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# Testicular Blood Flow: A Review of Hemodynamics, Thermoregulation and Clinical Applications in Domestic Animals

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## *ABSTRACT*

In species propagation, males play a crucial role, making testicular hemodynamic—referring to blood flow within the testicles—an essential parameter for evaluating fertility. It's important for testicular function, including sperm production and steroid production for behavioural response of the males. This article reviews testicular hemodynamic, along with the various tools to study it and to improve testicular blood flow with their clinical significance. The highly convoluted testicular artery, particularly prominent in farm animals like bulls and rams, facilitates heat dissipation, crucial for maintaining the lower body-temperature crucial for spermatogenesis. Testicular blood perfusion (TBP) is vital for nutrient and hormone exchange and thermoregulation, achieved through vascular mechanisms (pampiniform plexus, superficial vessels) and non-vascular mechanisms (sweating, cremaster muscle, tunica dartos muscle). Colour Doppler ultrasonography (CDU) is a valuable non-invasive tool for assessing TBP, measuring parameters like peak systolic velocity (PSV), resistive index (RI), and plasticity index (PI). Several factors viz., environmental (temperature, season), physiological (species, breed, age, sexual activity, disease), and technical (Doppler technique, transducer type, operator experience) factors influence TBF. The article explores methods to improve TBP in farm animals, such as GnRH/hCG administration, passive inhibin immunization, and melatonin or selenium-enriched probiotic supplementation.
***Keywords*:** Farm Animals, Heat Stress, Spermatogenesis, Testicular Artery, Testicular Hemodynamic, Thermoregulation

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## INTRODUCTION

Maintaining optimal testicular function is paramount for male fertility, a process intricately linked to efficient testicular hemodynamic. This article delves into the complex interplay of factors governing testicular blood flow (TBF), a process crucial for nutrient and hormone delivery, waste removal, and, critically, thermoregulation. The unique anatomical features of the testicular artery, particularly its extensive coiling in many species, play a significant role in

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heat exchange, highlighting the delicate balance between heat dissipation and the maintenance of a slightly hypoxic environment necessary for spermatogenesis (Ortiz-Rodriguez *et al*., 2017). However, this convoluted vascular architecture also contributes to increased vascular resistance, making the testes susceptible to ischemic damage under conditions of reduced blood flow (Trautwein *et al*., 2019).

Color Doppler ultrasonography (CDU) has emerged as a valuable non-invasive tool for assessing TBF, providing insights into testicular health through the measurement of parameters such as peak systolic velocity (PSV), resistive index (RI), and plasticity index (PI). However, the interpretation of CDU results requires careful consideration of numerous environmental, physiological, and technical factors that can influence TBF measurements. This review aims to examine the current understanding of testicular hemodynamic, exploring the key factors influencing TBF, and discussing potential strategies for improving TBF in farm animals.

## Testicular artery, blood flow to the testis and its importance

The abdominal aorta has a branch called the testicular artery. The funicular portion of the testicular artery, also known as the supraventricular artery (STA), is a funnel-shaped structure that goes down the inguinal canal and produces several irregular loops. These remarkable convolutions are crucial for heat dissipation, which lowers the testis' operating temperature (Srivastava *et al.,* 2024). All domestic animals have testicular artery coiling, albeit the degree of coiling varies from species to species. Stallions and camels have smaller coiling, whereas bulls have the most (up to 130 loops), followed by rams (80 loops), bucks (50 loops), and bucks. Interestingly, humans do not have this coiling (Gouletsou, 2017). Due to the coiled structure of testicular artery, it is very long. Among farm animals, rams have the longest testicular artery, reaching up to 400 cm (Table 1) (Elayat *et al*., 2014).

