



## Echocardiographic morphometry in healthy Bovine calves of upto 15 days of age

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### ABSTRACT

The present study was aimed to compare normal echocardiographic data in upto 15 days' old cow and buffalo calves. The study included 26 healthy calves; 12 buffalo and 14 cow calves. The calves were clinically examined and auscultated for healthiness of chest and heart. Most of the calves included were females {64.28% (n=9) cow and 91.67% (n=11) buffalo calves}. Most of the buffalo calves were cross bred of Murrah, while the cow calves included Sahiwal (n=3), Rathi (n=1) and crossbreds of Holstein Friesian (n=9) and Jersey (n=1). The average body weight of buffalo calves ( $28.25 \pm 6.59\text{Kg}$ ) was non-significantly higher than the cow calves ( $25.57 \pm 1.9\text{Kg}$ ). The echocardiography was done from right side in both long and short axis. The various parameters recorded in right parasternal short axis view were; Interventricular septum thickness in systole and diastole, Left ventricular internal diameter in systole and diastole, Left ventricular posterior wall thickness in systole and diastole, Right ventricular internal diameter in diastole, End Point Septal Separation and Right atrial diastole diameter. The calculated parameters from short axis view included the fractional shortening and ejection fraction. The parameters recorded in right parasternal long axis view included; left atrium and aorta diameter in diastole and its ratio, the blood flow velocity at the aorta and mitral valve. A significantly higher left and right atrial diameter in diastole was recorded in buffalo calves in comparison to cow calves. However, the inter-ventricular wall thickness in diastole and the left ventricular posterior wall thickness in systole were significantly lower in buffalo calves in comparison to cow calves. The average left atrium to aorta ratio in diastole was 1.3 in both the species. The study concludes that a data base for echocardiographic morphometry was generated for upto 15 days old healthy bovine calves, which in future might help in the diagnosis of congenital heart defects in bovine calves. The study revealed statistically significant differences in the echocardiographic morphometry of cow and buffalo calves. The interventricular septum in diastole was observed to be thicker in cow calves, while the left ventricle posterior wall thickness in systole was greater in buffalo calves. Additionally, the diameters of the right and left ventricles and atrium were higher in buffalo calves compared to cow calves.

**Keywords:** Buffalo, Calf, Congenital defects, Cow, Defects, Echocardiography, Healthy

Congenital heart defects (CHDs) are uncommon in calves, and is reported to be 0.17% (Van Nie 1966) to 2.7% (Leipold and Davis 1993) based on slaughter house findings with ventricular septal defects (VSD) (Buczinski *et al.* 2006) and Atrial septal defects (ASD) (Wronski *et al.* 2021) more common. Congenital abnormalities within cardiovascular system may arise due to genetic, environmental, infectious, toxicological, pharmaceutical, nutritional, or various other factors (Tou 2022).

Complications associated with CHD in cattle often include sudden death, stunted growth, chronic respiratory problems, heart murmurs (Buczinski *et al.* 2006) in the neonatal stage, and poor breeding performance (Mitchell and Schwarzwald 2016). Out of total congenital anomalies, 2.7% calves have been reported with cardiac malformations as well (Leipold *et al.* 1972).

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Since, presence of congenital cardiac malformations, lead to poor reproductive performance by the calf, if alive (Buczinski *et al.* 2006), it is important to rule out CHDs at an early age, especially if the calf is presented with another visible congenital anomaly. The primary diagnosis of congenital heart defects is made through physical examination and assessment of clinical signs, but echocardiography is the only confirmatory and non-invasive tool for CHDs. However, there is a dearth of reference values on the echocardiography in healthy bovine calves in existing literature. The present study was formulated to create a database for the echocardiographic measurements in cow and buffalo calves of upto 15 days of age and also to compare the data between the two species at this age.

### MATERIALS AND METHODS

The study was done from a period of August 2021 to July 2022 in the department of Veterinary Surgery and Radiology,

GADVASU, Ludhiana. Due ethical permission was taken for the Institutional ethical committee for the same.

