SUGGESSTED PROTOCOL

Sheep in Medical Research

Summary: This manuscript documents the technical sequence of managerial practices, with sheep as an experimental model in medical research. The farming approach to sheep management has been combined with a laboratory environment and subsequently to benefit the outcome of the science. The shepherd's calendar, peri operative protocols, pain management and rehabilitation programmes are reported. To assist with this marriage of diverse environments documentation throughout the holdings is consistent thus enabling traceability and animal health monitoring.

Disclaimer: information in this document has been collected from numerous sources over the past 3 years; however no references have been recorded. In addition, some of the information relayed was gained from experience in the commercial sheep farming sector. Images in this document are from actual cases and not recreated solely for the purpose of this document.

INTRODUCTION

The objective of this paper is to highlight the importance of excellent flock management and peri operative care of sheep used in medical research.

On performing a thorough review of available literature, it was apparent that there is little information available using sheep as the scientific model. Utilising advice from experienced personnel and information gleaned from courses, sheep specific regimes have been developed.

Sheep are a convenient large animal model as they are accessible and easily managed. The breed selected for *in-vivo* studies are female Blue faced Leicester x Suffolk. Animals undertaking surgery must be skeletally mature and within a specified weight range.

Anatomically sheep bone closely approximates that of humans and is regularly used as a model for evaluation of orthopaedic techniques.

This report is designed to disseminate information surrounding successful sheep management and peri-operative care. These working practices may have a positive impact on animal welfare and hopefully, enhanced the outcome of scientific results.

METHODS

The technical sequence of managerial practices, with sheep as an experimental model in medical research, were investigated. A gap analysis was produced to identify missing procedures and protocols, and then with the consent of the Named Veterinary Surgeon, appropriate actions were implemented.

Animals are provided by external contractors purchased from approved sources. All sheep purchased are female (ewe), 18 months old with approximate weights ranging between 65kg – 85 kg at the point of purchase.

Investigations were performed in accordance with UK regulations and together with the EU Directive 2010/63/EU guidelines.

Each phase of the management process was scrutinised, categorised by the normal sequence of events and documented as follows:

- Technician training and awareness

- Flock Management
- Peri operative care
- Critical Care
- Post-Operative analgesia
- Rehabilitation Programmes
- Documentation

RESULTS AND DISCUSSION

Technician Training

Animal care and welfare is the responsibility of all Technicians therefore rigorous training in their specific areas of expertise and continual updates are important to ensure the animal's wellbeing.

Flock management

A Flock health plan for both external and internal holdings were established, refer to Appendix 1 and Appendix 2 for in-depth information. Thus ensuring healthy animals from the point of purchase and ultimately able to tolerate surgical procedures ranging from 1 – 5 hours.

Fig. 1 Happy Sheep

Peri operative Care

Pre medication

The pre medication regime introduced renders the animal recumbent. Once recumbent the animal is transported to the preparation room.

The Administration the ketamine and xylazine Pre medication and the effects

Fig 2. Pre medication administered I/M

Fig.3. Immediately post pre-medication

Fig.4. 2-5 minutes post pre-medication

Fig. 5. 5+ minutes post pre-medication

Fig.6. The result of the pre-medication over approx. 10 minutes

This pre medication showed that less propofol was required at induction, reduced amounts administered by half.

Fig.6
Induction with Propoflo

However, reduced carbon dioxide levels were noted during surgery. This was addressed by first checking the sheep's breathing, secondly by checking the capnograph readings, then the depth of anaesthesia or by investigating the adequacy of the ventilators.

Intra Operative

The introduction of ventilators has had major impact on animal welfare intra and post operatively, especially where repeated anaesthesia was necessary to a study.

A Resuscitation Plan an essential part of emergency equipment.

Post-Operative

The administration of buprenorphine via CADD Legacy pumps has reduced post-operative handing. Analgesia is administered i/v at 4 hourly intervals for 48 hours, thus maintaining a level plane of pain relief. It was noted that the animals were more docile by administration of buprenorphine via this method kept

Fig.7 back pack pump filled with buprenorphine

The use Non-steroidal anti-inflammatory drugs (NSAIDS) for post-operative pain is under review. NSAID's, especially indomethacin, are notorious substances for the inhibition effect on cell regeneration, bone growth and fracture healing. Other NSAIDs having inhibiting effects on fracture healing include aspirin and ibuprofen. However, COX-2 NSAID's have revolutionised pain management and literature concerning modern selective anti-COX-2 agents is inconclusive, with their action on fracture healing probably being time- and dose-dependent.

