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Characterisation of testicular perfusion dynamics using digital pulse wave Doppler ultrasonography in indigenous cattle reared under coastal tropical climate

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Abstract: Testicular blood flow and haemodynamics play a critical role in maintaining spermatogenesis and thermoregulation in bulls. The assessment of the testicular blood flow provides detailed insights into the reproductive function in male animals. This study aimed to characterise the testicular perfusion pattern using pulse wave Doppler ultrasonography in the Shweta Kapila breed of indigenous cattle reared in their native habitat on the Indian west coast. Healthy Shweta Kapila bulls (n = 8) maintained under semi-intensive systems were examined. A digital Pulse Wave Doppler ultrasound system equipped with a linear array probe was used to assess peak systolic velocity (PSV), end diastolic velocity (EDV), resistive index (RI) and pulsatility index (PI) along the spermatic cord part of the testicular artery (sTA), marginal region (mTA), and intra-testicular branches (iTA) of the testicular artery. Mean PSV ranged from 4.93 cm/s to 12.32 cm/s, EDV ranged from 3.63 cm/s to 5.14 cm/s, RI ranged from 0.23 to 0.58 and PI ranged from 0.68 to 1.05 across different arterial segments. Significantly higher mean PSV values ($P < 0.05$) were recorded in the proximal sTA compared to distal arterial segments. RI demonstrated low within-bull variability and repeatability suggesting its potential as a reliable indicator of testicular perfusion. The stable and low intra-testicular RI values also suggest enhanced vascular adaptation and thermoregulatory efficiency. These findings establish the first baseline reference values for testicular perfusion and haemodynamics in native Shweta Kapila cattle. Pulse wave Doppler ultrasonography provides a reliable, non-invasive and real-time tool for evaluating vascular function and reproductive soundness in indigenous cattle reared under coastal tropical climate.

Keywords: breeding soundness; coastal climate; Doppler ultrasonography; indigenous cattle; testicular blood flow

India is home to one of the largest reservoirs of indigenous livestock genetic diversity, with several animal breeds uniquely adapted to distinct agro-climatic conditions. Among these, the Shweta Kapila breed, native to the Konkan coastal region, has gained prominence for its resilience to heat

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stress and diseases, modest nutrient requirements, and ability to perform efficiently under low-input systems (Shivankar et al. 2025). Reproductive characterisation of indigenous breeding bulls is crucial for enhancing reproductive efficiency and productivity especially in light of vanishing ecosystems, dwindling natural resources, and escalating climate change pressures. Bulls selected for breeding programmes must undergo a thorough breeding soundness examination to ensure optimal reproductive performance (Chenoweth and McPherson 2016). While a conventional evaluation depends on physical examination, scrotal biometry, and semen analysis, modern imaging techniques can provide additional and deeper insights into the testicular function. However, relevant information on the reproductive physiology of Shweta Kapila males remains limited, particularly regarding the testicular vascular dynamics in relation to climate adaptation and fertility under hot and humid tropical conditions.

Testicular perfusion plays a key role in thermoregulation and normal spermatogenesis by maintaining an optimal intra-testicular temperature gradient, typically about 4 °C to 6 °C below the core body temperature, which is vital for sperm production and viability (Pozor 2007). The testicular artery and its branches facilitate an efficient counter-current heat exchange to maintain the thermoregulatory gradient. Any disturbances in the testicular blood flow can impair spermatogenesis and testicular function, leading to subfertility or infertility. With advancements in diagnostic imaging, Doppler ultrasonography has emerged as a valuable non-invasive tool for assessing reproductive health in domestic animals (Pozor and McDonnell 2004). The advent of colour and pulse wave Doppler ultrasonography has revolutionised andrological evaluation by aiding a detailed assessment of blood flow parameters. In farm animals, Doppler-derived parameters are increasingly recognised as reliable indicators of vascular resistance and parenchymal perfusion (Gloria et al. 2018). These indices provide information about vascular resistance and impedance that cannot be obtained from B-mode ultrasonography alone.