The study involved 26 bovine calves (12 buffalo and 14 cow calves) of age upto 15 days. All the calves were healthy and accompanied their dam to the clinic. The body weight of the calves was recorded. All the calves were auscultated for any abnormal lung or heart murmur and only those found healthy were included. With the owner's consent, all the calves were subjected to echocardiography using a Wipro Logic F8 ultrasound machine with a 3.0 MHz phased array transducer with a small footprint.

The right side at 4<sup>th</sup> and 5<sup>th</sup> ICS, just behind the elbow area was shaved in standing position and the calves were casted on an ECHO table (with a cut-out for cardiac probe) in right lateral recumbency, with the forelimbs slightly stretched forward. No sedation was given to any calf for the examination. The following parameters were recorded in right short and long parasternal views in right lateral recumbency.

*Technique of echocardiography in right parasternal long and short axis:*

- Coupling gel was applied to the transducer.
- The apical beat was felt with the hand and the transducer was kept there with the probe mark facing cranially and laterally.
- The right parasternal long axis view was obtained. With a perfect view, the frame was frozen and required parameters were measured.
- Rotating the transducer, the probe mark was moved caudally from the lateral aspect until the papillary muscles were seen and the transverse view/short axis of the left ventricle was visible. (Mushroom view/mid-papillary view). Most of the short axis measurements (Fig.1) were taken here, after freezing the frame in B+M mode.
- Further, the transducer was mildly rotated in the same direction to see the mitral valve or the fish mouth view, and the EPSS was measured in B+M mode.
- Further, mild rotation of the transducer in the same direction, showed the cross section of aorta with the 3 semilunar valves visible (Mercedes Benz view).

The aorta and left atrium parameters were measured in long axis while all other parameters were recorded in short axis view.

In the right para-sternal short axis view (mushroom/mid-papillary view), the following parameters were recorded (Fig. 1)

1. LVIDd: Left ventricular internal diameter in diastole
2. LVIDs: Left ventricular internal diameter in systole
3. IVSd: Interventricular septum thickness in diastole
4. IVSs: Interventricular septum thickness in systole
5. LVPWs: Left ventricular posterior wall thickness in systole
6. LVPWd: Left ventricular posterior wall thickness in diastole
7. RVIDd: Right ventricular internal diameter in diastole
8. EPSS: End Point Septal Separation (Fig. 2)

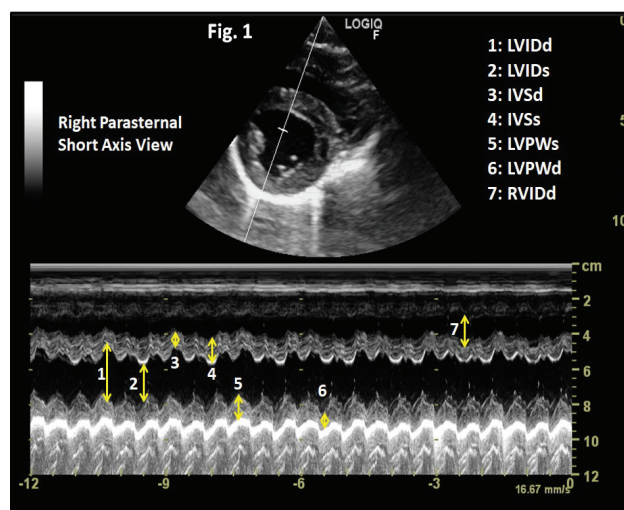


Fig. 1. Echocardiogram showing the parameters measured in right parasternal short axis view in calves.