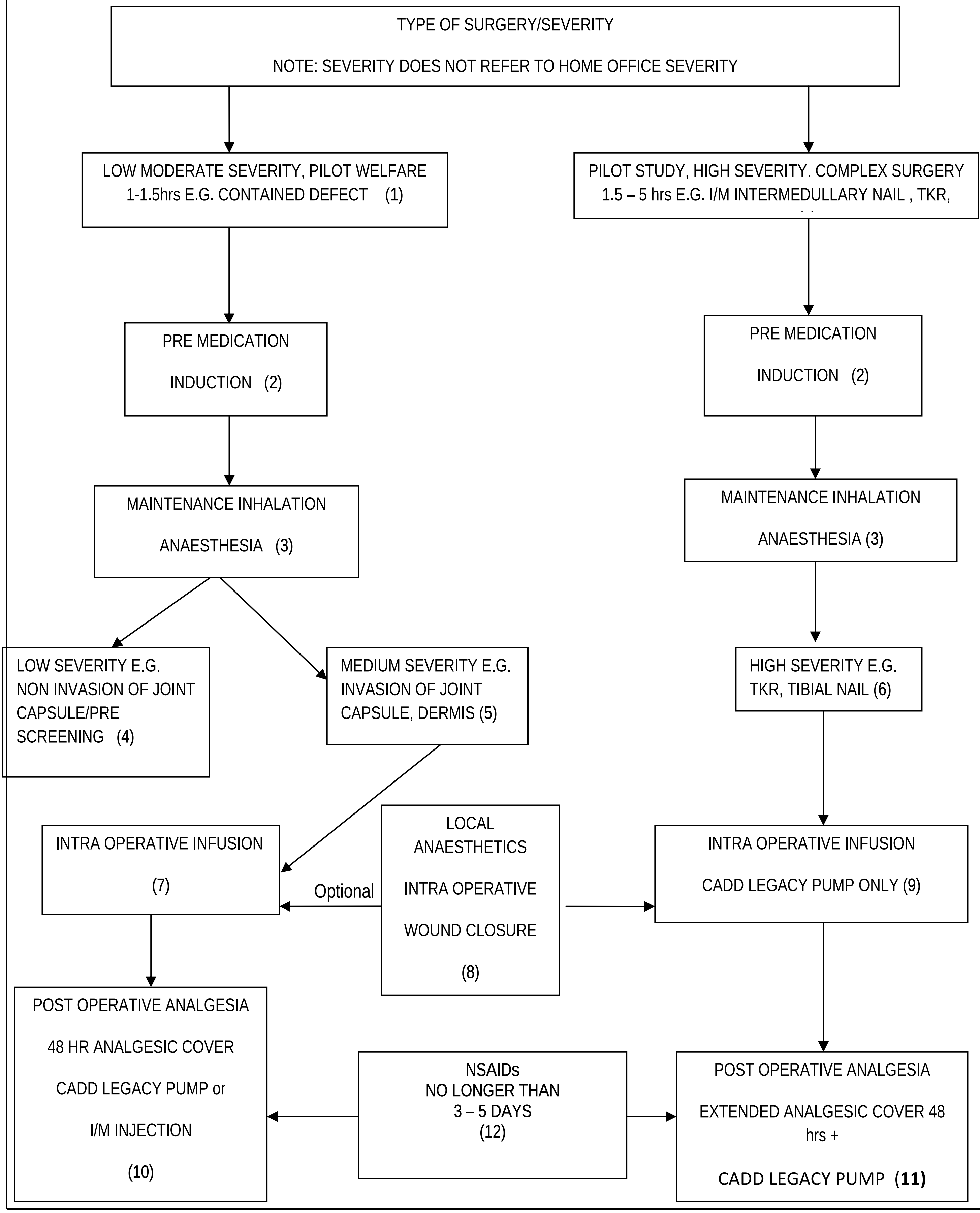
A decision making matrix has been developed to establish the most appropriate analgesic regime.

The decision making matrix below, demonstrates the areas considered when studies are being developed. The Matrix explains that for surgical procedures lasting 1-5 hours the pre medication and maintenance of anaesthesia is standard, however, beyond this point regimes differ.

