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Lucie Guilbaud[1], Nathalie Roux[1], Stéphanie Friszer[1], Raphaël Vialle[2], Charles Garabedian[1], Ferdinand Dhombres[1], Zoobia Shah[1], Bettina Bessières[3], Federico Di Rocco[4], Michel Zerah[5], Jean-Marie Jouannic[1]

[1] Department of Fetal Medicine, Armand Trousseau Hospital, APHP, Sorbonne University, UPMC Paris 6 University, Paris, France

[2] Department of Pediatric Orthopaedics, Armand Trousseau Hospital, Sorbonne University, UPMC Paris 6, Paris, France

[3] Department of Histology, Necker Hospital, APHP, Paris, France..

[4] Department of Pediatric Neurosurgery, Necker Enfants Hospital, APHP, Paris 5 University, Paris, France

Short title: *Fetoscopic suture of myelomeningocele in fetal lamb*

Corresponding author: Jean-Marie Jouannic

Address: Service de Médecine Fœtale de l’hôpital Armand Trousseau, 26 avenue du Docteur Arnold Netter, 75012 Paris, France

Phone number: +33631823271

Fax number: +33144735222

e-mail: jean-marie.jouannic@aphp.fr

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Abstract

Objective – To assess the feasibility and the effectiveness of a fetoscopic myelomeningocele (MMC) repair with a running single-suture using a two-port access in the sheep model.

Methods – 18 fetuses underwent surgical creation of a MMC defect at day 75. Fetuses were then randomized into three groups. Four fetuses remained untreated (control group). In other 14 fetuses a prenatal repair was performed at day 90: 7 fetuses had an open repair (oMMC) and 7 fetuses had a fetoscopic repair (fMMC) using a single-layer running suture through a two-port access. Lambs were sacrificed at term and histological examinations were performed.

Results – Hindbrain herniation was observed in all live lambs in the control group. A complete closure of the defect was achieved in all the lambs of the fMMC group. A complete healing of the defect and no hindbrain herniation were observed in all live lambs of oMMC and fMMC groups. Durations of surgeries were not statistically different between the oMMC and the fMMC groups (60min vs 53min, p=0.40) as the risk of fetal loss (fMMC: 1/7, oMMC: 3/7, p=0.56).

Discussion – Fetoscopic repair of MMC can be performed using a single-layer running suture through a two-port access and may be promising to reduce the risk of premature rupture of membranes.

Introduction

Myelomeningocele (MMC) is the most common central nervous system congenital anomaly, which induces serious neurological damage to the affected children (paraplegia, bladder incontinence, bowel dysfunction, neurocognitive and neurobehavioral deficits). The results of the Management of Myelomeningocele Study (MOMs) provided evidence that prenatal MMC repair reduces the need for ventriculo-peritoneal shunting at 12 months, improves motor prognosis and reduces the severity of Chiari 2 malformation associated with this congenital anomaly [1]. However, the open surgical approach described in the MOM study was associated with neonatal and maternal complications mainly including preterm deliveries (79% vs 15%, $p<0.001$), premature rupture of the membranes (PROM) (46% vs 8%, $p<0.001$) and uterine dehiscence at delivery (10%).

These complications have encouraged the development of a fetoscopic approach to antenatally repair MMC, a less invasive surgery which could minimize maternal morbidity, reduce the risk of premature delivery and allow vaginal delivery. The first fetoscopic MMC surgeries using dioxide insufflation were reported by Bruner *et al.* 18 years ago in four human fetuses, with disappointing results [2]. In the past two decades, indisputable improvements of the techniques used for fetoscopic repair for MMC have been achieved [3-5]. However, the contribution of this technique currently remains limited by a high rate of PROM and an associated risk of very preterm delivery [3,4,6,7]. Moreover, the effect of these techniques on neural structures is still a matter of debate.

