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ix. Bulleted statements (maximum 70 words)

x. Abstract

Objective: To determine the safety and efficacy of different neurosurgical techniques for closure of spina bifida (SB) in the fetal lamb model.

Method: Systematic review of studies reporting on fetal lambs undergoing induction and closure of SB compared to non-operated normal lambs (negative controls) and/or lambs not undergoing closure of the defect (positive controls). Primary outcomes were (1) survival at birth (safety) and/or (2) presence of Somatosensory Evoked Potentials on hind limbs and/or improvement in quantitative histological spinal cord findings and/or reversal of hindbrain herniation (efficacy).

Results: Out of 1311, 36 full-text articles were eligible. Nineteen were included for quality assessment. Due to high bias, only two adequately-powered studies were included in the final analysis. An open approach using a two-layer closure (muscle flap or acellular-dermal-matrix patch plus skin) was the only safe (patch+skin) and effective (both techniques) technique for prenatal closure in this animal model. No comparable level of evidence was identified for other techniques.

Conclusion: The experimental literature on prenatal SB closure underscores the lack of standardization. At present, there is animal experimental evidence that a 2-layer closure by hysterotomy is safe and effective. This technique is currently clinically used in a subset of patients. As new clinical techniques are introduced, it would seem logic to preclinically validate them against this experimental standard.

What's already known about this topic?

Surgical induction of SB in the fetal lamb induces some features of the clinical condition.

One experimental method was translated clinically and is one of the neurosurgical techniques later tested in a randomized clinical trial.

What does this study add?

A 2-layer open SB closure is safe and effective in the fetal lamb model.

There is no robust comparable evidence for other techniques.

INTRODUCTION

Spina bifida is one of the most common congenital anomalies of the central nervous system accounting for 5 per 10,000 live births in Europe and 3 per 10,000 live births in North America (1-4). Its most common form is an open defect called spina bifida aperta (SB). It is a non-lethal yet chronic and progressive disease with significant neurological morbidity. Its severity largely depends on the level of the defect (5-7). Affected children have various degrees of lower extremity paralysis, orthopedic disabilities, as well as bladder, bowel and sexual dysfunction (5). In addition to spinal cord dysfunction, children with SB almost invariably have an associated Chiari II hindbrain malformation and various degrees of ventriculomegaly. Though practices may vary, the latter is often treated by cerebrospinal fluid (CSF) shunting (5). SB presents as an *in utero* progressive disease, which is explained by the “two-hit” pathogenesis (8-14). First, there is a failure of neural tube closure by the 6th week of gestation. Second, continuous exposure causes damage to the neural elements, due to direct trauma and neurotoxic agents in the amniotic fluid. There is also an effect on the developing brain, evidenced by the development of ventriculomegaly and hindbrain herniation (HH) (9, 15-18). The latter is usually explained by CSF leakage at the level of the defect, leading to a “suction gradient” (18-20).

Obvious *in utero* progression is the rationale for fetal repair. The benefit of the latter was first experimentally proven by a landmark study by Meuli et al, using a latissimus dorsi muscle reversed rotation flap and skin closure of an earlier induced surgical defect in fetal lambs (10). The concept of fetal closure was subsequently clinically translated. The first attempts were by fetoscopy by the group from Vanderbilt University Medical Center, using a maternal split-thickness skin graft, Surgicel® and fibrin glue over the undissected lesion (21). This was abandoned due to fetal and maternal complications (22). The first case done by hysterotomy was published by Adzick et al., using placode dissection and skin closure (23). The group from the Children’s Hospital of Philadelphia later modified their technique, either closing the dura primarily or adding an Alloderm® patch for dural closure in large defects (24). They, as well as the Vanderbilt group, also used lumbodorsal fascial flaps and skin closure (referred to as “anatomical repair”, similar to what is done postnatally) later on (24, 25). Subsequently the concept of fetal surgery was tested and its safety and efficacy confirmed in a randomized controlled trial (Management of Myelomeningocele Study, MOMS) (12, 26, 27). The MOMS trial demonstrated that fetal surgery of SB via hysterotomy reduces the need for ventriculo-peritoneal shunting, reverses hindbrain herniation and

improves motor function. In that study, for most cases an anatomical repair was done. For larger defects dural replacement by Duragen® patch or skin substitutes by Alloderm® patch were done as well. Since, there has been an ongoing interest in alternative techniques as the current fetal surgical standard of care is not without risks for the mother and the fetus, mainly due to the hysterotomy and ensuing prematurity. Other teams explored fetoscopic surgery further, using a variety of neurosurgical techniques at times in combination with different patches (28-31). The place of fetoscopy remains controversial (32-36), and one of the topics of discussion is the efficacy of the neurosurgical repair.

