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Original Article

The Effects of Amniotic Fluid on the Histopathologic Changes of Exposed Spinal Cord in Fetal Sheep

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Background: **Experimental studies have shown that in myelomeningocele, the primary malformation is neural tissue damage resulting from exposure of neural tissue to amniotic fluid. In this study, the effects of amniotic fluid on histopathologic changes of exposed spinal cord in fetal sheep were evaluated.**

Methods: **In an experimental trial, 10 fetal sheep in two groups containing five subjects (group A) and five shams (group B) were studied. In the sheep at 90 – 100 days of gestation (term: 145 – 150 days) the lumbar skin was incised, paraspinal soft tissues were excised, laminectomy was performed at L2 - L4, and dura matter was opened. In group A, the dura matter was not dorsally closed and thus the spinal cord was left exposed to amniotic fluid, and in group B the skin was immediately closed. The lambs were delivered near term by cesarean section and were assessed clinically and morphologically.**

Results: **In group A, all lambs (n=5) had a complete or incomplete flaccid sensorimotor paraplegia and suffered from urine incontinence. Four lambs in this group were stool incontinent. In group B (n=4), only one lamb had paraparesis (*P*=0.048) and all lambs were urine and stool continent. In group A, all lambs had hypoplastic longitudinal muscles of the rectum but well-developed circular muscles. The anal sphincter muscles did not develop normally. In group B, all lambs had well-developed longitudinal and circular muscles and anal sphincter muscles developed normally (*P*=0.048). Histopathologic examination of the spinal cords showed edema, focal calcification, fibrosis, and capillary cell proliferation in group A, but in group B such changes were not seen. The number of ganglion cells was significantly higher in group B compared with group A (*P*<0.0005).**

Conclusion: **Exposure of spinal cord to amniotic fluid causes structural neural tissue damage that can be prevented by fetal surgery through repairs of myelomeningocele.**

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Keywords: Amniotic fluid • fetal surgery • sheep • spinal cord

Introduction

Myelomeningocele (MMC) is the hernia protrusion of the cord and its meninges through a defect in the vertebral arc, muscles, and skin. It is the most prevalent congenital disorder of the central nervous system.[1, 2]

MMC is accompanied by considerable morbidity and disability during the postnatal period and a lower rate of mortality due to its complications. Disabilities related to MMC include paraplegia or paraparesis, hydrocephalus, sexual dysfunction, bone and organ deformities, fecal and urine incontinence, and mental retardation.[2,3]

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Theories on neuralgic lesions in MMC include:

- Primary failure of spinal cord development; and
- Spinal cord lesion exposed to amniotic fluid (AF) leads to mesodermal migration and cordal histopathologic modifications.[4–6]

Examination of the fetal cord afflict with MMC is normal at mid-pregnancy in most cases. Since most of fetuses with MMC suffer from severe neurologic disorders of the lower extremities at birth, it is estimated that neuralgic lesion occurs during late pregnancy, which is probably the cause of spinal cord lesion due to exposure to toxic AF.[1,2,7,8]

The major sources of AF production are fetal urine and fetal lung liquid. Additionally, there is evidence that intrauterine fetal defecation does occur under physiologic conditions in humans. Analysis of human AF in different gestational ages has shown that enzymes such as disaccharidases, alkaline phosphatase, and trypsin are present in the human AF. These enzymes are absent in the AF of human fetuses with intestinal atresia or cystic fibrosis, suggesting that these gastrointestinal enzymes enter the AF as a result of fetal defecation.

In multiple studies it has been shown that gastrointestinal waste products in the AF rather than urinary waste products are responsible for histopathologic effect on exposed spinal cord to AF.[1,8]

In the present study, regarding the second theory, in an experimental trial on fetal sheep, the effects of AF on histopathologic modifications of the spinal cord are presented.

Materials and Methods

This experimental, clinical study was conducted on two groups of five sheep of Bakhtiari breed at the Veterinary Hospital of the Shahrekord Azad University, Shahrekord, Iran. Target population included pregnant female sheep aged two to three years from one generation that were considered healthy by the veterinarians.