Breed- and species-specific differences in testicular haemodynamics are well-documented. In bulls, Gloria et al. (2018) demonstrated consistent regional variations in blood flow velocities, while Favaro et al. (2020) correlated haemodynamic parameters with thermal stress, noting the increased supra-testicular blood flow with higher temperature-humidity index (THI) levels. Comparable findings have been reported in rams (Elbaz et al. 2019), stallions (Pozor 2007), and buffaloes (Vikram et al. 2021). Nevertheless, there remains a paucity of similar information in indigenous cattle breeds adapted to tropical coastal climate with high humidity and ambient temperature.

Understanding testicular haemodynamics and vascular physiology is crucial for the breeding soundness evaluation and success of sustainable breeding programmes, particularly in challenging coastal environments. This study, therefore, aims to characterise testicular perfusion parameters across key arterial segments using digital pulse wave Doppler ultrasonography and evaluate their physiological significance for the first time in indigenous Shweta Kapila bulls. The findings are expected to provide baseline haemodynamic information and foundation for the integration of vascular assessment into the comprehensive breeding soundness examination of native cattle breeds.

MATERIAL AND METHODS

Animals and experimental design. The study was conducted on eight clinically healthy Shweta Kapila bulls aged 4–6 years (mean age ± SEM: 5.25 ± 0.6 years), maintained in their native breeding tract in the Konkan region of India (latitude 15°49'N, longitude 73°91'E). The region experiences a hot and humid coastal climate, with average ambient temperatures ranging between 30 and 34 °C and relative humidity levels of 72–89% (THI: 74–84). All experimental animals (n = 8) were reared under a semi-intensive production system with uniform housing, feeding, and management practices. The mean body weight was 553.46 ± 24.16 kg and the mean scrotal circumference was 29.33 ± 0.96 cm. Selected bulls exhibited normal libido and scrotal symmetry. All experimental procedures adhered to the guidelines of the Committee for Control and Supervision of Experiments on Animals (CCSEA), Government of India, and the study was approved by the Institute Animal Ethics Committee (IAEC).

Ultrasonographic examination. Animals were restrained standing without sedation and the scrotum was cleaned, and coupling gel was applied

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to optimise image quality. Ultrasonographic examinations were performed using a Logiq® Book XP (GE Healthcare, WI, USA) equipped with a linear array probe (5–10 MHz). Examinations were performed by the same operator between 8:00 a.m. and 9.30 a.m. to minimise circadian variations. The initial B-mode ultrasonographic examination was performed to assess the testicular parenchyma and identify the testicular artery at three standardised locations bilaterally (Figure 1). Colour Doppler mode was employed to identify the spermatic cord portion of the testicular artery (sTA), marginal part of the testicular artery (mTA), and intra-testicular branches of the testicular artery (iTA) and to visualise arterial flow direction; subsequently, pulse wave Doppler mode was used to obtain spectral waveforms. The scan depth was standardised at 10 cm, images were acquired at 7.5 MHz transducer frequency, and the pulse wave Doppler sample volume was set to 1 mm. The Doppler sample gate was placed within the vessel lumen, and the angle of insonation (θ) was maintained below 60° to ensure accurate velocity measurements and to avoid the underestimation of the systolic peak (Gloria et al. 2018). The testicular artery was differentiated from accompanying veins based on the characteristic pulsatile arterial waveform on spectral Doppler examination. Measurements were taken bilaterally at three standardised locations. For each measurement site, at least three consecutive waveforms were recorded, and three representative measurements were averaged. Optimal scan and pulse wave images were frozen and recorded for analysis and interpretation. The measured pulse wave doppler parameters were peak systolic velocity (PSV, cm/s), end diastolic velocity (EDV, cm/s), and time-averaged maximum velocity (TAMAX). The resistive index (RI) was calculated using the formula: RI = (PSV – EDV)/PSV and pulsatility index (PI) was calculated as PI = (PSV – EDV)/TAMAX (Middleton et al. 1989). The systolic/diastolic ratio (S/D ratio = PSV/EDV) was computed as a metric for assessing vascular resistance and perfusion efficiency. All measurements were performed in triplicate at each location and the mean values were calculated for statistical analysis.

