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Published in:
Pediatric cardiology

DOI:
10.1007/s00246-021-02789-6

Published: 01/04/2022

Document Version
Peer reviewed version

Citation for pulished version (APA):
Faber, J. W., Buijtendijk, M. F. J., Klarenberg, H., Vink, A. S., Coolen, B. F., Moorman, A. F. M., Christoffels, V. M., Clur, S.-A., & Jensen, B. (2022). Fetal Tricuspid Valve Agenesis/Atresia: Testing Predictions of the Embryonic Etiology. *Pediatric cardiology*, *43*(4), 796-806. https://doi.org/10.1007/s00246-021-02789-6

Foetal Tricuspid Valve Agenesis/Atresia: Testing Predictions of the Embryonic Aetiology

Jaeike W. Faber MD MSc [a], Marieke F. J. Buijtendijk MD [a], Hugo Klarenberg MSc [b], Arja Suzanne Vink MD MSc (EBP) [c, d], Bram Coolen PhD [b], Antoon F.M. Moorman PhD [a], Vincent M. Christoffels PhD [a], Sally-Ann Clur MBBCh, MSc(Med), FCP(SA)Paed, PhD [d], Bjarke Jensen PhD [a,*]

Total word count: 3714

[a] Department of Medical Biology, Amsterdam Cardiovascular Sciences, Amsterdam University Medical Centres, Amsterdam, The Netherlands
[b] Department of Biomedical Engineering & Physics, Amsterdam University Medical Centres, Amsterdam, The Netherlands
[c] Department of Cardiology, Amsterdam University Medical Centres, Amsterdam, The Netherlands
[d] Department of Paediatric Cardiology, Emma Children's Hospital, Academic Medical Centre, Amsterdam University Medical Centres, Amsterdam, Netherlands.

Funding: This study was funded by an Amsterdam UMC grant 170421/2017.03.042 obtained by Antoon F. M. Moorman

Conflicts of interest/Competing interests: The authors have no conflicts of interest to declare that are relevant to the content of this article.

Ethics approval: Ethical approval was waived by the Amsterdam University Medical Centres medical ethics committee in view of the retrospective nature of the study. The study was performed in accordance with the ethical standards as laid down in the 1964 Declaration of Helsinki and its later amendments.

Consent to participate: Not applicable. Retrospective access and anonymised use of patient files did not require ethical approval according to the Amsterdam University Medical Centres medical ethics committee (project number W19_444 # 19.514)

Consent for publication: All authors consent to publication

Availability of data and material: The raw data files are available upon request.

Code availability: Not applicable.

Author's contributions: Conceptualization: Jaeike W. Faber, Antoon F. M. Moorman, Vincent M. Christoffels, Bjarke Jensen; Performance of the echocardiograms, prenatal diagnosis and management of the patients: Sally-Ann Clur. Methodology: Jaeike W. Faber, Sally-Ann Clur, Bjarke Jensen; Formal analysis and investigation: Jaeike W. Faber, Marieke F. J. Buijtendijk, Hugo Klarenberg, Arja Suzanne Vink; Writing - original draft preparation: Jaeike W. Faber,

Bjarke Jensen; Writing - review and editing: Jaeike W. Faber, Marieke F. J. Buijtendijk, Arja Suzanne Vink, Antoon F. M. Moorman, Vincent M. Christoffels, Sally-Ann Clur, Bjarke Jensen. Funding acquisition: Antoon F. M. Moorman; Resources: Bram Coolen, Vincent Christoffels; Supervision: Sally-Ann Clur, Bjarke Jensen, Vincent M. Christoffels

*Address for correspondence: Dr. Bjarke Jensen, Room L2-106, Meibergdreef 15, 1105 AZ, Amsterdam, The Netherlands, email: b.jensen@amsterdamumc.nl, telephone: +31(0)20 5664659, twitter: @BjarkeJensen4

Key words

Morphometry, development, congenital malformations, ultrasound

Abstract

Tricuspid valve agenesis/atresia (TVA) is a congenital cardiac malformation where the tricuspid valve is not formed. It is hypothesised that TVA results from a failure of the normal right-ward expansion of the atrioventricular canal (AVC). We tested predictions of this hypothesis by morphometric analyses of the AVC in foetal hearts. We used high-resolution MRI and ultrasonography on a post mortem foetal heart with TVA and with tricuspid valve stenosis (TVS) to validate the position of measurement-landmarks that were to be applied to clinical echocardiograms. This revealed a much deeper right atrioventricular sulcus in TVA than in TVS. Subsequently, serial echocardiograms of *in utero* foetuses between 12 and 38 weeks of gestation were included (n=23 TVA, n=16 TVS and n=74 controls) to establish changes in AVC width and ventricular dimensions over time. Ventricular length and width and estimated foetal weight all increased significantly with age, irrespective of diagnosis. Heart rate did not differ between groups. However, in the second trimester, in TVA, the ratio of AVC-to-ventricular width was significantly lower compared to TVS and controls. This finding supports the hypothesis that TVA is due to a failed right-ward expansion of the AVC. Notably, we found in the third trimester that the AVC-to-ventricular width normalised in TVA foetuses as their mitral valve area was greater than in controls. Hence, TVA associates with a quantifiable under-development of the AVC. This under-development is obscured in the third trimester, likely because of adaptational growth that allows for increased stroke volume of the left ventricle.

Introduction

The tricuspid valve normally develops in the right atrioventricular junction [1]. The aetiology of tricuspid valve atresia, or tricuspid valve agenesis [2, 3], is still considered to be poorly understood. A failure of normal atrioventricular canal (AVC) development is hypothesised to underlie TVA, but quantitative evidence to support this hypothesis is lacking [4].

