Canadian Journal of Cardiology 35 (2019) 1762–1771

Review

# Animal Models of Repaired Tetralogy of Fallot: Current Applications and Future Perspectives

Estibaliz Valdeomillos, MD,[a,b,c] Zakaria Jalal, MD, PhD,[a,b,c] Alexandre Metras, MD,[b,c,d] François Roubertie, MD, PhD,[b,c,d] David Benoist, PhD,[b,c] Olivier Bernus, PhD,[b,c] Michel Haïssaguerre, MD, PhD,[b,c,e] Pierre Bordachar, MD, PhD,[b,c,e] Xavier Iriart, MD,[a,b,c] and Jean-Benoit Thambo, MD, PhD[a,b,c]

[a] *Department of Pediatric and Adult Congenital Cardiology, Bordeaux University Hospital (CHU), Bordeaux, France*

[b] *IHU Liryc, Electrophysiology and Heart Modeling Institute, Fondation Bordeaux Université, Bordeaux, France*

[c] *INSERM, Centre de recherche Cardio-Thoracique de Bordeaux, Bordeaux, France*

[d] *Department of Pediatric and Adult Congenital Surgery, Bordeaux University Hospital (CHU), Bordeaux, France*

[e] *Department of Electrophysiology, Cardio-Thoracic Unit, Bordeaux University Hospital (CHU), Bordeaux, France*

## ABSTRACT

**Tetralogy of Fallot is the most common cyanotic congenital heart disease. Despite ongoing improvements in the initial surgical repair, there are lingering concerns regarding the long-term outcomes that may be complicated by right ventricular dysfunction, right ventricular dyssynchrony, and sudden cardiac death. The mechanisms leading to these late complications remain incompletely understood. Experimental animal models have been developed as preclinical steps to gain better insight into the pathophysiology of diseases and to develop new therapeutic strategies. This article summarizes the various types of experimental animal models of repaired tetralogy of Fallot published to date in the literature, with the aim of achieving a greater understanding of the deleterious mechanisms that may lead to these known late and sometimes lethal complications. In addition to analysing the type of animals that can be used according to a given study's objectives, needs, and constraints, the present review also evaluates**

## RÉSUMÉ

**La tétralogie de Fallot est la cardiopathie congénitale cyanogène la plus fréquente. Malgré les progrès réalisés en matière de réparation chirurgicale (la méthode employée en première intention), l'issue à long terme de la maladie, qui peut être compliquée d'une dysfonction ventriculaire droite, d'une dyssynchronie ventriculaire droite ou d'une mort cardiaque subite, demeure préoccupante. Les mécanismes menant à ces complications tardives ne sont toujours pas bien compris. Des modèles animaux expérimentaux ont été mis au point dans le cadre d'études précliniques afin de mieux comprendre la physiopathologie de la maladie et de concevoir de nouvelles stratégies thérapeutiques. Les auteurs résument les divers types de modèles animaux expérimentaux de tétralogie de Fallot réparée publiés à ce jour, dans le but de mieux comprendre les mécanismes délétères qui peuvent mener à ces complications tardives et parfois mortelles. En plus d'analyser le type d'animal qui peut être utilisé en fonction des**

Tetralogy of Fallot (TOF) is the most common cyanotic congenital heart disease resulting in long-term right ventricular (RV) failure, with a prevalence of 3.5 per 10,000 births.[1] Over the last decades, improvements in the approach of surgical correction have considerably increased survival.[2] Surgical therapy of TOF is highly successful, although the long-term course may be complicated by RV dysfunction, ventricular arrhythmias, and late sudden cardiac death (SCD).[3] These complications are a common denominator in RV outflow tract (RVOT) dysfunction. Currently, the mechanisms leading to these delayed adverse complications[4-6] are not yet completely understood, such that therapeutic possibilities are limited for these patients and only provide intermediary results.[7]

To better understand the mechanisms underlying the aforementioned complications and develop new therapeutic strategies, experimental animal models have been a key component of preclinical studies during the last 3 decades.

In this article, we will focus on experimental studies performed on animal models of repaired TOF. We will explore different mechanisms of dysfunction and their consequences with the aim of achieving a more comprehensive understanding

Received for publication March 28, 2019. Accepted July 3, 2019.

Corresponding author: Dr Estibaliz Valdeomillos, University Hospital of Bordeaux, Avenue Magellan, 33600 Pessac, Bordeaux, France. Tel.: +33-5-57-65-64-65; fax: +33-5-57-65-68-28.

E-mail: estibaliz.valdeolmillos@gmail.com

See page 1769 for disclosure information.

https://doi.org/10.1016/j.cjca.2019.07.622

the type of dysfunction that can be reproduced in our model according to the research objectives, as well as the different types of studies in which these models can be used. In view of all that, we propose a decision algorithm to create an animal model of repaired tetralogy of Fallot. This synthesis should furthermore help in the development of future studies and in the design of new experimental models, thus allowing greater insight into this disease, while not forgetting the ultimate goal of broadening future therapeutic measures to reduce the morbidity and mortality of this prevalent congenital heart disease.

objectifs de l'étude, des besoins et des contraintes, nous évaluons les types de dysfonctions qui peuvent être reproduites à l'aide de notre modèle en fonction des objectifs de la recherche, ainsi que les différents types d'études dans lesquelles les modèles peuvent être utilisés. À partir de toutes ces données, nous proposons un algorithme de décision permettant de créer un modèle animal de tétralogie de Fallot réparée. Cette synthèse pourra également servir à concevoir d'autres études et à mettre au point de nouveaux modèles expérimentaux pour mieux comprendre la maladie, sans oublier le but ultime d'élargir l'éventail des options thérapeutiques afin de réduire la morbidité et la mortalité associées à cette cardiopathie congénitale répandue.

of the different types of animal models that can be used to perform the targeted repaired TOF (rTOF) model. Sound knowledge of their strengths and limitations is essential to designing an ideal model in accordance with the various study objectives, needs, and constraints to develop future therapeutic strategies. In addition, we propose an algorithm to create the animal model of rTOF that is likely to be useful and might stimulate further research in this field.

## Methods

A systematic review of the literature was performed on original published studies of experimental animal models of TOF. The search was conducted on the PubMed, MEDLINE, and EMBASE (from 1985 to October 2018) databases. The search terms used were as follows: (tetralogy of Fallot) and (animal models) or (right ventricular function) and (animal models). Initially, the articles were screened by title and abstract. Only full articles, peer reviewed in English language, without time limitation, based on animal models with RVOT modification, either due to RV volume overload or RV pressure overload, or both, independent of the type of animal used, or the type of studies carried out, were reviewed. Exclusion criteria were articles written in other languages, case reports, studies that did not include animal models or those that studied a heart disease that did not meet the characteristics of our study.

