

# Animal Trials for Heart Valve Substitutes

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In the past, animal experiments have played an important part in the development of heart valve substitutes, and continue to serve the following roles. First, several animal trials must be conducted in order to obtain marketing approval for these class III medical devices. A series of rules introduced by the FDA and European Union (CE-mark), and based on ISO document 5840 (1), are laid down in a number of regulations and guidelines. A second reason to perform animal experiments is to observe and analyze valve function 'in vivo'. The influence of the presence of a heart valve prosthesis on heart function can also be studied and analyzed. Finally, animal studies can add to the knowledge of heart anatomy and function, allowing the development of better heart valve substitutes.

The format of animal experiments may be either acute (performed in intact animals or in isolated hearts) or chronic, and animal models which have been used for these investigations include dogs, goats, sheep, calves and pigs. Clearly, strict animal welfare should be maintained in all such studies.

The choice of an animal model should be based on the following criteria:

- The anatomy and function of the animal heart should resemble that of the human heart as much as possible.
- Physiological processes in animals - especially coagulation, wound healing, infection resistance and calcification - should be similar to those in humans.
- The size of the heart and the total body weight should be such that heart valve prostheses of similar sizes as in humans can be used.
- The use of (human) diagnostic tools (e.g. echo-Doppler equipment) should also be possible in the

animals utilized.

- The overall cost of animals (including price, food and housing) may be a factor in their use.
- The expertise of the investigators with a certain type of animal model may influence their choice.

The regulations laid down by the FDA (2) and CE-directorates are very similar, and based on the same ISO document 5840.

*Acute experiments* are only required if the chronic experiments include cases with non-orthotopic placement of the prosthesis. Three orthotopic implants must be performed, and hemodynamic studies over a range of cardiac outputs (CO) of >3 l/min should register mean and peak gradients over the prosthesis, effective orifice area (EOA), and the presence of stenosis and regurgitation. Data related to occluder motion should also be obtained. The report should mention the ease of handling (including packaging, sizing, etc.) and details on surgical procedure and implant techniques.

*Chronic experiments* should include six implants for a minimum of 20 weeks.

Mechanical and stented tissue valves must be implanted orthotopically, whereas stentless valves may be implanted non-orthotopically. In the latter case, the model must be justified, especially with regard to any calcification processes. In chronic trials, two reference valves must be included; these may be any comparable (similar type), FDA-approved valves. The animals must be evaluated preoperatively in terms of their general health and age (dental eruption). All procedures and techniques must be described, including postoperative care and housing. Laboratory tests include red blood cell count, white blood cell count, hematocrit, free hemoglobin, serum lactate dehydrogenase, haptoglobin, platelet- and reticulocyte counts, and serum levels of calcium and phosphorus. The explanted valves must be carefully described and studied, according to a separate protocol. The duration of implant and cause of death must be carefully registered. Post-mortem studies include gross examination of all organs, and histopathology of the heart, spleen,

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liver, and kidneys. Explanted valves should be photographed from both the inflow- and outflow sides. A description of gross appearance (e.g. tears, calcification) and histology of the leaflets is mandatory; tissue calcium and phosphorus contents should also be measured. Hemodynamic studies in experiments with an implant time of less than 20 weeks should include cine-angioventriculography. Regurgitation and occluder movement should be registered and quantified. No attention is paid to orientation of the prosthesis.

The shortcomings of the present regulations are the lack of any essential update in more than 20 years. An optimal, standard animal model should long have been selected. Varying techniques are allowed, and a degree of standardization seems important. Little or no attention has been paid to the fact that the growing animals used in these trials usually outgrow their prosthesis size rather quickly, and that this will lead to hypertrophy and abnormal flows and gradients. No standard reference valves have been selected. Hence, to improve this situation a standard model and reference valve should be chosen and standard techniques prescribed. It might be preferable that one experiment in the isolated heart be added to the requirements.

The porcine model (3) could very well be the system of choice, mainly because the porcine heart is similar to the human heart in both anatomy and function, while all physiological processes are comparable to human reactions and may even more pronounced in the pig. Moreover, the porcine model is easy to handle with some experience, especially with regard to anesthesia. Pigs are also relatively cheap to purchase and can be housed in a farm environment. In general, the porcine model also appears to be more predictive than either sheep or calf models, but should be properly evaluated in a randomized trial with other animal models.

In order to demonstrate the good qualities of the porcine model, some generalized experiences are worthy of mention. These have been derived from experiments, designed as six-month implants, after new valves had already obtained their pre-market approval (PMA) and the clinical trials had been started. Sheep or calf trials, according to FDA/CE-protocol had been successfully concluded. Twelve tissue valves - six stented, six stentless - all of which had been produced under exactly the same conditions, were compared. All stented valves showed early failure, at between 2.5 and 4 months, due to tears and severe calcification, whereas all stentless valves survived to 6 months and showed no gross abnormalities at explantation. The differences in pigs were much more marked than in the sheep trials (4).

Two mechanical bileaflet valves failed in the porcine model, due to valve hinge thrombosis, after 13 days

and 17 days respectively, though the sheep studies had not shown any thrombotic event. The clinical trial was discontinued, and 15% of the already implanted valves showed valve thrombosis eventually.

The *isolated porcine heart model* allows a good visualization and analysis of the valves' opening and closing behaviors, and permits study of the optimal orientation of a prosthesis (5,6). Hemodynamic studies are not interfered with by auto-regulatory mechanisms. All events, including simulated pathological conditions such as low output state or arrhythmias, are fully controlled. Disadvantages of the isolated heart model are the fact that it is suspended from the aorta, and that fiberscope observations are made through the wall of the left ventricle. At present, we are also investigating normal hearts, as in all animal experiments. All types of valves and annuloplasty rings can be studied in the isolated heart model. In fact, it has shown many details which could not clearly be shown with other diagnostic methods, including a lack of movement of the valve leaflet opposite the aorta in trileaflet porcine aortic or bovine pericardial bioprostheses.

It appears that, in the near future, there will be a need to seek alternatives to animal trials, as the European Union aims to ban all animal experiments by 2009, starting with evaluation trials for cosmetics. Bench testing using biological fluids may represent a future solution, but the presently available equipment does not provide constantly changing anatomy, flows and pressures resembling the physiological situation. Hence, this method is allowed only for durability testing.

Finally, since at present the "...results' of 'in-vitro' testing can not be transferred to humans" (FDA), the need for animal trials will continue at least for the next few years.

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