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Citation for published version:

Clutton, RE, Vettoratto, E, Schoeffman, G, Docherty, J, Burke, J & Gibson, AJN 2014, 'The perioperative care of lambs and ewes when the former undergo major experimental (scoliotic) surgery', *Laboratory Animals*, vol. 48, no. 1, pp. 27-35. <https://doi.org/10.1177/0023677213498718>

Digital Object Identifier (DOI):

[10.1177/0023677213498718](https://doi.org/10.1177/0023677213498718)

Link:

[Link to publication record in Edinburgh Research Explorer](#)

Document Version:

Peer reviewed version

Published In:

Laboratory Animals

Publisher Rights Statement:

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1 **The Perioperative Care of Lambs and Ewes When the Former Undergo Major**
2 **Experimental (Scoliotic) Surgery.**

3

4 **Short title: Perioperative Care of Lambs & Ewes**

5

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20 **Keywords:** anaesthesia, lambs, ewes, scoliosis, refinement

21

22 **Abstract**

23 The purpose of the study was to optimize postoperative comfort in lambs anaesthetized for
24 the surgical creation of scoliosis whilst maintaining the ewe-lamb relationship to minimize
25 rejection rates. The first management plan produced 6 scoliotic lambs but intraoperative
26 hypoventilation and hypovolaemia followed by postoperative dyspnoea, pain, monitoring and
27 nursing difficulties, hypothermia, hypoglycaemia and tympany were encountered. Three of
28 eight lambs (38%) were rejected by their ewes. Perioperative management was amended
29 which, combined with improved surgical technique, produced 16 scoliotic lambs. The lambs
30 recovered more rapidly (mean time to standing after discontinuation of anaesthesia was
31 reduced from 12 hours to 70 minutes) and appeared to be more comfortable. No rejections
32 occurred after the refinements were implemented.

33

34

35 Spinal curvatures develop in children when vertebral growth imbalances result in progressive
36 spinal deviations in the coronal (scoliosis) sagittal (lordosis / kyphosis) and axial (rotation)
37 planes^{1,2}. Untreated children develop thoracic insufficiency with cardiorespiratory failure and
38 ultimately death³. Optimal treatment involves harnessing growth⁴ rather than forcibly reducing
39 an existing deformation with instrumentation: the latter restricts pulmonary function and
40 retards growth, preventing younger children reaching their full height. Some recently-
41 introduced devices appear to function as growth harnesses⁵ but testing their pre-
42 commercialization performance requires a valid animal scoliotic model⁶. Despite the
43 questionability of using quadrupeds to study disorders in bipeds, calves, pigs, and goats have
44 been used to model vertebral column surgery for human beings. Sheep vertebra are
45 similarly-sized to those of children and, it is believed, provide a more valid biomechanical
46 model for studying human spinal disorders⁷.

47 Objections to the use of lambs and kids, whose rapid post-partum growth assures their
48 suitability as models for human skeletal growth disorders,⁸ may be countered by providing
49 optimal peri-operative care. Problems arose after the traumatic avulsion of ventral cervical
50 spinal cord nerve roots in unweaned lambs.⁹ There was a conflicting need to provide effective
51 analgesia and surgical conditions in physiologically immature animals that had a high
52 metabolic rate and body water content, yet low body fat, poorly developed thermoregulatory
53 and cardiovascular reflexes and a different response to drugs. Preserving the post-operative
54 ewe-lamb bond was also important. Anaesthetic techniques used in 8-week-old goats
55 undergoing scoliotic surgery have been reported but details of perioperative care are very
56 limited¹⁰ or absent¹¹.

57 Altered attitudes to human neonatal pain management¹² have important implications in the
58 peri-operative management of young laboratory ruminants, in which pain behaviours are less
59 ostentatious than those of other species. This biological adaptation which serves to reduce
60 the risk of predation probably leads to post-operative pain being under-diagnosed and under-
61 treated.

62 The creation of scoliotic lambs was expected to be challenging because of the degree of
63 surgical invasion, the anticipated duration, the fact that some animals would be younger than
64 those previously encountered⁹ and previously reported "serious" complication rates of 18%¹³ .
65 Earlier experiences also prompted reservations with the adequacy of analgesia described by
66 McCarthy et al in kids (4 IM flunixin doses [1.5 mg kg^{-1}] and a single [$50 \mu\text{g kg}^{-1}$] butorphanol
67 injection).¹⁰ Consequently, a more aggressive approach involving newer analgesic
68 techniques, combined with greater emphasis on the postoperative needs of both lambs and
69 ewes was planned. This paper describes features of perioperative management that
70 appeared to improve the welfare of lambs and ewes involved in major experimental
71 orthopaedic surgery.