**Table 1:** Morphometry of the testicular artery in domestic animals

| Species | Length of testicular artery (cm) | Loops/coils |
|---|---|---|
| Bull | 220 | 130 |
| Buffalo | 145-215 | - |
| Ram | 180-225 (up to 400 cm) | 80 |
| Buck | - | 50 |
| Stallion | 137-170 | <25 |

In the convoluted section of the testicular artery (STA), increased resistance leads to a reduction in intratesticular capillary pressure. This increase in resistance leads to a decrease in TBF (Trautwein *et al*., 2019). The reduced pressure creates a semi-hypoxic microenvironment within the seminiferous tubules (Ortiz-Rodriguez *et al*., 2017). When blood perfusion drops, the testis is vulnerable to ischemic injury because of vascular constriction (Samir *et al*., 2023). Although the low oxygen tension within the seminiferous tubules supports spermatogenesis, as it helps reduce damage caused by reactive oxygen species (Perumal *et al*., 2017; Bisla *et al*., 2021). Testicular blood perfusion is essential for testicular function, as it facilitates the exchange of nutrients, oxygen, hormones, and secretory products to and from the testes. Additionally, TBP plays a crucial role in maintaining testicular temperature.

## Thermoregulation of testis

The intricate process of testicular thermoregulation is essential for preserving ideal spermatogenesis, which requires a temperature that is marginally lower than body temperature. This is achieved through a combination of vascular and non-vascular mechanisms.

**Vascular Mechanisms:** The pampiniform plexus, a network of veins surrounding the testicular artery, plays a key role in counter current heat exchange. Warm arterial blood entering the testis loses heat to the cooler venous blood leaving the testis, thus cooling the arterial blood before it reaches the testicular tissue (Hansen, 2009). The tortuous nature of the testicular artery further enhances this heat exchange (Waites, 1973). Additionally, the extensive network of superficial testicular vessels contributes to heat dissipation.

**Non-Vascular Mechanisms:** In addition to behavioural adaptations like seeking shade, these processes involve physiological reactions like perspiration and changes in the location of the testicles in relation to the abdomen (Rizzoto and Kastelic, 2020). The cremaster muscle governs the scrotum›s posture in relation to the body, whereas the tunica dartos muscle, which is situated under the scrotal skin, controls the scrotal surface area (Hansen, 2009). The cremaster muscle contracts in cold weather to bring the testes closer to the body for warmth, and it relaxes in warm weather to allow the testes to hang away from the body for cooling (Morrell, 2020). Sweating is another way through which the thin, fat-free scrotal skin with its many sweat glands dissipates heat. The rugosity of the scrotal skin reduces surface area exposure to limit heat loss, while the testes can thermoregulate by contracting and shifting closer to the body in cold weather (Mariotti *et al*., 2011).

**Impact of Increased Temperature:** Maintaining the intratesticular temperature around 32°C is essential for normal spermatogenesis. Elevated environmental temperatures, regardless of humidity levels, can disrupt thermoregulatory mechanisms, impairing evaporative heat loss and resulting in elevated intratesticular temperature (Morrell, 2020). The elevated temperature leads to heightened testicular metabolism and oxygen requirements, causing hypoxia and the generation of reactive oxygen species, which adversely affect sperm production (Kastelic *et al*., 2017). The resulting decreased sperm quality is primarily due to hypoxia rather than hyperthermia itself. The severity and duration of the temperature increase determines the impact; mild increases may cause temporary reductions in sperm quality, while prolonged or substantial heating can lead to infertility or permanent cessation of spermatogenesis. The temperature humidity index (THI) must be taken into account when evaluating the effects of heat stress, in conjunction with seasonal influences (Llamas-Luceño *et al*., 2020). There have been documented breed-dependent reactions to THI, most likely as a result of variations in anatomy, endocrinology, or heat tolerance (Gloria *et al.,* 2021). Following thermal stress, sperm parameters typically recover in six to eight weeks; however, this recovery time is delayed by sustained or significant rises in testicular temperature. A useful technique for determining how high ambient temperatures affect testicular function is Color Doppler ultrasonography, which measures testicular hemodynamics.

