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## **Cell therapy for prenatal repair of myelomeningocele: a systematic review**

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# 1 Cell therapy for prenatal repair of myelomeningocele: a systematic 2 review

## 6 Summary

7 Myelomeningocele (MMC) is a spinal cord congenital defect that leads to paraplegia,  
8 bladder incontinence and bowel dysfunction. A randomized human trial demonstrated that *in*  
9 *utero* surgical repair of the MMC defect improves lower limb motor function. However,  
10 functional recovery remains incomplete. Stem cell therapy has recently generated great  
11 interest in the field of prenatal repair of MMC. In this systematic review we attempt to provide  
12 an overview of the current application of stem cells in different animal models of MMC.  
13 Publications were retrieved from PubMed and Cochrane Library databases. This process  
14 yielded twenty-two studies for inclusion in this review, experimenting five different types of  
15 stem cells: human embryonic stem cells, neural stem cells, induced pluripotent stem cells,  
16 human amniotic fluid stem cells, and mesenchymal stem cells (MSCs). Rodents and ovine  
17 were the two major species used for animal model studies. The source, the aims, and the  
18 main results were analyzed. Stem cell therapy appears to be a promising candidate for  
19 prenatal repair of MMC, especially MSCs. Further explorations in ovine and rodent models,  
20 reporting clinical and functional results, are necessary before an application in humans.

21  
22 **Keywords:** cell therapy • stem cell • myelomeningocele • fetal surgery • spina bifida •  
23 sheep

## 31 **Introduction**

32 Myelomeningocele (MMC) is one of the most frequent congenital defects diagnosed  
33 by prenatal ultrasound and is characterized by the protrusion of the spinal cord through a  
34 vertebral defect [1]. This non-lethal congenital defect generates paraplegia, sphincter  
35 disorders and neurological bladder. This open dysraphism is almost always associated with  
36 Chiari II malformation, and sometimes with hydrocephalus that may be responsible of  
37 cognitive disabilities [2].

38 Since 1990, it is admitted that MMC results from disarrangement during the  
39 development of the posterior neuropore, at the fourth week of gestation, and spinal cord  
40 injuries are worsened during gestation. This mechanism called “the two-hit hypothesis” was  
41 confirmed by the efficiency of prenatal repair of MMC [3]. The american randomized  
42 multicentered study “Management Of Myelomeningocele Study” (MOMS), comparing the  
43 prenatal and postnatal surgery of MMC, showed that the prenatal surgical repair of MMC  
44 allows improving motor functions and cerebral benefit, with reduction by half of the need for  
45 ventriculo-peritoneal shunt after birth.

46 In spite of these encouraging neonatal outcomes, the results are not totally  
47 satisfactory, since 55% of prenatally treated children were unable to walk independently at  
48 30 months of age [3,4]. New treatments thus have to be developed in order to improve  
49 clinical outcomes. The field of stem cell therapy appears as a promising approach, as cell  
50 engineering is known to provide a benefit in repair of tissue injury, such as their use in large  
51 skin burns and in cornea repair [5,6].

52 Despite many reviews on surgical fetal repair of MMC, to our knowledge, no study  
53 has systematically reported the current evidence on the effectiveness of stem cell on this  
54 topic. The purpose of this study was a systematical review about the use of stem cell therapy  
55 for prenatal MMC repair.

56

## 57 **Materials and Methods**

### 58 *Search strategy*

59 We performed this systematic review following the Preferred Reporting Items for  
60 Systematic reviews and Meta-Analysis (PRISMA) protocol [7]. Two independent reviewers  
61 (A.D. and L.G.) conducted the systematic search for relevant studies using the electronic  
62 PubMed and Cochrane Library databases with the following terms: (myelomeningocele OR

63 spinal OR spina bifida) AND (prenatal OR in utero OR fetal OR embryo) AND (stem cell(s)  
64 OR stromal cell(s) OR cell therapy) NOT (spinal cord injury). Publications from the earliest  
65 record of the databases to March 30, 2020, were selected (figure 1).