72

73 Animals

74

75 Twenty-six Scottish Blackface ewe-lambs were purchased with their ewes from a commercial
76 flock. Five weeks pre-purchase the ewes had been wormed and vaccinated against *Cl.*
77 *perfringens* and *tetani* (Lambivac, Intervet, UK). All were in good health based on physical
78 examination. The animals were acclimatized in purpose-designed indoor small ruminant
79 housing within the laboratory for >10 days before the first experiment. Housing was 2
80 opposing lines of 5 hurdled pens (1.5 x 2m) littered with barley or wheat straw. Each lamb
81 and its dam were confined in a single pen: water and hay were available *ad libitum* and the
82 ewe was provided with commercial pellets (0.5 kg day⁻¹ in 2 aliquots). Artificial lighting was
83 maintained from 08:00 until 23:30 each day. The study was approved by the University's
84 Ethical Review Committee and was licensed under the Animals (Scientific Procedures) Act
85 1986.

86

87 Material and Methods

88

89 Lambs were anaesthetized for the surgical creation of a right-sided scoliotic curve. Problems
90 encountered in the first 8 lambs (including 2 fatalities) required radical technical changes.
91 Consequently, the first 8 animals became regarded as pilot cases (and designated P(ilot) 1 –
92 8.

93 Surgery involved a dorsal midline skin incision made between T4 and L2 through which the
94 paraspinal muscles were retracted to expose the left lamina of T4-T6 and L1-L2. A 4 mm
95 braided synthetic Dacron tape (Abbott Spine, Austin, USA) was passed under the T5 lamina,
96 tunnelled submuscularly, passed under the L1 lamina and looped back to form a tether. This
97 was held under tension during the next surgical stage. Dissection bilaterally exposed the
98 caudal six ribs. On the right, 4 cm was resected sub-periosteally from each rib avoiding
99 breach of the thoracic pleura. On the left, the ribs were bound together with tape (3 mm

100 nylon, Ethicon, Ethicon UK, Livingston, UK) just distal to their angles. The combination of
101 the rib tether and final tightening and suture of the laminar ligament was intended to produce
102 a curvature with approximately a 25° scoliosis.

103

104 **Pilot Study**

105

106 Pre-anaesthetic medication was midazolam 0.5 mg kg⁻¹ (Hypnovel; Roche, Welwyn Garden
107 City, UK) and ketamine 5 mg kg⁻¹ (Vetalar V 10%; Pfizer, Sandwich, UK) injected into *m.*
108 *semimembranosus / semitendinosus*. Anaesthesia was induced with isoflurane (IsoFlo;
109 Abbott, Maidenhead, UK) using a Hall's pattern face mask and a Bain breathing system.
110 Total gas flow was 3 L minute⁻¹. When the palpebral reflex disappeared the trachea was
111 intubated under laryngoscopy with a suitable (5 or 6 mm cuffed) endotracheal tube (Portex
112 Blue Line; SIMS Portex Ltd, Hythe, UK). Anaesthesia was maintained with isoflurane
113 delivered in a 1:2 oxygen (O₂) : nitrous oxide (N₂O) mixture. The lungs were ventilated
114 mechanically (Blease-Manley, BME, Chesham, UK) to maintain normocapnia.
115 Neuromuscular blockade was produced with atracurium (0.5 mg kg⁻¹; Tracrium;
116 GlaxoSmithKline, Uxbridge, UK) to improve operating conditions and suppress ventilation.
117 Ringer's lactate solution (Vetivex 11; Dechra, Shrewsbury, UK) was infused (10 mL kg⁻¹ hour⁻¹
118 ¹) and during anaesthesia, heart rate (HR) the electrocardiogram (ECG) arterial pressure
119 (AP), end-tidal concentrations of isoflurane (F_E'ISO) and CO₂ (F_E'CO₂) and core temperature
120 were monitored along with pulse oximetry (Datex AS/3; Datex-Engstrom, Helsinki, Finland).
121 Analgesia was intravenous (IV) meloxicam (0.6 mg kg⁻¹; Metacam; Boehringer Ingelheim,
122 Bracknell, UK), epidural morphine (0.1 mg kg⁻¹; Morphine injection BP 1%; Martindale,
123 Romford, UK) and, or bupivacaine (Marcaine 0.5%; AstraZeneca, Luton, UK)
124 methylprednisolone (30 mg kg⁻¹; Solumedrone; Pfizer, Kent, UK) and buprenorphine
125 (Vetergesic; Animalcare, York, UK) given as needed. Lambs recovered in a straw-bedded
126 pen allowing close but limited contact with its ewe. Bottled milk replacer was offered at 3
127 hour intervals. The animals were observed continuously for the first 12 post-operative hours.

