Original Article

Effect of thoracic arthrodesis in prepubertal New Zealand white rabbits on cardio-pulmonary function

Federico Canavese[1,2], Alain Dimeglio[3], Davide Barbetta[4], Bruno Pereira[5], Sergio Fabbro[6], Federica Bassini[6], Bartolomeo Canavese[7]

Abstract

Background: This experimental study was aimed at evaluating the type of cardiac and pulmonary involvement, in relation to changes of the thoracic spine and cage in prepubertal rabbits with nondeformed spine following dorsal arthrodesis. The hypothesis was that T1-T12 arthrodesis modified thoracic dimensions, but would not modify cardiopulmonary function once skeletal maturity was reached.

Materials and Methods: The study was conducted in 16 female New Zealand White (NZW) rabbits. Nine rabbits were subjected to T1-T12 dorsal arthrodesis while seven were sham-operated. Echocardiographic images were obtained at 12 months after surgery and parameters for 2-dimensional and M-mode echocardiographic variables were assessed. One week before echocardiographic examination, blood samples were withdrawn from the animals' central artery of the left ear to obtain blood gas values. One week after echocardiographic assessment, a thoracic CT scan was performed under general anesthesia. Chest depth (CD) and width (CW), thoracic kyphosis (ThK) and sternal length (StL) were measured; thoracic index (ThI), expressed as CD/CW ratio. All subjects were euthanized after the CT scan. Heart and lungs were subsequently removed to measure weight and volume.

Results: The values for 2-dimensional and M-mode echocardiographic variables were found to be uniformly and significantly higher, compared to those reported in anesthetized rabbits. CD, ThK, and StL were considerably lower in operated rabbits, as compared to the ones that were sham-operated. Similarly, the ThI was lower in operated rabbits than in sham-operated ones.

Conclusion: Irregularities in thoracic cage growth resulting from thoracic spine arthrodesis did not alter blood and echocardiographic parameters in NZW rabbits.

Key words: Thoracic arthrodesis, New Zealand white rabbits, cardio-pulmonary function

Introduction

In most severe cases of untreated progressive early onset spinal deformities, respiratory insufficiency and pulmonary and cardiac hypertension can develop (cor pulmonale), characterizing the thoracic insufficiency syndrome (TIS), which can sometimes lead to death.[1-4]

[1]Department of Pediatric Surgery, Universitys Hospital Estaing, 1 Place Lucie et Raymond Aubrac, 63003 Clermont Ferrand, [2]Faculty of Medicine, University of Auvergne, 28 place Henri-Dunant BP 38 63001 Clermont-Ferrand, [3]Faculty of Medicine, University of Montpellier, 2 Rue de l'Ecole de Medecine, 34000 Montpellier, France, [4]Department of Life Sciences, University of Trieste, Animal Facility, Via Valerio 28, 34127 Trieste, Italy, [5]Department of Research and Innovation (CRCI), Biostatistic Unit, University Hospital of Clermont Ferrand, Clermont Ferrand, France, [6]Veterinary Clinic, Via Campos 192, 33030 Maiano (UD), Italy, [7]University of Udine, Via Palladio 8, 33100 Udine, Italy

Address for correspondence: Prof. F. Canavese,
Department of Pediatric Surgery, University Hospital Estaing, 1 Place Lucie et Raymond Aubrac, 63003 Clermont Ferrand, France.
E-mail: canavese_federico@yahoo.fr

Access this article online	
Quick Response Code:	Website: www.ijoonline.com
	DOI: 10.4103/0019-5413.128763

Rabbits *(Oryctolagus cuniculus)* are widely used for cardiovascular[5-7] and other experimental research.[8-12] Through an extensive collection of experimental data, several studies have allowed to determine reference values for rabbits and other laboratory species by investigating the morphology of some organs, the methods of collecting blood samples and analysing biochemical parameters, and several cardiovascular disorders.[13,14]

In this context, investigations can be completed by electrocardiographic and echocardiographic assessments. Electrocardiography and echocardiography are useful non-invasive methods in experimental and clinical settings.[15-18] Echocardiography can be used for *in vivo* evaluation of cardiac dimensions and performance. Doppler echocardiography provides additional information on cardio-circulatory hemodynamic status.[19] Reference values for various M-mode, flow Doppler, and tissue Doppler echocardiographic parameters have been reported in rabbits in the non-sedated state, as well as during various anesthetic and/or sedative combinations.[17,20,21]

This study aimed at describing the morphological changes in the thoracic cage and spinal column in New Zealand White (NZW) prepubertal rabbits, 6 weeks old, who were subjected to T1-T12 dorsal arthrodesis and were observed at the age of 13.5 months by computed tomography (CT) scan. This was done to investigate the type of cardio-pulmonary involvement, in relation to the changes of the thoracic spine and cage in prepubertal rabbits with non-deformed spine subjected to extended dorsal arthrodesis.

Our hypothesis was that T1-T12 arthrodesis would modify the thoracic cage dimensions at completion of growth, but not cardio-pulmonary function, as evaluated on the basis of blood gas samples and echocardiographic measurements and on the assessment of operated and sham-operated rabbits.

Materials and Methods

Animals

The study was conducted in 16 female NZW rabbits. Nine rabbits underwent an extra canal dorsal T1-T12 vertebral arthrodesis,[10,11,22-25] and seven were sham operated. All female rabbits underwent surgery at the age of 6 weeks. Subjects were housed in stainless steel cages in a controlled environment (temperature of 21°C with relative humidity of 40-50%, 10-15 changes of air per hour, and a light/dark cycle of 12/12 hours) and fed a standard pellet diet (2030 Teklad Global Rabbit Diet, Harlan Laboratories, Indianapolis, IN, USA) with water *ad libitum*.