Figure 1. Image of the transducer position during the acquisition of pulse wave Doppler ultrasonograms of the testicular artery in bulls

Statistical analysis. Data were analysed using GraphPad Prism v10.0 software (GraphPad Software Inc. 2023). Descriptive statistics were calculated and data were presented as mean ± SEM. Mean values were determined for each type of artery segment in both the testes (sTA, mTA and iTA). Data normality was verified using the Shapiro–Wilk test. Comparisons of Doppler parameters between different testicular arterial regions and between the right and left testis were performed using one-way analysis of variance (ANOVA) followed by Tukey's post-hoc test for multiple comparisons. Student's t-test was used for paired comparisons between the right and left testis. Pearson correlation analysis evaluated associations among different Doppler attributes. Repeatability of measurements within individual bulls was assessed using the coefficient of variation (CV). Values of $P < 0.05$ were considered statistically significant.

RESULTS

Selected bulls in the study were clinically healthy with normal scrotal conformation and testicular consistency on palpation. A B-mode ultrasonographic examination revealed the homogeneous echogenic testicular parenchyma with well-defined echogenic mediastinum testis in all animals. No focal lesions, masses, or abnormalities were detected either in testes or in epididymides. The testicular artery was identified at all three standard measurement locations using colour Doppler ultrasonography. A spectral Doppler examination revealed predominantly monophasic, low-resistance waveforms throughout its course, character-

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ised by a prominent systolic peak and a sustained forward diastolic flow, typical of organs requiring continuous perfusion (Figure 2).

Mean peak systolic velocity (PSV) values demonstrated a significant regional variation especially along the proximal part of the testicular arterial network (Table 1). PSV was significantly higher ($P < 0.05$) in the spermatic cord portion of the testicular artery (sTA) compared to the marginal (mTA) and intra-testicular (iTA) segments in both testes. Mean PSV values were comparable between the right and left testis at all three regions. When averaging bilateral measurements, overall mean PSV was 12.01 ± 0.35 cm/s for sTA, 5.03 ± 0.02 cm/s for mTA, and 4.98 ± 0.02 cm/s for iTA. The spermatic cord region of the testicular artery (sTA) exhibited the highest PSV corresponding to the higher vascular resistance.

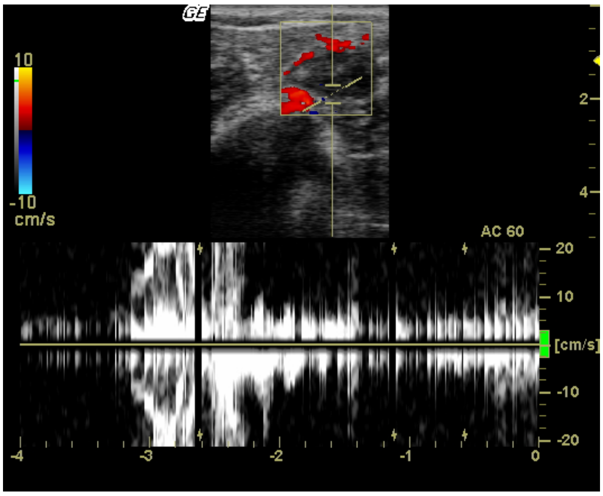

Figure 2. Representative pulse wave Doppler ultrasonogram of the testicular artery in bulls

EDV followed a similar pattern showing a progressive reduction from proximal to distal arterial segments with higher values recorded in proximal sTA and significantly lower but comparable and stable values in the distal segments (Table 1). When averaging bilateral measurements, overall mean EDV was 5.09 ± 0.16 cm/s for sTA, 3.83 ± 0.02 cm/s for mTA, and 3.72 ± 0.03 cm/s for iTA. Additionally, PSV and EDV were positively correlated ($r = 0.71$, $P < 0.05$), indicating the coordinated regulation of systolic inflow and sustained diastolic perfusion within the testicular arterial network.