128 P1-8 had a mean age of 4.6 (range 3-6) weeks and weight of 11 (9-17) kg. Operating
129 conditions were adequate in all cases but two lambs died. Haemorrhage occurred in P2, a
130 lamb weighing 7 kg, which lost 208 mL (50% circulating volume) over 2 hours. Infusing 100
131 mls colloid (Haemaccel; Intervet, Milton Keynes, UK) failed to prevent cardiac arrest. Post-
132 operative dyspnoea occurred in P3 in which asymmetric right thoracic and spasmodic right
133 abdominal wall movements obscured agonal breathing patterns. *Post mortem* examination
134 revealed total lung collapse on the left side where the tether had been over-tightened.

135 Other complications were: pain on midazolam / ketamine injection; difficulty in post-operative
136 pain assessment; nursing difficulties; prolonged recoveries from post-operative hypothermia
137 and inappetance. Intra-operative hypoventilation occurred in P1 - 5. Lambs P4, P5 and P6
138 were rejected by their ewes and required hand-rearing. Recovery times to first unassisted
139 standing ranged from 12 to 20 hours, No lambs from the pilot study were re-used.

140 **Main study**

141

142 Major changes to lamb and ewe management were made after the pilot study. Forty-eight
143 hours pre-surgery, the lambs' operation sites were clipped, sponged with surgical disinfectant
144 (Povidone Iodine; Vetasept, Animalcare, York, UK) and covered with conforming bandage
145 (Vetrap; 3M, Bracknell, UK) to familiarize the ewe with the lamb's post-operative appearance
146 and smell and so reduce rejection risk. Antibiosis was begun 24 hours pre-surgery with
147 intramuscular (IM) oxytetracycline (20 mg kg⁻¹, IM, Engemycin; Intervet, Milton Keynes, UK).
148 Two hours pre-surgery ewes were milked to provide an alternative postoperative food option
149 to proprietary milk replacer. Four hundred mL of maternal blood was also collected at this
150 time and stored at room temperature (Teruflex; Terumo, Egham, UK). Lambs were allowed
151 milk until separated from ewes for pre-anaesthetic medication. This was changed to
152 medetomidine (10 µg kg⁻¹, Domitor; Pfizer, Sandwich, UK) injected into the epaxial muscles.
153 The lambs were reunited with their ewes until they became recumbent.

154 When profound sedation and recumbency were present, anaesthesia was induced with
155 isoflurane or sevoflurane (Sevoflo; Abbott, Maidenhead, UK) delivered in 100% oxygen. After

156 intubation, anaesthesia was maintained with the same agent used for induction: end-tidal
157 isoflurane (FE'Iso) and sevoflurane (FE'Sevo) concentrations were measured and held at
158 1.8% and 2.8%, respectively¹⁴. The endotracheal tube was connected to a circle breathing
159 system in which a Penlon Nuffield 200 Ventilator (Penlon InterMed, Abingdon, Oxon, UK) with
160 paediatric adapter (Newton valve) was connected at the bag mount. Spirometry (Datex AS/3;
161 Datex-Engstrom, Helsinki, Finland) revealed that tidal volumes (V_T) of 15 – 20 mL kg⁻¹
162 delivered at a frequency (f_r) of 17 minute⁻¹ produced peak inspiratory pressures of 16 – 30
163 cms H₂O and eucapnia, i.e., PaCO₂ values of 4.7 – 6.0 kPa. Atracurium was given IV as
164 before¹⁵.

165 Before surgery, meloxicam 0.6 mg kg⁻¹ and morphine (0.5 mg kg⁻¹) were injected IV. A
166 constant rate infusion of ketamine (10 µg kg⁻¹ minute⁻¹) was delivered by syringe driver
167 (Graseby MS16A; Smiths Medical, Ashford, UK) after a loading dose (1 mg kg⁻¹) had been
168 given. Ketamine (0.3 mg kg⁻¹) was available in case inadequate anaesthesia or analgesia
169 was identified. Before wound closure, bupivacaine (1.5 mg kg⁻¹) was sprayed onto the
170 operation site using a mucosal atomization device (Wolfe Tory Medical, Waukesha, USA).

