

The Current State of In-vivo Pre-clinical Animal Models for Heart Valve Evaluation

Robert P. Gallegos, Pavel J. Nockel, Andrew L. Rivard, Richard W. Bianco

University of Minnesota Department of Surgery, Experimental Surgical Services and the Lillehei Heart Institute, Minneapolis, USA

For over 30 years, animal models have remained a central critical component in the pre-clinical safety evaluation of prosthetic heart valves developed for use in humans. Though many advances have been made in valve design, no ideal replacement prosthesis has yet been developed. As a result, valve manufacturers continue to address issues relating to thrombogenicity, structural integrity, fluid dynamics and calcification in their designs. Many animal models have been developed to examine these issues, including dog, pig, calf and sheep, yet no standard model has been accepted. Recently, the International

Standard Organization has provided guidelines in document 5840 to address cardiovascular implants. The aim of this report is to provide a summary of the current state of pre-clinical valve evaluation in animals. Changes in ISO 5840 will be addressed that have occurred between 1998 and the present date, and the role of current available animal models. The aim also is to provide rational guidance in the selection of appropriate animal models to match the purpose of valve implantation studies.

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The essential characteristics of the ideal valve prosthesis were outlined in the early 1950s (1). By definition, this valve would perfectly emulate the native biological valve it was meant to replace. This valve must be durable, allowing for minimal device-related failures, while capable of allowing normal physiologic hemodynamics with non-turbulent, unimpeded forward flow and no regurgitant flow. Both the hemodynamics and the material used to construct the valve should be biocompatible to allow for normal tissue healing, lack of infection, absence of thrombogenicity, or damage to circulating blood elements. Finally, the device would allow for technical ease in implantation in an anatomically correct position by any cardiac surgeon.

Considerable advancement in valve prosthesis design and manufacture has transpired since the first Starr-Edwards valve was introduced during the 1960s. Despite tremendous improvements however, the ultimate goal of valve manufacturers today remains the development of this ideal valve prosthesis. Mechanical

valves have proven to be durable and to provide adequate hemodynamic performance. Several design features have been incorporated to further improve hemodynamic profiles, such as supra-annular placement, with the intent of matching the effective orifice area (EOA) of the prosthesis to the patient's annular diameter. Regrettably, mechanical valve designs remain somewhat obstructive in nature, and material composition continues to require life-long anticoagulation in order to prevent thromboembolic complications. In contrast, bioprosthetic valves require no anticoagulation while offering a hemodynamic performance comparable to that of mechanical prostheses. Biological prostheses are limited, however, by abnormal accelerated collagen degeneration and calcification which results in significant regurgitation and/or stenosis. This phenomenon has been attributed to abnormal mechanical loading on the leaflets in stented valves. Efforts to better dissipate these mechanical forces have spurred new interest in low-profile stentless bioprosthetic valves and, certainly, much of the success in this new valve design must be attributed to the ongoing use of pre-clinical animal testing. Animal studies serve as effective means of assessing the degree of safety prior to use in humans, allowing for the identification of significant design faults before clinical implantation.

Address for correspondence:
Richard W. Bianco, University of Minnesota, Department of Surgery,
MMC 195, 420 Delaware Street SE, Minneapolis, MN 55455, USA
e-mail: bianc001@umn.edu

Changes in ISO standards for in-vivo pre-clinical valve testing

The use of experimental valve implantation studies in animals pre-dated the first clinical valve replacement. Today, animal models are used in validating valve designs and developing risk assessments before clinical studies. Reasonable guidance for pre-clinical valve implantation studies involving animal models can be gained from reviewing the document ISO-5840 (revised in 2005), as issued by the International Standards Organization. This document identifies the primary goal of pre-clinical in-vivo assessment, as the evaluation of performance characteristics of the heart valve substitute which are not assessable by in-vitro

testing. Characteristics required in formulating ideal animal studies as suggested by ISO-5840 are summarized in Table I. The guidelines mandate that all devices in an animal study be identical to that destined for clinical use with respect to design, manufacturing, sizing, packaging and sterilization.

The present authors' previous experience in the area of pre-clinical testing allowed for the observation that valve design must take into account the working conditions into which the valve is implanted (2). This is called 'site-specific' testing. Without site-specific testing, a device that functions well in the mitral position may not function well in the aortic position, due to higher afterload pressure. This scenario occurred with

Table I: Guidance pre-clinical evaluation summary.