#### 66 *Inclusion and exclusion criteria*

67 We included studies which met the following criteria: (a) designed as interventional  
68 studies; (b) studying animals who received stem cell therapy; (c) reports on clinical,  
69 histological, and/or immunohistochemical results. We excluded letters, editorials, replies from  
70 author, case reports, not systematical reviews and articles not published in English language.

#### 71 *Data extraction*

72 Data were extracted on first author, year of publication, number of cases, animal model,  
73 source of stem cell, way of administration and main results in the stem cell group (table 1).  
74 The same two reviewers excluded papers that were not appropriate for this review after  
75 screening based on the study title and/or on full-text studies.

76

## 77 **Results**

78

79 A total of 22 studies were found in the databases (table 1). Five different types of  
80 stem cells were used.

#### 81 *Human embryonic stem cells (hESCs)*

82 A cell-based therapy strategy for the treatment of MMC was first developed by Lee *et*  
83 *al.* [8,9]. They studied the closure capacity of a MMC-like defect by intra-amniotic injection of  
84 human embryonic stem cells (hESCs) in a surgical chick embryo model of MMC. The hESCs  
85 used for the repair were collected from frozen human blastocysts and injected as  
86 undifferentiated cells in the amniotic cavity, with a glucose phosphate-buffered saline vehicle.  
87 The authors showed that the lengths of the MMC defect were significantly shortened in the  
88 hESCs group as compared to the controls. They suggested that the hESCs mechanisms to  
89 improve MMC closure were the cell's paracrine activity and the mechanical bridging effect,  
90 but there were no experimentations to support this hypothesis.

#### 91 *Neural stem cells (NSCs)*

92 Fauza *et al.* introduced the use of neural stem cells (NSCs) engrafted in an ovine  
93 model [10]. A L1 to L5 defect was surgically created as previously described, with the aim to

94 evaluate the NSCs directly injected into the spinal cord, associated to its coverage with an  
95 acellular human dermal patch (AlloDerm) [11]. The 25 fetal lambs included were divided into  
96 three groups: NSCs injection and coverage with AlloDerm (n= 9), coverage with AlloDerm  
97 only (n=7) or no repair of the defect (n=9). NSCs were obtained from murine cerebellum and  
98 the repair was performed 20 days after the creation of MMC. After spontaneous vaginal  
99 delivery, the surviving lambs had a functional evaluation. No significant reduction of lamb  
100 paraparesis was observed in the group treated with NSCs and AlloDerm.  
101 Immunohistochemistry study demonstrated that NSCs were engrafted in the spinal cord.  
102 However, immunostaining of the spinal cords for immature neural stem markers and  
103 neuronal differentiation markers showed that these NSCs remained undifferentiated.  
104 Neurotrophic factors, such as glial cell line-derived neurotrophic factor (GDNF) and brain-  
105 derived neurotrophic factor (BDNF) were found both in the cytoplasm of the grafted cells and  
106 in the extracellular matrix surrounding the host cells, suggesting that both NSCs and host  
107 cells produced these factors.

108 The same team experimented rat's NSCs in a MMC rodent model [12]. The MMC was  
109 chemically induced by a single intragastric retinoic acid administration on embryonic day 10  
110 (E10). NSCs were obtained from dams amniotic fluid. They performed NSCs intra-amniotic  
111 injections on E17. At E21, they observed that NSCs were located on the neural placode,  
112 retaining an undifferentiated morphology, and predominantly located on exposed neural  
113 surfaces. Thus, they confirmed the feasibility of intra-amniotic injection of stem cells to repair  
114 MMC, in another model.

