Hindawi
Andrologia
Volume 2023, Article ID 5003366, 9 pages
https://doi.org/10.1155/2023/5003366

Research Article

Seasonal Alterations in Testicular Hemodynamics and Echotexture in Relation to Semen Quality in Buffalo Bulls

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Received 19 October 2022; Revised 15 November 2022; Accepted 6 December 2022; Published 6 February 2023

Academic Editor: Marta Olszewska

The present study is aimed at investigating the usefulness of the pulsed-wave Doppler ultrasonography for the characterization of testicular blood flow in buffaloes during the nonbreeding and breeding seasons. Ten Egyptian buffalo bulls were evaluated for changes in the blood flow (Doppler's indices: resistive index (RI) and pulsatility index (PI)) at the levels of supratesticular (STA) during the breeding (winter) and nonbreeding season (summer). The alterations in testicular parenchyma echotexture (pixel intensity (PIX) and echotexture heterogeneity (EH)) were assessed by computer analysis software. Circulating testosterone and nitric oxide (NO) concentrations and total antioxidant capacity (TAC) were measured colorimetrically. Sperm parameters including total and progressive motility %, viability %, morphology %, and concentrations were assessed. Results revealed lower values ($p < 0.05$) of RI (0.55 ± 0.02) and PI (0.82 ± 0.03) within the STA in the breeding period compared to the nonbreeding period (RI: 0.67 ± 0.03; PI: 0.99 ± 0.01). Higher ($p < 0.05$) PIX and EH were found in the nonbreeding season (PIX: 72.09 ± 1.81; EH: 14.99 ± 1.37) compared to the breeding season (PIX: 60.95 ± 1.51; EH: 11.75 ± 0.63). Concentrations of testosterone and TAC were not significantly changed. However, the concentrations of NO were greater ($p < 0.05$) during the breeding season (78.21 ± 0.87) compared to those of the nonbreeding one (59.74 ± 2.97). Total motility %, progressive motility %, viability %, and sperm concentrations were higher during the breeding season compared to that during the nonbreeding season ($p < 0.05$). In conclusion, seasonality alters testicular hemodynamics and echotexture, NO concentrations, and sperm quality in buffaloes without alterations in the testosterone and TAC levels.

1. Introduction

Buffalo (*Bubalus bubalis*) is a valuable dairy animal in Egypt (about 3.69 million heads) because it is the primary source of milk (44%) and red meat (39%) for the Egyptian economy and constitutes about 24.5% of the agricultural gross domestic products [1]. Compared to cattle, the reproductive performance of buffalo showed a seasonal pattern, and this seasonality depends on many factors such as geographical locations, breed, and nutrition [2–5]. Buffalo is characterized by lowered productive and reproductive performance, especially during the summer months [6–8].

Adequate blood flow is very important to testicular functions because of the limited oxygen concentration inside the seminiferous tubules that represent about 70%-80% of the testicular mass [9, 10]. Because a stable blood flow is critical to the function of the testis (steroids and sperm production), any decrease in testicular blood flow (TBF) may result in impairments in spermatogenesis [11] due to ischemic damage and defective energy metabolism of the testicular tissues [12]. Many reports have indicated close relationships between TBF and potential male fertility [12–15]. The pulsed Doppler ultrasonography is considered a helpful diagnostic aid to assess the reproductive performance and diagnose many infertility disorders of male domestic animals [14, 16, 17].

Moreover, studies that have assessed the effect of season on testicular hemodynamics are limited. To the authors' knowledge, no study has been performed to evaluate whether or not there are alterations in testicular hemodynamics in buffaloes between the breeding and nonbreeding seasons. Considering this issue, the current study is aimed at exploring the seasonal effect (breeding versus nonbreeding) on the TBF at the level of the supratesticular artery (STA) as assessed by the spectral Doppler ultrasonography in buffalo bulls. Also, it investigated the influence of the season on testicular echotexture, circulating testosterone, total antioxidant capacity (TAC), nitric oxide (NO), and semen quality.