Operative procedures and animal care were performed in compliance with the national and international regulations (Italian regulation D.L.vo 116/1992 and European Union regulation 86/609/EC). The protocol was examined and approved prior to the start of the study by the Director's Board of the Department of Life Sciences, Animal Facility, University of Trieste, Trieste, Italy, where this study was performed. The recommendations of the ARRIVE guidelines in animal research were also consulted and considered.[26]

Anesthesia

Surgery was performed under general anesthesia (GA) by an intramuscular injection of xylazyne 5 mg/kg (Virbaxil® 2%; Virbac Laboratories, Carros, France) and tiletiletamine-zolazepam 15 mg/kg (Zoletil® 100; Carros, France). Additional skin analgesia was obtained with a subcutaneous injection of 2% lidocaine hydrochloride (1 ml/animal). The postoperative period was eased by a subcutaneous administration of carprofen (Rymadil®, Pfizer Animal Health, West Dundee, Great Britain; 5 mg/kg twice a day over 5 days). Prevention of infection in the week following surgery was pursued by an intramuscular injection of enrofloxacil (Baytril® 5%, Bayer Animal Health, Kiel, Germany; 5 mg/kg twice daily).

Operative procedure

Nine rabbits were operated following a modified "Wisconsin" technique or extra canal dorsal T1-T12 vertebral arthrodesis, corresponding to posterior vertebral arthrodesis in bipeds.[22,23] A midline incision was made between the first and twelfth thoracic vertebrae. Once the muscular plane was reached, the *trapezium, latissimus dorsi*, *spinalis* and *longissimus lumborum et thoracis* were symmetrically retracted to allow a wide exposure of the vertebral laminae, spinous and transverse processes and the cranial and caudal facet joints of the thoracic vertebrae.[10,27] Two specifically designed C-shaped stainless steel bars, approximately 120 millimeters (mm) in length and 1.5 mm in diameter, were positioned laterally at the base of the spinous processes of the thoracic vertebrae and fixed with multiple 2/0 non-absorbable wire ligatures [Figure 1]. Multiple fragments of heterologous bone graft (Grafton DBM®, Osteotech Inc., Eatontown, NJ, USA) were placed on either side of the spinous processes of the thoracic spine to favour fusion [Figure 2]. Each operation lasted approximately 50 min.

Sham operation

Seven rabbits were sham operated. Access to the operating area was achieved exactly in the same manner as in those undergoing arthrodesis. After lavage with saline solution, the muscular plane and the subcutaneous plane were stitched with 2/0 suture, and the cutaneous plane with 3/0 suture, both absorbable (Vicryl, Ethicon Inc., United States). A sterile dressing was positioned on the surgical wound. To relieve pain, 5 mg/kg of carprofen were administered subcutaneously twice daily over 5 days. Prevention of infection, in the week following surgery, was pursued with an intramuscular injection of 5 mg/kg of enrofloxacin twice daily.

Blood gas analysis

One week before echocardiographic assessments, all animals underwent withdrawing of blood from the central artery of the left ear to obtain blood gas values using the i-STAT Portable Clinical Analyzer (Abbott Diagnostics, Abbott Park, IL, USA). Values of partial pressure of carbon dioxide ($PaCO_2$) in mmHg, partial pressure of oxygen (PaO_2) in mmHg, total carbon dioxide tension (TCO_2) in mmol/L, bicarbonates (HCO_{3-}) in mmol/L, oxygen saturation (saO_2) and pH were recorded.

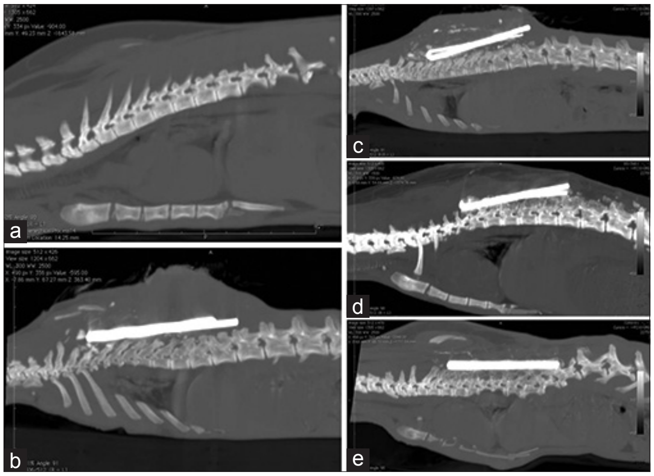

Figure 1: Lateral thoracic CT of sham operated (a) and operated rabbits (b-e). The head of the subjects is on the left hand side and the tail is on the right hand side. Evolution of the fusion during 12 months. From partial fusion (b) to completed fusion (e)

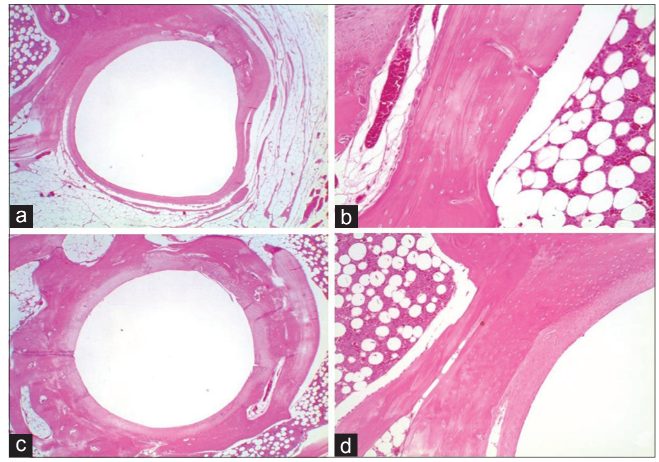

Figure 2: Histological pictures. Transverse sections. Bars have been removed prior to inclusion. Decalcified bone tissue: Organization around metallic bars used for arthrodesis (a and c). Particulates of the fusion mass (b and d)

Echocardiographic assessment

The 16 selected rabbits underwent echocardiographic evaluation at 12 months, when they had reached the age of 13 and a half months. At this age, somatic growth can be considered as fully completed and specifically, the species involved actually reaches skeletal maturity around 7-8 months of age.[27] Furthermore, the weight charts, which were updated and completed throughout the experimentation, show that both operated and sham operated rabbits reach a stable balance in total body weight at that age.

