AI (*P*/AI) was low [18,19]. The pregnancy rate was higher if ovulation occurred in response to the first GnRH injection in the OvSynch protocol [20]. Initiation of OvSynch on the 6−7th day of the estrous cycle resulted in an increased number of cows with a new accessory (CL) at the time of OvSynch (PGF2α), greater control of P4, subsequent luteolysis, antral age of the ovulatory follicle [20,21], and improved (*P*/AI) [22]. New protocols such as Presynch-OvSynch and Double-OvSynch have been developed to improve the timing of insemination and fertility following TAI [23−25].

2. The basics of B-mode ultrasound imaging

Ultrasound (US) is a common imaging tool used in veterinary medicine. Real-time ultrasonography has substantially expanded our understanding of reproductive biology through its application to the study of animal reproductive processes. US imaging has revealed new details regarding the structure of complicated reproductive processes in farm animals, including ovarian follicular dynamics, CL function, and fetal development [26,27]. The most well-known type of scanning is B-mode grayscale scanning, which produces actual images of anatomical structures. The sound beam is produced by a transducer placed in contact with the animal and acoustically coupled by gel [28]. After directing an ultrashort sound pulse into the animal, the transducer shifts to the reception mode. When the sound beams travel from one density of tissue to another, their velocity changes, creating an echo [29]. The stronger the echo, the greater the change in velocity. A small portion of these echoes is returned to the transducer, where the energy of the echoes is again converted into electrical impulses that are subsequently recorded by the US machine's computer [29]. The strength of the echo, the time required for the echo to return after the pulse, and the direction of the sound beam that was sent were all recorded. Using information from multiple echoes, the machine creates an image that represents the appearance of tissues when cut in the same plane on an anatomical specimen [30]. Diagnostic ultrasonography creates pictures of body structures using ultrasonic sound waves with frequencies ranging from 1.5 to 15 MHz depending on the pattern of echoes reflected from the tissues and organs being scanned [31]. The frequency of the transducer determines and is inversely related to the depth of tissue penetration of sound waves and image resolution. Thus, a 5.0 MHz transducer produces more tissue penetration but less image detail, whereas a 7.5 MHz transducer produces less tissue penetration but more image detail [32]. To examine the physiological interactions of the rectum and genitalia, transducers with frequencies ranging from 5.0 to 7.5 MHz are required. High frequencies are applied for judging early pregnancy or superficial anatomical features, such as the ovary and CL, while low frequencies are applied to investigate mid-to-late gestation [33].

The main current applications of US imaging in cattle are not only for the diagnosis of pregnancy but also to estimate the ovulation time as the end point. However, there are more applications and potential for this technology. A great deal of research has been conducted on follicle dynamics [27,34] during the estrous cycle [35] and early pregnancy [36].

3. Ultrasonographic evaluation of ovaries in dairy cows

The ultrasonographic anatomy of the ovaries of cows has been previously described [37]. US imaging of the ovaries showed that the cows exhibited two or three follicular waves during the estrous cycle. Ultrasonography allows the assessment of individual follicles during growth and/or regression over time and patterns of follicular development and investigates the gonadotropic control and hormonal role of the follicles [38]. Antral follicles of different sizes appear as non-echogenic structures, which can be distinguished from blood vessels in cross-sections by the elongated appearance of the vessels. Normal reflection from the liquid medium is absent. Follicles with a diameter of less than 2 mm are insignificant and may not be noticeable [37,39]. The dominant follicle selected for ovulation grows during the follicular phase, and ovulation occurs when the follicle reaches a size of 12−20 mm [40]. The majority of follicles reach their maximum size 36 h before ovulation [41]. Ovulation was indicated by the absence of a previously existing preovulatory follicle, which was later verified by the growth of the CL in the same ovary [42]. Follicle size during estrus was found to be strongly related to pregnancy rate. The pregnancy rate in estrus cows with follicle sizes less than 14 mm was considerably lower than that in cows with follicle sizes greater than 14 mm [43].