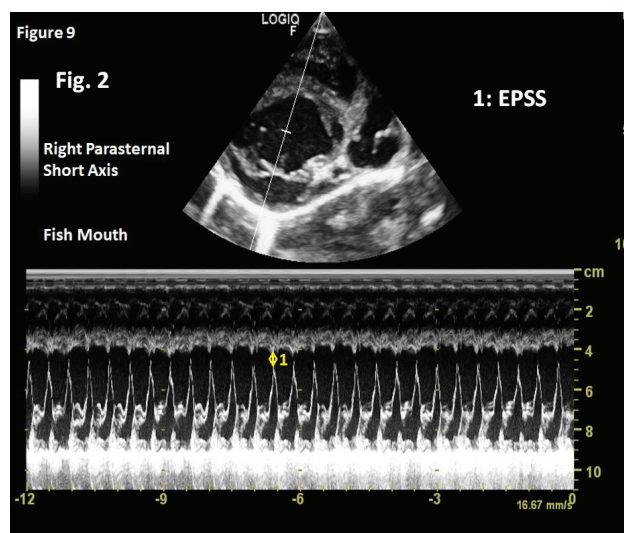


Fig. 2. Echocardiogram showing the end point septal separation (EPSS) in right parasternal short axis view (Fish Mouth) in calves.

The calculated parameters included:

1. Fractional shortening %:

$$FS\% = (LVIDd - LVIDs) / LVIDd \times 100$$

2. Ejection fraction %:

$$EF\% = SV / ED \text{ Vol} = (ED \text{ vol} - ES \text{ vol}) / ED \text{ vol} \times 100$$

$$ED \text{ vol} = 7 / (2.4 + LVIDd) \times LVIDd^3$$

$$ES \text{ vol} = 7 / (2.4 + LVIDs) \times LVIDs^3$$

$$SV = ED \text{ vol} - ES \text{ vol}$$

Where,

SV: stroke volume, ED vol: end diastole volume, ES vol: end systole volume

Parameters recorded in Right para-sternal long axis view (Fig. 3):

1. Aod (cm): Aorta diameter in centimeter
2. LAd (cm): Left atrium diameter in centimetre
3. RADd: Right atrial diastole diameter
4. LA/Ao ratio in diastole

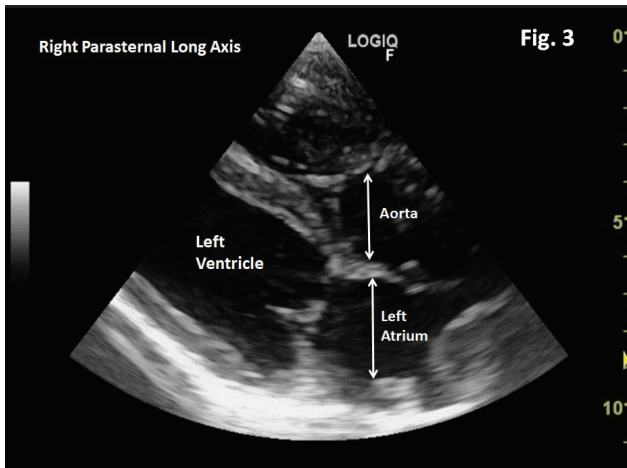


Fig. 3. Echocardiogram showing the parameters measured in right parasternal long axis view in calves.

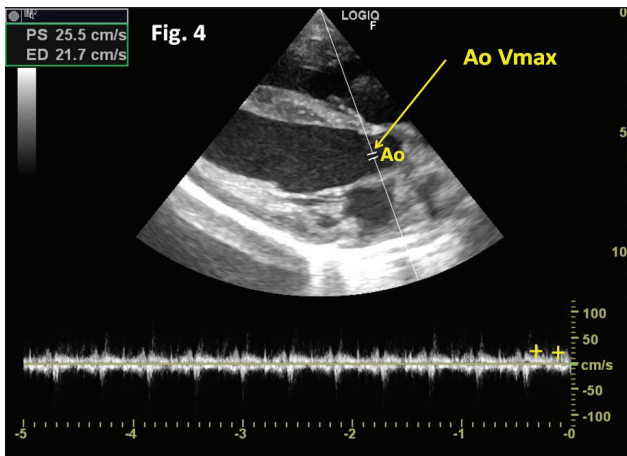


Fig. 4. Echocardiogram showing the measurement of velocity in Aorta in right parasternal long axis view in calves.