Parameter	Protocol
Species	Not specified (same species, similar gender, age, size) Minimum number of animals = 10
Site of implantation	Anatomical location intended for clinical use, i.e. site-specific (12)
Test valve	Clinical quality (clinically relevant size, design, manufacturing, sterilization, and packaging)
Control valve	Minimum number = 2 animals Passed ISO 5840 standards In clinical use ($\geq 10,000$ patients) at time of study
Duration	Safety study = 20 week survival (minimum 10 animals) Appropriate to study purpose Specified prior to implantation
Technique	Documented/reproducible Performed by trained personnel (GLP preferred)
Testing	Hemodynamic (echo, catheterization, angiography) Ease of handling Audible sound evaluation
Serial blood sampling	Preoperative (CBC, electrolytes, coagulation) 1 week postoperative (above plus free hemoglobin) Regular interval sampling At euthanasia
Pathologic examination	Macroscopic Animal (thromboembolic, pannus formation, inflammatory reaction) Device (structural damage, degeneration, calcification) Histological (thromboembolic, degenerative processes, inflammatory reaction)
Test report	Identify animal, valve Preoperative animal condition Detailed surgical and post-surgical course documentation Justification of any deviations from protocol Identity of investigators and institution

CBC: Complete blood count; GLP: Good Laboratory Practice.

the Sulzer-Carbomedics Photofix™ valve when placed in the mitral position in an ovine model. The Photofix valve, as tested in the aortic position, had significant leaflet erosion attributed at pathologic examination to the high aortic pressure (3,4). Site-specific testing is advantageous because the valve is tested in an orthotopic position with parameters similar to those to which it will be subjected after implantation in a human - that is, heart size, heart rate, cardiac output (CO), stroke volume and blood pressure. The ISO recognized the importance of site-specific testing and adopted it into the latest guidelines for pre-clinical testing of prosthetic replacement valves (2).

The duration of in-vivo studies is dependent upon establishing a basis for risk analysis for valve failure in animals of the same sex and age. The duration of study for ISO standards is currently 20 weeks following implantation, using at least 10 animals. At least two animals with a reference valve are used as controls. This time period allows for the longitudinal assessment of the risk of valve failure. The rationale for a risk-based approach is that a requirement-based approach cannot keep up with the speed of technological innovation. This risk-based approach involves cooperation and communication between the valve manufacturers and regulatory authorities. It is implied in this approach that the manufacturer will continuously strive for improvement in safety and performance of the implant, without having to depend upon the number of patient-years as evidence of effectiveness (Table II).

Risk management is a concept that involves assessing a valve design for the chance of failure based upon the core elements: risk assessment (which includes risk analysis and risk evaluation); risk control; and post-production monitoring. A risk analysis identifies hypothetical hazards derived from product testing, complaints, adverse events, recalls, audit observations, and other product or process deviations that are involved with the use, misuse, or abuse of the product. Risk analysis is then the sum of each estimate of the risks (5). Additional information about risk assessment and management is provided in the ISO 14971:2000 document.

Table II: Quality system regulation as is part of the 1996 FDA requirements.

Design and development plan
Design input
Design output
Design verification and validation
Design reviews
Design transfer
Design changes
Design history file (to incorporate the required documents)

Pathologic evaluation

An ISO-compliant study is not complete without a formal necropsy report. This includes bacteriological samples and photographs of the inflow and outflow tracts of the valve(s). There should be a gross pathological assessment of the valve as well as of the heart, lungs, liver, kidneys, brain and spleen. Representative samples of the organs, entire valve, and any pathology should be fixed, embedded, sectioned, and stained with hematoxylin and eosin. An important note contained in the ISO regulations states that entire heart block may be retained by the study sponsor, or electively processed for histopathology. The final necropsy summary report should include an animal history for all animals (full term and early death), gross and microscopic pathology results, and a summary by the study pathologist.