All echocardiographic operations were performed in the room where rabbits were stabulating with the actual advantage of keeping the subjects calm. Echocardiographic evaluation was performed without sedation or anesthesia and with all animals in a nonsedated and semi conscious state. Hair was removed in the right parasternal region of each rabbit. Animals were placed in a specially prepared concave basins made of elastic material, and were kept in lateral decubitus positions. They were calmed throughout the course of the examination by inducing a hypnotic state that favored their restraint during the echocardiographic examination. The animal was first laid in the dorsal decubitus position, then a delicate pressure was exerted on its ears while its eyes were closed with a slight pressure of the hand; the other hand was delicately placed on the abdomen, to maintain the decubitus position; simultaneously, the same word, specifically *pippo*, was repeatedly whispered in its ears'. The hypnotic state was induced within 3 to 5 min and lasted from 6 to 8 min, which was long enough to easily complete the echocardiographic examination. This approach was totally successful for all 16 subjects.

Examinations were carried out with the My Lab 30 CVX Vision device (Esaote, Florence, Italy). Phased array probes PA230/3.5 Mhz and convex CA123/5 Mhz were used. All echocardiographic acquisitions were made in sinus rhythm and only the right parasternal view of each rabbit's hemithorax was imaged echocardiographically.

Echocardiographic values were obtained from standard feline views[28] and measurements in diastole (d) and systole (s) included: Thickness of the interventricular septum (IVSd and IVSs) in millimeters (mm); left ventricular internal diameters in mm (DVS diastolic and DVS systolic); left ventricular free wall in mm (PPVS diastolic and PPVS systolic); contractile capacity of the left ventricle in mm (%FS: Fractional shortening defined as [100*(DVSd-DVSs)/DVSd]) of the left atrium in mm (LA); ejection fraction as a percentage (EF); diameter of the aortic wall (Ao) in mm; and LA/Ao ratio. The following dynamic parameters were also recorded: Maximal aortic outflow velocity (AO Vel_{Max}) in meters per second (m/s); maximal aortic outflow pressure gradient (GP Ao_{Max}) in mm of mercury (mmHg); maximal pulmonary outflow velocity in m/s (Po Vel_{Max}); and maximal pulmonary outflow pressure gradient in mmHg (GP Po_{Max}) [Figures 3-8].

Computed tomography scan measurements

Thoracic CT scan measurements were performed on a randomly selected sample of 11 rabbits 1 week after echocardiographic examination. We have assumed a usual 1:3 comparison between sham and operated rabbits. Three among seven sham rabbits were randomly selected and one operated rabbit was excluded because of poor quality CT scan measurements. For these reasons, we had a total of 11 CT scan measurements available for analysis (3 sham and 8 operated rabbits).

CT scan examinations were performed with a 16-slice CT scanner (©Aquilion 16, Toshiba Medical System

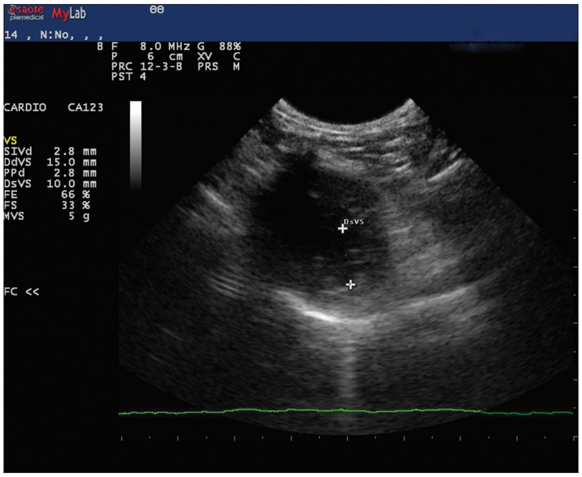

Figure 3: Echocardiographic image of sham operated rabbit. Right parasternal view, recording B-mode and Doppler values. Left ventricle in the short axis (LV, as VS in the figure)

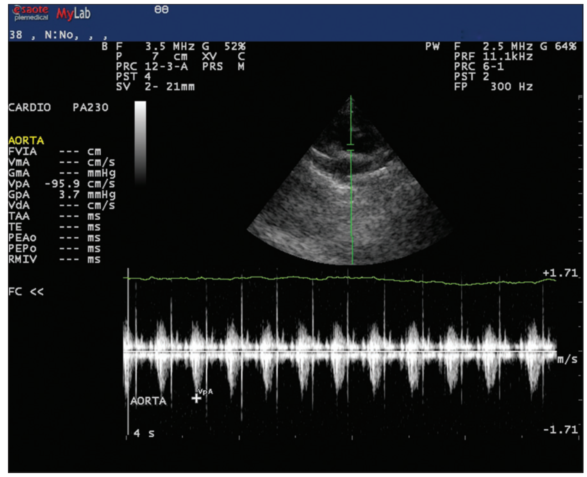

Figure 4: Echocardiographic image of sham operated rabbit. Right parasternal view, recording B-mode and Doppler values. Aorta; (Ao, as AORTA in the figure)

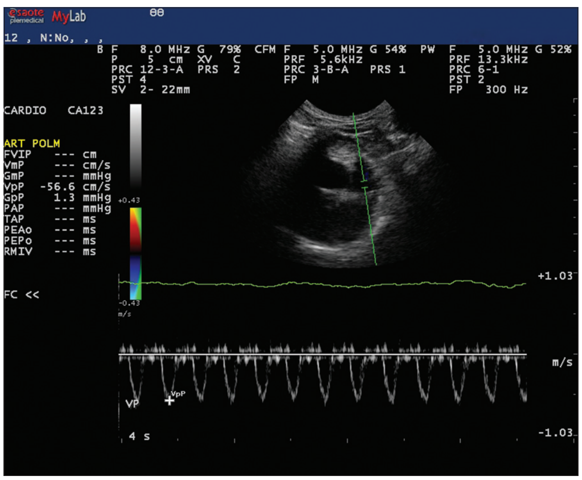

Figure 5: Echocardiographic image of sham operated rabbit. Right parasternal view, recording B-mode and Doppler values. Pulmonary artery (Po, as ART POLM in the figure)