Pregnancy and gestational age were determined by the veterinary radiologist through radiography and ultrasonography.

Surgical operations were conducted during the 90^{th} to 100^{th} days of gestation (term: 145 – 150 days). Half an hour before operation, one gram cefazolin IV and 100 mg indomethacin suppository were administered.

After general anesthesia, laparotomy was conducted through a left caudioventral incision of the abdomen. The uterus was palpated with hand and directed toward the operation field. The uterus was opened with cuter and the amniotic membrane was opened. The fetus was palpated and the location of L2 - L4 vertebrae was specified. The skin was incised, the paravertebral muscles were excised, laminectomy was done, and the dura was opened longitudinally so that the cerebrospinal fluid (CSF) could be specified. In group A (five fetal sheep), the skin was excised and the defect was left open so that the spinal cord could be exposed to the AF. In group B (five fetal sheep), to prevent the contact of spinal cord with the AF, the defect was covered only via the skin. AF was replaced by warm normal saline and the uterus was repaired by absorbable thread in one layer. Feeding was started eight hours after the surgery and cefazolin (1 g, IM) and indomethacin suppository (100 mg) were administered every eight hours. Cefazolin was continued for seven days and indomethacin was continued up to the time of cesarean section (CS).

Ten to fourteen days after the operation, fetal health was investigated by ultrasonography. The evaluations were continued at two-week intervals and up to the time of CS. On day 140 – 145 of gestation, the sheep underwent CS. In our study, one sheep died due to septicemia in group B. After birth, the newborns were clinically evaluated for 48 – 72 hours from the view point of superficial pain sensation (pressure with a needle); deep pain sensation in lower limbs (pressure exertion via hemostat); extremity and vertebral column deformities; and paraplegia and paraparesis (the evaluation of paraplegia and paraparesis was done by monitoring the animal in second and third day of its life in terms of movement in their lower limbs, standing on their feet, and walking. Lack of any movement in lower limbs called paraplegia and movement in lower limbs without any ability to standing and normal walking called paraparesis.

The evaluation of incontinency in paraplegic and paraparetic lambs was done by recurrent changing and monitoring the wet gauzes in urethra every 10 – 15 minutes. In lambs that had no sense or movement disorders in lower limbs, the evaluation was done by monitoring the normal pattern of urination at four- to six-hour intervals and by assessing whether the wool around

the urethral meatus is wet or dry. The wet gauzes indicated the incontinency.

The evaluation of defecation was done by monitoring the anus in terms of the way stool exits and its consistency for 24 – 48 hours. Continuous defecation with soft consistency and bowel peristalsis was called fecal incontinence. All these evaluations were done by the veterinarian.

The lambs were then slaughtered according to religious rules and the spinal cord at the defect site, thigh muscles, anal sphincter, and rectum were cut and kept in 10% formalin. Then, the specimens were transferred to the Pathology Department of Al-Zahra Hospital.

In this study, quantitative variables were reported as mean along with standard deviation. For precise statistical analysis Fisher's exact test was used.

Results

After birth all fetal sheep were clinically evaluated for deep and superficial pain sensation, deformity of limbs and vertebral column, paraplegia and/or paraparesis, and fecal and urine incontinence. Their spinal cord, rectum, anal, and thigh muscles were evaluated histopathologically. The following results were obtained:

In none of the two groups organ and extremity deformities were seen. In group A, all newborns suffered from paraplegia or paraparesis and in group B, a newborn (25%) had paraparesis, which was statistically significant (P=0.048).

None (0%) of the sheep in group A but one (25%) in group B had superficial pain, which was statistically significant (P=0.048). Deep pain perception was absent in four subjects in group A (80%); however, it was present in all subjects in group B (100%). This difference was statistically significant (P=0.04).