The CL is distinguished by its gray color and oval shape, which is distinct from other ovarian structures [44]. The CL develops through hypertrophy and luteinization of the granulosa and thecal cells [45]. The cross-sectional diameter, luteal area, and echogenicity of the CL have been linked to luteal structure and function [46,47]. The CL develops quickly, achieves a diameter of 10 mm in just 2 days, and reaches its maximum size by days 7−8 of the cycle. The largest diameter of the CL is between 20 and 25 mm. However, the development of luteal tissue cannot be observed immediately after ovulation. The young CL only becomes apparent on US imaging 2−4 days postovulation. The mean width of the CL is ~14 mm, and the mean CL length ranges between 18 and 21 mm [48].

The use of luteal features to increase the accuracy of pregnancy diagnosis in dairy heifers has been previously described [49]. Luteal size and echogenic features measured at various postbreeding dates may be effective in improving the accuracy of early pregnancy diagnosis in cows [50]. The size of the CL was the most distinguishing feature. This might be too large or too small. The smallest size recognized for a good quality CL was 1.5 cm. A CL greater than 1.5 cm in size produces a sufficient amount of progesterone for normal function. US can easily identify mature solid corpora lutea [51].

4. Ultrasonographic evaluation of the uterus in dairy cows

During US imaging, the uterine tissue in a nonpregnant cycling cow appeared as a hypoechoic structure. The uterus absorbs some US waves and reflects some of them as it is made of soft tissue [32]. In this manner, the uterus may be distinguished as a gray structure in either the cross-sectional or longitudinal section [33,52].

Ultrasonography can detect distinct changes in the tubular genitalia of females, including thickening of the uterine body, signs of increased vascularity and edema, and mucus buildup [53,54]. During the estrous cycle, the uterus undergoes morphometric and echotexture changes that correlate with circulating hormone levels. Days −4 to −1 (day 0 = ovulation) are known as proestrus and estrus, and they are characterized by increased uterine body thickness, accumulation of luminal fluid in the uterus first, then the cervix and vagina, and minimum curling of the uterine horns. Endometrial thickness (ET) is also found to increase during normal luteolysis and before ovulation by 1 day [55]. In contrast, diestrus (days 3−16) had the lowest uterine thickness, lowest luminal fluid, and lowest uterine horn tonicity. Endometrial echotexture differences are likely caused by plasma P4 levels [54,56]. Furthermore, cows with ET less than or equal to 8 mm had lower ovulation rates than cows with ET greater than 8 mm. Furthermore, cows with ET less than or equal to 8 mm had a lower percentage of pregnancies per AI. In an OvSynch regime, US measurement of ET 48 h after PGF2a treatment was a reliable indicator of successful ovulation and pregnancy [55].

5. Basic principle of Doppler ultrasound imaging techniques

The function of reproductive organs and structures can be ascertained by measuring blood perfusion using Doppler US imaging. In Doppler sonography, the moving reflectors that produce the returning echoes are primarily red blood cells and the static object is the transducer [29,57]. The Doppler shift is the difference between the frequencies of the transmitted (F1) and received (F2) US waves of the transducer (Doppler shift frequency = F2−F1) [58,59].

When a reflector is stationary concerning a linear-array transducer, or the red cells move directly across the US beam, the vessel is parallel to the transducer or perpendicular to the US beam [32]. Under these conditions, there was no shift between the transmitted and returned frequencies [58]. In this case, the Doppler shift frequency is not recorded. When the blood flow or red cell movement is directed toward or away from the transducer, the Doppler frequency is positive or negative, respectively, according to the Doppler equation $\Delta f = 2 \times f0 \times v \times \cos \alpha / c$, where f0 is the frequency of the transmitted US waves; v is the velocity of the reflector relative to the transducer; α is the angle between the US beam and the direction of movement of the reflector; and c is the speed at which the ultrasound waves are dispersed within a tissue [60]. Because the speed of the US waves in tissues is relatively constant at 1540 m/s, and if the frequency of the waves emitted from the transducer is known, the velocity of blood flow at a constant angle α can be calculated [58].

Note the difference between B-mode and Doppler imaging. That is, the strongest echoes in B-mode imaging occur when the transducer beams are perpendicular to or 90° to the reflective surface. However, a similar angle between the beams and blood flow or reflective surfaces from red cells yields insignificant Doppler signals [32,61].