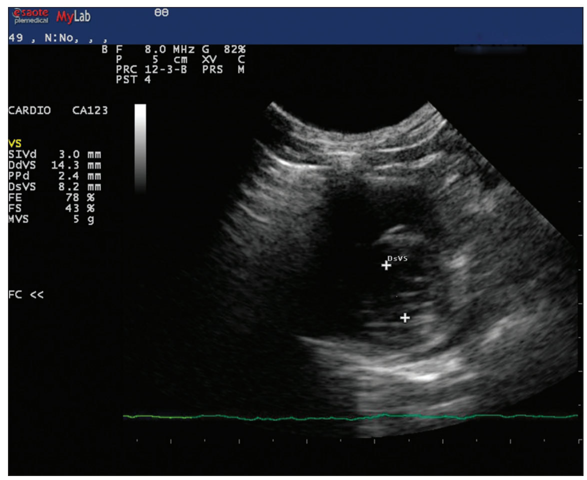

Figure 6: Echocardiographic image of operated rabbit. Right parasternal view, recording B-mode and Doppler values. Left ventricle in the short axis (LV, as VS in the figure)

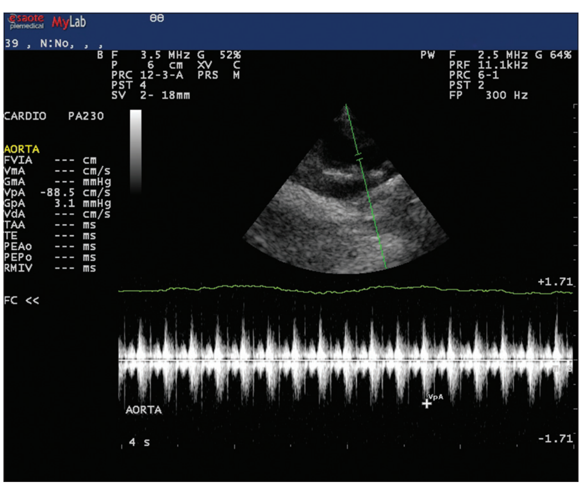

Figure 7: Echocardiographic image of operated rabbit. Right parasternal view, recording B-mode and Doppler values. Aorta; (Ao, as AORTA in the figure)

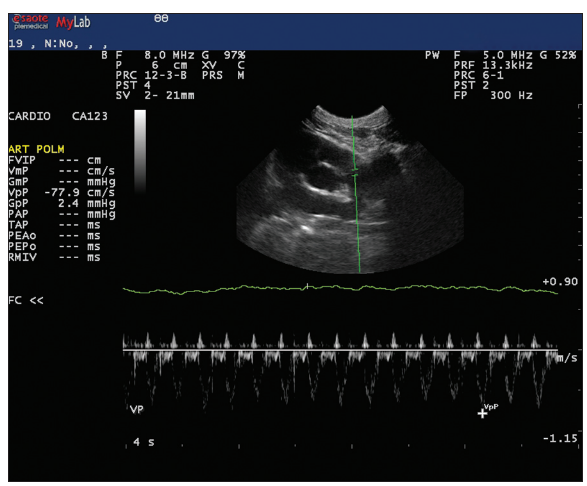

Figure 8: Echocardiographic image of operated rabbit. Right parasternal view, recording B-mode and Doppler values. Pulmonary artery (Po, as ART POLM in the figure)

Corporation, Tochigi, Japan). Tomography examinations were carried out in the supine position under GA. Measurements on the CT images were always carried out by the same operator to avoid interobserver error using OsiriX® imaging software, version 3.8.1 (©Antoine Rosset, 2003-2011, Geneva, Switzerland).

Thoracic dimensions

Chest depth (CD) was calculated from the ventral face of the vertebral body to the dorsal face of the sternum. Chest width (CW) connects the inner faces of two symmetrical ribs, and lies perpendicularly to CD, cutting it in half on the widest point of the thoracic cage. CD and CW calculations were then used to obtain the ThI, expressed by the ratio CD/CW of the thorax. Measurements were performed at T5 level. Thoracic dimensions are expressed in mm.

Thoracic kyphosis

Thoracic kyphosis (ThK), in degrees, was calculated from the upper border of T3 to the lower border of T10 following Cobb's method[29] and its application in clinical[30] and experimental[10] research was used [Figure 1].

Sternal length

Sternal length (StL) was calculated in mm from the cranial end of the tracheal cartilage to the caudal end of the xiphoid cartilage. StL was expressed in mm.

Heart and lung removal

Finally, the anesthetized rabbits were euthanized with a 26 mg/kg intravenous injection of embutramide-mebenzonio-tetracaine (Tanax®, Intervet Italia Srl, Peschiera Borromeo, Italy). After death, at the age 13 and a half months when their somatic growth was complete, they were jugulated.

Immediately after euthanasia and jugulation, the heart-lungs complex was removed. Heart and lungs were then weighed separately and their weight was recorded in grams (gr). The volume of heart and lungs was determined by displacement of the equivalent distilled water, with mass and values reported in milliliters (mL). Weight and volume of heart and lungs were obtained from specimens fixed in 10% buffered formalin and were ultimately useful to obtain the corresponding ratios heart/lungs (H/Ls), which were later submitted to statistical analysis.

Some samples of lung, fixed in 10% buffered formalin, were embedded in paraffin and microtome sectioned. Sections measuring 6-8 μm in thickness, were subjected to a routine histological staining technique.

Statistical analysis

Statistical analysis was performed using the STATA software, version 12 (StataCorp, College Station, TX, USA). Tests were two-sided, with a type I error set at $\alpha = 0.05$. Continuous data were presented as mean ± standard deviation (SD). The comparisons between operated/sham groups were analyzed using Student's *t*-test or Mann – Whitney test (*i.e.,* body weight, echocardiographic measurements, heart volume), with normality verified by the Shapiro-Wilk test and homoscedasticity by the Fisher-Snedecor test. Due to sample size, non-parametric tests were privileged. Mixed models were considered to take into account group as fixed effect and the intra-subject variability with rabbit as random effect (for analyses of CD, CW, ThI, ThK and StL). These models allow to avoid the multiple comparisons on a dependant dataset (for each thoracic vertebrae). Moreover, we did verify that sham ($n = 3$) and operated ($n = 8$) were not significantly different for weight ($P = 0.84$). The same analysis was realized to compare sham rabbits with ($n = 3$) and without ($n = 4$) CT scan measurements ($P = 0.72$) in order to avoid potential confounding bias in the analysis of CT scan measurements data.

