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Development of a Locomotor Rating Scale for Testing Motor Function in Sheep



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ABSTRACT

Background/Purpose: Research to cure paralysis associated with myelomeningocele (MMC) is ongoing using the fetal sheep model of MMC. Despite decades of research using this model, no standardized motor function assessment exists. The purpose of this study is to develop a sensitive and reliable locomotor scale for assessing the functional status of sheep.

Methods: Twenty lambs were used to develop and validate the locomotor scale. Lambs ($n = 15$) underwent a surgically created MMC defect at gestational age (GA) 75 days, followed by repair with various methods at GA 100. One lamb underwent a sham operation ($n = 1$). Normal lambs ($n = 4$) served as controls. All lambs were born via spontaneous vaginal delivery, and motor function was assessed for 24 hours. A locomotor rating scale was developed based on behavioral observations of lambs. Inter-rater reliability testing was performed to determine if the scale could be reliably applied by different raters.

Results: Observations led to the definition of 7 categories of locomotor recovery. A scoring system was developed to rank these categories. The scale captured a wide variety of neurologic outcomes. Inter-rater reliability revealed minimal variability between examiners (average standard deviation ± 0.431). The average score for all raters was within 1 point of the consensus score 100% of the time.

Conclusions: The sheep locomotor rating scale is capable of capturing subtle differences in neurologic function with minimal inter-rater variability. We propose a standardized rating scale for neurologic outcomes and believe this is a critical component for assessing the validity of experimental treatments to cure paralysis in MMC.

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The 2011 NIH/NICHD Management of Myelomeningocele Study (MOMS) was the first randomized controlled clinical trial to compare prenatal and postnatal closure of the myelomeningocele (MMC) defect using skin closure repair [1]. The trial demonstrated that prenatal repair was safe and improved associated hindbrain abnormalities, such as the Chiari 2 malformation. Prenatal repair was also shown to improve lower extremity motor function in some patients; however, 57% of children treated *in utero* had no improvement in the level of neurologic function and 58% remained unable to walk independently. While this study was the first to suggest that the devastating neurologic deficits seen in MMC can be improved, the disease is far from cured.

Research to improve distal motor function in MMC by augmenting *in utero* repair with a variety of novel treatments is ongoing, and the fetal sheep model of MMC is the most commonly used large animal model. This well-established animal model has been used for decades, and although the model represents a surgically created defect, the histologic

and clinical outcomes closely resemble the deficits seen in congenital human MMC [2].

Despite worldwide acceptance of the fetal sheep model as a reliable large animal model for prenatal MMC repair, no standardized locomotor rating system exists with which to consistently analyze functional results following delivery. In order to evaluate therapies that augment *in utero* repair to improve distal motor function, a uniform scale for locomotion assessment is essential. The Basso, Beattie, Bresnahan (BBB) Locomotor Rating Scale is a well-validated and widely used scale for locomotor testing in rodent spinal cord injury models [3]; it has been successfully adapted for use in a porcine model [4]. In this study, this locomotion rating system was adapted for use in sheep as a comprehensive and reliable measure of distal motor function.

1. Methods

1.1. Subjects

Twenty lambs were used to develop and validate the locomotor scale for sheep. A MMC defect was surgically created in fetal lambs as previously described [5,6]. In brief, each ewe underwent a survival laparotomy and hysterotomy at a gestational age of approximately 75 days.

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LIMB MOVEMENT				LOCOMOTION			
				STANCE WITH HELP/ ASSISTANCE		STEPPING Total #	FORLIMB-HINDLIMB COORDINATION
HINDLIMB				No	Yes	HINDLIMB	None Occasional Frequent
Hip	Knee		Ankle	Total Score			
L R	L R	L R	L R	SPONTANEOUS Hindlimb weight support		0	occ<50% freq>50%
0 0	0 0	0 0	0 0	No	Yes		
S S	S S	S S	S S	L R		1-4	CLEARANCE TEST
E E	E E	E E	E E				
0=none		S=slight		E=extensive		>5	Fail
0=0 points		S=1 point		E=2 points			
				SPONTANEOUS Standing		Pass	
				No	Yes		

Fig. 1. Locomotion Categories. The seven categories of locomotion were limb movement, stance with help, spontaneous hindlimb weight support, spontaneous standing, stepping, coordination, and hindlimb clearance.