## Species Wise Variation of Thermoregulation Under Varying Environmental Conditions

Studies using Color Doppler ultrasonography to assess TBF across various species reveal significant interspecies variations in response to environmental conditions. Seasonal changes in TBF are evident in some species like rams (Hedia *et al*., 2019) and bucks (Strina *et al*., 2016), with peak perfusion correlating with breeding seasons and testosterone levels. However, other species, such as dogs (Carrillo *et al*., 2012), show less pronounced seasonal effects on TBF. Furthermore, responses to thermal stress *vis-a-vis* TBF vary; while some species (bull) exhibit increased values with rising temperatures (Adwell *et al*., 2018; Rizzoto and Kastelic, 2020), others (Shiba buck) showed decreased TBF (Samir *et al*., 2018) or no significant change as in dogs (Henning *et al*., 2014). These differences emphasize the need for species-specific CDU reference ranges and reflect the interplay of physiological adaptations and environmental effects on testicular hemodynamics.

# FACTORS AFFECTING TESTICULAR BLOOD FLOW

Several factors influence testicular blood flow. These factors can be broadly categorized as environmental, physiological, and technical (Table 2). Understanding these influences is crucial for accurate interpretation of TBF measurements and proper clinical diagnosis.

## I. Environmental Factors:

Though body maintains homeostasis season and environmental temperature affects testicular blood flow in animals. Hence, variations attributable to such factors must be accounted for before final interpretation of TBF analysis.

**a)Temperature**: This is arguably the most significant environmental factor affecting TBF. The testis requires a temperature slightly lower than core body temperature for optimal spermatogenesis. Increased ambient temperature initially leads to increased TBF as a thermoregulatory mechanism to dissipate heat. However, prolonged exposure to high temperatures can cause decreased TBF and impaired spermatogenesis, leading to subminimal fertility. Studies have shown that short-term scrotal hyperthermia do not impact seminal parameters, TBF, and gonadal tissues in dogs (Henning *et al*., 2014). On the other hand, in dogs, artificial chilling of the testes can decrease scrotal blood flow without influencing TBF (Samir *et al*., 2023). The THI is a valuable metric for assessing the combined effects of temperature and humidity on heat stress and its impact on TBF. Different breeds exhibit varying sensitivities to THI (Arunpandian *et al*., 2023; Srivastava *et al* 2024), likely due to differences in anatomy, endocrinology, or resilience to heat. In bulls, exposure to scrotal insulation for extended periods alters testicular temperature, TBF velocity, and semen quality increasing semen discard rate (Bisla *et al*. 2022).

**b)Season**: Several species exhibit seasonal fluctuations in TBF, which are correlated with shifts in testicular volume, hormone levels, and semen quality. The lowest resistive index (RI) values of the supratesticular artery in rams indicate the highest TBF, which is recorded during the breeding season and significantly lowers during the non-breeding season. This correlates with changes in testosterone, estradiol, (Hedia *et al*., 2019) and seminal parameters like enhanced lipid peroxidation (Pande *et al.*, 2015; Perumal *et al.*, 2016) and sperm membrane damage (Bisla *et al.*, 2020; Kumar *et al.*, 2021). However, some studies have reported no significant seasonal effect on TBF in rams (Samir *et al.*, 2023). With changes in the ambient temperature, TBF in bucks falls in the summer as opposed to the winter (Strina *et al.*, 2016; Samir *et al.*, 2018).

## II. Physiological Factors:

Several physiological factors such as age, body weight, sexual maturity as well as activity affect the evaluation of testicular blood flow. Henceforth, a clinician must consider these factors as well before making final interpretation.

a) **Age**: Age-related changes in TBF were observed, with potential implications for fertility in older animals. In concurrence, studies in stallions and rams have shown age-related effects on TBF (Pozor and McDonnell, 2004; Elbaz *et al.*, 2019).

b) **Body Weight**: Body size and weight can influence TBF, with larger animals potentially having higher absolute blood flow values, although the relative flow per unit of testicular tissue may not differ significantly.

c) **Sexual Maturity**: Pubertal differences in TBF have been reported in dogs and rams (El-Sherbiny e*t al.*, 2022).

d) **Sexual Activity**: Increased sexual activity or frequent ejaculation can positively affect TBF in stallions, possibly due to increased demand for sperm production. However, ejaculation can also influence Doppler parameters, therefore a period of sexual rest before examination is recommended. Studies in rams have shown conflicting results regarding the effect of ejaculation on TBF (Gouletsou *et al.*, 2003; Ahmadi *et al.*, 2012).