TVA occurs in approximately 1 in 10,000 live born children [5]. It is characterised by the absence of a valve and luminal continuity between the right atrium and right ventricle [6]. This means that the circulation to the lungs is severely impaired. If left untreated, survival of the neonate is dependent on patency of the foramen ovale, ductus arteriosus and, or, the presence of a ventricular septum defect. Normally, early in the embryonic phase, till approximately 1 month of development, the AVC myocardium surrounds a lumen which is located over the developing left ventricle and ventricular septum (Figure 1a) [7]. Over time, the AVC changes position and broadens laterally, thereby becoming more ellipsoid [8, 9]. It is then divided by the atrioventricular cushions [10]. These cushions remodel so that towards the end of the embryonic period, in the second month of development, the atrioventricular lumen is divided into a distinct right and left atrioventricular junction that is separated by the myocardialised cushions and interventricular septum (Figure 1b) [2, 11–14]. The extent of expansion can be quantified [15]: when the relative width of the AVC over the total ventricular width has been measured in embryonic humans and other animals with two ventricles, a relative widening of the AVC was observed during the period of septation. After septation, the atrioventricular junction was approximately one-half the width of the ventricles. In contrast, in pre-septation embryos and animal species with an unseptated single ventricle such as lizards and snakes, this relative widening did not occur and the AVC was relatively narrow with a width of one-third of that of the ventricle (Figure 1c) [15]. Therefore, the relative AVC width seems to be a measure of the degree of rightward expansion of the AVC during ventricular septation in normal embryonic development, providing a valuable means of studying the aetiology of TVA.

We hypothesise that the AVC in TVA is smaller than normal. To the best of our knowledge, quantitative measurements of the AVC have only been performed on ultrasounds from normal foetuses [16–18] and there is no data available on adaptive growth of the hearts of foetuses with TVA. Therefore, through this study, we provide quantitative data on atrioventricular and ventricular dimensions based on clinical ultrasound examination of foetuses with TVA or tricuspid stenosis (TVS) during the second and third trimester of

pregnancy and compared these to foetuses with structurally normal hearts. To select appropriate landmarks for these measurements, post-mortem TVA and TVS hearts were examined carefully using different imaging modalities.

TVA was clinically defined as there being no measurable flow between the right atrium and right ventricle. TVS was defined as there being a substantially smaller than normal forward flow detectable with pulsed and/or colour Doppler over the valve in the presence of a smaller tricuspid valve compared to the mitral valve.

Materials and Methods

This retrospective case-control study consists of an *ex vivo* and *in vivo* part. The Medical Ethics Committee of the Amsterdam University Medical Centres waived the need for ethical approval and the need to obtain consent for the collection, analysis, and publication of the retrospectively obtained and anonymised use of patient files.

Ex vivo

In order to determine the appropriate anatomical landmarks to use on clinical foetal echocardiographic images, we obtained post-mortem hearts of a TVA and TVS case obtained from the Becker collection of cardiac specimens from the Amsterdam University Medical Centres/Academic Medical Centre which were imaged using ex vivo magnetic resonance (MR) and ultrasound imaging.

The TVA heart was of a male foetus that died in utero in 1989 at 38 weeks of gestation of an unknown cause. The heart exhibited TVA. The baby also had trisomy 21.

The TVS heart was of a male neonate that died in 1994 of hypoxia at 1.5 days post-partum after closure of the arterial duct. The heart showed dilated atria, a small tricuspid ostium and dysplastic changes of the tricuspid valve. The right ventricle was hypertrophic with a narrowing of the outflow portion culminating in a severe pulmonary valve stenosis. However, the pulmonary trunk was normally developed.

Magnetic Resonance Imaging

Each post-mortem heart was rinsed in tap water for 72 hours to remove hydrophobic compounds that could reduce the cavity fluid-tissue contrast before being scanned in a 3T MR scanner in a water-filled plastic container (Ingenia, Philips Healthcare, Best, The Netherlands). The container with the post-mortem heart was positioned into the vendor supplied with a 16-channel knee-coil. High resolution 3-D isotropic T1 weighted Turbo Field Echo images were acquired with 0.3mm isotropic resolution. Field-of-view and matrix size were adapted to the size of each heart. Other image parameters were as follows: repetition time = 9.98ms, echo time = 4.16ms,

echo train length = 10, flip angle = 20°, number of averages = 1. Postprocessing was performed by a medical doctor on a remote workstation immediately following MR imaging.

Echocardiograms

Each post-mortem heart was additionally scanned using a WS80A Elite ultrasound system (Samsung Medison, co ltd, Seoul, South Korea) with a CV1-8A volumetric transducer, suitable for transabdominal obstetric ultrasound imaging. Cardiac settings appropriate to the size of the heart were selected. Transducer frequency ranged from 38 to 84Hz. The hearts were fully submerged overnight so as to reduce the number of air bubbles in the water and thus minimise the presence of air between the transducer and the heart. The water level allowed at least 5cm between the heart and the transducer. B-mode imaging was used to obtain a four chamber view of each heart.

In vivo

For the *in vivo* part, we retrospectively screened the hospital database for available echocardiograms of foetuses evaluated at the Amsterdam University Medical Centres – Academic Medical Centre between 1999 and 2020 as part of routine clinical management. We included all cases diagnosed with TVA and TVS during this period. In addition, healthy controls were randomly selected from the same time period. The control group comprised patients that participated in a normal heart study that was running concurrently, as well as patients referred for foetal echocardiography on suspicion of a heart defect, which were found to have a structurally normal heart. All echocardiograms had been made and diagnosed by the same paediatric cardiologist (S.A. Clur) in a standardised fashion [19]. As the inclusion of patients covered several years, at least three different ultrasound machines were used over the study period. The recordings made before 2008 were stored on video tapes that were converted to digital files before the four chamber view still for the measurements was selected.

Measurements and additional data

From the included echocardiograms, a four chamber view at ventricular end-diastole was obtained. On each four chamber view still, the following measurements were performed: I: the width of the left atrioventricular junction (mitral valve opening); II: the width of the fibrous body where the valve leaflets anchor; III: the width of the right atrioventricular junction (tricuspid valve opening); IV: maximal width of the ventricles; V: the maximal distance between apex and fibrous body; VI: the inner surface area of the ventricles, including the interventricular septum (Figs. 2-3).

