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Original Article

Transpericardial 3D Echocardiography in the Sheep Model, Facilitating Real Time Anatomical Assessment for Planning and Guiding Transcatheter Mitral Valve Replacement and Repair

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Abstract
Introduction—Symptomatic mitral regurgitation (MR) is associated with high morbidity and mortality which can be ameliorated by surgical valve replacement or repair. Despite this, many patients are excluded from surgery. Transcatheter mitral repair and replacement has emerged as a promising therapeutic option for the treatment of severe MR. The majority of these procedures rely on high definition three dimensional (3D) transesophageal echocardiographic (TEE) real time imaging. The sheep model is the model of choice for preclinical studies of transcatheter mitral valve (MV) replacement and repair. However due to the thoracic conformation TEE studies in those animals are suboptimal.
Methods and Results—In this study we tested and compared the feasibility of conventional TEE and newly described transpericardial 3D echocardiography (TPE) for assessment of the MV as a tool to plan and possibly guide transcatheter/transapical procedures in the sheep model. The conventional TEE was challenging and produced incomplete data in all of the animals. In contrast, the TPE method clearly depicted the left atria and ventricle, MV apparatus, LVOT, aortic valve and proximal ascending aorta facilitating high resolution 3D rendering in all of the animals.
Conclusion—The value of TEE in the sheep model is limited and could not be reliable for complete assessment of the LV and mitral valve apparatus. Whereas, TPE produce reliable and similar imaging to TEE in the human patients which could allow animal testing of devices designed to be delivered in humans.

Keywords—Transesophageal 3D echocardiography, Mitral interventions, Ovine animal model.

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ABBREVIATIONS

TPE	Transpericardial echocardiography
TEE	Transesophageal echocardiography
MR	Mitral regurgitation
MV	Mitral valve
LV	Left ventricle
3D	Three dimensional
LA	Left atrium
LVOT	Left ventricular outflow tract
PMs	Papillary muscles
AAP	Auricular appendage
LVB	Left ventricular band
CS	Coronary sinus
OF	Oval fossa

INTRODUCTION

Mitral regurgitation (MR) is the most prevalent form of valve disease in Western countries. The current estimated prevalence of moderate to severe MR in the United State is 2 to 2.5 million, and is expected that this number will rise to 5 million by 2030.[11] Surgical open heart mitral valve repair or replacement is the gold standard treatment for MR. However up to 50% of patients with severe symptomatic MR are not referred to surgery, mainly because of advanced age, comorbidities, and left ventricle (LV) dysfunction.[4,10] The observation of this unmet need that significant number of patients with severe MR were not referred to MV surgery despite the emergence of minimally invasive MV surgery[8] led to the need for development

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of different, less invasive, trans-catheter approaches aiming at treating MR. Advancements in the development of new state of the art ultrasound machines with real-time three-dimensional (3D) echocardiographic capability facilitated the development and echo-guided of new interventional approaches and devices for the repair and replacement of damaged mitral valve.

The sheep model is currently accepted as the model of choice for testing human chronic cardiac implants such as cardiac valves. The advantage of sheep over other large animal models related to their limited somatic growth which avoids important adverse effects like paravalvular leakage and increase in cardiac output.[3] Normal cardiovascular physiological parameters of sheep approximate those of humans in blood pressure, heart rate, cardiac output, and intracardiac pressure.[1] In addition, the anatomy of adult heart provides valve orifice diameters that are similar to humans.[12] In general, sheep as experimental animals are widely available, allow for easy handling, long term husbandry and reliable clinical monitoring. However, due to different topography of the chest wall, sheep have more anterior heart position in the chest compared to humans. Furthermore, the heart base and left atrium (LA) are not in close contact with the oesophagus rendering transesophageal echocardiographic (TEE) investigations incomplete and unpredictable.