The time period between pulses allows the reception of the returning echoes before sending another pulse. The operator controls this period by changing the pulse repetition frequency (PRF) of the Doppler US waves. In most systems, PRF is related to the velocity scale and is regulated by varying the range of velocities sampled [61]. When the targeted vessels are proximal to the transducer or the blood flow is high, a high-PRF setting is applied. When the vessels were far from the transducer or the flow was slow, a low PRF value was applied. A low value increases the possibility of aliasing artifacts [32,62].

Doppler ultrasonography uses three primary modes, color, spectral, and power Doppler, each of which can be used to analyze distinct blood parameters.

6. Color mode of the Doppler ultrasound imaging

Color-flow Doppler US has the potential to improve the diagnostic and predictive abilities of large-animal veterinarians [32,58]. Blood-flow color signals are displayed on a B-mode (grayscale) image, allowing the blood-flow velocities and perfusion to be visually examined on an image plane. Color spots (signals) or pixels can be alternatively assigned numerical values [63]. When the B-mode and color flow imaging are combined, they provide real-time anatomical details and physiological blood flow information. As a result, this technique has the potential to provide a novel perspective on reproductive structures and functions [58].

The coloration of either red or blue depends on the position of the probe used to illustrate the blood flow in one direction or the other. The blue coloration indicates that the flow of blood moves away from the probe, whereas the blood flow toward the probe is shown in red. Each pixel's color (e.g., red vs. orange; dark blue vs. light blue) shows a frequency shift for a specific area [64,65].

The degree of vascular perfusion in a structure (such as a preovulatory follicle, endometrium, or embryo proper) may be a good indicator of the current condition of the structure and its potential for success in the future. Color-flow analysis of the vasculature could assist the clinician in making decisions regarding the ovulatory potential of the follicle, expected time of ovulation [66,67], functionality of the CL [51,68], quality of the oocyte (indicated by the follicle vasculature) [69], suitability of the endometrium for receiving a natural or transferred embryo [70], and detection of abnormal or disturbed blood flow [71].

7. The spectral or pulsed mode of Doppler ultrasound imaging

In the spectral mode, blood flow in a given vessel can be monitored by placing a sample-gate pointer on the B-mode or color mode image of the lumen of the vessel. In addition, an angle cursor was used to represent the angle of intersection of the US beams with the direction of blood flow (insonation angle) [32,72]. The instrument computes blood velocity using the Doppler-shift frequency and insonation angle. The focused results from the placement of a sample gate in an artery are displayed on the screen using a graphic spectrum that represents the changing velocities over time and depicts the arterial pulses generated by each cardiac cycle [32,60,73]. Peak systolic velocity, end-diastolic velocity, and time-averaged maximal velocity (TAMV) were computed and presented for a specific cardiac cycle. Doppler indices (resistance index [RI] and pulsatility index [PI]) were used to determine the frequency shift ratios. An increase in RI or PI values indicates an increase in resistance and a decrease in perfusion of artery-supplied tissues [74].

The magnitude of the Doppler-shift frequencies is represented as velocities (cm/s) on the vertical axis, and time is represented on the horizontal axis if the angle between the Doppler beam and the direction of blood flow is known. Positive Doppler frequencies (blood flow toward the transducer) are exhibited above the baseline, while negative signals (blood flow away from the transducer) are displayed below the baseline [75]. The position of the spectrum relative to the baseline can be inverted at the discretion of the operator. Furthermore, the operator can change the range of velocity detection by adjusting the baseline [58].

8. The power Doppler of Doppler ultrasound imaging

In the Power Doppler mode, the power of the Doppler signal backscattered from red blood cells is displayed as a function of the number of blood cells moving within a preselected region of interest within a distinct time. The color was superimposed onto a grayscale B-mode image. However, no directional information on blood flow is obtained, but Power Doppler is a more sensitive indicator of blood perfusion and is thus useful in the detection of small vessels containing sluggish blood flow [76].

9. Blood flow in the female reproductive tract during estrous cycle

To investigate the blood flow in the artery of interest using color Doppler sonography, it must be located.