2. Materials and Methods

This study was carried out on Egyptian buffalo bulls (*Bubalus bubalis*), a species characterized by fluctuations in the potential fertility among seasons; being high during autumn and winter (breeding season) and low during spring and summer (nonbreeding season) [4].

2.1. Animals and Experimental Design. Ten Egyptian buffalo bulls, weighing 480 ± 60 kg, and aged 4.5 ± 0.88 years were included at the beginning of the present study. Bulls were managed under natural photoperiodic conditions at a dairy buffalo farm located in the Kalyobia governorate, Egypt (30.41°N, 31.21°E) during a characteristic period of the low (summer season; June to August) and high (winter season; December to March) reproductive potential in buffalo [2, 18]. Animals were fed a balanced ration of a commercially prepared concentrate (16% crude protein/dry matter) daily. Wheat straw and sweet clover were also provided as bulky feed material. Salt licks and tap water were accessible *ad libitum*. Buffalo bulls were regularly immunized against important infectious diseases such as foot and mouth disease and enterotoxaemia. Upon a full physical examination, bulls were apparently free from any evidence of disease before starting the study. All bulls were exposed to a thorough andrological assessment (breeding soundness examination (BSE)) to verify the absence of reproductive disorders before the start of the study [19]. The bulls were well trained, had high libido, and were proven to be good fertile (based on the BSE protocol including semen assessment) before the study. The procedures of this study were performed following the Guidelines of Cairo University for the Use and Animals Care (VetCU20092022480).

In the current study, ultrasonographic assessment of the blood perfusion to the testis and testicular echotexture was performed once every two weeks for three consecutive months during the nonbreeding (June to August; summer season) and breeding seasons (December to March; winter season). Concurrently, blood samples were collected to determine the concentrations of testosterone, TAC, and NO. Variations in climatic data including temperatures, temperature humidity index (THI), and daylight length were reported between the breeding and nonbreeding seasons based on Egypt Meteorological Agency (Table 1).

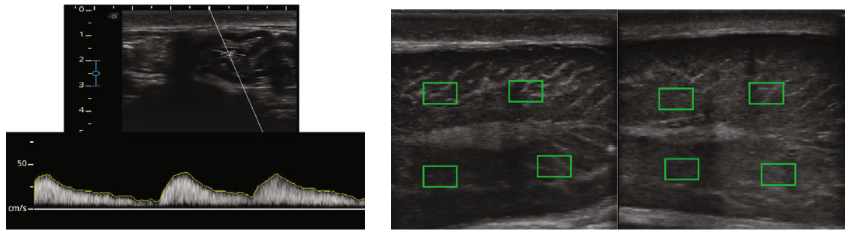
Table 1: Climatic data (mean ± SEM) during the study (breeding versus nonbreeding season) based on Egypt (30.41°N, 31.21°E) Meteorological Agency (http://ema.gov.eg/wp/).

Parameter	Breeding season	Nonbreeding season
Minimum temperature (°C)	12.19 ± 0.25	$25.59 \pm 0.21^{***}$
Maximum temperature (°C)	21.65 ± 0.41	$36.97 \pm 0.23^{**}$
Temperature-humidity index	20.35 ± 1.87	$32.50 \pm 1.19^{**}$
Mean daylength (h)	10.50 ± 0.23	$13.67 \pm 0.18^{*}$

Climatic data were recorded within the breeding season (winter; from December to March) and nonbreeding season (summer; from June to August) in this study. $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$.