The combined mean resistance and perfusion indices across three testicular arterial segments are presented in Table 2. The resistive index (RI) also demonstrated a progressive and significant decline ($P < 0.05$) from the spermatic cord portion to distal arterial segments. Importantly, RI demonstrated a considerably lower individual variability compared to PSV and EDV. Within-bull coefficient of variation for RI measurements at the same location was less than 10%, indicating good repeatability. The S/D ratio (PSV/EDV) decreased progressively from proximal sTA segment to distal mTA and iTA segments (Table 2). Overall, mTA and iTA had lower RI (~0.24) and S/D ratio (~1.32), indicating lower vascular resistance and more continuous flow, typical of deeper sustained parenchymal perfusion.

In addition to RI, pulsatility index (PI) values showed a progressive decline along the course of the testicular artery with higher values recorded

Table 1. Bilateral comparison of testicular perfusion parameters measured at different segments of testicular artery in Shweta Kapila bulls ($n = 8$)

Parameter	Testis	sTA (mean ± SEM)	mTA (mean ± SEM)	iTA (mean ± SEM)
PSV (cm/s)	right	12.32 ± 0.36^a	5.11 ± 0.02^b	4.93 ± 0.03^b
	left	11.70 ± 0.34^a	4.94 ± 0.03^b	5.02 ± 0.02^b
EDV (cm/s)	right	5.14 ± 0.17^a	3.91 ± 0.03^b	3.80 ± 0.03^b
	left	5.03 ± 0.16^a	3.75 ± 0.02^b	3.63 ± 0.03^b
RI	right	0.58 ± 0.02^a	0.23 ± 0.01^b	0.24 ± 0.01^b
	left	0.57 ± 0.02^a	0.24 ± 0.01^b	0.28 ± 0.01^b

Values are presented as mean ± SEM

[a,b]Means bearing different superscripts within a row differ significantly ($P < 0.05$)

EDV = end diastolic velocity (cm/s); iTA = intra-testicular branches of the testicular artery; mTA = marginal region of the testicular artery; PSV = peak systolic velocity (cm/s); RI = resistive index; sTA = spermatic cord portion of the testicular artery

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Table 2. Mean resistance indices and perfusion metrics across three testicular arterial segments in Shweta Kapila bulls (n = 8)

Region	RI	S/D ratio	TAMAX (cm/s)	PI
sTA	0.58 ± 0.02^{a}	2.36 ± 0.07^{a}	6.59 ± 0.21^{a}	1.05 ± 0.03^{a}
mTA	0.235 ± 0.01^{b}	1.31 ± 0.05^{b}	1.46 ± 0.02^{b}	0.82 ± 0.01^{b}
iTA	0.26 ± 0.01^{b}	1.34 ± 0.05^{b}	1.81 ± 0.02^{b}	0.68 ± 0.01^{b}

^{a,b}Values bearing different superscripts within a column indicate significant differences (P < 0.05)

Values represent the mean of right and left testicular measurements

PI = pulsatility index; RI = resistive index; S/D ratio = systolic/diastolic ratio; TAMAX = time-averaged maximum velocity (cm/s)

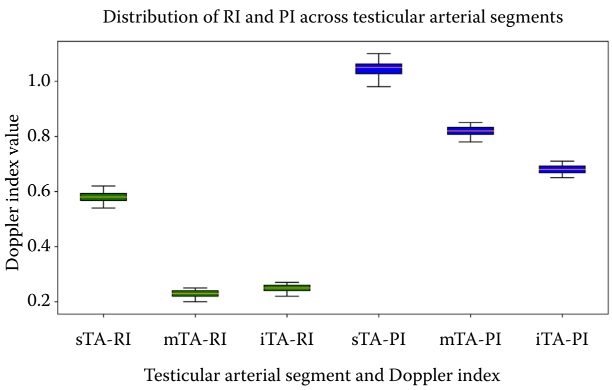

Figure 3. Combined boxplot illustrating the distribution of resistive index (RI; green boxes) and pulsatility index (PI; blue boxes) in different testicular arterial segments of Shweta Kapila bulls assessed using pulse wave Doppler ultrasonography

in the proximal sTA and lower values in the distal segments (Figure 3). When averaging bilateral measurements, PI was 1.05 ± 0.03 for sTA, 0.82 ± 0.01 for mTA, and 0.68 ± 0.01 for iTA. No significant bilateral differences (P > 0.05) were observed for PI values between the right and left testis. Interestingly, pulsatility index (PI) demonstrated a positive correlation with RI (r = 0.78, P < 0.05), confirming their shared dependence on distal vascular impedance.

