Neurological Outcomes after Human Umbilical Cord Patch for In Utero Spina Bifida Repair in a Sheep Model

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Abstract

Objectives The objective of our study was to test the hypothesis that in utero repair of surgically created spina bifida in a sheep model using cryopreserved human umbilical cord (HUC) patch improves neurological outcome.

Methods Spina bifida with myelotomy was surgically created in timed pregnant ewes at gestational day (GD) 75. The fetuses were randomly assigned to unrepaired versus HUC and treated at GD 95 and then delivered at GD 140. Neurological evaluation was performed using the Texas Spinal Cord Injury Scale (TSCIS), bladder control using ultrasound, and the hindbrain herniation.

Results Three lambs without the spina bifida creation served as controls. There were four lambs with spina bifida: two were unrepaired and two underwent HUC repair. The control lambs had normal function. Both unrepaired lambs had nonhealed skin lesions with leakage of cerebrospinal fluid, a 0/20 TSCIS score, no bladder control, and the hindbrain herniation. In contrast, both HUC lambs had a completely healed skin defect and survived to day 2 of life, a 3/20 and 4/20 TSCIS score (nociception), partial bladder control, and normal hindbrain anatomy.

Conclusions Cryopreserved HUC patch appears to improve survival and neurological outcome in this severe form of the ovine model of spina bifida.

Keywords
- neural tube defect
- umbilical cord
- regenerative healing
- sheep spina bifida model
- spina bifida repair

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In utero spina bifida repair at midgestation has proven to decrease the morbidity and mortality compared with the postnatal repair.[1] The primary goals of in utero spina bifida repair are to create a barrier against continuous exposure to the amniotic fluid to the spinal cord and to prevent leakage of cerebral spinal fluid, and thus preserve spinal cord function and prevent Chiari II malformation.[2] Despite this, 58% of children who underwent primary in utero closure were unable to ambulate at 30 months of age, and 8% of children needed a surgical release of a tethered cord before 12 months of age.[1] This lack of improvement has been attributed to the scar formation leading to spinal cord tethering at the repair site,[3] which is associated with long-term neurological complications requiring multiple surgeries in later life.[4,5]

It remains unclear whether it is beneficial to perform the intrauterine repair with a patch system to create not only a watertight barrier but to reduce scar formation and inflammation. Both of these mechanisms may prevent damage to the spinal cord.[6] Recently, we have reported that cryopreserved human umbilical cord (HUC) provides a watertight barrier and helps regenerate the skin defect, preserves spinal cord anatomy, and prevents hindbrain herniation in a sheep model of surgically created spina bifida.[7] The rationale of adopting HUC as a patch is based on the promising clinical outcome of cryopreserved amniotic membrane used in cornea, skin, and for the repair of tendon and ligaments[8] to deliver anti-inflammatory and antiscarring effects.[9–11] HUC, like an amniotic membrane, also exert similar therapeutic actions but is thicker when flattened as a sheet.[12] Herein, we provide additional evidence to support the notion that the aforementioned histological healing results[7] are correlated with clinical benefit in the preservation of neurological functions in a sheep surgical model of spina bifida. The study was performed as a pilot study to demonstrate the feasibility of using the HUC patch to study the neurological outcomes in a myelotomy spina bifida model.

Methods

The study protocol was approved by the Institutional Animal Care and Use Committee at the University of Texas McGovern Medical at Houston (protocol AWC-12–007). All animal care was in compliance with the Guide for the Care and Use of Laboratory Animals.

Creation of Spina Bifida Model

Timed-pregnant sheep with twin or triplet gestations verified by ultrasound were obtained from K-Bar Livestock, L.L.C. (Bastrop, TX) for the creation of the sheep model of spina bifida as previously reported.[13,14] Briefly, the first surgery was performed on gestational day (GD) 75 (term: 145 days) under general anesthesia with the animal placed in a supine position with left lateral tilt. Under sterile conditions, a laparotomy was performed using a midline incision followed by hysterotomy to expose the lower fetal lumbar/sacral spine. As described previously,[7,13,14] the spina bifida defect was surgically created by removing the skin to create a defect of 4 cm $\times$ 4 cm. The paraspinous muscles and posterior lamina of the vertebra at 4 to 5 levels between L3 and L6 vertebral levels were also removed to expose the spinal cord. Myelotomy was performed by incising the meningeal coverings of the spinal cord using an 18-gauge needle and the central canal at the midline of the spinal cord was entered to allow the egress cerebrospinal fluid (CSF). After the fetus was repositioned back into the amniotic cavity, the uterine incision was closed in two layers using 2–0 Vicryl suture (Ethicon Inc., Somerville, NJ). The procedure was then repeated on the second fetus in the remaining uterine horn. After completion of the procedure, the uterus was placed back into the abdomen and the skin and fascial incisions were closed. The fetuses that did not undergo spina bifida creation served as controls.