2.2. Assessment of Testicular Hemodynamics by the Pulsed Doppler Ultrasonography. Ultrasonographic examinations were achieved by the same proficient examiner using the pulsed-wave Doppler device (SonoScape E1 Vet, SonoScape Medical Corp., China) just after blood sampling throughout the study. The Doppler device was supplemented with a linear transducer (7–14 MHz transducer). All settings of the ultrasound scanner (brightness, contrast, focus, and gain) were standardized and fixed for all scanning procedures. The high pass filter was adjusted at 50 Hz, and the gate of the Doppler angle (≤60°) with the examined vessels was adjusted constantly at 1.5 mm. Buffalo bulls were examined in a standing position without being sedated or tranquilized to avoid its influence on TBF. Animals were restrained in a stanchion. Without the necessity to shave the hair on the scrotum, the transducer was applied to the scrotal testes after covering them with enough amount of ultrasonographic gel to enhance the ultrasonographic imaging and minimize the presence of air spaces for proper scanning.

The testicular artery in buffalo bulls is highly coiled at the area of the supratesticular portion [20]. Because the veins are convoluted around the testicular artery, some misinterpretations may be occurred between the testicular artery and veins by Doppler's assessment. For proper assessment, the blood flow within the veins is relatively constant without a clear spectral pattern; however, the blood flow within the artery has a spectral waveform indicating the arterial pulse (systole and diastole) in each cardiac cycle. After observing the largest cross section of STA using the B-mode ultrasonography and adjusting the angle and gate, the pulsed-wave Doppler was activated to characterize the spectral waveforms of the blood flow within the STA (Figure 1(a)). After that, these velocity parameters were recorded: peak systolic velocity (the maximum blood flow velocity; PSV, cm/s), end-diastolic velocity (lower velocity of blood flow; EDV, cm/s), and time-averaged maximum velocity (abbreviated as TAMAX, cm/s). Moreover, Doppler's indices including the resistive index (calculated automatically using this equation: $RI = (PSV - EDV)/PSV$), and pulsatility index (calculated automatically using this equation: $PI = (PSV - EDV)/\text{mean velocity}$) were recorded. About three to five shots were recorded for each parameter along different segments of the STA (located proximal to the testis and at the distal end of the spermatic cord) to verify the blood perfusion of the testis and minimize the variabilities of the measured Doppler velocities at the STA due to its high-convoluted conformation. In this regard, all ultrasonographic examinations were carried out at fixed times (09 : 00 AM) and digitally saved for further analysis. Three trials for blood flow assessment were performed before the start of the study to accommodate buffaloes for the experimental procedures and minimize the possible effect of animal behavior (nervous temperament) on the studied parameters.



(a) (b)

Figure 1: The spectral pattern of the blood flow to the testis at the examined supratesticular artery (a) of buffalo bulls assessed by pulsed Doppler's ultrasonography. Assessment of changes in the echotexture of testicular parenchyma (pixel intensity and echotexture heterogeneity) in bulls by B-mode ultrasonography and assessed by computer-image analysis software (b).

2.3. Assessment of the Echotexture of Testicular Parenchyma. For proper assessment of testicular echotexture including the pixel intensity (PIX) and echotexture heterogeneity (EH), the transducer was applied gently (without pressure) on the testis until the obvious appearance of the testis mediastinum (being the mediastinum in the center). Good B-mode images of the testicular parenchyma were recorded and used for further digital computed assessment by a computer image analysis software program (ImageJ, USA National Institutes of Health) as previously described [21–23]. Briefly, four rectangular spots (10 mm × 5 mm) within the homogenous parenchyma of the testis and laterally to the mediastinum testes (on both sides, Figure 1(b)) were selected and exposed to the software to calculate the PIX and EH. The PIX of the testicular parenchyma is representing the mean pixel values within the selected spots of the parenchyma based on a reverse gray shades scale (from one as a black shade to 255 as a white shade).

