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

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Title: *Animal models of repaired tetralogy of Fallot: current applications and future perspectives*

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BRIEF SUMMARY

This article summarizes all experimental animal models of repaired tetralogy of Fallot published to date, with the aim of achieving a greater understanding of the deleterious mechanisms that may lead to late complications associated with this defect.

After more than a decade of work in this field, our group aimed to provide an overview of the different types of animal protocols published, as well as their main results, emphasizing their current applications and future perspectives.

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 which 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 analyzing the type of animals that can be used according to a given study's objectives, needs and constraints, the present review also evaluates 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 utilized. 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 in order to reduce the morbidity and mortality of this prevalent congenital heart disease.

KEY WORDS

Animal model; tetralogy of Fallot; right ventricular dysfunction; ventricular arrhythmias; congenital heart disease; repaired tetralogy of Fallot.

ABBREVIATIONS AND ACRONYMS

CPB: Cardiopulmonary bypass

LV: Left ventricle

PAB: Pulmonary artery band

PPVI: Percutaneous pulmonary valve implantation

PR: Pulmonary regurgitation

rTOF: repaired tetralogy of Fallot

RV: Right ventricle

RVOT: Right-ventricular outflow tract

SCD: Sudden cardiac death

TAP: Transannular patch

INTRODUCTION

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 births¹. Over the last decades, improvements in the approach of surgical correction have considerably increased survival². 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 right ventricular outflow tract (RVOT) dysfunction. Currently, the mechanisms leading to these delayed adverse complications⁴⁻⁶ are not yet completely understood, such that therapeutic possibilities are limited for these patients and only provide intermediary results⁷.

In order to better understand the mechanisms underlying the aforementioned complications as well as develop new therapeutic strategies, experimental animal models have been a key component of pre-clinical studies during the last three decades.

In the present article, we will focus on experimental studies performed on animal models of repaired tetralogy of Fallot. We will explore different mechanisms of dysfunction and their consequences with the aim of achieving a more comprehensive understanding of the different types of animal models that can be utilized in order to perform the targeted repaired TOF (rTOF) model. A 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 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 animals 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?

1. Type of animal

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

1.1. Large mammalian models of rTOF

Large mammals have been proposed as a good model of human physiology, since they more closely resemble humans with a high percentage of genetic conservation⁸. 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 pre-clinical studies of rTOF⁹. However, it is important to consider the anatomical and functional differences between these species in order 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 humans¹².

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 to that of humans¹³.

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 due to the early

appearance of cardiac injuries, however the latter becomes a disadvantage in chronic disease models. The ovine model has slower growth which allows assessing disease evolution over time. Thus, depending on the time-frame of the study, such differences will influence the decision in choosing one species or another¹⁴.

Another relevant issue to consider is the lack of 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 utilized due to ethical concerns, logistical difficulties and the high costs involved⁹.

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 which differs from that of humans¹⁵.

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

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 practice⁹.

1.2. 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 which allow analyzing 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 limitation⁹. (*Table 1*)

2. 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*)

2.1. 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}.

In order to create the RV overload, various surgical procedures have been performed in porcine, ovine and murine models including pulmonary valvulotomy, transannular patch (TAP)^{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.

2.2. 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 remodeling³³.

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 TeflonTM membrane deployed across the pulmonary valve³⁸.

2.3. 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 block (RBBB) as well as electromechanical ventricular delays are generated^{39,40}, leading to macroreentry circuits and monomorphic ventricular tachycardia⁴¹.

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 a RVOT scar without having to perform an RBB ablation^{42,43}. However, there are also certain groups, which directly perform radiofrequency ablation of the right bundle branch without incurring a RVOT scar in canine models⁴⁴.

2.4. Repaired Fallot model

In order 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 LV dysfunction, as observed in an adult population of repaired TOF⁴⁵. Other groups have since designed animal models with similar characteristics^{46,47}.

3. Surgical procedures vs. Transcatheter techniques

In the 80s, the first model with RV dysfunction was performed by PAB to create a pressure overload and atrial septal defects to create a volume overload⁴⁸. 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 a tightening of the snares, the authors performed a 2-cm arteriotomy in the main pulmonary artery and excised two 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 CBP.

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 three 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 infundibulum⁴⁵. (*Figure 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 and coll.*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 TeflonTM membrane across the pulmonary valve.

