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Application of Color Doppler Ultrasound in Egyptian Buffalo Reproduction

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THE Color Doppler ultrasonography is widely utilized in equines, dairy cattle, and small ruminants as a safe, non-invasive means of monitoring reproductive performance. Non-intrusive color Doppler ultrasonography was utilized to evaluate high-risk pregnancies and the health of the fetus. The alterations that take place in farm animals after giving birth are thought to be crucial in predicting future fertility. B mode ultrasonography has been applied in several studies on postpartum buffaloes. Color Doppler ultrasonography is a non-invasive technique that can be used before, during, and after delivery. Using the uterine and umbilical arteries, it is possible to evaluate the perfusion of the uteroplacental and fetoplacental blood flow in buffaloes. The motion of the transmitter and receiver affects the frequency of an ultrasonic pulse, which is a phenomenon called the Doppler Effect, and was initially described by Christian Doppler. Nowadays, the use of color Doppler ultrasonography has followed the same pattern as that of cows and other animals. Doppler ultrasound application's basis is likened to cows. The blood supply to the uterus is remarkably similar. Recent research have described the features of vaginal blood circulation in buffaloes throughout gestation and the early puerperium in addition to the uterine blood flow. The impact of vaginal blood circulation on buffalo fertility needs to be studied in the future. We highlight the different benefits of non-invasive color Doppler ultrasound applications in the buffalo's reproduction.

Keywords: Color Doppler, Buffaloes, Puerperium, Vaginal blood flow, Blood flow volume.

Introduction

The use of color Doppler ultrasound as a secure, non-intrusive method to assess reproductive efficiency of horses, milk cows, and smaller ruminants is common [1–6]. Non-invasive color Doppler ultrasound was largely used to evaluate the health of the fetus and high-risk pregnancies [7–10]. Postpartum alterations are considered to be important in predicting future fertility in farm animals [11-13]. Numerous studies on the use of B mode ultrasound in postpartum

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buffaloes have been published. [14–16]. Doppler ultrasonography is a non-invasive method that may be utilized both throughout gestation and after giving birth [5,12,17]. Examining the uterine and umbilical arteries will allow you to judge the uteroplacental and fetoplacental circulations' perfusion, respectively [٥,12,17]. The correlation between the motions of the transmitter and receiver affects the frequency of an ultrasonic wave. Christian Doppler is the one who originally discovered the phenomena, which is eventually called the Doppler Effect (DOPPLER 1842) (cited by Elmetwally et al.) [5].

Doppler sonography evaluations can fall into one of three categories:

1. The first, qualitative, is based mainly on wave assessment and includes the existence or lack of end-diastolic blood flow as well as how it relates to earlier and later peak systolic blood flow. Moreover, it involves wave features that are higher or lower than the baseline as well as a pathologically or physiologically elevated or reduced end-diastolic blood flow velocity [5,12].

2. The second method, which is semi-quantitative, often relies on reports of blood flow metrics that are unaffected by insolation angle. Peak velocity (PV) and time-averaged maximum velocity (TAMV) values are calculated during the cardiac cycle after the Doppler indices PI (pulsatility index) and RI (resistance index) are registered. These Doppler indices can be used by researchers and medical professionals to gauge the degree of vascular perfusion [3, 4].

3. The quantitative analysis includes evaluating the target blood vessel diameter and examining blood flow variables that requires altering the Doppler angle between the Doppler beam and the blood vessel, such as blood flow volume (BFV) [4,12].

Imaging ultrasound types:

- Real-time B-mode: [18] presented the interfaces that produce echo in a two-dimensional diagnostic ultrasonography view; The brightness (B) of the spot is modulated to indicate the echo's intensity, and the angular location of the transducers and the transit times of the sonic pulse and its echo are used to calculate the echo's position [19]. A dynamic anatomical diagnosis is possible due to the wealth of information that B-mode ultrasonography offers in a short amount of time. A two-dimensional picture in a quick-to-generate succession of photographs provides a concept of real-time motion structure analysis. The signals are continuously sent, received, and analyzed, updating the picture of the organ [20]. The most common use of method-mode ultrasonography is the assessment of the reproductive system in cattle and other big animals, such as the water buffalo [21-23].

- A-Mode: Using a line chart with magnitude and depth as the axes, the amplitude mode generates a one-dimensional display of echo amplitudes at various depths. The main purpose of the A-mode ultrasound is to evaluate the lean and fat parts of meat animals [24, 25]. Real-time B-mode represents the most often employed technique, however A-mode has also been utilized to detect pregnancy [25].

- M-Mode: Motion mode ultrasound is a kind of B-mode ultrasound that is used to assess moving structures, such as the heart [19]. A simple line graph featuring depth and time as the axes is used to represent the change of reflector depth over time [25].

2. Doppler: According to Ginther and Utt, (2004) [26], Doppler ultrasonography detects blood flowing toward, away from, or in an oblique direction from the probe (red indicates blood flow toward the probe; blue indicates blood flow away from the probe). Doppler is used in medicine to assess blood flow in the fetal heart, the corpus luteum, and ovulation, among other things [2,10,27].

Types of ultrasound probes: many transducers for ultrasound imaging are available according to the distribution of piezoelectric crystals.

- Any device that changes the form of energy is referred to as a transducer. To produce ultrasonic waves, ultrasound transducers transform electrical power into mechanical power. They also transform the acoustic energy of echoes into electrical energy. There are three classifications for transducer arrays [25].

- *Linear array*: Throughout the length of the transmitter, the rectangular electric crystals are stacked in linear arrays side by side. The two-dimensional picture and the examination field are shown on the screen as a rectangle. A transrectal linear-array scan's rectangular field is directed longitudinally with regard to the animal. Sequential linear array devices are the most common type of ultrasonic scanners (transducers) used to investigate large animals' reproductive tracts (Fig. 1 L).

- Sector (sectorial) array: A sector array is the term used to describe the pie-shape inspection field and picture. For projecting beams across confined areas, such as between ribs, sector transducers are helpful. The laser penetrates the tissues through a tiny window before spreading out.