Standard animal models

A variety of animal species has been used for valve implantation studies, with standardized models reported in dogs, pigs, calves and sheep (6-14). Each model has inherent advantages and limitations. Ultimately, none of these animals completely replicates the human anatomy, biochemistry and physiology. As such, any information garnered from animal studies must be evaluated with respect to the idiosyncrasies of the model chosen. ISO-5840 acknowledges this problem by clearly stating that no universally accepted animal model yet exists. The investigator is simply urged to justify their choice in the animal model used for each specific study. Although this was intended to allow for broad utilization of models to better approximate the human condition, it has resulted in significant controversy, with the validity of results from pre-clinical models being called into question (15). It is the goal of this report to discuss the state of current models, so as to unify all researchers in an effort to strengthen belief in pre-clinical testing.

A concise review of the literature was conducted using a Medline query to determine the present use of large-animal models for valve research. Key words utilized in the search included: heart valve; aortic; mitral; pulmonic; mechanical; tissue; bioprosthetic; tissue engineering; animal; dog; calf; pig; sheep; and years 1998-2004. A total of 70 studies conducted in large animals was reported in the years 1998-2004, involving a total of 1,011 animal subjects (Figs. 1 and 2). The studies reported had various end-points that included: concept; calcification; thrombosis; cavitation; and safety. Valve types included current clinically approved mechanical and tissue valves, in addition to research with tissue-engineered, polyurethane, mechanical, and tissue devices. A review of animal species involved

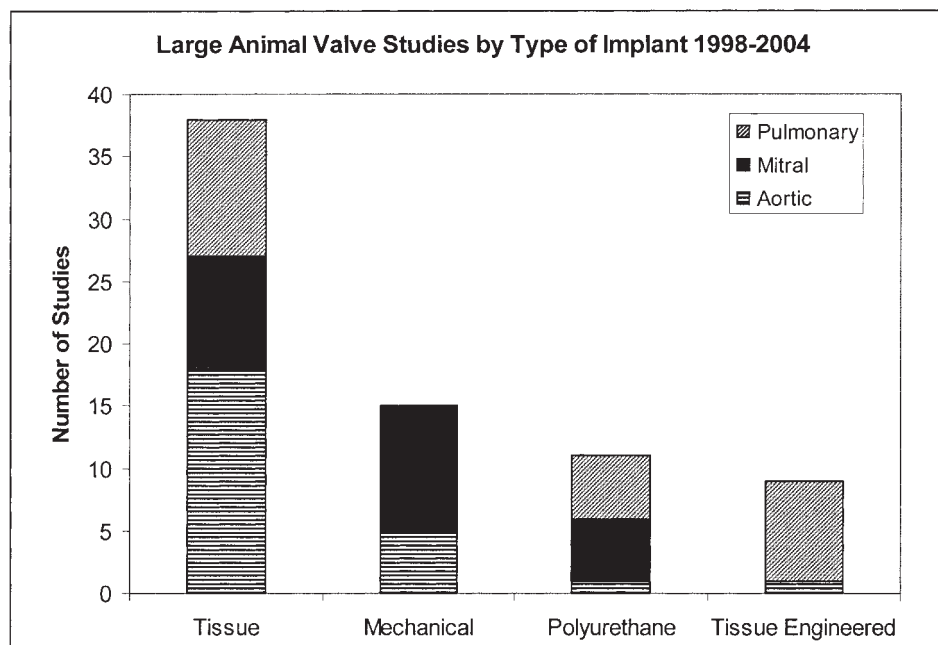


Figure 1: Reported pre-clinical valve studies in animal models by type of implant.

Valve type	No. of studies	Total studies by site		
		Aortic	Mitral	Pulmonary
Tissue*	37 (52.8)	18 (72)	9 (37.5)	11 (45.8)
Mechanical	15 (21.4)	5 (20)	10 (41.6)	-
Polyurethane	9 (12.8)	1 (4)	5 (20.8)	5 (20.8)
Tissue-engineered	9 (12.8)	1 (4)	-	8 (33.3)
Total studies [†]	70	25 (34.2)	24 (32.8)	24 (32.8)

Values in parentheses are percentage of total studies.

*Tissue valves include stented and stentless valves.