Repair of the Spina Bifida Defect

The fetuses that survived to the repair phase after the creation of the defect were randomly assigned to the unrepaired group versus repair using HUC. The fetuses assigned to HUC repair, underwent this procedure on GD 95, that is, approximately 3 weeks after the initial surgery. HUC (AmnioGuard; TissueTech Inc., Miami, FL) was processed from donated full-term human placentas after cesarean delivery recovered in compliance with American Association of Tissue Banks and the good tissue practices set forth by the Federal Drug Administration. After removing the umbilical vessels, the umbilical cord was flattened to create a patch of various sizes. The HUC patch was sutured to the skin edge of the spina bifida defect in the fetus using 4–0 Monocryl (Ethicon Inc.) in a continuous running locking fashion. The unrepaired fetuses were managed expectantly.

Delivery by Cesarean Section and Neonatal Care

All fetuses were delivered at GD 139 to 142 days by planned laparotomy and hysterotomy under general anesthesia or spinal anesthesia with sedation. After delivery of the fetus, the cord was clamped and cut. The fetuses were transitioned to the room air by stimulation and drying. The lambs were kept alive for 2 days after delivery for neurological assessment. The ewe was euthanized immediately after cesarean delivery.

Clinical Outcome Assessment

The clinical assessment of the hind limbs function was performed by videotaping the examination. The masking of treatment assignment of each lamb was performed by covering the lower spine of the lamb with a bandage of 10 cm $\times$ 10 cm. Three independent examiners, blinded to the treatment assignment of lambs, reviewed the videos to evaluate neurological outcomes using Texas Spinal Cord Injury Scale (TSCIS).[15]

Neurological Assessments

Neurological assessments were first performed by standard clinical neurological examination techniques using the TSCIS (►**Table 1**). This scale allows for a combined score gait, proprioceptive positioning (knuckling) and nociception up to 10 points per limb. A maximum overall score of 40 can be assessed for all four limbs.[15,16] The gait was assessed by tail walking (holding the lamb upright by the tail or using a sling near the lower spine area) if the lamb is not able or unwilling to voluntarily ambulate. Scores of 0 to 6 are assigned based on the presence and clinical

Table 1 Texas spinal cord injury score

Gait[a]
0 = No voluntary movement seen when supported
1 = Intact limb protraction with no ground clearance
2 = Intact limb protraction with inconsistent ground clearance
3 = Intact limb protraction with consistent ground clearance (> 75%)
4 = Ambulatory, consistent ground clearance with moderate paresis–ataxia (will fall occasionally)
5 = Ambulatory, consistent ground clearance with mild paresis–ataxia (does not fall, even on slick surfaces)
6 = Normal gait
Proprioceptive positioning[b]
0 = Absent response
1 = Delayed response
2 = Normal response
Nociception[c]
0 = No deep nociception
1 = Intact deep nociception, no superficial nociception
2 = Nociception present

[a]Ground clearance refers to the ability to lift the limb off of the ground when it is being protracted.
[b]Proprioceptive positioning is performed by supporting the lamb's weight and gently placing the dorsum of the hoof on the ground. A delayed response is indicated by a greater than 2 seconds lag between hoof placement and correction.
[c]Deep nociception is measured by cross-clamping the distal limb with hemostats. Superficial nociception is tested by pinching the interhoof webbing with hemostats.