e) **Testicular Laterality**: Most studies have found no significant difference in TBF between the right and left testes in bucks and stallions (Samir *et al.*, 2018 & 2020; Mandour *et al.*, 2020). However, some studies in Awassi rams have reported slightly higher RI and PI values in the right testicular artery compared to the left (Hedia *et al.,* 2020). This may be related to asymmetry in testicular volume or orientation.

f) **Diseases and Reproductive Disorders**: Various diseases, particularly those causing fever, can alter TBF due to increased body temperature. Conditions like varicocele and testicular torsion significantly reduce TBF and compromise testicular function. Testicular ischemia, resulting from reduced TBF, can lead to testicular damage and impaired spermatogenesis.

## III. Technical Factors:

For an andrologist to make clinical interpretation using USG (Fig. 1 & 2), following evaluation of TBF awareness of certain technical factors influencing outcome of technique involved is essential. These factors are outlined here:

**a) Doppler Technique**: The specific Doppler technique used (e.g., pulsed-wave, Color Doppler, Power Doppler) and the parameters measured (e.g., peak systolic velocity (PSV), end-diastolic velocity (EDV), mean velocity (MV), resistive index (RI), pulsatility index (PI)) can influence the results and therefore final inference. Doppler indices (RI and PI) are less angle-dependent than velocity measurements and are often preferred for assessing TBF, especially in vessels with tortuous courses.

b) **Arterial Segment Examined**: The segment of the testicular artery examined (e.g., supratesticular artery (STA), middle testicular artery (MTA), and intratesticular arteries (ITA) significantly affects TBF measurements. Certain studies (Trautwein *et al.*, 2019) have shown variations in Doppler parameters along the course of the testicular artery, with higher resistance in the proximal STA and lower resistance in the MTA and intratesticular arteries. The MTA is often considered a more suitable segment for TBF assessment due to its accessibility and straighter course of movement.

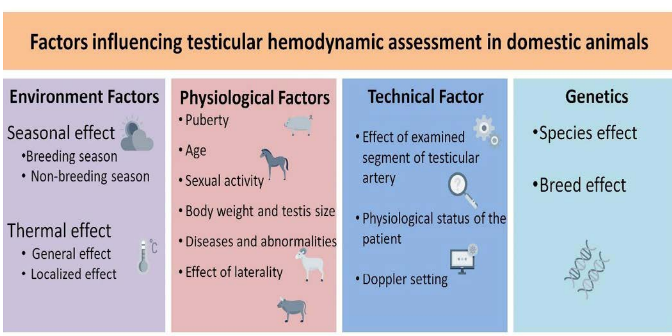
**c) Transducer Type and Frequency**: The type and frequency of the ultrasound transducer used can influence image quality and measurement accuracy. Linear transducers are often preferred for evaluating the STA, while higher-frequency transducers may be better for visualizing intratesticular arteries.

**d) Doppler Angle**: Accurate velocity measurements are angle-dependent, requiring careful adjustment of the Doppler angle to ensure parallel alignment with blood flow. Doppler indices are less affected by the angle.
**e) Operator Experience**: The experience and skill of the operator significantly influence the quality of the ultrasound images and the accuracy of TBF measurements.

## IV. Genetic Factors

**Species and Breed**: Baseline TBF values vary significantly across species and breeds. Therefore, establishing species-specific reference ranges is essential for accurate interpretation of Doppler ultrasound findings. In agreement, breed differences in TBF response to thermal stress have been observed in bulls (Junior *et al*., 2018).