Results

Table 1 shows the mean values of partial pressure of carbon dioxide ($PaCO_2$) in mmHg, partial pressure of oxygen (PaO_2) in mmHg, total carbon dioxide tension (TCO_2) in mmol/L, bicarbonates (HCO_{3-}) in mmol/L, oxygen saturation (saO_2) as a percentage and pH. There is no significant difference in the values of operated and sham-operated animals ($P > 0.05$).

All echocardiographic measurements (M-mode, 2D, Doppler echocardiography) were easily recorded with all rabbits in the non-sedated state. Table 2 provides mean values ± standard deviation (SD) of echocardiographic parameters for operated and sham-operated rabbits. Values are not significantly different between the two groups ($P > 0.05$), except for %FS ($P = 0.04$). Moreover, these echocardiographic measurements were found to be significantly higher compared to those previously reported in anesthetized healthy rabbits, except for %FS and EF [Table 3].[17,18]

Table 1: Mean values of partial pressure of carbon dioxide ($PaCO_2$) in mmHg, partial pressure of oxygen (PaO_2) in mmHg, total carbon dioxide tension (TCO_2) in mmol/L, bicarbonates (HCO_{3-}) in mmol/L, oxygen saturation (saO_2) and pH

Parameter	Mean±SD	
	Operated	Sham operated
$PaCO_2$ (mmHg)	29.2±2.6	29.2±1.7
PaO_2 (mmHg)	90±18.2	98.3±24.5
TCO_2	22.78±4.18	21.33±2.34
HCO_{3-} (mmol/L)	21.8±4.02	20.53±2.36
saO_2 (%)	96.89±2.37	97.83±1.17
pH	7.46±0.06	7.45±0.06

SD = Standard deviation, No statistically significant difference was found (P>0.05)

Mean values ± SD of CD, CW, ThI, ThK, and StL are provided in Table 4. These values demonstrate the existence of two different types of chest shape 12 months after surgery. CD ($P = 0.02$), ThK (=0.01), and StL ($P = 0.02$) were reduced in operated subjects compared to sham-operated rabbits. ThI was lower in operated rabbits compared to sham-operated subjects, and it was significantly different ($P < 0.001$). In contrast, CW did not show any statistically significant difference ($P = 0.26$).

Table 2: Mean values±standard deviation of echocardiographic parameters for operated and sham-operated rabbits

Parameter	Mean±SD	
	Operated	Sham operated
IVSd (mm)	3.06±0.47	3.39±0.33
IVSs (mm)	3.9±0.33	4.29±0.32
LVIDd (mm)	16.68±1.22	15.74±1.55
LVIDs (mm)	11.49±2.15	11.44±1.13
LVPWd (mm)	3.49±0.41	3.53±0.28
LVPWs (mm)	4.02±0.65	4.23±0.39
SF (%)*	36±4.67	29.71±3.17
LA (mm)	10.82±0.66	10.66±0.95
EF (%)	68.78±6.2	60.71±6.33
Ao (mm)	8.16±0.8	7.63±0.43
LA/Ao	1.29±0.11	1.31±0.13
Ao Vel_{Max} (m/s)	0.72±0.14	0.79±0.12
GP Ao_{Max} (m/s)	2.22±0.76	2.73±0.85
Po Vel_{Max} (m/s)	0.66±0.11	0.68±0.1
GP Po_{Max} (m/s)	1.84±0.58	1.93±0.62

SD=Standard deviation, IVSd and IVSs, thickness of the interventricular septum in diastole and in systole, respectively, LVIDd and LVIDs, left ventricular internal diameter shorth axis in diastole and in systole, respectively, LVPWd and LVPWs, thickness of the ventricular posterior wall in diastole and in systole, respectively; FS= fractional shortening, LA=Left atrial diameter, EF=Ejection fraction, Ao=Aorta diameter, LA/Ao=Ratio left atrial diameter and aorta diameter, Ao=VelMax, maximal aortic outflow velocity, GP AoMax, maximal aortic outflow pressure gradient: Po VelMax, maximal pulmonary outflow velocity, GP PoMax, maximal pulmonary outflow pressure gradient. Values are not significantly different between the two groups of rabbits (P>0.05), except for %FS (P=0.04)*

Table 3: Comparison of M-mode and doppler echocardiographic values in operated rabbits *versus* reported reference values

	Operated	Fontes-Sousa	*P* value
IVSd (mm)	3.06±0.47	2.65±0.31	0.03
IVSs (mm)	3.9±0.33	3.63±0.34	0.04
LVIDd (mm)	16.68±1.22	13.51±1.05	<0.001
LVIDs (mm)	11.49±2.15	8.64±0.82	0.004
LVFWd (mm)	3.49±0.41	2.25±0.29	<0.001
LVFWs (mm)	4.02±0.65	3.15±0.38	0.003
SF (%)	36±4.67	36.01±4.31	0.99
LA (mm)	10.82±0.66	7,49±1.14	<0.001
EF (%)	68.78±6.2	69.58±5.33	0.72
Ao (mm)	8.16±0.8	6.57±0.46	<0.001
LA/Ao	1.29±0.11	1.15±0.19	0.006
Ao Vel_{Max} (m/s)	0.72±0.14	0.86±0.12	0.02
GP Ao_{Max} (m/s)	2.22±0.76	-	-
Po Vel_{Max} (m/s)	0.66±0.11	0.78±0.12	0.01
GP Po_{Max} (m/s)	1.84±0.58	-	-

IVSd and IVSs, thickness of the interventricular septum in diastole and in systole, respectively; LVIDd and LVIDs, left ventricular internal diameter short axis in diastole and in systole, respectively; LVPWd and LVPWs, thickness of the ventricular posterior wall in diastole and in systole, respectively; FS=Fractional shortening, LA=Left atrial diameter, EF=Ejection fraction, Ao=Aorta diameter, LA/Ao=Ratio left atrial diameter and aorta diameter, Ao VelMax=Maximal aortic outflow velocity, GP AoMax=Maximal aortic outflow pressure gradient: Po VelMax=Maximal pulmonary outflow velocity, GP PoMax=Maximal pulmonary outflow pressure gradient

At 12 months after surgery, mean body weight was 5353 ± 497 gr (range, 4807 to 6200) in operated and 5266 ± 703 gr (range, 4440 to 6102) in sham-operated animals: At this time, the two groups of rabbits had nearly identical body weight and showed no significant differences ($P = 0.87$).