There is an emerging need to develop a transesophageal-like imaging technique which will simulate the real-time three dimensional echocardiographic study in human patients and facilitate innovative interventions and implantation of medical devices in the sheep model. To overcome the difficulties we developed transpericardial approach which allow for complete three-dimensional echocardiographic study to plan, guide and assess transcatheter mitral valve repair and replacement.

METHODS

The animal studies were conducted in the Lahav Research Institute (LRI), Israel. The study was performed following application-form review by the National Council of Animal Experimentation and after receiving their approval that the study complies with the rules and regulation set forth.

Twelve female indigenous sheep (Assaf) aged 12 to 24 months weighing 65 to 75 kg were used and prepared using standardised protocols. Prior to the procedure all animals were examined by a veterinarian to exclude existing cardiac problems and signs of infection. Monitoring known from human cardiac surgery was applied.

All echocardiographic examinations were performed by the same imaging specialist (AA*) using Epic 7 (Phillips Medical Systems) and a transesophageal (X8-2t) 2–7 MHz pure Wave Crystal transducer.

Prior to surgery, TEE examination of the left side of the heart and mitral apparatus was attempted and scrutinised on the anesthetised animals (Table 1). Thereafter, minimal midline sternotomy was performed in order to expose the LV apex and a ten centimetre longitudinal incision was made through the pericardium. TEE probe tip was immersed in ultrasound transmission gel and covered with a sterile probe cover. The tip of the TEE was flexed 20° to the left then anteroflex by approximately 60 to 90° (Fig. 1a). Then the tip of the TEE probe was advanced from the apex within the pericardial sac towards the left side of the base of the heart in such a way that it formed intimate contact with the dorsal left atrial (LA) wall (Figs. 1b and 1c). Our starting point for the initial pre-procedure echocardiogram multiplanar angle was determined as approximately 130° producing an image similar to the TEE mid esophageal long axis left ventricular outflow view (LVOT) in the human subjects (Fig. 2). Once a clear image was achieved the transducer was anchored to the edge of the sternotomy with a suture or clamp then a comprehensive perioperative echocardiographic examination of the left ventricle and mitral apparatus was performed.

During the echocardiographic examination the visibility of specific anatomical structures was assessed: Visibility of mitral valve (MV) and papillary muscles (PMs), left atria (LA), auricular appendage (AAP), left ventricle (LV) endocardial outline and presence of left ventricular bands (LVB), shape of the cardiac apex of the LV (pointed, rounded, elliptical), ventricular formation of the apex (right, left, or both ventricles). Thereafter, all animals underwent transapical implantation of tri-leaflet bovine pericardial experimental valve contained in a self-expanding annular nitinol frame.

The objective of this paper is to describe and assess feasibility of the proposed transpericardial 3D echocardiographic examination for assessment of the MV and as a tool to plan and possibly guide transcatheter/ transapical procedures.

RESULTS

The conventional TEE was challenging and produced incomplete data in all of the animals. The acoustic window was too small, mostly through the aortic arch providing reasonable image of the AO valve and LVOT and only partial image of the LV and

TABLE 1. Comparison between TEE and TPE.

		Visibility of MV leaflets		Visibility of PM		Visibility of LV wall and apex		Visibility of AO valve and LVOT		Visibility of L ventricular band	
Ovine subject	Weight (kg)	TPE	TEE	TPE	TEE	TPE	TEE	TPE	TEE	TPE	TEE
1	65.5	+	±	+	−	+	−	+	+	+	−
2	66	+	±	+	±	+	±	+	+	+	−
3	68	+	−	+	−	+	−	+	−	+	−
4	69	+	±	+	−	+	−	+	±	+	−
5	71	+	±	+	−	+	−	+	±	+	−
6	74	+	±	+	±	+	±	+	+	+	±
7	68.8	+	±	+	−	+	−	+	±	+	−
8	67	+	±	+	±	+	−	+	+	+	−
9	74.6	+	±	+	−	+	−	+	±	+	−
10	72	+	±	+	−	+	−	+	±	+	−
11	67	+	±	+	±	+	±	+	+	+	±
12	67.6	+	−	+	−	+	−	+	−	+	−

