

VARICOSITY AND PREGNANCY INDUCED BLOOD FLOW CHANGES IN THE CRANIAL TIBIAL VEIN IN BUFFALOES: B-MODE AND DOPPLER ULTRASOUND STUDY

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ABSTRACT

The study was aimed to assess the effect of varicosity and pregnancy on the blood flow parameters in 22 cranial tibial veins of 17 buffaloes using B-mode and Doppler ultrasonography. Out of these, five buffaloes were suffering from unilateral hind limb varicosity and 12 were clinically healthy non-pregnant (n=6) or in advanced stages of pregnancy (n=6). The study highlights a significant increase in the Doppler blood flow parameters of varicosity affected veins in comparison to clinically healthy cranial tibial veins, with highest percentage change in volume flow/minute. A significant lower vessel diameter and end diastole velocity of the cranial tibial vein was also recorded in healthy advanced pregnant compared to that of healthy non-pregnant buffaloes.

Keywords: ultrasound, varicose, bovine, saphenous, pregnant, cranial tibial

INTRODUCTION

Varicosity is the condition of veins which may develop on the tail (Tyagi and Singh, 2001; Kulkarni *et al.*, 2005), teat and udder (Tyagi and Singh, 2001; Rambabu *et al.*, 2009; Larde *et al.*,

2013), fore limb, hind limb, vulva, and scrotum in bovine animals (Tyagi and Singh, 2001). Clinically, varicose veins may appear enlarged, twisted and tortuous. The etiopathogeny of varicose veins is not clearly defined but, as per the medical literature, it may be congenital or pregnancy induced and is more commonly reported in females than in males (Barros Junior *et al.*, 2010). Persistent pressure on the walls of the vein, make them stretched and less flexible. This leads to weakening of valves which causes leaking of blood backward, stasis and dilation of vein resulting in varicosity.

Anatomically, the cranial tibial vein (CTV) is joined by dorsal pedal and the dorsal metatarsal vein. Proximally, CTV joins with caudal tibial vein and drains blood into the femoral vein along with the saphenous vein in bovine (Pasquini *et al.*, 2003). Although varicosity of cranial tibial vein and its lower veins has been reported in buffaloes (Tyagi and Singh, 2001), but scanty work to study its pathogenesis or treatment has been done. In humans, Doppler ultrasonography of varicose veins is done to study the pattern of incompetence at superficial and deep venous junctions, incompetent perforators and their distribution to aid in the selection of surgical or non-surgical treatment of the patients (Irodhi *et al.*, 2011). Medical literature correlates limb varicosity with pregnancy (Barros Junior *et al.*, 2010). Limb varicosity in buffalo is

sometimes life threatening as the affected vein might get injured or burst due to high pressure of blood in it. The preliminary work on the objective documentation of varicosity affected cranial tibial vein could be of value in assessing severity of disease by determining the changes in the blood flow patterns as compared to that of normal vein. As per author's knowledge, there is paucity of literature on the B-mode and Doppler ultrasonography of normal or varicosity affected cranial tibial vein in bovine animals. Considering the above facts, the present study was planned with the following objectives:

1. To evaluate the difference in the Doppler blood flow parameters of varicosity affected cranial tibial vein and the contra lateral healthy vein of the varicosity affected buffaloes.

2. To evaluate the effect of pregnancy on the blood flow parameters of cranial tibial vein.

3. To compare the change in the blood flow parameters of the contra lateral clinically healthy CTV of varicosity affected buffaloes and clinically healthy non-varicosity affected buffaloes.

MATERIALS AND METHOD

Twenty-two CTVs were evaluated using B-mode and Doppler ultrasonography in 17 adult she-buffaloes. The study included: CTV of clinically healthy non-gravid buffaloes (Group 1, n=6), clinically healthy gravid (third trimester) buffaloes (Group 2, n=6), varicosity affected gravid buffaloes (Group 3a, n=5) and normal contra lateral CTV of varicosity affected gravid buffaloes (Group 3b, n=5). The buffaloes in Group 3 were suffering from unilateral varicosity in hind limb (Figure 1). Signalment, history of trauma and clinical examination findings were recorded.