significance of movement, for example, limb protraction. Proprioceptive positioning (also referred to as knuckling) was measured by a postural reaction test by placing the dorsum of the manus or pes on a nonsticky surface while the lamb's ventrum was supported with one hand. It was scored as normal (score = 2) if they were able to correct the limb immediately. Lambs that replaced the hoof, but took a prolonged period of time to do so (> 2 seconds or had difficulty doing so were referred to as delayed (score = 1). An absent response was scored as zero. Nociception was assessed by applying a painful stimulus to the limb and observing the lamb for physiological retraction or behavioral (orientation toward the stimulus, vocalization, licking) responses.[16,17] Superficial nociception (soft tissue pain) was tested by applying a hemostat to the interdigital webbing.[18] If no superficial nociception was detected, deep nociception (bone or joint pain) was evaluated by cross-clamping a nail bed, digit, or the distal limb with a hemostat.[18] Lambs were scored as having normal nociception (score = 2), no superficial nociception (score = 1), and no deep and superficial nociception (score = 0). The TSCIS was scored twice at 3 and 6 hours after birth on day 1, and was scored on day 2 in the morning at 10 AM.

Bladder Assessment

Bladder volumes were measured using ultrasound two-dimensional (2D) grayscale images in three dimensions.[19] The bladder volume was calculated using the volume for an ellipsoid (volume = $4/3 \times \pi abc$, where a, b, and c are the radius dimensions of the ellipsoid).[19] The bladder volume was serially measured every 5 minutes during and immediately after feeding until spontaneous voiding occurred. The maximum dimensions were used as the prevoid bladder volume, while the dimensions measured immediately after voiding was used as the postvoid volume. If there was constant leakage of urine without spontaneous voiding, volumes at 1-hour intervals were chosen to calculate the pre/postvoid volume ratios.

Euthanasia and Harvest of Tissues

After the neurological assessment, the lambs were intubated and general anesthesia was administered. Euthanasia was performed by exsanguination under anesthesia. Following thoracotomy pericardial sac was entered and the left ventricle was catheterized to infuse1,000 units of intravenous heparin administered into the circulation. Subsequently, the right atrium was incised to allow for bleeding. Through the left ventricular cannula, 10% normal buffered formalin (NBF) was infused until the bleeding through the right atrium was clear. The head and neck were separated from the remaining body and the defect site was excised fixed with 3 cm margin of tissue. The tissues were further fixed in 10% NBF.

Magnetic Resonance Imaging of Lamb Heads

The neuroanatomy of the calvarium and upper cervical spine was assessed after magnetic resonance imaging (MRI) using a 7T/30 USR MRI scanner (Bruker BioSpin; Karlsruhe, Germany) with a water-cooled gradient coil system (Model BGA 20 S2; 30 cm i.d.). The transmission and reception were based on the vendor-supplied birdcage resonator with 155 mm i.d. using ParaVision (PV 5.1) as the scanner's operating system. A pilot scan was used to place the head in the center of the magnet.

Then the images were acquired by rapid acquisition and relaxation enhancement (RARE)[20] with the following specifications: Effective echo time 36 ms, repetition time 9 seconds, RARE factor 6, number of averages 9, total scan time 2 hours, field of view 70 mm × 70 mm, matrix 233 × 233, spatial resolution 0.3 mm, and slice thickness 1 mm (total of 70 slices). Images were obtained in sagittal and coronal orientations including fat suppression and saturation slices. Images were exported into digital imaging and communications in medicine format and were analyzed using Ositix HD (Pixmeo SARL, Geneva, Switzerland) by a pediatric neuroradiologist (S. P. P) who was blinded to the assignment of the laboratory for qualitative assessment of the characteristic findings of hindbrain malformation.

The data are presented as descriptive statistics. The interrater agreement for each component of the TSCIS scores each hind limb was performed using kappa statistics between the examiners. The bladder volumes are presented as a mean and standard deviation. Inferential statistics was performed to compare the bladder volumes using two-way analysis of variance (ANOVA) with posthoc analysis. Prism 6 (GraphPad Software, Inc., La Jolla, CA) was used for analysis and graphs. A p value < 0.05 was considered as significant.

Results

There were a total of seven lambs included in the study. Four lambs that had the creation of the defect survived to the repair stage: two underwent HUC repair and two were left unrepaired. There were a total of three lambs in the study that served as controls without the spina bifida.