MV mitral valve, *PM* papillary muscle, *LV* left ventricle, *AO* aorta, *LVOT* left ventricular outflow tract, *TPE* transpericardial echocardiography, *TEE* transesophageal echocardiography.
+ Clearly visible, − Not visible, ± Partially visible.

mitral apparatus (Table 1) in all of the animals. The image was not clear and frequently disappeared with inflation of the lungs. The PM, endocardial outline and LV were only partially and inconsistently visible and frequent twerking of the probe was necessary to keep the limited acoustic window. The apex of the heart was not clearly visible to allow choosing the transapical access point. It was concluded that conventional TEE is not a reliable examination for thorough assessment of the mitral valve apparatus and planning transcatheter procedures in the ovine model.

For evaluation of the MV using the transpericardial (TPE) approach initially the long axis left ventricular outflow view (LVOT) was obtained by choosing imaging angle of approximately 130° (Fig. 2a). The position of the probe was then adjusted so the LV apex was clearly visible (true long axis). The image was similar to the mid esophageal long axis LVOT view (120°) in the human patient (2B), clearly depicted the LVOT, aortic valve, proximal ascending aorta as well as the P2 and A2 scallops of the MV. Then switching to simultaneous multiplane imaging (*x*-plane mode) the long axis LVOT image on the left was used as reference view and the right side image is the plane produced by the electronic beam steering on the reference image. Live *x*-plane and correct electronic beam steering allowed simultaneous acquisition and display of full resolution long axis LVOT and orthogonal long axis mitral bicommissural 2D and color real time image planes where both anterior and posterior PMs were clearly visible in all animals and the coronary sinus (CS) is identified in cross section in the atrioventricular groove (Fig. 3a). Further steering of the electronic beam towards the aortic root produce a slightly oblique long axis four chamber image with the interatrial septum and oval fossa (OF) clearly identifiable. The *x*-plane real time image allowed detailed evaluation of the general appearance, movement and motility also with color Doppler while altering the image on the right using the electronic beam steering on the reference long axis LVOT image, as well as providing clear image of the LV wall, it's endocardial outline and apex which could assist in choosing the correct transapical access point. The TPE clearly demonstrated the LV wall, endocardial outline and LVB in all of the animals. All of the cardiac apexes were pointed and formed entirely by the LV.

Once the optimal *x*-plane 2D orthogonal images were acquired it was possible to convert into wide-angle 3D volume in live mode with a focused wide sector (3D zoom) which produce a wide sector view of the MV apparatus from the LA through the mitral annulus to the papillary tips. For ''Surgeon's view'' of the MV we included the AO valve in the zoom box then rotated the 3D live image to position the AO valve at 12 o'clock. This view was excellent for displaying the MV, large portion of the LA, AAP and the atrial septum (Fig. 4a).

DISCUSSION

Surgical MV repair or replacement are standard therapies for mitral regurgitation (MR), however, many elderly patients are deemed to high risk for surgical intervention.[9] Percutaneous repair strategies are under investigation as alternative options in high-risk populations.[2,6] Many of the recent transcatheter