Experimental models have and continue to play a critical role before clinical implementation of any new fetal surgery technique. Logically neurosurgical techniques are best explored in a large animal model. The fetal lamb has been the one most used (10, 37). Yet also that model has undergone evolution over time in an effort to reproduce as much as possible the clinical phenotype. Essentially the neural tube is exposed by a wide resection of skin, paravertebral muscles, lamina and dura over the fetal lumbar region (37). Later on a myelotomy, i.e. posterior mid-sagittal incision of the spinal canal, was added to increase the occurrence of HH (11, 19). Herein we aimed to systematically review that experimental literature, to determine the safety and efficacy of different fetal neurosurgical techniques when applied to the SB fetal lamb model.

METHODOLOGY

Study design

This systematic review was conducted in line with the guidance provided in the PRISMA (Preferred Reporting Items for Systematic reviews and Meta-analyses) and the SyRF (CAMARADES-NC3Rs preclinical Systematic Review & meta-analysis Facility) statements (38, 39). The protocol was based on the SyRCLE guidelines (Systematic Review Centre for Laboratory animal Experimentation) and published on the SyRF Protocol Registry website (39, 40). Two authors (L.J. and F.D.B) independently performed all the steps described below. Any disagreement concerning a specific article or interpretation of the data was resolved by discussion and consensus or, if required, by consulting a third author (E.D).

Information Sources and search technique

In March 2017, we searched Medline via PubMed, Web of Science, Embase and Scopus databases to identify relevant articles published in English without time limit. Subsequently, we conducted a search of the gray literature (200 first hits in Google Scholar). Free text and Medical Subject Headings (MeSH) terms used and combined were (1) sheep/ovine/lamb, (2) fetal therapy/fetal surgery/fetoscopy/therapy/open fetal surgery, and (3) myelomeningocele/spina bifida/myeloschisis/spinal dysraphism. The term “fetus” was not used as a fourth independent term in order not to decrease the number of hits hence miss any article. Endnote (Clarivate Analytics, Boston, MA, USA) was used to eliminate type-I (among different databases) and type-II (in different journals/issues) duplicate reports (41). Finally, a hand search of the reference lists of all eligible articles was conducted to identify further relevant papers.

Eligibility Criteria and study selection

We included all original peer-reviewed studies aiming at determining the safety and/or efficacy of SB closure in the fetal lamb model. These studies had to assess the outcomes of fetal lambs that underwent prenatal SB induction and closure and were compared to outcomes of non-operated normal lambs (negative controls) and/or unclosed SB lambs (positive controls). Studies were included irrespective of sheep breed, technique of induction of SB (with or without myelotomy (19, 37), closure technique and type of approach (via hysterotomy [referred to as “open”] or fetoscopy).

We excluded articles where the body of the text was not in English, that were on other topics, clinical studies, review articles or medical book chapters, studies using other animal models, veterinary sheep case-reports, conference/congress abstracts, letters to the editor or correspondence. Studies using the fetal lamb model with only a skin defect or using the SB model that was left unclosed hence without treatment group, were also excluded. Studies passing the first screening were read in full. We then excluded articles with suboptimal methodology. This included studies where the SB defect was induced and closed simultaneously during the same procedure and where there were no control groups to compare to the experimental group. We finally excluded studies that did not explicitly aim at determining safety and/or efficacy of a new technique in the SB fetal lamb model but rather feasibility of a new assessment method or a new technique. As displayed in the flowchart, a stepwise study selection was performed (figure 1).

Data collection

A standardized form was used to extract data from the included safety and/or efficacy studies (supplementary table 1). Primary outcome for safety was survival at birth. For efficacy, the composite primary outcomes were objective and quantitative assessments of the spinal cord or the brain. This included the presence of either Somatosensory Evoked Potentials (SEP) in the hind limbs and/or improvement of quantitative histological spinal cord findings and/or reversal of HH. Secondary outcome measures were assessed at birth, including the rate of complete closure of the SB defect, presence of macroscopically visible fluid leakage over the lumbar scar, sensory-motor deficit based on neurological examination, non-quantitative histology of the spinal cord, histology of brain, rectum and bladder, imaging of the brain, spinal cord and urinary tract and urodynamic investigation. Closure of the defect was categorized as either being a “repair”, i.e. with prior dissection around the defect followed by skin repair or grafting, or “coverage” when the defect was covered yet without prior dissection (figures 2-3). Studies were categorized as including negative controls (normal lambs) and/or positive controls (unclosed SB; figure 2).

