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Review Article

Color Doppler and testicular hemodynamics: Applications in assessing fertility in domestic animals

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Abstract

Doppler imaging in farm animals remains limited and is mainly used in female reproduction, with less focus on males. In males, testicular blood perfusion (TBP) is vital as it supplies oxygen, nutrients, and regulatory factors. This is especially important for the seminiferous tubules, which form most of the testicular mass and have high metabolic demands despite low oxygen levels. Color Doppler ultrasonography is a recent non-invasive tool that enables assessment of testicular blood flow in different species. Over the last two decades, numerous studies have demonstrated its value in evaluating TBP for andrological assessment, infertility diagnosis, and monitoring in farm animals. TBP is now considered a promising marker of male fertility. However, Doppler measurements can be influenced by factors such as breed, season, laterality, and the site of measurement, which must be considered for accurate diagnosis. Research has also explored strategies to improve TBP, aiming to enhance testicular function and fertility through therapeutic interventions. This review will give insights about Doppler ultrasonography, its applications, limitations and future prospects.

Keywords: Blood flow, Color Doppler ultrasonography, Doppler indices, Fertility, Testicular blood perfusion

Highlights

- Testicular blood flow affects semen quality and is a vital fertility marker.
- Color Doppler is a non-invasive tool for testicular hemodynamics and disorders.
- Wider use is promising but limited by cost, expertise, and lack of standardization.

INTRODUCTION

Assessing the fertility of male animals is crucial for understanding their reproductive and productive capabilities. Various methods have been employed to evaluate the reproductive potential of male animals, as it holds significant importance in gauging their overall fertility performance. Conventional techniques such as measuring scrotal circumference, assessing semen quality, and conducting endocrine assays are widely employed to identify testicular dysfunction in different animal species. In the context of animal reproduction, especially among ruminants, a selected few males demonstrating superior reproductive efficiency are chosen to mate with numerous females within the same breeding system. (Barth & Waldner, 2002; Camela et al., 2019). Hence, it is crucial to thoroughly evaluate the reproductive efficiency of the male. Various methods exist for assessing the breeding potential of male farm and companion animals.

Breeding Soundness Evaluation (BSE) is a vital and widespread practice in domestic animals aimed at forecasting the reproductive capabilities of males. It allows for the identification and removal of infertile males from the breeding program, while favouring the selection of top-performing individuals as sires (Singla et al., 2017; Tibary et al., 2018). Current guidelines for Breeding Soundness Evaluation (BSE) primarily focus on factors like reproductive history, overall health, physical examination, scrotal circumference, morphology, motility of sperm (Eilts et al., 1988; Griffin, 2000; Pinho et al., 2018) and rarely endocrine analysis of hormones like FSH and LH in the case of stallions (Douglas & Umphenour, 1992). Therefore, assessing semen quality stands out as a crucial aspect in the process of selecting males for breeding purposes. Physical examinations like palpation of the testis have been ineffective in accurately evaluating testicular parenchyma

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(Tibary et al., 2018). The importance of testicular blood flow dynamics is closely linked to the counter-current heat exchange mechanism within the pampiniform plexus, which facilitates efficient cooling of arterial blood before it reaches the testes, thereby maintaining optimal temperature for spermatogenesis and making blood flow velocity and vascular resistance critical parameters. More commonly, B-Mode ultrasonographic assessment of reproductive organs is regularly employed due to its safety and non-invasiveness. This technique is valuable in identifying a healthy testicular parenchyma and serves as a crucial tool for evaluating testicular biometric parameters, which have been linked to fertility potential (Eilts et al., 1988; Boukhliq et al., 2018; Pinho et al., 2018). In the Indian context, the application of Color Doppler ultrasonography offers considerable potential for advancing reproductive management in livestock species. The selection of breeding bulls for artificial insemination programs relies heavily on conventional semen evaluation, which may not fully reflect underlying testicular function. Incorporation of Doppler-derived indices such as resistive index (RI) and pulsatility index (PI) enables objective assessment of testicular perfusion, providing functional insights into spermatogenic activity and endocrine status. This approach is particularly relevant under tropical and subtropical conditions prevalent in India, where thermal stress and environmental fluctuations can impair testicular hemodynamics.