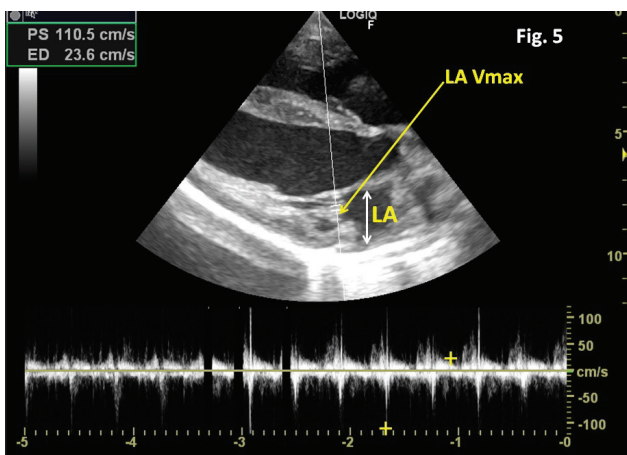


Fig. 5. Echocardiogram showing the measurement of velocity in Left Atrium in right parasternal long axis view in calves.

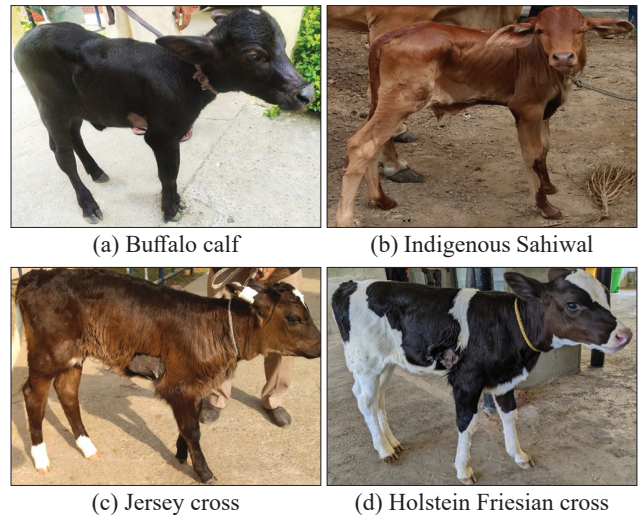


Fig. 6. Photographs of the various breeds of calves used in the study; Holstein Friesian cross (a), Sahiwal (b), Jersey cross (c) and Murrah cross (d).

- 5. The Vmax (m/s) velocity of aorta (Fig. 4)
- 6. The Vmax (m/s) velocity of Left Atrium (Fig. 5)

*Statistical Analysis:* The data generated were subjected to statistical analysis using Microsoft Office Excel, 2007. The mean and the standard deviation of all the numerical parameters were calculated in all the calves. The student t-test was applied to test the significance of differences in the echocardiographic parameters in the two species at 5% or 1%. The subjective data was compared using percentage, absolute and relative. The Pearson’s correlation test was applied wherever applicable.

RESULTS AND DISCUSSION

A total of 14 cow calves born of artificial insemination of less than 15 days of age (mean ± SD 8.64 ± 3.15) underwent echocardiography to establish the normal reference data. The calves included 5 males and 9 females. Most of the calves (n=9, 64.28%) were Holstein Friesian (HF) crossbred (Fig. 6a), while 3 were Sahiwal, one Rathi (Fig. 6b) and one Jersey cross (Fig. 6c). Most of the calves were from the 3<sup>rd</sup> parity of their dam (n=7, 50%), followed by those in 2<sup>nd</sup> parity (n=5, 35.41%), one in 6<sup>th</sup> and one in 1<sup>st</sup> parity. The average body weight of the calves was 25.57 ± 1.90 Kg. The calves of HF cross were non-significantly heaviest among other breeds. The average body weight of the male calves (24.7 Kgs) was non-significantly less than that of females (26.05 Kg), possibly attributed to the practice of not rearing male calves in non-cow beef-eating countries.