171 A 22 gauge cannula was placed in the auricular artery for collecting blood for gas analysis (i-
172 STAT, i-STAT, Abbott Diagnostics, Dartford, UK) and monitoring AP. A human ear-lobe
173 probe attached to the tongue was used for pulse oximetry (SpO₂). The oesophageal and
174 rectal temperatures were monitored with thermistors advanced *per os* to the heart base and
175 *per rectum* respectively. An attempt to maintain normothermia was made by increasing the
176 ambient temperature to 22-24°C. A warming pad, and a heat and moisture exchange filter
177 (Hydro-Therm II HME, Intersurgical Ltd, Wokingham, UK) were also used.

178 A 22 gauge cannula was placed in either the cephalic or a lateral saphenous vein and
179 Ringer's lactate solution infused at 10 mL kg⁻¹ hour⁻¹. Arterial hypotension (mean AP < 55
180 mm Hg) was treated with ephedrine (0.1 mg kg⁻¹ IV, ephedrine hydrochloride injection 3%;
181 Martindale Pharmaceuticals, Brentwood, UK) or dextran (4 ml kg⁻¹; Dextran 40; Baxter
182 Healthcare, Thetford, UK).

183 After venous access was established blood was taken and cross-matched with a sample
184 collected earlier from the ewe using a technique described by the Animal Health Trust, UK
185 (http://www.aht.org.uk/cms-display/diag_clinpathis1.html).

186 Lacrilube (Allergan Pharmaceuticals, Westport, Ireland) was placed hourly into the
187 conjunctival sacs for corneal protection.

188 Haemorrhage was quantified by weighing bloodied swabs on a precision laboratory balance
189 (PGW153e; Milton Keynes, UK). One mL blood was taken to weigh 1.3 g. This value is
190 greater than that reported for sheep (1.08 g¹⁶) but was applied to compensate for
191 evaporative plasma water losses that were likely to have occurred before measurement.

192 The blood volume lost (V_e) through surgical suction was estimated by recording the volume in
193 the suction jar (V), measuring *in vivo* and *in vitro* haematocrits (Hct) and applying the
194 equation: $V_e = (in\ vitro\ Hct / in\ vivo\ Hct) \times V$.

195 Surgery was performed with lambs in the prone position supported by a vacuum-pad (Buster
196 vacusupport; Krusse A/S, Marslev, Denmark) shaped to contour the animal's abdomen in a
197 way that minimized intra-abdominal pressure and so reduced surgical haemorrhage.

198 Cefuroxime (20 mg kg⁻¹ IV; Zinacef; GlaxoSmithKline, Uxbridge, UK) was given immediately
199 before surgery and continued at 8 hour intervals for 4 days. Metronidazole (20 mg kg⁻¹;
200 Metronidazole 0.5%; Baxter Healthcare, Thetford, UK) was also given for 5 post-operative
201 days.

202 When surgery ended, ketamine and inhaled anaesthetic administration were discontinued and
203 the trachea extubated once laryngeal reflexes were forceful. The leads for physiological
204 monitoring were left *in situ* whilst the lamb was transferred from the operating table to a sling
205 (Fig.1). The slung lamb and multichannel monitor were then moved into a room next to the
206 operating theatre where O₂ was available and where the lamb's ewe waited in a divided stall.
207 The sling was positioned in the stall in a way that allowed partial contact between the two
208 animals. The lamb's section was bare-floored but warmed with infra-red lamps and the sling
209 was draped with bubble wrap under which a proprietary hair drier blew warm air. The ewe's
210 section of the stall was littered with straw and equipped with food and water bowls.

211 Monitoring physiological variables continued in recovery along with fluid infusion. Oxygen
212 was delivered by mask only when pulse oximetry and other signs convincingly indicated its
213 need. Arterial samples were taken for blood-gas analysis whenever abnormal breathing
214 patterns coincided with SPO_2 values < 0.9 . Bottled ewe's milk or milk replacer was offered at
215 hourly intervals for the first 3 hours, when venous blood samples were taken to monitor blood
216 glucose levels (One-Touch Ultra 2; LifeScan Inc, CA, USA).

217 Signs of post-operative pain (plaintive bleating; bruxism, depression, disinterest in the ewe;
218 trembling, reflex "cringing" on attempts to stand) were treated with morphine (0.5 mg kg^{-1} IV)
219 or buprenorphine ($20 \mu\text{g kg}^{-1}$ IM) and, or ketamine ($0.3 \text{ mg kg}^{-1} \text{ hour}^{-1}$) depending on the
220 timing of previous doses.

221 Monitoring and O_2 delivery continued until the lamb could stand within the sling when the
222 arterial cannula was removed. Fluid infusion, maintained at $10 \text{ mL kg}^{-1} \text{ hour}^{-1}$ during recovery
223 was discontinued when bottled milk was imbibed readily. At this time, the barrier between
224 stalls was removed and the ewe allowed access to the lamb. The animals' behaviours were
225 monitored directly for at least 20 minutes after this. At least one venous cannula was retained
226 for the first 24 post-operative hours.

