In Utero Repair of Experimental Myelomeningocele Saves Neurological Function at Birth

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● In a previous series of fetal sheep experiments, the authors demonstrated that midgestational exposure of the normal spinal cord to the amniotic space leads to a myelomeningocele (MMC) at birth that closely resembles human MMC phenotypes in terms of morphology and functional deficit. The present study tested whether delayed in utero repair of such evolving experimental MMC lesions spares neurological function. In 12 sheep fetuses, a spina bifida–type lesion with exposure of the lumbar spinal cord was created at 75 days' gestation (full term, 150 days). Four weeks later, the developing MMC lesions were repaired in utero for seven fetuses (five fetuses died before this time). Of those that had repair, three were delivered near term by cesarean section, and four died in utero or were aborted. All survivors had healed skin wounds and near-normal neurological function. Despite mild paraparesis, they were able to stand, walk, and perform demanding motor tests. Sensory function of the hindlimbs was present clinically and confirmed electrophysiologically. No signs of incontinence were detected. Histologically, the exposed and then covered spinal cord showed significant deformation, but the anatomic hallmarks as well as the cytoarchitecture of the spinal cord essentially were preserved. These findings show that timely in utero repair of developing experimental MMC stops the otherwise ongoing process of spinal cord destruction and "rescues" neurological function by the time of birth. Because there is evidence that a similar secondary damage to the exposed neural tissue also occurs in human MMC, we propose that in utero repair of selected human fetuses might reduce the neurological disaster commonly encountered after birth.

INDEX WORDS: Myelomeningocele, spina bifida, neural tube defect, spinal dysraphism, fetal surgery.

MYELOMENINGOCELE (MMC) is one of the most common and devastating congenital malformations in humans.[1,2] The related disabilities include sensorimotor paraplegia or severe paresis, urine and stool incontinence, vesicoureteric reflux, sexual dysfunction, and a variety of skeletal deformations. Most patients have hydrocephalus as well as parenchymal brain anomalies, and many children exhibit impaired intelligence and cognitive development despite optimal care.[2-5] Currently, there is no therapy that significantly improves the neurological deficit after the baby is born.

The etiology of MMC is unknown and probably multifactorial.[6-8] The most widely accepted pathogenesis is a disorder of neurulation during the embryonic development of the spinal cord.[7,9] However, a recent study performed in human fetuses with MMC provided evidence that the primary malformation may be mesodermal, not neural. The hypothesis is that failed closure of the posterior vertebral column leaves the spinal cord openly exposed to the amniotic space, and mechanical and/or chemical factors cause progressive alterations in utero, which lead to MMC at birth (Hutchins, unpublished data).[10,11]

In a previous series of experiments, we tested this hypothesis in fetal sheep and demonstrated that midgestational exposure of the normal lumbar spinal cord to the intrauterine environment leads to MMC-type pathology at birth, which shares many of the morphological and functional characteristics of human MMC.[12] These findings strongly support the theory that some forms of human MMC could be exclusively attributable to secondary damage to a primarily normal or near-normal spinal cord. Yet it is important to state that the in utero acquired injury to the openly exposed neural tissue is likely to be a main factor causing the massive loss of neurological function in all forms of MMC, regardless of the pathogenesis that leads to exposure of the neural tissue. Moreover, our first study prompted the hypothesis that prenatal coverage of developing MMC lesions would stop the progressive neural tissue destruction and improve the neurological outcome at birth. The aim of the present study was to test whether in utero repair of evolving experimental MMC spares neurological function at birth.

MATERIALS AND METHODS

Fetal Sheep Surgery

For surgical procedures (creation of spina bifida–type lesions, in utero repair, and cesarean section) we used standard techniques,

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previously described in detail.[13] Briefly, the ewe underwent general halothane/oxygen anesthesia, the uterus was exteriorized through an infraumbilical midline laparotomy, and the fetus was exposed via hysterotomy. After the fetal surgical procedure, the fetus was returned to the uterus, and amniotic fluid volume was restored with warm sterile saline. The hysterotomy was closed with a TA-90 stapler (US Surgical Corp, Norwalk, CT), the maternal laparotomy was closed in layers, and the ewe was returned to her stall. Pregnancy was continued until near term, and serial ultrasound examinations were performed to determine fetal viability.