Finally, two groups studied percutaneous pulmonary valve implantation (PPVI) after different periods of PR. In 2012, *Smith et al.*³¹ deployed a stent (22 mm CPTM) in the pulmonary valvular annulus and after three months of free PR, a pulmonary valve (Medtronic MelodyTM) was mounted in the CPTM 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

1. 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 left ventricle (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 contractility³⁸. These findings by *Kuehne and coll.* were subsequently confirmed by other studies^{29,50}.

In order to describe the early RV remodeling after chronic RV overload and pressure overload, new porcine and ovine models were created showing functional and structural RV remodeling. In addition to the previous hemodynamic findings, several teams observed a significant RV fibrosis which 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/m² and 45 mL/m² for diastolic and systolic volumes, respectively, indicating that dilatation per se rather than PR duration prior to valve replacement is a major determinant.

2. 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 remodeling 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 remodeling involve a proliferation of the extracellular matrix and changes in ion channel expression^{47,51–53}.

3. 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.* in 2009⁵⁴, 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, in order to study the feasibility and the effects of cell therapy using intramyocardial injections of human MesP1p/SSEA-1p cardiogenic mesodermal cells. They observed that cell therapy appears to confer benefits with regard to overloaded RV tissue remodeling and arrhythmic susceptibility.

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 ventricles⁵⁵.

4. 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 patient⁵⁷.

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 fulfills 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, in order 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 function¹⁵. 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, since they do not include the anatomic substrate, such

as malalignment of the ventricular septum, ventricular septal defect or subvalvular pulmonary stenosis⁴⁵. Neither do they consider the potential effect of prenatal exposure to altered hemodynamic conditions⁵⁹. 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 study².

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 (*Central Illustration-Figure 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 right bundle branch block or 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 remodeling, 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 haemodynamic studies and testing new devices^{25,50,61}. Experiments using only infantile animals have the advantages of being higher cost-effective compared to chronic models and of allowing acute evaluations (either haemodynamic 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 for Fallot patients; animal models of rTOF have evolved in parallel, as mentioned above, sometimes anticipating surgical changes, with the aim of improving future perspectives of Fallot patients.

In 2010, our team characterized a chronic porcine model which mimicked essential parameters of postoperative TOF⁴⁵. The latter has been validated as a reliable long-term model of RV dysfunction and dyssynchrony, with echocardiographic and ECG measurements comparable to 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 repair⁵⁵. Finally, in our most recent study, we focused our investigation on electrophysiological remodeling 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 remodeling after TOF repair includes several levels of regulation that may lead to delayed complications (*Figure 3*)⁵¹. Following the same research protocol, we have recently showed a marked remodeling of left ventricular structure in rTOF pre-clinical model that precedes LV dysfunction and it is likely to contribute to ventricular arrhythmias and sudden cardiac death in patients with rTOF⁶⁰. 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.

CONCLUSION

The long-term outcome of patients with repaired tetralogy of Fallot 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 over-pressure.

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, in order to gain better insight and thus develop future therapeutic strategies enabling to reduce morbidity and mortality in TOF.

This study is, to the best of our knowledge, the first that systematically and exhaustively have reviewed the existing animal models of repaired Fallot published so far (*Supplementary Table*) and proposes a decision algorithm for its creation.

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

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

RV: Right ventricle, rTOF: repaired tetralogy of Fallot.

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

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

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

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

PR: pulmonary regurgitation; PS: Pulmonary stenosis; RVOT: right ventricular outflow tract

FIGURE TITLES AND LEGENDS

FIGURE 1. *Step by step surgical rTOF 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 DacronTM patch is sewed (black arrow). F: Gore-Tex band is placed around main pulmonary artery distally to DacronTM patch (black arrow). *rTOF: repaired tetralogy of Fallot.*

CENTRAL ILLUSTRATION- FIGURE 2. *Creating an animal model of repaired tetralogy of Fallot.* 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 in order to obtain the type of animal model desired, thereby enabling to conduct the various investigations in the model according to the needs of the study. *rTOF: repaired tetralogy of Fallot.*

FIGURE 3. *RV activation in rTOF porcine model.* Representative activation maps from a Sham and a rTOF RV with 5 ms-spaced isochrones. Multiple areas of slowed conduction were observed (yellow arrows) throughout the rTOF RVs. Adapted from *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;103:347–354.*

RV: Right ventricle, rTOF: repaired tetralogy of Fallot.

Figure 1

Figure 2: Central Illustration