Understanding the testicular arterial network

The testicular artery originates from the aorta, courses along the inguinal canal, and creates multiple irregular loops referred to as supra-testicular arteries (STAs). The extensive coiling of the vessels facilitates the dissipation of heat, leading to a decrease in the operational temperature of the testis (Kastelic et al., 1997). Bulls exhibit a notably high degree of coiling in their testicular artery, with approximately 60 to 130 loops, while rams and goats (80 and 50 loops), other animal species typically have fewer than 25 loops. Horses show a comparatively lower degree of coiling (Setchell, 1978) and in humans, the testicular artery lacks coiling, remaining straight (Kormano & Reijonen, 1976). Differences among species in the extent of convolution and the diameter of the testicular artery, along with its association with the veins of the pampiniform plexus, may be influenced, at least in part, by variations in abdominal-testicular temperature gradients across different species (Harrison, 2009). In ruminants, upon exiting the pampiniform plexus, the testicular artery divides into two branches: one leading to the testis and the other to the epididymis (Gouletsou, 2017). The testicular branch, referred to as the marginal portion or marginal testicular artery (MTA), travels along the posterior edge of the testis in ruminants. The MTA is notably lengthy in relation to the size and position of the testis in these animals. It exhibits a degree of tortuosity, particularly in rams and bucks, when compared to bulls (Samir et al., 2023). At the ventral pole of the testis, the testicular artery divides into lateral and medial branches. Subsequently, these lateral and medial branches further separate into dorsomedial and dorsolateral directions, giving rise to the structure known as the "tunica arteriosatestis", from the tunica arteriosa, parenchymal branches originate, traversing through the testicular parenchyma and extending towards the mediastinum (Elayat et al., 2014). At the mediastinum, they turn back parallel to centripetal branches as centrifugal branches (Polguj et al., 2015). In equines, the testicular artery takes a wavy course adjacent to the epididymal border, known as the marginal segment of the testicular artery. It runs in proximity to the caudal pole of the testis and along the unattached edge, either as a single branch or as two or three branches (Pozor, 2007). In dogs, the coiled segment, referred to as the STA, is situated toward the cranial aspect of the testis. Simultaneously, the MTA follows a linear path extending to the caudal pole of the testis, emitting numerous branches on both sides. These branches, in turn, give rise to terminal vessels that penetrate the testicular parenchyma (Harrison & Weiner, 1949).

Importance of testicular blood perfusion (TBP)

The adequate functioning of the testis relies on TBP, serving as the primary route for the regulated exchange of essential nutrients, oxygen, and hormones. Furthermore, TBP plays a pivotal role in maintaining the optimal testicular temperature (Junior et al., 2018). The high vascular resistance characteristic of blood flow to the testis results in a low intratesticular capillary pressure, creating a semi-hypoxic environment that is crucial for spermatogenesis, safeguarding sperm cells from potential damage caused by oxygen radicals (Ortiz-Rodriguez et al., 2017). Nevertheless, the susceptibility of the testes to ischemic damage becomes evident in the presence of vascular occlusions, leading to oxidative stress. This ischemic condition can lead to an increase in damaged spermatozoa, rendering them more prone to apoptosis (Bergh et al., 2001). Hence, the timely detection of alterations in TBP is imperative for the early and accurate diagnosis of various testicular

pathologies. The assessment of TBP focuses on the testicular artery, particularly the supratesticular artery (STA), which exhibits high vascular resistance, leading to a lower intratesticular capillary pressure compared to other organs (Bergh & Damber, 1993). Under acute hypoxic conditions, Spermatogenesis has adapted to thrive, affording sperm cells a protective advantage against damage from oxygen-free radicals (Max, 1992; Aitken, 1999; Setchell et al., 2006). Despite the benefits of low oxygen tension for spermatogenesis, the testis becomes susceptible to ischemic damage when blood perfusion diminishes due to vascular restrictions (Kay et al., 1992).

What is Doppler ultrasonography (USG)?