Creation of Spina Bifida–Type Lesion

In 12 fetal lambs, at 75 days' gestation (full term, 150 days) a spina bifida–type lesion was created surgically, according to the recently reported technique.[12] Briefly, the normal and undamaged spinal cord (between L-1 and L-4) was directly exposed to the amniotic space by excising the skin, paraspinal musculature, posterior vertebral column (ie, laminectomy), and dorsal portion of the dura (Fig 1).

Repair of MMC Lesion

The repair operation was performed at 100 days' gestation, using a latissimus dorsi flap (Fig 2). The technical details are described elsewhere.[14] Essentially, the muscle was dissected through a flank incision between the scapula and iliac crest. The humeral origin, the proximal vascular pedicle, and the paraspinal insertions were transected, leaving the muscle attached to the posterior iliac crest and the distal vascular pedicle. The skin around the lesion was mobilized and a subcutaneous tunnel created from the flank incision to the back lesion. The muscle flap was flipped over, pulled through the tunnel, and placed over the lesion, which otherwise was not manipulated. The flap was sutured to the tissue adjacent to the MMC. The skin over the back wound and the flank incision was sutured closed.

Clinical Evaluation

The lambs were delivered by cesarean section at 145 days' gestation (full term, 150 days). The newborns were assessed clinically on days 1, 2, and 3 of life by the same veterinarian according to a standardized protocol.[15] During each examination, the general health status, mental status, and basic neurological function were assessed. The latter included cranial nerves, spinal reflexes, postural reactions, gait, and posture. Pain perception of the forelimbs, hindlimbs, face, and rump was assessed by needle prick (superficial pain) and by pinching with a hemostat (deep pain). The animal was observed for micturition and defecation during the entire study period. The examinations were videotaped to enable comparison with observations collected previously for unrepaired MMC lambs and normal control lambs.[12]

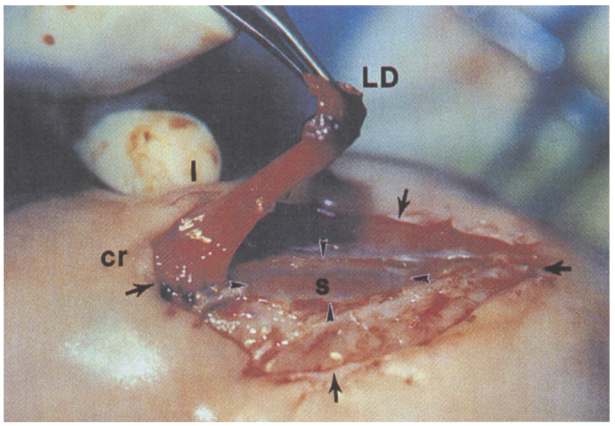

Fig 2. Intraoperative view of repair. The left latissimus dorsi muscle flap (LD) is placed over the evolving MMC lesion (arrowheads; s, spinal cord). The skin has been mobilized (arrows). cr, cranial, l, left side of the fetus.

Recording of Somatosensory-Evoked Potentials (SEP)

SEP represents the response of the somatosensory cortex to stimulation of peripheral nerves and requires intact neural conduction through the dorsal column pathway of the spinal cord.[16] To conclusively demonstrate sensory transmission to the brain, cortical SEP to bilateral fore- and hindlimb stimulation were recorded. On postnatal day 3, the lambs underwent general anesthesia with intravenous Diprivan (Stuart Pharmaceuticals, Wilmington, DE). Cortical SEP were recorded to bilateral stimulation of both posterior tibial and ulnar nerves with techniques adapted from those used routinely for clinical SEP evaluation in humans.[17] Latencies and amplitudes of the initial negative and positive waves of the cortical response were compared with values obtained from a control group of six normal neonatal lambs (Yingling, unpublished data). Unpaired t tests (two-tailed) were used for all statistical comparisons.