Quality appraisal

Risk of bias assessed study quality of all eligible studies using a checklist of 14 items adapted from the SYRCLE's Risk of Bias tool (9 first items) and the CAMARADES (Collaborative Approach to Meta-Analysis and Review of Animal Data from Experimental Studies) quality checklist (5 last items) (39, 42).

Based on the SYRCLE guidelines, studies were defined as having high risk of bias when they would demonstrate high selection bias ($\geq 2/3$ items), high attrition and reporting bias (2/2 items) and/or when they would be underpowered (table 1). Selection bias depended on 3 items: (1) absence of standardization of surgical induction and/or closure (inadequate sequence generation), (2) inadequate SB induction and repair differing from the initial model (37), i.e. with SB skin defect $< 4 \times 4$ cm at induction and/or with < 21 days interval between induction and closure (dissimilar baseline characteristics) and (3) absence of random group allocation (inadequate allocation concealment). To demonstrate whether studies were adequately powered for the primary outcome, we calculated the sample size using Chi-square test and 2-sided Fisher's exact test to compare group proportions with 5% significance level and 80% power (43, 44). We determined the required numbers per group by using the highest number computed by the two tests.

Data analysis

Data were combined using descriptive analysis and further analyzed for all outcomes. Studies with high risk of bias were ruled out from final quantitative analysis.

RESULTS

Study Selection and data extraction

Our literature search identified 1311 publications. Of these, the vast majority (n=1275, 97%) were excluded on reading the title and/or abstract (figure 1). This left 36 (3%) studies for full-text evaluation and data extraction. Based on the aforementioned criteria, we excluded 17 more studies because of simultaneous induction and closure of the lesion (n=5), absence of controls (n=5) and a feasibility study design (n=7; figure 1, supplementary table 2).

Study Characteristics and Risk of Bias

Nineteen (1.4%) studies were included in the qualitative analysis. This encompassed 17 studies evaluating SB closure technique via open approach and 2 studies via fetoscopic approach. Quality assessment revealed 17 studies with high risk of bias (figure 1, table 1). This was due to high selection bias (n=13), high attrition and reporting bias (n=2) and underpowered study design (n=2) (figure 1, table 1). The surgical techniques of induction and closure of these 24 studies are detailed in table 2 and figures 2 and 3.

Firstly, high selection bias was mainly caused by an inadequate SB induction (table 1, item 2; table 2). Actually, in those studies were the skin defect was $<4 \times 4$ cm, the spontaneous closure rate among SB lambs with unclosed lesions was 17-80% instead of 0% when the skin defect was $\geq 4 \times 4$ cm (figure 2; table 3) (10, 11, 45-52). Animals that also had a myelotomy at the induction of SB were apparently less likely to have spontaneous closure (17-56%) as opposed to those who did not have a myelotomy (22-80%). Furthermore, with a defect $<4 \times 4$ cm and additional myelotomy, the HH rate at birth was lower (22-50%) than larger lesions with myelotomy ($\geq 85\%$) (11, 45-47). Another study with high selection bias, due to a <21 -day interval between induction and repair, was excluded from the comparison as it had high attrition and reporting bias and was underpowered (table 3) (53). This 3-week interval is necessary to obtain a $\geq 85\%$ rate of HH (instead of $\leq 50\%$ after 2 weeks) when a myelotomy is performed at SB induction (figure 2C-D) (11, 19, 47).

Secondly, two studies without high selection bias but with high attrition and reporting bias were also put aside (54, 55).

Finally, two studies were removed from final analysis because they were underpowered. Watanabe et al. had only one lamb in the positive control group (56). The study by Wang et al. allegedly aimed to demonstrate superiority of a 3-layer SB closure with or without mesenchymal stromal cells (57). For such comparison, the sample size should have been of at least 10 surviving lambs per group to detect an expected difference between 0% for independent ambulation in the group without cells and 67% in the group with cells.

Analysis of outcome data

The quality assessment left two (0.2%) studies with a low risk of bias for final data analysis. Results are displayed in table 4. In the first study by Meuli et al., SB was adequately induced without myelotomy (figure 2A-B), and the lesion was repaired in two layers using a latissimus dorsi muscle reversed rotation flap sutured to the tissue surrounding the defect and skin closure with sutures (figure 3A-C) (10). Their primary outcome (independent ambulation) and one of their secondary outcomes (SEPs) had comparable results, 0% in the unrepaired lambs and 100% in the repaired group. Even if there is no mention of a formal sample size calculation, this study is in retrospect well-powered for both outcomes as a minimum of 3 lambs alive at birth was required. Consequently, this 2-layer flap technique is effective because it showed significant improvement in primary (SEPs) and secondary outcomes, i.e. neurological examination, skin closure and histology of spinal cord. As the survival rate at birth was not reported in this paper, statements on safety cannot be made.