The details of fetuses with respective sheep and their allotment are listed in ►**Table 2**. Sheep 1 had a triplet gestation, of which two fetuses underwent spina bifida creation survived to repair stage. These were randomly assigned to unrepaired and repair with HUC. The remaining fetus without the spina bifida creation served as an internal control. All three fetuses survived to delivery. In sheep 2, there was a singleton fetus that underwent spina bifida creation and survived to the repair stage, which was randomly assigned to the unrepaired group. Sheep 3 had a triplet gestation and two of these fetuses underwent creation of the defect. Only one of the two fetuses with spina bifida survived to the repair stage. This fetus was randomly assigned to HUC repair. The other fetus without spina bifida served as internal control. Both of these fetuses survived to delivery. Sheep 4 had one fetus without spina bifida, which was delivered at term and served as control.

Outcomes at Delivery

The findings of the defect site including the histology of the lambs with spina bifida have been presented in our recent publication.[7] Among the unrepaired group, both lambs transitioned to the room air with drying and stimulation. One of the lambs had leakage of CSF from the nonhealed defect site measuring 20 mm × 10 mm. At 3 hours after birth, the lamb developed irregular heart rate, apnea, and hypothermia and

Table 2 Clinical characteristics of included lambs that survived to delivery

Sheep no.	Fetus	Group	Defect size at harvest (mm: height × width)	Sex	Texas spinal cord injury scale Hind limbs only								Bladder control Pre/postvoid residual volume ratio (%)	MRI Hindbrain herniation
					Gait (Max = 6 per limb)		Proprioception (Max = 2 per limb)		Nociception (Max = 2 per limb)		Combined hind limb score			
					Right	Left	Right	Left	Right	Left				
1	1	Unrepaired[a]	10 mm × 20 mm	Female	NA	NA	NA	NA	NA	NA	NA		100%	Yes
	2	HUC	Completely healed	Female	0	0	0	0	2	2	4		45%	No
	3	Control	NA	Female	6	6	2	2	2	2	20		3%	No
2	1	Unrepaired	40 mm × 4 mm	Female	0	0	0	0	0	0	0		100%	Yes
3	1	HUC	Completely healed	Female	0	0	0	0	1	2	3		32%	No
	2	Control	NA	Male	6	6	2	2	2	2	20		2%	No
4	1	Control	NA	Female	6	6	2	2	2	2	20		5%	No

Abbreviations: MRI, magnetic resonance imaging; NA, not applicable; HUC, cryopreserved human umbilical cord.
[a]This uncovered lamb demised 3 hours after birth from cardiorespiratory failure (irregular breathing), lethargy, and irregular pulse rate, and temperature irregularity. The MRI of the head showed severe hindbrain herniation with complete absence of cerebrospinal fluid in the calvarium.

expired. Resuscitative efforts were performed for 30 minutes. The other unrepaired spina bifida lamb had a skin defect also remained nonhealed and measured 40 mm × 4 mm, with leakage of fluid. In contrast, the skin defect of one lamb in the HUC group was completely healed with regeneration of hair and keratinization, while that of the other HUC lamb was also healed but without keratinization and hair growth. Furthermore, compared with the normal controls (►**Fig. 1A**), unrepaired lambs had severe contractures bilaterally of the hip, knee, and ankle joints with knee joints positioned against the abdominal wall (►**Fig. 1B**). On the elevation of the lower spine, the lower extremities lifted off the ground. In contrast, there were minimal contractures of all three joints of the both lower extremities in the repaired lambs (►**Fig. 1C**).

Texas Spinal Cord Injury Scale

The normal control lambs were assigned the maximal combined score of 20 for both limbs on day 2. The forelimbs assessment in all lambs was normal for gait, proprioception, and nociception. The hind limbs scores for all lambs are described in ►**Table 2**. In the unrepaired lambs, TSCIS scores were assessed in the only surviving lamb and the scores were 0 for both hind limbs. In contrast, one of the lambs repaired with HUC responded to superficial stimulation with a withdrawal response in both hind limbs. In the other HUC lamb, one hind limb showed a response to superficial painful stimuli while the other hind limb showed a response to deep stimulation only. The proprioceptive response was for the hind limbs 0 in both HUC-repaired lambs. The reproducibility of the TSCIS score was performed for 36 individual limb assessments from 6 lambs for each examiner: 12 for gait, 12 for proprioception, and 12 for nociception. The agreement between three examiners was 94%, which was statistically significant with a $p < 0.001$ (Kappa statistic).