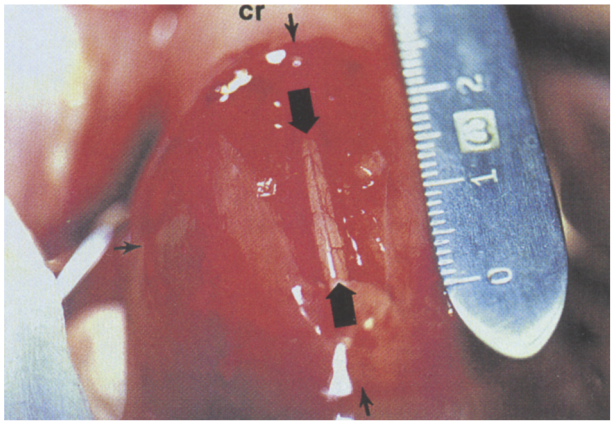

Fig 1. Overview of spina bifida–type defect created at 75 days' gestation. The normal and undamaged spinal cord is openly exposed over a length of 2 cm, between lumbar levels 1 and 4 (thick arrows). Thin arrows denote the skin defect. cr, cranial.

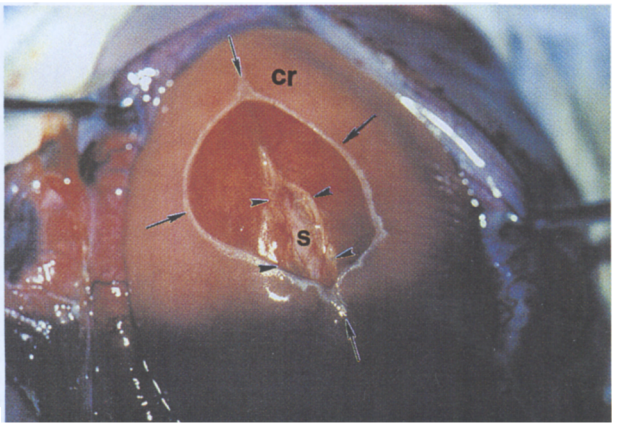

Fig 3. Developing MMC lesion at 100 days' gestation, before repair. Arrows indicate the large skin defect. Arrowheads denote the bony spina bifida defect. Note the slight herniation of the spinal cord (s) out of the spinal canal. cr, cranial.

Morphological Assessment

Immediately after SEP measurements, the animals were killed with Beuthanasia-D (Schering, Kenilworth, NJ) and perfused via a tracheal tube inserted in the ascending aorta. We used standard phosphate-buffered saline solution (1.5 L per animal) for washout and 10% formalin solution (3 L per animal) for fixation. The entire spine en bloc and the brain were removed intact. The brain was examined for hydrocephalus, and the spine (including surrounding soft tissues) was sectioned into blocks (T-12–S-2) and examined histologically. Serial cross sections were stained with Gomori's trichrome.

RESULTS

Creation of Spina Bifida–Type Lesion; Repair of Developing MMC

All 12 fetuses (100%) survived creation of the spina bifida–type lesion. During the 4-week interval between wounding and repair, five fetuses (42%) died or were aborted. In none of these animals had the back wound healed, and gross inspection showed an evolving MMC.

At 100 days' gestation, the repair operation was performed successfully in the remaining seven animals (58%). These animals had unhealed lumbar back wounds of different sizes (in some, the initial defect had grown with the animal; in others, there was moderate wound contraction) (Fig 3). The defects were entirely covered with a thin and almost transparent fibrous peel, and the exposed spinal cords appeared flattened and herniated, slightly or moderately, out of the spinal canal. No major destruction of the neural tissue could be seen.

Of the animals that underwent repair (n = 7), two were determined to be dead at the time of ultrasound follow-up (and fetectomized 5 days and 4 weeks postoperatively), two were aborted 3 days and 6 days postoperatively, and the remaining three were delivered at 145 days' gestation by cesarean section.

During necropsy, the four prematurely harvested animals were found to have closed/healed skin wounds; the muscle flap tissue covered the entire former defect and looked similar to adjacent muscles. There were no signs of infection or bleeding at the repair site. These findings were confirmed histologically, and the formerly exposed spinal cords showed significant flattening of the anterior-posterior dimension and widening of the lateral dimension. Grossly, the cytoarchitecture of the cords was intact.

Clinical Evaluation

At term, the three survivors had healed skin wounds and near-normal neurological function. Even though the animals were delayed neurologically for several hours (compared with normal neonates), they were able to stand, walk, and perform demanding motor tests such as stair climbing. The strength of the hindlimbs was reduced slightly. The motor coordination developed later, and to a slightly lesser extent than that of control lambs. Sensory function was present in the hindlimbs, and the reaction to noxious stimuli was similar to that obtained from the forelimbs. There were no signs of urine or stool incontinence. The clinical diagnosis of these animals is best summarized as mild paraparesis.