#### 115 *Induced pluripotent stem cells (iPSCs)*

116 Saadai *et al.* studied neural crest stem cells (NCSCs) derived from human induced  
117 pluripotent stem cells (iPSCs), in the fetal lamb model of MMC, within a biocompatible  
118 surgical scaffold [13]. The MMC defect was surgically created at 75 days of gestation, and  
119 repaired 25 days later in two lambs, with no control group. After sacrifice, 10 days prior to  
120 term, an immunohistochemistry analysis was performed on the lamb's spinal cords, showing  
121 the survival and the integration of the NCSCs in the spinal cord at the level of the MMC  
122 lesion.

123 In the retinoic acid-induced rat model, Kajiwara *et al.* experimented a three-  
124 dimensional (3D)-skin using iPSCs, derived from human amniotic fluid [14]. The 3D-skin was  
125 correctly transplanted on the MMC defect in the 20 fetal rats operated. The rationale of these  
126 authors was that the coverage of the MMC lesion using a 3D-skin enables less invasive and

127 earlier fetal intervention. Their results showed only 20% of complete coverage at birth, two-  
128 days after the 3D-skin graft.

### 129 *Human amniotic fluid stem cells*

130 Abe *et al.* experimented intra-amniotic injections of xenologous human amniotic fluid  
131 CD117-positive stem cells (hAFSCs), in the retinoic acid-induced rat model at E17 [15]. Their  
132 results showed that hAFSCs-treatment promotes skin coverage of the cutaneous defect. The  
133 authors also observed significant increased tubulin  $\beta$ -III areas in the spinal cords of hAFSCs  
134 group which could correspond to an induced neurogenesis in the MMC lesion.

### 135 *Mesenchymal stem cells*

136 Lee *et al.* experimented human bone marrow-derived mesenchymal stem cells (BM-  
137 MSCs) in the surgical chick embryo model of MMC [16]. Four groups were compared in their  
138 study: intraamniotic injection of human BM-MSCs, or human NSCs, or human foreskin  
139 fibroblasts (HFF), and an untreated control group. Their results showed a better reclosure of  
140 the MMC lesion in the BM-MSCs group.

141 Li *et al.* also experimented BM-MSCs in four studies, in the acid retinoic rodent model,  
142 previously described [17-20]. BM-MSCs were dam allogenic bone-marrow derived and  
143 injected directly into the defective region of the spinal cord.

144 In their first study, Real Time PCR (RT-PCR) analysis in spinal cords revealed that  
145 BDNF and Neural Growth Factor (NGF) expressions were significantly higher in the MSC-  
146 treated group compared to the control group [17]. Immuno-reactivity of BDNF and NGF was  
147 localized to both the GFP-labeled cells and in the adjacent host spinal cells, consistent with  
148 the study on NSC previously described [10]. A significant reduction of the expression of  
149 Caspase-3 and Bcl-2 in the BM-MSCs group was interpreted by the authors as a reduction of  
150 apoptosis after BM-MSCs transplantation.

151 In another study, they found an increased expression of Epidermal Growth Factor  
152 (EGF) and Fibroblast Growth Factor (FGF) by RT-PCR after transplantation of BM-MSCs  
153 [18]. EGF was not detected by immunofluorescence in the cultured cells but in the  
154 transplanted cells, suggesting an intrinsic response increasing growth factor expression.

155 In a third study, they observed an increased expression of the transcription factors  
156 Brn3a and Runx1 in the spinal cord, which could potentially promote the differentiation of the  
157 transplanted MSCs and the surrounding cells into sensory neurons in the spinal cord [19].

158 The same BM-MSCs were then engrafted in a chitosan-gelatin scaffold and surgically  
159 seeded on the MMC-like defect, and compared to a control unrepaired group, in the  
160 previously described MMC chemical rat model [20]. The authors concluded that their scaffold  
161 allowed the survival and the migration of the GFP-labelled BM-MSCs in the defective region  
162 of MMC. They also concluded that the co-localization of Nestin and Tubulin with GFP-BM-  
163 MSCs in the spinal cord was related to the differentiation of BM-MSCs into neural stem cells  
164 and into neurons.