10. The location of the uterine and vaginal artery

As illustrated in Fig. 1, the location of the uterine artery in dairy cows depends on the location of the abdominal aorta dorsally at the level of the pelvic inlet [57,77]. After the origin of one of the external iliac artery was found, the transducer was moved caudally to locate the internal iliac artery on the desired side. Subsequently, the umbilical artery was found to originate from the internal iliac artery. The uterine artery originated from the umbilical artery. The uterine artery is movable in the mesometrium compared with the more firmly attached external iliac artery, which can be useful in identification. The artery can be traced until it begins to branch [57,78,79].

The vaginal artery arises from the external iliac artery around the hip joint [32,80]. It was scanned within 5 cm of its dissociation from the internal iliac artery in the pelvic cavity ipsilateral to the dominant follicle.

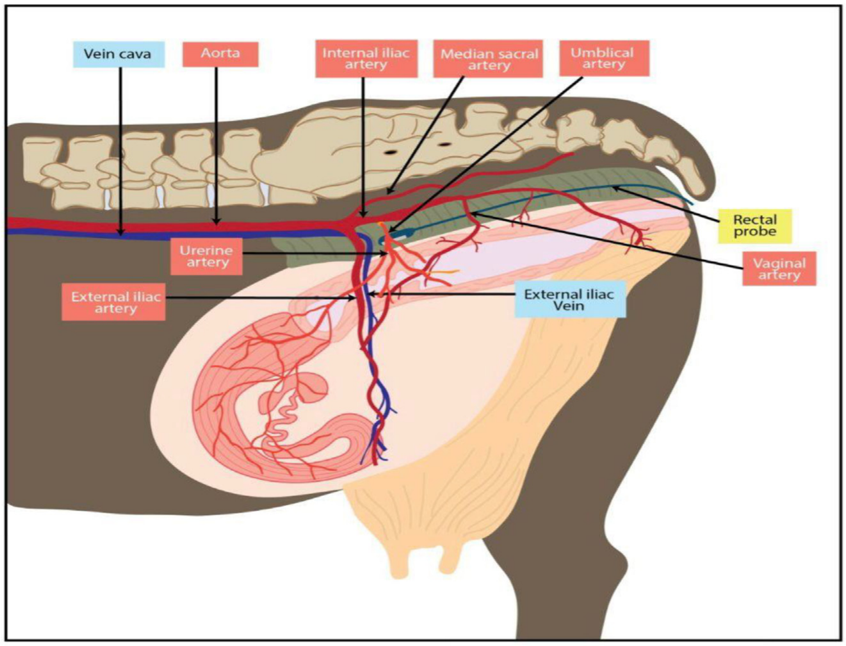

Fig. 1. Diagrammatic representation of a cow's pelvic region and the location of the ultrasound transducer during a Doppler sonographic examination of the uterine and vaginal arteries [77].

11. The uterine artery blood flow during the estrous cycle

In clinical research, Doppler ultrasonography has been widely used to assess alterations in uterine blood flow (UBF) during the estrous cycle [78,79,81] and synchronization protocols [77,82]. The overall reduction in UBF, as expressed by an increased resistance index of the uterine artery, is a marker of infertility and lower pregnancy rates, resulting in a very low survival rate and poor outcome; however, good uterine vascular perfusion is required for good pregnancy rates and embryo nourishment [83]. The presence of an ovulatory follicle or CL, as well as the day and phase of the cycle, influences not only ovarian but also uterine artery blood flow [84].

UBF in cows reflects a certain pattern during proestrus and estrus when time-averaged maximum velocity values are at their highest. During diestrus, the blood flow velocity is nearly constant. The lowest RI and greatest TAMV values were recorded between days −3 and −1 of the estrous cycle, whereas the highest RI and lowest TAMV values were determined on day 0 (the day of ovulation) and day 1 [78]. When compared with estrus and ovulation in Sahiwal cows, diestrus cows have a significantly lower RI of uterine arteries, a measure of blood flow, and a significantly higher PI [81]. The plasma concentrations of estrogens and P4 are related to changes in UBF velocity during the estrous cycle [77,79].

An increase in UBF may be observed during the first 21 days of pregnancy [85]. Interestingly, the location of the conceptus during early gestation is related to a local increase in endometrial vascularization that may be observed on Doppler ultrasonography [86].

12. The ovarian blood flow during bovine estrous cycle

Blood perfusion to the ovarian structures has been established in several studies using color Doppler ultrasonography for more than 20 years in various species [59,87,88].