**Table 2 :** Factors affecting testicular blood flow assessment in domestic animals




# ROLE OF COLOR DOPPLER ULTRASONOGRAPHY IN TESTICULAR HAEMODYNAMICS

Doppler ultrasonography has emerged as the preferred technique for assessing blood flow to different organs. Because it incorporates information on both the anatomical and dynamic flow parameters, it is one of the most straightforward and accurate methods for estimating blood flow (Strina *et al*., 2016). Ultrasonography, particularly Color Doppler ultrasonography, offers several advantages in assessing male reproductive health. Its primary advantage is its non-invasive nature, which eliminates the risks linked to invasive procedures. Because of the high metabolic rate and low oxygen concentration in the seminiferous tubules, TBP is a crucial component of testicular function that can be accurately measured using this technique (Pozor *et al*., 2014). By measuring parameters like time average maximum velocity (TAMV), end-diastolic velocity (EDV), peak systolic velocity (PSV), resistive index (RI) and pulsatility index (PI) of the testicular arteries (Viana *et al*., 2018), ultrasonography can detect subtle changes in blood flow indicative of reproductive disorders and subfertility. This allows for early detection and intervention, improving the chances of successful treatment. Furthermore, ultrasonography can monitor the effectiveness of treatments by tracking changes in TBP over time. This can be incorporated into breeding soundness examinations (BSE) to enhance the evaluation of male reproductive potential. Finally, it is useful in studying seasonal variations in testicular function across the species.

## Methods to improve testicular hemodynamics in farm Animals

Though there are several procedures to improve upon the TBF in various domestic species a few significant protocols are briefly discussed here. After administering a single dose of GnRH or hCG, Shiba goats in Egypt showed positive stimulatory effects on TBP and testis volume, though hCG had a quicker effect than GnRH (Samir *et al*., 2015). A single dose of hCG (6000 IU) given to stallions enhanced testicular perfusion as soon as 1 hour after delivery (Pozor *et al*., 2006). More recently, in another study in the shiba goats, following passive inhibin immunization, result in significant increase in blood circulation inside the testicular arteries (via changes in the values of doppler indices) (Samir *et al*., 2020). Shiba goats' TBP is raised with a single dose of s/c slow-release melatonin treatment. These results were in line with improvements in semen quality and higher levels of the metabolites E2, IGF-1, and nitric oxide (Samir *et al*., 2020). TBP elevations may enhance male fertility by having a beneficial impact on spermatogenesis. A previous study that focused on goats also revealed ground-breaking findings about the stimulatory effects of a food supplementation with selenium and probiotics on blood flow in the testicular and common carotid arteries. Additionally, concentration of various circulating hormones (FSH, LH, IGF-1, and testosterone), some haematological parameters, and antioxidant indicators were all enhanced by selenium-enriched probiotics (Mandour *et al*., 2020).

Though hormonal intervention has significant effect on TBF, it has its limitations and species-specific variations. To address such issues with GnRH/hCG administration, passive inhibin immunization, and melatonin/selenium supplementation in enhancing testicular blood flow across various species, further research is needed to optimize the procedure. Species-specific responses vary significantly, as evidenced by differing seasonal TBF patterns and responses to thermal stress.

The different therapies aiming at raising TBP and, consequently, enhancing testicular function should, in general, be of major value as a therapeutic agent to raise male farm animals' reproductive status. It may be beneficial to include this as a crucial step in improving future treatment plans for illnesses linked to testicular problems.

## CLINICAL SIGNIFICANCE OF TESTICULAR BLOOD FLOW IN MALE REPRODUCTION

**Diagnostic Applications:** Conditions such as testicular tumors in dogs and varicocele and torsion in stallions can be successfully detected with CDU, as it enables the visualization of blood vessels and the assessment of blood flow patterns to pinpoint these issues (Pozor and McDonnell, 2004; Pozor, 2007). However, the technique provides an overview of perfusion rather than detailed velocity parameters (Viana *et al*., 2018; Pugliesi *et al*., 2014, 2019). While precise velocity measurements are angle-dependent and require straight vessels, Doppler indices like the pulsatility index (PI) and resistive index (RI) offer angle-independent assessments of TBF, even in tissues with tortuous vasculature. These indices are valuable in assessing testicular function and identifying potential infertility problems in males, although they do not directly measure fertility of the affected animals.

**Assessment of Reproductive Potential:** TBF assessment via CDU (Fig. 1 & 2) provides insights into the reproductive potential of males, serving as a rapid and non-invasive tool for diagnosing and monitoring infertility issues. While semen analysis remains the gold standard for assessing testicular functioning and fertility, TBF evaluation offers a complementary approach providing additional information for an accurate diagnosis. Changes in TBF can indicate underlying issues affecting testicular function, ultimately impacting fertility. Studies have explored the correlation between TBF parameters and semen quality in various species such as bulls (Gloria *et al*., 2018; Junior *et al*., 2018), bucks (Samir *et al*., 2015; Samir *et al*., 2018; Strina *et al*., 2016; Mandour *et al*., 2020), rams (Batissaco *et al*., 2013; Hedia *et al*., 2019), stallions (Pozor *et al*., 2014; Ortega Ferrusola *et al*., 2014; Ortiz-Rodriguez *et al*., 2017), and dogs (Zelli *et al*., 2013).