The mitral valve area (MV_{area}) was calculated by assuming a circular shape of the opening. It was used as a proxy for potential left ventricular inflow volume. AVC width was calculated as the sum of the left and right atrioventricular junction width plus the width of the fibrous body (see Figures 2-3, S1). Additionally, the foetal heart rate and the body weight, (based on Hadlock's formula using biparietal diameter, head circumference, abdominal circumference and femur length measured at the time of the echocardiogram) [20, 21], were retrieved from the patient files.

The investigator performing the initial and intra-observer measurements (10%) (J.W. Faber) was blinded to identity, age, and diagnosis of the foetus and was unblinded only after all measurements were completed.

Statistics

The *ex vivo* data is presented descriptively. The *in vivo* data were analysed with R version 3.6.1 [R Foundation for Statistical Computing, Vienna, Austria]. If there was more than one ultrasound study per individual per timepoint, the maximal value of each of the measurements for that timepoint was taken for statistical analysis. For the relative AVC width, the maximal values for valve, fibrous body, and ventricular width were used to calculate the ratio of maximal AVC width over ventricular width. Baseline characteristics and echocardiographic parameters were presented as median (interquartiles). The data was stratified into four gestational age groups: ≤140 days (20+0 weeks), 141-150 days (20+1 – 21+3 weeks), 151-200 days (21+4 – 28+4 weeks) and >200 days (28+4 weeks). Each gestational age group included one measurement per foetus. Differences between the three diagnostic groups (i.e. TVA, TVS, and controls) were analysed using a one-way ANOVA test. A Bonferroni correction was done to correct for multiple testing. A p-value <0.001 was considered to be statistically significant.

We performed linear mixed-effect-model analyses to estimate the average age trends for relative AVC width, absolute AVC width, ventricular width, and MV_{area}. All available echocardiograms per foetus were included. The age trends were allowed to vary smoothly by gestational age (fixed-effect) via restricted cubic splines. Knots were placed at four fixed quantiles of the predictor's distribution as suggested by Stone [22]. We allowed the intercept (i.e. value at birth) to differ per patient, and assumed a multivariate normal distribution (random effect). Trends were allowed to differ by diagnosis. Sampling uncertainty was quantified via 95% confidence intervals and p-values. A p-value <0.05 was considered to be statistically significant.

Intra-observer variability for the absolute parameters expressed as the intra-class correlation coefficient (ICC) for multiple measurements based on a two-way consistence model

according to Ciccheti and Fleiss [23, 24]. Bland-Altman analyses were performed to assess the standard error and the limits of agreement (LoA)[25].

Results

Ex vivo

When post-mortem hearts with TVA and TVS were compared macroscopically (Figure 2a-b) the TVA heart showed a deeper right atrioventricular groove than the TVS heart. On images from the *ex vivo* echocardiography (Figure 2c) and, in particular, MRI (Figure 2d), it was clear that the right atrioventricular groove in the stenotic heart was somewhat shallow. The groove extended to the hinge-point of a parietal leaflet of the tricuspid valve. In contrast, in TVA there was no valve and the atrioventricular groove was so deep as to be juxtaposed to the crest of the ventricular septum. This difference in the depth of the atrioventricular groove was then taken into account in setting the boundary for the tricuspid valve (Figure 2e). The comparison of echocardiograms to MRI scans showed that the placement of atrioventricular landmarks at borders of high to low contrast (lumen to myocardium) on the echocardiograms were easier to objectify than placing the atrioventricular landmarks within annulus fibrosus where atrial myocardium meets ventricular myocardium. The high signal intensity of the muscle sometimes obscured the lower intensity of the annulus fibrosus on the echocardiogram stills.

In vivo

Population characteristics

A total of 113 foetuses were included; 23 TVA, 16 TVS and 74 controls. The median number of echocardiograms per foetus was 1.5 (IQR=1.0). The dataset covered 12 to 38 weeks of gestation. In Table 1, we show the baseline characteristics and echocardiographic parameters of the included foetuses stratified by gestational age group. As expected, weight increased with gestational age, but did not differ between the three groups based on diagnosis. The heart rate was relatively consistent over the gestational age groups without significant differences between TVA, TVS and controls. Remarkably, although not statistically significant, TVA had lowest absolute AVC at all age groups compared to TVS and controls. The absolute differences in ventricular width were less pronounced between the TVA foetuses and the TVS and control foetuses, resulting in a smaller absolute AVC width over the total ventricular width ratio at all age groups, which was most notable in the gestational ages of 141-150 days (20+1 – 21+3 weeks).

Trend-analyses

The relative AVC width differed between the three diagnostic groups (p=0.06). Figure 4a shows that TVA foetuses had a significantly lower relative AVC width compared to TVS and controls between the gestational ages of approximately 120-175 days of gestation (17+1 – 25+0 weeks) even though there was no significant effect of gestational age on the relative AVC width within each diagnostic group (p=0.07 for TVA, p=0.44 for TVS and p=0.07 for controls).

To see whether the difference in relative AVC width was due to a difference in absolute AVC width or absolute ventricular width, we examined the age trend of these parameters. Absolute AVC width changed significantly over time in the total cohort (p<0.001), but with no significant difference between the three groups (p=0.33). Figure 4b, shows that, although not significant, TVA foetuses have a smaller absolute AVC width at all gestational ages compared to TVS and controls. The ventricular width also showed significant growth in the total cohort (p<0.001), but again no difference between the three diagnostic groups was seen (p=0.24) (Figure 4c).

There was a significant growth in MV width and MV_{area} over time in the total cohort (both p<0.001) with, for MV_{area}, a significant difference in growth between the three groups (p=0.002). For MV width, the trend analysis did not show a significant difference between the three groups (p=0.28) but as can be appreciated from Figure 5, the TVA foetuses had a significantly larger MV_{area} and MV width compared to controls after approximately 150 days (21+3 weeks) of gestation.

Intra-observer variability

There was a fair (0.40-0.59) to excellent (0.75-1.00) intra-observer agreement of the measurements of linear dimensions and area (Table 2, Figure S1).