[†]Three studies involved multiple sites of implantation such that the total study number (n = 70) is not equal to the total number of different sites tested (n = 73).

revealed that three species - sheep (78%), pig (9.5%) and calf (3.8%) - accounted for almost 95% of the subjects utilized. The sheep model has rapidly become the preferred and most utilized model, for numerous reasons. To justify the use of these animal models, each will be discussed in detail, excluding the primate model. The primate model is an inappropriate valve replacement model because the annulus diameter of commonly available primates is not of a size that would allow the implantation of prosthetic valves of a size relevant to human implants.

Canine model

The use of dog models for valve implantation is noteworthy primarily for historical perspective, as usage of this model has been quite limited recently (see Fig. 2). Significant success with this model was reported by the mid-1960s (16-26), and the model remained

dominant into the mid-1980s, with numerous investigators (including the present authors' laboratory) experiencing few complications (7). However, reports of increasing incidence of infection and thrombosis raised concern (6,27). In addition, increasing government regulations and social concerns have made the model less favorable. Possibly most significant is the greater tendency towards thrombosis and much lower CO observed in the canine model relative to humans (Table III)

Porcine model

Interest turned towards the use of the pig in the midst of growing concerns that the sheep model was inadequate for the evaluation of valve-related thrombosis complications. Supporting this concern was the voluntarily discontinuation of the Medtronic Parallel™ valve after an usually high incidence of valve-related

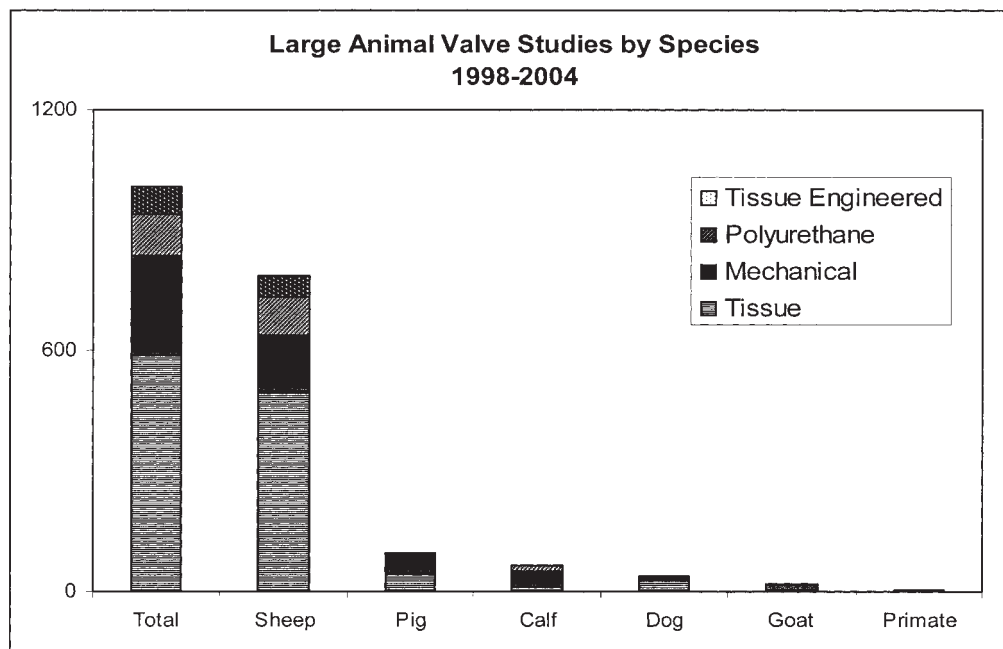


Figure 2: Reported pre-clinical animal models by species and valve type.

Valve type	Total	Total no. of subjects in study					
		Sheep	Pig	Calf	Dog	Goat	Primate
Tissue*	591 (58.4)	495 (83.7)	47 (7.9)	12 (2.0)	29 (4.9)	5 (0.8)	3 (0.5)
Mechanical	248 (24.5)	145 (58.4)	45 (18.1)	39 (15.7)	4 (1.6)	15 (6.0)	-
Polyurethane	106 (10.4)	92 (86.7)	-	14 (13.2)	-	-	-
Tissue-engineered	66 (6.5)	56 (84.8)	4 (6.0)	-	6 (9.0)	-	-
Total	1,011	788 (78)	96 (9.5)	65 (6.5)	39 (3.8)	20 (2.0)	3 (0.2)

Values in parentheses are percentage of total studies.