## Which Animal, Which Dysfunction, Which Approach?

### Type of animal

The anatomic and pathophysiological cardiovascular characteristics differ according to the species used. The choice of the model should be based primarily on the study's objectives, and hence the importance of being familiar with the specificities of the various animal models and their possible applications in the studied field.

**Large mammalian models of rTOF.** Large mammals have been proposed as a good model of human physiology, because they more closely resemble humans with a high percentage of genetic conservation.[8] Because of their similarities with humans in terms of heart size, coronary anatomy, and vascular physiology, swine, ovine, and canine models have most often been used in preclinical studies of rTOF.[9] However, it is important to consider the anatomic and functional differences between these species to choose the most appropriate model for the study's objectives.

Coronary circulation in pigs has no anastomoses between vascular branches, whereas the coronary circulation in dogs can be extensively collateralized.[10,11] Conversely, the electrophysiological properties of the various species have been extensively studied for which certain particularities need to be considered. The distribution of the Purkinje fibers in swine extends over nearly the entire transmural distance from endocardium to epicardium, and, consequently, ventricular activation differs markedly from that in humans. However, Purkinje fibers and cardiac activation sequences in dogs are quite similar to humans.[12]

In terms of functionality, the contractile and relaxation kinetics of sheep cardiomyocytes are also similar to humans. In addition, the resting heart rate as well as systolic and diastolic pressure in sheep is also comparable with that of humans.[13]

Another difference to consider is growth. The swine model features a rapid growth and reaches an adult human size at 4-6 months. Its rapid growth represents an advantage because of the early appearance of cardiac injuries; however, the latter becomes a disadvantage in chronic disease models. The ovine model has slower growth that allows assessing disease evolution over time. Thus, depending on the timeframe of the study, such differences will influence the decision in choosing one species or another.[14]

Another relevant issue to consider is the lack of a significant difference between species with regard to RVOT and pulmonary branch anatomy, which are similar to humans.

Although primate models would typically represent the best surrogate of man, they are rarely used because of ethical concerns, logistical difficulties, and the high costs involved.[9]

The main limitations with large mammals are the ethical, practical, and financial difficulties. In addition, they have longer gestation times and lifespan, and their reproduction rate is lower. They are moreover less suitable than small animals for genetic selection and the production of transgenic strains and generate less spontaneous disease models. Another inconvenience is the quadrupedal posture that conditions a cardiac orientation that differs from that of humans.[15]

Although large mammals can be used for *in vitro* studies, they are rarely used because of their high cost and the fact that they rarely offer any particular advantage over similar preparations obtained from small mammals.

**Table 1.** Advantages and limitations of the animal models used in the study of RV dysfunction

| Animal model | Advantages | Drawbacks | References |
|---|---|---|---|
| Large mammalian models of rTOF (porcine, ovine, and canine models) | Greater resemblance to the human heart<br>Longer lifespan<br>Long-term studies<br>Similar techniques and interventions to those in humans | Ethical constraints<br>Higher cost<br>More difficult to handle and maintain<br>Longer gestation time and lower reproduction rate<br>Smaller samples<br>Less suitable for genetic manipulation | Graves et al.[8]<br>Hearse et al.[9]<br>Hamlin et al.[10]<br>Yong et al.[11]<br>Smith et al.[12]<br>Milani-Nejad et al.[13]<br>Camacho et al.[15] |
| Small mammalian models of rTOF (murine and rabbit models) | Lower cost<br>Higher availability<br>Easy to handle and maintain<br>High reproduction rate and short lifespan<br>Short-term studies<br>Substantial sample size, highly cost effective<br>Easy to manipulate genetically | Disparity with the human heart and its physiology<br>Special technical requirements (small size) | Hearse et al.[9]<br>Patten et al.[16]<br>Ou et al.[17]<br>Van den Akker et al.[18]<br>Urashima et al.[19]<br>Reddy et al.[20] |

rTOF, repaired tetralogy of Fallot; RV, right ventricle.

The main advantages of large mammalian models are that they allow chronic studies to be undertaken. Regarding devices and interventional cardiology, these models also allow performing similar techniques and interventions as those used in human clinical practice.[9]

**Small mammalian models of rTOF.** The main small mammal models used for the study of rTOF are rat, rabbit, guinea pig, and mouse. Most of these experimental model types have been used in *in vitro* studies.[16,17]

One of the main advantages is their high reproduction rate and the short lifespan that allow analysing the natural history of the disease within a short time span. On the other hand, their genetic similarity with humans enables the study of certain alterations and their correspondence with clinical diseases. Technological advances in animal models such as mice models have allowed pinpointing specific alterations in the genome. These mice models based on genetic alterations have facilitated the study of rTOF and their genetic determinants.[18-20]

Small mammals are an ideal experimental model to achieve a considerable sample size in a highly cost-effective manner. However, the main drawback is their disparity with the human heart and their physiology. In addition, the technical challenges related to surgical procedures represent a considerable limitation[9] (Table 1).

## Type of dysfunction

Ideally, any animal model should faithfully reproduce the structural and functional characteristics of the human pathology studied. However, rTOF encompasses a wide spectrum of potential postoperative lesions ranging from isolated severe RVOT obstruction to free pulmonary regurgitation (PR). Accordingly, diverse experimental models can be distinguished depending on the type of postoperative sequelae performed (Table 2).

**Table 2.** Main characteristics according to the type of dysfunction depending on the postoperative sequelae performed in rTOF models

| Dysfunction | Surgical methods | Percutaneous methods | Species | References |
|---|---|---|---|---|
| RV volume overload | Pulmonary valvulotomy<br>Transannular patch<br>External plication of the pulmonary valve | Stenting the pulmonary valvular annulus | Ovine model<br>Porcine model<br>Murine model | De Vroomen et al.[25]<br>Gray et al.[26]<br>Yerebakan et al.[27]<br>Mori et al.[28]<br>Reddy et al.[20]<br>Agger et al.[29]<br>Kuehne et al.[30]<br>Smith et al.[31]<br>Ersboell et al.[32] |
| RV pressure overload | Pulmonary artery banding | Self-expanding nitinol stent fitted with a thin intraluminal Teflon membrane deployed across the pulmonary valve | Porcine model<br>Rabbit model | Lambert et al.[34]<br>Minegishi et al.[35]<br>Bove et al.[36]<br>Hodzic et al.[37]<br>Kuehne et al.[38] |
| RVOT scar | Pulmonary valvulotomy or pulmonary artery banding + RVOT scar | Radiofrequency ablation of the right bundle branch without incurring an RVOT scar | Porcine model<br>Canine model | Zeltser et al.[42]<br>Kaltman et al.[43]<br>Chiu et al.[44] |
| rTOF model | Pulmonary valvulotomy + pulmonary artery banding + RVOT scar (longitudinally over the infundibulum) | | Porcine model | Thambo et al.[45] |

rTOF, repaired tetralogy of Fallot; RV, right ventricle; RVOT, right ventricular outflow tract.