Electrophysiological Evaluation

All animals had normal SEP in the hindlimbs and forelimbs (Fig 4). For ulnar stimulation, the latencies of the primary negative wave (mean of left + right sides, ±standard deviation) were 16.92 ± 0.91 milliseconds (ms) in the controls and 18.32 ± 1.15 ms in the animals that underwent repair (t = 1.60; not significant). For the following positive wave, the latencies were 23.78 ± 2.77 ms in the controls and 25.14 ± 0.89 ms in experimental animals (t = 0.95; not significant). The mean peak-to-peak amplitude of the ulnar response was 1.40 ± 0.98 μV in the control group and 1.03 ± 0.47 μV in the experimental group (t = 0.63; not significant). For posterior tibial stimulation, the latencies of the initial cortical negativity were 30.22 ± 3.10 ms in the controls and 30.73 ± 3.42 ms in the experimental group (t = 0.16; not significant). The latencies of the following positivity were 38.50 ± 4.78 ms in the control group and 39.01 ± 2.68 ms in the experimental group (t = 0.8; not significant). The mean peak-to-peak amplitude of the PTN response was 0.41 ± 0.17 μV in the controls and 0.61 ± 0.15 μV in the experimental group (t = 1.56; not significant). These findings show that sensory

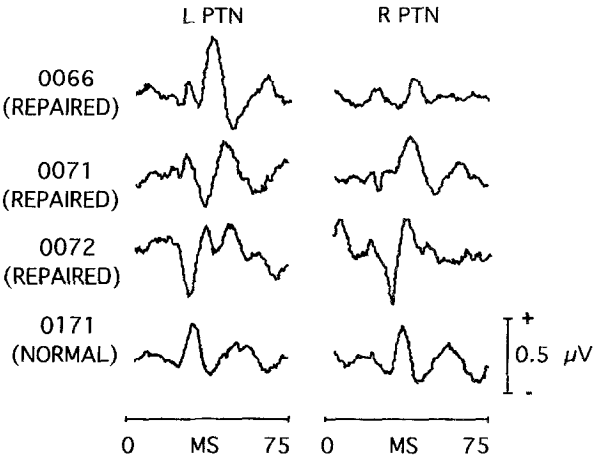

Fig 4. Somatosensory-evoked potentials to left (L PTN) and right (R PTN) posterior tibial nerve stimulation. Shown are the responses from the three animals that survived after repair (0066, 0071, and 0072) and from one normal control lamb (0171). All animals had clear responses to stimulation of each side; the responses from the experimental animals are in the normal range (see text). MS, milliseconds; μV, microvolts. Plus sign denotes positivity; minus sign denotes negativity.

transmission from forelimbs and hindlimbs through the dorsal column–medial lemniscal pathways was not impaired in either group of animals.

Morphological Assessment

Histologically, all surviving animals exhibited similar features. The formerly exposed and then covered spinal cord was flattened in its dorsoventral axis and widened in its lateral axis. The central canal was mildly dilated. The hallmarks of the spinal cord (gray and white matter, ventral and dorsal horn) were clearly discernable. The cellular architecture of the cord, nerve roots, and spinal ganglia was well preserved (Figs 5 and 6). In a few areas, especially the dorsal aspect of the spinal cord, minimal loss of neural tissue was detected. The latissimus dorsi flap was viable and demonstrated moderate muscle atrophy. The flap covered the surgically created former defect entirely and had developed a thin fibrous capsule that bound it to the underlying connective tissue and to the overlying healed skin (Fig 5). For comparison, a cross section through a normal lumbar spine is shown (Fig 7).