The second study by Bouchard et al. was very comparable, yet fetal lambs had a myelotomy at the time of SB induction (figure 2C-F), and the lesion was closed in two layers using an acellular dermal collagen matrix rather than a flap (figure 3A,D; table 4) (11). HH was the primary outcome measure. There was no formal mention of a power analysis, yet in retrospect the numbers in the study were sufficient to demonstrate efficacy. They detected a 74% difference in reversal of HH at birth (15% of the 13 unrepaired animals and 89% of the 9 survivors in the repaired group had a normal hindbrain position). Also, motor outcomes significantly improved. Survival rates were 93% (13/14) and 90% (9/10) in both groups. Based on these differences, the 2-layer matrix open repair can also be considered safe and effective.

DISCUSSION

Against the background of continuing interests to improve outcomes of fetal SB repair by animal experimentation prior to their clinical introduction, we aimed to systematically review the animal experimental data. For that purpose, we determined the safety and efficacy of different fetal surgical closure techniques for SB in the fetal lamb model. There is at present experimental evidence that a 2-layer closure via hysterotomy is safe and effective. There is no such evidence for other techniques. In view of the introduction of fetoscopic SB closure techniques, there is currently no experiment testing a 2-layer closure similar to open surgery, neither is there any evidence of safety and efficacy of any alternative fetoscopic technique.

We learned several lessons from this literature search into preclinical fetal surgical research related to SB. First, our systematic review shows the lack of standardization in most of the published studies and highlights the need for standardized methodology when performing animal studies to assess the safety and efficacy of fetal SB repair. Second, it demonstrates the relevance of the exact nature of the lesion being induced. There is variation in the timing of induction (range: 60-81 days), yet the most striking observation was that the method of induction is directly related to the *in utero* effects. Assuming the clinical condition typically includes CSF leakage and HH at birth, this is best experimentally reproduced by creating a large enough defect associated with a myelotomy i.e. a posterior mid-sagittal incision of the spinal canal. For instance, skin defects $<4 \times 4$ cm at induction have a tendency to close spontaneously in 17-80% of the lambs (10, 11, 45-52). This is associated with an absence of HH at birth in 0-78% of cases (11, 45-47). Along the same lines, it seems that HH is best reproduced when a myelotomy is performed (85%) as compared to when it is not (0%) (11). The lack of these features caused a high risk of selection bias in 13/19 studies. This bias justifies the eventual exclusion of these studies from final analysis of safety and efficacy. We have therefore decided for our experimental programs to standardize induction of the defect by performing (1) a circular $\geq 4 \times 4$ cm skin and subcutaneous fascia resection, (2) bilateral subtotal paraspinal muscle resection, (3) ≥ 5 -level laminectomy, (4) ≥ 5 -level posterior durotomy, and (5) ≥ 5 -level mid-sagittal posterior myelotomy. That method should give a 100% persistence of the defect as well as $\geq 85\%$ presence of HH at birth. Standardizing the lesion for most effective reproduction of the condition is in line with the ARRIVE (Animal Research Reporting In Vivo Experiments) guidelines (44).

Third, this review investigated the outcomes of SB coverage via hysterotomy. Again, 13 studies had high risk of bias mainly due to inadequate SB induction, yet apart from that, they tested a broad range of repair or coverage techniques, in one to three layers (table 2). Most used was a two-layer technique (n=9), either with a muscle flap (n=2), native dura (n=1) or a patch (n=6) with skin closure. This left only two studies with appropriately induced lesions, closed by a two-layer repair via open approach, with either a muscle flap or an acellular collagen matrix patch, covered by skin closure, which were demonstrated to be safe (patch plus skin technique only) and effective (both techniques). To our knowledge, none of the two experimental methods has been translated into clinical practice. The technique using the rotation muscle flap was only applied in a feasibility study on aborted human fetuses with SB (58). Yet a modification using primarily dural closure or lumbodorsal myofascial flaps was performed from the onset of the clinical experience (24, 25). The other technique was also not used clinically. Another modification using Alloderm® or Duragen® patch for the dura was done in a subset of patients with large dural defects from the pre-MOMS and MOMS cohorts respectively, yet in an unknown number of patients and at the surgeon's discretion (12, 24, 59). Other clinically used techniques include the use of Alloderm® patch or Integra® dermal regeneration template as a skin patch for large skin defects (12, 60-62). These materials have to our knowledge not been experimentally tested.