Bladder Function Assessment

For the normal controls, the prevoid bladder volume was 10.4 ± 3.6 mL (range: 7.8–14.5 mL, $n = 3$) while the postvoid residual volume measured within 5 minutes of voiding was 0.38 ± 0.13 mL (range: 0.25–0.5 mL), yielding a ratio between postvoid residual volume to prevoid volume of 3.3 ± 1.5% (range: 2–5%). The frequency of urination was every 20 to 45 minutes on day 1, and every 2 to 3 hours on day 2, usually after a feeding episode. For both unrepaired lambs, there was a constant trickle of urine suggesting overflow continence immediately after delivery with a mean bladder volume measurement of 7.25 ± 2.5 mL (range: 5.5–9 mL). We were able to obtain only one measurement of 9 mL in the fetus that demised 3 hours after delivery. The other unrepaired spina bifida lamb had residual bladder volumes of 5.5 mL, with no evidence of spontaneous voiding. For the lambs repaired with HUC, there was a spontaneous voiding at a frequency similar to normal control lambs. The prevoid volumes were 5 and 12 mL, with a mean of 8.55 ± 4.6 mL ($n = 2$) and the postvoid residual volumes were 2.4 and 3.8 mL, with a mean of 3.1 ± 0.99 mL yielding a ratio between postvoid residual volume to prevoid volume of 32 to 45%, respectively. The difference in bladder volume between the groups was statistically significant (►**Fig. 2**). Two-way ANOVA; a p-value < 0.001; posthoc analysis significant difference between control versus unrepaired $p < 0.001$, unrepaired versus HUC repaired $p = 0.01$, and control versus HUC repaired $p < 0.01$.

Head Magnetic Resonance Imaging Findings

All three control lambs had normal intracranial anatomy with the presence of CSF around the cerebellum and extracerebral space (►**Fig. 3 A, D**, and **G**). Both unrepaired lambs exhibited a hindbrain herniation with the cerebellar tonsils below the level of the foramen magnum. In addition, there was a decreased or complete absence of CSF in the intracranial space, resulting in overcrowding of the posterior fossa and invisible lateral ventricles (►**Fig. 3B, E**, and **H**). In contrast, the HUC-repaired lambs had intracranial anatomy similar to normal controls with a normal hindbrain and the normal appearing lateral ventricles (►**Fig. 3 C, F**, and **I**).

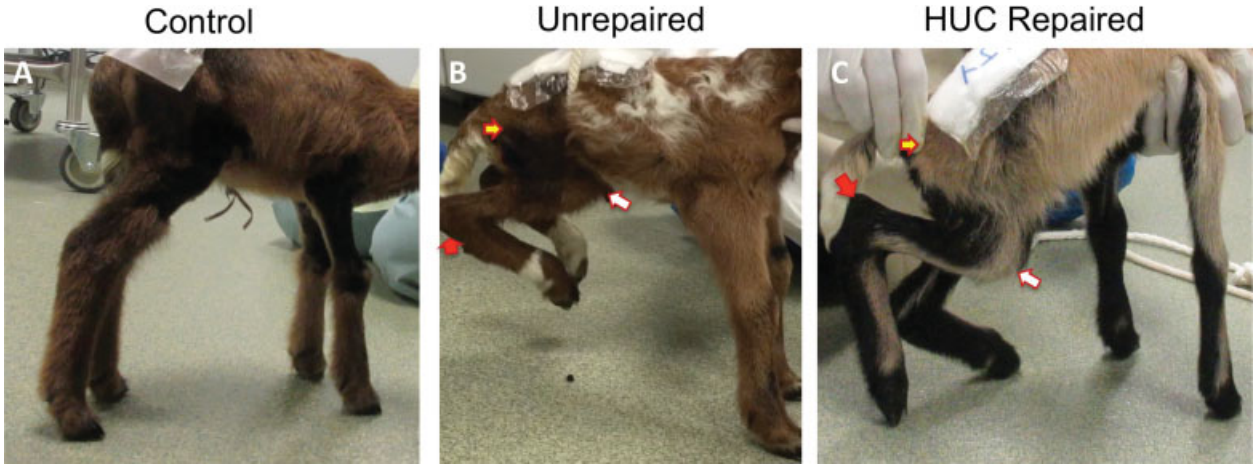

Fig. 1 Comparing the hind limbs posture in a standing position. (**A**) Control lamb with all four limbs on the floor and extended position. (**B**) Unrepaired spina bifida lamb with lower spine elevated using a sling showing severe contractures. The hip joint (yellow arrow), knee joint (white arrow), and ankle joint (red arrow) (all three) had severe contractures. (**C**) HUC-repaired spina bifida lambs had minimal contractures at hip joint (yellow arrow), knee joint (white arrow), and ankle joint (red arrow). HUC, human umbilical cord.