Discussion

In this study, we have presented evidence that TVA young foetuses have a smaller relative AVC width compared to TVS and controls, supporting the hypothesis that agenesis of the tricuspid valve is likely a failure of rightward expansion of the embryonic AVC. This was most prominent between 120 and 175 days of gestation (17+1 – 25+0 weeks). After 150 days of gestation (21+3 weeks) this coincided with a larger MV_{area} in the TVA cases. The deep atrioventricular groove in TVA, as found in our *ex vivo* investigation and as previously reported [3], together with the relatively smaller AVC width in early foetal development, fits with the embryonic aetiology of TVA as described in previous anatomical studies [2, 14, 26].

The TVS foetuses followed the same growth of the AVC as controls which fits with a normal initial development of the junctional orifices but with a valvular defect. Given this finding, the barrier between the right atrium and the right ventricle in TVS is most likely composed of valvular connective tissue [27, 28]. In contrast, the similarly positioned barrier in TVA is much more complex [29], consisting of the myocardial floor of the right atrium [30] and shoulder of the right ventricle and, in-between these, the fibro-fatty tissues of the insulating plane and maybe even branches of the right coronary artery [11]. The scope for potential future surgical interventions may therefore be more limited and challenging in TVA compared to TVS since there is no junction in TVA that can ever be mechanically expanded [31–33]. At the moment, postnatal surgical options for tricuspid stenosis are dependent on the size and function of the tricuspid valve and right ventricle and may include a univentricular path, a 1,5 repair, and in some cases no surgery depending on the degree of the stenosis and the size of the RV. A univentricular path is required for tricuspid atresia.

We saw no differences in heart rate, or foetal growth based on estimated foetal weight between diagnostic groups, showing that the cardiac output in both groups was at least sufficient to maintain similar growth rates. This is different to a previous report, though the difference can possibly be explained by the relatively low number of included older foetuses with TVA [34]. Given that there was no difference in heart rate between diagnoses, nor a sufficiently great difference in cardiac output to cause growth impairment in the TVA group as compared to the TVS and control groups, the left ventricle in TVA likely generated a greater stroke volume in order to provide sufficient cardiac output to maintain normal growth. Indeed, we found that the MV width and MV_{area} in TVA were enlarged, suggesting that the left ventricle accommodated to the increased blood-flow over the MV. The cases with TVS showed an intermediate MV width and MV_{area} trendline compared to the foetuses with TVA and controls, evidence of the effect that a restricted right atrioventricular junction has on the mitral valve. The mitral valve adaptation became pronounced around 150 days (21+3 weeks) which could reflect the increasing importance of the right ventricular function in the second trimester which has to be compensated in order to maintain a similar total cardiac output [35, 36].

Limitations

Due to the retrospective study setting, the video acquisition was not aimed to generate near-to perfect four chamber view images. Therefore, the still frames on which the measurements were performed might be influenced by imaging angle even though we selected the best four chamber images available. When several echocardiograms per time-point were available, only the largest

values were used to attempt a correction of variations in imaging angle. This is based on the assumption that a tilt of the heart would reduce its maximum dimensions. Measurements of several ventricular dimensions within the same individual could, therefore, have been derived from different echocardiograms where the position of the foetus was likely to be different. This could explain some of the large variation between individual data points. In calculating the MV_{area}, we assumed an equal, circular left atrioventricular junction in all foetal hearts. Similarly, because we could not obtain sagittal views from the included foetuses, the effects of differences shape of the atrioventricular junction in an anterio-posterior direction between conditions could not be assessed.

Tricuspid atresia is an uncommon cyanotic heart defect and the cases were included over more than 20 years. In that time there have been significant technical improvements in ultrasonography. The echocardiograms where made using different ultrasound machines during the study period which might have influenced maximal resolution of the videos and subsequent selected stills.

Only two specimens were used for the *ex vivo* analysis. This investigation successfully established suitable landmarks on ultrasound images that could also be used for the *in vivo* analysis. However, the qualitative findings in these two cases, while fitting with the hypothesis of AVC malformation underlying TVA, cannot be extrapolated to the disease groups as a whole due to variability of the presentation of TVS and TVA in terms of extent of tricuspid valve malformation and overall cardiac morphology.

Conclusion

We provide quantitative evidence that the AVC in TVA develops abnormally, in support of the hypothesis that TVA results from a failed rightward expansion of the AVC. Adaptive remodelling of the left heart in TVA becomes pronounced in the third trimester.

Acknowledgements

We thank Roelof-Jan Oostra for providing access to the Becker collection.