Two sheep studies evaluated fetoscopic SB coverage using a similar coverage methods in two layers (silastic or Matriderm® covered with CoSeal® bioglue) (63, 64). Safety was comparable to what was observed in the two studies included in the final analysis. However, both studies did induce a more moderate lesion, which may temper the effect of the simulated SB condition. Apart from that limitation, one fetoscopic experiment did not report detailed outcome measures (64). Finally, both studies were prone to attrition and reporting bias. This prompts us to plead for additional experimental research on this approach, or any modification one considers, using standardized induction and closure and standardized outcome measures, as well as controls that are repaired using a validated two-layer technique by hysterotomy. We think such experiments should be done prior to wide clinical implementation of any presumed technique, and we are currently doing so. At present, as fetal surgery is widely spreading, surgeons start using new neurosurgical techniques or modify them, without formally testing them in ideal experimental conditions (28-31, 65).

This study and its interpretation have several limitations. First, the eventually selected studies still have some bias and were less than ideally prospectively designed. Second, there are many limitations to experimental models. The fetal lamb model is a non-malformative

model where surgical induction is done after normal primary neurulation. Therefore, it only mimics the second out of two hits that cause clinical SB (8-14). Despite standardization, surgical induction and closure of the lesion may on itself cause direct damage and their quality may be relatively heterogeneous. These limitations could be overcome by using other large animal models of naturally occurring SB such as Pugs and English bulldogs. In those animals, the prevalence of SB is as high as 38.2% and 2.4%, respectively (66, 67). Third, the embryology and anatomy of the fetal lamb, uterus and placenta differs from humans, which in turn may impede extrapolation or testing of specific techniques or instrumentation. Finally, it should be realized that the most widely clinical technique consisting of hysterotomy, and a mimic of clinical postnatal repair (consisting of appropriate dissection and primary closure with the logical anatomical structures such as dura, paraspinal myofascia and skin) has neither been experimentally tested (12, 59, 62). The reason for that is that it is technically impossible to perform myofascial closure in the SB fetal lamb model, because the creation of the model requires excision of the paravertebral muscles and fascia. Of course, the safety and efficacy of the current anatomical technique can meanwhile not be denied, as it is the only one supported by level-I evidence (12). It would seem logic that ultimately any technical innovation, whether it is on closure techniques, graft materials or other access routes, should be clinically tested against the “gold” standard.

In summary, there is animal experimental evidence that a two-layer repair of surgically induced SB by hysterotomy is safe and effective. A similar neurosurgical technique is currently clinically used in a subset of patients. As new clinical techniques are introduced, it would seem logic to preclinically validate them against this experimental standard in the SB fetal lamb model. Such experiments should use standardized methods for both timing of the experiments, i.e. induction and closure of the lesion, and objectively evaluating the effects of surgical closure (68, 69).

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Figure 1 - Study flow diagram adapted from the PRISMA 2009 flow diagram

Figure 2 – Technique of induction of spina bifida in the fetal lamb model

A) Induction of the lesion, consisting of a $\geq 4 \times 4$ cm skin and myofascial resection, ≥ 5 -level laminectomy, and ≥ 5 -level durectomy. In this case, without ≥ 5 -level myelotomy. Gestational age: 75 days (term, 145 days).

B) Artist impression of A).

C) Same as A), yet with ≥ 5 -level myelotomy, i.e. posterior mid-sagittal incision of the spinal canal.

D) Artist impression of C).

E) Lesion as above in C), 25 days later at 100 days, prior to closure.

F) Artist impression of a surgically induced open spina bifida-type of lesion.

Drawings by Myrthe Boymans (www.myrtheboymans.nl) for and copyright by UZ Leuven, Belgium.

(SB, spina bifida)

Figure 3 – Techniques of closure of spina bifida in the fetal lamb model

A) Surgically induced open spina bifida-type of lesion repaired at 100 days and overlaid by skin.

Artist impression of this lesion overlaid:

B) in a single layer by skin closure

C) in two layers, i.e. a (latissimus dorsi) muscle flap and skin closure

D) in two layers, i.e. an (acellular dermal matrix) dural patch and skin closure

E) in a single layer using a patch. Bioglue has also be used for that purpose (not shown).