DISCUSSION

These results show that timely in utero repair of evolving experimental MMC lesions stops the otherwise ongoing process of spinal cord destruction and "rescues" neurological function by the time of birth. It is important to state that this conclusion can only be drawn in light of our previous studies. Recently we showed that the same type of spinal cord exposure used in this study, but without repair, leads to progressive alterations of the exposed neural tissue and results in a "classical" humanlike MMC at birth.[12] Those newborn lambs had a fluid-filled, cystic back lesion with the severely altered neural remnants exposed on the dorsal aspect of the cystic sac. Functionally, they were paraplegic and incontinent. Even histologically, the type of neural tissue damage and the configuration of the meninges were similar to findings recently reported for human MMC (Hutchins, unpublished data).[10,11] Our experiments were designed to approximate an eventual in utero repair of the naturally occurring human MMC. Therefore, the surgical induction of MMC was performed at the earliest suitable time in this animal model (75 days' gestation). The time point of repair (100 days' gestation) was chosen for three reasons. First, it is significantly delayed in relation to the time point of spinal cord exposure, and thus simulates the "untreated phase" in the human malformation during which a presumably limited neural damage occurs. Second, spinal cord exposure from day 75 through day 100 of

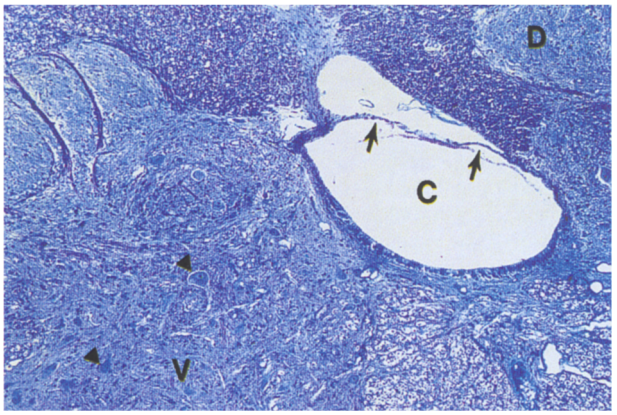

Fig 6. Photomicrograph of the dorsal part of the spinal cord of an animal who underwent repair (same section as in Fig 5). The cytoarchitecture of the cord is preserved. The central canal (C) is dilated, mildly distorted, and has focal loss of ependyma (arrows). D, dorsal horn; V, ventral horn. Arrowheads denote normal motor neurons. (Gomori trichrome stain, original magnification ×200.)

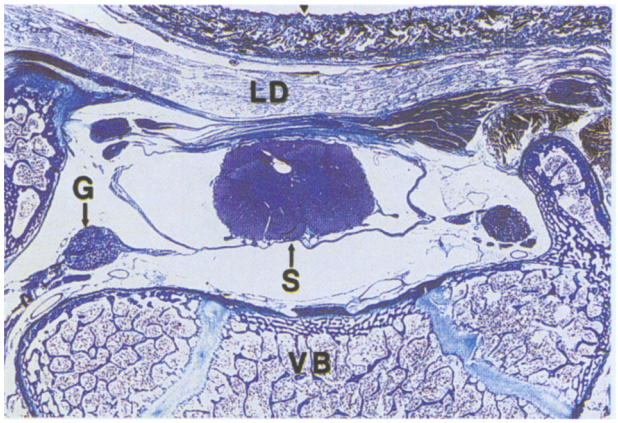

Fig 5. Photomicrograph (overview) of cross section through the repair site. The spinal cord (S) is moderately deformed (compared with the normal shape), but the anatomic hallmarks of the cord as well as the spinal ganglia (G) are preserved (for comparison, see normal spinal cord in Fig 7). VB, vertebral body. The skin (arrowhead) is healed, and the viable muscle flap (LD) covers the entire defect. (Gomori trichrome stain, original magnification ×10.)

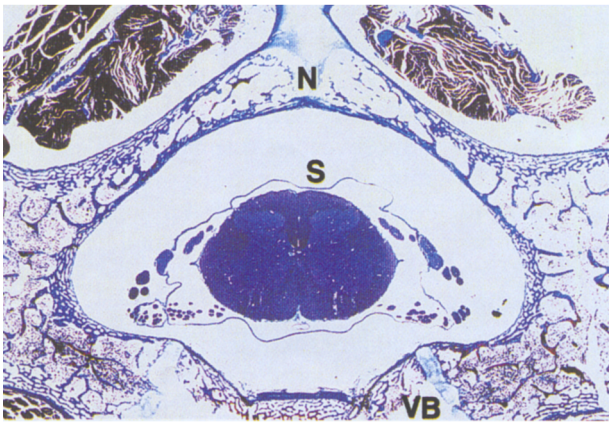

Fig 7. Photomicrograph of cross section through the normal lumbar spine of a neonatal lamb. VB, vertebral body; N, neural arches; S, spinal cord. (Gomori trichrome stain, original magnification ×10.)