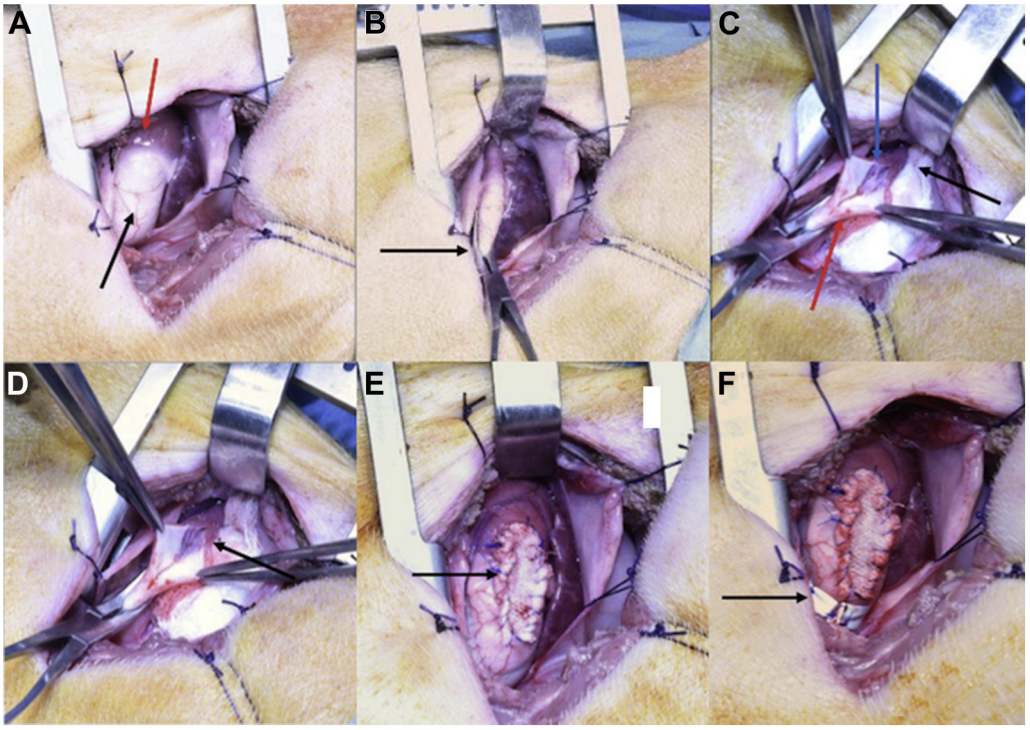


**Figure 1.** Step-by-step surgical repaired tetralogy of Fallot swine model creation. (**A**) After a left thoracotomy, pericardiotomy exposes the right ventricular infundibulum (**red arrow**) and main pulmonary artery (**black arrow**). (**B**) Longitudinal side clamp (pulmonary artery and infundibulum) across the pulmonary annulus (**black arrow**). (**C**) Longitudinal transannular arteriotomy (**red arrow**) with small infundibulotomy (**black arrow**). Pulmonary valve leaflets (**blue arrow**) are exposed. (**D**) Pulmonary valve leaflets are resected (**black arrow**). (**E**) Transannular Dacron patch is sewed (**black arrow**). (**F**) Gore-Tex band is placed around the main pulmonary artery distally to Dacron patch (**black arrow**).

**RV volume overload.** This model represents the RV volume overload caused by a PR resulting from RVOT reconstruction, typically performed using a large transannular patch during TOF repair. Chronic isolated severe PR has been shown to result in RV dilation,[21,22] as well as an increased incidence of SCD.[23,24]

To create the RV overload, various surgical procedures have been performed in porcine, ovine, and murine models including pulmonary valvulotomy, transannular patch,[25-28] and external plication of the pulmonary valve.[20,29] In addition, in various instances, PR has been created through percutaneous procedures in porcine models by stenting the pulmonary valvular annulus.[30-32] These techniques will be comprehensively described in a dedicated section below.

**RV pressure overload.** As a result of the residual postoperative RVOT obstruction, a model of chronic RV pressure overload can be created, leading to progressive RV function impairment and RV hypertrophy. Initially, RV systolic function is preserved with RV pressure overload, although diastolic dysfunction may occur as a consequence of myocardial hypertrophy and remodelling.[33]

The most common procedure used to achieve the RV pressure-overload model has been pulmonary artery banding (PAB).[34-37] This procedure has been performed in porcine models as well as rabbit models. Likewise, transcatheter techniques have been used for this purpose in porcine models as well as a self-expanding nitinol stent fitted with a thin intraluminal Teflon membrane deployed across the pulmonary valve.[38]

**RVOT scar and right bundle branch block.** As a result of closing the ventricular septal defect and alleviating the RVOT obstruction, atrial and ventricular scars, right bundle branch (RBB) block (RBBB), and electromechanical ventricular delays are generated,[39,40] leading to macroreentry circuits and monomorphic ventricular tachycardia.[41]

All surgical groups used porcine models to position the scar over the infundibulum without interruption of the pulmonary valve apparatus. As demonstrated previously, it is possible to reproduce an animal model that presents RV dysfunction and biventricular dyssynchrony with RBBB by performing a pulmonary valvulotomy or PAB together with an RVOT scar without having to perform an RBB ablation.[42,43] However, there are also certain groups, which directly perform radiofrequency ablation of the RBB without incurring an RVOT scar in canine models.[44]

**Repaired Fallot model.** To reproduce a true rTOF model that encompasses all of the aforementioned features, our group introduced a porcine model that reproduces the essential parameters of postoperative Fallot, including (1) RV pressure overload, (2) RV volume overload, and (3) RVOT scarring around the patch. This allowed achieving chronic left

ventricle (LV) dysfunction, as observed in an adult population of repaired TOF.[45] Other groups have since designed animal models with similar characteristics.[46,47]

## Surgical Procedures vs Transcatheter Techniques

In the 1980s, the first model with RV dysfunction was performed by PAB to create a pressure overload and atrial septal defects to create a volume overload.[48] The first surgical technique to create PR in a large animal model was proposed in 1998 by Shiota et al.[49] by excising the pulmonary valve leaflets in an ovine model. Later, Mori et al.[28] improved the technique using cardiopulmonary bypass (CPB), through a small RV infundibulotomy, in which the right anterior pulmonary valve cusp was radially incised in their ovine model.