Meanwhile, ovarian activity and hormone production are linked to an enhanced circulatory supply to ovarian tissues [89,90]. In farm animals, blood flow through the lutael and preovulatory follicles has been used to evaluate reproductive performance [58,64,91].

According to previous studies, an adequate blood supply is necessary for follicles to achieve dominance, as high vascularization is only seen in dominant and preovulatory follicles but not in atretic follicles [87,90]. Higher steroid hormone concentrations in the follicular fluid have also been associated with follicular blood flow [92,93].

Moreover, color Doppler ultrasonography-examined dairy cows showed positive correlations between follicle vascularity and follicular estradiol:

progesterone ratio [92]. The percentage of estradiol-active follicles increased in the same research when follicular blood flow was identified, and favorable correlations between estradiol concentrations and follicle size were observed. Similar positive relationships appear between blood perfusion of the preovulatory follicle and the diameter, vascularization, and progesterone-releasing capacity of the subsequent CL [93]. These results indicate that follicular vascularization can be used to evaluate follicular function. They also discovered that cumulus−oocyte complexes produced by follicles with detectable blood flow were of higher quality than those produced by follicles without detectable blood flow.

The functional state of the bovine (CL) must be accurately determined before administration of prostaglandins [59,88]. Transrectal palpation, which is based on the substantial positive correlation between luteal size (LS) and P4 levels, is used to evaluate the functional state of a CL [88]. Luteal blood flow (LBF) is a key factor in the regulation of P4 levels and can be reliably assessed using transrectal Doppler sonography. The fact that steroid precursors are delivered to the CL through blood supply and P4 release depends on good LBF can be used to explain the strong relationship between LBF and P4 [94].

13. Color flow Doppler imaging in the reproductive management

Noninvasive color Doppler US imaging plays a crucial role in the reproductive management of dairy cows.

14. Monitoring follicular and luteal dynamics

Color flow Doppler imaging is used to evaluate the quantity and distribution of blood flow within the ovary, serving as an indirect indicator of its functionality. This application is particularly valuable in identifying the presence of luteal tissue, including the early developing CL, tumors, or luteinized cysts [95,96], and in making indirect assessments of CL function, such as progesterone secretion. Visualizing blood flow in the CL (CLBF) serves dual purposes: for studies investigating ovarian physiology and for informing reproductive management decisions [97].

A CL with insufficient Doppler signals (colored pixels), indicative of inadequate blood supply, may be deemed nonfunctional; however, it could still exhibit normal-size parameters (diameter or area) [97]. Indeed, blood flow has been suggested to be more appropriate than size for CL function evaluation [98] because CL vascularization plays a key role in regulating luteal function [99].

Evaluations of CLBF could, consequently, be useful for detecting pregnancy failures or predicting pregnancy rates after embryo transfer or AI. Color Doppler US is used to enhance the prognosis of successful gestation by measuring luteal activity using CL blood perfusion during ET. Several studies using the FTET program have revealed a connection between gestational success and luteal blood perfusion [100,101]. Compared with females with lower blood perfusion in the CL, cows and heifers with higher blood perfusion in the CL exhibit higher serum P4 concentrations and improved reproductive success [102].

Enhanced follicular vascularization has been linked to improved pregnancy rates, with studies in both women and cattle demonstrating its predictive value for reproductive outcomes. In women undergoing in vitro fertilization and embryo transfer, higher follicular blood perfusion measurements have been associated with increased chances of pregnancy success [103,104]. Similarly, in cattle, color Doppler assessment of follicular blood perfusion revealed greater preovulatory blood flow in heifers that achieved pregnancy than in those that did not [71].

Furthermore, heightened vascular perfusion of preovulatory follicles is correlated with improved IVF outcomes, resulting in a higher rate of cleaved oocytes [105]. Pancarci *et al.* [106] also observed that follicles with detectable blood flow, as evaluated by color Doppler ultrasonography, yielded higher quality cumulus−oocyte complexes than those without detectable blood flow. Collectively, these findings underscore the positive association between increased follicular vascularization and enhanced follicular function and oocyte quality as well as improved outcomes in assisted reproductive technologies and pregnancy rates in cattle [106].