Doppler ultrasonography is now the preferred technique for assessing the blood supply to different organs. This technology has proven invaluable to researchers and veterinarians, enabling the exploration of both the anatomical and physiological foundations of testicular functions (Ginther, 2007). It is one of the easiest and accurate method for estimating blood flow, as it integrates information about anatomy and dynamic flow characteristics (Strina et al., 2016). It works on the principle of Doppler Effect, the Doppler Effect was first given by Austrian physicist Christian Johann Doppler in 1842. When ultrasound interacts with stationary surfaces lacking movement, the reflected echoes share the same frequency as the emitted waves. In contrast, when these waves encounter mobile structures like blood cells, the frequency of the reflected waves deviates from that of the emitted waves, resulting in the recognizable Doppler Effect (Bollwein et al., 2016). This can be positive when the frequency of the returning signal is higher than the transmitted frequency, indicating blood flow toward the transducer, as well as negative, when the returning frequency is lower than the transmitted frequency, indicating movement of red blood cells away from the transducer (Zagzebski, 2005). Ther are two types of Doppler (Rubio et al., 2014), the first one being Continuous, which does not discriminate depth of field, and the second being Pulsed in which one can choose the depth of the field to study. Doppler signal can be represented by three distinct modes – first one being Color Doppler - It provides information about the existence and direction of blood flow, capturing frequency variations through red and blue (Rubio et al., 2014; Bollwein et al., 2016; Oglat et al., 2018), second one being Spectral Doppler - Examines the velocity of blood movement within a vessel (Rubio et al., 2014; Bollwein et al., 2016; Oglat et al., 2018) and the last one being Power Doppler - It gauges the quantity of cells traversing a vessel within a specific timeframe. Pulsed-wave Doppler is applied in regions characterized by sluggish blood flow (Markey et al., 1995; Barbosa et al., 2014).

Assessment method of Color Doppler ultrasonography (CDU)

Appropriate restraint of animals during examination is very important during examination. Large animals are typically assessed while standing and may require restraint using a trevis. The use of tranquilization can be considered to minimize the risk of injuries from animal to operator (Samir et al., 2021). An adequate dosage of xylazine is sufficient, as it does not cause significant alterations in testicular hemodynamics in stallions (Pozor et al., 2004). Small ruminants and other small animals can be examined in a standing position or recumbent (Samir et al., 2021). Palpation should be carried out firstly and cleaning of the site may be done, especially in small ruminants, and an appropriate quantity of gel may be useful during examination. Evaluating testicular hemodynamics in the STA poses greater challenges compared to the MTA (Samir et al., 2015). Veins encircle the STA, creating a vascular cone. Hence, prior to evaluating Doppler parameters of the artery, it is essential to distinctly differentiate between the artery (having regular peaks on the spectral image) and veins (Velasco & Ruiz, 2020). Initially, the vascular structures are identified, and the largest longitudinal section of the testicular artery is observed using grayscale B-mode ultrasonography. Subsequently, the Doppler assessment of TBP is conducted by activating the spectral wave mode, and Doppler velocimetric parameters are measured (Samir et al., 2015, 2018). To mitigate the potential influence of diurnal rhythm on testicular hemodynamics, it is advisable to conduct examinations at a consistent time each day. Additionally, standardizing ultrasonic settings and ensuring an angle of insonation within the range of 40°-60° are crucial considerations (Pozor & McDonnell 2004; Samir et al., 2021).

Doppler parameters: Blood flow in the testicular vessels can be assessed by Doppler indices such as Resistive Index(PSV- EDV/PSV); Resistance to the flow of blood in a pulsatile vascular system and Pulsatility Index (PSV- EDV/TAMV); Index used to assess the resistance in a pulsatile vascular system and Doppler velocities PSV Peak systolic velocity (The highest velocity of blood flow during systole), EDV (Lowest velocity of blood flow during diastole) and TAMV

Table 1. Mean RI and PI values for testicular haemodynamics in different domestic animals

Species	Vessel/Segment	Mean RI	Mean PI	References
Dogs	Supratesticular artery	0.51±0.06	0.82±0.23	Gloria et al. (2020)
Dogs	Marginal artery	0.44±0.04	0.61±0.07	Gloria et al. (2020)
Dogs	Intratesticular artery	0.32±0.06	0.43±0.10	Gloria et al. (2020)
Bulls	Supratesticular artery	0.39±0.20	0.45±0.16	Fávaro et al. (2020)
Bulls	Supratesticular artery	0.41±0.01	0.33±0.01	Junior et al. (2018)
Rams	Supratesticular artery	0.64±0.04	1.3±0.2	Camela et al. (2019)
Rams	Supratesticular artery	0.45±0.02	0.64±0.05	Hedia et al. (2020)
Goats	Supratesticular artery	0.45±0.03	0.63±0.05	Samir et al. (2020)
Goats	Supratesticular artery	0.49±0.01	0.69±0.02	Samir et al. (2018)
Stallion	Supratesticular artery	0.80±0.05	2.28±0.45	Ortiz-Rodriguez et al. (2017)