In 2003, Gray et al.[26] further developed the technique to be used on the beating heart without CPB, which was a significant challenge and allowed reducing the study costs. After encircling the caval veins and the main pulmonary artery with snares, followed by tightening of the snares, the authors performed a 2-cm arteriotomy in the main pulmonary artery and excised 2 leaflets of the pulmonary valve. Significant PR was achieved in all intervention lamb models, although the method obviously bears a risk of lethal bleeding.

In 2005, Zeltser et al.[42] designed a porcine model with mixed RVOT lesions by left thoracotomy, combining pulmonary valvulotomy together with performing a longitudinal incision across the pulmonary annulus, and excising 2 pulmonary valve leaflets, with PAB or RVOT scar. All procedures were performed by placement of a side-biting vascular clamp over the infundibulum, without CPB.

The potentially highest cost-effective porcine model was proposed by Agger et al.[29] by external suture plication of the pulmonary valve leaflets to create PR, placing single sutures through the wall of the pulmonary trunk and around the hinge points of the pulmonary valve leaflets.

In 2010, our group created the first animal model (swine model) that combined the 3 main characteristics of rTOF: (1) RV volume overload from PR achieved by means of longitudinal placement of a vascular clamp across the pulmonary valve annulus without obstruction of the RV outflow tract. A 2-cm incision was then made longitudinally across the pulmonary annulus; (2) RV pressure overload, via PAB by a loose Goretex tape partially occluding the main pulmonary artery placed 1 cm distal to the valve annulus; (3) RV outflow tract scar, in which a 1-cm full-thickness incision was made longitudinally over the infundibulum[45] (Fig. 1).

Aside from a few anecdotal studies, the most notable concept of creating PR by the transcatheter technique was suggested by Kuehne et al.[30] in 2003 whereby a self-expanding nitinol stent (18-20 mm) was deployed in the pulmonary valve annulus of a swine model. The amount of PR achieved by this method was higher than in most clinical series. Two years later, Kuehne et al.[38] developed a porcine model of RV overload and pressure overload by placing a self-expanding nitinol stent (18-20 mm) fitted with a thin intraluminal Teflon membrane across the pulmonary valve.

Finally, 2 groups studied percutaneous pulmonary valve implantation (PPVI) after different periods of PR. In 2012, Smith et al.[31] deployed a stent (22 mm CP) in the pulmonary valvular annulus, and after 3 months of free PR, a pulmonary valve (Medtronic Melody) was mounted in the CP stent in 6 piglets. Later, in 2013, Ersboell et al.[32] used the same technique in 36 piglets but with differential duration of PR after which PPVI was performed (Table 3).

## One Model, Different Possibilities

### Hemodynamic studies

The first decisive hemodynamic study of RV dysfunction was conducted in a swine model in 2003 by Kuehne et al.[30] They established for the first time that chronic RV volume overloading causes progressive RV dilatation with concomitant compression of the LV and ultimately alters biventricular systolic function, RV contractility, and LV diastolic performance. Two years later, the same group conducted a study to determine the effects of chronic combined pulmonary stenosis and insufficiency on RV and LV function in a swine model. The authors observed that chronic pressure and volume overload impairs biventricular systolic pump function and diastolic compliance but preserves RV myocardial contractility.[38] These findings by Kuehne et al.[38] were subsequently confirmed by other studies.[29,50]

To describe the early RV remodelling after chronic RV overload and pressure overload, new porcine and ovine models were created showing functional and structural RV remodelling. In addition to the previous hemodynamic findings,

**Table 3.** The main advantages and drawbacks of surgical and percutaneous procedures in rTOF models

| Procedure | Advantages | Drawbacks | References |
|---|---|---|---|
| Surgical | Control over the injury to be performed: adjustable PR or PS<br>More reproducible<br>Possibility of performing RVOT scar | Higher risk of postoperative complications (infections, thoracotomy problems, etc.)<br>Higher morbidity and mortality<br>Need for skilled surgeon/surgical staff | Gray et al.[26]<br>Agger et al.[29]<br>Zeltser et al.[42]<br>Thambo et al.[45] |
| Percutaneous | Lower morbidity and mortality<br>Less invasive | Less control over the injury to be performed<br>Need for a hemodynamics laboratory<br>Procedural complications (coronary impingement, device embolization, venous thrombosis, infective endocarditis) | Khuene et al.[30,38]<br>Smith et al.[31]<br>Ersboell et al.[32] |

PR, pulmonary regurgitation; PS, pulmonary stenosis; RVOT, right ventricular outflow tract.