**Fig. 1.** Ultrasonographic examination of the testicles of a bull using a linear probe.1: Transducer (ultrasound probe); 2: Right testis.

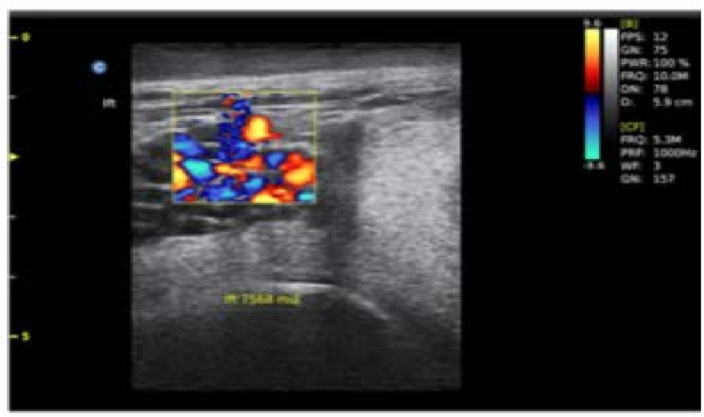


**Fig. 2.** Color Doppler ultrasonographic image showing blood perfusion at the testicular vascular cone.

### FUTURE DIRECTIONS

Future research in testicular hemodynamics should prioritize several key areas. First, establishing robust, species-specific reference ranges for Doppler parameters (PSV, EDV, RI, PI) is crucial, requiring large-scale studies accounting for age, season, breed, and body weight to improve diagnostic accuracy. Standardization of ultrasonographic techniques, including transducer selection, artery segment measured, and angle correction methods, is equally important to ensure consistent and reliable results across different laboratories and researchers. Exploring advanced techniques like contrast-enhanced ultrasonography could offer more detailed insights into microcirculation, although their cost-effectiveness is a limiting factor. Further investigations into the impact of environmental (heat stress, seasonality) and physiological (age, breed, body size, sexual activity) factors on TBP is necessary to fully understand their influence on male fertility. Moreover, strengthening correlations between Doppler parameters and comprehensive semen analyses (concentration, motility, morphology, DNA integrity) will improve the predictive value of CDU in assessing fertility potential. Finally, CDU's role in monitoring the efficacy of therapeutic interventions (pharmacological, nutritional, management strategies) for improving testicular function and treating reproductive disorders in males needs further exploration. Comparative studies across species will also be valuable in understanding interspecies variations and developing species-specific diagnostic criteria and treatment strategies. In summary, a multi-faceted approach encompassing standardization, advanced techniques, and a deeper understanding of influencing factors is needed to advance the field and improve the clinical application of testicular hemodynamics research.

## CONCLUSIONS

This review highlights the critical role of testicular hemodynamics in maintaining testicular function and male fertility in domestic animals. In breeding males' critical evaluation of TBF can provide insight in certain pathological conditions such as subfertility, presence of testicular torsion as well as testicular neoplasm in addition to providing valuable information about the structural and functional aspects of the testis. Though Color Doppler ultrasonography provides a valuable non-invasive tool for assessing TBP, accurate interpretation requires consideration of numerous environmental, physiological, and technical factors influencing measurements. Further research is needed to establish species-specific reference ranges, standardize techniques, and strengthen correlations between Doppler parameters and semen quality to enhance the clinical utility of CDU in assessing and managing male reproductive health. Ultimately, a multidisciplinary approach integrating advanced imaging techniques, physiological studies, and clinical evaluations will be vital for advancing our understanding of testicular hemodynamics and its impact on male reproductive health.

# CONFLICT OF INTEREST

Author declares no conflict of interest.

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