We described, in the past, a simplified technique of fetoscopic MMC coverage using a patch secured with surgical adhesive in sheep [8]. Although we demonstrated that the fetoscopic MMC coverage using a two-port access is a quick feasible intervention, the results at birth on the MMC lesion closure and the hindbrain herniation were disappointing. Fontecha *et al*., in association with Belfort, obtained better results with a similar technique using a different patch and glue in sheep [9, 10]. However, they did not choose to apply this technique in human and they experimented the MMC suture rather than MMC coverage with a patch for their clinical study [5]. Their results with their two-port technique are excellent as regards to the rate of PROM and gestational age at birth but disappointing as regards to the relatively low rate of Chiari malformation reversal.

The purpose of our study was to assess the feasibility and the effectiveness of a fetoscopic myelomeningocele repair with a running single-suture using a two-port access after dioxide insuflation in the sheep model.

Material and methods

This study protocol was approved by the French national committee on animal research (APAFIS#2845-2015100520053611v10) and all animals received care in strict compliance with institutional guidelines, and guidelines for the provision of standard care to laboratory animals.

This experimental study was performed in four-year-old pregnant ewes (Pre-Alp), 24-hours fast. All surgical procedures were performed under general anesthesia (induction with intravenous thiopental (10 mg/kg), endotracheal intubation and maintenance with isoflurane 2%) and sterile conditions. Fetal anesthesia was achieved by transplacental passage of the medications. For all interventions, ewes were administered nalbuphine (0.2 mg/kg, intramuscular) for pre and postoperative pain control. Intravenous cefamandole was administered as preoperative surgical prophylaxis, and cefamandole was also instilled into the amniotic fluid before hysterotomy closure. Duration of each surgery was recorded.

Creation of MMC defect

A myelomeningocele lumbar defect was created at 75 days in 14 fetal lambs, as previously described [11,12]. Briefly, low para-mammary incision and hysterotomy were performed to expose the fetal back. The lumbar skin and the paraspinal muscles were removed using an electric bovie (60W) to expose the spinal cord. A complete laminectomy from L1 to L5 was performed. The dorsal portion of the dura was excised between the origins of the dorsal roots from L1 to L5 and a midline myelotomy was performed to enter the central canal and observe a cerebro-spinal fluid (CSF) leak. The defect measurements average were 4.9±0.4 cm by 4.5±0.4 cm. The fetus was then returned to the uterine cavity, and intrauterine warm lactated Ringer's solution was administrated before hysterotomy closure, using a continuous running suture (2-0 Vicryl®). Maternal abdominal wall and skin were closed using 1-0 Vicryl®.

Correction of MMC defect

The MMC defect repair was performed at gestational age 90 days. Fetuses were then randomized into three groups. No further intervention was performed in the control group. An open repair of the defect (oMMC) was performed in 7 fetuses and a fetoscopic repair (fMMC) was performed in 7 fetuses at day 90. In order to reduce operative time, neither the skin edges nor the edges of the placode were revised in both groups. For all fetuses, the size of the defect prior to the repair was similar to the one measured at the time of the creation in all fetuses.

In the oMMC group, the MMC was repaired using a two-layer suture, after exposure of the MMC lesion through a hysterotomy of 5cm. The muscles were closed using 6-0 mononylon-interrupted sutures and the skin was closed using 5-0 mononylon-interrupted sutures. Hysterotomy was closed with a running suture of 2-0 Vicryl® before reintegration of the uterus and closure of the maternal wall.