227 Once the lambs were standing unaided and interacting normally with the ewe they were
228 removed from the sling, but most initially did not suck normally and required some bottled
229 ewe's milk or replacer. An observer was continuously present during the first hour of
230 recovery but withdrew thereafter to monitor the animals on CCTV.

231 Meloxicam was injected once daily for 3 - 7 days and buprenorphine was given according to
232 individual needs. If the latter was ineffective, IM morphine at doses up to 0.5 mg kg^{-1} were
233 used instead. The ewe-lamb pairs were allowed into a grassed paddock from the third post-
234 operative day if the weather was fine,

235

236 **Results**

237 The revised technique produced 18 scoliotic lambs, a more rapid recovery from anaesthesia¹⁴
238 (70 – 90 minutes to stand) and a briefer interval until successful re-unification with no lamb
239 rejection. Two lambs died however from peri-acute post-operative *Clostridium perfringens*
240 endotoxaemia¹⁷. The median age of animals studied was 4 weeks (range 3 - 6) weeks and
241 their body mass 12 ± 2.3 kg (means \pm standard deviation [SD]).

242 Medetomidine ($10 \mu\text{g kg}^{-1}$) injection produced little reaction and profound sedation within 15
243 minutes in all lambs. Mask induction was well-tolerated and effective; tracheal intubation was
244 normally completed in 2-3 minutes¹⁴. The anaesthetic produced adequate surgical conditions
245 in all lambs with a median (min-max) recorded FE'Iso of 1.8% (1.5 – 2.1). Equivalent values
246 for sevoflurane (FE'Sevo) were 2.8% (2.5 – 3.1)¹⁴.

247 The Penlon ventilator achieved normocapnia in all lambs without complication or modification.
248 Mean (\pm SD) f_r of 17 ± 3 breaths minute^{-1} and a median (min-max) peak inspiratory pressure
249 (PIP) of 22 (16-30) $\text{cms H}_2\text{O}$ resulted in a median V_T of 16 (15 - 20) ml kg^{-1} and mean PE'CO₂
250 values of 5.62 ± 0.6 kPa. When inspired O₂ concentrations were > 90% the mean (\pm SD)
251 arterial tension of O₂ (PaO₂) was 55.2 ± 12.4 kPa.

252 Haemodynamic variables were stable in all cases although all but one was hypotensive.
253 Ephedrine and, or dextran were effective on all occasions. After treatment for hypotension,
254 the mean intraoperative HR was 128 ± 23 beats minute^{-1} while systolic, mean and diastolic
255 AP were 88 ± 11 , 67 ± 12 and 56 ± 12 mm Hg, respectively.

256 Hourly arterial blood analysis revealed a mean (\pm SD) arterial pH of 7.43 ± 0.05 , base excess
257 (BE) of 4.7 ± 3.2 mmol L^{-1} and HCO₃⁻ of 29.0 ± 3.2 mmol L^{-1} . Median (min-max) lactate and
258 glucose were 1.5 (0.72-2.95) and 5.75 (2.3-15.2) mmol L^{-1} , respectively.

259 No *in vitro* reactions were observed on mixing blood from 18 ewe-lamb pairs, and no adverse
260 effects were observed in the single lamb requiring transfusion (which received 25 mL kg^{-1} of
261 maternal blood at $0.5 \text{ ml kg}^{-1} \text{ hour}^{-1}$ rising to $5 \text{ ml kg}^{-1} \text{ hour}^{-1}$ after 30 minutes).

262 The mean (\pm SD) rectal temperature recorded at end-surgery was $38.69 \pm 0.5^\circ\text{C}$

263 As the study progressed, reduced surgery times caused a corresponding reduction in
264 anaesthesia time, with mean values of 125 ± 24 mins and 214 ± 45 mins (from induction to
265 the discontinuation of isoflurane) respectively. No further problems were encountered with
266 post-operative dyspnoea and post-operative blood-gas values indicated adequate pulmonary
267 function and levels of ventilation.

268 Signs of post-operative pain were observed in most lambs during early recovery and were
269 treated with variable success. The intensity and duration of pain signs diminished as surgery
270 became briefer and less traumatic. Post-operative monitoring, fluid and O₂ administration,
271 general nursing and maintaining normothermia were greatly facilitated by the sling (Figure 1).
272 Lambs and ewes were re-united on average 70 minutes (range: 20 – 637) after anaesthesia
273 was discontinued.