A defect was created in the fetal lamb (n = 15) by removing the dorsal lamina of six lumbar vertebrae and exposing the underlying spinal cord. The dura over the exposed cord was excised. Twenty-five days later, a second survival laparotomy and hysterotomy was performed. A variety of experimental treatments were used to repair the defect, producing various gradations of motor recovery. Normal lambs (n = 4) without surgically created defects served as controls; one ewe, pregnant with one lamb (n = 1), underwent laparotomy and hysterotomy without defect creation at gestational age of 75 days to control for any possible effects of surgery on the lamb's motor function.

Ewes were allowed to carry the pregnancy to term; all lambs were delivered via spontaneous vaginal delivery. All animals surviving to term with spontaneous vaginal delivery and complete neurologic assessment were included in the analysis. Any aborted fetuses or animals euthanized prior to vaginal delivery were excluded. All animal protocols were approved by the University of California, Davis Institutional Animal Care and Use Committee, animal care was in compliance with the Guide for the Care and Use of Laboratory Animals, and all facilities were accredited by the Association for the Assessment and Accreditation of Laboratory Animal Care International [7].

1.2. Locomotor Testing

After birth, lambs were assessed in person at two time points by a minimum of two examiners. Open field testing was performed in the birthing pen shortly after birth. Lambs were then allowed to adjust to

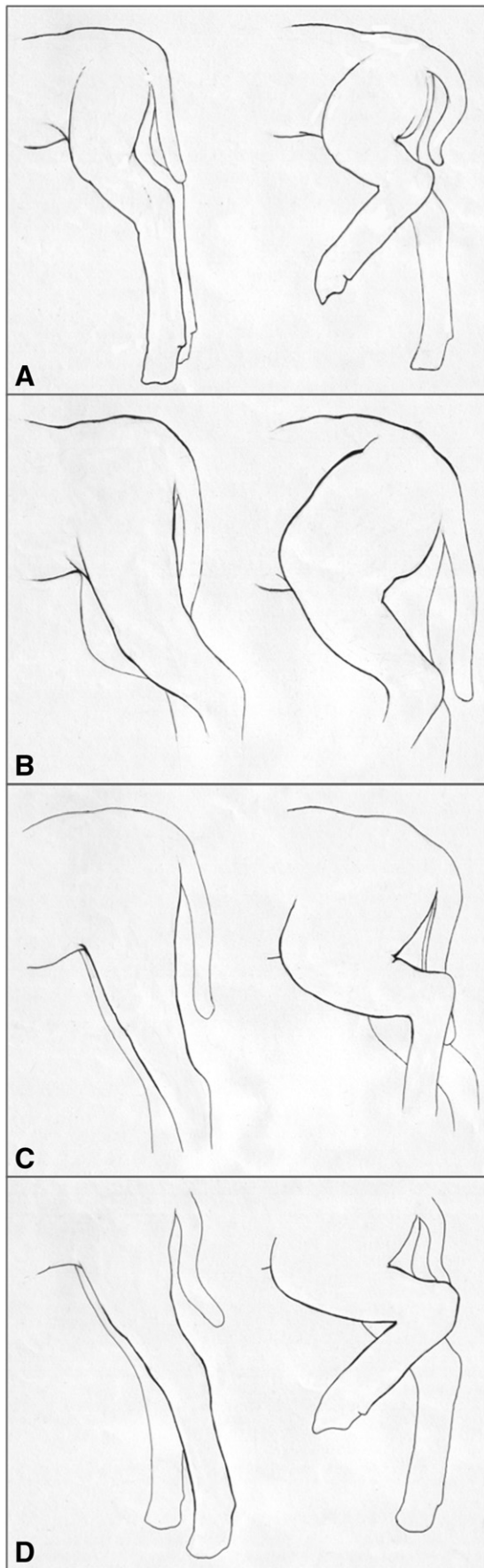
the environment for 24 hours; after this time period, locomotor testing was performed again. Lambs capable of spontaneous standing and/or ambulation were allowed to locomote without interference. If unable to stand spontaneously, lambs were first observed, then assisted to stand by lifting the lamb into standing position with all limbs in contact with the ground. Any ability to stand unassisted or ambulate after being assisted to stand was observed without interference. Lambs unable to stand even after assistance were evaluated by assessing movement in each joint of each hindlimb. All voluntary hindlimb movements were recorded; movements attributed to reflex stimulation or elicited by the examiner were excluded. Each lamb was recorded for at least 5 minutes with a video camera.