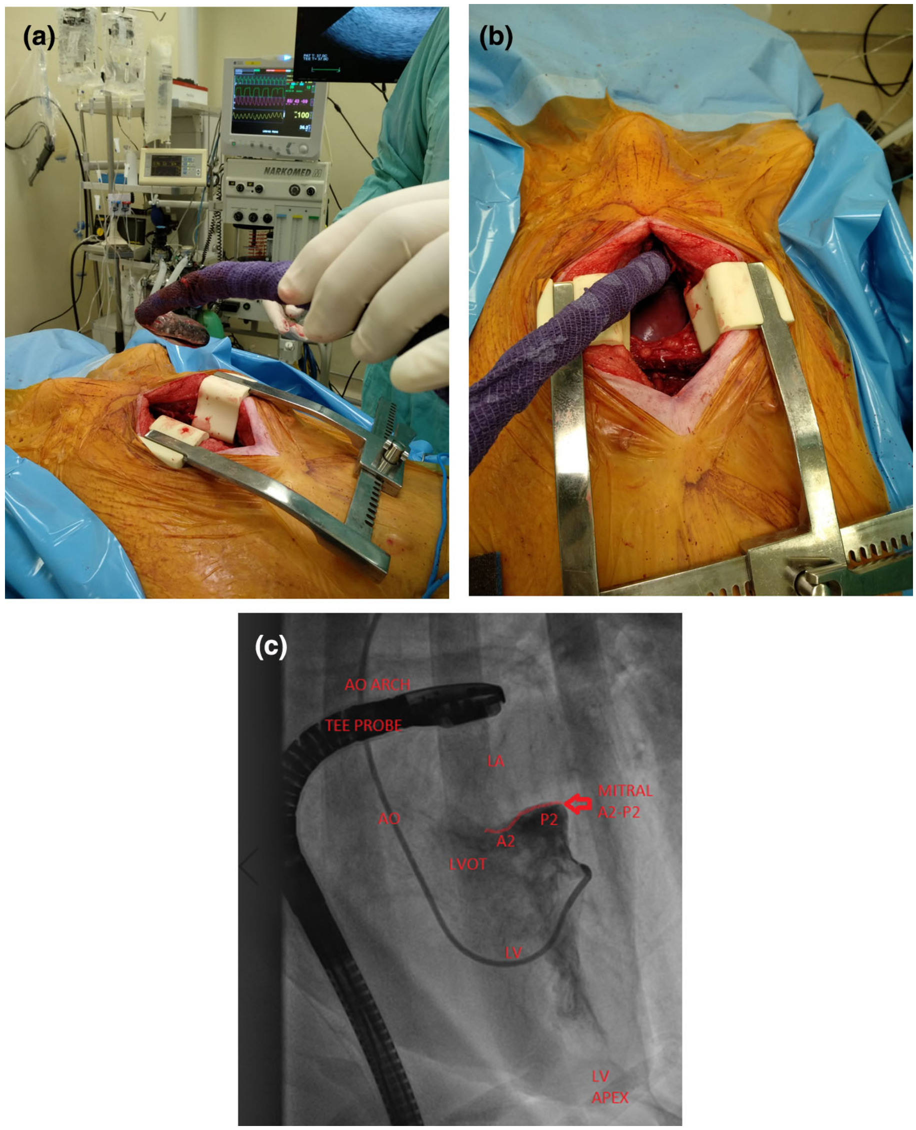

FIGURE 1. Set-up: The animal is in dorsal recumbency with minimal midline sternotomy and a longitudinal cut through the pericardium exposing the LV apex. The covered TEE probe is flexed 20° to the left and anteroflexed by approximately 90°. (a) The tip of the TEE probe was advanced from the apex within the pericardial sac towards the left side of the base of the heart in such a way that it forms intimate contact with the dorsal left atrial (LA) wall. (b), (c) Left ventricular angiogram demonstrate the shape and position of the TEE probe in relation to the LA, MV and LV. The straight red line mark the mitral outline at P2-A2 scallops.

procedures rely on excellent real-time 3D imaging of the beating heart by the new fully sampled Matrix-Array TEE transducers. The 3D real-time imaging capability of the newly developed ultrasound machines allow for adequate patient selection, guid implant delivery and deployment and facilitate accurate post implantation assessment.[5,7]

The sheep model is consider the model of choice to demonstrate proof-of-concept for new cardiac interventions and devices such as prosthetic valve replacement.[3] In the first stage of our study we noted that the value of TEE in the sheep model is limited and could not be reliable for complete assessment of the LV and mitral valve apparatus, hence, we were unable to use TEE as a reliable tool for patient selection and device implantation. It is likely that due to the different thoracic conformation and cranial cardiac position there is only minimal contact between the LA and the esophagus (Fig. 5). Additionally, inflation of the lungs can leed to further displacement of the esophagus away from the LA and loss of accoustic window.