- Convex array: A convex array scanner is a curved scanner that creates a field that resembles a sector with a resolution similar to a linear array. Convex transducers are excellent for transvaginal oocyte aspiration, rectal exams, and transabdominal imaging in small animals and small ruminants (Fig. 1 R).

- *Ultrasonographic Imaging of the ovaries and reproductive tract:*

Water buffalo is a short-day breeding species with a seasonal polyestrous, mono-ovulatory cycle. They have seasonal changes in their estrus, conception, and calving exhibitions. Although having a comparable anatomical structure to cattle and having a similar reproductive system, there are several important variations to consider [28-31]. Zambrano-Varón, (2015) [32] noticed that water buffalo tubular genitalia are usually more muscular and firmer than cow tubular genitalia, and the uterine horns seem to be more coiled. Comparing the uterus to a cow, it is one to two centimeters shorter in length (two to four centimeters). As comparison to cows, water buffaloes have smaller cervixes (3-10 centimeters in length, one and a half to six centimeters in diameter), and the cervix seems to be more convoluted. Water buffaloes have three cervical folds on average. Additionally, during standard rectal exams of non-pregnant buffalo females, the wide ligament appears to be stiffer in comparison to cows, making it challenging to entirely retract and expose the uterine horns.

When compared to domestic cattle, the buffalo ovary is smaller and lighter in weight (2.5 cm vs. 3.7 cm of length and 3.9 grams vs. 8.5 grams of weight, respectively) [32]. The CL's morphological appearance has been described as well [27, 33, 34]. As deeply surrounded by the ovarian stroma as cattle, but smaller in size [35, 36]. Diagnostic ultrasonography can be used to assess morphological alterations in the uterus during the reproductive cycle, postpartum uterine involution [37, 38], and [39], ovarian biometry, and non-pregnant female pathological conditions [40].

It is critical to highlight that in order to perform sonographic investigations correctly, the clinician must have a thorough understanding of the anatomy and placement of anatomical structures, as well as the clinical skills to perform rectal examinations for the recognition of reproductive structures [20]. An ultrasound examination, on the other hand, does not require rectal palpation.

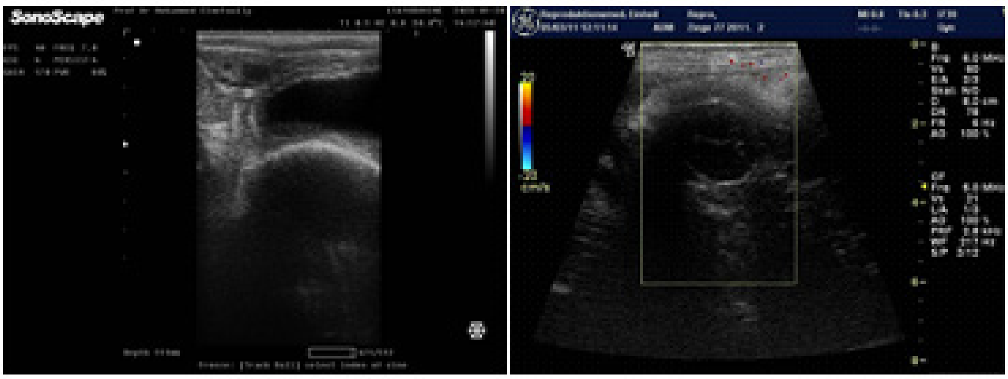

Fig.1. Representation of the ultrasound beam. Left panel: Linear array, and right panel: Convex array. A broadband probe could be utilized at frequencies between 2.0 and 10 Megahertz (MHz) thanks to manufacturing innovations that have made it possible to create transducer crystals with a wide frequency range. Sector and convex transducers generally operate in the 2.0 to 6.0 MHz frequency range, whereas linear probes often operate in the 5.0 to 10 MHz range.

The examiner must completely clear the rectum of feces before inserting the lubricated transducer. The transducer must be positioned longitudinally, toward the uterus. The probe should be gradually inserted into the cervix, uterine horns, and lateral direction forward towards the ovaries [41, 42]. The transducer should be positioned dorsal to the tissues while looking at the genital tract. Depending on where the probe is placed, the cervix, uterine body, and uterine horns can be seen on the screen in a longitudinal or transverse cross-section [43]. It's crucial to keep the distance between the probe and the tissues being examined as little as possible when examining the uterus. In order to obtain a high-quality image while causing the least amount of damage to the rectal mucosa, this necessitates gently pressing the uterus with the probe [42].

Ultrasound imaging of the uterus

When a longitudinal portion of the uterus is identified, it has been proposed that the uterine horn be split into four or five segments to describe the location of an embryo or a specific pathogenic change. Based on how far the probe is placed from the longitudinal axis, there are different cross sections of the uterine horn. Physiological changes are measured using variables such echogenicity, vascular structures, uterine wall edema, fluid buildup in the uterine horn lumen, and more (Fig. 2). Because of excessive edema and uterine tonality, the uterine wall becomes more echogenic during estrus (Fig. 3). Non-echogenic areas within the uterine wall are characterized as vascularity and edema, and they are closely linked with estrogen-dominated days of the estrous cycle. On rare occasions, fluid can accumulate physiologically in the uterine lumen throughout estrus in female buffaloes [37] as it occurs in cattle [45].