The numbers in brackets relate to the analgesic and the method of delivery illustrated in the drugs key provided

DECISION MAKING MATRIX

ANAESTHESIA AND ANALGESIA



Drug Key

1. Pre-medication

Xylazine (0.1mg/kg) and Ketamine (10mg/kg) given as a single intramuscular injection. The reversal agent, atipamezole (0.025mg/kg), Clamoxyl LA (15mg/kg), Buprenorphine ((0.3mg/ml)

2. Induction anaesthetics

Propofol given to effect via an intravenous injection

3. Maintenance anaesthetics

Anaesthesia maintained using up to 5% isoflurane, up to 5% oxygen and 2% nitrous oxide mixture

4. Analgesia

None administered post operatively

5.6.7.9. Intra operatively CRI:

6mls of lidocaine and 0.12ml ketamine placed into 1 litre bag of saline 0.9% (NaCl). The intra-operative fluid rate is 10ml/kg/hr.

8. Local anaesthetics

llidocaine or bupivicaine

Wound closure

10 ml of Bupivicaine is instilled into the joint to provide 8-12 hours of sensory denervation post-operatively.

10. Post operative analgesia

48 hrs of Buprenorphine cover 0.3mg/kg/I/M

11. Post operative analgesia 48 hours +

CADD Legacy Pump administering Buprenorphine i/v every 4 hours

12. NSAID’s

Carprofen 4mg/kg s/c 3-5 days

Rehabilitation programmes

Programmes have been established to enable ease of animal handling pre and post operatively, refer to Appendix 3. These sessions are the sheep equivalent of physiotherapy to aid and record recovery.

Documentation

The administrations of routine treatments on the external holdings are documented i.e. anthelmintic treatments, Heptavac P+. These documents record dose rates, batch numbers, date of administration, expiry dates and signatures.

CONCLUSION

Owing to the implementation of improved protocols the following conclusions can be deduced:

1. Technician awareness and training is of paramount importance in the interests of animal welfare

2. Flock management and Health Plans require continual evaluation. Health issues arise, e.g. blue tongue. Ailments and diseases need to be monitored as weather conditions and environment dictate.

3. Peri operative care

    - The pre medication renders the animal recumbent dramatically reducing stress levels. In addition has health & safety benefits.

    - The amount of induction agent (Propofol) has reduced by half.

    - Local anaesthetics are used routinely intra operatively and at closure providing 8 – 12 hours pain relief.

    - Intra operative infusions of Ketamine and Lidocaine has been successfully used in longer, more involved surgery.

    - The introduction of constant rate infusion pumps, for the delivery of analgesics intra operatively, have helped maintain the animal's normal physiological parameters whilst under anaesthesia.

    - CADD-Legacy Pumps for the administration of analgesia post operatively have proved advantageous to animal welfare. This method appears to keep the sheep more docile.

4. The use of NSAID's for the management of post-operative pain management is under review.

5. Rehabilitation Programmes have been successfully implemented for surgery that has required them.

6. Documentation

    - Post-operative Observations and monitoring are particularly important for Pilot and Welfare Studies. In addition to an observation form, "welfare narratives" are written as a continual assessment of the animal's recovery and to document other findings.

- All treatments administered on the external holdings are documented for traceability.

7. Teamwork is a key aspect to the success of a project.

Appendix 1

Manual for the Routine Treatment of Sheep on the External Holdings incorporating a flock Health plan

Source of stock

The sheep should be sourced from either private or livestock sales by external contractors. For traceability animal movements should be documented in the Movement Record book in accordance with current legal requirements.

To assist in the control of disease, wherever possible purchase sheep where the health status is known and documentation is provided. Collect from the vendor as much information as possible on the recent history of the flock, flock management and what treatment/vaccinations the animals have received.

All purchased stock should be free from the following diseases:

- Caseous Lymphadenitis (CLA)
- Jaagsiekte
- Maeda Visna
- Sheep scab

Newly Purchased Sheep

On arrival onto the farm holdings all sheep require a 4 - 6 week quarantine period. During this period animals must receive individual identification and must be health checked by a qualified vet. The NVS or NACWO should be informed of any ill sheep or suspected problems.

If the health status of the new arrivals is unknown all routine husbandry tasks should be performed as if no treatment has been received.

These are as follows:

- Treatment with Cydectin for scab,, keds and lice.
- Vaccination programme for clostridial diseaseand Pasteurella pneumonia.
- Foot care: footbath in either formalin or zinc sulphate. The frequency of treatment may vary dependent upon the ailment and advice should be sought from NVS.
- Faecal sample including for Liver fluke and administer an antihelmintic recommended by the NVS currently Cydectin and Levamisole consecutively, then resample after 6 weeks.
- Keep records of all veterinary treatments.