165 Fauza *et al.* experimented fetal MSCs of rats in the rodent acid retinoic induced-  
166 model [21-25]. In their five studies, they experimented stem cell using intra-amniotic  
167 injections, described as Trans-amniotic Stem Cell Therapy (TRASCET) technique.

168 The first study experimented amniotic fluid MSCs (afMSCs) of normal rat dams [21].  
169 The fetuses were divided into three groups: intra-amniotic injection of afMSCs, intra-amniotic  
170 injection of saline solution (PBS), or no injection. All the injections were performed on E17  
171 and the analysis at E21. At birth, MMC was present in 46% of the afMSC group, 68% of the  
172 PBS group and 80% of the untreated group ( $p=0.03$ ). They also observed that the MMC  
173 defect coverage was thicker in the afMSC-group, as compared to the two other groups. GFP-  
174 labelled stem cells were found more frequently engrafted in bone structures and rarely in  
175 neural tissue. A second study with the same protocol showed a brainstem displacement  
176 significantly smaller in the afMSC group than the untreated group on Magnetic Resonance  
177 Imaging (MRI) examination [22]. In this study, no statistical difference was found on the  
178 cerebellar displacement. The same team compared placenta-derived mesenchymal stem  
179 cells (pMSCs) to afMSCs, in the same rat model [23]. No difference was found on the  
180 histological examination of the MMC defect coverage between the two groups. The kinetic of  
181 afMSCs, labeled with a luciferase reporter gene, was analyzed by luminometry in a fourth  
182 study [24]. Luminometry was positive exclusively in the placenta, umbilical cord, spleen, bone  
183 marrow, hip bones, defect, and brain, result interpreted by the authors as a potential a  
184 hematogenous path for homing. TRASCET was then applied to the surgical rabbit model  
185 using GFP-labeled afMSCs obtained from normal rabbit [25]. Three groups were compared:  
186 intra-amniotic injection of afMSCs suspension, intra-amniotic injection of saline solution, or  
187 no injection. Histological examination of the defect showed coverage by a neoskin,  
188 significantly thicker in the afMSCs group. No stem cells were found in the neoskin but some  
189 were observed in the bone marrow.

190 Farmer *et al.* experimented xenogenous human placental-derived mesenchymal  
191 stromal cells (pMSCs), collected from human early gestation placenta tissue, in the surgical  
192 ovine model of MMC [26-30].



193 First, they compared GFP-labeled pMSCs seeded into a hydrogel delivery vehicle to  
194 repair MMC-like defect in six lambs, with six control lambs, repaired with a vehicle-only [26].  
195 In both groups, an extracellular matrix patch was secured over the defect before skin closure.  
196 After spontaneous delivery, the lambs were evaluated by a validated scale, i.e. the Sheep  
197 Locomotor Rating scale (SLR) [31]. The lambs in the pMSC-treated group had a significantly  
198 higher SLR score at birth, with 4/6 lambs able to walk independently, against 0/6 in the  
199 untreated group. Immunocytochemical analysis revealed that pMSCs had a paracrine activity  
200 *in vitro*, with secretion of factors implicated in angiogenesis, chemotaxis, extracellular matrix  
201 remodeling, and the innate immune response. The histological analysis demonstrated an  
202 increase in “large neuron density” in pMSC-treated lambs at the lesion epicenter, but no  
203 GFP-labeled pMSC was found into the spinal cord or surrounding tissue.

204 The same pMSCs were then experimented using a FDA-approved vehicle: a porcine  
205 small intestine submucosa derived extracellular matrix (SIS-ECM) [27]. Fetal MMC defects  
206 were repaired at 93 days of gestation, 25 days following the creation, either by SIS-ECM  
207 seeded with pMSCs (n=8), or by SIS-ECM with no added cells (n=6). The authors observed  
208 no statistical difference on the SLR score, but concluded to a clinically significant  
209 improvement of motor function in the treated group with 5/8 lambs able to walk over obstacle,  
210 compared to 1/6 in the control group (p=0.04). In this study, pMSCs were transduced with  
211 GFP, but no cell tracking was reported.

