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A Decade of Experience with the Ovine Model of Myelomeningocele – Risk Factors for Fetal Loss

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Abstract

INTRODUCTION: The ovine model is the gold standard large animal model of myelomeningocele (MMC), however, it has a high rate of fetal loss. We reviewed our experience with the model to determine risk factors for fetal loss.

METHODS: We performed a retrospective review from 2009–2018 to identify operative factors associated with fetal loss (early fetal demise, abortion or stillbirth). Operative risk factors included gestational age at operation, operative time, reduction of multiple gestations, amount of replaced amniotic fluid, ambient temperature, and method of delivery.

RESULTS: MMC defects were created in 232 lambs with an overall survival rate of 43%. Of the 128 fetuses that died, 53 (42%) had demise prior to repair, 61 (48%) aborted, and 14 (11%) were stillborn. Selective reduction of multiple gestations in the same uterine horn was associated with increased fetal demise (OR 3.03 (95% CI 1.29–7.05, p=0.01)). Later gestational age at MMC repair and Cesarean delivery were associated with decreased abortion/stillbirth (OR 0.90 (95% CI 0.83–0.90, p=0.03) and OR 0.37 (95% CI 0.16–0.31, p=0.02)), respectively.

CONCLUSION: Avoiding selective reduction, repairing MMC later in gestation, and performing Cesarean delivery decrease the rate of fetal loss in the ovine MMC model.

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Statement of Ethics

The University of California Davis Institutional Animal Care and Use Committee (IACUC) approved all animal protocols, and all animal care was in compliance with the Guide for the Care and Use of Laboratory Animals. All facilities used during the study period were accredited by the Association for the Assessment and Accreditation of Laboratory Animal Care International.

Disclosure

The authors have no conflicts of interest to declare.

Keywords

fetal surgery; myelomeningocele; spina bifida; animal disease models; fetal research

1. Introduction

Myelomeningocele (MMC) results from failure of neural tube closure in early gestation and leads to lower extremity paralysis, bowel and bladder dysfunction, and hindbrain herniation (1). Clinical and experimental evidence suggested that the paralysis associated with MMC was partly due to intrauterine chemical and mechanical trauma and, therefore, was partially preventable (2). Following initial evidence in small and large animal models of MMC, the Management of Myelomeningocele Study (MOMS) confirmed that *in utero* surgical repair improves lower extremity function in children with MMC (3–6). However, 58% of children treated with *in utero* surgery were still unable to walk independently, leading to further translational studies to augment the *in utero* surgery in the ovine model (7, 8).

The ovine model is the gold standard large animal model for studying *in* utero treatment of MMC due to anatomic size allowing for surgical instrumentation, pregnancies with multiple gestations, gestation length allowing study of disease pathogenesis and treatment, and relative tolerance of multiple uterine surgeries (9). However, the ovine model is limited by expense and high rates of fetal loss (4, 10–12). Previous studies have reported fetal loss following both *in utero* surgical MMC model creation and surgical MMC repair (11, 13, 14). Given our experience with the ovine model over the past decade, we sought to evaluate the risk factors for fetal loss. We hypothesized that lower ambient temperatures at parturition and Cesarean delivery were associated with lower rates of fetal loss.

2. Materials and Methods

2.1 Time-mated ewes

Time-mated ewes (*Ovis aries*) were obtained from two vendors during the study period: 2009 to 2018. The vendors tested ewes for *coxiella burnetii* (Q fever) prior to mating and confirmed pregnancies with ultrasound. Ewes were delivered to the University of California, Davis regardless of the number of gestations 7 to 14 days prior to surgery for acclimation and repeat Q fever testing. Prior to surgery, ewes were fasted for 12 hours. Ewes and lambs were housed pre- and post-operatively in a natural indoor, open-air barn environment.

The University of California Davis Institutional Animal Care and Use Committee (IACUC) approved all animal protocols, and all animal care was in compliance with the Guide for the Care and Use of Laboratory Animals. All facilities used during the study period were accredited by the Association for the Assessment and Accreditation of Laboratory Animal Care International.