Twelve healthy buffalo calves, primarily crossbreds of Murrah (Fig. 6d) with some local breeds, underwent echocardiography, except one purebred Murrah calf. The

Table 1. Table showing the various echocardiographic values in healthy cow calves in right parasternal short axis view

	IVSd (cm)	IVSs (cm)	LVIDd (cm)	LVIDs (cm)	LVPWd (cm)	LVPWs (cm)	RVIDd (cm)	FS %	EF %
Mean	1.15	1.34	3.34	2.17	0.81	1.18	1.48	35.86	66.11
SD	0.25	0.26	0.47	0.53	0.15	0.22	0.44	9.44	12.14

group consisted mostly of females (91.67%), and one male. The average age of the buffalo calves was  $9.42 \pm 5.73$  days, and they were distributed across different maternal parities. Natural service contributed to 16.67% (n=2) of births, while the majority (83.33%) were born through Artificial Insemination (AI). The average body weight of the calves was  $28.25 \pm 6.59$  kgs, with the male calf slightly heavier than the average female calves. Notably, one female calf, a pure Murrah breed, was the heaviest among all. The reported body weight of healthy buffalo calves at birth is notably higher than that of cow calves, irrespective of gender, for both males and females (Bhatti *et al.* 2009). The weight difference can vary based on factors such as the specific breed of buffalo and cow, genetic factors, and environmental conditions.

The mean and SD of the values in right parasternal short axis and long axis for cow calves in depicted in Table 1 and 2. While comparing the right para-sternal short axis parameters of cow and buffalo calves (Table 3), the IVS distance/thickness during diastole was significantly ( $p < 5\%$ ) higher in cow calves (Fig. 7), however, in systole it was non-significantly higher. The IVS thickness is the measurement of the muscular wall between the heart's left and right

ventricles. Changes in this thickness can indicate various cardiac disorders, such as hypertrophic cardiomyopathy (HCM), which involves abnormal thickening of the heart muscle and is rare in bovine (Machida *et al.* 1996).

The radiographic assessment of heart for cardiovascular defects is limited to the size and shape of the cardiac silhouette (Farrow 1999). Comparative radiographic morphometry of thorax in healthy buffalo and cow calves of upto one month of age has been described in literature (Verma *et al.* 2023) which can be utilized to assess the size and position of heart in calves suspected to be suffering from congenital defects.

The LVID in diastole was significantly ( $p < 5\%$ ) higher in buffalo calves in comparison to cow calves, however, it was non-significantly higher during systole (Fig. 8). The LVID assesses the size of the heart's pumping chamber, with alterations serving as crucial indicators for evaluating heart function and detecting conditions such as hypertrophy or dilation. While it's speculated that the higher body weight of buffalo calves compared to cow calves may contribute, no supporting literature was found for these results.

The LVPWd was almost similar in both the species but was significantly ( $p < 5\%$ ) higher in systole in cow calves

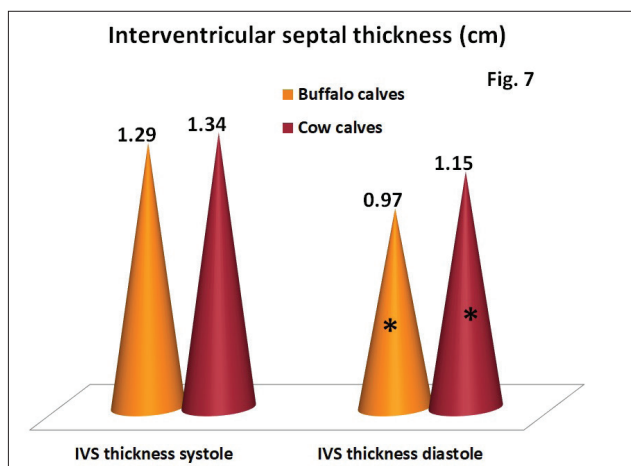


Fig. 7. Bar Graph showing the Interventricular septal (IVS) thickness in cow and buffalo calves during diastole and systole.