15. Doppler ultrasonography for early pregnancy diagnosis

In routine reproductive management, the early detection of conception failure is crucial for improving reproductive efficiency. Color Doppler imaging has been proposed as a method to identify nonpregnant animals by assessing corpus luteum blood flow (CLBF) [68]. As a functional CL is essential for establishing pregnancy, the absence of CLBF on specific days of the estrous cycle can serve as a direct indicator of nonpregnancy [107]. Matsui and Miyamoto (2009) [5] suggested that a decrease in CLBF around 19−21 days post-AI is indicative of nonpregnancy, and proposed that color Doppler flow imaging could facilitate accurate early pregnancy diagnosis in cattle [5,36]. In essence, the concept is to identify normal-sized but nonfunctional corpora lutea through color Doppler imaging, which signifies conception failure. Incorporating CLBF evaluation into routine reproductive practices may enable early resynchronization of nonpregnant animals, leading to reduced days open and calving intervals [108].

16. Physiological basis for estrus synchronization

16.1. Bovine estrus cycle

The mammalian female arousal stage that leads to ovulation is known as the 'estrus' stage. Ovulation is the period when females are more receptive to mating. This time period is usually referred to as the 'heat period' [109]. The hallmarks of the estrous cycle might be thought of as repeated ovarian alterations that occur during folliculogenesis, ovulation, luteogenesis, and luteolysis [106]. It occurs throughout the year in bovine species (annual polyestrous) and is only hindered during pregnancy, lactation, nutritional problems, and pathological circumstances such as a persistent CL.

The four phases of the estrous cycle were proestrus, estrus, metestrus, and diestrus. Under the combined action of pituitary hormone synthesis (follicle-stimulating hormone (FSH) and luteinizing hormone (LH)), the follicle grows and estrogen output gradually increases during proestrus until the follicle transforms into a preovulatory follicle [110]. During the beginning of estrus, this preovulatory follicle secretes a significant amount of estrogen, which is connected to changes in the genital tract and female behavior [95,111]. Excess vaginal mucus, vulvar edema, frequent urination, and discomfort are obvious indications of estrus [96]. The estrus phase of sexual responsiveness takes ~16−18 h. The metestrus period lasts from the termination of estrus to the fifth day of the estrous cycle [97], and it occurs when ovulation occurs 24−48 h after the start of estrus or 10−16 h after the end of estrus [98]. Due to the obvious effect of estrogen, cows have substantial capillary dilatation of the endometrium and rupture of uterine capillaries that may occur, resulting in the discharge of bloody mucus 1−3 days following estrus, indicating metestrus hemorrhage [99]. The diestrus phase is a progesterone phase of the estrous cycle and occurs when the CL is functionally active, producing progesterone, which is responsive to the action of PGF2α. This phase runs from the fifth to the 17th day of the estrous cycle; therefore, it is the longest duration phase. The CL is a temporary endocrine organ that is active during diestrus in cycling animals and during gestation. By the action of progesterone, the cervix becomes closed, and the uterus becomes more flaccid, with less immunological resistance and greater gland activity [100]. Around the 15th day of the estrous cycle, when there is more activity of the CL, maximum values are observed in the serum progesterone concentration [101].

16.2. The endocrine changes during the estrous cycle

Gonadotropin-releasing hormone (GnRH), (the gonadotropins (FSH and LH)), ovaries (estrogen, progesterone, and inhibin), and the uterus PGF2a all contribute to the regulation of the estrous cycle. Positive and negative feedback are the ways in which these hormones communicate with one another [102,103].