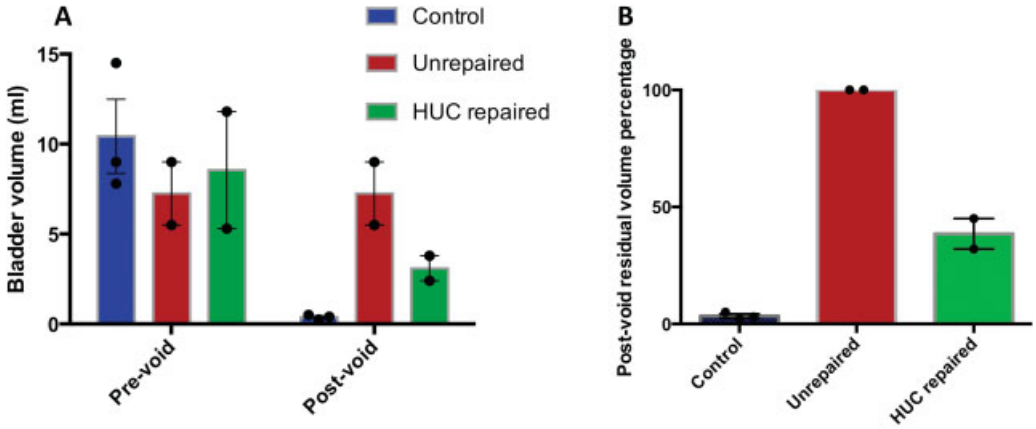

Fig. 2 Graphical representation of the prevoid and postvoid bladder volumes. (**A**) Absolute volumes as measured using a 2D ultrasound and using volume of an ellipsoid formula. There was no difference in the unrepaired spina bifida lambs. (**B**) Postvoid residual volume percentage measured as differences between prevoid/postvoid bladder volumes. 2D, two-dimensional.