274 The animals' behaviours normalized rapidly in the first 20 minutes after re-uniting. No
275 rejections occurred in the second group and no lambs were unable or disinclined to suck
276 naturally. Only 1 lamb in 18 required additional morphine and no cases of tympany were
277 observed. All lambs were behaving, suckling and moving normally within 48 hours of
278 recovery. This process was accelerated by allowing ewe-lamb pairs access to pasture.

279 Modest abdominal gas distension was detected during recovery and relieved by trochar (P2)
280 and stomach tube (P8).

281 Discussion

282 Management changes during the study's course were successful on the basis of lower
283 morbidity, mortality, and ewe rejection rates with more rapid and more comfortable
284 recoveries. Therefore, the changes were regarded as a refinement of methods described
285 previously⁹. Nevertheless, two lambs unexpectedly died of Clostridial enterotoxaemia¹⁷. It is
286 possible that lamb - in addition to ewe vaccination may have provided higher levels of
287 protection after colostral immunity had waned¹⁸ and prevented losses in the main study.

288 Maximizing the ewe's involvement in her offspring's peri-operative care reduces the cost of
289 human assistance. However, this relies on the early and complete acceptance of the lamb.
290 Any human intervention, such as that required for analgesic administration, will obviously
291 threaten the ewe-lamb bond. In this study, effective analgesia was prioritized on practical, as
292 well as welfare grounds; postsurgical lumbar pain could conceivably have reduced the lamb's
293 ability to stand and "bunt" the udder to promote milk "let-down". The literature does not help
294 solve this problem. The report of a caprine scoliosis model (McCarthy et al. 2010) limited
295 management details to drugs and doses for anaesthesia and post-operative analgesia (*vide*
296 *supra*) but provided no information on the comfort achieved and did not describe the dams'
297 role. Other reports of scoliotic 4 – 8 week-old kids gave no information on analgesics or the
298 role of the dam^{11, 13},

299 In our study, the anaesthetic provided adequate surgical conditions and uncomplicated
300 recoveries. Medetomidine injected IM caused less reaction than the ketamine – midazolam
301 mixture probably because ketamine causes muscle damage¹⁹. Ventilation was more easily
302 controlled with the Penlon Nuffield 200. The Blease-Manley device required a "controlled
303 leak" to restrict peak inspiratory pressures to 20 cms H₂O when gas flows and ventilator
304 settings were producing normocapnia. The "leak" - an adjustable pressure limiting valve
305 incorporated into the inspiratory limb - needed constant adjustment and waste-gas
306 scavenging. Despite this, limiting lung inflation pressure to 20 cms H₂O failed to achieve
307 normocapnia in P1 and P2, even when gas flows exceeded the minute volume of ventilation
308 (VM) calculated for lambs using Purves' equation²⁰ i.e., $VM (L \text{ minute}^{-1}) = 0.123 + 0.261.x$

309 (where x is body mass [kg]). Using the Penlon with a rebreathing (circle) system lowered
310 costs because gas flow and requirements were reduced. Importantly, rebreathing reduced
311 heat loss caused by breathing dry and cold gases.

312 Measures taken after P2's demise from haemorrhagic shock, i.e., contouring the surgical
313 mattress and refining surgical technique, prevented further losses from hypovolaemia. Only
314 one lamb went on to require a maternal blood transfusion. This animal lost approximately 25
315 mL kg⁻¹ blood during surgery which exceeded the 15 mL kg⁻¹, that would be expected to
316 compromise circulation in a conscious adult sheep²¹. A literature review conducted after P2's
317 loss failed to confirm meloxicam's potential anticoagulant effect and so the drug was not
318 withheld from subsequent cases.

319 Haemolytic tests are preferred for grouping sheep blood, except factor D²² (Nguyen &
320 Bunch, 1980) whose antigens are detected by agglutination²³. The lack of commercial blood-
321 typing cards or gels for sheep precluded the straightforward characterization of ewe and lamb
322 blood in the current study so transfusion reaction risks were reduced by cross-matching blood
323 of each ewe-lamb pair. No adverse *in vitro* reactions were seen in 18 cross-matches, and no
324 adverse reactions occurred in the single lamb receiving transfusion.

325 The fatal postoperative dyspnoea occurring in P3 has also been reported in caprine scoliotic
326 models with post-operative respiratory failure in 8%¹³ and 7%¹¹ of animals. In the main part of
327 the current study, complications were avoided by more judicious tensioning of the surgical
328 tether. It would still be expected that rib resection and tethering on the convexity and
329 concavity of the curve respectively would significantly inhibit ventilatory function. In essence
330 the lamb had a flail chest wall segment on one side and limited capacity to 'over-expand' the
331 lung on the other. Although not measured the dead space would increase, possibly by as
332 much as 50%. Ventilatory function was also monitored more effectively using hourly arterial
333 blood-gas analysis in preference to continuous pulse oximetry.