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Figure legends

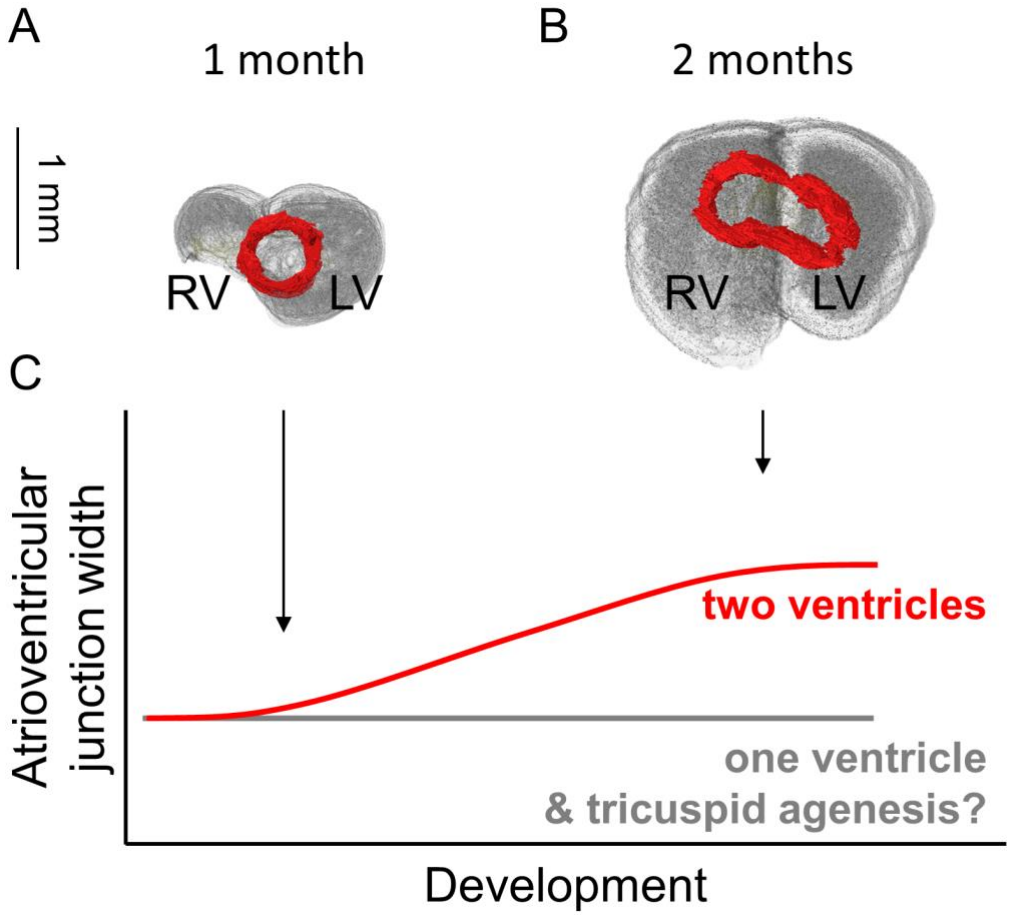

Fig. 1 Development of the AVC *in embryo*

A: In early human embryonic development the AVC is circular and overlies only the interventricular septum and the left ventricle (LV). **B**: Towards the end of human embryonic development, the AVC has become ellipsoid and extends rightward to also overlap the right ventricle (RV). The models were derived from previously published data [8]. **C**: Schematic overview of relative AVC width in species with two ventricles, in which the relative width can be seen to increase over time, and those with a single ventricle in which the relative AVC width remains constant. Adapted from [15].

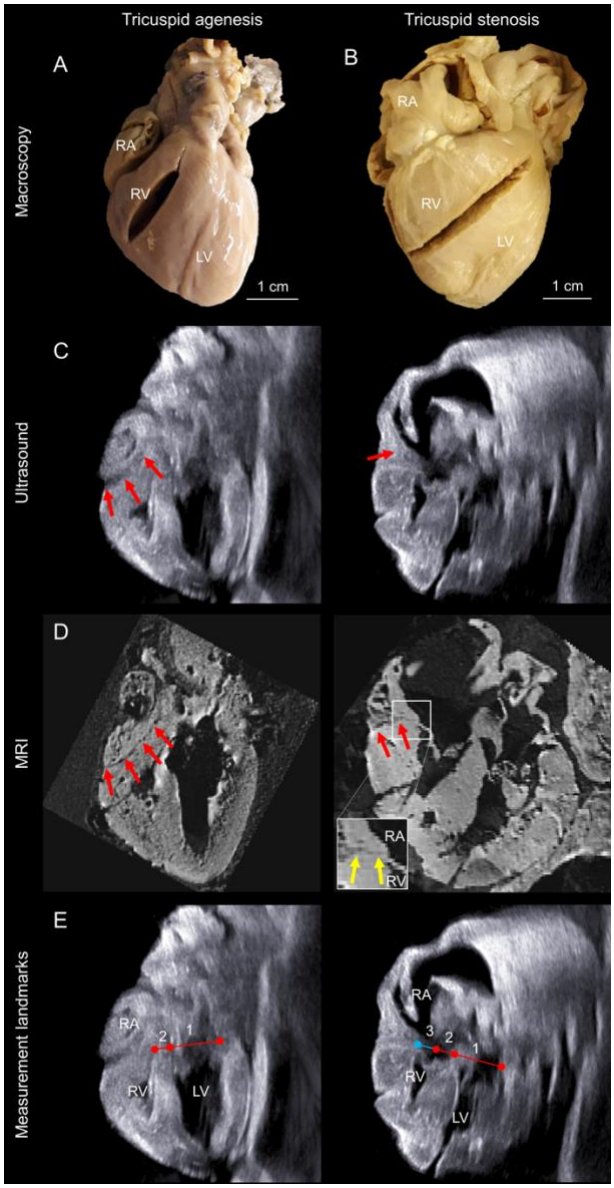

Fig. 2 TVA and TVS *ex vivo*

A: Foetal heart, 31 weeks gestational age, diagnosed with TVA. The heart was cut during the post mortem examination . **B**: Neonatal heart, 1.5 days old, diagnosed with TVS and pulmonary valve stenosis. The heart was cut during the post mortem examination. **C**: *Ex vivo* echocardiogram of the same hearts as in A and B. Red arrows point towards the right atrioventricular groove. **D**: *Ex vivo* MRI of the same hearts as in A and B. Red arrows point towards the right atrioventricular groove which, in the case of TVA, extends to the level of the interventricular septum and therefore deeper than can be appreciated from the echocardiogram. In TVS the right atrioventricular groove extends towards the insulating plane as indicated with yellow arrows (insert). **E**: Landmarks (dots) for the *in vivo* measurement of the mitral valve width (1), interventricular septal width (2) and tricuspid valve width (3). LV: left ventricle, RA: right atrium, RV: right ventricle.