*Tissue valves include stented and stentless valves.

thrombosis was observed in clinical use, even after no such complications were observed in animal studies (28). Goodman et al. added to this concern by reporting findings suggesting that sheep platelet activity was significantly lower than that of humans (29). These authors went on to report that pig platelets appeared to have similar in-vitro activity as the human counterpart, concluding that the pig model may be useful for testing blood-material interaction. Several additional reports suggested that the pig may indeed be a viable alternative to the sheep model (30,31). As a result, significant effort was invested so as to overcome several limitations that had previously hindered use of the pig model, primarily that of infection and clotting. These included refinement of surgical and bypass techniques, chronic venous access with the Port-a-Cath system, and rigorous anticoagulation (8).

Unfortunately, long-term replacement of the mitral

valve in the porcine model for the purpose of evaluating valve-related thrombosis was found to have limited utility (8). This is because maintenance of appropriate levels of anticoagulation was found to be highly variable between animals, resulting in a high incidence of hemorrhagic complications. In addition, marked pannus formation not only hindered valve leaflet movement and closure, but also contributed to the tendency for thrombosis. Finally, a high incidence of perivalvular leak was observed and attributed to normal somatic growth in the pig.

The Yucatan mini-pig is a model that has not been extensively used because of the high cost associated with purchasing this strain. However, it may be a suitable option for valve studies in the future. The normally fast growth of swine, high variability in anticoagulation regimens between animals, and the numerous potential mechanisms of thrombus produc-

tion are prohibitive for the use of this animal model in the long-term safety evaluation of valve implants as they relate to issues of thrombosis. Nevertheless, the pig model remains adequate for acute studies involving valve implantation - that is, feasibility studies of single cusp, or percutaneous valves.

Calf model

A recent report touted the development of a new growing calf model as a replacement for the sheep model, given the above-mentioned shortcomings with thrombosis (15,29). In actuality, the calf model was first used extensively in the 1980s for the purpose of calcification studies (9-11). A review of the literature reveals that this model is not currently utilized significantly (6.5%), primarily because it shares many of the limitations of the pig model. Somatic growth is rapid in the growing calf, and this contributes to both anatomic and hemodynamic alterations that undermine the validity of long-term follow up studies. Anatomically, the calf valve aortic annulus tends to be larger than that of humans (see Table III). As a result, outflow obstruction follows implantation with valves sized for clinical relevance, and any additional growth may contribute to the development of perivalvular leak, as was observed in the pig model (32). Additionally, rapid somatic growth in the calf is associated with dramatic elevation in CO well above the maximum observed in humans (Fig. 3).

In fact, if the calf model were to be used for standard six-month trial, CO would be expected to be almost three-fold greater than that of the adult human. This natural increase in CO was felt to be advantageous by Gregoric and colleagues, who suggested that "... without high cardiac output it is difficult to assess any device related hemodynamic changes." (33). However,

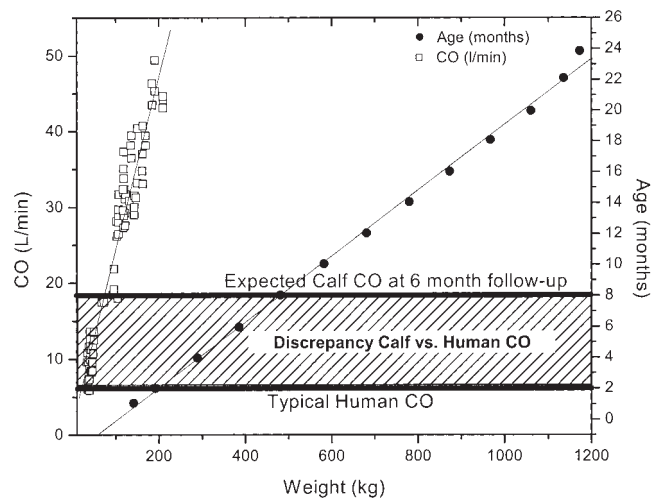


Figure 3: Cardiac output (CO) (□) and age (●) as a function of weight in the growing calf (adopted from reference (58) and (59)). Assuming that a calf is aged 2 months at the time of valve implantation, note the discrepancy disparity (hash lines) between the typical adult human CO and expected calf CO six months later at the end time point of a valve study.

the calf model not only represents a high CO environment but also a stenotic model consistent with patient-prosthesis mismatch. It is well known that a valve's EOA significantly impacts on the incidence of heart failure symptoms, adverse cardiac events, preoperative mortality, left ventricular impairment, and is associated with short- and long-term mortality (34-40). It is therefore not surprising that left ventricular hypertrophy and cardiac failure will be expected to follow valve implantation in the stenotic calf model when the CO is tripled over the six-month follow up period. Indeed, Gregoric reported symptoms of congestive

Table III: Key cardiac parameters for various species used in valve implantation studies.