2.2 Maternal and fetal anesthesia

Anesthesia was induced with intravenous ketamine (4 mg/kg) and propofol (5 mg/kg). Ewes were orotracheally intubated and mechanically ventilated to achieve approximately 7 ml/kg

tidal volume. General anesthesia was maintained with isoflurane (1.75–3%). Antibiotic prophylaxis with ceftiofur (1.1–2.2mg/kg IV) was administered pre-operatively. Fetal anesthesia was provided via maternal-fetal circulation of isoflurane. In the event of fetal movement during the operation, the isoflurane concentration was increased, and the operation was paused until the cessation of fetal movement. Anesthetic technique was consistent throughout the review period.

2.3 Myelomeningocele defect creation

An ultrasound was performed to confirm fetal viability prior to the MMC operation. The number of gestations was estimated at the time of pre-operative ultrasound, though intraoperative confirmation was required. Defects were surgically created at 75 ± 7 days of gestation based on initial research that the MMC defect created at 75 days of gestation had less spontaneous healing than at 60 days (10). Following maternal laparotomy and hysterotomy, the MMC defect was created by removing the skin, paraspinal muscles, and posterior vertebral lamina and dura overlying 6 lumbar segments as previously described (Figure 1) (7). This large defect avoids complete healing of the defect prior to repair. A defect creation score is documented to account for injury to the spinal cord at the time of the MMC defect creation (12). No myelotomy was performed in this series of experiments as the focus was specifically on motor function rather than the consequences of hindbrain herniation. Amniotic fluid was replaced with a recorded volume of warm normal saline, and antibiotics (1 million units of penicillin and 100 mg of gentamicin) were added to the amniotic fluid prior to hysterotomy closure.

During the majority of the study period, multiple gestations in the same uterine horn were reduced through a second hysterotomy in order to minimize growth restriction and on the assumption that it would improve fetal survival in the experimental animals. Thus, only one defect and repair was performed in each horn of the uterus.

2.4 Myelomeningocele repair

MMC repairs were performed at 100 ± 7 days of gestation based on initial reports of fetal MMC repair in the model (4, 13). Innate healing from the time of MMC defect creation was documented with a validated score (12). Repair strategies varied during the study and included closure of skin only or application of autologous amniotic membrane, nanofiber scaffold patch, collagen hydrogel with or without neural crest stem cells or placenta derived mesenchymal stem cells (PMSCs), or extracellular matrix (ECM) patch with or without PMSCs (8, 15–20).

2.5 Delivery and lamb resuscitation

From 2009 to 2015, lambs were delivered via spontaneous vaginal delivery (SVD). Following an observation of dystocia and increased stillbirth in some lambs born via SVD, starting in 2016, lambs underwent Cesarean delivery at term (146 days of gestation) if they had not previously been born via SVD. Cesarean delivery was performed at term in order to limit negative effects of prematurity. Following Cesarean delivery, lambs required resuscitation due to maternal-fetal anesthesia. The lambs' oropharynx were suctioned, followed by endotracheal intubation and ventilation, until the lambs were breathing

spontaneously and demonstrated adequate head control. Additionally, lambs were dried with towels and warmed with a heated air blanket. Lamb hind limb motor function was scored from 0 to 15 with a validated Sheep Locomotor Rating Scale shortly after birth and 24 hours later (21). The vast majority of the Cesarean deliveries during the study were terminal procedures for the ewes. Lambs were then bottle fed until the study endpoint. Anecdotally, we have had success with achieving ewe-lamb bonding after survival Cesarean delivery by placing amniotic-fluid soaked towels on the ewe's nares and by exposing ewes to the newly delivered lambs during the anesthesia emergence and recovery period.

Following each operation, ewes received buprenorphine (.005-.1 mg/kg IM) every 12 hours for the first 48 hours for pain control. Ceftiofur (1.1–2.2 mg/kg IM) was administered daily for five days post-operatively.