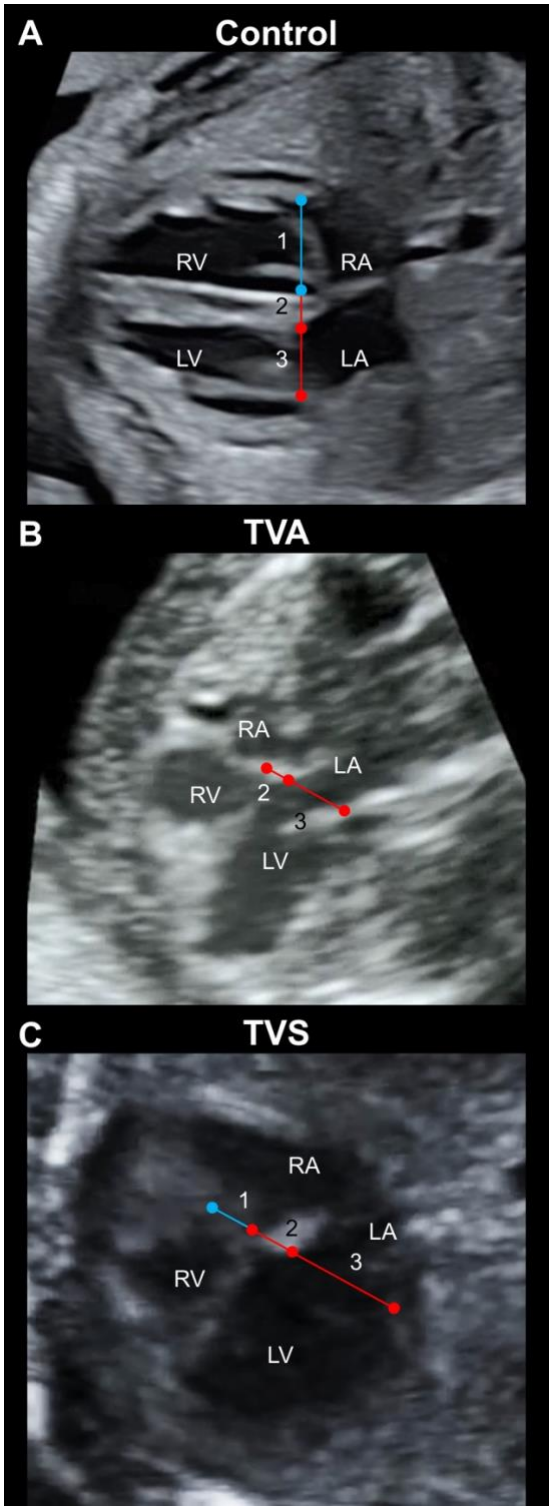

Fig. 3 TVA and TVS *in vivo*

A: Clinical ultrasound of a foetal control heart of 215 days of gestation (30+5 weeks). **B**: Clinical ultrasound of a foetal heart with TVA of 93 days of gestation (13+2 weeks). **C**: Clinical ultrasound of a foetal heart with TVS of 137 days of gestation (19+4 weeks). Landmarks (dots) indicate mitral valve width (1), interventricular septal width (2) and tricuspid valve width (3). LA: left atrium, LV: left ventricle, RA: right atrium, RV: right ventricle.

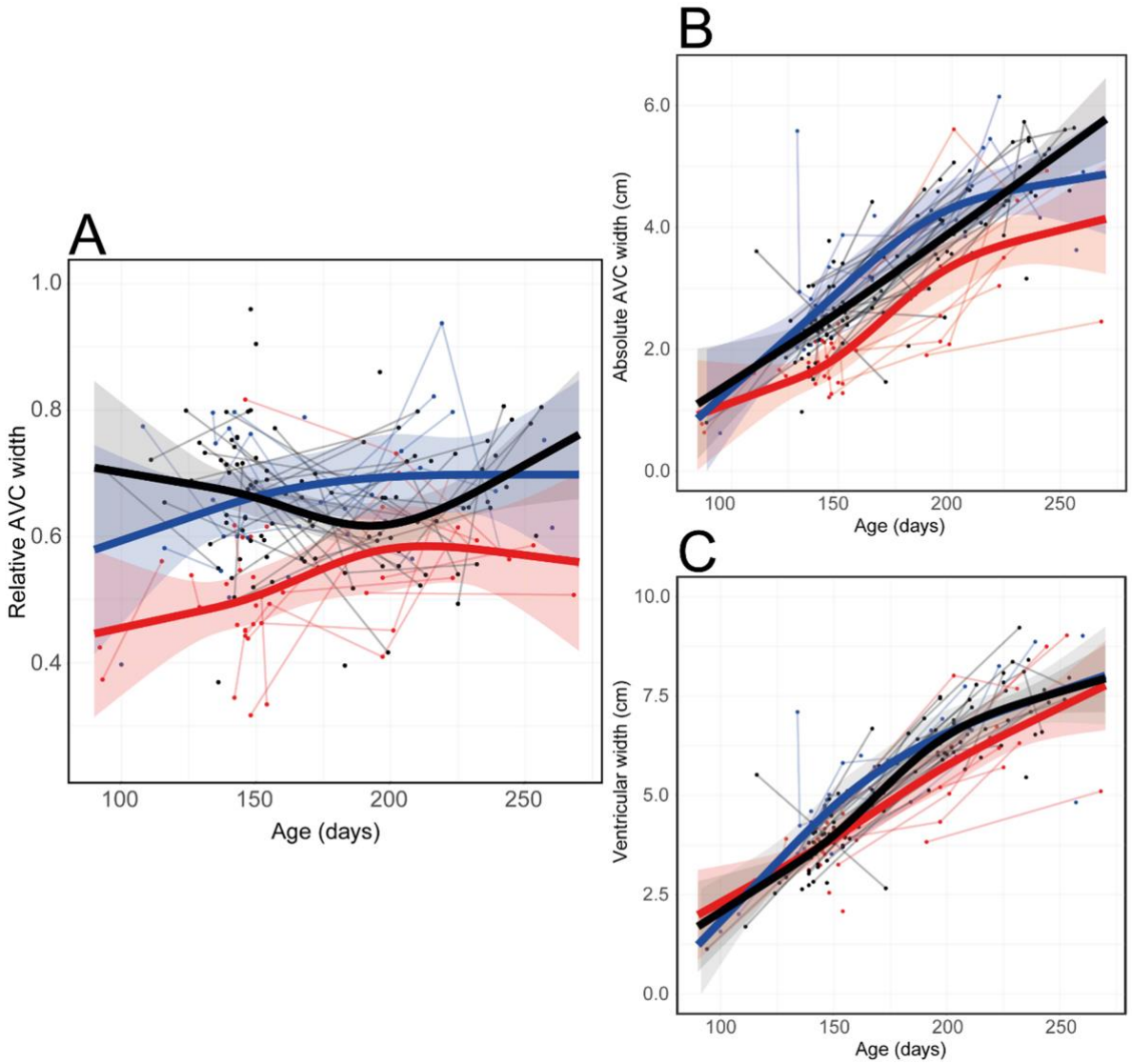

Fig. 4 Atrioventricular canal and ventricular width in TVA, TVS and controls

A: Gestational age-related changes in AVC width over total ventricular width. **B**: Gestational age-related changes in absolute AVC width. **C**: Gestational age-related changes in absolute maximal ventricular width. TVA cases are indicated in red, TVS in blue, and controls in black. Individual measurement trends are shown in the background of the average trend. The shaded areas are 95% confidence intervals.