Ultrasound imaging of the ovaries

The transmitter must always be placed in direct connection with the ovary to eliminate artifacts caused by the transmitter not coming in contact or coming into contact with other tissues during an ultrasound examination of the ovary (Fig. 4). To determine the features that are present at a specific period and produce a high-quality picture, the veterinarian must move the ovary and transmitter [41,42]. For example, when viewing an image on the screen, you will notice that the follicle has

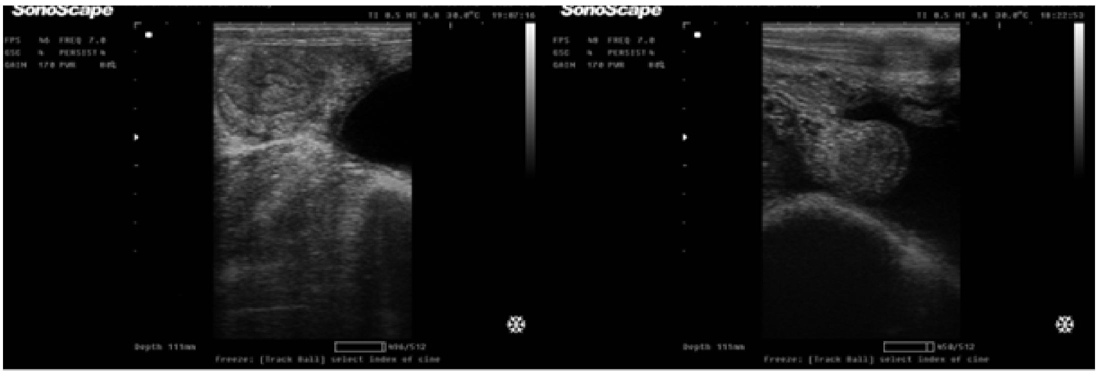

Fig. 2. Cross section in the buffalo uterine horn at day 14 of estrus cycle at 10-11 or 8-9 clock in relation to the urinary bladder.

Fig. 3. Cross section of uterus at estrus (left) and the contralateral ovary with dominant follicle (right).

Fig. 4. Ovaries in buffalo with smaller follicles at 12 (left) and 11 (right) o'clock in relation to the urinary bladder.

Fig. 5. Ovaries with CL in cows (left) and in buffalo (right)

a round shape, usually smaller than 8mm, and contains a non-echogenic fluid that is enclosed in an echogenic wall (Fig. 4). Nevertheless, the corpus luteum (CL) is an echogenic structure that develops at a diameter of 13–16 mm visibly dependent on the day of the estrous cycle (it shows as a grey structure on the monitor) (Fig. 4). In buffalo, CL is relatively smaller than in cows, and they don't have a tendency to extrude from the ovarian parenchyma, making it challenging to clearly identify them by rectal palpation, as previously mentioned (Fig. 5). The presence of a CL is essential in order to analyze the buffalo's cyclicity, begin estrus synchronization techniques, embryo transfer treatment methods, transfer of embryos, or evaluate anovulatory states and real anestrus. [35].

B-mode ultrasound imaging

In order to view deep bodily systems for physiological and pathological research, such as the female reproductive system in numerous animal species, ultrasound imaging exploits the reflecting high-frequency ultrasonic waves. Ultrasonic frequencies applied in diagnostic ultrasound range from one to ten million Hertz [46, 47]. In general, the deeper the tissue is penetrated by ultrasonic waves, the lower the ultrasonic frequency[18].

Mid to late gestation is evaluated with low frequencies, whereas earlier gestation or superficial anatomical characteristics like the ovary and corpora lutea are investigated at high frequencies. In diagnostic ultrasound, piezoelectric crystals, composed of quartz crystals, are utilized to transform electric power to acoustic waves and the other way around [48]. In the crystals, which are connected to electrodes, certain particles charged positively while others charged negatively. Crystals align themselves in the presence of electric current, causes the particles to change dimensions. Because of this phenomenon, it is called the piezoelectric effect[49, 50].

The sound waves produced by electric current conversion have very similar properties as depth sound in oceanographic analyses of the ocean bottom. Various acoustic resistance is reflected to the transducer when ultrasonic waves that are directed at particular organs, like the uterus, strike the surface of the tissue. The transducer then converts the rebounded ultrasound waves into recognizable echoes on the screen, expressing an image of the tissue[51, 52]. The thickness of the piezoelectric crystals typically determines the transducer's target frequency. The piezoelectric elements grow thinner with increasing transducer frequency[53-55].

Depending on the degree of ultrasonic wave reflection, the color of the ultrasonic echoes displayed on the monitor varies greatly, ranging from black to white. Similar acoustic and visual characteristics apply to the ultrasound beams used in B-mode sonography. When many ultrasound beams are transmitted and received in the same plane, two-dimensional ultrasound images are produced[56, 57]. Moreover, the visual brightness of the displayed echoes is frequently related to their amplitude [58]. Air and bone frequently obstruct the passage of ultrasonic waves [59].

Fluids that do not reflect ultrasonic waves, such as those found in embryonic vesicles and ovarian follicles, typically appear black and are classified as non-echoic. The ruminant cervix and other thick tissue ultrasound pictures, on the other hand, show the opposite. These pictures are known as hyperechogenic or echogenic. Images of soft tissues often exhibit varying hues of grey depending on echogenicity [60].

Principles and techniques of color Doppler sonography

When B-mode ultrasonography was established in the 1980s, it significantly improved bovine reproduction by enabling the first noninvasive view of the internal reproductive organs, which was a milestone in both clinical and academic studies. Although this method may be used to evaluate the morphological aspects of an organ, it fails to offer information regarding the function of the organ, such as blood flow. The perfusion of the cattle reproductive tract was initially examined experimentally using intrusive techniques [2, 61, 62]. Throughout the past 20 years, there has been a rise in the application of color Doppler ultrasound for blood flow analyses in bovine reproduction. [63, 64]. This has led to new knowledge and insight into the physiology and pathology of the female genital tract. [65, 66]. A Doppler shift occurs when the frequency of ultrasound waves reflected by moving objects, such as red blood cells, differs from the waves that were originally sent. Positively, when red blood cells are traveling in the direction of the transducer, the frequency of reflected waves is greater than emitted waves, resulting in a positive shift. The Doppler shift turns negative as blood cells move away from the transducer since the frequency of emitted waves is higher than the frequency of reflected waves. [2,5, 17].

Using Color Doppler to Assess Vascularity:

In order to assess ovarian blood flow before to in vitro embryo development, color Doppler ultrasonography was initially used in human-assisted reproduction in the 1990s [67-70]. According to more recent studies using the pulsatility index, women with unexplained infertility exhibited reduced uterine and ovarian arterial blood perfusion during the luteal phase[71,72]. Women's ovarian stroma's lack of arterial perfusion led to decreased oocyte recovery, decreased organ volume, decreased follicle size (>14 mm), and subpar in vitro embryo development[73,74].