(The average velocity of blood flow over time) (Viana et al., 2013, Bollwein et al., 2016). RI and PI from Doppler ultrasound quantify vascular resistance in arteries like the testicular, renal, or supratesticular. Values vary by species, vessel, health status, and laterality, with lower RI/PI indicating better flow (e.g., fertility-linked in testes). Table 1 shows the compiled mean/upper limit values from studies on healthy domestic animals. Elevated values of Resistive Index (RI) and Pulsatility Index (PI) indicate increased vascular resistance in blood flow, leading to decreased blood perfusion and vice versa (Bollwein et al., 2016). Doppler indices, specifically Resistive Index (RI) and Pulsatility Index (PI), serve as more accurate indicators of TBP (Pozor et al., 2004). Large vessels tend to show greater pulsatility in blood flow compared to smaller vessels (Hassan et al., 2022). Among these indices, RI stands out as the most precise predictive parameter for identifying abnormal waveforms (Rifkin et al., 1987). Increased values of Doppler indices within the blood flow of testicular arteries are commonly associated with ischemic or degenerative processes occurring in the testes (Jee et al., 1997).

Interrelationships between Doppler ultrasonography, fertility and spermatogenesis

Male fertility has considerable importance in animal husbandry. The conventional semen analysis tests have limited effectiveness as it is based on the underlying assumption that an ejaculate must contain a specific quantity of motile, morphologically normal spermatozoa to fulfil the minimum criteria for sperm reaching the oviduct and achieving successful oocyte fertilization (Rodríguez-Martinez, 2013). Since these tests are subjective, objective evaluations using CASA are performed (Michos et al., 2017). In humans, Pinggera et al. (2008) proposed that males with an RI value greater than 0.6 could be indicative of a pathological sperm count. In another study in varicocele patients, a correlation was observed wherein there was an inverse relationship between the progressive motility of spermatozoa and the RI of the intraparenchymal arterial blood flow (Rehman et al., 2019). In animal stallions with epididymoorchitis show reduced RI values in the STA owing to hyperemia, conversely, in ageing males experiencing degenerative changes, RI values of the STA were found to be elevated due to increased resistance in the testicular tissue (Pozor & McDonnell, 2004). Stallions with chronic non-strangulated inguinal hernia were documented to have elevated values of Doppler parameters, particularly the RI of the MTA (Ortega-Ferrusola et al., 2014). CDU is a reliable and non-invasive method for measuring prostatic blood flow, offering valuable insights for the clinical assessment of Benign Prostatic Hyperplasia (BPH) and monitoring the impact of hormonal interference treatment, BPH is characterized by higher Peak Systolic Velocity (PSV) and End-Diastolic Velocity (EDV) values compared to those in individuals with normal prostates (Polisca et al., 2013). It can also be employed for the diagnosis of various tumours in dog, like Sertoli cell tumour, seminoma, in which there is a rise in the TBP (Bigliardi et al., 2019). Zelli et al. (2013) showed a negative correlation between PI and RI with both the overall progressive motility and the percentage of membrane-intact sperm. In bulls, a correlation was observed between the RI of MTA and various sperm parameters, including the total sperm count in the ejaculate, immature sperm, and teratoid sperms (Gloria et al., 2018). Batissaco et al. (2014), showed that in rams, there was no identified correlation between sperm motility and hemodynamic characteristics, however, an association was observed between the percentage of total sperm defects and the RI and PI. Currently, the reason for the positive correlation between RI and sperm count is not yet explainable, but since testicular arteries are targets for androgens,

and in infertile males these arteries are narrow, so this anatomical pattern may be used as markers for spermatogenesis dysfunction (Pinggera et al., 2008).