2.4. Biochemical Assessment of Testosterone and NO Concentrations and TAC. Blood samples (10 ml) were withdrawn from the bulls by jugular venepuncture, centrifuged at 1350 g for 20 min, and the obtained serum was stored at -20°C until the biochemical analyses. Serum testosterone concentrations were measured following the procedures of commercial kits (ELISA testosterone kit, BioCheck, Inc., Foster City, USA). Intra and interassay coefficients of variation of testosterone were 3.9% and 4.7%, respectively, and the sensitivity was 0.05 ng/ml. Concentrations of NO (μmol/L) were measured using a commercial kit (Biodiagnostic company, Dokki, Egypt) using a spectrophotometer (at wavelength 540 nm). The NO inter and intra-assay coefficient of variations were 6.8% and 5.4%, respectively, with assay sensitivity of 0.225 μmol/L in nitrites form. Assessment of the changes in the concentrations of TAC (mM/L) was done following the procedures of a commercial kit (Biodiagnostic, Dokki, Egypt) at a wavelength of 505 nm of the spectrophotometry as described previously [24, 25]. The inter and intra-assay coefficient variations of TAC were 2.7% and 8.2%, respectively.

2.5. Evaluation of Parameters of Semen. Data regarding the semen quality of the examined buffalo bulls during the nonbreeding and breeding periods was retrieved based on the assessment of the semen samples. All the bulls were in the same sexual regimen during the period of the study. Semen samples were collected once every two weeks (9 : 00 AM) during the studied periods using an artificial vagina (settled prewarmed at 42.50°C). Then, the samples were transferred directly to the laboratory and kept in an adjusted (25-30°C) water bath until the conventional semen assessment procedures with the aid of a heated-stage (38°C) microscope (Olympus, Tokyo, Japan) as previously described [18, 26]. In brief, the semen sample was diluted (1.20) with sodium citrate dihydrate 2.9% as an extender. The diluted semen samples were subjectively analyzed using microscopic inspection for the total motility and progressive motility percentages. In addition, percentages of sperm viability and morphology were assessed using the technique of eosin-nigrosin staining (about 200 sperm). Sperm concentrations were evaluated using the Neubauer hemocytometer.

2.6. Statistical Analysis. The obtained results related to the hemodynamic changes in the STA (RI, PI, PSV, EDV, and TAMAX), testicular echotexture (PIX and EH), and biochemical assessment (testosterone, TAC, and NO), and parameters of semen samples are presented as means ± SEM. The normality of the data was tested using the Kolmogorov-Smirnov test. Regarding the measured parameters of testicular hemodynamic and echotexture of the testis, no significant differences were found between the right and left testes; therefore, the data were pooled. The mean values of studied parameters were statistically compared among the nonbreeding and breeding seasons by the repeated measures t-test (the number of examinations and samples collected for all animals is 60/each season; there are 6 examinations and samplings dates in each season × number of bulls (6 × 10 = 60)). Correlations between semen parameters and the echotexture of testicular parenchyma were studied using the correlations coefficient. Furthermore, differences in climatic data between seasons were statistically compared. GraphPad Prism5 was used for the statistical comparisons of all the studied parameters. $p < 0.05$ value was considered significant.

3. Results

As scanned by the pulsed Doppler sonography, the blood flow within the STA has wave-like appearances, mostly monophasic and nonresistive spectral waveforms (Figure 1(a)). Significant lower values of RI (0.55 ± 0.02) and PI (0.82 ± 0.03) of the blood flow within the STA (Figure 2) were found in the