Despite similarity in age, weight, and length of infertility, this persists. However, color Doppler has been used in large domestic animal reproduction studies after its success in human reproductive research [64]. by examining the ovarian structures' morphology and physiology, particularly with regard to cattle: corpora lutea and follicles [75-77].

Color Doppler ultrasonography in veterinary practice is typically restricted owing to expense or convenience, although it has been applied in cows to calculate pregnancy outcome in recipients intended for embryo transfer, demonstrating data to show that its use would result in cost reductions for the producer. [78-80]. It was also possible to anticipate the progesterone production as well as the vascularity of the corpus luteum after ovulation based on the ovulatory follicle's blood flow [81,82]. Even though Doppler ultrasonography in cattle reproduction management has been delayed, when it is available, it still contributes crucial data to reproductive science in both animal and human health. When using color Doppler ultrasonography, postpartum buffaloes' uterine and vaginal blood flow can be observed in ways that would be missed in brightness mode alone. When appropriate, information from other species is compared to the findings of this study.

Evaluation of blood flow

With the use of Doppler equipment, which produce a so-called Doppler wave on a two-dimensional graph when frequency varies as a function of time, it is feasible to assess arterial blood perfusion during cardiac cycles [2, 10, 83]. In ultrasound devices with color Doppler capability, color-coded Doppler changes can be noticed. Red often reflects positive shifts (blood flow in the direction of the transducer), whereas blue typically represents negative shifts (blood flow away from the transducer) [16, Fig. 6]. Power mode [2, 84] is a more complex method

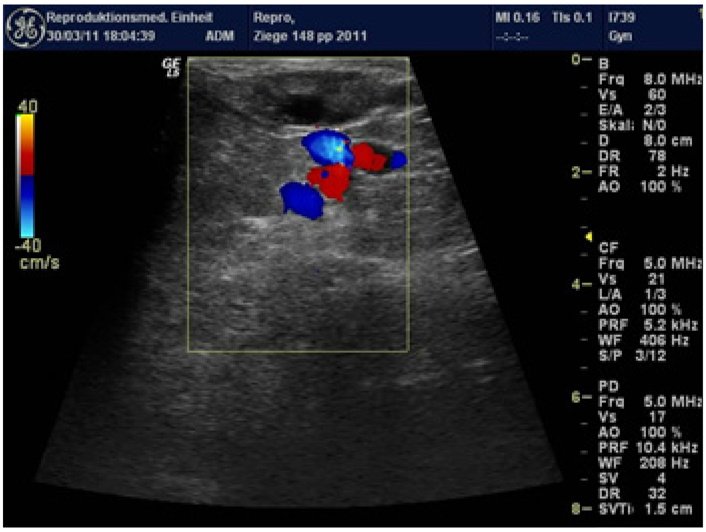

Fig. 6. Blood flow in vessels detected by color Doppler mode. Red shows blood flow moving toward the ultrasound probe and blue shows blood flow moving away from it.

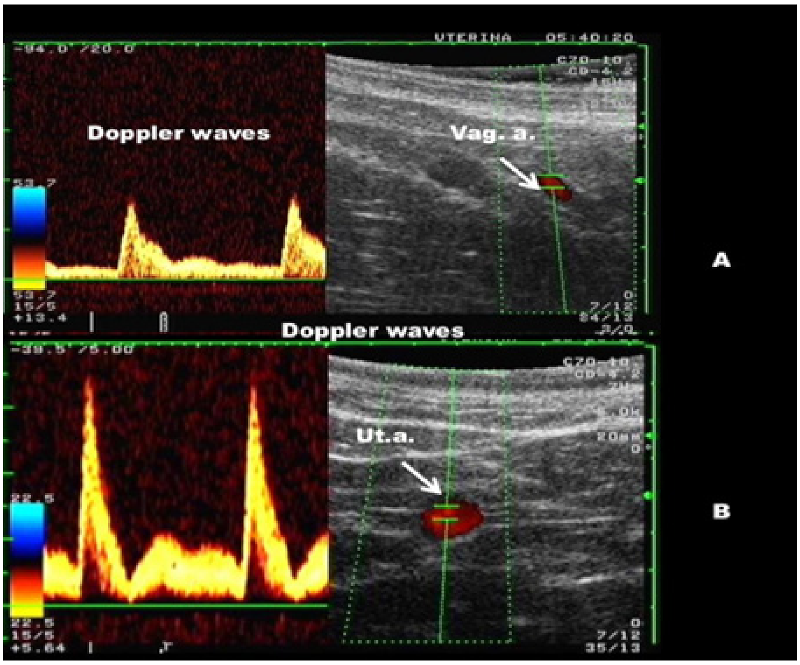

Fig.7. Time-dependent changes in frequency shift of an artery for the duration of cardiac cycles detected by Doppler spectral mode.

of displaying blood flow. Unlike conventional approaches, this methodology measures blood flow intensity rather than blood flow velocity (i.e., the number of RBCs moving through a vessel per time unit). Bright foci in B-mode are displayed on the screen as the blood cells. For displaying exceptionally low blood perfusion, such as follicular blood circulation, this method is preferable to the conventional color Doppler technique (Fig. 7).

The so-called Doppler indices are used to semi-quantitatively measure blood flow in certain arteries. These indices do not track blood flow; rather, they display the resistance to blood flow in vessels that are far from the vessel under examination. Rising values result in rising blood flow resistance, and vice versa [3, 4,17]. The relative values used to calculate the Doppler indices are the highest (S), lowest (M), end (D), or time-averaged mean frequency shift (TAMF) during one cardiac cycle [3,4: Fig. 8].

Nevertheless, because this index by design assumes the maximum value, it is impossible to differentiate blood flow with an end-diastolic flow that goes to zero [17]. Such blood flow is examined using the pulsatility index (PI). The PI is applied to tissues with high flow resistance when diastolic blood backflow occurs. The PI computes the mean velocity across the cardiac cycle by dividing the whole distance from the top to bottom of the systolic peak. Its formula is PI = (S - M)/TAMF. (Fig. 8).