Fecal incontinence was seen in four subjects in group A (80%); however, none of the subjects in group B had fecal incontinence. Statistically, the difference between these two groups was significant (P=0.04).

Urine incontinence was seen in all subjects in group A (100%); however, no such case was seen in group B. Therefore, the difference between the two groups was significant (P=0.008).

In histopathologic examination of thigh, rectal, and anal muscles, atrophy of thigh muscles, longitudinal rectal muscles, and sphincteric muscles was evident in all subjects of group A. In group B, however, all muscles had normal development; therefore, the difference between the two groups was statistically significant (P=0.008) (Figures 1 and 2).

Microscopic study of spinal cord at the defect site from the view point of ganglion cells showed that in group A the number of ganglion cells at each field of microscope was 4.8±0.74 and in group B was 14.5±0.59. This showed a significant difference between the two groups (P<0.0005, Figures 3 and 4).

In group A, pathologic investigations of spinal cord revealed edema, focal calcification, fibrosis,

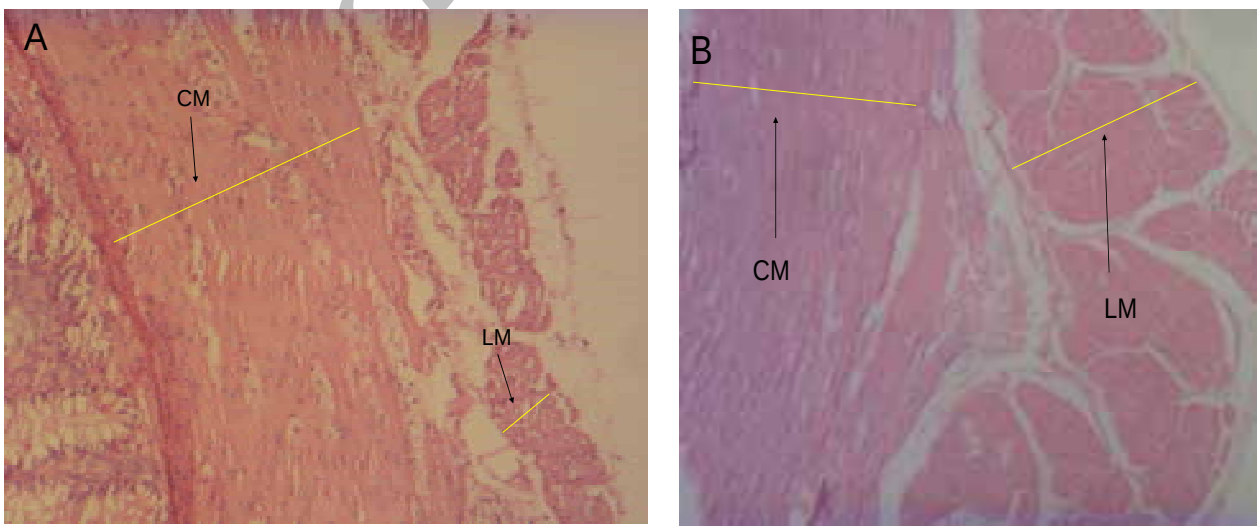

Figure 1. Microscopic findings for muscles in the rectal wall (hematoxylin-eosin staining ×100. **A**) An unrepaired lamb. Circular muscle (CM) is well-developed, but longitudinal muscle (LM) is undeveloped. **B**) Both CM and LM are well-developed.