Radiography of the metatarsal region was done in buffaloes having history of injury. In Group 3 buffaloes, lateral aspect of both limbs, from mid tibia to mid metatarsal region was prepared for ultrasonography (Figure 2). Similar site of the right hind limb was prepared for ultrasonography in Group 1 and 2 buffaloes. The buffaloes were restrained in a cattle crate in standing position. The B mode and Doppler ultrasonography was done using a 7 to 12 MHz linear multi-frequency transducer on Wipro Logiq III expert ultrasound machine with in-built function for color flow and pulsed wave Doppler.

The B-mode scanning was aimed to visualize the morphology of the vein and valves. The vein was scanned in the longitudinal view and the color-flow mode was switched-on to visualize the flow of blood within the vessel lumen. Gain and pulse repetition frequency (PRF) adjustments were made to minimize color flow aliasing. The MD cursor was placed at the site where pulse Doppler was desired. When the flow was visualized, the pulsed-wave mode was switched-on. The angle of incidence was kept at 60° and the other parameters such as sample volume and PRF were set, as required. A mean of three values, taken at three different sites in the distal tibial region, were used for statistical analysis. Variables of Doppler study including peak systole (PS) cm / s, end diastole (ED) cm / s, TA Max cm/s, pulsatility index (PI), resistive index (RI), acceleration (Acce) cm / s² and TA Mean cm/s were automatically determined by the machine software upon freezing the frame. The diameter of the vessel from the intima to the intima and distance of the vessel from the transducer was determined electronically using in-built caliper of the machine. The diameter of the vessel was further used by the machine software to calculate volume flow in ml/minute.

The results of Doppler ultrasonography in Group 1, 2, 3a and 3b were statistically analyzed for mean and standard deviation (SD) using SPSS 16.0 software. Difference between the values of Doppler parameters of vessels in Group 1 and 2, Group 3a and 3b, Group 1 and 3b, Group 2 and 3b were tested using 2-tailed Student's t-test at 0.05 and 0.01 level of significance (p). The percent change in the Doppler values of varicosity affected and its contra lateral vein was also calculated.

RESULTS AND DISCUSSION

The buffaloes of Group 3 had a mean \pm SD body weight 538 ± 59.75 kg (range 450 to 660 kg) which was comparable to that of buffaloes in Group 1 and 2 (Table 1). The buffaloes in Group 3 were adult, aged between 6 and 10 year and were in third trimester of pregnancy. Feeding, urination and defecation were normal in all the buffaloes. The duration of varicosity ranged from one month to one year. Three buffaloes had a history of injury in the affected limb. Clinical examination revealed involvement of cranial tibial, dorsal pedal and dorsal metatarsal vein, extending up to mid tibial region. The affected limbs were painful on palpation and blood could be felt gushing up and down in the veins.

The skin was hard, dry and keratinized in the ventral portion of the limb, but in between the folds of the bulge, it was moist. In humans also venous hypertension is associated with skin pigmentation, lipodermatosclerosis and ulcerations (Irodhi *et al.*, 2011). The buffaloes showed partial weight bearing at rest and mild lameness from affected limb while walking. The veins were more engorged and tortuous, in the lower limb near hoof, leading to bulging of skin. This bulging

had led to a moist area under the fold, which was infested with maggots in four buffaloes. Maggots had lead to kicking and irritation in the buffaloes, thus injuring the limb further. The varicosity was limited to hind limbs only, but not to same side of the body. Buffaloes of Group 3a had varicosity in left (n=3) or in right hind limb (n=2). Lateral radiograph of the metatarsal region revealed mild periosteal reaction (n=2) or lytic changes and cortical thickening (n=1).