2.5 Statistical analysis

Results are reported as medians (interquartile range). To assess operative factors associated with fetal loss, we used a generalized logistic model while accounting for correlation among lambs from the same ewe. Fetal demise was defined as loss prior to MMC repair. Abortion was defined as fetal loss subsequent to MMC repair and prior to 138 days of gestation, and stillbirths were lambs that were born dead after 139 days of gestation or that died during birth. Analysis was performed in SAS version 9.4 (SAS Institute Inc., Cary, NC, USA) with significance set at $p<0.05$.

3. Results

3.1 Summary of animal numbers

From 2009 to 2018, a total of 142 ewes were operated on, 71% of which had multiple gestations (mostly twins, with up to five fetuses). 225 MMC defects were created and 172 MMC repairs were performed. 53 of the 225 fetuses had MMC defects only due to fetal demise prior to repair. A total of 41 fetuses that were part of a multiple gestation pregnancy were reduced. Additionally, 5 control lambs were used to define normal imaging and histologic parameters.

3.2 Operative results

The median gestation age for MMC creation was 77 days of gestation (IQR 77–79). The median operative time for defect creation for each fetus was 34 min (IQR 28–41) with a median ewe operative time of 124 min (IQR 98–144). The median saline added to each uterine horn during creation was 700 ml (IQR 500–850). For MMC repair, the median gestational age was 101 days of gestation (IQR 99–105) with a median time between defect creation and repair of 26 days (IQR 22–28). The median operative time for MMC repair of each fetus was 50 min (IQR 39–64) with a median ewe operative time of 164 min (IQR 128–201). The median saline added at repair was 1000 ml (IQR 750–1260). Cesarean delivery was performed on 43.8% (n=60) of ewes at a median gestational age of 146 days (IQR 140–147).

3.3 Ewe complications

Seven ewes were euthanized. These were due to partial fascial dehiscence secondary to superficial surgical site infection (n=3), postoperative respiratory complications (n=1), pregnancy toxemia (n=1), straddle injury (n=1), and post-operative weight loss (n=1).

3.4 Fetal loss

There were 2 fetal demises prior to defect creation. Following defect creation, 128 fetuses were lost due to fetal demise (n=53, 41%), abortion (n=61, 48%), or stillbirth (n=14, 11%). All surviving fetuses subsequently underwent MMC defect repair. The rate of fetal survival following MMC defect creation (76.4%) was significantly higher than survival following MMC defect repair (56.4%) ($p<.001$) During the study period, the overall survival rate was 43%, which varied by academic year (Table 1). Academic year, which accounts for operator involvement, was not associated with fetal loss (OR 0.97 (96% CI 0.84–1.11)).

The only operative factor during defect creation associated with increased fetal loss was performing a selective reduction in a single uterine horn with multiple gestations (OR 3.03 (95% CI 1.29–7.05, p=0.01)). Other operative factors during defect creation including gestational age, ewe and fetal operative time, and amount of saline added to the uterus were not significantly associated with fetal loss (Table 2).

The interval timing of MMC repair was significantly associated with fetal loss. A longer interval between defect creation and repair was associated with decreased fetal loss (OR 0.82 (95% CI 0.73–0.91, $p<.001$)). For every additional day between defect creation and repair there was a 0.82 odds of fetal death. Similarly, later gestational age at repair was associated with decreased fetal loss (OR 0.90 (95% CI 0.83–0.90, p=0.03)). For every additional day of gestation at time of MMC repair, there was a 0.90 odds of fetal loss. Neither operative time for the fetus or ewe nor the volume of added saline was associated with fetal loss during MMC defect creation or repair.

The method of delivery was also significantly associated with fetal loss. For those lambs reaching term, Cesarean delivery was associated with a decreased fetal loss (OR 0.37 (95% CI 0.16–0.31, p=0.02)). Ambient outdoor temperature and gestational age at delivery were not associated with fetal loss (Table 2).