After removal of the heart-lung complex, the mean heart weight was 10 ± 1.6 gr (range, 7.2 to 11.5) in operated animals and 10.5 ± 1.4 gr (range, 8.6 to 12.1) in sham-operated rabbits. Mean heart volume was 9.3 ± 1.8 mL (range, 6.4 to 11.9) in operated and 10 ± 2.5 mL (range, 6.7 to 13.4) in sham-operated rabbits. Mean lung weight was 18.6 ± 2.9 gr (range, 14.3 to 22.6) in operated and 17.5 ± 2.7 gr (range, 14.8 to 22) in sham-operated subjects. Mean lungs volume was 21.1 ± 4.7 mL (range, 16 to 29.5) in operated and 19.2 ± 3.1 mL (range, 15.7 to 23.5) in sham-operated animals. The compared values above are not homogenous and need not to be taken into consideration, given the disparity of some single values of heart and lung parameters depending on the specific total body weight. Whereas, the most reliable comparison among the corresponding ratios heart (H)/lungs (Ls) of operated and sham-operated does not highlight significant differences. However, H/Ls volume ratio showed 10% of reduction in operated (0.52 ± 0.13) when compared with the sham operated (0.62 ± 0.12) ($P = 0.14$).

Histological observations of the lungs highlight pathological specific involvements, such as atelectasics, congested and interstitial patterns, equally distributed between operated and sham operated. Such cases are not investigated for possible differences between operated and sham operated, because such evidence may be possible in the rabbits, as outlined in veterinary anatomical pathology.[31]

Discussion

The main finding of our study was that T1-T12 arthrodesis would modify some thoracic cage dimensions at completion

Table 4: Mean values±SD of chest depth, chest width, thoracic index, thoracic kyphosis and sternal length

Parameter	Mean±SD		*P* value
	Operated	Sham operated	
CD (mm)	40.14±2.91	44.49±1.84	0.02
CW (mm)	60.94±1.84	60.22±4.66	0.26
ThI	0.661±0.058	0.74±0.035	0.001
ThK (°)	6±3.739	28±2	0.01
StL (mm)	103.05±3.46	110.83±1.5	0.02

SD = Standard deviation, CD = Chest depth, CW = Chest width, ThI = Thoracic index, ThK = Thoracic kyphosis, StL = Sternal length. Values are expressend in mm of growth, but not modify cardio-pulmonary function, as from evaluations based on blood gas samples and H/Ls and echocardiographic measurements.

Overall, the thoracic cage of operated rabbits grew less than that of sham operated animals. Although mean CD, ThK, ThI and StL were reduced in operated rabbits, the irregular chest growth did not alter echocardiographic values. Operated rabbits showed a tendency to have greater PO_2 reduction and PCO_2 increase compared to sham operated ones, thus highlighting a possible modification of gas exchanges. Those growth irregularities did not alter blood and echocardiographic parameters. Furthermore, %SF was significantly higher in operated rabbits. Similarly, the ratio H/Ls did not show statistical differences eventhough H/Ls volume ratio showed 10% of reduction in operated rabbits.

Rabbits can be used for cardiovascular, orthopaedic, and other experimental research, mainly as they are small yet large enough to allow physiological, medical, and surgical procedures.[9,10,12,20,32-36]

Data concerning echocardiography in rabbits and other animals under sedation, anesthesia, or in non-sedated state are currently available. Some of those studies suggested the need for sedation or anesthesia to allow the ultrasonographer to obtain adequate two-dimensional, M-mode, flow Doppler and tissue Doppler images for quantitative measurements[17] [Figures 3-8]. Dupras *et al*. found that mean arterial pressure and CO_2 arterial pressure were reduced after ketamine-midazolam-xylazine and tiletiletamine-zolazepam-zylazine anesthesia.[36] More recently, Fontes-Sousa *et al*. demonstrated that when sedated with a combination of ketamine and midazolam rabbits had a tendency to have an increased heart rate.[17,20] Our study shows that adequate echocardiographic images in non-sedated rabbits could be obtained at 12 months after T1-T12 dorsal arthrodesis [Figures 1 and 2] and values for two-dimensional, M-mode and spectral Doppler echocardiographic parameters [Tables 2 and 3] were found to be significantly reduced compared to those reported previously in anesthetized rabbits.[20,32]

Rabbits were housed in stainless steel cages, in compliance with national and international animal care regulations. However, the size of the cages and the subsequent relative inactivity of the animals may have influenced the clinical picture by limiting the effects of surgery in operated rabbits and by negatively influencing physiological results in sham-operated animals.[17,33-36]

Moreover, it is possible that chronicity is another factor of some impact; in other words, we had allowed for more than 12 months to pass post-operatively the reported changes we have seen would have progressed even further, potentially enough to show statistical significance.

The irregular growth of vertebral bodies is the basis of a distorted development, both in human bipeds and in quadrupeds. Ottander[37] showed it in pigs, Veliskakis and Levine[38] and Coleman[39] demonstrated it in dogs. Kioschos *et al*., in a skeletally immature dog model confirmed that the presence of dorsal vertebral arthrodesis leads to a significant reduction of dorsal kyphosis, even without implanted material, as a consequence of the ventral growth of vertebral bodies which therefore become asymmetric.[40]

More recently, Canavese *et al*. evaluated the consequences of disturbed growth of vertebral bodies on the development of the ribs, sternum, and lungs.[10,11] They found that experimental fusion in young rabbits performed at the critical T1-T6 segment of the thoracic spine produces hypoplasia of the entire upper thorax, including ribs, sternum, vertebral bodies and lung volume. They concluded that development of the thoracic cage and intra-thoracic organs is a complex process that requires perfect synergy among the various components of the rib-vertebral-sternal complex. Alterations to any of these elements affect and change the development and growth of the others. Therefore, to preserve thoracic motility and permit a normal development of the respiratory tree, treatment should not only focus on the spine but also consider the rib-vertebral-sternal complex as a whole.[10,11,24,25,41]