212 In order to establish a cost-effective study, they used the same protocol on the  
213 chemically induced rodent model [28]. The fetal rodents were either repaired with different  
214 seeding densities of pMSCs onto the SIS-ECM, or using SIS-ECM only. The authors  
215 concluded that the pMSCs-treated group had a significantly smaller degree of spinal cord  
216 compression, whatever the pMSCs density. Their results also suggested that pMSCs  
217 improve the coverage of the MMC defect, by reducing the density of apoptotic cells in the  
218 spinal cord.

219 Farmer’s team pursued their work by studying the effects of different seeding  
220 densities of human pMSCs, compared to the use of SIS-ECM only, in the ovine model [29].  
221 Their results showed that the pMSC-groups had a significantly higher median SLR score and  
222 a higher neuron density than the SIS-ECM-only, whatever the pMSCs density group. In their  
223 last study, they compared the effectiveness of three different lines of pMSC and they  
224 identified a way to facilitate the selection of the optimal pMSC lines, using an *in vitro*  
225 neuroprotection assay [30].

226

## 227 **Discussion**

228 Through this systematic review, stem cell therapy appears to be a promising option to  
229 improve prenatal repair of MMC. The challenge in humans is to provide an efficient and safe  
230 therapy which may improve the results of the prenatal surgical repair. We thus have to  
231 determine which are the most effective cells and the most suitable mode of administration.

### 232 *Stem cells types*

233 Despite their attractive pluripotency and their high capacity of proliferation, hESCs  
234 have an obvious legal restricted use and their use is ethically controversial. Moreover,  
235 undifferentiated hESCs are known to induce teratoma and thus will never been used in  
236 humans without preliminary differentiation [32]. NSCs could be pertinent in this application  
237 because they are a source of glial scar astrocytes with beneficial functions, including  
238 preserving tissue integrity and supplying neurotrophic support for surviving neurons [33].  
239 However, these cells prepared from neural tissue are difficult to obtain, which also raises  
240 ethical issues together with potentially infectious risk concerns. iPSC-NSCSc imply fastidious  
241 methods of production with uncertain efficiency in cell replacement therapy [34]. In addition,  
242 iPSCs are not entirely safe for human application because they are randomly epigenetic-  
243 transformed after reprogramming and can accumulate mutations after transplantation [35,36].

244 MSCs appear to be the best candidate for cell therapy in MMC prenatal repair. They  
245 are easily obtained, in large quantities. They have the ability to facilitate recovery from spinal  
246 cord injury, and are capable of producing trophic factors [37-42]. Moreover, MSCs are the  
247 only type of cells to have demonstrated an improvement in motor function in the experiments  
248 of Farmer *et al.* [26-30]. Despite the use of xenogenic graft, no graft versus host reaction  
249 were observed in their experiments. This result is likely explained because of the  
250 immunomodulatory properties of MSCs and because the fetal immune system can induce a  
251 donor-specific immune tolerance, with a process of self-education [43,44].

252 This hypothesis is corroborated by the results of stem cell therapy in humans spinal  
253 cord injury (SCI). Clinical trials demonstrated that the use of MSCs in SCI improve motor  
254 function and quality of life for at least one year [45]. Moreover, these cells are known to  
255 promote repair and functional repair by enhancing neuroprotection, immunomodulation, axon  
256 regeneration and sprouting [46]. However, further investigations are necessary to resolve  
257 issues such as ideal source and type of stem cell, way of delivery and safety associated to  
258 stem cell-based therapy before being ranked as a gold standard in humans.