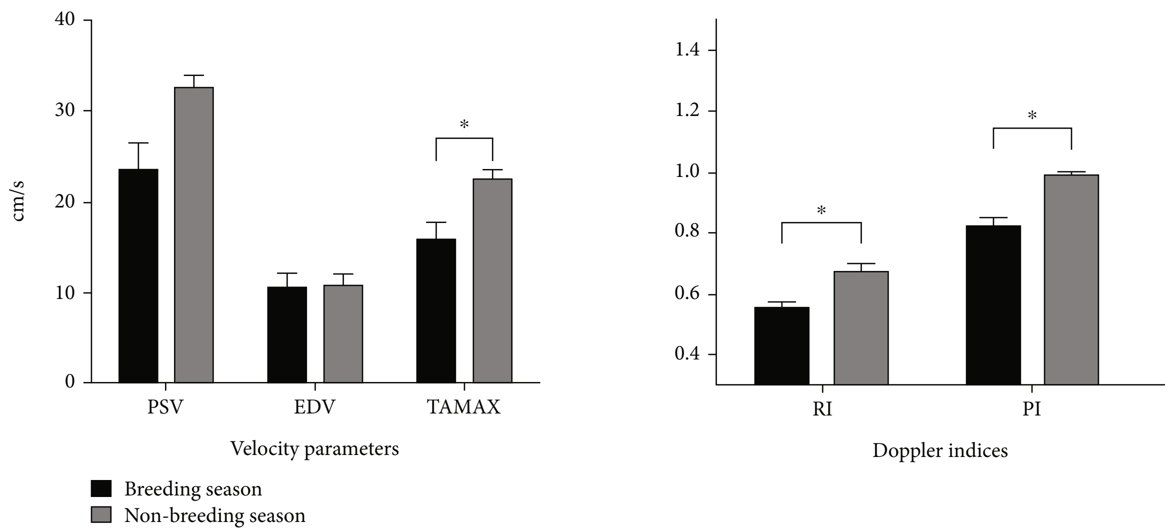


Figure 2: Parameters of blood flow at the level of the supratesticular artery (PSV: peak systolic velocity; EDV: end-diastolic velocity; TAMAX: time-averaged maximum velocity; RI: resistive index; PI: pulsatility index;) as imaged by pulsed-wave Doppler's ultrasonography in 10 buffalo bulls during the breeding and the nonbreeding season. *Values are significant at least at $p < 0.05$. Notes: total number of Doppler's examinations for all buffalo bulls during each season is 60 (once every two weeks for three consecutive months on ten buffalo bulls during each season).

breeding season compared to the nonbreeding season (RI: 0.67 ± 0.03; PI: 0.99 ± 0.01). Lower TAMAX of the STA blood flow was found in the breeding season (15.88 ± 1.79 cm/s) compared to the nonbreeding season (22.41 ± 1.15 cm/s) ($p < 0.05$). However, other velocity parameters of the blood flow showed nonsignificant differences between the breeding and nonbreeding season in the STA ($p < 0.05$). Regarding testicular echotexture (Figure 3(a)), significant decreases in the PIX (60.95 ± 1.51) and EH (11.75 ± 0.63) were found in the breeding season compared to the nonbreeding season (PIX: 72.09 ± 1.81; EH: 14.99 ± 1.37).

Results of the semen quality are presented in Figure 3(b). Higher ($p < 0.05$) total motility %, progressive motility %, viability %, and sperm concentrations were found during the breeding period compared to the nonbreeding one, while nonsignificant alterations were found in the total abnormalities % between the seasons ($p < 0.05$). Although concentrations of testosterone and TAC were not significantly changed, the concentrations of NO were greater ($p < 0.05$) during the breeding season (78.21 ± 0.87) compared to those during the nonbreeding season (59.74 ± 2.97) (Table 2). In this regard, high correlations ($p < 0.05$) were found between the PIX of testicular parenchyma and total motility (%), progressive motility (%), total abnormalities (%), and sperm concentration (Table 3).

4. Discussion

Doppler's ultrasonography is a useful and noninvasive tool for the assessment of the blood flow to the testis in various species of farm animals such as rams [27, 28], stallions [14, 29], goat bucks [15, 30–33], and dogs [34]. In the present study, we used the pulsed Doppler ultrasonography for the characterization of TBF in buffaloes. In addition, we fully monitored the changes in the echotexture of testicular parenchyma during the nonbreeding and breeding seasons. Such data is very important because it gives, for the first time, information about the normal hemodynamics and echotexture of the testis during the breeding transition in buffaloes. The measured parameters are considered good reference values for the physiological status of the testis [12, 21]. These reference values could be potentially used in the future as diagnostic markers for the assessment of the pathological changes in the fertility potential in buffaloes.