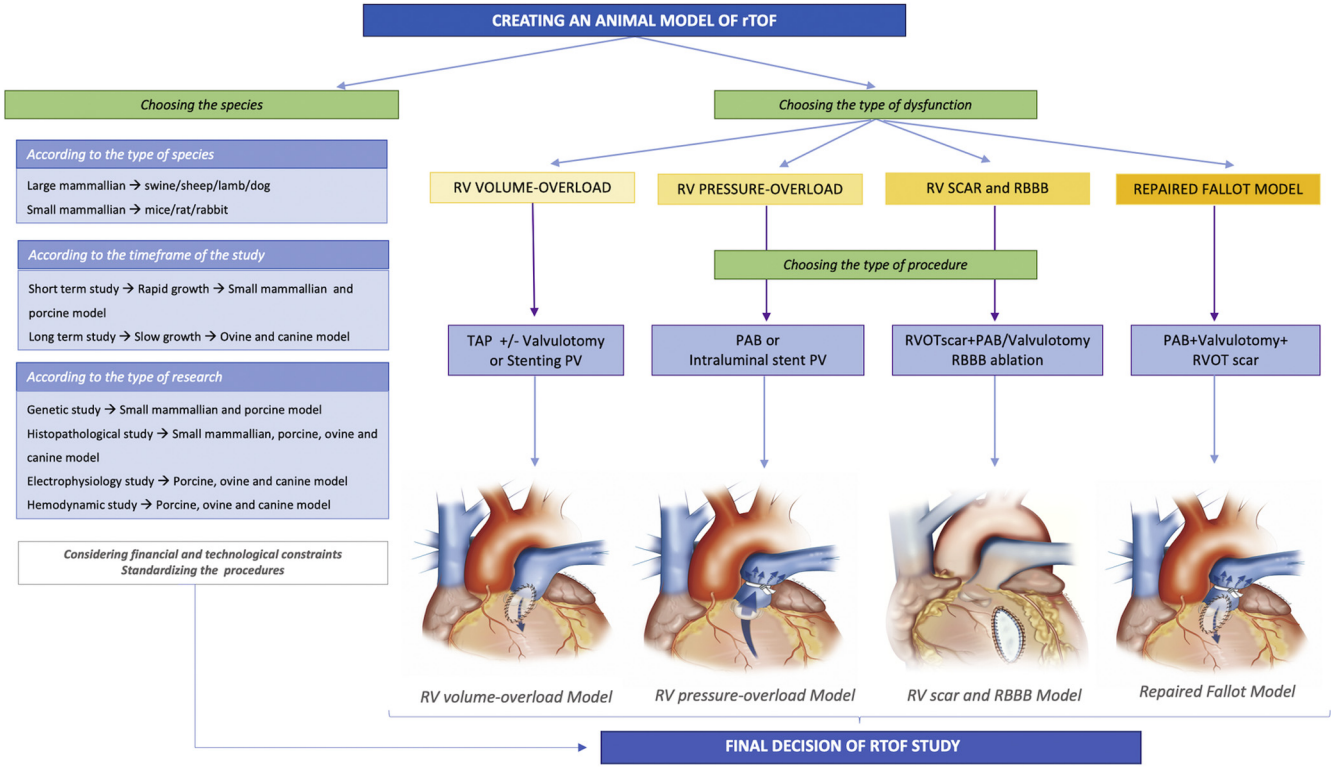


**Figure 2.** Creating an animal model of repaired tetralogy of Fallot (rTOF). This illustration summarizes the approach used to perform an animal model of rTOF according to a given study's objectives and requirements. Steps include choosing the type of animal to use, followed by determining the type of dysfunction to be created to obtain the type of animal model desired, thereby enabling us to conduct the various investigations in the model according to the needs of the study. PAB, pulmonary artery banding; PV, pulmonary valve; RV, right ventricle; RVOT, RV outflow tract; TAP, transannular patch.

several teams observed a significant RV fibrosis that was correlated with QRS prolongation.[32,34,36,50]

In recent years, the interest in determining the optimal timing of valve replacement and the recovery capacity of RV has increased, in which new percutaneous porcine models have appeared such as that described by Ersboell et al.[32] They determined that the main predictor of non-recovery, measured in a porcine model, was RV dilatation above 120 mL/m$^2$ and 45 mL/m$^2$ for diastolic and systolic volumes, respectively, indicating that dilatation per se rather than PR duration before valve replacement is a major determinant.

## Electrophysiological studies

In 2003, Gray et al.[26] investigated for the first time the *in vivo* and *in vitro* electrophysiological properties of the RV in a lamb model of chronic PR. They showed that chronic PR leads to inhomogeneity of RV activation, alters myocardial calcium cycling, reduces conduction velocity, and increases intracellular resistivity, contributing to the development of arrhythmias. Later, Zeltser et al.[42] demonstrated that QRS prolongation was a risk factor for ventricular arrhythmias in porcine models and that an increased RV end-diastolic pressure was the sole variable that predicted ventricular tachycardia or fibrillation inducibility.

In the last decade, the advent of new technologies has allowed characterizing the electrophysiological remodelling of the RV in a preclinical porcine, ovine, and canine models of rTOF. Heterogeneous repolarization and slow and discontinuous conduction were found to predispose to re-entries and to generate a proarrhythmic substrate. In addition, mechanisms associated with this remodelling involve a proliferation of the extracellular matrix and changes in ion channel expression.[47,51-53]

## Biomolecular studies

Over the last decade, the use of rTOF models has been extended to the field of biomolecular and genetic engineering, with the aim of seeking new therapeutic strategies. One of the first ovine models used for this goal was designed by Yerebakan et al.[54] in 2009, in which the authors evaluated the feasibility and efficacy of autologous umbilical cord blood mononuclear cell transplantation on RV function in an ovine model of chronic RV volume overload. They demonstrated that these cells enhance diastolic properties with a probable underlying mechanism of increased angiogenesis.

Ten years later, Lambert et al.[46] created a swine model reproducing RV dysfunction secondary to chronic pressure-volume overload, to study the feasibility and the effects of cell therapy using intramyocardial injections of human MesP1β/SSEA-1β cardiogenic mesodermal cells. They observed that cell therapy appears to confer benefits with regard to overloaded RV tissue remodelling and arrhythmic susceptibility.

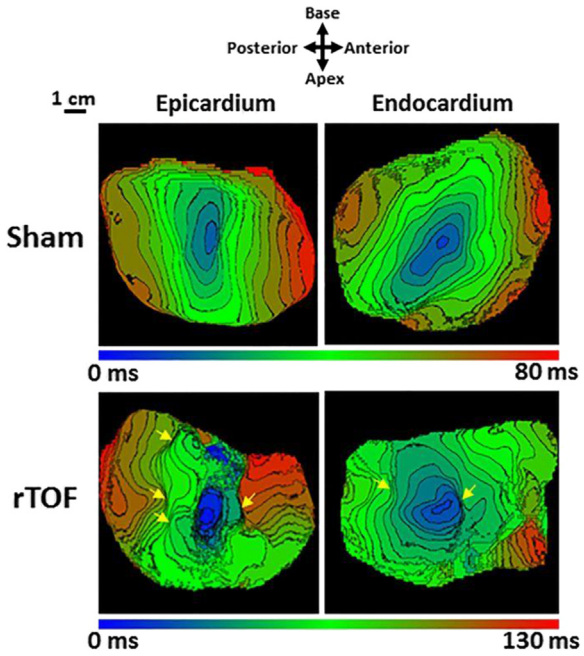


**Figure 3.** Right ventricle (RV) activation in the repaired tetralogy of Fallot (rTOF) porcine model. Representative activation maps from a Sham and an rTOF RV with 5 ms spaced isochrones. Multiple areas of slowed conduction were observed (**yellow arrows**) throughout the rTOF RVs. Adapted from Benoist et al.[51] with permission from BMJ Publishing Group Ltd & British Cardiovascular Society.

The first report on global gene expression profiling in the heart of a long-term swine model of rTOF was carried out by our team in 2015. We found that arrhythmia-related genes (*MYO5B*, *KCNA5*) were among the most deregulated genes in TOF ventricles.[55]

### Testing new devices

In September 2000, Bonhoeffer et al.[56] were the first group to successfully perform the first PPVI procedure for the nonsurgical implantation of a biological valve in the pulmonary position in a lamb model. A fresh bovine jugular vein containing a native valve was sutured into a vascular stent after which the device was hand-crimped onto a balloon catheter, inserted percutaneously and finally deployed in the position of the native pulmonary valve of the lamb. A few months later, the same group performed the first successful PPVI in a human patient.[57]

Later in 2011, Robb et al.[50] designed a new method for the treatment of RVOT dilatation and distortion in an ovine model. They successfully implanted a Melody valve into both the right and left branch pulmonary arteries, leading to a significant reduction in PR.

In keeping with the above, Kim et al.[58] investigated the feasibility of a large-diameter self-expandable valved stent and the durability of the valve after $>$ 6 months in an ovine model. They created a nitinol wire—based, self-expandable valved stent with leaflets made from porcine pericardium and implanted the stent in the main pulmonic valve. Implanted stents showed well-preserved valve leaflets with functional competence in the mid-term results.