4. Discussion

This is the largest reported series using the surgically-created ovine model of MMC. The overall survival rate – from the time of MMC defect creation to live birth – in this study was 43%. Reduction of multiple gestations, decreased time between MMC defect creation and repair, and earlier gestational age at MMC repair were associated with increased fetal loss. Contrary to the hypothesis, ambient temperature at parturition was not significantly associated with fetal loss. However, Cesarean delivery was associated with decreased fetal loss. After transitioning to routine Cesarean delivery, the survival rate improved from 40 to 46%.

The reported rate of survival in this study is within the range of previously published rates ranging from 25 to 87% (5, 10, 11, 14, 22, 23). Some variation in these published rates can be explained by small experimental series, inclusion of sham controls, and inclusion of maternal deaths. Our practice of multiple gestation fetal reduction and a large six lumbar level MMC defect creation may explain our increased rate of early fetal demise, however, the explanation of our higher rate of late abortion and stillbirths remains unclear. While transitioning to Cesarean delivery, as was performed in all but one of the published studies, has improved our survival, we continue to have a large number of stillbirths. One explanation of higher rates of stillbirth may be our practice of performing Cesarean delivery near term at 146 days of gestation, while other investigators have chosen an earlier delivery at 140 days with better survival and potentially fewer deaths from dystocia (11, 14, 24).

Fetal loss was increased with the selective reduction of multiple gestations and with earlier MMC repair. After historically reducing multiple gestations in the same uterine horn to minimize growth restriction, we have since stopped this practice due to its association with fetal demise. As the placental structure for sheep is cotylendonary and therefore overlaps between multiple gestations in the same horn, reduction of a fetus may result in regression of the cotyledons in the same horn, negatively affecting the other gestation. The association of earlier MMC defect repair and fetal loss remains unclear, though this may be due to cumulative stress of fetal surgical intervention and membrane disruption.

A number of plausible risk factors for fetal loss are not captured in this study including fetal blood loss and type of treatment. We did not record fetal blood loss because the amount is minimal, saturating significantly less than a single 4×4 gauze. Based on visual correlates, we estimate our fetal blood loss to be 1–2 ml per MMC defect creation and less than 1 ml per MMC repair (25). The lamb fetoplacental blood volume at the time of defect creation is approximately 22 ml based on their average 0.18 kg weight, which corresponds to a 16 or 17-week human fetus (26). Less than 10% total blood volume hemorrhage, which is caused in MMC defect repairs, should be tolerated well given that human fetuses replace half of any lost blood volume within 30 minutes (27).

We did not find any evidence of operator experience on fetal loss. Academic year, which best accounts for the learning curve of general surgery research fellows during surgeries, was not associated with fetal loss. Anecdotally, we have observed that new learners tend to increase the duration of each of the procedures, however operative time was not associated with fetal loss.

Infectious causes of abortion in sheep include *Campylobacter* sp, *Chlamydia* sp, *Toxoplasma* sp, *Listeria* sp, *Brucella* sp, *Salmonella* sp, border disease virus, and Cache Valley virus (28). While our practice is to send any fetuses with suspected pathology for testing, none of the aforementioned infectious agents have been documented. One fetal death occurred following a fungal infection (species not reported), which may have resulted from amniotic fluid contamination.

Chorioamniotic membrane separation is a known risk of fetal surgery in humans associated with premature rupture of membranes and early delivery (29–31). Membrane separation in

humans is more commonly seen when performing hysterotomy at an earlier gestation (29, 31). In our ovine model we try to minimize the separation, however, we do not have a documented spectrum of separation, and therefore did not account for its effects in this study.

5. Conclusion

The fetal ovine model of MMC is complicated by high rates of fetal loss, which can be minimized by not performing reductions, performing MMC defect repairs later in gestation, and performing Cesarean deliveries.