Associations between TBP and the seminal attributes

Several studies show an association between TBP and seminal attributes. In humans, RI is recognized as the earliest indicator for detecting different reproductive disorders in men. For instance, patients with oligospermia exhibited a notable increase in RI values, reaching up to 0.7, while normospermia men demonstrated a decrease to levels below 0.5 (Pinggera et al., 2008), another study shows that the testicular volume, closely linked to semen parameters, may serve as a clinical predictive factor for semen quality in males (Le et al., 2020). In Bovines, Gloria et al. (2018) discovered that assessing the RI at the MTA could serve as a readily applicable parameter for evaluating the quality of spermatogenesis in both young bulls and normal adults. In rams, a rise in Ec2 (white pixels) and Ec3 (grey pixels) may suggest a decline in seminal quality, while tubular density and lumen area could serve as indicators or predictors of favourable seminal quality (Carvajal-Serna et al., 2022). Ultrasonography can also be used as a predictive tool for assessing changes in the sperm quality of donor rams exposed to varying frequencies of semen collection in reproduction centres (Montes-Garrido et al., 2022). For Dorper rams, evaluating echo textural features of the epididymis and accessory sex glands, along with testicular morphometry using B-mode ultrasonography and considering serum testosterone concentrations, offers more valuable insights into the advancement of sexual maturation and the current semen quality compared to relying on the estimation of blood flow indices (Camela et al., 2019). For stallions, infertile stallions demonstrated elevated values of RI and PI at the level of MTA (Ortiz-Rodriguez et al., 2017). RI and PI values measured at the STA exhibited positive correlations with both the total number of dead sperms and those lacking mitochondrial activity, however no significant differences in blood flow parameters were reported between fertile and sub-fertile stallions at this location (Pozor et al., 2004). For bucks, Samir et al. (2020) reported decreases in Doppler indices of blood perfusion within the STA and MTA, occurring simultaneously with improvements in semen quality parameters following the administration of melatonin. In the context of sperm abnormalities, Gloria et al. (2020) stated that in testes exhibiting elevated levels of primary sperm abnormalities, a higher pulsatility index was observed in comparison to testes with normal spermatogenesis, specifically in the supratesticular artery, marginal artery, and intratesticular artery. Likewise, the resistive index was found to be significantly higher in the same vessel tracts in testes with abnormal spermatogenesis compared to those with normal spermatogenesis. Similarly, in a case study involving a Beagle dog with normal libido and semen volume but experiencing asthenozoospermia, oligozoospermia, and teratozoospermia in the ejaculates, higher values of RI and PI were observed in the MTA and intratesticular vessels of the affected testis compared to the healthy one (Luño et al., 2020).

Various factors affecting TBP

Effect of thermal factor on TBP: In many mammals, the positioning of the testes within the scrotum, external to the body, serves the purpose of regulating the intratesticular temperature to be slightly lower than the core body temperature. The testis employs a complex thermoregulatory system, consisting of both vascular and non-vascular components (Samir et al., 2023). Vascular elements include the pampiniform plexus, facilitating counter-current heat exchange (Hansen, 2009), and non-vascular include sweating, location of scrotum (Rizzotov & Kastelic, 2020). Elevated temperatures, have the potential to disrupt the thermoregulatory mechanism of the testes that may lead to a disruption of heat loss from the scrotal surface, ultimately causing an elevation in intratesticular temperature (Morrell, 2020). An elevation in testicular heat levels leads to an increase in testicular metabolism and oxygen requirements. This, in turn, induces hypoxia and the generation of reactive oxygen species, which significantly influence sperm production (Kastelic et al., 2017). In bulls with a higher temperature gradient, also demonstrated better testicular blood flow and improved semen attributes, indicating that efficient thermoregulation and perfusion support reproductive function under thermal stress (Lakhanpal et al., 2026), also subjecting the testes to experimental warming, reaching a temperature of 40 °C, led to a pronounced and significant increase in TBF (Rizzoto et al., 2020). Similarly, in rams, direct application of heat to the testis induced a notable increase in TBF, reaching up to 26% (Mieusset et al., 1992). When the testis was artificially cooled, it resulted in significant reductions in scrotal blood flow, without any discernible effect on TBF (Glode et al., 1984). Thermal stress induces an elevation in cortisol levels within the animal's body, disrupting spermatogenesis through endocrine mechanisms,

particularly in the release of luteinizing hormone (LH) and testosterone (Prabhakar et al., 1990).