1.3. Scale Development

A preliminary checklist of motor patterns was developed from adaptations of the rodent BBB Locomotor Rating Scale [3], the porcine neurologic motor scoring system [4], and observations of locomotion in normal lambs. Motor function behaviors were added, modified, or removed after observation of all animals. Based on these observed behaviors, the sheep locomotor rating (SLR) scale was compiled. An assessment sheet was created to reflect this checklist and to facilitate accurate documentation of motor function at the time of functional assessment (Fig. 1).

SEVERE	Grade 0: complete paraplegia, no movement of any joints
	Grade 1: total of 1-3 points for joint movement
	Grade 2: total of 4-6 points for joint movement
	Grade 3: total of 7-9 points for joint movement
MODERATE	Grade 4: total of 10-12 points for joint movement
	Grade 5: capable of stance with help, ≥4 joints with slight movement
	Grade 6: capable of stance with help, ≥4 joints with extensive movement
	Grade 7: capable of spontaneous hindlimb weight support, ≥4 joints with slight movement
	Grade 8: capable of spontaneous hindlimb weight support, ≥4 joints with extensive movement
MILD	Grade 9: capable of stance with help, capable of 1-4 steps
	Grade 10: capable of stance with help, capable of ≥5 steps with no or occasional forelimb-hindlimb coordination
	Grade 11: capable of stance with help, capable of ≥5 steps with frequent forelimb-hindlimb coordination
	Grade 12: capable of standing up spontaneously on hindlimbs, capable of 0-4 steps
	Grade 13: capable of standing up spontaneously on hindlimbs, capable of ≥5 steps with no or occasional forelimb-hindlimb coordination
NORMAL	Grade 14: capable of standing up spontaneously on hindlimbs, capable of ≥5 steps with frequent forelimb-hindlimb coordination, not able to pass hindlimb clearance test
	Grade 15: capable of standing up spontaneously on hindlimbs, capable of ≥5 steps with frequent forelimb-hindlimb coordination, able to pass hindlimb clearance test

Fig. 2. 15-point Scoring system. A description of locomotor patterns attributed to each score ranging from complete paraplegia with no joint movement (0) to normal locomotion (15).



1.4. Scale Validation

The validity of the SLR scale was evaluated by its ability to capture a wide range of neurologic recovery. The ideal locomotor scale should be able to distinguish the full range of motor function, from complete paralysis, through individual movements around the hind-limb joints, weight support, standing, stepping, and coordination. Mild, moderate, and severe motor deficits should be able to be clearly described, as opposed to solely differentiating between ambulatory and non-ambulatory. Thus, scores should extend across the entire scale range.

The SLR scale was also tested for reliability using inter-rater reliability testing. After an initial training session reviewing the operational definitions, the behavioral categories, and subcategories to be assessed, all five raters reviewed videos for each animal. Any differences in scores were discussed and a group consensus score was reached for each animal. Next, all five reviewers, blinded to treatment and live clinical scores, independently watched 3 minute video segments of 6 animals with mild, moderate, and severe motor deficits. Each rater scored each video independently. Scores were compared for consistency, and the average score and standard deviation was calculated for each lamb video. Inter-rater reliability was measured by calculating the mean standard deviation for all scores and comparing average scores to consensus scores.

2. Results

2.1. Animal Outcomes

Only lambs surviving to term and born via spontaneous vaginal delivery ($n = 20$) were included in this analysis. The average gestational age at birth was 145.6 days (range: 133–153 days). All lambs survived for 24 hours. Immediately following delivery, newborn lambs often demonstrated weakness and lacked coordination; this improved significantly within 24 hours for lambs with mild neurologic deficits. One lamb became significantly weaker over the course of 24 hours. Twelve lambs required bottle-feeding due to an inability to nurse. One lamb sustained a tibial fracture associated with trauma during delivery that was discovered after euthanization. There were no other complications.

2.2. Scale Development

Motor function was scored using a 15-point rating system (Fig. 2). Group discussions were used to derive the final features used to describe the range of motor function exhibited by the cohort of observed animals. The scale assessed seven categories; these categories were ranked by order of clinical importance during recovery (Fig. 1). For example, extensive joint movement was scored higher than slight movement, and the ability to stand spontaneously was scored higher than standing with help. Scores were based on the best performance for each lamb. For most animals, best performance was demonstrated at the second time point, twenty-four hours after birth.