Peak systolic frequency shifts are denoted by S, minimum diastolic frequency shifts are denoted by M, and time-averaged maximum frequency shifts are denoted by TAMF [85].

For a quantitative assessment of blood perfusion, the blood flow volume (BFV) is calculated through using time-averaged maximum velocity and the cross-sectional area of the arteries [4,12,84,86]. Since the ovarian corpora lutea and follicles are supplied by multiple blood vessels, follicular and corpus luteum blood flow (LBF: Fig. 9) is almost always assessed by examining the area of approximately of colored pixels. In cross-sectional B-mode photographs of these structures or in power mode when using computer-aided image analysis tools, the colored area in proportion to the overall area [2,66].

Transrectal Localization of the Uterine Artery

The aorta is first located (Fig. 10) and scanned in its caudal region to locate the confluence of the internal iliac artery. This is done in order to evaluate vascular perfusion in the uterine artery using transrectal color Doppler sonography. Branches of the uterine artery and the rudimentary umbilical artery may be found in the distal direction. The primary blood artery supplying the

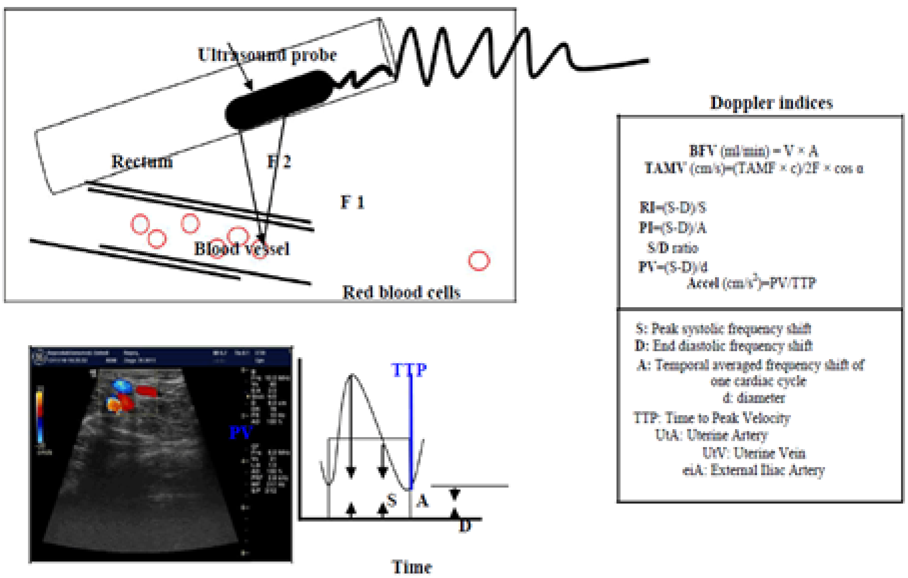

Fig. 8. Semi-quantitative evaluation of blood flow by determination of the resistance to blood flow using the Doppler indices RI and PI. D, end diastolic frequency shift; M, minimum frequency shift; S, systolic frequency shift; TAMF, time averaged maximum frequency shift.

Fig. 9. Blood flow (colored area) within the corpus luteum wall detected by power mode. The white line shows the borderline between the follicle and the ovarian tissue.

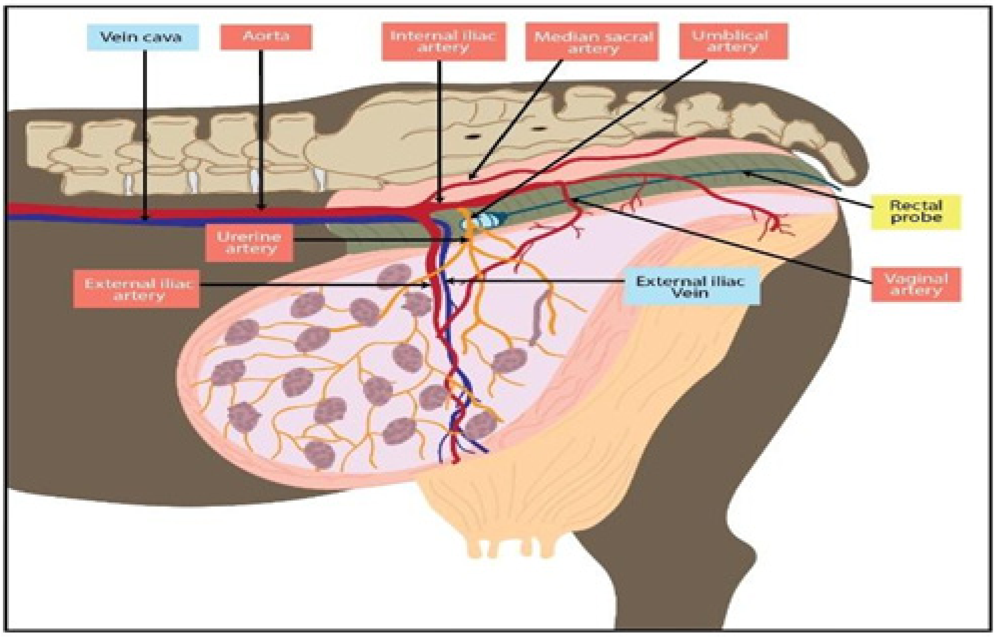

Fig. 10. Schematic presentation of the pelvic area of a cow and the position of the ultrasound transducer during Doppler sonographic examination of the uterine artery.

uterus in non-pregnant cows has a diameter of up to 5.0 millimeters [1,63].

Uterine Blood Flow

Estrous Cycle

The time-averaged maximum velocity values during proestrus and estrus, when the uterine vascular perfusion in cattle peaks, can be used to identify this pattern. Vascular perfusion remains significantly diminished in diestrus for an extended length of time [1,87]. The fluctuations in uterine blood flow rate during the estrous cycle and the plasma levels of estrogens and progesterone are somewhat correlated (P4). These results imply that additional factors, in addition to sexual steroid hormones, also influence uterine vascular perfusion. They remain unidentified. [63].