The aim of this study was to describe and evaluate our proposed transpericardial echocerdiographic procedure for the real time 3D anatomical assessment of the MV, LV and associated structures in the ovine model.

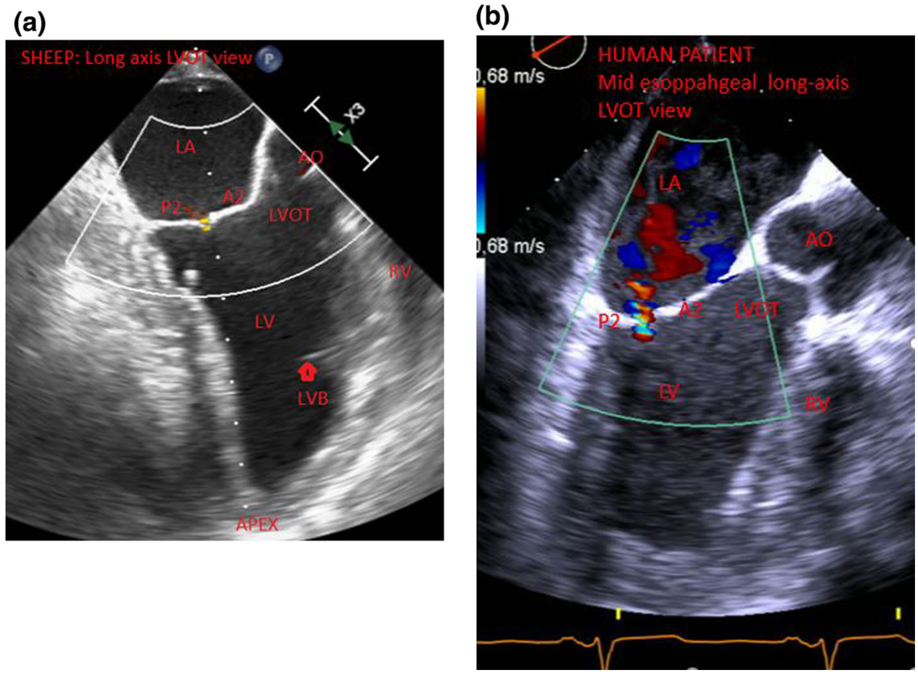

FIGURE 2. For initial evaluation of the mitral valve in the sheep model, the 2D long axis left ventricular outflow view (LVOT) was obtained by choosing imaging angle of 135° (a). The position of the probe was then adjusted so the left ventricular apex was clearly visible (real long axis). The image (a) is similar to the mid esophageal longitudinal LVOT view (120°) seen in human patients (b), clearly depicting the LVOT, aortic (AO) valve, left ventricle (LV), left ventricle band (LVB), LV apex as well as the P2 and A2 scallops of the MV. A color Doppler box is cantered on the MV.