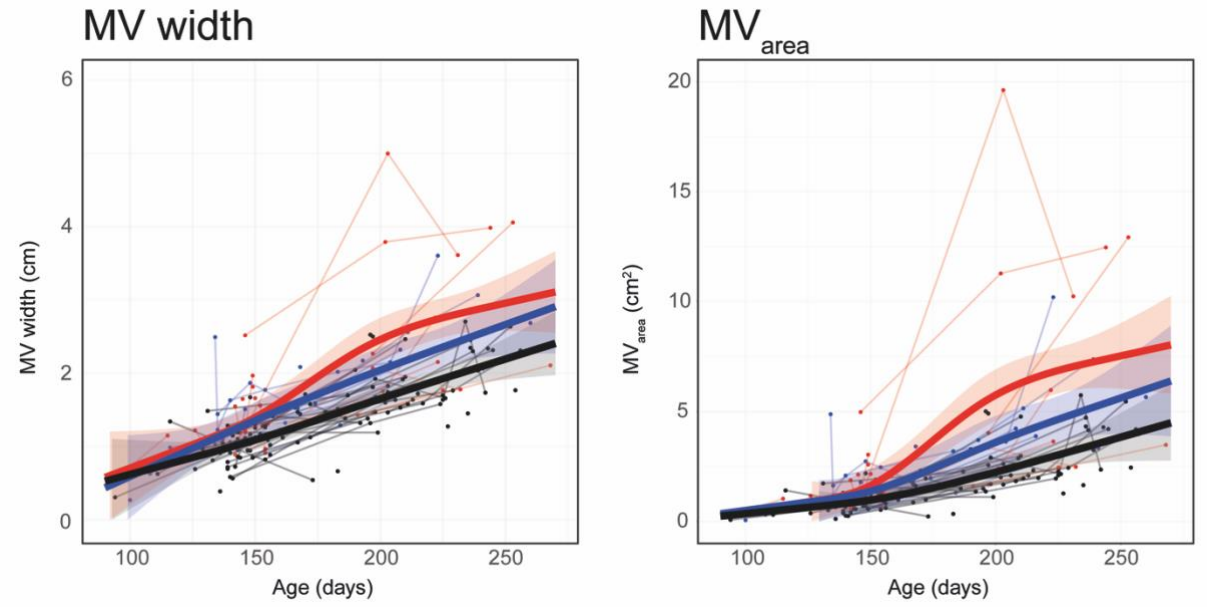

Fig. 5 MV size in TVA, TVS and controls

Gestational age-related changes in mitral valve width and calculated mitral valve area. TVA in red, TVS in blue, and controls in black. The shaded areas are 95% confidence intervals.