The first category assessed bilateral hindlimb movement. This category was sub-divided into assessments of movement at each joint for each limb: hip, knee, and ankle (Fig. 3). Joint movements were characterized as no movement, slight movement (defined as movement of the joint through less than half the range) or extensive (defined as movement of the joint through greater than or equal to half the range). Slight movements were awarded 1 point per joint, while extensive movements were given 2 points per joint for a maximum possible 12 points. Starting positions must be considered because a slight

Fig. 3. Lamb joint movements. Drawings of full range of motion for the lamb hindlimb (A) and open (left image) and closed (right image) position for each individual joint: hip (B), knee (C), and ankle (D) joints. Extensive movements were $\geq 50\%$ of the joint range of motion; slight movements were $< 50\%$ of the joint range of motion.

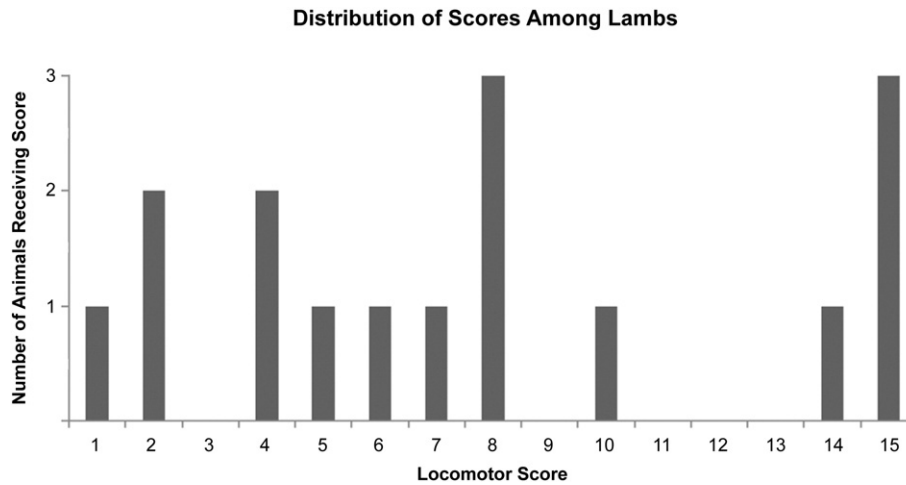


Fig. 4. Locomotion score distribution. Experimental lambs ($n = 16$) displayed an extensive range of locomotor recovery with a wide distribution of locomotion scores.

movement may result in a fully closed or opened joint if the initial position was partially bent [3].

The next behavioral categories (in order of progressive motor function) assessed the ability to stand with help, to demonstrate spontaneous hindlimb weight support, and to stand spontaneously. Standing with help was defined as the ability to stand unassisted for any length of time after initially being assisted by the experimenter. Spontaneous hindlimb weight support is defined as support of the body through the hindlimb as evidenced by the ability to lift the hips off the ground. Of note, an animal lifting its hips off the ground with the exclusive use of its forelimbs is not considered to be capable of hindlimb weight support.

Stepping, or the ability to ambulate, is the next category assessed. Lambs capable of standing (either with help or spontaneously) were evaluated for their ability to step. A step is defined by weight support, forward limb advancement, followed by re-establishment of weight support. The total number of steps taken was counted; steps do not need to be continuous to be counted. Lambs able to walk 5 or more steps were evaluated for the frequency of forelimb-hindlimb coordination and were scored as none, occasional (<50% of the time), or frequent ($\geq 50\%$ of the time). One forelimb advance for every hindlimb advance when moving at a constant speed is considered normal coordination.

Lastly, animals capable of spontaneous standing and ambulation ≥ 5 steps with frequent coordination were further discriminated by the hindlimb clearance test. To perform this test, animals were observed walking over a 4"×4" wooden bar in an open field as described by Navarro et al. [4]. Ability to walk over the bar qualifies as a pass. If the

animal is unable to clear the bar, the test is failed. Based on these observations, each lamb was scored using a 15-point rating system.

2.3. Scale Validation

Scale validity was evaluated by the ability to capture a wide range of neurologic recovery; a valid scale should utilize the entire breadth of the rating scale without clustering animals around certain scores. All normal lambs without a surgically created defect ($n = 4$) were capable of ambulation and demonstrated normal locomotion. However, in one lamb the clearance test was not performed (average clinical score of 14.75). The remaining sixteen lambs demonstrated a wide range of clinical scores ranging from 1 to 15 (Fig. 4). A score of 0–4 represented a severe motor deficit, a score of 5–9 was associated with a moderate deficit, a mild motor deficit was characterized by a score between 10 and 14, and normal locomotion was defined as a score of 15. Five lambs (31.2%) were categorized as having severe motor deficits, 6 as having moderate deficits (37.5%), and 5 as having mild or no deficits (31.2%), demonstrating a fairly even distribution of scores (Fig. 5).