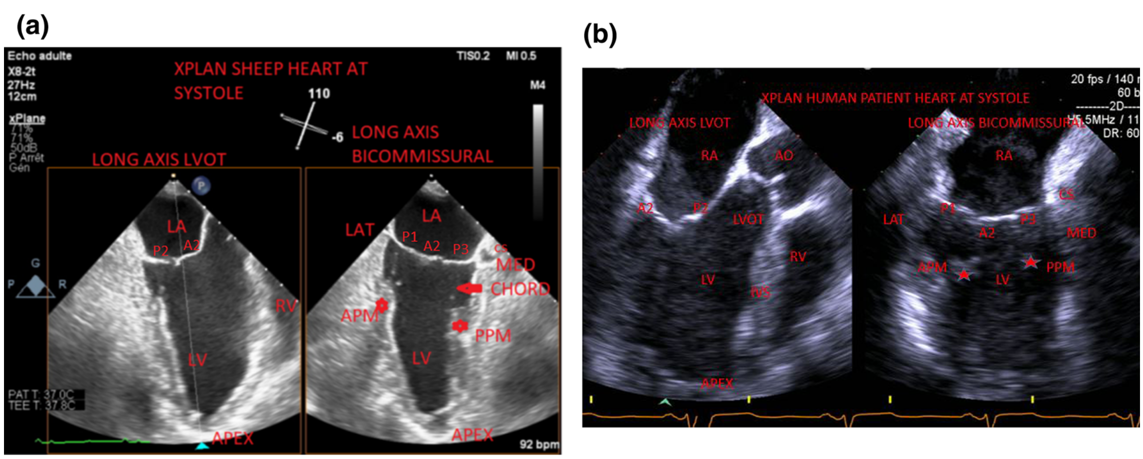

FIGURE 3. Simultaneous multiplane imaging (*x*-plane mode) at systole in the sheep model (a) and from mid esophageal TEE in the human patient (b). The long axis LVOT image on the left is used as the reference view while the right side image is the plane produced by the electronic beam steering on the reference image (faint interrupted white line). Correct electronic beam steering produced simultaneous acquisition and display of full resolution long axis LVOT and orthogonal long axis mitral bicommissural 2D real time image planes. The following structures are clearly visible in the sheep TPE and human patient TEE images: anterior (APM) and posterior (PPM) papillary muscles, coronary sinus (CS), lateral (LAT) and medial (MED) commissures, mitral leaflets (P1, P2, P3, A2), left ventricle outflow tract (LVOT), aortic valve (AO) and left ventricle (LV) wall and apex.

A major drawback of the procedure is the requirement of sternotomy and incising the pericardial sac. However, this is a standard surgical procedure in all of the transapical approaches[13] and many of the translational "humanised" sheep models. Furthermore, the sheep are inherently deep chested having conical heart with pointed apex rendering comprehensive epicardial intraoperative echocardiography limited.

We were able to adequately position the TEE probe within the pericardial sac in contact with the dorsal wall of the LA in all of the animals without any ill effect. It seemed that the pericardial sac facilitated intimate contact and minimal movement of the tip of