FSH is the primary hormone influencing the follicular phase. It induces follicular growth; when the follicle grows in diameter, it is recognized as a dominant follicle [104,105] that secretes estrogen and inhibin [107]. Under adenohypophysis, inhibin causes negative feedback, which lowers the concentration of FSH [108,112]. This makes it insufficient for the development of other subordinate follicles. The dominant follicle becomes highly responsive to LH [113] and continues to grow. In contrast to the estrous cycle during which the follicles develop, the change in FSH dependence [114] for LH [115] occurs in the presence of LH receptors in granulosa cells [116,117] and theca cells, allowing growth of the dominant follicle in an environment with a lower concentration of FSH [118]. An increase in estrogen secretion by the preovulatory dominant follicle associated with decreased progesterone serum concentration, which occurs during regression of the CL, induces hypothalamic secretion of GnRH [119]. GnRH induces a preovulatory LH peak [120]. After ovulation, LH is at a concentration lower than that responsible for ovulation, inducing luteinization of follicular cells and forming the CL [121]. Thus, FSH is mainly responsible for the recruitment and selection of the dominant follicle, and the exposure of a preovulatory dominant follicle to the frequencies of LH pulses is the key to the final maturation and ovulation [122−124]. During ovulation, the luteal phase is characterized by an increase in serum levels of progesterone secreted by the CL [125]. At this stage, even with a high progesterone concentration, follicular waves continue to cycle, which is required for ovulation to occur [126,127]. To maintain gestation, the CL produces progesterone during the luteal phase of pregnancy. Besides, progesterone suppresses the release of gonadotropins throughout pregnancy, thereby delaying the onset of estrus [127,128]. On day 16 of the estrous cycle, there was no maternal recognition of pregnancy by interferon-tau secretions. Regression of the CL occurs by the action of prostaglandins secreted by the cow's uterus [129,130], which decreases serum levels of progesterone [131]. The prostaglandin secreted by the uterus arrives at its place of action through the contra-current mechanism between the uterine vein and ovarian artery [132,133].

16.3. What does follicular dynamics mean?

Ultrasonographic monitoring of follicular dynamics in cattle, along with endocrine profiles, allows the clinical characterization of the estrous cycle in cows [134]. The bovine estrous cycle includes two to three follicular waves [135,136]. Four follicular wave cycles can occur, but they are uncommon [137,138]. A new follicular wave typically begins immediately after ovulation. Four or five antral follicles emerged from the pool of follicles under the influence of FSH [95,139]. These emerging follicles grow to a diameter of 4 mm [140]. After 2 or 3 days, one of these follicles establishes dominance through the secretion of estradiol and inhibin, forcing subordinate follicles to undergo atresia and regression [95,119].

The dominant follicle continues to expand and creates LH receptors. Because of the blockage of an LH surge in the presence of a functioning CL (luteal phase, high progesterone levels), ovulation is not possible, and the dominant follicle develops atresia [110,119]. Another follicular wave emerges as a result of the loss of endocrine function and regression of a prominent follicle from the previous wave, which reduces the negative feedback on FSH. In a cycle with two follicular waves, the second wave appeared between days 9 and 10 of the cycle (day 0 is the day of ovulation). After luteolysis, the dominant follicle from this wave continued to expand and finally ovulated in response to an increase in LH [141]. In a three-wave cycle, the second follicular wave appears between days 8 and 9 and ends when the dominant follicle regresses. The third follicular wave then appeared on days 15 or 16, with the dominant follicle continuing to develop until ovulation. The duration of dominance in the first follicular wave determines the number of follicular waves in a cycle. Three-wave cycles (23 days) are often longer than two-follicular wave cycles (20 days) [142,143]. Studies characterizing the follicular wave pattern in cattle have allowed the development of strategies to control follicular development, leading to synchronization programs. The general principle for synchronization of the follicular wave and ovulation is to control the emergence of the follicular wave and establish a new dominant follicle in a predictable manner. Although follicular wave control can be achieved using estrogens and progesterone, the use of the former is forbidden in several countries [144]. The dominant follicle of the first follicular wave ovulated within 2 or 3 days when PGF2a was administered 5 or 8 days after ovulation but within 5 days when PGF2a was administered 12 days after ovulation [145]. Currently, most synchronization programs are based on a combination of GnRH, PGF2α, and P4 [3].

Ethics approval

This review article is a part of Heba Sharawy master student. The Ethical Animal Care and Use Committee of the Faculty of Veterinary Medicine, Mansoura University, approved all procedures performed on the cows (M/158).

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Authors' contributions

ME and HB, HS develop the theory and follow up the progress in the review article with the other coauthors. AM, AS, and F A, care and help in the B-mode US basics. SS, AH, and YL supervised the basics for Doppler imaging and demography for the vasculature of the genitalia. All authors contributed to the final manuscript either in writing and\or editing.

Availability of data and materials

The data that support the results of this study are available on reasonable request.

Conflicts of interest

There are no conflicts of interest.

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