Medical literature supports multiple theories for the occurrence of limb varicosity with pregnancy and its disappearance with dead fetus or delivery (Barros Junior *et al.*, 2010). But, in buffaloes, limb varicosity is usually huge and the animals are always in some stage of pregnancy except for a few weeks in a year. Moreover, the telephonic follow up did not reveal resolution of varicosity, though some improvement in the wounds was noticed after calving. Mechanical compression of the uterus on the iliac veins and inferior vena cava, especially in the last trimester of pregnancy, hormonal theory, increase in pelvic circulation during pregnancy, hereditary predisposition, structural alterations in the wall of vessel and venous valve alterations are some of the possible etiologies for the limb varicosity explained in medical literature. Etiology of varicose veins may be post thrombotic or congenital or acquired arteriovenous fistulae (Barros Junior *et al.*, 2010). However, in the present study, the etiology could not be ascertained as the buffaloes were not kept with the same owner throughout the life and were presented in the advanced stage of varicosity. But, history of trauma in three buffaloes of this study suggests the condition may be secondary or acquired.

Table 1. Comparison of signalment and Doppler ultrasonography variables (Mean \pm SD) of cranial tibial vein in healthy non-gravid (Group 1), healthy gravid (Group 2), varicose (Group 3a) and normal contra lateral vein of varicosity affected buffaloes (Group 3b).

S. No.	Variables	Group 1(n=6)	Group 2(n=6)	Group 3b(n=5)	Group 3a (n=5)	% Change in Group 3a compared to 3b
		Mean \pm SD Range	Mean \pm SD Range	Mean \pm SD Range	Mean \pm SD Range	
1	Body weight (Kg)	495.00 \pm 55.77 (450.00 to 600.00)	571.83 \pm 84.29 (450.00 to 650.00)	538.00 \pm 59.75 (450.00 to 660.00)	538.00 \pm 59.75 (450.00 to 660.00)	Not applicable
2	Gestation period (Months)	Not applicable	6.67 \pm 1.03 (6.00 to 8.00)	6.40 \pm 1.34 (5.00 to 8.00)	6.40 \pm 1.34 (5.00 to 8.00)	Not applicable
3	Age (Year)	5.50 \pm 1.05 (4.00 to 7.00)	5.17 \pm 1.17 (4.00 to 7.00)	7.80 \pm 1.48 (6.00 to 10.00)	7.80 \pm 1.48 (6.00 to 10.00)	Not applicable
4	Peak Systole velocity (PS) cm / s	10.36 \pm 4.27 (5.46 to 15.91)	8.04 \pm 2.39 (5.90 to 12.28)	14.76 \pm 5.09 ^s (9.55 to 21.19)	127.01 \pm 45.12 ^{**} (52.69 to 166.26)	760.50
5	End Diastole velocity (ED) cm / s	8.19 \pm 3.39 (4.75 to 13.06)	4.28 \pm 1.68 [^] (2.38 to 6.39)	8.45 \pm 4.06 (4.07 to 12.43)	76.45 \pm 28.29 ^{**} (26.68 to 97.72)	804.73
6	TA max cm / s	7.88 \pm 5.05 (1.80 to 14.34)	5.02 \pm 2.01 (2.41 to 8.57)	9.37 \pm 5.58 (3.31 to 14.59)	91.56 \pm 32.59 ^{**} (36.65 to 123.49)	877.16
7	Pulsality Index (PI)	1.97 \pm 2.01 (0.27 to 4.86)	2.45 \pm 1.20 (0.98 to 4.34)	1.55 \pm 1.24 (0.43 to 2.90)	1.04 \pm 0.63 (0.45 to 2.02)	-33.55

* Significant difference between Group 3a & 3b, at p<0.05, ** Significant difference between Group 3a & 3b, at p<0.01.

[^] Significant difference between Group 1 & 2 at p<0.05, ^s Significant difference between Group 2 & 3b at p<0.05,

^{††} Significant difference between Group 1 & 3b at p<0.01

Table 1 (continued). Comparison of signalment and Doppler ultrasonography variables (Mean \pm SD) of cranial tibial vein in healthy non-gravid (Group 1), healthy gravid (Group 2), varicose (Group 3a) and normal contra lateral vein of varicosity affected buffaloes (Group 3b).