In the fMMC group, MMC defect repair was performed using fetoscopy with carbon dioxide insufflation. Because of the interposition of digestive structures in the ewe, these fetoscopic interventions were performed on an exteriorized uterus, after maternal laparotomy. An 11Fr introducer (Terumo®, Tokyo, Japan) was placed into the amniotic cavity using a Seldinger approach, under ultrasound guidance. Amniotic fluid was then partially removed and partial amniotic carbon dioxide low-pressure (10mmHg) insufflation was performed, as previously described [2,3]. After exposition of the fetal lumbosacral region, a laparoscopic 11mm balloon trocar (Kii® Advanced fixation sleeves, Applied Medical) was inserted into the amniotic cavity. The EndoSticth™ (Covidien) device was inserted through this second trocar to perform a single-layer running suture of the skin edges of the MMC defect using 4-0 barbed mononylon suture (V-loc® 4-0) (fig.1). This barbed suture comprises a loop at its extremity which allows to easily tighten the initial knot, and the subsequent running stitch is automatically blocked with no need neither to hold the suture between each stitch nor for a final knot. After the insufflated gas was removed, warmed lactated Ringer's solution with cefamandole was infused to restore a normal amniotic fluid index. The trocar insertion sites were closed using 2-0 Vicryl stiches and the uterus was reintegrated before the maternal wall closure.

Delivery, euthanasia and pathological evaluation

The lambs were delivered by cesarean section at 138 days' gestation (full term, 142 to 152 days, average 147 days) and sacrificed using thiopenthal (10mg/kg) and pentobarbital (140mg/kg) umbilical vein injections. The lamb skull and cervical spine were opened, and brain and cervical spinal cord were removed *en bloc* and post-fixated for 3 weeks in a 4% zinc formaldehyde solution. Macroscopic and microscopic studies were performed to assess the presence of hindbrain herniation. The hindbrain herniation was defined as the descent of the cerebellar vermis below the level of the foramen magnum. A macroscopic examination of the MMC area was performed to evaluate the defect healing and to look for any CSF leakage. The MMC area was then post-fixated in a 10% formaldehyde solution for three weeks before. A microscopic histological examination was performed to precise defect healing.

The main purpose of this study was to compare the effectiveness of the repair intervention defined as the correction of the hindbrain herniation and the complete closure of the skin over the MMC defect at birth. Secondary outcomes were represented by the operating duration of the procedures and the fetal loss rates. Statistical analyses were performed using the Chi-square test or Fisher's exact test, as appropriate. Statistical significance was set at a $p<0.05$.

Results

Pregnancy outcomes

Among the 18 fetuses involved in this study, 13 were alive and delivered at term: 3/4 (75.0%) in the control group, 4/7 (57.1%) in the oMMC group, and 6/7 (75.7%) in the fMMC groups (Fig.2). In the control group, a fetal death was diagnosed during the cesarean section, at term. In the oMMC group, three ewes had bleeding at 133 days of gestation. A fetal death was diagnosed in all these three cases. In the fMMC group, one ewe had a spontaneous labor at 130 days of gestation and vaginally delivered a stillborn lamb.

MMC defect was created in 18 lambs at a mean of gestational age of 74.6 ± 0.4 day's gestation. There was no fetal loss between the creation of the defect and the repair of the MMC defect in the 14 fetuses that had repair surgery at a mean gestational age of 89.2±0.8 days.

Effectiveness of the MMC repair surgeries

A complete suture of the lesion was achieved in both repaired groups. At birth, the four lambs of the control group presented no closure of the MMC defect and a hindbrain herniation at postmortem examination. All the lambs in the two repair groups (oMMC and fMMC) had a complete closure of the defect without leakage of cerebro-spinal fluid, and no case of hindbrain herniation was observed at post mortem examination. Histological examination confirmed the macroscopic findings. In the two repair groups, the scar of the defect consisted of collagenous connective tissue and skin, covering the spinal cord (fig.3). No case of tethered cord was observed neither in the 4 live lambs of the oMMC group, nor in the 6 live lambs of the fMMC group.

Complications of the MMC repair surgeries

Durations of surgeries were not statistically different between the oMMC group (60 min [45-90 min]) and the fMMC group (53 min [45-61 min]) (p=0.40). There was no significant difference between the two repair groups in the fetal loss rate (fMMC: 14%, oMMC: 43%, p=0.56).