In humans, untreated progressive early onset spinal deformities have been associated with deformed spine, short trunk, short staure and disproportionate body habitus. The inability of the thorax to ensure normal breathing and the accompanying serious respiratory insufficiency is characterized by the thoracic insufficiency syndrome. This clinical picture can be linked to costo-vertebral malformations, neuromuscular disease, syndromes such as Jeune and Jarcho-Levin, or to 50% fusion of the thoracic spine before the age of.[7,3,4,10,11,24,25,41]

As the spinal deformity progresses, not only spinal growth is affected, but also the size and shape of the thoracic cage are modified. As a ''domino effect'', the distortion of the thorax will eventually interfere with lung development and cardiac function, leading those children to develop thoracic insufficiency syndrome and cor pulmonale which can be lethal in most severe cases.[3,41]

Karol *et al*. reported that a thoracic spine height of 18 to 22 cm or more is necessary to avoid severe respiratory insufficiency and it is needed for sufficient lung volume development.[4] They showed that children undergoing precocious spinal fusion exhibit reductions in thoracic depth and shorter T1-T12 segments compared to normal subjects. In addition, they proved that high thoracic spinal fusions (T1-T6 segment) in children with early onset spinal deformities resulted in shorter thoracic spine and worse pulmonary function tests than low thoracic spinal fusions (T7-T12 segment). The forced vital capacity may decrease by 50% of the predicted volume if more than 60% of the thoracic spine (eight or more thoracic vertebrae), is fused before eight years of age.[4] In their clinical work, Karol *et al*. confirmed some of the previously published experimental findings.[2-4,10,11,24,25,41] However, our results show that a precocious surgery of a non-deformed spine does not induce cardiopulmonary complications as severe as those found in patients with thoracic insufficiency syndrome. These data can be considered as a positive contribution to promoting conservative means of treatment in prepubertal patients to support the expansion of the thoracic cage, lung growth and cardiac function. Although quadrupeds do not develop scoliosis[24,25,37-39,41] it is possible that moderate and harmonious experimental chest volume reduction in bipeds should be differentiated from severe and deregulated distortion and deformation of the trunk secondary to spontaneously progressive spinal deformities. Scoliosis remains at the heart of severe chest deformities and it is therotically possible that to develop clinical pictures similar to thoracic insufficiency syndrome a deformed spine and a reduced thoracic spine height must develop progressively and simultaneously.

To conclude, our experimental model does not support the hypothesis that a precocious arthrodesis of a non-deformed spine could be source of cardiopulmonary complications severe enough to reproduce a clinical picture comparable to TIS.

References

1. Swank SM, Winter RB, Moe JH. Scoliosis and cor pulmonale. Spine (Phila Pa 1976) 1982;7:343-54.
2. Campbell RM, Smith MD, Mayes TC, Mangos JA, Willey-Courand DB, Kose N, *et al*. The characteristics of thoracic insufficiency associated with fused ribs and congenital scoliosis. J Bone Joint Surg Am 2003;85:409-20.
3. Goldberg CJ, Gillic I, Connaughton O, Moore DP, Fogarty EE, Canny GJ, *et al*. Respiratory function and cosmesis at maturity in infantile-onset scoliosis. Spine (Phila Pa 1976) 2003;28:2397-406.
4. Karol L, Johnston C, Mladenov K, Schochet P, Walters P, Browne RH. Pulmonary function following early thoracic fusion in non neuromuscular scoliosis. J Bone Joint Surg Am 2008;90:1272-81.
5. Bras-Silva C, Fontes-Sousa AP, Moura C, Areias JC, Leite-Moreira AF. Impaired response to ET (B) receptor stimulation in heart failure: Functional evidence of endocardial endothelial dysfunction? Exp Biol Med (Maywood) 2006;231:893-8.
6. Lange M, Smul TM, Blomeyer CA, Redel A, Klotz KN, Roewer N, *et al*. Role of the beta1-adrenergic pathway in anesthetic and ischemic preconditioning against myocardial infarction in the rabbit heart *in vivo*. Anesthesiology 2006;105:503-10.
7. Barraud D, Faivre V, Damy T, Welschbillig S, Gayat E, Heymes C, *et al*. Levosimendan restores both systolic and diastolic cardiac performance in lipopolysaccharide-treated rabbits: Comparison with dobutamine and milrinone. Crit Care Mede 2006;35:1376-82.
8. Mehta HP, Snyder BD, Callender NN, Bellardine CL, Jackson AC. The reciprocal relationship between thoracic and spinal deformity and its effect on pulmonary function in a rabbit model: A pilot study. Spine (Phila Pa 1976) 2006;31:2654-64.
9. Magit DP, Maak T, Troiano N, Raphael B, Hammouri Q, Polzhofer G, *et al*. Healos/recombinant human growth and differentiation factor-5 induces posterolateral lumbar fusion in a New Zealand White rabbit model. Spine (Phila Pa 1976) 2006;31:2180-8.
10. Canavese F, Dimeglio A, Volpatti D, Stebel M, Daures JP, Canavese B, *et al*. Dorsal arthrodesis of thoracic spine and effects on thorax growth in prepubertal New Zealand white rabbits. Spine (Phila Pa 1976) 2007;32:E443-50.
11. Canavese F, Dimeglio A, Granier M, Stebel M, Pittioni E, Cavalli F, *et al*. Arthrodesis of the first six dorsal vertebrae in prepubertal New Zealand white rabbits and thoracic growth to skeletal maturity: The role of the "Rib-Vertebral-Sternal complex". Minerva Ortop Traumatol 2007;58:369-78.
12. Mehta HP, Snyder BD, Baldassarri SR, Hayward MJ, Giuffrida MJ, Entezari V, *et al*. Expansion thoracoplasty improves respiratory function in a rabbit model of postnatal pulmonary hypoplasia: A pilot study. Spine (Phila Pa 1976) 2011;35:153-61.
13. Martin MW, Darke PG, Else RW. Congestive heart failure with atrial fibrillation in a rabbit. Vet Rec 1987;121:570-1.
14. Marco I, Cuenca R, Pastor J, Velarde R, Lavin S. Hematology and serum chemistry values in the European brown hare. Vet Clin Pathol 2003;32:185-98.
15. Szabuniewicz M, Hightower D, Kyraz JR. The electrocardiogram, vectocardiogram and spatiocardiogram in the rabbit. Can J Comp Med 1971;35:107-14.
16. Cheitlin M, Alpert J, Amstrong W. ACC/AHA guidelines for the clinical application of echocardiography. Circulation 1997;95:1686-774.
17. Fontes-Sousa AP, Moura C, Carneiro CS, Teixeira-Pinto A, Areias JC, Leite-Moreira AF. Echocardiographic evaluation including tissue Doppler imaging in New Zealand white rabbits sedated with ketamine and midazolam. Vet J 2009;181:326-31.
18. Noszczyk-Nowak A, Nicpon J, Nowak M, Slawuta P. Preliminary reference vaues for elecrocardiography, echocardiography and myocardial morphometry in the European brown hare (Lepus europaeus). Acta Vet Scand 2009;51:6.
19. Porciello F, Fruganti G, Avellini G. Echocardiography in dogs, cats and horses. Milano, Italy, Poletto Ed. 2004.
20. Fontes-Sousa AP, Bras-Silva C, Moura C, Areias JC, Leite-Moreira AF. M-mode and Doppler echocardiographic reference values for male New Zealand white rabbits. Am J Vet Res 2006;67:1725-9.
21. Stypmann J, Engelen MA, Breithardt AK, Milberg P, Rothenburger M, Breithardt OA, *et al*. Doppler echocardiography and tissue Doppler imaging in the healthy rabbit: Differences of cardiac function during awake and anaesthetised examination. Int J Cardiol 2007;115:164-70.
22. Resina J, Ferriera-Alves A. A technique of correction and internal fixation for scoliosis. J Bone Joint Surg Br 1977;59:159-64.
23. Drummond DS. Harrington instrumentation with spinous process wiring for idiopathic scoliosis. Orthop Clin North Am 1988;19:281-9.