In the current study, values of the Doppler indices of the STA were notably greater than those reported in *Bos taurus* bulls in previous literature [35, 36]. These differences might be attributable to the anatomical characteristic differences between the scrotum in buffalo and *Bos taurus* bulls. Buffalo possesses a less efficient evaporative cooling system in the skin due to lowered sweating ability [4] compared to those in *Bos taurus* bulls (buffaloes dissipate heat poorly by sweating because the skin of buffalo has one-sixth of the density of sweat glands that skin of cattle has). In addition, buffalo bodies can absorb a great proportion of solar radiation because of their dark skin and sparse coat or hair. Collectively, these anatomical characteristics could result in variations in testicular hemodynamics than those reported in cattle bulls. Therefore, heat stress is one of the major constraints on the reproductive potential of buffalo bulls, and it is aggravated when it is accompanied by increases in relative humidity [4, 37].

Limited studies have been carried out to evaluate the effect of seasonality on TBF. In this study, increases in Doppler's indices of the examined STA were found in the nonbreeding season (summer months) compared to those in the breeding season. Doppler's indices (RI and PI) are good indicators of the resistance to blood flow in the examined vessel because of their negative correlations with tissue

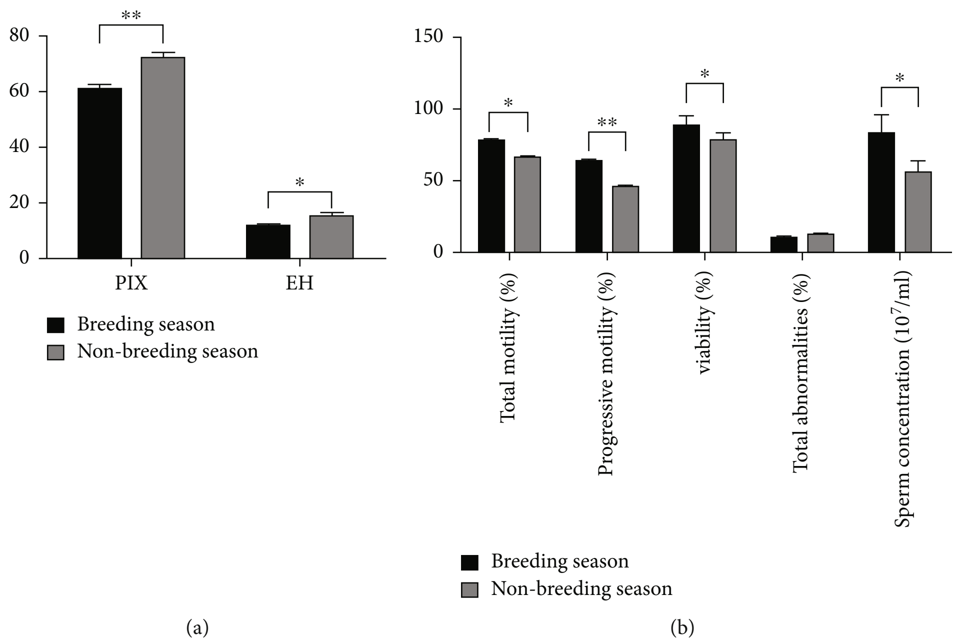


Figure 3: (a) Changes in the parameters of testicular echogenicity (PIX: pixel intensity; EH: echotexture heterogeneity) of 10 buffalo bulls as imaged by B-mode ultrasonography and assessed by computer analysis software during the breeding and the nonbreeding season. $^{*}p < 0.05$; $^{**}p < 0.01$. Notes: total number of ultrasonic assessments of echotexture of testicular parenchyma for all buffalo bulls during each season is 60 (once every two weeks for three consecutive months on ten buffalo bulls during each season). (b) Changes in the studied semen parameters in buffalo bulls during the breeding and the nonbreeding season. $^{*}p < 0.05$; $^{**}p < 0.01$. Notes: total number of collected samples for all buffalo bulls during each season is 60.

Table 2: Changes in the concentrations of total antioxidant capacity (TAC (mM/L)), nitric oxide (NO (umol/L)), and testosterone (ng/ml) in 10 buffalo bulls during the breeding and the nonbreeding season.