### 259 *Ways of administration*

260 Different ways of administration have been proposed so far: delivery directly into the  
261 spinal cord, intra-amniotic injection, or *via* a vehicle, surgically seeded. Injections into the  
262 spinal cord could be considered too invasive with additional injuries that cannot be excluded.  
263 However, if the safety of this way of administration was demonstrated, it would be a relevant  
264 option, authorizing early and repeated ultrasound-guided procedures, which is relevant  
265 regarding the fact that MMC worsens from the first trimester of pregnancy [2]. Intra-amniotic  
266 injections would present similar interests and are easier to perform with no risk of secondary  
267 injuries. However, Fauza *et al.* observed that after intra-amniotic injection, MSCs were found  
268 in the umbilical cord, placenta, spleen and brain, a result which is difficult to understand  
269 knowing the physiology of fetal circulation, thus requiring further investigations [24]. Stem  
270 cells can also be grafted on a patch and surgically seeded on the MMC-like defect. This  
271 surgical technique has already been experimented in human fetal surgery for simple  
272 coverage and have the advantage to control stem cell homing and engraftment directly on  
273 the defect.

#### 274 *Animal models and endpoints to study stem cell therapy in the prenatal treatment* 275 *of MMC*

276 Through this systematic review, four different animal models have been studied. The  
277 chick model is interesting because of its cost effectiveness but the defect is difficult to create  
278 and to compare to humans. The rodent model obtained with retinoic acid is also inexpensive,  
279 and the multiple fetuses gestations make studies statistically more powerful. However,  
280 authors reported up to 15.6% of pups with no macroscopically visible defect [47]. Thus, it  
281 seems inappropriate to choose the MMC closure at birth as endpoint with this model.  
282 Furthermore, retinoic acid is a teratogenic agent with a potential impact on all organs. The  
283 short time of gestation implying a short duration between treatment and birth could induce a  
284 bias in the treatment's evaluation. The surgical rabbit model causes the same issue because  
285 the MMC defect is created late in gestation [25]. Farmer *et al.* worked in the ovine model,  
286 which represents the more widely used model for fetal surgery because of the similarities to  
287 the human MMC malformation [11,26,27,29,30,48]. Moreover, the neurological examination  
288 of lambs is now standardized with the SLR, a validated score in the MMC ovine model [31].

289 The aim of stem cell therapy on prenatal repair of MMC is to improve the spinal cord repair  
290 during fetal surgery, in order to enhance the functional improvement of the prenatally treated  
291 children. The clinical evaluation of animals at birth is therefore the most relevant approach.  
292 Other parameters, such as immunohistochemical assays, cells survival, their integration and  
293 paracrine activities, in particular secretion of growth factors, can be used to study the

294 mechanisms of spinal cord repair. In our opinion, the induced cutaneous closure of the defect  
295 and the correction of the Chiari II malformation are not satisfactory endpoints for stem cell  
296 therapy studies because they are currently obtained in humans by simple coverage of the  
297 defect [3,4,11,48].

298

### 299 *Efficiency of stem cell therapy*

300 These studies show that stem cells, and particularly MSCs, could have a beneficial effect  
301 for the antenatal treatment of MMC, both on the healing of the MMC defect and on the motor  
302 function. Unfortunately, Farmer's team is the only one to have shown a functional benefit of  
303 stem cell therapy in prenatal myelomeningocele surgery [26-30]. This could be explained by  
304 the fact that the other teams did not choose functional assessment endpoints. It may be  
305 related to the high cost and complex organization involved in keeping animals alive and  
306 studying them for at least 24 hours. It seems therefore necessary to focus all research efforts  
307 on the study of MSCs in order to show the reproducibility of the results of Farmer *et al.* and to  
308 consider an application in humans.

309 The various results obtained are in favor of a benefit related to a paracrine activity, with  
310 secretion of growth and neuroprotective factors such as BDNF, NGF, EGF, FGF. At present,  
311 the pathophysiology of MMC, and in particular the mechanisms of destruction of the spinal  
312 cord, remain poorly understood. It is therefore probably premature to say that the paracrine  
313 effect of stem cells could halt the mechanism of the spinal cord destruction and that cell  
314 therapy alone might be sufficient as an antenatal treatment for this malformation. On the  
315 other hand, it is certain that the fetal repair of MMC brings a benefit, but also obligatory  
316 secondary surgical lesions, related to the dissection of the spinal cord. Because of their  
317 paracrine properties, stem cells could limit these side effects of surgery. Thus, their effect  
318 seems of great interest, as an adjunctive treatment to the fetal surgery.

