Welfare in a chronic model of cardiac disease in sheep

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Heart disease is a major cause of death and disability worldwide. Changes in the electrical and mechanical function of the heart following a myocardial infarction or heart attack can over time lead to life-threatening disturbances in heart rhythm or to heart failure which untreated can be potentially fatal.

The Cardiac Structure and Function Group from the Auckland Bioengineering Institute have over the past 4 years been developing large and small chronic animal models of cardiac disease to meet obligations under a programme grant from the Health Research Council of New Zealand.

A myocardial infarction is induced in adult sheep by occlusion of distal diagonal branches of the left anterior descending coronary artery using fluoroscopy. The sheep subsequently recover and the chronic effects of the myocardial infarction are studied in a second surgical intervention several months later. My contribution to this programme has been in developing and carrying out the animal care regime throughout these studies. This review will reflect on the steps taken to ensure a better quality of life for the sheep in our care within the term of our study.

The Cardiosurgical Laboratory is operated as a semi-autonomous facility within the overall framework of the Vernon Jansen Unit at the University of Auckland. It is well equipped for a wide range of cardiovascular and other surgical procedures providing anaesthesia, fluoroscopy, ultrasound, defibrillator, monitoring and mapping systems.

Sheep are sourced from a local farm where they have been subject to standard routine farming practices. Their acclimatisation to life in the laboratory begins on the farm. Sheep are offered the pelleted diet firstly in the field and then in the confined space of yards for 2-3 weeks before transport to the laboratory. Sheep quickly adapt to this complete diet and will consume a daily ration of 2 kg. The farmer transports the sheep to the University along with companion animals in a covered vehicle providing shelter from the weather and adequate ventilation.

In this project we are utilising fluoroscopic imaging techniques used in current clinical practice. The thoracic anatomy of sheep as a cardiac research model is not typically well suited for fluoroscopy due to their deep chest cavity and wide sternum, most unlike humans. The Lincoln Longwool sheep has an adult bodyweight of 40-60 kg and is a fine-boned breed and thus provides good quality images using fluoroscopy. Of British origin, this breed was first imported into New Zealand in the early 1860s. They are described as a hardy animal from a farming perspective, but we have found this breed to also have a remarkably calm temperament, which is a great advantage from a research perspective.

Housing sheep in an indoor laboratory environment requires that the social needs of these animals are given equal consideration to their physical needs such as nutrition, husbandry and hygiene. As a prey species, sheep rely strongly on their senses of sight, hearing, smell and touch. With this in mind, floor pens with sawdust and shavings as bedding best meet our requirements for both the experimental design and animal welfare. We are fortunate to have recently

moved into a new purpose-built unit where the floor pens have been well designed and constructed. They allow sheep to be individually penned within constant visual contact with others, or adjoining pen doors to be opened allowing more contact in a shared space. We are able to use both options on a group-by-group basis and each has its merits. It is important to ensure that in the shared pen environment no sheep is disadvantaged in competition for food and that there is no bullying by any dominant individual. Considered use of the two options in the floor pen environment ensures that all sheep remain settled throughout their stay.

Adult sheep appear to have very little interest in items provided for environmental enrichment unlike other species such as pigs but do enjoy some variety in diet such as hay, which we occasionally provide.

Following arrival into the laboratory and within the week before surgery, sheep quickly acclimatise to the indoor environment and settle into the daily routine of feeding and twice weekly bedding changes. Sheep experience hours of day and night along with 20 minutes of programmed dawn and dusk with lighting set on a 12/12 cycle. Room temperature is programmed and recorded daily to ensure a 16-18°C range is maintained. Feed given and left over is weighed daily, and water intake and urine and faeces output are monitored and recorded by animal technicians.

The animal technicians are the key influence in the environmental and social conditions that determine the well-being of our sheep housed in the laboratory. When sheep first arrive it is vitally important we take the time to develop a social bond with the sheep in our care. In a laboratory environment, sheep rely strongly on a daily routine. They recognise familiar technicians by sight and smell and learn not to feel threatened. They often exhibit attention-seeking behaviour especially where food is concerned, such as leaning out of their pens and will often sniff your hand in their own time. I have no doubt that this time spent quietly interacting with the sheep pays off substantially, providing research models that cope better with later surgical intervention and the following post-operative care. Pre-anaesthetic fasting of food for 24 hours (water available ad libitum) and surgical manipulation may disturb this trust, but it is rapidly regained when initially well established.

Before anaesthesia sheep are weighed to calculate drug dosage as well as to provide a baseline weight for future reference. Induction of anaesthesia requiring access to the cephalic vein in the foreleg is best achieved by using the "shearing" position with the sheep sitting on its hindquarters with support given to its back and head by the handler's legs and body. Sheep appear to sit comfortably in this position for short procedures. An intravenous catheter is secured in place for administration of intravenous fluids and drugs. Following induction, sheep are intubated and ventilated with inhaled anaesthetic. They are positioned on an X-ray cradle fitted with a heated blanket positioned on the X-ray table. An electrocardiogram (ECG) is continuously monitored from four limb leads and anaesthesia is monitored and titrated using palpebral eye reflexes, pinch reflex, heart rate and blood pressure. End-tidal CO_2 is also monitored and used to regulate the ventilator rate and tidal volume.