DISCUSSION

This study provides the first comprehensive assessment of testicular perfusion pattern and haemodynamics in indigenous Shweta Kapila bulls using digital pulse wave Doppler ultrasonography. The findings also establish baseline reference values for testicular perfusion and demonstrate the potential clinical utility of this technique in native cattle reared under coastal tropical conditions. The predominantly monophasic, low-resistance waveform pattern observed in Shweta Kapila bulls is consistent with findings reported in other ruminant species (Gonzalez-Bulnes et al. 2010; Samir et al. 2015; Hedia et al. 2020; Velasco and Ruiz 2020; Elbaz et al. 2022). This contrasts with the biphasic, resistive waveforms reported in stallions (Pozor and McDonnell 2004), likely reflecting differences in testicular positioning and vascular anatomy between species. In the present study, PSV was significantly higher (P < 0.05) in the proximal sTA compared to the distal segment, consistently with the high-resistance proximal flow pattern. The mean PSV values of Shweta Kapila bulls fall within the range reported for other cattle breeds, though direct comparisons are limited by breed differences and methodological variations. Junior et al. (2018) reported breed-specific differences in the mean velocity between Aberdeen Angus, Brangus, and Nellore bulls, with values ranging from approximately 13 cm/s to 18 cm/s while Favaro et al. (2020) found the mean velocities of 7.53 cm/s to 13.63 cm/s in Braford bulls depending on seasonal factors. An individual bull variation in PSV was notable, particularly in the sTA segment where values ranged from 8.59 cm/s to 14.41 cm/s among bulls. Gloria et al. (2018) also demonstrated consistent regional variations in testicular blood flow velocities in bulls. This variability might be attributed to the convoluted course of the vessel at this location, making the precise angle correction challenging despite the careful probe positioning.

In the present study, the mean EDV values followed a progressive decline from sTA but the values were stable across the marginal parts (mTA) and intra-testicular part of the testicular artery (iTA). The sustained diastolic flow within distal segments of testicular arteries indicates optimal nutrient and

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oxygen delivery to seminiferous tubules. The substantially higher PSV and EDV values in the sTA compared to distal segments likely reflect the measurement of the main arterial trunk before extensive branching. The convoluted course of the sTA presents technical challenges for Doppler angle correction, contributing to the higher individual variability observed at this location. This finding supports previous recommendations that distal arterial segments may provide more reliable and repeatable measurements (Gloria et al. 2018).

The observed variability in PSV and EDV measurements in the sTA of Shweta Kapila bulls is consistent with findings reported by Bollwein et al. (2006), who highlighted the importance of individual baseline values due to a considerable inter-animal variation in the testicular blood flow. Their findings suggest that a comprehensive vascular assessment incorporating multiple parameters may provide more reliable information about the testicular function. The progressive reduction in PSV and EDV observed in our study aligns with findings in bulls (Gloria et al. 2018) and other species (Trautwein et al. 2019) and this phenomenon reflects on fluid dynamics in branching vascular systems. The positive correlation between PSV and EDV further indicates that systolic inflow and diastolic perfusion are dynamically coordinated within the testicular vascular network. As the testicular artery divides into smaller branches, the total cross-sectional area increases, resulting in reduced flow velocity and low-resistance perfusion and this arrangement optimises the nutrient and gas exchange at the capillary level.