319 If the benefits of stem cell therapy in the prenatal MMC surgery are confirmed, we could  
320 also imagine an application to the postnatal treatment of MMC, both for immediate post  
321 surgical effect, but also to prevent a secondary Tethered Cord Syndrome (TCS). TCS is a  
322 common complication after post-natal MMC surgery that affects 10 to 30% of children and  
323 can induce lower-limb motor deficits, sensory deficits, bladder dysfunction, and  
324 musculoskeletal deformities [49]. It has recently been shown that pro-inflammatory and pro-  
325 apoptotic mediators are involved in the physiopathology of this syndrome [49]. The use of  
326 stem cell therapy in this indication seems therefore particularly relevant.

327

## 328 **Conflicts of Interest**

329 The authors declare no conflict of interest.

330

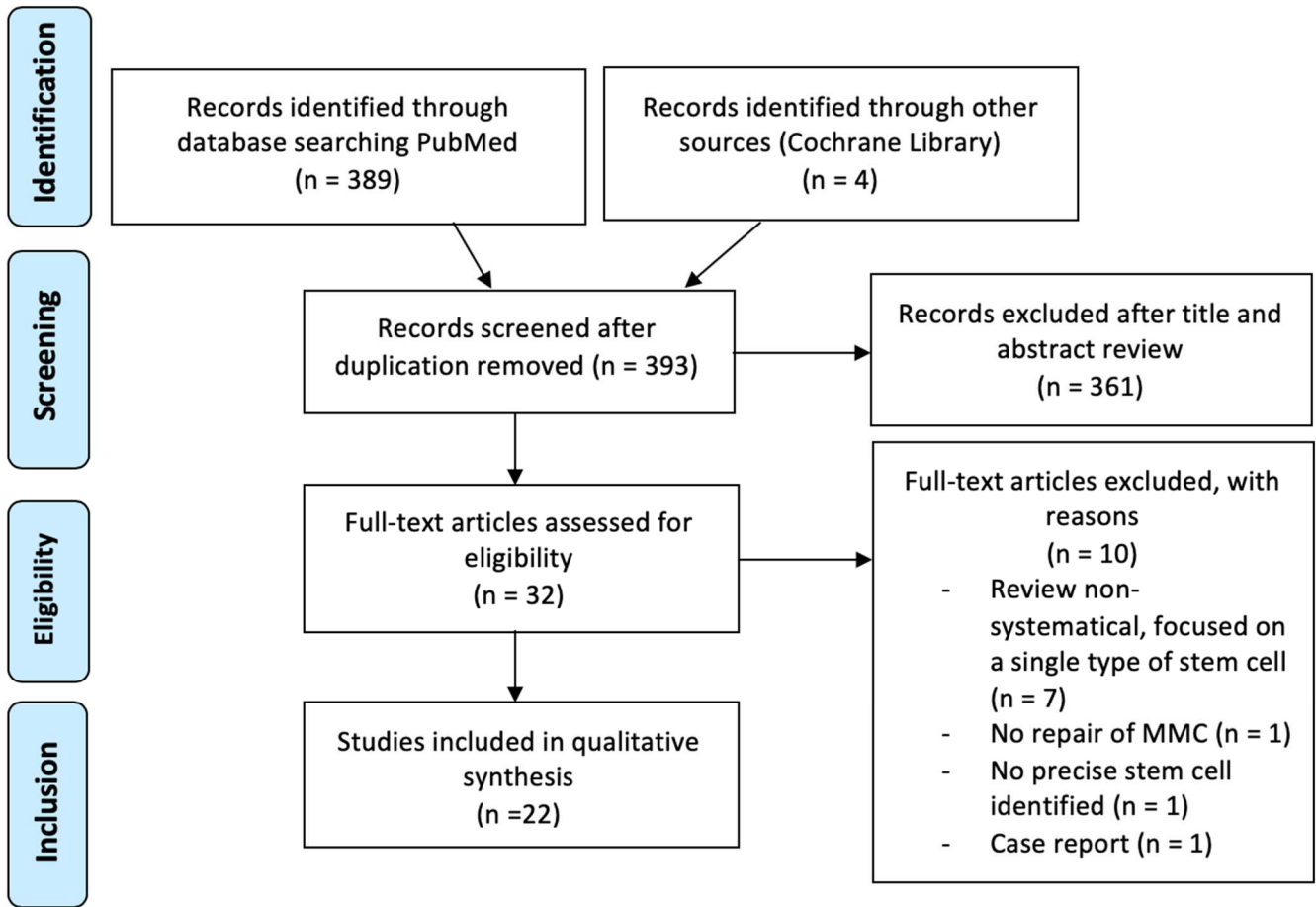
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467 **Figure 1** – The PRISMA flow diagram of the study selection process.  
 468 PRISMA: Preferred Reporting Items for Systematic reviews and Meta-Analysis protocol  
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**Table 1-** Types of animal models, stem cells, and main results of the studies included.