Housing

During the winter months or extreme weather conditions, the sheep maybe housed in outside pens around the main barns. Access to the covered areas will be provided to shelter the sheep in extreme conditions. The sheep maybe housed for approximately 3-4 months depending on weather conditions and state of the grassland. While an acceptable stocking density is considered to be 10 sheep per acre this does not apply when sheep are housed for winter. Bedding should be of deep-bedded straw. The stocking density should be in accordance with Defra code of Practice.

Grassland

There are currently 17 fields in use and licensed for holding sheep. The fields will not be designated to stock or surgical animals to allow free rotation of the fields.

When the grasslands are capable of supporting livestock the sheep will be turned out to pasture. The stocking density of 10 sheep per acre will be observed, however, the stocking density may vary according to weather conditions.

Grassland management will be employed by field rotation and should work alongside the worming regime to avoid parasitic burden. Strip grazing may be employed on the larger fields

Nutrition

During winter months the sheep's diet should consist of either:

Good quality hay, haylage, Fodder beet, mineral licks and/or concentrates.

During summer months simple grazing is all that is required.

Access to water will be provided at all times.

It is advisable to body score the sheep on a monthly basis, thus allowing the weight and condition of the sheep to be closely monitored. The feed rations maybe altered accordingly to maintain the required optimum body score of 3.

Routine treatments.

Worming

Parasite control in sheep has changed in recent years in light of the increasing resistance problem of parasites to modern wormers. The sheep should be allowed to stand for 12 hours, to allow the stomach contents to be emptied, and then wormed according to manufacturer's guidelines. This includes worming the sheep by individual weight or if not then giving the dose for the largest in the group to all sheep. Dosing guns should be checked prior to dosing for delivery of the correct dose. Once wormed the sheep should be returned to their original pasture for 24 hours to allow reinfection with susceptible worms. The sheep must not be transported to new pasture and then wormed.

Faecal Sampling

Current recommendations advise regular stool sampling to evaluate the faecal egg count (FEC). A 6 weekly representative stool sample should be sent for evaluation, the sample should consist of 10 individual samples of at least 3g weight (5g where Fluke is tested for as well) these will be mixed by the lab and they should be fresh. A similar sample (faeces from several sheep pooled together) should also be tested 1 week after worming. This must be sent out immediately to a veterinary laboratory for analysis within 48 hours of sampling. If a high number of eggs are found, over 500 eggs per gram of faecal matter, then the sheep must be wormed. If exceptionally high numbers of eggs are counted, especially after recent worming, the NVS must be alerted, and the NACWO informed. If egg counts are low then the period between worming may be extended until the threshold level of 500 eggs/gm is reached. This method accepts that sheep will carry a parasitic burden but at a low enough level not to effect health and may reduce the likelihood of resistance. The sheep should also be wormed as they enter winter housing.

Foot care

It is imperative that the animals receive a high standard of health care at all times and the importance of good foot care is crucial.

Although the sheep are split into 2 groups, animals that have undergone surgery and stock animals, the same level of care should be employed. All animals should be foot trimmed as required and foot bathed every 4-6 weeks.
In preparation for surgery the sheep will have their hooves inspected prior to transportation to

the IVB holding, any animals with foot care problems will remain on the farm holdings. All animals returning to the farm holdings, from the IVB holding, will be inspected prior to transportation. Any animals with foot care problems will remain in the IVB holding unless otherwise advised by the NVS or NACWO.

There are various different substances for footbaths. The manufacturer's instructions must be adhered to, as different products will attract different recommendations.

Lameness should be controlled by foot trimming, Engemycin spray and/or foot bathing. In the event of any unusual ailments or serious problems the NVS and NACWO should be informed.

The use of a large calendar or diary to mark treatment days for various groups is highly recommended.

Shearing

All sheep on the holdings require shearing annually between May – August and should only be performed by trained personnel.

The external contractors are responsible for the collection and disposal of wool according to British Wool Marketing Board regulations.

General Health Checks

Whether the animals are out at grass or housed, they should be inspected a minimum of twice a day (morning and evening) and whenever performing routine husbandry tasks.

Particular attention should be paid to the body score of the animal. A condition score of 3 should be maintained throughout the year.

The NACWO should be informed of any minor treatments that may be required through out the year e.g. brisket sores, mild mastitis. More severe problems e.g. fly strike, severe mastitis, the NVS should be informed.

Any sudden death that occurs, a post mortem will be performed whether it is a stock or study animal.

Monthly status reports

Written reports on the health status and welfare of the flock, detailing specific incidents, must be submitted to NACWO or Deputy at the end of every month. Specific issues must be reported verbally to NACWO or Deputy as they arise.