Pregnancy

Within the initial three weeks following insemination, pregnant cows have changes in their uterine blood flow. However, early pregnancy cannot be detected with a single Doppler sonographic examination of uterine blood flow since uterine blood flow greatly varies not only across but also among animals. It is uncertain why uterine vascular perfusion changes during early pregnancy, similar to estrous cycles. [88,89].

Monitoring uterine perfusion once a month throughout the gestation suggests a considerable increase in BFV, according to Bollwein et al. 2002 [1]. Throughout the first eight months of pregnancy, the RI drops, after which it very much stays the same until calving. The uterine vessels' transition into a low-resistance system with changes in tone and width is what causes the reduction in blood flow resistance. Each week, Panarace and his bandmates put on a show [6].

When the notch, a protodiastolic incisure with in uterine artery's Doppler waves, was examined using Doppler sonography on both uterine arteries between twenty second and the twenty sixth weeks of pregnancy, it was found to have vanished. The researchers figured that this occurrence would be considered as a sign of robust placental development since the persistence of the notch during the last trimester in pregnant women is thought to be a symptom of a fetus with insufficient blood supply [90]. It may be inferred that the uterine arteries had reached their maximum blood flow capacity because there were no changes in BFV throughout the final seven days before to delivery. End-of-pregnancy uterine perfusion was associated with birth weight of calves [91].

Puerperium

The first four days after calving are when uterine perfusion alterations are most perceptible, according to [92]. The substantial drop in uterine size and weight throughout this time period and the changes in uterine perfusion are related. Whereas the PI increases until the 28th Day after parturition, the BFV decreases as the puerperium goes on [93]. This is due to the caruncular vascular bed's changes having been finished about day 30 following delivery. Notwithstanding the fact that uterine involution is finished on day 47 following birth clinically and histologically, [93,94]. After calving, the BFV and PI both continued to decline until Days 65 and 86, respectively. This reveals that uterine vascular bed alterations take longer to manifest than endometrial histological changes. The largest PI in women also occurred 40–50 days after the uterine involution was complete [96], with this occurrence being common. Uterine involution is adversely affected by metritis and retained fetal membranes (RFM), two puerperal uterine diseases [97]. Hence, puerperal uterine disorders have an effect on uterine blood flow. Cows with RFM showed higher RI values of the uterine arteries in the days leading up to calving as a consequence of the malfunction of the fetomaternal adherence physiologic ablation process [91]. Compared to unaffected cows, cows with metritis have lower PI levels and increased BFV of the uterine arteries on Day 8 after birth. Beyond Day 45 postpartum, the BFV in animals with uterine problems remains steady, but the BFV in healthy cows continues to decrease [91]. In cows with uterine diseases, the delayed uterine involution is thought to be due to altered blood flow characteristics. Together with uterine involution, transrectal Doppler sonography can be used to evaluate the indirect contractility of the uterus. According to the intensity of the contraction, the uterine arteries narrow in women, increasing vascular resistance and decreasing blood flow velocity [98]. As a result, healthy cows who get an injection of oxytocin on Day 2 after calving see a drop in BFV and a rise in PI. Animals with RFM did not react to the oxytocin challenge, indicating that their uteri are not able to respond to oxytocin at this time in these cows. [99].

Ovarian Blood Flow

Follicular Blood Flow

Color Doppler ultrasound was used to examine FBF at various points in the estrous cycle [100]. One day before follicle selection, small follicles (diameter >2.5 mm) with visible blood flow had bigger diameters than those without it. Prior to diameter deviation, the future dominant follicle (DF) shows an increase in FBF. The DF has a higher probability and volume of blood flow following deviation as compared to the second-largest follicle. While [101] found that blood supply appears to be important for achieving and maintaining follicular dominance [102], the differential in blood flow between the DF and the second-largest follicle is thought to be the result rather than the cause of follicular deviation. As a persistent histologic trait, the dominant follicle's theca layer is more vascular than the atretic follicle's, enabling better transport of nutrients and gonadotropins. [103]. According to the research, monitoring FBF can be a useful tool for predicting follicle survival following deviation and spotting probable DF at an early stage of development. Counting the number of follicles with detectable blood flow may also aid in predicting the super ovulatory response. [104].

A functional link between FBF and plasma levels of estradiol (E2) and luteinizing hormone during the periovulatory period has been demonstrated [105]. Blood flow is hardly detectable in a tiny region around the base of the follicle prior to the luteinizing hormone surge, although FBF steadily increases along with plasma E2 concentrations. During the luteinizing hormone spike, blood flow at the base of the follicle rises sharply and peaks right before ovulation [76], but blood flow to the apex falls. It is believed that increasing blood supply to the preovulatory follicle will boost the generation of gonadotropin and facilitate follicular rupture[106]. Contrary to preovulatory follicles with good blood flow, atretic (anovulatory) follicles are characterized by a lack of visible blood flow. As a result, color Doppler ultrasonography evaluations of FBF can be utilized to spot healthy follicles and determine how close an ovulation is. It's likely that the association between FBF and follicular E2 concentration results from E2's capacity to quickly cause blood channel dilation by improving the bioavailability of nitric oxide [101]. The first ovarian follicular wave's DF always has a bigger diameter, superior steroidogenesis, and more blood flow than the second wave's DF [107].

Also, when comparing the two for distinguishing follicular and luteal cysts, color Doppler ultrasound is preferable than B-mode ultrasonography for choosing the proper therapy. Blood flow more reliably signals active luteal tissue than wall thickness, based on the accuracy of recognizing luteinized follicles using B-mode (61.5%) and color Doppler ultrasonography (92.3%) [108].

The effective establishment of pregnancy in cattle has been shown to be positively correlated with the preovulatory follicle's blood flow at the moment of artificial insemination (AI) [109]. Although the precise mechanism is unknown, it is possible that lower oocyte mitochondrial oxidative phosphorylation accounts for the lower conception rate in cows with lower FBF. On the other hand, accelerated in vitro cleavage of the retrieved egg and following embryo development are associated with higher preovulatory follicle blood circulation [109].