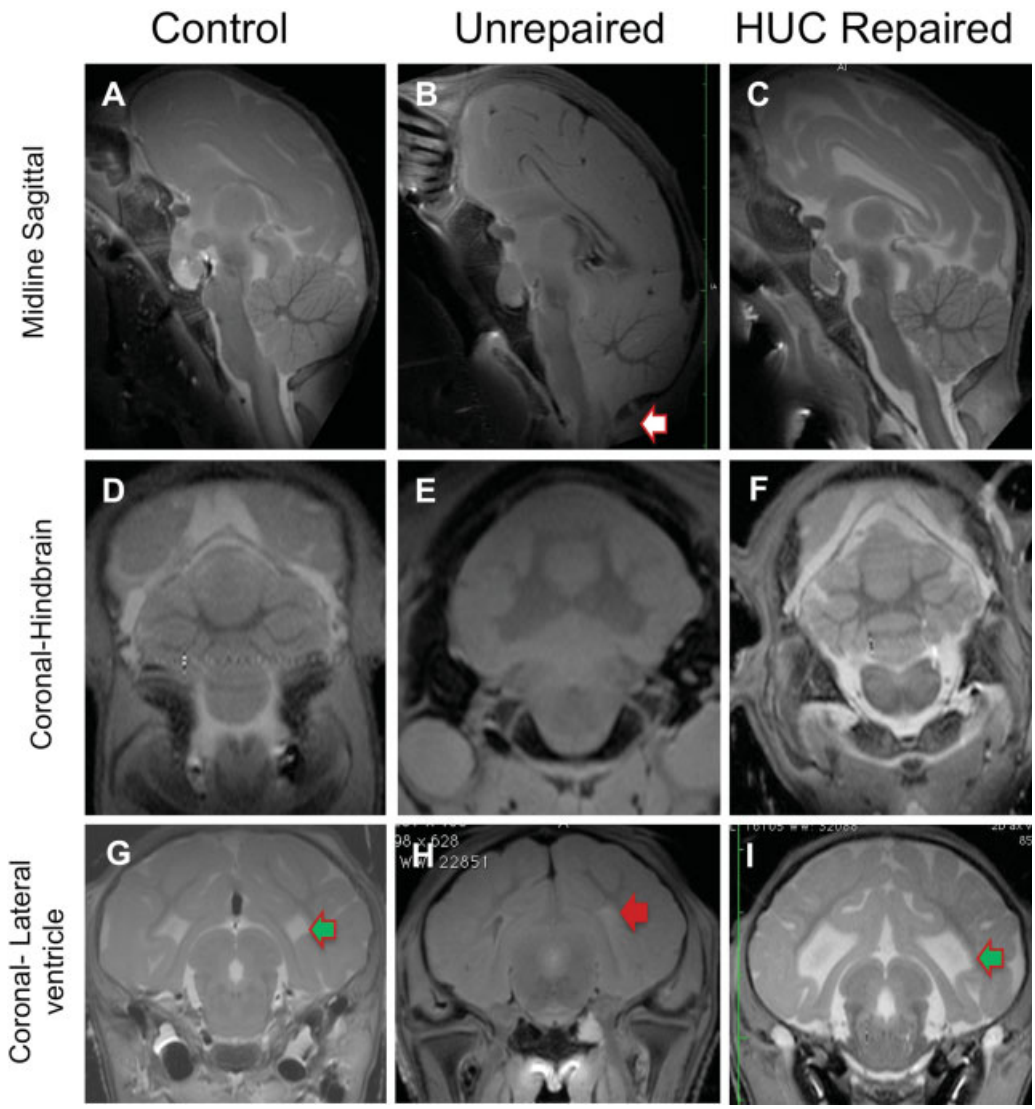

Fig. 3 Comparison of head MRI between control, unrepaired, and HUC-repaired groups. (**A–C**): Midline sagittal views, (**D–F**): coronal view of hindbrain, and (**G–I**): coronal view at lateral ventricles. Controls showed normal cerebral spinal fluid around the cerebrum and in the lateral ventricles (green arrow in the ►**Fig. 3G**) and in the posterior fossa. The cerebellum was above the level of foramen. In the unrepaired group, there was a complete lack of cerebrospinal fluid around the brain tissue and in the lateral ventricles (red arrow in ►**Fig. 3H**). The cerebellar tonsil was herniated through the foramen magnum (white arrow in ►**Fig. 3B**). The HUC repaired spina bifida lambs intracranial anatomy was comparable to the controls and there was normal cerebrospinal fluid and in the lateral ventricles (green arrow in ►**Fig. 3I**). HUC, human umbilical cord.

Discussion

The major finding of our study was that the HUC patch prevents hindbrain herniation and preserves partial bladder and partial sensory motor function in a myelotomy spina bifida animal model. The hindbrain herniation findings in the current study noted on MRI are similar to the findings seen with midline sagittal sections of the hindbrain in our recent investigation.[7] Cumulatively, including our previous publication, 8/8 (100%) lambs with surgically created spina bifida that then underwent HUC repair demonstrated the absence of hindbrain herniation.[7] In contrast, we noted lack or absence of CSF in the lateral ventricles and around the brain in the unrepaired lambs. The unrepaired spina bifida lamb that demised 3 hours after delivery had the most severe hindbrain herniation along with the complete absence of CSF in the calvarium. The lack of ventriculomegaly despite hindbrain herniation has been previously noted by other researchers in the sheep model of spina bifida.[21] However, in human, the spina bifida is associated with enlargement of lateral ventricles.[22] The explanation for the difference remains unknown.

In our study, we followed the method first reported by Meuli et al[13] to surgically create a spina bifida lesion at midgestation in sheep. In addition, similar to what has been advised by Paek et al[23] and Bouchard et al,[21] we created a myelotomy to allow egress of the CSF to cause an hindbrain herniation. Finally, we performed the surgical repair 3 weeks after the initial creation to allow sufficient exposure of the spinal cord to the amniotic fluid. Furthermore, the uncovered defects were randomly assigned to repair or no repair to reduce the assignment bias. Furthermore, in one of our ewes, there were all three interventions: control, unrepaired spina bifida, and HUC-repaired spina bifida. This served as an internal validity of our findings.