Discussion

This experimental study demonstrates that fetoscopic repair of MMC using a single-layer running suture through a two-port access under carbon dioxide insufflation can effectively be achieved in the fetal lamb. In all cases, we observed a complete watertight closure of the defect and a reversal of hindbrain herniation at birth.

Kohl *et al.* and Pedreira *et al.* published their results of MMC repair in humans, using carbon dioxide insufflation fetoscopy, through a percutaneous approach using three or four trocars [3,4]. Unfortunately, they reported extended operation duration and very high rates of PROM, mitigating the potential benefit of this technique [6,7]. For these two groups, the defect was covered by a patch before the closure. Belfort *et al.* very recently published their results on a carbon dioxide insufflation fetoscopy in human fetuses [5]. They chose to facilitate the procedure by exposing the maternal uterus by laparotomy. The fetoscopic procedure used a two-port access in 10 "standardized" cases. The closure of the defect was achieved using interrupted stiches, with no dissection of the placode and no coverage of the placode with a patch. In these 10 cases, they recorded extended operation durations but they did not observe any PROM. These encouraging results for the risk of PROM might be explained by the reduction of number of trocars. As Quintero *et al.* reported in twin-twin transfusion syndrome treatment, the risk of PROM decreased with the use of a reduced number of trocars [13]. Similarly, it is known that extended operating time increases the risk of PROM in fetal surgery [14]. The excellent results achieved by Belfort *et al.* in terms of pregnancy prolongation need to be balanced by the moderate rate of 60% of hindbrain herniation reversal observed in these 10 cases at birth. In line with this, a cerebrospinal fluid leakage (CSF) from the defect was observed in 10% of their cases at birth. Our results confirmed the feasibility of the MMC defect suture through a two-port access after carbon dioxide insufflation. Furthermore, this technique achieved a watertight closure of the defect with a reversal of hindbrain herniation in all the cases and no CSF leakage at birth. These results provide additional evidence that, in lambs, a single-layer running suture might be sufficient to prevent hindbrain herniation. In addition, a running suture using barbed suture might be more appropriate than interrupted stitches as used by Belfort *et al.* to achieve a complete watertightness of the lesion.

Another benefit of our simplified technique is represented by an operative time that appears to be two to three times shorter as compared to the fetoscopic repair techniques currently performed in humans. In our study, the mean operating time was 53 minutes (45-61), and was similar to the one we recorded for open repair. Kohl *et al.* and Pedreira *et al.* in human studies reported mean operating times of 223 minutes (45-315) and 242 minutes (153-331) respectively, using a three or a four trocars access [3,4]. Belfort *et al.* reported a mean operating time of 246 minutes (206-233) using two trocars [5]. This reduction of the operating time is potentially partly explained by the easier access to the lamb fetus as compared to the human fetus, because of the thinner uterine wall and the cotyledonary placentation in sheep. However, the important reduction of the operating time with the surgical technique we described could not be only related to species differences and we believe that this technique can also reduce the operating time in humans. We already know from other fetoscopic procedures for fetal surgery, that the rate of premature rupture of membranes (PROM) is related with the operating duration [14]. Our results in sheep are consistent with these observations and the risk of fetal loss following the fetoscopic single-layer suture seems to be lower than the one we observed in open repair.

The fetoscopic MMC repair technique we described does not include the use of an inert patch or surgical glue before suture. The rational for using a patch in human studies is to favor protection of the neural cord from amniotic fluid environment and to prevent the tethered cord that may require postnatal surgical revision. Pedreira *et al.* cover the placode with a biocellulose patch while Kohl *et al*. used a collagen patch or a teflon patch [3,4]. In the Pedreira series, no surgeries for tethered cord were reported at 1 year of life. This outcome was not reported by Kohl *et al.* Belfort *et al.* opted not to use any patch for their fetoscopic repair technique and among the 10 cases they reported, no surgeries to treat tethered cord were needed [5]. Our results in sheep are consistent with the results of Belfort et *al.*. The rational for patch placement before suturing the defect remains a matter of debate. One might argue that if the suture is watertight, the neural tissues are no longer exposed to harmful effects of the amniotic environment. The other advantage of our technique is represented by the fact that we did not use surgical glue to obtain complete watertightness of the defect. This is also of importance as glue was incriminated for potential neurotoxicity [15].