The RI values of Shweta Kapila bulls are comparable to those reported in other breeds. The observed gradient of decreasing RI from sTA to iTA corroborates previous findings by Pozor (2007) and Favaro et al. (2020), highlighting the progressive reduction in vascular resistance along the arterial tree. Interestingly, low and stable RI values in marginal and intra-testicular segments reflect adaptive vascular modulation and efficient perfusion essential for spermatogenesis and thermoregulation in humid climates (Menegassi et al. 2015). Doppler ultrasonography provides an instantaneous, non-invasive assessment of the testicular vascular dynamics, potentially detecting the subclinical dysfunction before affecting the semen quality – a limitation of traditional breeding soundness examination (Elbaz et al. 2019). Notably, RI demonstrated a remarkably low within-bull variability compared to velocity parameters, consistent with observations across species (Pozor and McDonnell 2004; Pinggera et al. 2008). This low variability suggests that significant deviations from baseline RI may indicate pathological changes. Interestingly, the lower RI value correlates with better thermoregulation, enhanced oxygen delivery, and improved sperm motility and testosterone levels (Ortiz-Rodriguez et al. 2017), while elevated RI corresponds to degenerative changes (Gloria et al. 2020), reinforcing its diagnostic potential. Independence of the RI value from the insonation angle also enhances its clinical utility as a reliable indicator of testicular perfusion and for early identification of subclinical and perfusion-related dysfunctions.

A higher S/D ratio indicates greater resistance in the downstream vascular bed, as a lower end-diastolic velocity (EDV) relative to peak systolic velocity (PSV) suggests restricted flow. In the present study, the S/D ratio decreased progressively from proximal sTA segment to distal mTA and iTA segments (Table 2), indicating a transition from the high-resistance conduit flow to the sustained and deeper parenchymal perfusion crucial for thermoregulation and spermatogenesis under tropical conditions. The PI values in the present study further support the physiological integrity of testicular perfusion in Shweta Kapila bulls. The highest PI was recorded in the sTA segment, followed by the mTA segment, while the lowest PI was observed in the intra-testicular segment (Figure 3). Higher PI with greater dispersion in the spermatic cord region reflects its role as a high-resistance conduit vessel, whereas the lower and more stable PI values in the distal segments (0.68–0.82) indicate reduced downstream resistance and sustained diastolic perfusion at the parenchymal level. Doppler parameters can vary along different segments of the testicular artery, with progressive reductions in vascular resistance observed from the supra-testicular to intra-testicular portions (de Souza et al. 2014). Similar arterial gradients in PI have been reported in bulls, stallions and rams, where low intra-testicular PI values were associated with efficient spermatogenic activity and normal testicular function (Zelli et al. 2013; Ortiz-Rodriguez et al. 2017). The relatively low intra-testicular PI values observed in bulls reared under coastal climatic conditions also suggest effective vascular adaptation and thermoregulatory efficiency in this indigenous breed.

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Overall, no statistically significant differences were observed between the right and left testis for any Doppler parameter at any measurement location ($P > 0.05$), suggesting normal symmetric testicular perfusion in healthy Shweta Kapila bulls. The absence of significant differences in Doppler parameters between the right and left testis is consistent with most published studies in bulls (Gloria et al. 2018; Claus et al. 2019). However, some studies in dogs have reported laterality effects, potentially related to anatomical differences in the arterial origin and course (de Souza et al. 2014). Collectively, these findings establish the first baseline reference values for testicular perfusion and haemodynamics in indigenous Shweta Kapila cattle. Pulse wave Doppler ultrasonography represents a valuable, non-invasive and real-time tool for evaluating vascular function and reproductive soundness in cattle reared under coastal tropical climate. Further research involving larger sample sizes and comparative studies between fertile and sub-fertile bulls would define diagnostic thresholds and provide more robust reference ranges. Longitudinal studies on individual bulls over extended periods would also allow an assessment of age-related changes in testicular perfusion and provide deeper insights into thermoregulatory mechanisms and seasonal effects in this breed.

CONCLUSION

This study provides the first comprehensive characterisation of testicular blood flow in the indigenous Shweta Kapila breed of cattle using digital pulse wave Doppler ultrasonography and establishes baseline reference values for testicular perfusion and haemodynamics in this breed under coastal tropical conditions. Velocity parameters exhibited a greater variability in the proximal segment, likely influenced by anatomical convolution and angle correction, whereas the low and stable intra-testicular RI values suggest effective vascular modulation and adaptive thermoregulation in this breed under coastal tropical climate. Collectively, these findings support the utility of the Doppler-based testicular haemodynamic assessment for reproductive evaluation protocols and provide a foundation for future studies investigating perfusion-related fertility indicators and environmental adaptability in tropical cattle breeds.

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Conflict of interest

The authors declare no conflict of interest.

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