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(SB, spina bifida)

Table 1 – Quality assessment of included studies using a checklist of 14 items adapted from the SYRCLE's Risk of Bias tool (items 1 to 9) and the CAMARADES quality checklist (items 10-14)

A study was awarded a maximum of 1 point for each numbered item: 1 indicated low risk of bias and 0 high risk of bias. Studies filled the criteria of:

- Absence of selection bias: (1) adequate sequence generation (Was the allocation sequence adequately generated and applied, i.e. randomly and/or in a standardized fashion?); (2) similar baseline characteristics (Were the groups similar at baseline, i.e. with SB skin defect $\geq 4 \times 4$ cm and with a ≥ 21 days interval between creation and closure? Were they adjusted for confounders in the analysis?); (3) Allocation concealment (Was the allocation to the groups adequately concealed?)
- Absence of performance bias: (4) random housing (Were the animals randomly housed during the experiment?); (5) Performance blinding (Were the caregivers and/or investigators blinded from knowledge which intervention each animal received during the experiment?)
- Absence of Detection bias: (6) random outcome assessment (Were animals selected at random for outcome assessment?); (7) detection blinding (Was the outcome assessor blinded?)
- Absence of Attrition bias: (8) complete outcome data (Were incomplete outcome data adequately addressed?)
- Absence of Reporting bias: (9) no selective outcome reporting (Are reports of the study free of selective outcome reporting?)
- Absence of other sources of bias: (10) precise animal species; (11) well-powered study (sample size calculation/justification number of animals used); (12) description of primary and secondary outcomes; (13) compliance with animal welfare regulations/ethical committee approval; (14) statement of potential conflict of interests.

(AJPr, American Journal of Perinatology reports; USOG, journal UltraSound in Obstetrics & Gynecology)

Items	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Absence of bias of	selection			performance		detection		attrition	reporting	Absence of other sources of bias				
Meuli et al. 1995	1	1	0	0	0	0	0	1	1	0	1	1	0	0
Meuli et al. 1996	1	1	0	0	0	0	0	0	0	0	1	0	0	0
Yingling et al. 1999	1	1	0	0	0	0	0	0	0	0	1	0	0	0
Paek et al. 2000	0	0	0	0	0	0	1	0	0	0	0	1	1	0
Bouchard et al. 2003	1	1	0	0	0	0	0	0	1	1	1	1	1	0
Yoshizawa et al. 2003	0	0	0	0	0	0	0	1	0	0	0	1	1	0
Yoshizawa et al. 2004	0	0	0	0	0	0	0	1	0	0	0	1	1	0
Sanchez e Oliveira et al. 2007	1	0	0	0	0	0	0	1	0	1	0	0	1	1
Eggink et al. 2008	0	0	0	0	0	0	0	1	1	1	0	0	1	0
Abou-Jamra et al. 2009	0	0	0	0	0	0	0	0	0	1	0	0	1	1
Fontecha et al. 2009	0	0	0	0	0	0	0	0	0	1	1	1	1	1
Encinas et al. 2011	0	0	0	0	0	0	1	0	0	0	0	0	1	0
Fontecha et al. 2011	0	0	0	0	0	0	1	0	0	1	1	1	1	0
Peiro et al. 2013	0	0	0	0	0	0	0	0	0	1	1	1	1	1
Guilbaud et al. 2014	1	0	0	0	0	0	1	0	0	1	0	0	1	0
Wang et al. 2015	1	1	0	0	0	0	1	0	0	1	0	1	1	1
Papanna et al. 2016 - AJPr	0	0	0	0	0	0	1	0	0	0	0	1	1	1
Papanna et al. 2016 - USOG	0	0	0	0	0	0	1	1	1	0	1	1	1	1
Watanabe et al. 2016	1	1	0	0	0	0	1	1	1	0	0	0	1	1

Table 2 – Surgical techniques of the 24 included studies with high risk of bias

Alloderm® is a Human cadaver Acellular Dermal Matrix (HADM); AJPr, American Journal of Perinatology reports; USOG, journal UltraSound in Obstetrics & Gynecology.