S. No.	Variables	Group 1 (n=6)		Group 2 (n=6)		Group 3b (n=5)		Group 3a (n=5)		% Change in Group 3a compared to 3b
		Mean \pm SD Range	Mean \pm SD Range	Mean \pm SD Range	Mean \pm SD Range	Mean \pm SD Range	Mean \pm SD Range			
8	Resistive Index (RI)	0.79 \pm 0.79 (0.17 to 1.87)	0.70 \pm 0.25 (0.37 to 1.11)	0.46 \pm 0.12 (0.31 to 0.58)	0.52 \pm 0.21 (0.31 to 0.82)				13.04	
9	Acceleration cm / s ²	30.81 \pm 33.48 (6.78 to 98.11)	112.99 \pm 135.84 (22.28 to 348.25)	38.68 \pm 8.64 (27.59 to 50.04)	927.27 \pm 647.73* (219.37 to 1720.92)				2297.29	
10	TA mean in cm / s	3.89 \pm 2.33 (0.83 to 7.42)	2.57 \pm 0.99 (0.94 to 3.97)	4.22 \pm 3.47 (0.05 to 7.58)	53.71 \pm 18.45** (23.02 to 69.45)				1172.75	
11	Volume flow in ml / min	116.03 \pm 89.17 (30.51 to 280.09)	51.57 \pm 25.97 (17.60 to 93.99)	62.57 \pm 61.64 (0.74 to 132.06)	5489.47 \pm 4594.95* (1647.45 to 11908.94)				8673.33	
12	Vessel diameter in cm	0.74 \pm 0.11 (0.62 to 0.90)	0.59 \pm 0.14 [^] (0.41 to 0.79)	0.57 \pm 0.062 ^{††} (0.47 to 0.63)	1.58 \pm 0.56* (1.04 to 2.31)				177.19	
13	Distance of vessel from the transducer in cm	0.77 \pm 0.38 (0.16 to 1.34)	0.68 \pm 0.20 (0.46 to 1.00)	0.67 \pm 0.10 (0.57 to 0.63)	0.62 \pm 0.13 (0.50 to 0.84)				-7.46	

* Significant difference between Group 3a & 3b, at p<0.05, ** Significant difference between Group 3a & 3b, at p<0.01.

[^] Significant difference between Group 1 & 2 at p<0.05, [§] Significant difference between Group 2 & 3b at p<0.05,

^{††} Significant difference between Group 1 & 3b at p<0.01

B-mode ultrasonography

In standing position, the CTV in Group 3a, was superficial and distended, but, in Group 3b, 2 and 1 it was mildly palpable at the distal end only. The lateral aspect of CTV was scanned for ultrasonography, as the lower limb was very painful. The CTV of Group 3a was in-collapsible than that of other groups. Maximum of three valves were seen in the Group 3a, which were very flaccid and were lying close to the vessel wall with mild fluttering (incompetent valves). The valves were not able to close the vessel lumen completely. The number of valves visualized in Group 3b, 1 and 2 ranged from 1 to 3 with only one valve seen in 12 out of 17 CTVs. The increase in the lumen diameter and the in-collapsible nature of the vein in Group 3a might be the reasons for better visualization of valves compared to that of Group 1, 2 and 3b. Studies on human patients reveal agenesis or hypoplasia of the iliac-femoral valve, which supports the hydrostatic pressure of a blood column from the heart to the inguinal region, may result in unilateral varicosity (Junior *et al.*, 2010). The failure of check valves in the perforating veins, allows high pressure generated in the deep veins by the muscular contractions to be transmitted directly to the unsupported superficial veins (Irodhi *et al.*, 2011).

The mean \pm SD vessel diameter of Group 3a buffaloes was 1.58 ± 0.56 cm (range 1.04 to 2.31) which was significantly ($p=0.012$) higher than that of Group 3b (Figure 3). A 177.19 % change in vessel diameter of CTV in Group 3a than that of Group 3b was recorded (Table 1). The CTV wall was significantly ($p=0.008$) thicker in Group 3a (0.31 ± 0.029 cm) than that of Group 3b (0.08 ± 0.02 cm) due to keratinization of the wall. Sacculations were seen in the varicose vein while a smooth, uniform diameter was observed in Group 3b, 1 and 2

buffaloes. Chronic stasis of blood leads to dilatation and sacculations in the affected vein resulting in the increase in wall thickness and diameter of the vein (Junior *et al.*, 2010). No echogenic mass or thrombi within the vessel lumen were detected in any of the varicosity affected CTV. Thrombosed tarsal vein in cattle due to metastatic abscessation has also been reported to be incompressible and had an increase in vessel diameter which progressively reduced to normal within 5 month (Kofler *et al.*, 1996; Kofler and Kubber-Heiss, 1997).