Parameter	Species				
	Human	Porcine (52)	Bovine (53)	Ovine (52)	Canine (52)
Aortic annulus size (mm)*	26.4 ± 3.15	26.6 ± 1.84	33.7 ± 2.74	25.8 ± 1.29	33 ± 4.8
CO (l/min)	6.0 ± 2.0	2.36 ± 0.9	See Fig. 3	5.2 ± 1.4	2.5 ± 0.1
LVEF (%)	69 ± 8 (55)	59 ± 1	44 ± 7	52 ± 8	42 ± 7
EDV (ml)	115 ± 54 (55)	54 ± 4	682 ± 137	45 ± 7	42 ± 12
Heart rate (bpm)	66 ± 9 (56)	112 ± 3	97.3 ± 3.1	98 ± 7	98 ± 17
Systolic BP	116 ± 12 (55)	116 ± 13	137 ± 9	85 ± 3	124 ± 15
Diastolic BP	73 ± 8 (55)	70 ± 3 (57)	108 ± 15	58 ± 15	78 ± 15

Numbers in parentheses relate to references for cardiac parameters and values.

*Aortic annulus size was determined by Sands et al. (54) from an anatomical comparison of human, porcine, bovine, ovine and canine valves.

BP: Blood pressure; CO: Cardiac output; EDV: End-diastolic volume; LVEF: Left ventricular ejection fraction.

failure in two of nine (22%) animals receiving mechanical implantation that were followed for four months (33). Ultimately, these authors concluded that trileaflet mechanical valves were superior, because cardiac failure was associated only with bileaflet and not trileaflet mechanical implants.

The present authors agree that the improved performance of the trileaflet valve in Gregoric's report is attributed to its significantly greater EOA (R.W. Bianco et al., unpublished data). However, a review of the data provided by Gregoric reveals that the majority of animals were actually in cardiac failure, given that the mean CO was only 11 ± 3 l/min when the expected value would be in the range of 18-20 l/min, based on animal weight and study duration (see Fig. 3). Clearly, valve replacement in the calf model is representative of the patient-prosthesis mismatch condition, rather than an ideal in-vivo model for replacement human valves. In the context of clinical use, it is important to avoid patient-prosthesis mismatch because mismatch is strongly predictive of congestive heart failure following aortic valve replacement as well as reoperation (41,42). The choice of animal model to study the replacement valve's hemodynamic characteristics for regulatory approval should, ideally, reduce or eliminate confounding factors such as patient-prosthesis mismatch. Therefore, the calf model is not an ideal animal for testing prosthetic valves in a pre-clinical setting because of the somatic growth. The calf model is best suited for acute studies in which somatic growth would not bias results, or perhaps as a model of high-output congestive heart failure.

Sheep model

Sheep models represent by far the most utilized animal models in pre-clinical valve studies, accounting for 78% of the animals used over the review period. The present authors have found that juvenile sheep of adequate size for valve replacement can be obtained without difficulty from vendors. Use of the sheep model is best realized by a brief review of the current literature. Models of juvenile, adolescent and adult sheep are available for the study of both mechanical and tissue valves for the purpose of long-term safety evaluation (12-14). Calcification studies that accurately predict clinical function are readily completed in the juvenile sheep model, in animals aged between three and six months (43-45).