Parameter	Breeding season ($n = 60$)	Non-breeding season ($n = 60$)
TAC (mM/L)	1.46 ± 0.16	1.67 ± 0.24
NO (umol/L)	78.21 ± 0.87	59.74 ± 2.97*
Testosterone (ng/ml)	1.67 ± 0.62	0.76 ± 0.17

*Values are significant at least at $p < 0.05$. Notes: n is the total number of collected samples for all buffalo bulls during each season (once every two weeks for three consecutive months on ten buffalo bulls during each season).

Table 3: Correlation coefficients between the studied semen parameters and testicular echotexture (pixel intensity and echotextural heterogeneity) and in buffalo bulls during breeding and nonbreeding seasons ($n = 120$).

Semen parameters	Pixel intensity	Echotextural heterogeneity
Total motility (%)	-0.651*	0.214
Progressive motility (%)	-0.843*	-0.152
Viability (%)	-0.357	0.095
Total abnormalities (%)	0.551*	0.221
Sperm concentration (10^9/ml)	-0.673*	0.189

n is the number of samples or measurements in 10 buffalo bulls in both seasons. $^{*}p < 0.05$ for the correlations.

blood perfusion [38–41]. When the RI and PI values decrease, the resistance to the blood flow decrease [38, 42, 43], and this indicates an increase in the blood flow (carrying oxygen and nutrients) to the respective organ. These findings agreed with previous reports on male goats [32] and rams [13]. Seasonal variations of TBF are most often attributed to differences in the spermatogenic and endocrine functions of the testis ([13, 44]) and may be the cause of seasonal alterations in the testicular function [45]. Therefore, various treatments that target the enhancement of the potential fertility in males should also address the improvement of TBF [12], especially during low reproductive seasons [46–48].

Indeed, buffaloes have lower physiological adaptation to the thermal effect than cattle. Although the temperature of the buffalo body is slightly lower than that of cattle, heat is poorly dissipated in buffaloes because the skin of buffalo is usually black, heat absorbent, and has a low density of sweat glands than cattle skin [4].

The effect of thermal factors on TBF in farm animals was highlighted in several studies [13, 32, 49–51]. In bulls, significant increases in TBF were found in response to increases in ambient temperature (from 5°C to 35°C) [49]. Experimental warming of the testes (to 40°C) in both *B. taurus* and *B. indicus* bulls resulted in an extreme increase in TBF in a way to provide enough oxygen and meet the increased metabolic demands of the testes and avoid hypoxia [51]. However, the present study showed an increased TBF in the winter (low ambient temperature) compared to the summer months (high ambient temperature). Our findings are in the agreement with that reported on goats [32] and rams [13]. Indeed, the nature of the thermal stress should consider whether the thermal factor is restricted to the testes (i.e., local effect by warming the testis or insulation) or is a general effect resulting from exposing the animal to high environmental or experimental temperature [28]. Another reason for this discrepancy might be attributable to whether the testicular temperature surpassed the body temperature or not. The difference in animal adaptability (species or breeds) to thermal stress may be another factor that should be considered [52]. In dogs, for example, no changes in the TBF were reported when the average temperature of the scrotal surface increased [50]. Also, after the artificial cooling of dog testicles, marked reductions in scrotal blood flow was observed without any effect on the TBF [53]. However, in rams, TBF increased up to 26% by directly applying heat to the testis [54]. Various morphological and anatomical differences in the testicular artery were found among the bulls' breeds that may influence the response of TBF to thermal factors [55].