24. Canavese F, Dimeglio A, Granier M, Beraldo P, Bonnel F, Stebel M, *et al*. Influence de l'arthrodèse vertébrale sélective T1- T6 sur la croissance thoracique: Étude expérimentale chez des lapins New Zealand white prépubertaires. Rev Chir Orthop Reparatrice Appar Loc 2008;94:490-7.
25. Canavese F, Dimeglio A, D'Amato C, Volpatti D, Granier M, Stebel M, *et al*. Dorsal arthrodesis in prepubertal New Zealand white rabbits followed to skeletal maturity: Effect on thoracic dimensions, spine growth and neural elements. Indian J Orthop 2010;44:14-22.
26. Kilkenny C, Browne WJ, Curthill IC, Emerson M, Altman DG. Improving Bioscience Research Reporting: The ARRIVE Guidelines for Reporting Animal Research. PloS Biol 2010;8:e1000412.
27. Barone R, Comparative anatomy of domestic animals, Vol. 1-6. Paris, France, Vigot Frères Eds., 1996-2004.
28. Thomas WP, Gaber CE, Jacobs GJ, Kaplan PM, Lombard CW, Moise NS, *et al*. Recommendations for standards in transthoracic two-dimensional echocardiography in the dog and cat. Echocardiography Committee of the Specialty of Cardiology, American College of Veterinary Internal Medicine. J Vet Intern Med 1993;7:247-52.
29. Cobb JR. Outline for the study of scoliosis. Instr Course Lect 1948;5:261-75.
30. Nachemson AL, Peterson LE. Effectiveness of treatment with a brace in girls who have adolescent idiopathc scoliosis: A prospective, controlled study based on data from the BRACE study of the Scoliosis Research Society. J Bone Joint Surg Am 1995;77:815-22.
31. Marcato PS. Patologia sistematica veterinaria. Bologna, Edagricole; 2002.
32. Muders F, Elsner D. Animal models of chronic heart failure. Pharmacol Res 2000;41:605-12.
33. Kavinsky CJ, Umeda PK, Levin JE, Sinha AM, Nigro JM, Jakovcic S, *et al*. Analysis of cloned mRNA sequences encoding subfragment 2 and part of subfragment 1 of alpha- and beta-myosin heavy chains of rabbit heart. J Biol Chem 1984;259:2775-81.
34. Ezzaher A, el Houda Bouanani N, Crozatier B. Force–frequency relations and response to ryanodine in failing rabbit hearts. Am J Physiol 1992;263:H1710-5.
35. Hasenfuss G. Animal models of human cardiovascular disease, heart failure and hypertrophy. Cardiovasc Res 1998;39:60-76.
36. Dupras J, Vachon P, Cuvelliez S, Blais D. Anesthesia of the New Zealand rabbit using the combination of tiletamine–zolazepam and ketamine–midazolam with or without xylazine. Can Vet J 2001;42:455-60.
37. Ottander HG. Experimental progressive scoliosi in a pig. Acta Ortop Scand 1963;33:91-7.
38. Veliskakis K, Levine DB. Effect of posterior spine fusion on vertebral growth in dogs. J Bone Joint Surg Am 1966;48:1367-76.
39. Coleman SS. The effect of posterior spinal fusion on vertebral growth in dogs. J Bone Joint Surg Am 1968;50:879-96.
40. Kioschos HC, Asher MA, Lark RG, Harner EJ. Overpowering the crankshaft mechanism: The effect of posterior spinal fusion with and without stiff transpedicular fixation on anterior column growth in immature canines. Spine 1996;21:1168-73.
41. Canavese F, Dimeglio A. Normal and abnormal spine and thoracic cage development. World J Orthop 2013;4:167-74.

How to cite this article: Canavese F, Dimeglio A, Barbetta D, Pereira B, Fabbro S, Bassini F, Canavese B. Effect of thoracic arthrodesis in prepubertal New Zealand white rabbits on cardio-pulmonary function. Indian J Orthop 2014;48:184-92.

Source of Support: Nil, **Conflict of Interest:** None of the authors of this paper have a financial or personal relationship with other people or organisations that could inappropriately influence or bias the content of the paper.