Overall, sheep closely mimic the human anatomy and physiology, having similar annulus size, equivalent heart rate and CO, and little somatic growth. In addition, sheep are widely available, and few social limitations are encountered with their use for scientific purposes. The sheep coagulation system is readily managed with minimal anticoagulation in a fashion

that allows for standard protocols, without the need for individualized dosing that might bias results. Opponents of the sheep model argue that the calf model (or in the past, the pig) would better predict thrombosis. However, supporters of this argument can only state that in-vitro anticoagulate (sodium citrate or acid-citrate-dextrose) platelet activity is reduced in sheep relative to pig and human (29). In his report, Goodman commented that, "This is not to say that the sheep is an inappropriate model, since non-anticoagulated sheep platelets do form thrombi in vivo on carbon heart valves."

Goodman's observation is supported by recent reports suggesting that care must be taken on evaluating platelet function when sodium citrate is used, as calcium chelating significantly reduces platelet aggregation (46). Also, platelet function appears to be distinctly different under static and flow conditions, calling into question the validity of applying results obtained using Goodman's method to pre-clinical in-vivo animal testing where blood flows across the valve (46). Furthermore, overall coagulation activity and platelet function - as tested by the xylem clot signature analysis - revealed hyper responsive platelets in the dog, and an increased tendency of overall clotting activity under flow conditions in the calf when compared to humans (46). Overall, the platelet-mediated homeostasis time (PHD) and clotting time (CT) were significantly ($p < 0.01$) shorter in the dog and calf when compared to sheep and human, indicating that both the dog and calf are overly aggressive with respect to coagulation activity. Conversely, no significant difference was noted between human and sheep, allowing these authors to conclude that the sheep model is best suited for the study of blood compatibility with devices (46). Finally, it should be noted that sheep can be placed on standardized anticoagulation regimes that can be followed with commonly available human measures of anticoagulation. The same cannot be stated in the calf, where it has been shown that commonly used human anti-coagulation tests are not valid (46,47).

Discussion

Stress or iatrogenic models of valve disease are currently a growing field in the use of animal models. There is a lack of suitable models in this important area for the evaluation of cardiovascular therapies, beyond that of valve replacement. Until now, it has been possible to use the ovine model to produce a graded stenosis by banding the aorta in young animals (48). The induction of aortic supra-annular stenosis, as well as aortic valvular stenosis, is also routinely performed in canine and porcine models (49,50). Furthermore,

mitral valve regurgitation in the canine is possible by placement of a shunt or by incision of the chordae tendineae (51). How these various animal models of human disease will be used in the future for valve replacement studies is currently unknown. The primary purpose of animal models in prosthetic valve replacement studies is to examine valve performance carefully and systematically. At the present time, there are no FDA guidelines for the pre-clinical testing of prosthetic heart valves in a chronic atrial fibrillation model; however, it is anticipated that an adequate animal model of atrial fibrillation will lead to a significant changes, including directed valve testing to prevent thromboembolic events. The present authors prefer the sheep model as the species of choice, because it involves relatively easy handling and husbandry. Moreover, the cardiac anatomy allows implantation of the aortic and mitral valves using standard operating procedures and survival for extended periods of time. Stentless, stented, and mechanical valves can be placed into both the aortic and mitral positions in the ovine model, using standard valve replacement techniques.

In conclusion, pre-clinical testing in animal models remains a powerful central tool in the development of new heart valve devices. The power of site-specific testing has been demonstrated previously, and the aim of this report was to focus on available animal models for pre-clinical testing. Although no single animal model exactly replicates all human parameters, the sheep model most accurately simulates most characteristics of the human anatomy and physiology. In addition, the results of recent studies have suggested that coagulation in the sheep may more closely reflect that of human coagulation than was previously believed. Most importantly, the greatest repository of data from 'gold standard' devices - such as the St. Jude Medical bileaflet mechanical valve - involved use of the sheep model. Ultimately, the sheep is the most utilized and the preferred model for long-term safety evaluation of heart valve devices. Other available models - such as the dog, pig and calf - are better suited for acute studies to evaluate design concepts. Certainly it must be recalled that the primary goal of pre-clinical animal testing in compliance with ISO standards is to identify heart valve substitute performance problems that were not assessable by in-vitro testing. Unfortunately, animals are only models of human disease, and it is possible that not all problems with devices will be discovered during the pre-clinical phase. However, the use of standard sheep models completed in laboratories that operate under Good Laboratory Practice conditions will provide data of high quality. This will allow for comparison to be made with historical data, potentially reducing the

number of animals required, while increasing the ability to recognize design problems.

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