The major limitation of our fetoscopic MMC repair technique is represented by the diameter of the EndoSticth™ device that needs to be inserted into a 10mm diameter trocar. This device is easy to handle but it is too wide considering the size of a fetal MMC lesion and the endoscope's diameter. The development of an appropriate sized device would also reduce the size of the uterine defect thus reducing the associated-risk of PROM. [16]. In addition, the 4-0 barbed mononylon suture we used is the smallest suture currently available adapted to the EndoSticth™. Although we did not observe any complication with this barbed mononylon suture, we believe that a 5-0 barbed mononylon suture will be more appropriate to fetal tissues. Further miniaturization of the EndoSticth™ device is thus mandatory in this fetal surgery.

In conclusion, our experimental study showed that a fetoscopic single-layer suture using a two-port access with carbon dioxide insufflation is feasible and effective to repair MMC lesion in sheep. This technique achieves a complete and watertight closure of the defect with a short operative time. Future developments of devices dedicated to fetal surgery would allow the introduction of this simplified technique for endoscopic surgery for antenatal treatment of myelomeningocele in humans.

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Legend's figures

Figure 1 – Endoscopic view of the MMC defect coverage at the 90th days'gestation (fMMC group).

After the initial knot, the single-layer running suture is automatically blocked, using a 4-0 barbed mononylon suture (V-loc® 4-0) with the EndoSticth™ device (Covidien).

Figure 2 – Survival rates after fetal creation and correction of MMC defects in fetal lambs

Figure 3 – Microscopic view of the MMC lesion complete closure at birth in a lamb that had fetoscopic suture of the MMC defect: transverse section through the centre of the MMC defect completely closed, with widely open vertebral arches, posterior horns lesions and a complete coverage of the defect by newly formed collagenous connective tissue (arrow) and skin (double arrows). SC: spinal cord, V: vertebra.