Effect of season on TBP: The majority of farm animals exhibit noticeable seasonal reproductive patterns, leading to variations in ovulation frequency, spermatogenic activity, gamete quality, and sexual behaviour within most species (Chemineau et al., 2007). Seasonal breeders typically experience a rise in ejaculate volume during the breeding season, compared to the non-breeding season. The peak TBP was observed in the breeding season, spanning from September to March, and notably decreased from April to June. Simultaneously, there was a positive impact on testosterone, oestradiol, and semen parameters during the breeding season (Hedia et al., 2019). In a recent study, effective testicular thermoregulation and blood perfusion at the vascular cone emerge as critical markers for assessing and improving semen quality under summer stress in bulls (Lakhanpal et al., 2025). In bucks, a decline in the mean values of the RI of the testicular artery was observed during the summer months, with the highest RI value being detected in November (Strina et al., 2016). The possible reason for this change in blood flow might be photoperiod, which is considered the principal factor influencing reproduction in small ruminants. For stallions, Boyd et al. (2006) showed that testicular perfusion experienced an elevation during the breeding season, and this increase was found to be correlated with the rise in testosterone concentrations, and this might be due to an increase in the diameter of the testicular artery and an increase in the blood flow mean velocity.

Effect of age, body weight and breed on TBP: Various studies show an increase in TBP with age, in a recent study by Hedia and El-Shalofy. (2022), lower values of RI and PI were reported for STA in mature rams (2.5 years old) and aged rams (7.5 years old) compared to younger counterparts (1 year old). In heavy horses, a greater blood perfusion, as indicated by lower Doppler indices values, was observed in comparison to small horses (Ortega-Ferrusola et al., 2014). There have been few studies conducted on breed variations in TBP (Junior et al., 2018; Rodrigues et al., 2020), in a study by Rodrigues et al. (2020), it was reported that there were significantly higher values of PI and RI observed in the testicular arteries of Nelore breed bulls in comparison to Caracubulls. This could be due to the presence of more sweat glands in the scrotal skin and a higher testicular artery length.

Effect of location of evaluation and testicular side on TBP: Since the results of TBP evaluation are affected by the location of evaluation, one of the most important aspects is to identify an appropriate location. In bulls, the PSV, EDV, and RI values of the STA were significantly higher than those in the MTA and were also higher in the MTA as compared with those in the intratesticular branches (Gloria et al., 2018). The testicular artery originating from the aorta, a vessel known for its high resistance to blood flow, accounts for the high velocities observed at the Supratesticular Artery (STA) in comparison to other segments near the entry of the testis and the intratesticular branches. Meanwhile, in stallions, the values of the mean RI in the marginal aspect of the artery were only slightly lower than those in the convoluted aspect (Gacem et al., 2020). These variations are thought to be influenced by the positioning of the testes along their long axis, characterized by a horizontal orientation in horses. In dogs, the RI and PI values decreased significantly from proximal supratesticular artery to intratesticular artery (Trautwein et al., 2019), it might be due to the high vascular dichotomization as a result of the artery branching as it enters the testicular parenchyma. Variations in testicular volumes between the left and right testes have been documented. Hedia et al. (2020) stated that RI and PI were slightly increased in the right testicular artery when compared with the left one. The variations in RI and PI values could be associated with the asymmetry in volume or orientation between the right and left testes.

Effect of various agents on TBP: Elevated testicular blood perfusion (TBP) is closely linked to improved male fertility, primarily through enhanced delivery of oxygen, nutrients, and hormonal signals that support spermatogenesis. A range of pharmacological and nutritional interventions have demonstrated the ability to modulate TBP, particularly under stress conditions and during non-breeding seasons. Antioxidant-based approaches show consistent benefits. L-carnitine improves testicular hemodynamics in heat-stressed rams, likely by reducing oxidative stress and supporting mitochondrial function (El-Sherbiny et al., 2022a). Complementary evidence indicates improved sperm motility, mitochondrial activity, and fertility at optimal doses, along with enhanced spermatogenesis following toxic insult (Kooshesh et al., 2023; Masoudi et al., 2026). Melatonin similarly enhances TBP and semen quality, with increases in Doppler velocities and intratesticular vascularization reflecting improved perfusion (Samir et al., 2020; Salama et al., 2022). Its