## Discussion

Determining the best experimental model of a human condition requires a model that fulfils the purpose of a given study, with the property of being highly reproducible, cost-effective, and relatively easy to perform. The importance of designing a good experiment not only requires the ability to identify the most appropriate animal model and species to complete the research work and achieve expectations but also the ability to balance, as much as possible, the conflict between its financial and technological constraints.

Likewise, it is necessary to consider the crucial importance of standardizing the various procedures used, to obtain relevant and reproducible results that can be compared with other findings.

Nevertheless, it should be taken into consideration that animal models can closely reproduce the electrophysiologic and mechanical abnormalities found in patients with this disorder, but they are not a perfect surrogate for the human being.

The main limitations in experimental studies with animal models are linked to the differences between human and experimentally induced disease, both in terms of genetic regulatory mechanisms and factors that influence cardiovascular function.[15] Moreover, TOF is not an entity, but rather a broad spectrum of congenital heart defects, and results are contingent on the type of dysfunction displayed by the animal model, thus rendering it difficult to make a direct comparison between different existing conditions. In addition, most of the models are not a perfect replica of rTOF from the physiological point of view, because they do not include the anatomic substrate, such as malalignment of the ventricular septum, ventricular septal defect, or subvalvular pulmonary stenosis.[45] Neither do they consider the potential effect of prenatal exposure to altered hemodynamic conditions.[59] Another challenge in creating an animal model is the timeframe. The follow-up period in the majority of studies does not exceed 6 months, and although this period is quite long in terms of animal study standards, the natural lifespan of the large animals is longer. Therefore, this timeframe does not always resemble the period observed in humans to develop elcometromechanical complications derived from the disease under study.[2]

In view of all that has been mentioned so far, and despite the limitations, we propose an algorithm to create the animal model of rTOF that best fits the study objectives, needs, and constrains (Fig. 2). This decision algorithm is based on the systematic review of the literature that we have made in this article and in our own experience. The objective of it is to provide keys when creating an animal model of rTOF, based initially on the type of animal to choose, according to the type of study that is going to be carried out, whether it is an *in vitro* study or *in vivo* study. Likewise, considering which method we will perform in our model, either surgical procedure or transcatheter technique, to create the type of dysfunction desired, either RV volume overload, RV volume overpressure, RVOT scar, and RBBB or the repaired Fallot model that encompasses all of the features of TOF, to finally accomplish the different studies according to our objectives in our animal model of rTOF.

Many of rTOF models are applied on adult animals, as one of the main goals of these studies is to evaluate chronic cardiac

remodelling; however, studies using only infantile animal models are relatively scarce, but they can be of great interest. Infantile animal models have been dedicated preferably to acute hemodynamic studies and testing new devices.[25,50,60] Experiments using only infantile animals have the advantages of being higher cost effective compared with chronic models and of allowing acute evaluations (either hemodynamic conditions or device testing).

Future perspectives in this field are promising. In the same way as operative techniques of TOF have been developed successfully over the years, including transatrial repair of the ventricular septal defect and transatrial relief of RVOT obstruction or even pulmonary valve sparing, improving long-term outcomes of Fallot patients, animal models of rTOF have evolved in parallel, as mentioned above, sometimes anticipating surgical changes, with the aim of improving the future perspective of Fallot patients.

In 2010, our team characterized a chronic porcine model that mimicked essential parameters of postoperative TOF.[45] The latter has been validated as a reliable long-term model of RV dysfunction and dyssynchrony, with echocardiographic and electrocardiogram measurements comparable with adult patients after TOF repair. After validating the model, we first demonstrated that biventricular stimulation significantly improved RV and LV function by alleviating electromechanical dyssynchrony.[6,52,53] More recently, using the same porcine model, we characterized the global gene expression profile in the heart after TOF repair.[55] Finally, in our most recent study, we focused our investigation on electrophysiological remodelling after TOF repair. Using high-resolution optical mapping of isolated perfused RV wedges in a pig model mimicking rTOF, we highlighted certain tissular and cellular electrophysiological modifications that may partially explain susceptibility to ventricular arrhythmias. These data show that RV remodelling after TOF repair includes several levels of regulation that may lead to delayed complications (Fig. 3).[51] Following the same research protocol, we have recently showed a marked remodelling of the left ventricular structure in the rTOF preclinical model that precedes LV dysfunction and it is likely to contribute to ventricular arrhythmias and SCD in patients with rTOF.[61] Future research perspectives will include a particular focus on the potential reversibility of the previously characterized lesions after treating RVOT dysfunction, either surgically or percutaneously.

## Conclusions

The long-term outcome of patients with rTOF is the development of RV and LV dysfunction, ventricular arrhythmias, and SCD. The mechanisms leading to these delayed adverse complications are not fully understood, resulting in therapeutic options being severely limited. The present analysis may contribute in providing key elements for facilitating the study of the aforesaid long-term complications occurring in rTOF, and possibly in other types of congenital heart diseases involving chronic RV overload and overpressure.

The development of animal models notably allows clarifying the hemodynamic, electrophysiological, genetic, and biochemical basis of the disease process, as well as the investigation of novel therapies such as new devices for PPVI and stem cell therapy.

The creation of new animal models is needed to face the ongoing challenges of this particular disease, to gain better insight and thus develop future therapeutic strategies enabling us to reduce morbidity and mortality in TOF.

This study is, to the best of our knowledge, the first that systematically and exhaustively has reviewed the existing animal models of repaired Fallot published so far (Supplemental Table S1)[18-20,25-32,34-38,42-47,49-55,58,60-66] and proposes a decision algorithm for its creation.

## Funding Sources

This work was supported by the French Government as part of the "Investments of the future" program managed by the National Research Agency (ANR) (Grant reference ANR-10-IAHU-04). E.V. was supported by a research grant from the Spanish Foundation Alfonso Martín Escudero.

## Disclosures

The authors have no conflicts of interest to disclose.

## References

1. Egbe A, Uppu S, Lee S, Ho D, Srivastava S. Changing prevalence of severe congenital heart disease: a population-based study. Pediatr Cardiol 2014;35:1232-8.

2. Sesllar S, Robinson M. Understanding sudden death risk in tetralogy of Fallot: from bedside to bench. Heart 2017;103:333-4.

3. Gatzoulis MA, Balaji S, Webber SA, et al. Risk factors for arrhythmia and sudden cardiac death late after repair of tetralogy of Fallot: a multicentre study. Lancet 2000;356:975-81.