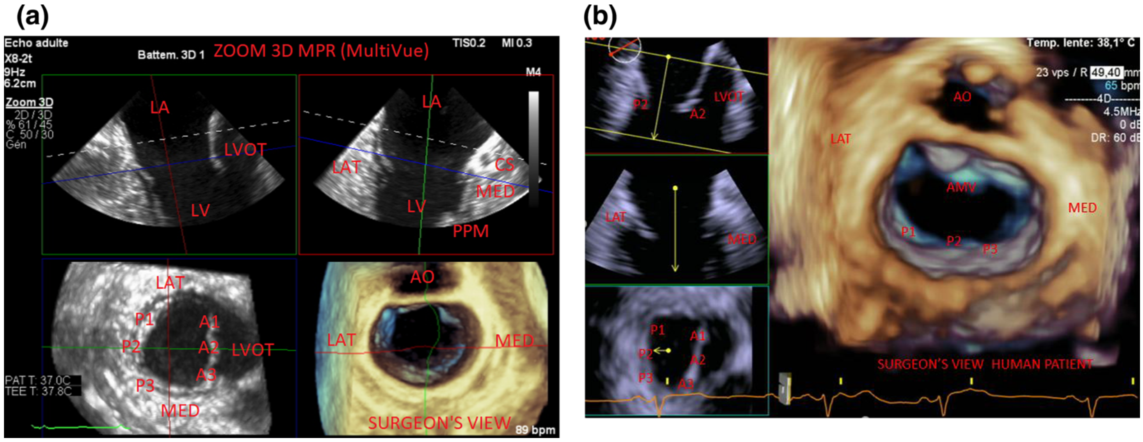

FIGURE 4. Surgeon's View of the mitral valve (MV) at diastole in the sheep model (a) and from mid esophageal TEE in the human patient (b). Once a perfect *x*-plane images of the LV and mitral apparatus is acquired one can adjust the Zoom box on both images to include all the structures of interest (mitral apparatus), then pressing on the 3D Zoom button produce live 3D image of the MV as viewed from the left atrial aspect (Surgeon's View). The aorta is in the midline at the top of the image (12 o'clock position) and the lateral commissure is on the left (LAT) as well as the auricular appendage. In order to achieve the most anatomically correct image one cane use real time multiplanar reconstruction (MPR) and cropping (MultiVue). Leaflet segments are categorised as 1, 2, and 3, with 1 denoting the lateral segments, 2 the middle segments, and 3 the medial segments. AML; anterior mitral leaflet.

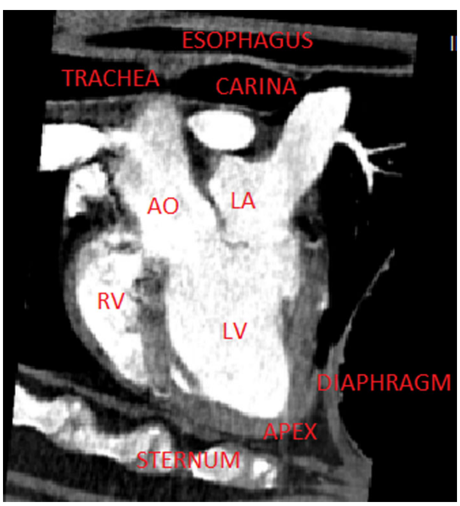

FIGURE 5. CT angiography depict long axis three chamber LVOT view of the left ventricle of a sheep with contrast filled left and right ventricles, left atria (LA), pulmonary vein (PV) and aortic arch (AO). The air filled trachea and carina are located dorsal to the heart base and the esophagus which is filled with small volume of air is located further dorsal, away from the LA. This CT image demonstrate the lack of contact between the LA and thoracic esophagus which may complicate any attempt for TEE.

the TEE probe with respect to the chosen contact site. Due to the close proximity of the probe to the LA and the triangular or pyramidal shape of the sector scan it was not possible to visualise small part of the lateral boundaries of the dorsal left atrial wall. One should also make sure that the probe is wrapped snugly with a sterile cover and that the tip of the probe is not compressing the LA leading to hemodinamic impairment. Additional possible disadvantages are decreased near-field resolution and possible accoustic shadowing by implanted devises positioned within the LA, close to the probe. Regardless, in all of the animals we were able to clearly depict the left AAP, OF, whole mitral apparatus,PMs, LV wall and apex.

Due to the intimate contact of the probe with the LA wall the initial 2D image was of a superior quality in all of the animals facilitating the use of multiple 3D acquisition modes such as *x*-plane (lateral, elevation and rotation), color *x*-plane on all scan planes, live 3D with or without color, live 3D zoom (with or without color) and full volume live 3D wide angle. Interestingly, there was no evidence of probe over-heating even after prolonged 3D exam. This may be attributed to better heat conduction within the pericardial sac.

We used the long axis LVOT (Fig. 2a) as the reference image (*x*-plane) as it depict the most important anatomical landmarks (LVOT, mitral leaflets A2-P2 scallops, aortomitral continuity and anterior MV hinge point) for positioning and anchoring many of the prosthetic valves and annuloplasty devices. On the LVOT long axis view the LV pointed apex is best visible to transect with the electronic beam (*x*-plane) producing two simultaneous orthogonal long axis images of the apex which are essential to plan accurate transapical access point and guide transapical wire through the MV. We found that with real time steering of the electronic beam on the LVOT long axis reference image (*x*-plane) we could acquire high resolution 2D orthogonal or angulated images (right *x*-plane box) of the beating heart and use it for a complete assessment of the cardiac left side.