Furthermore, [110] report obtaining cumulus-oocyte complexes (COCs) of higher quality from FBF-positive follicles compared to FBF-negative follicles, which may be related to a more estrogenic environment in the follicle or a greater supply of dietary and hormonal materials to COCs in the follicular fluid. As a result, measuring FBF is a helpful technique for predicting the retrieval of high-quality COCs from cows. COCs produced from follicles containing FBF do not necessarily result in an increased chance of pregnancy because the presence of FBF does not always guarantee effective COC maturation.

The fact that ovarian vascular perfusion is greater in ovaries with a DF than in ovaries without a DF or a CL is proof that there is a link between ovarian vascular perfusion and follicle formation. The increased blood supply for the expanding CL also increases the blood supply to the DF if the DF is present in the same ovary or particularly close by. Even while evaluating FBF, luteal blood supply demands must be taken into consideration since, when the DF and the CL are present in the same ovary, one ovarian artery branch serves both structures [111].

Luteal Blood Flow

The association between LBF and P4 is stronger when the entire estrous cycle is taken into consideration [112, 113] than the connection between the cross-sectional area of the CL (luteal size [LS]) and P4. The strong relationship between LBF and P4 is explained by the fact that steroid precursors are delivered to the CL by blood flow and that P4 release is also dependent on sufficient LBF. Blood flow rises together with luteal volume, plasma P4 concentrations, and the early CL (Days 2–5) [105], which suggests active angiogenesis and sound luteal growth.

The CL experiences the highest blood flow rates per unit of tissue of any organ as it grows into one of the body's most vascularized organs [114]. As a rise in vascular flow to the bovine CL is associated with a rise in plasma P4 concentrations, LBF appears to be a valuable tool for determining early corpus luteum activity.

There are moderate to significant positive relationships between luteal volume and P4 concentrations in plasma and luteal tissue from Days 9 to 12 of the mid-luteal phase [113,115]. Decreased luteal volume is typically linked to decreased circulatory P4 concentrations [116]. As a result, the number of luteal cells appears to be a key factor in how much P4 the bovine CL produces. The magnitude of the CL is substantially connected with LBF and plasma P4, and the somewhat significant relationship between LBF and P4 during the mid-luteal phase is most likely driven by LS rather than LBF. Plasma P4 is not connected with relative LBF, which is the quotient of LBF and LS used to refute the impact of LS on LBF. Consequently, in the CL of the mid-luteal phase, it is not feasible to measure luteal P4 production using blood flow.

LBF first increases in cows with spontaneous luteolysis during the late luteal phase (17^{th} and 18^{th} Days), then decreases the next day. 5 A decrease in LBF occurs simultaneously with a decrease in P4, and two to three days later, a decrease in LS [112]. Before a noticeable reduction in LS (structural luteolysis) can be observed, LBF produces functional luteolysis, which is demonstrated by declining P4 concentrations. The function of the CL during the regression phase is better described by LBF than by LS, despite the fact that during the mid-luteal phase plasma P4 only correlates with LS and not with LBF. LBF is useful for contrast CLs that are simultaneously developing (functional) and regressing (nonfunctional) and that are the same size because of the strong relationship between LBF and P4 in the early and late luteal phases.

It is believed that LBF assessment might be used to identify early pregnancy in cattle since it decreases when the CL regresses. In a recent study, [112] cows that had undergone artificial insemination were afterwards classified as pregnant (embryo with heartbeat on the 25^{th} Day after estrus), nonpregnant (interestrus interval 15–21 days), or having clearly lost an embryo (interestrus interval >25 days). Day 15 following AI, a critical period in the progression of pregnancy in cattle, is when [117] noticed that LBF was considerably greater in pregnant cows than in nonpregnant or unbred cows, underscoring the distinct physiologic characteristics of pregnant and cyclic animals' CLs. On Day 18, luteal regression in nonbreeding, non-gestational cows results in a significant decrease in LBF, LS, and plasma P4.

A meaningful pregnancy diagnosis cannot be made during the first three weeks of pregnancy using LS evaluation by B-mode ultrasonography since the functional regression of the CL happens before the morphologic regression. Nevertheless, using color Doppler ultrasonography to assess LBF on Days 19 to 21 after insemination improves the detection of early pregnancy because LBF more precisely captures how the CL functions during the late luteal phase than LS [112]. LBF reliably decreases in non-pregnant cows approximately 19 days after ovulation, despite the fact that it is frequently observed in pregnant cows from Days 16 to 23 following ovulation [76]. While being more useful for identifying non-pregnant cows than pregnant cows, LBF measurement has not been shown to be a valid diagnostic tool for identifying early pregnancy due to its low sensitivity [118]. Nevertheless, a recent study found that using color Doppler ultrasonography to diagnose pregnancy 20 days after AI, a substantial majority of cows had high sensitivity and a low rate of false negatives. LBF on Days 19 to 21 of early pregnancy is greater in pregnant cows than in non-pregnant cows, which allows for the early detection of non-pregnant cows [77].

While the LBF of cows that miscarry is identical to that of pregnant cows up to the 13^{th} Day post-estrus (and does not rise after that), it rises later in pregnancy, likely as a result of conceptus signaling [112]. On the other hand, plasma P4 concentrations do not change until Day 18 following estrus between cows who are pregnant and those that have lost their embryos, indicating that LBF may be a more reliable indicator of embryonic loss than plasma P4. At least 3 days following the diagnosis of fetal demise, luteal regression is discovered between Days 25 and 40 [119].