The standard deviation of scores from all examiners was calculated to estimate inter-rater reliability. The mean standard deviation for all individuals was ± 0.431 , demonstrating the reliability of the scoring system among trained examiners. Furthermore, the average score for all raters was within 1 point of the consensus score 100% of the time.

3. Discussion

The MOMS trial showed exciting promise that disease outcomes in MMC can be improved with fetal intervention and ignited hope that research will be able to improve distal motor function in children born with MMC [1]. The standard large animal model used for such research is the fetal lamb model of MMC, which has been extensively characterized and shown to exhibit histologic and neurologic deficits comparable to those seen in congenital MMC in humans [2,5,6,8]. Despite decades of research using this model, a means of consistent functional assessment is lacking.

Several studies have reported performing thorough neurologic exams in the fetal sheep model, but the reported data focus on the incidence of paraplegia [9–13]. While the ultimate goal of MMC research may be to cure paralysis, the gradations of functional recovery are more complex and require more nuanced categorization than simply ambulatory versus non-ambulatory. Functional recovery should be analyzed and reported completely and consistently so that small, but important, improvements in distal neurologic function are not overlooked. Furthermore, the grading scale should be uniform in order to facilitate generalizability and data interpretation. This study proposes

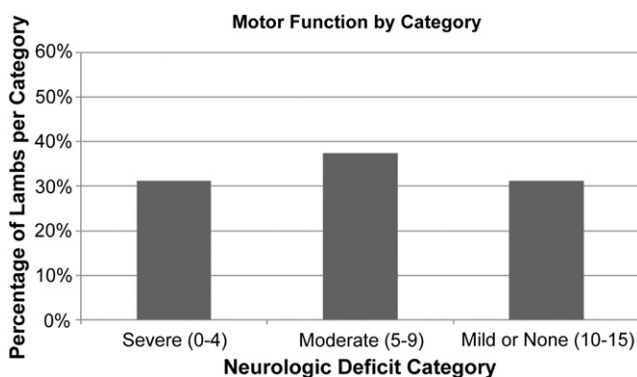


Fig. 5. Distribution of motor function deficit severity. Scores were classified as mild, moderate, or severe neurologic deficit. Five lambs (31.2%) were categorized as severe motor deficits, 6 as moderate (37.5%), and 5 as mild or no deficit (31.2%) for an even distribution of scores.

a standardized system for locomotor scoring for this disease model that is capable of capturing subtle differences in distal neurologic function.

The importance of a standardized, complete neurologic assessment is undeniable. Participants in the MOMS trial were evaluated using two standard systems for assessment of neurologic function- the Bayley and Peabody motor scales [1]. These scales facilitated the discovery that children repaired *in utero* were more likely to exhibit a level of function two or more levels higher than expected given the anatomy of their defect, were more likely to be able to walk without assistance, and had better parent-reported mobility. While the majority of these children were unable to ambulate, their neurologic improvements were still significant. Even small improvements in motor outcome may drastically reduce morbidity for children with MMC.

The rating scale described here provides a comprehensive analysis of motor function for lambs. The scale is reliable, accurate, capable of capturing a wide range of neurologic outcomes, and able to distinguish subtle differences among lambs. Furthermore, the entire assessment can be performed in a matter of minutes. In this study, no animals with scores of 11–13 were observed. While this is a large group for a study using large animals, the number may be too small to observe all scores. Additionally, it is possible that improvements in treatment efficacy will increase the number of animals in this mild deficit range.

Prior research in the model has demonstrated various degrees of histologic improvement with different experimental treatments [2,14]. While spinal cord preservation is an important component of disease treatment, the ultimate goal is improved neurologic function regardless of the appearance of the spinal cord. For this reason, motor outcome was the major focus of this study. An area of future investigation will be to correlate histologic appearance with functional outcomes.

In conclusion, this study has further characterized the established fetal sheep model of MMC by developing a reliable clinical assessment tool for distal motor function. The SLR scale can capture subtle differences in locomotor function and has minimal inter-rater variability. The neurologic deficits seen in the lambs in this study are similar to those previously described; however, the SLR scale provides a more complete assessment of locomotor function. A complete, standardized grading system for neurologic outcomes in the fetal sheep model is

not only feasible (as demonstrated here), but is critical to advancing research toward the ultimate goal of curing paralysis in MMC.

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