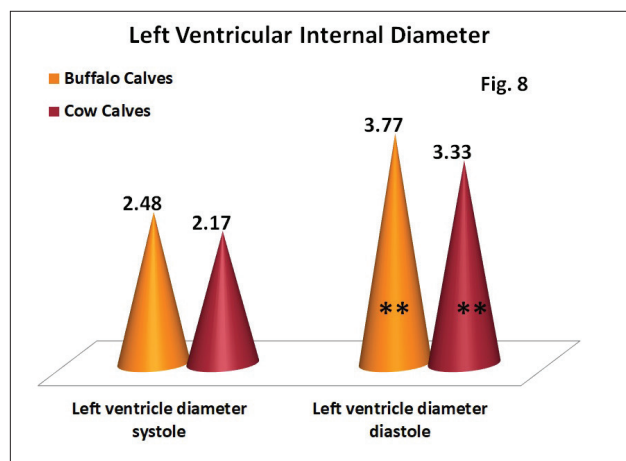


Fig. 8. Bar Graph showing the Left ventricular internal diameter (LVID) in cow and buffalo calves during diastole and systole.

Table 2. Table showing the various echocardiographic values in healthy cow calves in right parasternal long axis view.

	Aod (cm)	LAd (cm)	RADd (cm)	LA/Ao	Ao Vmax (m/s)	LA Vmax (m/s)	EPSS (cm)	Regurgitation
Mean	1.74	2.25	2.19	1.314	0.57	0.68	0.34	None
SD	0.34	0.38	0.31	0.21	0.19	0.17	0.16	

Table 3. Table showing the various echocardiographic values in healthy buffalo calves in right parasternal short axis view

	IVSd (cm)	IVSs (cm)	LVIDd (cm)	LVIDs (cm)	LVPWd (cm)	LVPWs (cm)	RVIDd (cm)	FS %	EF %
Mean	0.98	1.29	3.77	2.48	0.88	1.45	1.53	34.67	63.89
SD	0.17	0.21	0.37	0.50	0.21	0.32	0.20	9.49	12.19
ttest Cow & buffalo calves	0.04	0.59	0.01	0.14	0.32	0.02	0.72	0.75	0.65

Table 4. Table showing the various echocardiographic values in healthy buffalo calves in right parasternal long axis view.

	Aod (cm)	LAd (cm)	RADd (cm)	LA/Ao	Ao Vmax (m/s)	LA Vmax (m/s)	EPSS (cm)	Regurg-itation
Mean	1.76	2.33	2.57	1.34	0.55	0.77	0.36	None
SD	0.28	0.44	0.31	0.28	0.19	0.20	0.17	
T test cow & buffalo calves	0.91	0.63	0.004	0.76	0.71	0.20	0.74	

in comparison to buffalo calves (Fig. 9). Left ventricular posterior wall thickness is the measurement of the thickness of the muscular wall on the back side of the left ventricle in the heart. Variations in this thickness can signal various cardiac conditions, such as hypertrophy (thickening of the heart muscle), endocarditis (Buczinski *et al.* 2006) or other abnormalities that may impact the heart's function. Difference in the value may be due to the species variation.

In inference, the walls of ventricles are thicker in cow calves in comparison to buffalo calves while the diameter of left ventricle is higher in buffalo calves. Even the right ventricle internal diameter was non-significantly higher in buffalo calf. There was no significant difference in the fractional shortening % and the ejection fraction % among the two species.