gestation leads to limited spinal cord alterations.[12] Third, 100 days' gestation in the sheep corresponds to roughly 26 weeks' gestation in the human, a typical time at which human fetal surgery is performed for life-threatening malformations.[18]

In our model, these timing considerations proved to be accurate; at the time of repair, we found evolving MMC lesions with relatively mild alterations of the exposed spinal cord, including various degrees of flattening and slight herniation out of the spinal canal, but massive neural tissue destruction was not yet present. Even though this experimental model is subject to some biological variability, our previous experience[12] strongly suggests that repair late in gestation would have only minimally spared neurological function, because most neural destruction would have occurred already.

Previous reports have described surgical creation and repair of spina bifida–type defects in fetal animals.[19-21] Spinal cord exposure via laminectomy followed by immediate coverage of the spine defect was performed in monkey and pig fetuses. In rat fetuses, late gestational spinal cord exposure with immediate and delayed (delay, 24 hours) wound closure was carried out. These studies have shown that coverage of the spine defect after fetal laminectomy is basically feasible and not associated with neurological deficit, provided the postoperative course is uneventful. However, none of the studies addressed the crucial biological dynamics of a significantly procrastinated repair after creation of a spina bifida–type lesion in a large animal model with long-term gestation. As shown in the present study, to conclusively demonstrate the benefit of prenatal MMC repair, the experimentally induced MMC must be repaired at an intermediate stage of development, and the repaired animals' neurological outcome at birth must be significantly better than that of the nonrepaired animals.[12]

What are the clinical implications of this study? One concern is that the animal model defect does not exactly replicate the human malformation. For example, the experimental MMC is induced surgically, at midgestation, and is not the result of a developmental error during embryogenesis. Also, our model does not produce any of the features often associated with human MMC, such as hydrocephalus, Arnold-Chiari malformation, and parenchymal abnormalities of the brain and spinal cord.[3] Another issue is the high fetal death/abortion rate in this study; however, there is evidence that the fetal loss is not related to the type of intervention, but rather is intrinsic to the ovine animal model used.[14] These reasons indicate that successful experimental repair alone is insufficient evidence to embark on human fetal MMC repair. Yet other lines of evidence support the hypothesis that prenatal intervention for human MMC might be beneficial.

Human MMC fetuses with well-preserved and differentiated spinal cord tissue at the level of the lesion have been described. It is conceivable that early prenatal repair of such lesions could preserve neurological function.[22] On the other hand, several investigators have reported that obvious trauma to the exposed neural tissue has occurred in human fetuses, neonates, and infants with MMC.[10,22,23] These findings are consistent with our own observations (Hutchins et al, Meuli et al, unpublished data) and corroborate our theory of a secondarily acquired spinal cord injury that is potentially preventable. Moreover, prenatal coverage of MMC may arrest the neural destruction, and the plasticity of the developing nervous system also might allow rearrangement of function.[24] Preserved neural tissue might provide the necessary matrix for late-developing cerebrospinal projections that would be lost without repair.[25] Recently, functional recovery after bridging of segmental spinal cord defects with fetal spinal cord grafts has been reported for adult rats.[26] Similar regenerative forces might drive axonal outgrowth and neural connections across or within an MMC lesion once the chronic neural trauma is stopped. Ultrasonography of human fetuses with very large MMC lesions showed normal leg movements until week 17 of gestation, indicating that motor function was present initially and lost only later in gestation.[27] Finally, cesarean section ("latest possible fetal surgery") before the onset of labor improves neurological function in humans by avoiding mechanical trauma to the exposed neural tissue caused by labor and the passage through the birth canal.[28,29]

Our experimental results together with the abovementioned considerations suggest that in utero repair of human MMC may save neurological function at birth. Although many fetuses with nonlethal neural tube defects are still carried to term,[30] and although human fetal surgery is a reality for potentially fatal malformations, issues such as preterm labor after fetal surgery, ethics of fetal surgery for nonlethal malformations, and the natural history of intrauterine MMC development and selection criteria for the "ideal" candidate must be appropriately addressed before fetal surgery for MMC can be envisioned.[18] Thereafter, the proposed approach to this crippling condition might become a revolutionary alternative to the currently dismal choices of abortion, early postnatal death, or lifelong multiple disabilities.

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