(SB, spina bifida aperta; GA, mean gestational age in days; I, induction; C, closure; NS, non-specified; SD, standard deviation; LD, Latissimus dorsi muscle; SIS, small intestinal submucosa biomatrix; SI, Silastic patch; SM, Silastic and Marlex patch; pLA, poly L-lactic acid patch; PLC-PPG, PolyL-Lactide-co-Caprolactone and PolyPropylene Glycol and sodium acetate; AM, autologous Amniotic Membrane patch; HUC-A, human umbilical cord-AmnioGuard patch)

No. of full-text article	Size SB skin defect	GA at induction (days)	GA at closure (days)	Interval (days)	Technique of SB fetal closure (approach & closure)	
Meuli et al. 1995	4x4cm	75	100	≥ 21	Open	2 layers Latissimus dorsi muscle flap + skin repair
Yingling et al. 1999	4x4cm	75	100	≥ 21	Open	
Paek et al. 2000	NS (oval)	75	100	≥ 21	Open	1 or 3 layers Alloderm® patch coverage or Dura + muscle + skin repair
Yoshizawa et al. 2003	NS (oval)	75	100	≥ 21	Open	1 or 3 layers Gore-Tex® patch coverage or Alloderm® patch coverage or Dura + muscle + skin repair
Yoshizawa et al. 2004	NS (oval)	75	100	≥ 21	Open	
Sanchez e Oliveira et al. 2007	1.5x3cm	NS (72-78)	NS (93-105)	≥ 21	Open	2 layers Alloderm® or cellulose patch + skin repair
Eggink et al. 2008	3x3cm	NS (72 or 79)	NS (86 or 93)	< 21	Open	1 layer Collagen patch coverage
Abou-Jamra et al. 2009	2x3cm	75 (74-78)	102 (98 - 112)	≥ 21	Open	2 layers Cellulose patch + skin repair
Fontecha et al. 2009	NS (oval)	75	95	< 21	Open	2 layers SI or SM patch + CoSeal® glue coverage
Encinas et al. 2011	NS	75	100	≥ 21	Open	≥1 layers CoSeal® glue coverage or NS repair
Fontecha et al. 2011	NS (oval)	75 (SD 5.8)	101 (SD 5.6)	≥ 21	Fetoscopy	2 layers SI patch + CoSeal® glue coverage
Peiro et al. 2013	NS (oval)	75	95	< 21	Fetoscopy	2 layers SI or collagen (Matriderm®) patch + CoSeal® glue coverage
Guilbaud et al. 2014	3x5cm	74 (73–75)	89 (88-90)	< 21	Open	2 layers Dura + skin repair
Wang et al. 2015	4x4cm	77 (73-81)	103 (97-107)	≥ 21	Open	3 layers collagen + extracellular matrix patch (Oasis®) + skin repair
Papanna et al. 2016 - AJPr	4x4cm	75	95	< 21	Open	1 layer HUC-A patch coverage
Papanna et al. 2016 - USOG	3x4cm	75	95	< 21	Open	
Watanabe et al. 2016	4.1x4.5cm	72	100	≥ 21	Open	1 or 2 layers Sponge (gelatin or gelatin/collagen) + gelatin sheet repair

Table 3 – Comparison of outcomes at birth of unclosed spina bifida fetuses between inadequate and standard surgical inductions

A bilateral paraspinal muscle resection was performed in all studies after skin resection and before laminectomy. Even if durectomy was not performed in some studies, a durotomy (incision of dura mater) was done.

Eligible studies after subanalysis	Skin resection	Laminectomy	Durectomy	Survival	Complete skin closure	Hindbrain herniation
Unclosed SB lambs without myelotomy						
Standard technique Meuli et al. 1995	4x4cm circle	4 levels (L1-4)	Yes (L1-4)	NS	0% (0/3)	NS
Eggink et al. 2008	3x3cm approx.	3 levels (L3-5)	No	71% (5/7)	80% (4/5)	0% (0/5)
Abou-Jamra et al. 2009	2x3cm	NS	No	Unclear 56% (9/16)	22% (2/9)	NS
Fontecha et al. 2009 *	NS (oval)	3 levels	No	83% (n=5/6)	0% (0/5)*	Unclear 100% (5/5)*
Fontecha et al. 2011 *	NS (oval)	3 levels	No	67% (4/6)	0% (0/5)*	100% (4/4)*
Peiro et al. 2013 *	NS (oval)	3 levels	No	80% (4/5)	0% (0/4)*	100% (4/4)*
Unclosed SB lambs with myelotomy						
Standard technique Bouchard et al. 2003	4x4cm circle	5 levels (L1-5)	Yes (L1-5)	93% (13/14)	0% (0/13)	85% (11/13)
Paek et al. 2000 **	NS (oval)	4 levels (L1-4)	Yes (L1-4)	Unclear 37.5% (3/8)	Unclear ** 12.5% (1/8)	Unclear ** 100% (3/3)
Sanchez e Oliveira et al. 2007	1.5x3cm	4 levels	No	17% (4/23)	17% (1/6)	NS
Encinas et al. 2011	NS	NS	NS	60% (9/15)	56% (5/9)	22% (2/9)
Guilbaud et al. 2014	3x5cm	5 levels (L1-5)	Yes (L1-5)	43% (6/14)	17% (1/6)	50% (3/6)
Papanna et al. 2016 USOG ***	3x4cm	3 levels (L4-6)	Yes (L4-6)	100% (2/2)	0% (0/2) ***	100% (2/2) ***

* These 3 papers from the same group were excluded from the comparison as they had high risk of bias (especially attrition and reporting bias) and their results (100% of hindbrain herniation without myelotomy) have never been reproduced by another group.