combined antioxidant and endocrine actions make it particularly useful during seasonal reproductive decline. Nutritional interventions further support TBP regulation. Curcumin supplementation in Baladi bucks during the non-breeding season improved TBP, testosterone, nitric oxide (NO) levels, and semen quality (El-Sherbiny et al., 2022b), suggesting a vasodilatory mechanism mediated by NO. Likewise, vitamin D_3 has been shown to restore testicular structure, reduce apoptosis and fibrosis, and enhance protective receptor expression under pathological conditions (Liu et al., 2018). Hormonal modulation also influences TBP. In buffalo bulls, human chorionic gonadotropin (hCG) increased supratesticular artery vascularization and improved endocrine profiles, supporting steroidogenesis and overall testicular function (Abdelnaby, 2022). Overall, TBP is highly responsive to antioxidant, nutritional, and hormonal interventions. While Doppler-based findings consistently indicate improved perfusion, further integration with molecular and fertility outcomes is needed to confirm long-term reproductive benefits.

Limitations of Color Doppler USG

The application of Color Doppler ultrasonography in andrological evaluation is constrained by several practical and technical factors. Limited availability under field conditions, high equipment cost, and the need for trained personnel remain major challenges to its widespread use (Viana et al., 2013). From a methodological perspective, Doppler assessment is more reliable in large, straight vessels, whereas evaluation of testicular blood flow is complicated by the convoluted course of the supratesticular artery and its close association with the pampiniform plexus. This anatomical complexity makes differentiation between arterial and venous signals difficult during examination (Velasco & Ruiz, 2020). Accuracy of measurements is further influenced by animal-related factors. Movement during scanning can disrupt probe positioning and affect signal acquisition. In addition, stress and handling conditions may alter testicular hemodynamics, leading to variability in Doppler indices. Moreover, Doppler parameters are highly operator-dependent and influenced by instrument settings such as angle of insonation, gain, and pulse repetition frequency. Variations in these factors, along with physiological influences such as breed, season, and testicular size, can contribute to inconsistencies in interpretation.

Advanced imaging for vascular flow detection

Doppler techniques such as Color Doppler Imaging (CDI), Spectral Doppler Imaging (SDI), and Power Doppler Imaging (PDI) are widely utilized in routine clinical practice for characterizing tumor and organ vascularity, as well as confirming vascular patency. These tools offer effective quantitative and qualitative assessments of the vascular bed. However, their utility is constrained when it comes to evaluating micro vessels and vascular beds with slow flow. The newer platforms include (Revzin et al., 2019) firstly the B flow imaging**:** This technique, observed in real-time grayscale ultrasound, showcases the movement of intravascular echoes without relying on Doppler methods. It enables the visualization of blood flow by selectively amplifying signals from mobile blood cells. Additionally, it is accessible in a B-flow color mode, enhancing the representation of blood flow dynamics**.** Secondly Microflowimaging: This method allows the visualization of microvasculature and detects signals from slow-moving blood flow. It exhibits heightened sensitivity to subtle or weak blood flow within tissues**,** third is the Superb microvascular imaging (SMI): It allows to observe blood flow in low-speed and narrow-diameter blood vessels. SMI (Superb Microvascular Imaging) has the capability to eliminate motion artifacts without filtering out the faint signals originating from small vessels, thereby achieving heightened sensitivity. However, it cannot be utilized to determine the direction of the flow, and lastly Contrast-enhanced US: it involves injecting an intravenous contrast agent composed of microbubbles of gas enhances the scattering of ultrasound waves, resulting in increased vascular contrast. This mechanism is comparable to the use of intravenous contrast media in CT and MRI imaging. Recently, Contrast-Enhanced Ultrasound (CEUS) has been employed to analyse the pattern of blood flow in canine testicles following chemical sterilization through the injection of calcium chloride (CaCl2) in alcohol. The results indicate a discernible alteration in vascularization compared to the blood flow pattern in normal testes (Cicirelli et al., 2022).

Conclusion

Enhancing reproductive performance in male animals is of paramount importance for animal husbandry, which highlights the crucial role of TBF assessment in male health management. With technological advancements, Doppler Ultrasound has become a key tool for detecting and characterizing blood flow in clinical practice. However, it underscores the importance of experienced and knowledgeable operators for the effective use of this technique in diagnosing and monitoring vascular conditions. A comprehensive understanding of Doppler modes,

image optimization, and artifact recognition is crucial. Further this technique can be utilized for breeding soundness examination and also for monitoring therapeutic outcomes after treatment.

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