The vessel diameter of healthy CTV in advanced pregnant buffalo of Group 3b ($p=0.009$) and Group 2 ($p=0.058$) was found to be significantly less than that of non-pregnant buffaloes in Group 1 (Figure 3). This decrease in CTV diameter was in contrast to Boivin *et al.* (2000) findings in human females where the diameter of competent and incompetent veins increased in third trimester compared to first trimester which returned back to initial values in puerperium. This could be attributed to the difference in the standing posture of humans (bipedal) and bovine animals (quadrupedal).

Color Doppler ultrasonography

Venous reflux with a mixture of red and blue color was visualized in CTV of Group 3a (Figure 4), while minimum or no color flow was observed in CTV of Group 3b, 1 and 2. Venous reflux is the retrograde flow of blood in the veins caused by absent or incompetent valves (Irodhi *et al.*, 2011). Even the spectral flow was missing in certain veins of healthy animals which were not included in the present study. It was noticed that partial weight bearing improved the blood flow in healthy CTV (Figure 5). In human patients also, the Doppler ultrasound examination of the limb veins is preferred in standing position, while supporting weight on the contra lateral extremity (Irodhi *et al.*,

2011). But, in buffaloes it was difficult to make the animal support weight on one hind limb.

Differences in the values of doppler parameters of CTV between various groups

All the velocities, PS ($p=0.005$), ED ($p=0.006$), TA max (0.005), Acce ($p=0.036$) and TA mean ($p=0.004$) were recorded to be significantly higher in the vein of Group 3a than that of Group 3b (Figure 6). The volume flow/minute in the vein of Group 3a was significantly higher ($p=0.035$) than in Group 3b which was also highest in percent change (8673.33%) among all the Doppler variables recorded in the present study (Figure 7). Detailed mean \pm SD Doppler values with percent change are given in Table 1.

A significant increase in ED ($p=0.038$)

and vessel diameter ($p=0.058$) was observed in the CTV of Group 1 than that of Group 2 which might be related delayed emptying of the blood due to pregnancy and less vessel diameter. However, except vessel diameter no significant change was observed in the Group 1 and 3b.

Among pregnant buffaloes (Group 3b and 2), only PS was found to be significantly ($p=0.038$) higher in Group 3b however, other Doppler variables were comparable.

Along with the tibial, the dorsal pedal and the dorsal metatarsal veins are more affected being more ventrally located. Till now surgical intervention is not reported as a treatment modality in varicosity of cranial tibial vein in buffaloes. Though, varicosity of coccygeal veins can be treated through tail amputation at an early stage



Figure 1. Photograph showing hind limb varicosity in a buffalo.

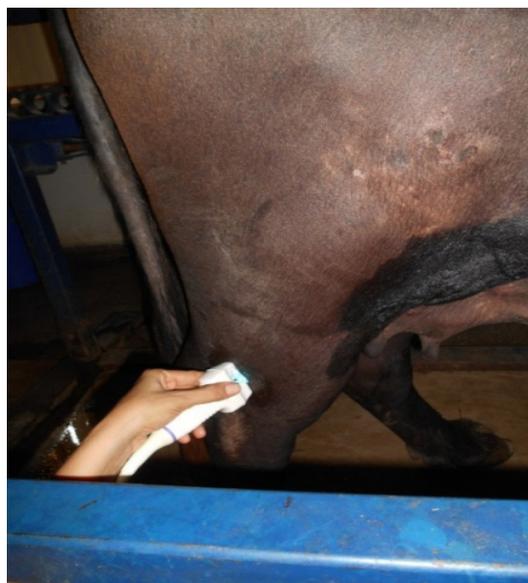


Figure 2. Photograph showing localization of CTV in buffalo.