4. Uebing A, Gibson DG, Babu-Narayan SV, et al. Right ventricular mechanics and QRS duration in patients with repaired tetralogy of Fallot: implications of infundibular disease. Circulation 2007;116:1532-9.

5. Davlouros PA, Kilner PJ, Hornung TS, et al. Right ventricular function in adults with repaired tetralogy of Fallot assessed with cardiovascular magnetic resonance imaging: detrimental role of right ventricular outflow aneurysms or akinesia and adverse right-to-left ventricular interaction. J Am Coll Cardiol 2002;40:2044-52.

6. Bordachar P, Iriart X, Chabaneix J, et al. Presence of ventricular dyssynchrony and haemodynamic impact of right ventricular pacing in adults with repaired tetralogy of Fallot and right bundle branch block. Europace 2008;10:967-71.

7. Boucek MM, Waltz DA, Edwards LB, et al. Registry of the International society for heart and lung transplantation: ninth official pediatric heart transplantation report 2006. J Heart Lung Transplant 2006;25:893-903.

8. Graves JA. Background and overview of comparative genomics. ILAR J 1998;39:48-65.

9. Hearse DJ, Sutherland FJ. Experimental models for the study of cardiovascular function and disease. Pharmacol Res 2000;41:597-603.

10. Hamlin RL. Animal models of ventricular arrhythmias. Pharmacol Ther 2007;113:276-95.

11. Yong SL, Wang QK. Animal models for cardiac arrhythmias. Methods Mol Med 2006;129:127-48.

12. Smith CR, Hamlin RL, Crocker HD. Comparative electrocardiography. Ann N Y Acad Sci 1965;127:155-69.

13. Milani-Nejad N, Janssen PM. Small and large animal models in cardiac contraction research: advantages and disadvantages. Pharmacol Ther 2014;141:235-49.

14. Smith AC, Swindle MM. Preparation of swine for the laboratory. ILAR J 2006;47:358-63.

15. Camacho P, Fan H, Liu Z, He JQ. Large mammalian animal models of heart disease. J Cardiovasc Dev Dis 2016;3:30.

16. Patten RD, Hall-Porter MR. Small animal models of heart failure: development of novel therapies, past and present. Circ Heart Fail 2009;2: 138-44.

17. Ou L, Li W, Liu Y, et al. Animal models of cardiac disease and stem cell therapy. Open Cardiovasc Med J 2010;4:231-9.

18. Van den Akker N, Molin D, Peters P, et al. Tetralogy of Fallot and alterations in vascular endothelial growth factor—a signaling and notch signaling in mouse embryos solely expressing the VEGF120 isoform. Circ Res 2007;100:842-9.

19. Urashima T, Zhao M, Wagner R, et al. Molecular and physiological characterization of RV remodelling in a murine model of pulmonary stenosis. Am J Physiol Heart Circ Physiol 2008;295:1351-68.

20. Reddy S, Zhao M, Hu D, et al. Physiologic and molecular characterization of a murine model of right ventricular volume overload. Am J Physiol Heart Circ Physiol 2013;304:1314-27.

21. Zahka KG, Horneffer PJ, Rowe SA, et al. Long-term valvular function after total repair of tetralogy of Fallot: relation to ventricular arrhythmias. Circulation 1988;78(suppl):III14-9.

22. Ebert PA. Second operations for pulmonary stenosis or insufficiency after repair of tetralogy of Fallot. Am J Cardiol 1982;50:637-40.

23. Marie PY, Marcon F, Brunotte F, et al. Right ventricular overload and induced sustained ventricular tachycardia in operatively "repaired" tetralogy of Fallot. Am J Cardiol 1992;69:785-9.

24. Gatzoulis MA, Till JA, Somerville J, Redington AN. Mechanoelectrical interaction in tetralogy of Fallot. QRS prolongation relates to right ventricular size and predicts malignant ventricular arrhythmias and sudden death. Circulation 1995;92:231-7.

25. De Vroomen M, Cardozo R, Steendijk P, van Bel F, Baan J. Improved contractile performance of right ventricle in response to increased RV afterload in newborn lamb. Am J Physiol Heart Circ Physiol 2000;278: H100-5.

26. Gray R, Greve G, Chen R, et al. Right ventricular myocardial responses to chronic pulmonary regurgitation in lambs: disturbances of activation and conduction. Pediatr Res 2003;54:529-35.

27. Yerebakan C, Klopsch C, Prietz S, et al. Pressure-volume loops: feasible for the evaluation of right ventricular function in an experimental model of acute pulmonary regurgitation? Int CardioVascThorac Surg 2009;9: 163-8.

28. Mori Y, Rusk RA, Jones M, et al. A new dynamic three-dimensional digital color doppler method for quantification of pulmonary regurgitation: validation study in an animal model. J Am Coll Cardiol 2002;40: 1179-85.

29. Agger P, Hyldebrandtb JA, Nielsena EA, Hjortdal V, Smerup M. A novel porcine model for right ventricular dilatation by external suture plication of the pulmonary valve leaflets—practical and reproducible. Interact Cardiovasc Thorac Surg 2010;10:962-6.

30. Kuehne T, Saeed M, Gleason K, et al. Effects of pulmonary insufficiency on biventricular function in the developing heart of growing swine. Circulation 2003;108:2007-13.

31. Smith J, Goetze JP, Søndergaard L, et al. Myocardial hypertrophy after pulmonary regurgitation and valve implantation in pigs. Int J Cardiol 2012;159:29-33.

32. Ersboell M, Vejlstrup N, Nilsson JC, et al. Percutaneous pulmonary valve replacement after different duration of free pulmonary regurgitation in a porcine model: effects on the right ventricle. Int J Cardiol 2013;167: 2944-51.

33. Gaynor SL, Maniar HS, Bloch JB, Steendijk P, Moon MR. Right atrial and ventricular adaptation to chronic right ventricular pressure overload. Circulation 2005;112:I212-8.

34. Lambert V, Capderou A, Le Bret E, et al. Right ventricular failure secondary to chronic overload in congenital heart disease: an experimental model for therapeutic innovation. J Thorac Cardiovasc Surg 2010;139: 1197-204.

35. Minegishi S, Kitahori K, Murakami A, Ono M. Mechanism of pressure overload RV hypertrophy in infants rabbits. Heart J 2011;52:56-60.

36. Bove T, Vandekerckhove K, Bouchez S. Role of myocardial hypertrophy on acute and chronic right ventricular performance in relation to chronic volume overload in a porcine model: relevance for the surgical management of tetralogy of Fallot. J Thorac Cardiovasc Surg 2014;147:1956-65.

37. Hodzic A, Bobin P, Mika D, et al. Standard and strain measurements by echocardiography detect early overloaded right ventricular dysfunction: validation against hemodynamic and myocyte contractility changes in a large animal model. J Am Soc Echocardiogr 2017;30:1138-47.