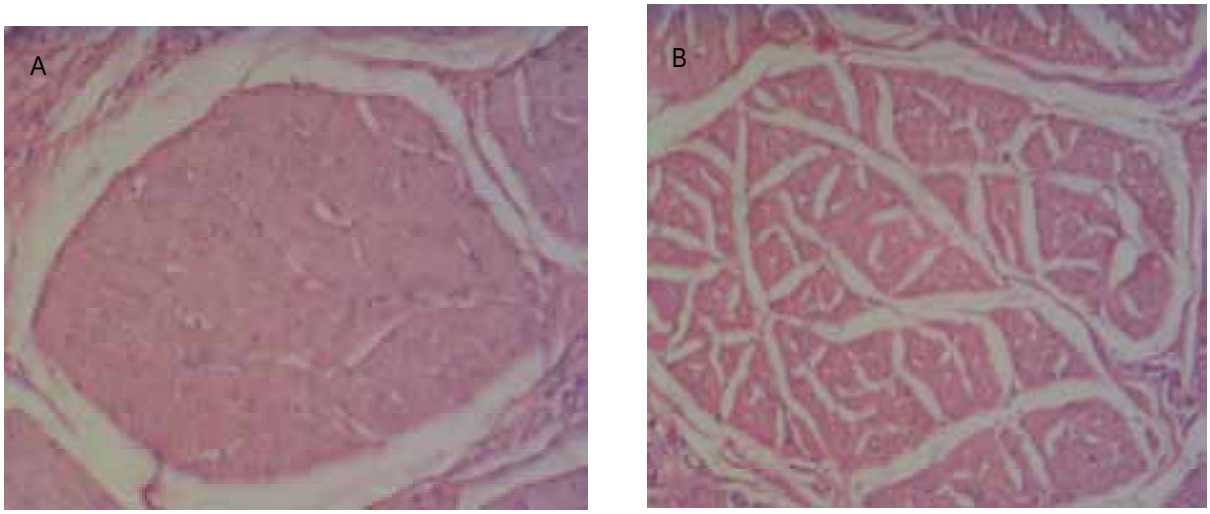

Figure 2. Microscopic findings of anal sphincter muscle in the lambs that underwent repair (A) and in the untreated lambs (B). The high density of muscle fibers in the A lambs and low density of muscle fibers in the B lambs are seen.

and proliferation of the capillary cells, while in group B no such modifications could be observed.

Discussion

Exposure of spinal cord to AF in patients suffering from MMC can lead to spinal lesion. Also, numerous studies have shown that AF is more toxic toward the end of gestational period.[1,2,7,8]

In most patients with MMC, paraplegia is seen.[1,2,5,9] This case was seen in our study on animals as well. In patients with MMC, some associated disorders including deformity of extremities such as club foot and vertebral column deformities can be seen.[2,3,5,8] In our study, however, such disorders were not observed.

Histologically, pia and dura matters located on the spinal cord at the defect site are uncovered which gradually via fibrous tissue become connected to the defect edges and, therefore, the subarachnoid space will become closed. Thus due to lack of dura matter at the back of the spinal cord, the posterior part of the spinal cord will remain open and in contact with the AF.[1] In group B, posterior part of the spinal cord was covered by skin and, therefore, it was prevented from contact with the AF. Once spinal cord becomes exposed to the AF, neurons death follows and number of ganglions will decrease.[1] In our study as well, the number of ganglion cells in the spinal cord in group A in comparison with group B had significantly decreased.

In a study conducted by Meuli et al. on fetal sheep, it was shown that if the exposed spinal cord was covered at the beginning of gestation, it would