Endocrine changes during the periparturient period in buffaloes

The peripartum stage is crucial in the reproductive cycle of the buffalo. In dairy cattle and buffaloes, inadequate nutrition is a key contributor to periparturient issues, metabolic disorders, reduced milk supply, and prolonged calving intervals, all of which have an impact on reproductive performance, productivity losses, and profitability. Postpartum fertility is a significant economic factor in buffalo reproduction [120]. Because reproductive success is important in defining and/or uterine environment [121,122]. Based on a comparison of the hormonal profiles provided above, an endocrine mechanism beginning parturition in the buffalo animal has been identified. Undoubtedly, a key role is played by the significant rise of corticosteroids that occurs about day 12 before delivery. The luteolytic activity of this hormone, which induces a fast drop in plasma progesterone concentrations 7 days prior to delivery, is most likely connected to its mode of action in initiating parturition [28,123]. The increased placental estradiol-17p production at day 10 before delivery was also stimulated by the heightened corticosteroid concentration at this time, which led to increased PGF 2a release a few hours later. Rising levels of placental oestradiol17p also resulted in a high estrogen/progesterone ratio, which was necessary for activating the formation of oxytocin receptors [124].

There is a noticeable increase in mammary gland activity right before parturition, which results in an energy deficit and enhanced lipomobilization from body reserves both before parturition and during the early stages of breastfeeding [125,126]. Although while homeostatic mechanisms work to keep blood parameters within normal ranges, the increased metabolic demands of pregnancy and breastfeeding cause changes in metabolites and hormones [127]. While these changes do not necessarily indicate disease, they can render pregnant animals physiologically unstable and more susceptible to certain metabolic ailments than they are at other times of life, which decreases output [128,129]. Maintaining physiological balance or the stability of the environment inside the animal is necessary for controlling homeostasis. The planned or coordinated control of body tissue metabolism necessary to preserve a physiological condition is referred to as homeostasis [130,131]. The peripartum stage of a buffalo's life is especially crucial [132] because it requires them to physically adapt to the increased energy and dietary needs for milk production. [120,133].

From day 60 prepartum (47.29 2.03 pg/ml) until the day of calving (146.79 3.55 pg/ml), the pooled mean plasma estradiol-17 (E2) concentration grew progressively and considerably in normal parturient buffalo cows [120]. The mean plasma estradiol level abruptly and considerably decreased on day 15 postpartum, according to the authors (21.07 1.39 pg/ml). On day 15 postpartum, the mean plasma estradiol level (21.07 1.39 pg/ml) decreased dramatically and abruptly. [134,135].

The elevated plasma progesterone concentrations during the first two months of pregnancy may be related to the creation of accessory luteal tissues as a result of ovulatory cycles [136,137]. This is known to occur often in cattle during this pregnant phase [138]. Progesterone is produced by the placenta, adrenal glands, and corpus luteum in cows. Its main purpose is to continue the pregnancy. Progesterone plays a role in lactation initiation and mammary gland development [139–141]. The progesterone level in Holstein cows on the day of insemination was, on average, 0.225 ng/ml, according to Lopes et al. [2007] [142]. High amounts of progesterone have been seen in cows throughout pregnancy, however Edqvist et al. [1978] [143] report that a gradual decline starts on the 60th day before to calving. A month before to calving, the blood plasma of primiparous and multiparous Holstein cows had an average progesterone content of 3.69 ng ml-1. [144,145]. Acute alterations were observed 24-48 hours before calving [143]. Higher corticosteroid and PGFM plasma concentrations remained for about 7 days postpartum, leading to fetal membrane evacuation after about 1 day and complete regression of the corpus luteum within about 10 days [133,138].

Conclusions

Color transrectal According to the results of multiple studies in buffaloes, doppler sonography is a valuable tool for the non-invasive measurement of vaginal perfusion in cows during different phases of the estrous cycle, gestation, and puerperium. It gives more details about the physiological and pathological processes that affect the uterus and ovaries and could result in a novel approach to the treatment of reproductive disorders. Up till now there may be a limitation for using the color Doppler machines for farm inspection Due to the large size of the equipment, this method could not be used for farm inspections until a few years ago. However, the costs of high-quality hand-carried, battery-powered color Doppler equipment are currently too costly for them to be profitably utilized in cow or buffalo reproduction. Due to the substantial drop in ultrasound equipment prices, particularly in the last several years, the authors are optimistic that the color Doppler approach will soon be useable even by the bovine practitioner.

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Conflict of interest

There are no competing interests. Funding statement Self-funding

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تطبيق الموجات فوق الصوتية دوبلر الملونة في تكاثر الجاموس المصري

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تستخدم الموجات فوق الصوتية الملونة على نطاق واسع في الخيول والأبقار الحلوب والمجترات الصغيرة كوسيلة آمنة وغير جراحية لتقييم الكفاءه الإنجابية. تم استخدام الموجات فوق الصوتية الملونة دوبلر لتقييم الجنين وحالات الحمل عالية الخطورة. تعتبر التغييرات التي تحدث في حيوانات المزرعة خلال فترة ما بعد الولادة حاسمة في التنبؤ بالخصوبة في المستقبل. تم نشر العديد من الأبحاث حول استخدام الموجات فوق الصوتية في الجاموس بعد الولادة. يعد استخدام الموجات فوق الصوتية دوبلر الملون طريقة غير جراحية يمكن استخدامها أثناء الحمل وبعد الولادة. يمكن استخدام الشرايين الرحمية والسرية لتقييم نشاط الدورة الدموية الرحمية والجنينية. يختلف تردد الموجات فوق الصوتية باختلاف حركة المرسل والمستقبل. كان كريستيان دوبلر أول من وصف هذه الظاهرة التي أصبحت تعرف باسم تأثير دوبلر. في الآونة الأخيرة ، انتقل استخدام الموجات فوق الصوتية دوبلر الملونة في نفس الاتجاه كما هو الحال في الأبقار والأنواع الأخرى. ولكن أيضًا الدراسات الجديدة المنشورة تصف خصائص نضح الدم المهبلي طوال فترة الحمل والنفاس المبكر في الجاموس. يجب أن تبحث الأبحاث المستقبلية في تأثير تدفق الدم المهبلي على الخصوبة في الجاموس و تقييم المخاطر المحتملة

الكلمات الدالة: دوبلر الملون ، الجاموس ، النفاس ، تدفق الدم المهبلي ، حجم تدفق الدم.