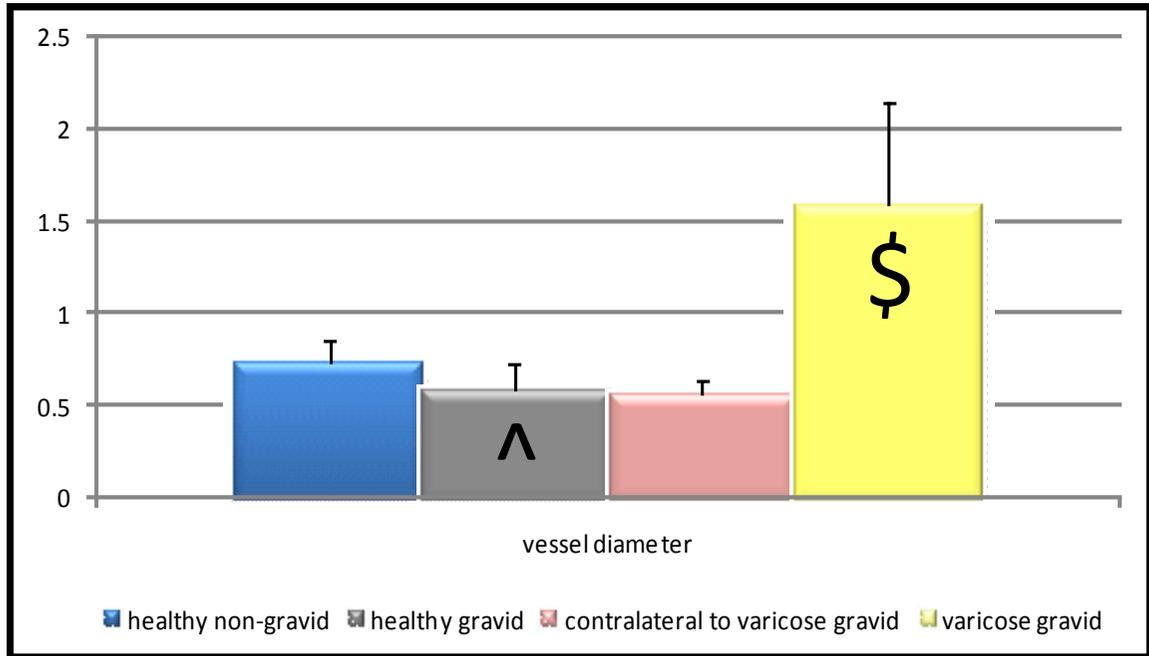


Figure 3. Bar graph showing comparison between the vessel diameter of CTV in buffalo. (^= significance between healthy gravid and non-gravid at $P < 0.05$, \$ = significance between varicose and contralateral healthy CTV at $P < 0.05$).

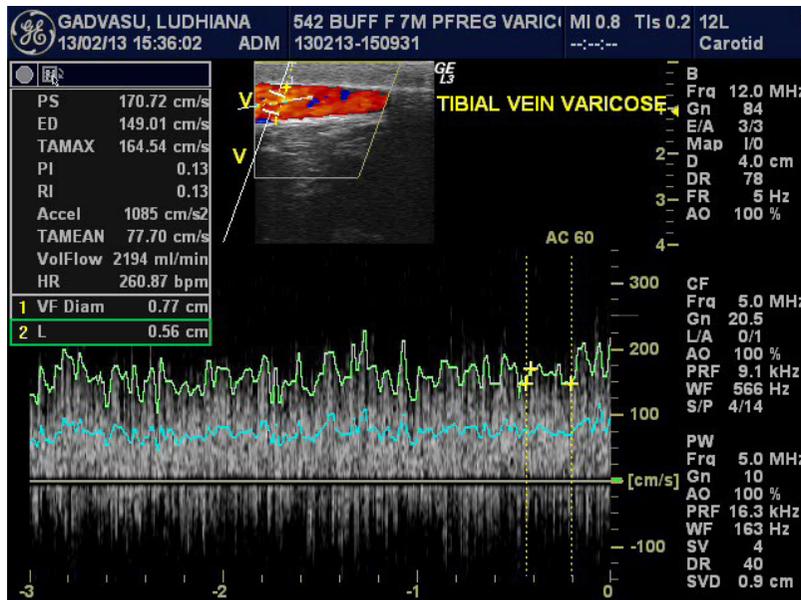


Figure 4. Spectral display showing Doppler scan of varicose CTV in a buffalo.