Figure 1

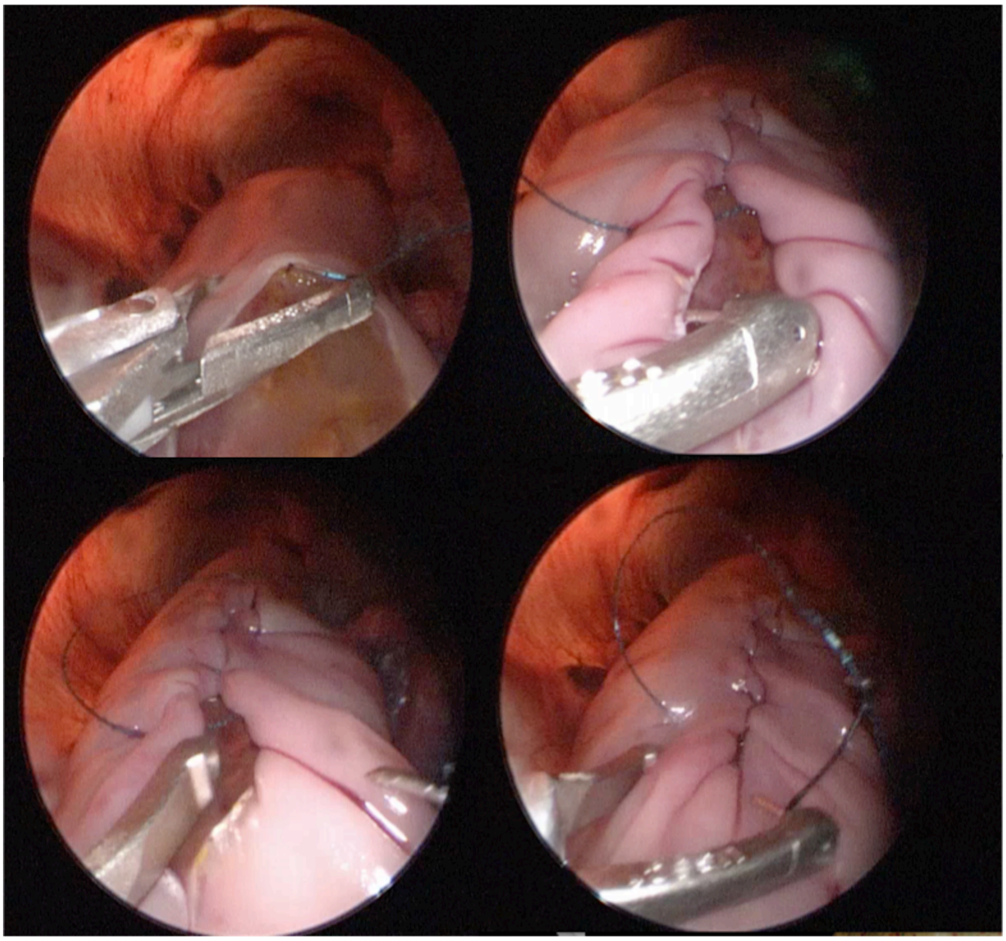

Figure 2

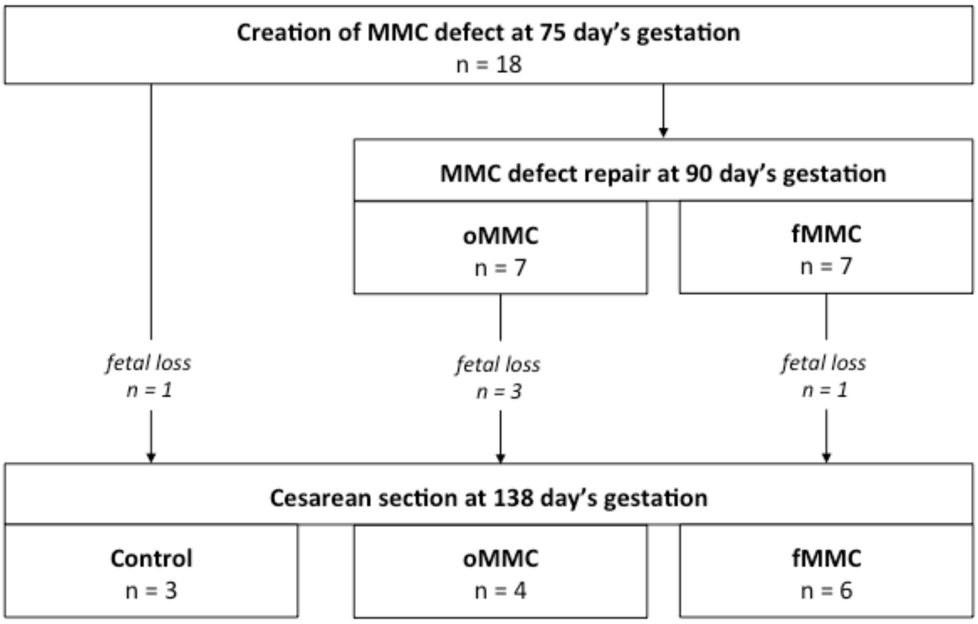
Creation of MMC defect at 75 day's gestation
n = 18
MMC defect repair at 90 day's gestation
oMMC
n = 7
fMMC
n = 7
fetal loss
n = 1
fetal loss
n = 3
fetal loss
n = 1
Cesarean section at 138 day's gestation
Control
n = 3
oMMC
n = 4
fMMC
n = 6

Figure 3