Appendix 2: Table to show Diseases/Ailments that will affect sheep going onto a study

Disease/Ailment	Cause	Symptom	Prevention	Treatment
Respiratory Diseases: Bacterial Pneumonia-Pasteurellosis Parasitic Pneumonia	Pasteurella haemolytica Lungworm	High temperature Rapid breathing Nasty hard dry cough Bronchitis giving rise to severe coughing. Discharge from the eyes and nose	Remove all draughts Vaccination Avoid stress particularly in the autumn when the disease is most likely to break out Routine Worm dosing	Broad spectrum antibiotics Good ventilation Reduced Stocking Rates Faecal samples
Internal parasites: Roundworms Nematodirus infection Liver fluke	Intensification of stocking Sudden onset of diarrhoea Fasciola heptaca	Diarrhoea or scouring Listless Ill thrift Anaemia Weight loss Stand about, go off food, unwilling to move Swollen belly Very thin Odema underneath the jaw Mucous membranes of the eye are pale and anaemic	Good grassland management Knowledge of the life cycle of the worms As above Faecal egg sampling Injection	Anthelmintic dosing As above Anthelmintic dosing
Foot conditions: Foot Rot	Fusiformis necrophorus Fusiformis nedosus Spirochaeta penortha Acutely contagious	Mild inflammation between the digits A break appears between the skin and hoof horn The fusiformis causes death of the	Foot trimming. Foot bathing Clean bedding & regular Disinfecting	Foot trimming. Foot bathing Clean bedding & regular Disinfecting

		tissues and this leads to a ticking discharge		
Foot Abscess	Fusiformis necrophorus	Severe lameness Hot swollen and painful and the digits are spread	Good husbandry	Antibiotics Warm saline Poultice
Skin, Eye and gum conditions Mycotic Dermatitis Pink eye/Conjunctivitis	Intensive conditions with high stocking rates Poor ventilation	Blink repeatedly Tear stained face Membranes are red and inflamed	Low stocking rates Adequate trough space	Orbenin
Confirmation:				
Weight Loss >10% within one week				
CCN	Vitamin B1 deficiency caused by metabolic disurbance	Walk in continuous circles or just stand motionless. Appear to be blind, staggering and swaying as the condition progresses After a few hours they pitch forward on their side or brisket, throw their heads back and kick their legs as though in a fit The legs often stiffen, sudden noise will trigger off the violent leg kicking	Vitamin supplement containing thiamine fed to the entire flock	Vitamin B1 administered I/V Plus long acting antibiotic
Listeria	Listeria monocytogenes	High temperature Loss of appetite Circling Pushing against obstacles	Avoid feeding silage/haylage	Penicillin Streptomycin terramycin

Appendix 3

Rehabilitation programmes

Sheep must be acclimatised to animal technicians.

Sit in pen with sheep nuts for up to one hour each day and gently encourage animals to approach and take feed from the bucket/scoop or from technician's hand.

Where possible carry out routine activities such as weighing and foot trimming in the stock barn where the sheep are housed, so they become accustomed to daily activities.

Once acclimatised to technicians encourage sheep to follow a technician around a circuit in the new barn ever other day. The circuit should include the pallets and hurdles situated a suitable distance apart.

Post-operative rehabilitation regime

48hrs post operatively

Sheep are to be encouraged to stand when the analgesic injections are given at 8am, 1pm, 5pm and 12am and if able, depending on surgical procedure, put some weight on the operated limb encouraging blood circulation and to reduce the risk of bloat.

After the first 48hrs

Depending on the progress of each sheep allow out of the pen and encourage them using diet to walk around animal room once a day until analgesic regime is complete.

Once analgesia regime is complete return to the smaller pens in the study/new barn.

The day after the sheep have been returned to the study barn, whilst completing daily routine observations, allow the sheep out for a walk within the study/new barn, complete this exercise every day.

Two – three weeks post-surgery

Exercise the sheep by encouraging them to walk around the exercise circuit in the new barn. Repeat the circuit two - four times per session. Complete the session two - three times a week depending on progress of sheep and guidance from the NVS.

Four - six weeks post-surgery

Move the sheep into a larger pen.

Add pallets and low hurdles made from a soft material to exercise circuit to extend range of motion. The circuit should be completed four - six times three times a week depending on progress of the sheep and guidance from NVS.

Once the hurdles and levels are in place continue with the circuit for up to 12 weeks post surgery. Stance, ambulation and swelling should be monitored once a week throughout this stage.

From 12 weeks post-surgery animals can be kept in a field. The sheep will be checked daily by the keeper and once a month by NACWO. Stance and ambulation observations should be completed once a month.