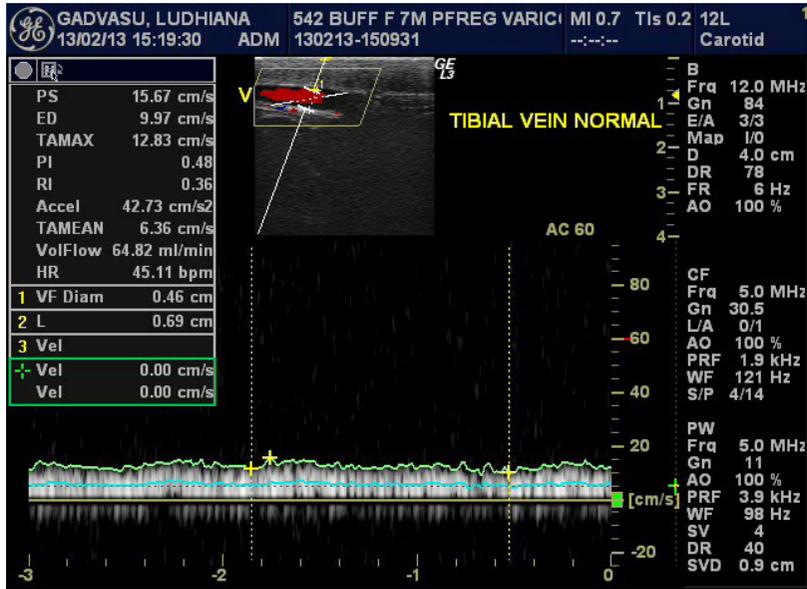


Figure 5. Spectral display of healthy CTV (contra lateral to the varicose CTV) in a buffalo.

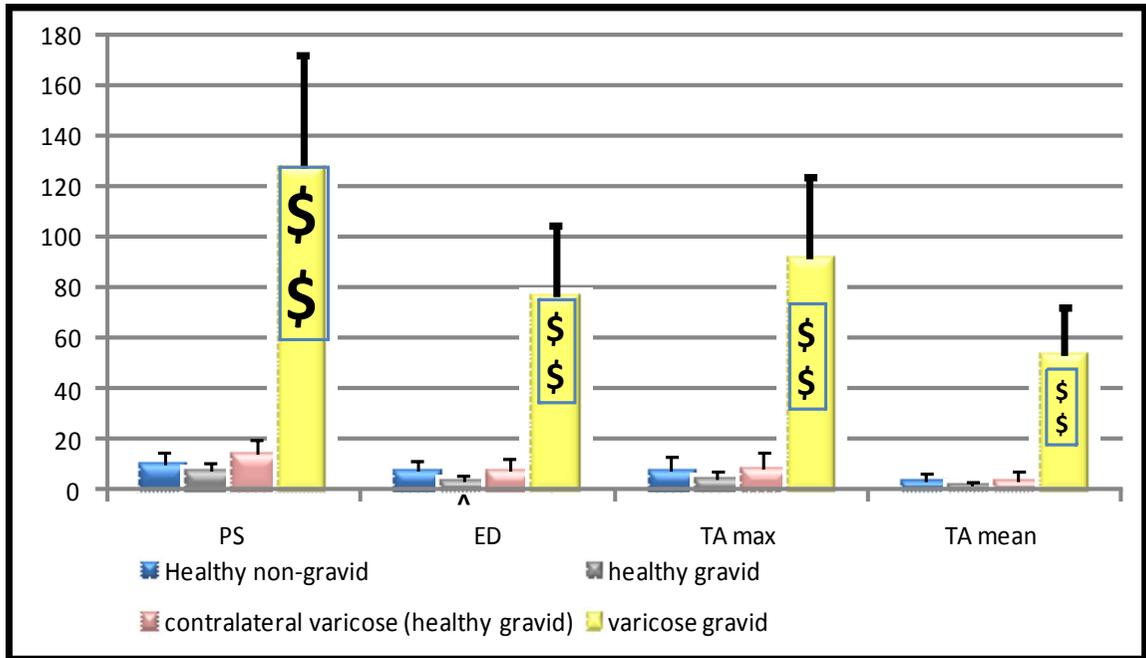


Figure 6. Bar graph showing comparison between the velocities of CTV in buffalo. {^= significance at 5% between healthy gravid and non-gravid, \$\$= significance at 1 % level of significance between varicose and healthy CTV (gravid, non-gravid and contra lateral)}.