Surgical time is kept as short as possible. The right groin area is prepped and draped and an incision made to access the femoral artery for insertion of the catheter used to induce the myocardial infarction. This catheter is small and the amount of trauma to the surrounding tissue is minimal. Following induction of myocardial infarction, the incision is infiltrated with local anaesthetic before closure with simple interrupted sutures. Sutures are left in place for 7-10 days. All surgical procedures are completed under strict aseptic technique. Whilst under anaesthesia, sheep are ear-tagged with a unique study number for future identification.

Post-operative care begins in the theatre. Once spontaneous respiration is re-established, the endotracheal tube is kept in place whilst respiration rate is monitored. Most sheep are extubated before returning to the floor pens. They are placed on their chest with all legs folded underneath to assist in standing when ready and are able to hold their own head up.

Sheep are brought into the laboratory in groups of three with the intention that there will always be a familiar companion when another is removed from the group for even the shortest period of time. In the unfortunate circumstance that not all sheep return to the pen room after surgery, a mirror is positioned in the neighbouring pen to provide the illusion of a companion. Sheep will treat their own reflection as a stranger but this perceived company in the neighbouring pen appears to be reassuring.

Monitoring is essential for both good animal welfare and a good research outcome. In order to ensure comprehensive recovery care we have established an extensive post-operative monitoring sheet. The sheet is simple in design and divided into three sections: *From a distance; Close observation*; and *On handling*. Space is provided for morning and afternoon observations and most boxes require a simple yes/no answer. *From a distance* observations are made on activity, posture, respiration and vocal activity. *Close observation* comment is made on inquisitiveness, food and water intake and urine and faeces output. For *On handling* the surgical wound and sutures are checked, medication noted if administered and bodyweight recorded at least weekly.

The extensive post-operative monitoring of the sheep makes it essential that these interactions are minimally stressful, particularly in the post-operative period when high stress for an animal with a recent myocardial infarction has the potential to induce abnormal heart rhythms which in some cases may be fatal. Our initial familiarisation routine is central to ensuring good behavioural and welfare outcomes, and also a more successful experimental outcome.

Given the opportunity, sheep in a paddock will spend up to 8 hours grazing each day. It became apparent from our initial studies that post-operatively our sheep were well and showed no sign of impaired function following the myocardial infarction. It was therefore arranged for these study sheep to be returned to a farm where they could graze in the field whilst being monitored daily by the property owner who fortunately is also an experienced animal technician. We believe this provides the best care option for our sheep.

Sheep immediately recognise grass yet retain a keen interest in the pelletted diet. This daily expectation on the part of the sheep provides the means for close daily observation. Sheep are returned to the facility 12-14 weeks later, when chronic changes as a result of the myocardial infarction are well established, for terminal studies.

In these later studies the structural changes that have occurred in the heart wall, particularly in the border zone where the healed infarct joins healthy heart muscle, are investigated. The altered mechanical and electrical function is studied and mapped from both the inner and outer wall of the left ventricle.

An Endocardial Solutions Inc Ensite multi-electrode diagnostic balloon catheter with 64 non-contact electrodes is inserted through the femoral artery and positioned in the left ventricle using fluoroscopy. The proximal end of this catheter has an electrical connection to the Ensite 3000 Workstation. This is a sophisticated cardiac mapping system in current clinical use and is used to record and map the electrical activity from the inner wall or endocardium of the left ventricle.

An array of 25 plunge needles each containing 13 electrodes is also inserted into the left ventricle wall across the infarct border zone. The spread of electrical activity across the heart wall with each cardiac beat is measured by both these high resolution methods in both natural sinus rhythms and in paced heart rhythms.

These results combine to produce an electrical activation map of the left ventricle wall following a myocardial infarction. Subsequently these maps are superimposed on the detailed 3D reconstruction of the heart geometry and left ventricular wall microstructure around the infarct border zone obtained from high field magnetic resonance imaging (MRI) and extended-volume laser scanning confocal microscopy imaging. Analysis of the data allows for determination of the relationship between the myocardial microstructure and both normal and abnormal electrical function in the heart.

In conclusion, sheep are one of the most apprehensive of animals used in research and are instinctively fearful by nature. Understanding their biology and behaviour as well as developing some trust assists our group with their husbandry and handling whilst reducing sources of stress, improving the safety and welfare for both animals and technicians working with them. This ultimately leads to success in experimental studies ensuring the research has positive outcomes in terms of furthering the development of knowledge around both human and animal cardiac health and disease.

This paper was presented by the winner of the 2010 ANZCCART New Zealand Animal Care Technicians' Career Award