In all of the animals the cardiac apexes were pointed and formed entirely by the LV. Such anatomy may lead to a limited transapical access point for tran-

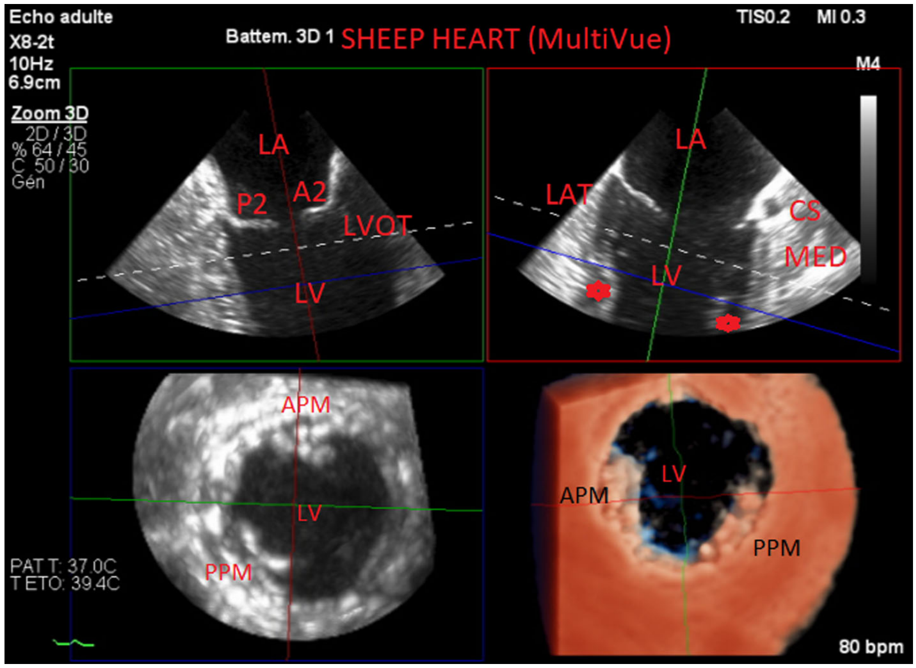

FIGURE 6. Multiplanar reconstruction (MPR) and cropping (MultiVue), in the sheep model, focused on the tip of the papillary muscles provide a real time short-axis 3D best imaging plane for identification of the location, number, and orientation of the papillary muscles and attached chordae.

scatheter procedures which should be planned meticulously. LVB were identified in all the animals. The bands were variable in thickness and morphology and should be taken into account when planning and performing transapical procedures.

Once we acquired optimal *x*-plane long axis orthogonal 2D images (anterior-posterior and bicommissural) of the mitral valve (Fig. 3a) it was possible to use 3D zoom by defining a 3D box to include the MV and aortic root for construction of an en face atrial view of the MV (surgical view) (Fig. 4a). Using the 3D zoom dual display also portray the ventricular perspective and can be important in assessing the subvalvular structures, PMs tip and LVOT. Thereafter one can use precise cropping or multi planner reconstruction (MPR) [MultiVue-Phillips] for exact cross sectional anatomical assessment and alignment (Fig. 6). Most importantly is the notion that entire investigation of the LV and mitral apparatus could be performed without altering the position of probe within the pericardial sac allowing for simultaneous fluoroscopic guided procedure.

In conclusion, although heart anatomy is very similar among mammals there is significant difference in thoracic conformation between sheep and humans. Such differences limit the usefulness of TEE in the sheep model. To overcome this problem we used transpericardial echocardiographic examination (TPE) producing reliable and similar imaging to TEE in the human patients (Figs. 2b, 3b and 4b) which could allow animal testing of devices designed to be delivered in humans. This is a preliminary study and additional studies should be performed for anatomical and Doppler measurements as well as assessment the usefulness of TPE for guiding and analysing transcatheter procedures.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

ETHICAL APPROVAL

This study was approved by the local Animal Care Committee. Animals received humane care in compliance with the Principles of Laboratory Animal Care formulated by the National Society of Medical Research and the Guide for the Care and Use of Laboratory Animals.

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