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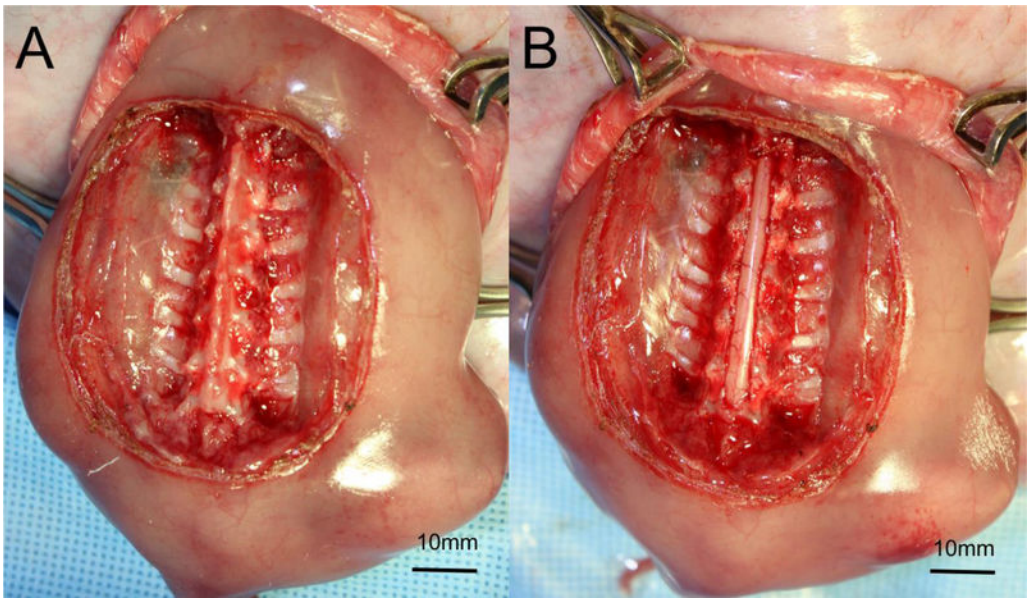

Figure 1. Myelomeningocele defect.

The MMC defect is created by removal of skin, subcutaneous tissue and paraspinal muscles from L1 to L6 **(A)**. A posterior laminectomy exposes the spinal cord **(B)** before the dura is removed.

Table 1.

Survival rate by academic year.

Survival rate ranged from 26-100% from 2009 to 2017 (p=0.063)

Academic Year	2009	2010	2011	2012	2013	2014	2015	2016	2017
Number of Lambs	3	23	7	18	35	15	36	39	49
Survival Rate	100%	48%	57%	33%	26%	27%	56%	41%	49%

Table 2.
Factors associated with fetal demise or abortion.

Reduction of multiple gestations, decreased time between MMC defect creation and repair, and earlier gestational age at MMC repair were associated with increased fetal loss. Cesarean delivery was associated with decreased fetal loss.

Defect Creation:	**Median (IQR)**	**Odds Ratio (95% CI)**	**p value**
Gestational age	77 days (77–79)	1.06 (0.92–1.22)	0.43
Fetus operative time	34 min (28–41)	1.01 (0.99–1.03)	0.29
Ewe operative time	124 min (98–144)	1.00 (0.99–1.02)	0.19
Volume of added saline	700 ml (500–850) N (%)	1.00 (0.99–1.00)	0.42
Reduction of multiple gestation	41 (14.8)	3.03 (1.29–7.05)	0.01 *
Defect Repair:	**Median (IQR)**	**Odds Ratio (95% CI)**	**p value**
Days since defect creation	26 days (22–28)	0.82 (0.73–0.91)	<0.001 *
Gestational age	101 days (99–105)	0.90 (0.83–0.90)	0.03 *
Fetus operative time	50 min (39–64)	1.07 (0.96–1.20)	0.25
Ewe operative time	164 min (128–201)	1.01 (0.99–1.01)	0.18
Volume of added saline	1000 ml (750–1260)	1.00 (0.99–1.00)	0.13
Delivery:	**Median (IQR)**	**Odds Ratio (95% CI)**	**p value**
Ambient high temperature	75°F (66–86)	1.00 (0.97–1.02)	0.70
Gestational age	146 days (140–147) N (%)	0.94 (0.87–1.03)	0.18
Cesarean delivery	60 (43.8)	0.37 (0.16–0.31)	0.02 *

* $p<0.05$