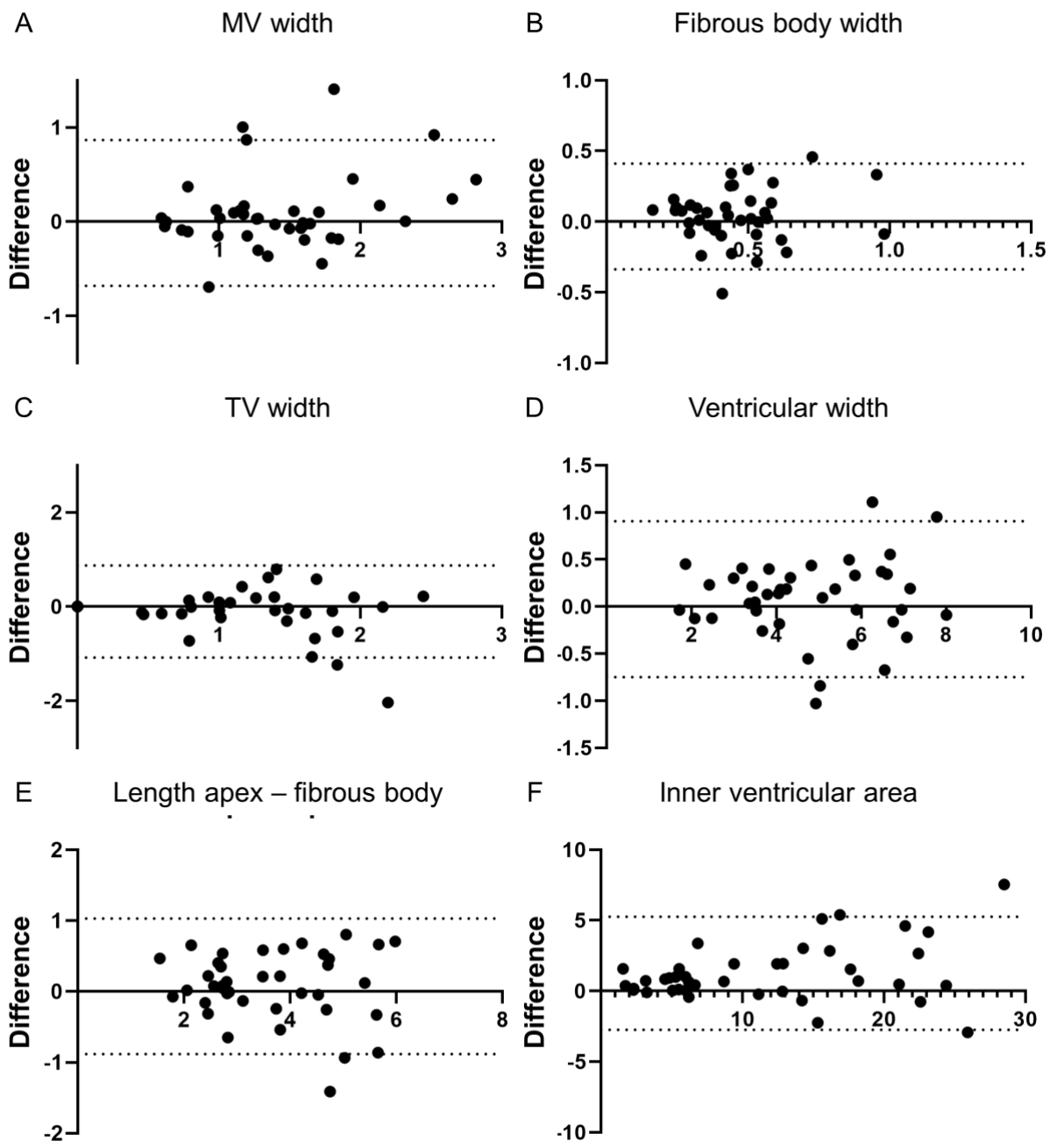

Fig. S1 Intra-observer variability

Bland-Altman plots of the measurements performed on the *in vivo* ultrasounds.

Table legends

Table 1 Characteristics of the investigated groups

Measurements derived from echocardiograms subdivided in four gestational age groups. R AVJ: right atrioventricular junction, L AVJ: left atrioventricular junction, AVC: atrioventricular canal. P<0.001 is considered to be significant

Table 2 Intra-observer variability

Intraclass correlation coefficients (ICC) on 40 re-measured echocardiograms. CI: confidence interval, LoA: limits of agreement.

Tables

Table 1

Age group	Diagnosis		Weight (g)	Heart Rate (bpm)	Length apex - fibrous body (cm)	Ventricular width (cm)	R AVJ width (cm)	L AVJ width (cm)	Absolute AVC width (cm)	Relative AVC width
≤140 days	Control	n=19	268 ± 61	152 ± 14	2.5 ± 0.7	2.8 ± 1.1	0.8 ± 0.4	0.8 ± 0.3	1.9 ± 0.7	0.7 ± 0.1
	TVA	n=6	303 ± 52	120 ± 51	2.0 ± 0.7	2.5 ± 0.9	0 ± 0	0.9 ± 0.4	1.3 ± 0.5	0.5 ± 0.1
	TVS	n=7	292 ± 42	157 ± 13	2.7 ± 0.9	3.6 ± 1.9	0.8 ± 0.8	1.1 ± 0.7	2.2 ± 1.6	0.6 ± 0.1
	P-value		*0.350*	*0.833*	*0.774*	*0.290*	*0.721*	*0.190*	*0.770*	*0.288*
141-150 days	Control	n=23	371 ± 55	143 ± 15	3.2 ± 0.5	3.8 ± 0.6	1.1 ± 0.3	1.0 ± 0.3	2.5 ± 0.5	0.7 ± 0.1
	TVA	n=12	387 ± 70	150 ± 8	3.1 ± 0.5	4.0 ± 0.6	0 ± 0	1.4 ± 0.5	1.9 ± 0.4	0.5 ± 0.1
	TVS	n=5	347 ± 88	151 ± 11	3.0 ± 0.5	4.1 ± 0.8	0.9 ± 0.1	1.2 ± 0.4	2.6 ± 0.5	0.7 ± 0.1
	P-value		*0.854*	*0.092*	*0.594*	*0.268*	*0.002*	*0.039*	*0.292*	*0.115*
151-200 days	Control	n=33	751 ± 318	144 ± 9	4.2 ± 1.1	5.3 ± 1.2	1.3 ± 0.4	1.4 ± 0.5	3.2 ± 0.8	0.6 ± 0.1
	TVA	n=8	597 ± 474	144 ± 11	2.8 ± 0.9	3.6 ± 1.1	0 ± 0	1.3 ± 0.2	1.8 ± 0.4	0.5 ± 0.1
	TVS	n=9	745 ± 271	143 ± 8	4.2 ± 0.8	5.8 ± 0.7	1.4 ± 0.4	1.7 ± 0.3	3.6 ± 0.6	0.7 ± 0.1
	P-value		*0.747*	*0.765*	*0.365*	*0.939*	*0.278*	*0.172*	*0.797*	*0.088*
>200 days	Control	n=28	1869 ± 641	139 ± 12	5.6 ± 0.8	7.0 ± 0.9	1.8 ± 0.4	1.9 ± 0.5	4.5 ± 0.8	0.7 ± 0.1
	TVA	n=8	1886 ± 866	139 ± 10	4.9 ± 1.0	6.5 ± 1.4	0 ± 0	2.8 ± 1.3	3.7 ± 1.2	0.6 ± 0.1
	TVS	n=8	1880 ± 572	135 ± 11	5.7 ± 1.0	6.8 ± 1.3	1.6 ± 0.6	2.2 ± 0.3	4.6 ± 0.7	0.7 ± 0.1
	P-value		*0.957*	*0.413*	*0.987*	*0.467*	*0.036*	*0.071*	*0.746*	*0.214*

1 Table 2

	Intra-observer variability	
	ICC (95% CI)	Mean (± 95% LoA)
MV width (cm)	0.77 (0.61-0.87)	0.092 ± 0.774
Fibrous body width (cm)	0.54 (0.27-0.72)	0.037 ± 0.374
TV width (cm)	0.79 (0.64-0.88)	-0.104 ± 0.978
Ventricular width (cm)	0.97 (0.94-0.98)	0.079 ± 0.828
Length apex - fibrous body (cm)	0.92 (0.86-0.96)	0.075 ± 0.956
Inner ventricular area (cm^2)	0.97 (0.94-0.98)	1.261 ± 4.000
AVC width (cm)	0.84 (0.72-0.23)	0.025 ± 1.384

2