334 While nursing and monitoring reunited lambs required additional staff for ewe restraint, early
335 reunification was prioritized. However, identifying the ideal time for this was complicated by
336 difficulty in interpreting the ewes' intent. This in turn was complicated by the presence of

337 people. Such difficulties contributed to three rejections during the pilot study. Lambs began
338 vocalizing in early recovery which elicited vocal responses in the ewes and prompted re-
339 unification. However, some lambs vocalized whilst recumbent, which caused two ewes to
340 vigorously "paw" their offspring in an apparent attempt to prompt standing and movement, but
341 with a force that threatened the surgical site. In contrast, the ewes of **P3** and **4** allowed
342 suckling within 2 – 3 hours of recovery from anaesthesia while **P5's** dam persistently head-
343 butted its offspring, which had to be removed and nursed manually. It became apparent
344 during the recovery of **P6** that continuous human presence contributed to ewe anxiety and
345 probably lamb rejection.

346 Measures taken to reduce maternal rejection, i.e., familiarizing ewes with their lambs' post
347 operative state, minimizing post-operative human interference using CCTV and re-uniting
348 slung lambs as soon as possible were successful because no rejections occurred in the main
349 study. That rejections were not encountered in another study using ewes of a different breed⁹
350 suggests a problem with the mothering characteristics of the Scottish Blackface. However,
351 some pilot lambs of the current study were if anything over-mothered, i.e., were vigorously
352 "pawed" by their dams.

353 The potential variation in mothering qualities within and between breeds coupled with
354 variation in the robustness of lambs complicates the choice of any specific breed for
355 experimental surgical studies. However, on the basis of the experiences described, ewe
356 familiarization, early reunification and remote postoperative surveillance are strongly
357 recommended.

358 The sling as shown in Figure 1 provided numerous benefits. Unconscious lambs could be
359 moved from the operating table whilst attached to the breathing system and physiological
360 monitors, saving time and allowing uninterrupted monitoring. By restricting lamb movement,
361 the physiological monitors, fluid and O₂ administration were resistant to disruption. The sling
362 allowed more intimate access to the lamb by the ewe whilst protecting the former from over-
363 attention. By supporting the lamb, the sling probably relieved tension in the paraspinal
364 muscles and so reduced pain both at rest and during attempts to stand that would otherwise

365 have been necessary. By elevating the animal from the floor, the sling assisted in keeping
366 vascular access points clean and improved the ergonomic efficiency of nursing. The
367 attendants did not have to repeatedly kneel and the animal and instrumentation could be
368 moved more easily. The sling's design also contributed to restoring normothermia.

369 Assessing postoperative pain was difficult and there was variation in perceived comfort
370 levels in animals receiving similar analgesics. Signs of discomfort were usually presaged by
371 generalized shivering early in recovery. Most lambs bleated before rejoining their ewes,
372 when they became silent (indicating that separation may have been causative) but those in
373 discomfort continued to be depressed, reluctant to feed and disinterested in the
374 surroundings. Bleating could be stopped by re-infusing ketamine or injecting morphine or
375 buprenorphine, but whether this was the result of sedation or enhanced analgesia was
376 impossible to determine. Strong, sustained bleating had the dubious benefit of
377 demonstrating that the surgically-created flail segment was of limited functional significance.

378 Under non-laboratory conditions, vocal behaviours between ewes and lambs is breed-
379 dependent and serves several purposes including location after separation²⁴. Lambs also
380 vocalize in response to acute pain caused by castration and tail docking although this does
381 not always correlate with apparent intensity and its expression varies greatly amongst
382 individuals²⁵. However, there appears to be little information on the relative contribution of
383 separation *versus* pain to vocalization during castration or docking which is unfortunate as
384 such information would have been of considerable use in the current study. Until this
385 becomes established, we propose that the absence of postoperative vocalization in separated
386 or reunited lambs is no assurance of an absence of pain.

387 Other (non-vocal) signs of pain lambs related to husbandry procedures, e.g., castration, have
388 been extensively described in lambs.²⁶ These, however were of limited use in the current
389 study because the pain was different as it arose from more extensive musculoskeletal injury
390 inflicted over a greater area.