Testicular echotexture is a computer software image analysis of the parenchyma of the testis including an assessment of the PIX and uniformity. Much literature indicated the usefulness of the assessment of testicular echogenicity in assessing the pubertal status and its correlation to the semen traits and the potential fertility of bulls and rams [21, 56–58]. However, according to the authors' knowledge, the current study is the first that addresses the influence of season on testicular echotexture in buffalo bulls. In the current study, decreases in the values of PIX found during the breeding season may be in part attributable to increases in testicular blood perfusion (through decreasing in the values of RI and PI of the STA). Increases in TBF may lead to increases in intratesticular fluids that in turn decrease the echotexture PIX. Our assumption agrees with that reported previously in rams [59]. The reduced testicular blood flow (as evidenced by increases in the values of RI and PI) that was reported during the nonbreeding season of the current study was accompanied by reduced parameters of the semen quality. These findings agreed with those reported in rams [60]. Importantly, the present study found high correlations between the PIX of testicular parenchyma and total motility (%), progressive motility (%), and sperm concentration. Similar findings were reported in rams throughout the year [57, 61]. On the contrary, no correlations were found between semen quality and the PIX of testicular parenchyma in bulls [58]. Many previous studies indicated the reduced semen parameters during the nonbreeding summer season in buffalo due to increasing the temperature-humidity index, together with relatively poor nutrition [2–4, 18, 62].

In the current study, the concentrations of TAC and testosterone revealed nonsignificant changes between the breeding and nonbreeding seasons. Our findings agreed with the finding of a previous report [63]. However, significant increases in the concentrations of NO were noted during the reproductive season than in the nonbreeding season. These increases in the NO might be incorporated, at least in part, in the increases in the blood perfusion to the testis during the breeding season. Nitric oxide has local physiological roles to regulate the transfer of nutrients and oxygen, hormones, and other agents by the testicular vessels [64, 65]. Previous studies also reported the crucial role of NO in the regulation of testicular blood flow owing to its powerful vasodilatory effect [64–67]. The explanation of the differences in the levels of NO between the nonbreeding season and the breeding season was out of the scope of the present study. However, it could be attributed to the oxidative stress conditions that might be evoked during the nonbreeding season. Indeed, the testis is very liable to suffer from ischemic damage when blood perfusion is decreased due to any vascular restriction as the result of inadequate nutrient supply [11]. Herein, exposing the testis to thermal stress could increase the testicular metabolism and oxygen demands, resulting in hypoxia and the formation of reactive oxygen species, which impairs spermatogenesis [68–70]. Increases in the ambient temperature during the nonbreeding season might lead to increases in the free radicals (superoxide anion and hydroxyl radicals) [71]. These radicals enhance the conversion of the NO to peroxynitrite and, in turn, reduce its bioavailability [72]. Despite being nonsignificant alterations were found in the levels of TAC between the two seasons. Yadav et al., [73] indicated the involvement of nitric oxide synthase (NOS) genes in the amelioration of thermal stress to keep the integrity and homeostasis of blood mononuclear cells during different seasons in goats. Furthermore, NO could be able to elicit various vasodilator and cutaneous adaptive responses to thermal stress conditions due to its pivotal role in the circulatory and immune systems inside the animal body [52, 73].

5. Conclusion

Overall, these findings supported the hypothesis of our study that seasonality alters parameters related to the testicular hemodynamics and echotexture concomitantly with alterations in the semen quality in Egyptian buffalo bulls. Therefore, it could be used as helpful reference values for assisting in diagnosing different infertility problems and for further enhancement of productivity in buffalo. However, further studies may be needed to investigate the relationship between testicular hemodynamics and the potential fertility of buffalo herds on a large scale (for example pregnancy rate) among seasons.

Data Availability

The data are available by the corresponding author upon a reasonable query.

Conflicts of Interest

The authors stated no conflict of interest in this study.

Authors' Contributions

Haney Samir was involved in the conceptualization, experimental plan, ultrasonographic examination, hormonal and biochemical analyses, data curation and validation, statistical analyses, and manuscript writing. Hossam R. El-Sherbiny was involved in the conceptualization, Doppler examination, biochemical analysis, and manuscript reviewing and editing. Amr S. El-Shalofy participated in the design and procedures of the study, hormonal assay, and review and critical editing of the manuscript.

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