This is the first study to use the TSCIS scale to quantify sensory and motor function in a spina bifida animal model. The scale has been validated for four-legged animals, such as dogs with spinal cord injury.[15,24] This scale incorporates proprioception and nociception in addition to gait. Brown et al[25] used a locomotor scale in 20 lambs, of which 15 had a surgically created spina bifida, and showed reproducibility to quantify hind limb motor function. However, the locomotor scale does not incorporate proprioception and nociception pathways, which are important parts of spinal cord function. In our study, the lack of complete recovery in the TSCIS was secondary to the inability for the spinal cord to regenerate after myelotomy where the spinal cord is intentionally damaged during the surgical creation of the spina bifida. This phenomenon has been well observed in spinal cord injury in the mammalian animals.[26,27] The relative preservation of the nociception reflexes despite a complete lack of locomotor function and proprioception was unexpected. The pain fiber pathway in the spinal cord could explain the findings. Pain fibers travel into the spinal cord, crosses to the opposite side in front of the central canal at the same level and ascend through the lateral and anterior spinothalamic tracts.[28] During the myelotomy, which is performed posteriorly in the midline of the spinal cord, the lateral and anterior parts of the spinal cord are untouched. The sensory and proprioception pathways ascend on the same side of the spinal cord through medial lemniscuses of the posterior column, which are damaged during the creation of the defect.

Spina bifida affects bladder function due to inadequate bladder wall development and thus leads to poor bladder filling and increased postvoid residual volume[29] causing lifelong morbidity in more than 90% of children.[30,31] Both of these manifest with increased postvoid residual volumes requiring intermittent bladder catheterization.[32,33] In this study, we evaluated postvoid residual volumes in the lambs using 2D ultrasound. The repair of the spina bifida reduced the postvoid residual volume by 50% compared with a lack of spontaneous voiding in the unrepaired lambs. The improvement noted in the urinary function could be secondary to preservation spinal cord tissue in the repaired lambs at the S2–S4 level where the micturition reflex occurs, which is below the site of the spina bifida level of L2–L6.[7,34] However, in the unrepaired, the lower spinal cord may be further damaged due to continued exposure to the amniotic fluid.

We had anticipated a higher degree of spinal cord function preservation in the HUC repaired lambs due to its known anti-inflammatory, antiscarring, and regenerative properties. This hypothesis was based on the identifying heavy chain hyaluronic acid (HC-HA)/pentraxin 3 (PTX3) as the relevant tissue characteristic from amniotic membrane, similar biological composition, and properties as HUC, responsible for the aforementioned actions. HC-HA/PTX3 is formed by the tight association between PTX3 and HC-HA complex, which consists of high molecular weight HA covalently linked to the heavy chain 1 of inter-α-trypsin inhibitor through the catalytic action of tumor necrosis factor-stimulated gene-6.[35–39] We have gathered strong data to support the notion that HC-HA/PTX3 is a novel matrix responsible for the anti-inflammatory, antirejection, and antiscarring actions[35–39] clinically observed in the surgical procedure of amniotic membrane transplantation for treating many ocular surface diseases.[10,11,40] Our recent data suggest that this HC-HA/PTX3 complex is more abundantly present in the HUC[12] and can directly modulate quiescence of epithelial stem cells.[41] Lack of regeneration of the damaged part of the spinal cord in the HUC needs to be further investigated.

Future studies should test the HUC patch repair compared with conventional repair with myelotomy. In addition, further testing of HUC patch is required in a functional spina bifida model without myelotomy.[13,42] Incorporation of a combination of the TSCIS and locomotor scales should be considered in future studies. If confirmed in humans, HUC may be a promising biomaterial to promote regenerative wound healing for the correction of fetal spina bifida and other developmental abnormalities. The utility of such a patch system can be further expanded if it can be delivered via a minimally invasive approach that would negate the maternal risks associated with laparotomy and hysterotomy.

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Scheffer C. G .Tseng, MD, PhD and his family members are more than 5% shareholders of TissueTech, Inc., Miami, FL, which procures and processes human placenta into

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