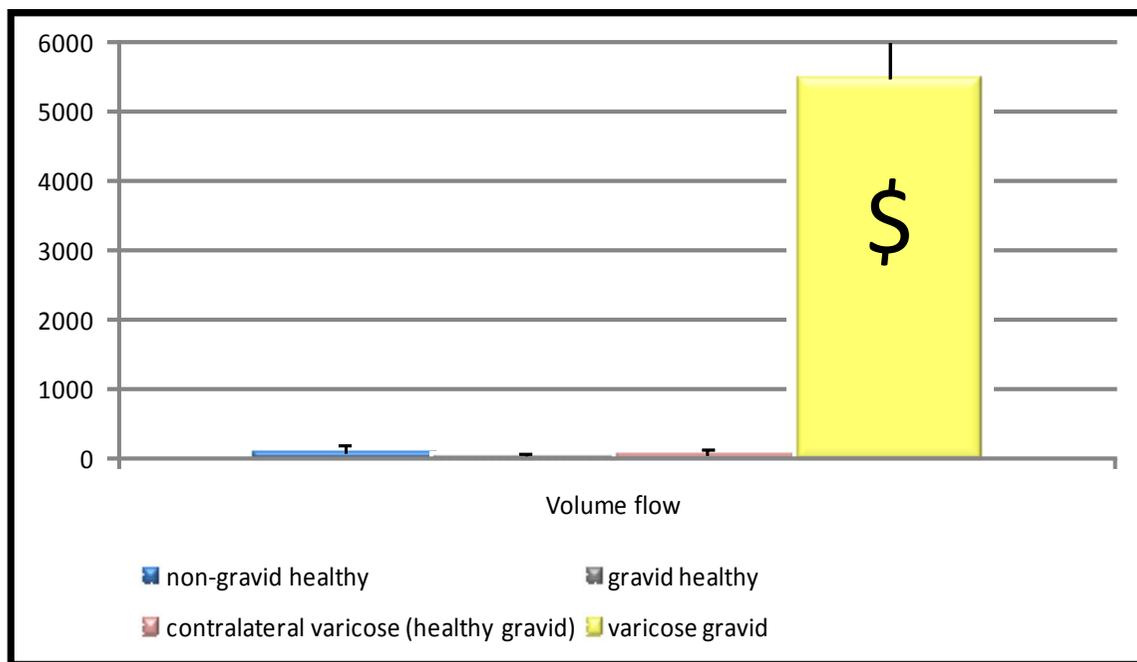


Figure 7. Bar graph showing comparison between the volume flow of CTV in buffalo. (\$= significance at 5% level of significance between varicose and healthy CTV (gravid, non-gravid and contra lateral).

in buffaloes. In medical science, there are various modalities available for the treatment of varicosity. In humans long standing jobs are considered to be an inciting cause for varicosity. Now days in India, buffaloes are not left loose for pasture feeding; but are tied to one post and have very little space for movement. Long standing posture, pregnancy, injury to the limb or any congenital defect may be the reason for varicosity. The study highlights that since, a large volume of blood is stacked in the varicosity affected limb and the animal is in continuous pain and irritation, there is a need to understand the predisposing factors and etio-pathogenesis of the condition and to formulate effective preventive or therapeutic protocol for varicosity of CTV in buffaloes.

CONCLUSION

The study concludes that pregnancy lowered the diameter and ED of CTV in buffaloes. A significant increase in Doppler blood flow parameters occurred in varicosity affected CTVs in comparison to clinically healthy veins in buffaloes.

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