391

392 Aggressive analgesia did not appear to cause problems with no signs of overdose or adverse
393 reactions observed. However, some lambs responded disappointingly to extradural drugs.
394 For example, **P7** received 2 mL (0.25 mg) bupivacaine mixed with 0.2 mg kg⁻¹ morphine
395 before recovery yet looked depressed and adopted a torticollic position. Two additional
396 epidural injections (same drugs and doses) were without apparent effect. However, 6 hours
397 later the lamb made a full and rapid recovery, behaving normally thereafter. Improved
398 responsiveness of lambs to analgesics in the main, compared with the pilot study, probably
399 resulted from an improved surgical technique and associated reduction in surgery time.
400 Probably the aggressive polymodal pain therapy, with some elements given pre-emptively,
401 also helped.

402 The measures we adopted to limit hypothermia were successful in the main study. This was
403 important because the post-operative hypothermia that occurred in all the pilot lambs
404 appeared to retard recovery. Cranial nerve reflex recovery only began once re-warming
405 achieved rectal temperatures > 35°C. We observed that withholding oxygen from the
406 inspired breath in lambs with rectal temperatures between 32 and 35°C resulted in
407 tachycardia, rapid falls in SpO₂ and parallel rises in FE'CO₂. Hypothermia probably
408 contributed to cardiac arrest in **P3** because its *ante mortem* rectal temperature was 34.4°C.
409 In the pilot study, in which lambs were unslung, attempts to achieve post-operative
410 normothermia were frustrated because the conscious animals moved away from IR heat
411 sources (and displaced the rectal thermistor probe), whilst the unmoving, sedated animals
412 were predisposed to hypothermia through inactivity. The latter presumably also had a
413 thermoregulatory reflex depression. It should be noted that the periodic recording of rectal
414 temperature using an electronic thermometer provoked signs of discomfort in some lambs
415 and anxiety in ewes. This warrants consideration being given to the use of telemetric devices
416 in future studies.

417 Finally, the variability in the ewes' readiness to suckle and the lambs' inclination to imbibe
418 complicated the maintenance of stable post-operative blood glucose levels. Our recorded
419 values ranged from 2.3 - 15.2 with a mean of 5.7 mmol L⁻¹. The published blood glucose
420 values for 4 – 8 week-old lambs range of 3.7 – 4.8 mmol L⁻¹ 27 suggests that most of our were

421 hyperglycaemic. This perhaps was not unexpected. The postsurgical stress response in pre-
422 term humans²⁸ and lambs²⁹ involves plasma glucose surges that parallel plasma cortisol
423 levels and broadly reflect surgical trauma²⁷. These factors prevailed against attempts to
424 maintain normoglycaemia and so the objective re-focused on ensuring that the lambs
425 periodically drank milk, with a rationale that this would promote sucking and ewe-lamb
426 bonding, whilst providing glucose in the animals that were hypoglycaemic. Consequently,
427 bottled ewes' milk, or milk replacer (approximately 180 mL) was provided at 3 hourly intervals
428 to lambs which were not allowed or unable to suckle. Lambs refusing the bottle received 40%
429 glucose (15 ml) IV over 10 minutes. Concerns with osmotic diuresis were dispelled by
430 infusing crystalloid solution at supra-maintenance levels. The cause of the noted abdominal
431 gas distension was unknown as the two animals were managed no differently from those
432 recovering without gastro-intestinal disturbances.

433 Normal ewe and lamb behaviours were almost completely restored when the animals were
434 allowed onto pasture. Under these conditions, lambs that had surgery only three days earlier
435 displayed sucking and play behaviours that were indistinguishable in terms of complexity and
436 vigour than those demonstrated by lambs which had not undergone surgery. Exposure to a
437 grassy environment also appeared to accelerate the restoration of normal ewe - lamb
438 behaviours which in some cases appeared to be subdued by indoor conditions.

439 In conclusion, experiments involving major surgery on lambs compromise the welfare of both
440 the lambs and their ewes. We have outlined specific measures that will limit complications
441 and increase the likelihood of rapid and comfortable recoveries. Ewe and possible lamb
442 vaccination against Clostridial disease is strongly recommended

443

444 **Acknowledgements:** The study was funded by the Medical Research Council, UK. MRC
445 G0700918
446

447

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554 **Figure 1: Legend.** A purpose-built sling for recovering lambs after major surgery. The frame
555 accommodates differently-sized subjects. Height is readily adjusted (long slider). The
556 frame's width ensures the sling is taut enough for sternal support, but does not restrict
557 breathing. Short slider adjustment allows the unconscious lamb's head to rest in a way
558 allowing single-handed tracheal intubation when necessary. The frame was 5 cm box
559 stainless steel and could be heat sterilized. The sling was washable linen toweling. All
560 measurements in cms.