** These 2 papers from 2 groups were excluded from the comparison as they were underpowered (only 2 animals per group).

*** Paper also excluded from comparison. The rate of HH at birth is unclear due to attrition and reporting bias. In fact, one fetus had its SB defect spontaneously closed at the time of repair (100 days) and was excluded from analysis. If this fetus had been allocated to this unrepaired SB group and had survived until birth, the HH rate would have been 75% (3/4)

(SB, spina bifida aperta; HH, hindbrain herniation; NS, non-specified; L, lumbar spinal level; USOG, USOG, journal UltraSound in Obstetrics & Gynecology)

Table 4 – Demographics and outcomes of spina bifida fetal closure in the spina bifida sheep model

Included studies	Meuli et al. 1995	Bouchard et al. 2003
Demographics		
Sheep breed	NS	Western Cross
Number of fetal SB inductions (75d)	NS	34
Myelotomy	No	Yes (n=28)
Number of fetal SB repairs (100d)	NS	14
Approach	open	open
Type of closure	repair	repair
Number of layers	2 layers Latissimus dorsi muscle flap + skin closure	2 layers Acellular dermal matrix patch (XenoDerm®) + skin closure
Negative control group (normal lambs)	N=10 (for neurological examination)	N=8
Positive control group (unclosed SB)	N=3	N=14
Experimental group (closed SB)	N=3	N=14
Primary outcomes		
Survival at birth		
Normal lambs	NS	NS
Unrepaired SB lambs	NS	13/14 (93%)
Repaired SB lambs	NS	9/10 (90%) *
SEP on hind limbs		
Normal lambs	NS	NS
Unrepaired SB lambs	0/3 (0%)	NS
Repaired SB lambs	3/3 (100%)	NS
Absence of HH at birth		
Normal lambs	NS	NS (100%)
Unrepaired SB lambs	NS	2/13 (15%)
Repaired SB lambs	NS	8/9 (89%)
Histology of lumbar spinal cord		
Normal lambs	NS	NS
Unrepaired SB lambs	Abnormal cytoarchitecture: extreme flattening and erosion of dorsal portion or complete loss neural tissue.	Loss of neural tissue; disruption of neural bundles, areas of cord necrosis.
Repaired SB lambs	Good preservation of cytoarchitecture with discernible landmarks: mild flattening of spinal cord and mild dilatation of central canal	NS (proximal to lesion, normal in structure and myelination; no anomalies or dilatation of the central canal)
Secondary outcomes		
Closure of lumbar skin at birth		
Normal lambs	NS	NS
Unrepaired SB lambs	0/3 (0%)	0/13 (0%) †
Repaired SB lambs	3/3 (100%)	NS
Presence of lumbar SB leakage at birth		
Normal lambs	NS	NS
Neurological examination		
Normal lambs	Normal (no deficit)	NS
Unrepaired SB lambs	3/3 (100%) sensory-motor paraplegia, stool-urine incontinence	13/13 flaccid sensory-motor paraplegia, stool-urine incontinence
Repaired SB lambs	3/3 (100%) near-normal hind limb function & no stool-urine incontinence. Able to walk, stand, stair climbing.	9/9 improvement with partial weight-bearing on the hind limbs; stool-urine incontinence
Histology of brain		
Normal lambs	NS	No hydrocephaly
Unrepaired SB lambs	No hydrocephaly	0/13 (0%) hydrocephaly; normal brain/body weights
Repaired SB lambs	No hydrocephaly	0/9 (0%) hydrocephaly; normal brain/body weights
Histology of rectum and bladder		
Normal lambs	NS	NS
Imaging of the brain and spinal cord		
Normal lambs	NS	NS
Urodynamics		
Normal lambs	NS	NS

* Exclusion of 4/14 created fetuses that were harvested (autopsy) before term delivery.

† cystic sacs

(SB, spina bifida aperta; SC, spinal cord; HH, hindbrain herniation; SEP, Somatosensory Evoked Potentials; SLR score, Sheep Locomotor Rating on neurological examination; NS, non-specified)

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