38. Kuehne T, Gleason BK, Saeed M, et al. Combined pulmonary stenosis and insufficiency preserves myocardial contractility in the developing heart of growing swine at midterm follow-up. J Appl Physiol 2005;99: 1422-7.

39. Nollert G, Fischlein T, Bouterwek S, et al. Long-term survival in patients with repair of tetralogy of Fallot: 36-year follow-up of 490 survivors of the first year after surgical repair. J Am Coll Cardiol 1997;30:1374-83.

40. Murphy JG, Gersh BJ, Mair DD, et al. Long-term outcome in patients undergoing surgical repair of tetralogy of Fallot. N Engl J Med 1993;329: 593-9.

41. Morwood JG, Triedman JK, Berul CI, et al. Radiofrequency catheter ablation of ventricular tachycardia in children and young adults with congenital heart disease. Heart Rhythm 2004;1:301-8.

42. Zeltser I, Gaynor JW, Petko M, et al. The roles of chronic pressure and volume overload states in induction of arrhythmias: an animal model of physiologic sequelae after repair of tetralogy of Fallot. J Thorac Cardiovasc Surg 2005;130:1542-8.

43. Kaltman J, Gillespie M, Seymour T, et al. Substrate characterization of ventricular tachycardia in a porcine model of tetralogy of Fallot using noncontact mapping. Pacing Clin Electrophysiol 2007;30:1316-22.

44. Chiu S, Huang S, Chang C, et al. The role of mechanical-electrical interaction in ventricular arrhythmia: evidence from a novel animal model for repaired tetralogy of Fallot. Pediatr Res 2011;70:247-52.

45. Thambo J-B, Roubertie F, De Guillebon M, et al. Validation of an animal model of right ventricular dysfunction and right bundle branch block to create close physiology to postoperative tetralogy of Fallot. Int J Cardiol 2012;154:38-42.

46. Lambert V, Gouadon E, Capderou A, et al. Right ventricular failure secondary to chronic overload in congenital heart diseases: benefits of cell

therapy using human embryonic stem cell-derived cardiac progenitors. J Thoracic Cardiovasc Surg 2015;149:708-715.e1.

47. Chiu S, Tsai C, Lin L, et al. Repolarization alternans and ventricular arrhythmia in a repaired tetralogy of Fallot animal model. J Am Heart Assoc 2015;4:e002173.

48. Marino TA, Kent RL, Uboh CE, Fernandez E, Thompson EW. Structural analysis of pressure versus volume overload hypertrophy of cat right ventricle. Am J Physiol Heart Circ Physiol 1985;249:379.

49. Shiota T, Jones M, Chikada M, et al. Real-time three-dimensional echocardiography for determining right ventricular stroke volume in an animal model of chronic right ventricular volume overload. Circulation 1998;97:1897-900.

50. Robb JD, Harris MA, Minakawa M, et al. Melody valve implantation into the branch pulmonary arteries for treatment of pulmonary insufficiency in an ovine model of right ventricular outflow tract dysfunction following tetralogy of Fallot repair. Circ Cardiovasc Interv 2011;4:80-7.

51. Benoist D, Dubes V, Roubertie F, et al. Proarrhythmic remodelling of the right ventricle in a porcine model of repaired tetralogy of Fallot. Heart 2017;103:347-54.

52. Thambo JB, Dos Santos P, De Guillebon M, et al. Biventricular stimulation improves right and left ventricular function after tetralogy of Fallot repair: acute animal and clinical studies. Heart Rhythm 2010;7:344-50.

53. Roubertie F, Eschalier R, Zemmoura A, et al. Cardiac contractility modulation in a model of repaired tetralogy of Fallot: a sheep model. Pediatr Cardiol 2016;37:826-33.

54. Yerebakan C, Sandica E, Prietz S, et al. Autologous umbilical cord blood mononuclear cell transplantation preserves right ventricular function in a novel model of chronic right ventricular volume overload. Cell Transplant 2009;18:855-68.

55. Charron S, Roubertie F, Benoist D, et al. Identification of region-specific myocardial gene expression patterns in a chronic swine model of repaired tetralogy of Fallot. PLoS One 2015;10:e0134146.

56. Bonhoeffer P, Boudjemline Y, Saliba Z, et al. Transcatheter implantation of a bovine valve in pulmonary position: a lamb study. Circulation 2000;102:813-6.

57. Bonhoeffer P, Boudjemline Y, Saliba Z, et al. Percutaneous replacement of pulmonary valve in a right ventricle to pulmonary-artery prosthetic conduit with valve dysfunction. Lancet 2000;356:1403-5.

58. Kim G, Lim H, Kim Y, et al. Novel self-expandable, stent-based transcatheter pulmonic valve: a preclinical animal study. Surg Today 2014;173:74-9.

59. Anderson RH, Spicer DE, Giroud JM, Mohun TJ. Tetralogy of Fallot: nosological, morphological, and morphogenetic considerations. Cardiol Young 2013;23:858-66.

60. Dickstein M, Yano O, Spotnitz H, Burkhoff D. Assessment of right ventricular contractile state with the conductance catheter technique in the pig. Cardiovascular Res 1995;29:820-6.

61. Dubes V, Benoist D, Roubertie F, et al. Arrhythmogenic remodeling of the left ventricle in a porcine model of repaired tetralogy of Fallot. Circ Arrhythm Electrophysiol 2018;11:e006059.

62. Basquin A, Pineau E, Bonnet D, et al. Transcatheter valve insertion in a model of enlarged right ventricular outflow tracts. J Thorac Cardiovasc Surg 2010;139:198-208.

63. Robb JD, Harris MA, Minakawa M. An ovine model of pulmonary insufficiency and right ventricular outflow tract dilatation. J Heart Valve Dis 2012;21:247-52.

64. Ishimaru K, Miyagawa S, Fukushima S, et al. Functional and pathological characteristics of reversible remodeling in a canine right ventricle in response to volume overloading and volume unloading. Surg Today 2014;44:1935-45.

65. Kopic S, Stephensen S, Heiberg E, et al. Isolated pulmonary regurgitation causes decreased right ventricular longitudinal function and compensatory increased septal pumping in a porcine model. Acta Physiol 2017;221:163-73.

66. Godart F, Bouzguenda I, Juthier F, et al. Experimental off-pump transventricular pulmonary valve replacement using a self-expandable valved stent: a new approach for pulmonary incompetence after repaired tetralogy of Fallot? J Thorac Cardiovasc Surg 2009;137:1141-5.

## Supplementary Material

To access the supplementary material accompanying this article, visit the online version of the *Canadian Journal of Cardiology* at www.onlinecjc.ca and at https://doi.org/10.1016/j.cjca.2019.07.622.