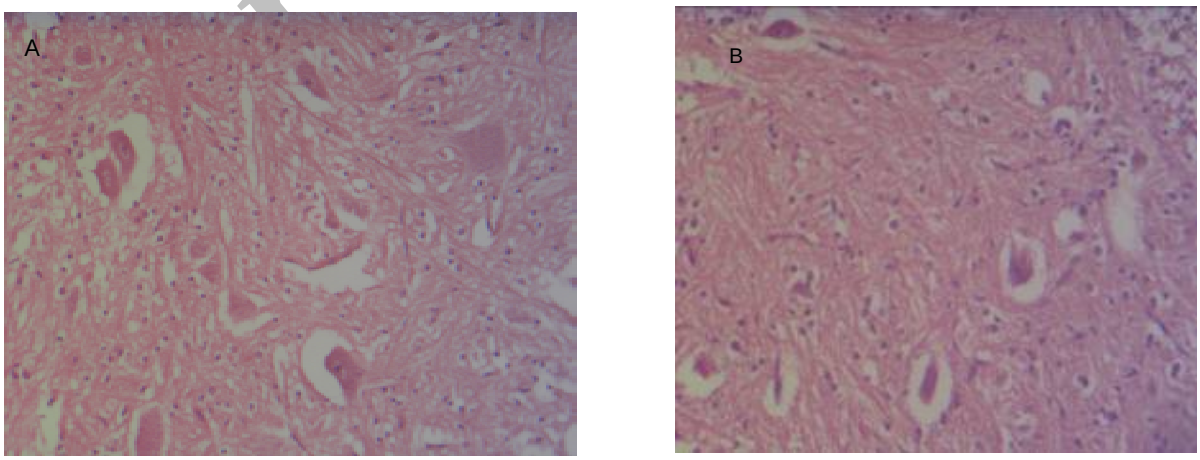

Figure 3. Microscopic feature of spinal cord in **A**) treated lambs show higher neural cell count and **B**) untreated lambs show decrease of neural cell count.

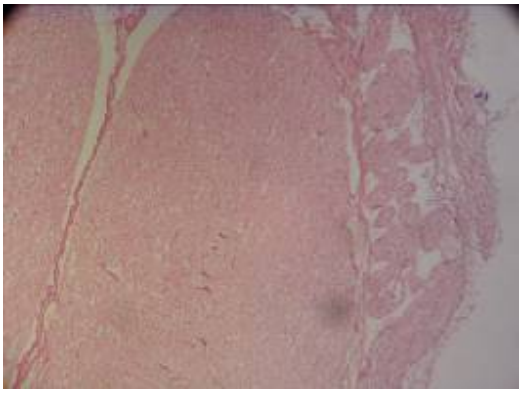

Figure 4. Microscopic feature of spinal cord of untreated lambs show fibrosis (hematoxylin-eosin staining × 100).

be protected from further contact with the AF and, thus, progressive destruction of spinal cord and loss of its function would be decreased.[1] Through a similar mechanism in our study in group B, spinal cord exposure to AF was prevented by a skin cover. This had led to prevention of spinal cord lesions and its disordered functioning. All sheep in group A suffered from paraplegia or paraparesis, but only one subject in group B suffered from paraparesis, which led to a significant difference between the two groups.

Hirose et al. conducted laminectomy on fetal donkeys and provoked MMC in them. In one case he left the defect open and in the other group the defect was immediately repaired. After birth, in subject with nonrepaired MMC, paraplegia and incontinence were seen, while in the repaired group no such disorders were seen.[10] Our results also confirm these findings.

In the study by Yoshizawa et al. on operated fetal sheep, it was shown that in sheep with untreated MMC longitudinal rectal muscles had not developed and were atrophic; however, circular muscles were developed. In the treated fetal sheep, both longitudinal and circular rectal muscles were developed.[9] In our study, such modifications were seen with a significant difference between the two groups.

In another study conducted by Yoshizawa et al. on fetal sheep, it was shown that provoking MMC and then covering it with skin led to normal development of the anal sphincteric muscles. In fetal sheep with MMC in which spinal cord has been left exposed to the AF, anal sphincteric muscles had not fully developed and signs of atrophy were seen.[9] Similar changes were seen in our study with a significant difference between the groups A and B.

Olguner et al. through a study performed on chicken showed that in all those in which MMC had been provoked, pathologic changes of the spinal cord including edema, focal calcification, fibrosis, and proliferation of capillary cells existed.[8] In our study on fetal sheep (group A), these changes in spinal cord were seen as well.

Our study showed that the main pathogenic factor in spinal lesions and neuromuscular disorders in patients with exposed spinal cord was exposure of spinal cord to the AF and the toxic effects of this fluid on the spinal cord and that fetal surgery could prevent MMC complications.

Also, this study showed that in sheep with exposed spinal cord whose defects were covered by skin, normal development of thigh, rectal, and anal sphincter muscles was possible.

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