The aorta and the left atrium diameter were non-significantly higher in buffalo calves in comparison to cow calves (Table 4). The right atrium diameter was significantly ( $p < 1\%$ ) higher in buffalo calves (Fig. 10). The measurement of the right atrium in diameter refers to assessing the size of the right atrium. Changes in the diameter of the right atrium can be indicative of various cardiac conditions, including conditions affecting the heart's ability to pump blood efficiently or alterations in blood flow dynamics such as right atrial enlargement and right ventricular dysfunction.

The left atrium to aorta ratio was more than one in both the species and on an average was 1.3 in both the species. The velocity of the blood flow in aorta was non-significantly

higher in cow calves while the velocity of left atrium flow was non-significantly higher in buffalo calves. The velocity of the blood flow through the shunt (ventricular septal defect) can be utilized as a prognostic factor for life in few animal species (Reef 1995). The EPSS was similar in both the species and no regurgitation was recorded in any of the calf during echocardiography.

A segmental approach to the 2D echocardiography has been recommended for healthy as well as calves suffering from cardiac congenital defect. By, segmental approach, it is meant that the individual heart chambers are first identified and studied, followed by the atrio-ventricular connections (tri and bicuspic valves), ventriculo-arterial connections (pulmonary vein and the aorta) (Hagio *et al.* 1987).

A baseline echocardiographic dataset for buffalo and cow calves of up to 15 days of age was established. The study revealed statistically significant differences in the echocardiographic morphometry of cow and buffalo calves. The interventricular septum in diastole was observed to be thicker in cow calves, while the left ventricle posterior wall thickness in systole was greater in buffalo calves. Additionally, the diameters of the right and left ventricles and atrium were higher in buffalo calves compared to cow calves. The normal echocardiographic database in upto 15 days old calves, proved the fact that the heart of cow and buffalo calves is not same by birth but the relevance of these normal values in diagnosing congenital heart defects still need to be explored.

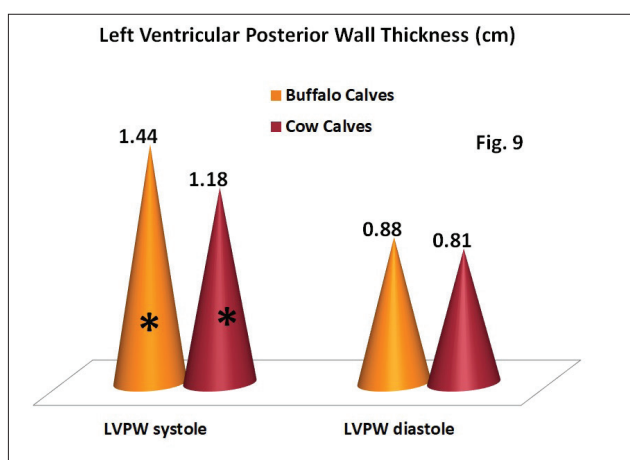


Fig. 9. Bar Graph showing the Left ventricular posterior wall thickness (LVPW) in cow and buffalo calves during diastole and systole.

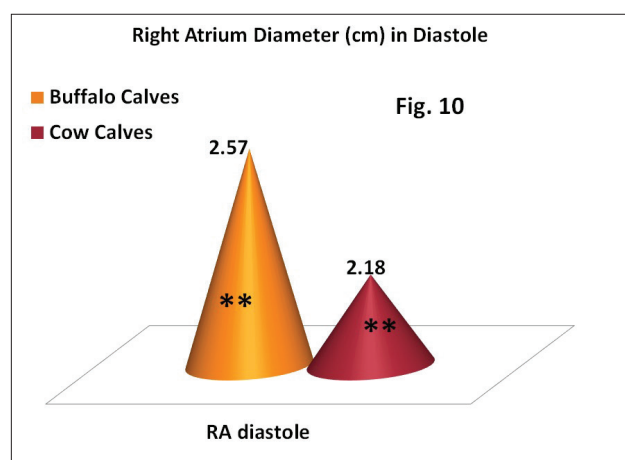


Fig. 10. Bar Graph showing the Right Atrium diameter in cow and buffalo calves during diastole.

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