First author	Number of cases (n)	Animal model	Type of stem cell	Source of the stem cells	Way of administration	Main results in the stem cell group
Lee <sup>8,9</sup>	15 / 63	Chick	hESCs	Xenologous – human blastocysts	Intra-amniotic injection	Lengths of the MMC defect were significantly shorter in the hES group
Fauza <sup>10</sup>	9	Ovine	NSCs	Xenologous – murine cerebellum	Injection into the defective region spinal cord	No significant reduction of lamb paraparesis with NSCs NSCs and endogenous host cells produced GDNF and BDNF
Turner <sup>12</sup>	15	Rodent	NSCs	Allogenic – rodent AF	Intra-amniotic injection	NSCs located on the neural placode, predominantly located on exposed neural surfaces
Saadai <sup>13</sup>	2	Ovine	NCSCs - iPSCs	Xenologous – human fibroblasts	Surgically seeded, within a biocompatible scaffold	Survival and integration of NCSCs-iPSC in the spinal cord
Kajiwara <sup>14</sup>	20	Rodent	Keratinocytes - iPSCs	Xenologous – human AF	Surgically seeded	20% of complete coverage at birth
Abe <sup>15</sup>	116	Rodent	hAFSCs CD117-positive cells	Xenologous – human AF	Intra-amniotic injection	Promote skin coverage of the MMC lesion Induced neurogenesis
Lee <sup>16</sup>	30	Chick	MSCs and NSCs	Xenologous – MSCs: human BM NSCs: human	Intra-amniotic injection	Better reclosure indice
Li <sup>17-19</sup>	136 11 10	Rodent	MSCs	Allogenic – rodent BM	Injection into the defective region spinal cord	Higher expression of BDNF and NGF and reduction of expression of Caspase-3 and Bcl-2 Increased expression of EGF and FGF by RT-PCR Increased number of Brn3a+ neurons in total neurons
Li <sup>20</sup>	134	Rodent	MSCs	Allogenic – rodent BM	Surgically seeded, within a chitosan-gelatin scaffold	Co-localization of Nestin and Tubulin with GFP-BM-MSCs BM-MSCs found in the scaffold and in MMC defect
Dionigi <sup>21</sup>	87	Rodent	MSCs	Allogenic – rodent AF	Intra-amniotic injection	Thicker defect coverage, with a paucity of adnexa
Dionigi <sup>22</sup>	28	Rodent	MSCs	Allogenic – rodent AF	Intra-amniotic injection	Smaller brainstem displacement
Feng <sup>23</sup>	115 (P) & 73 (AF)	Rodent	MSCs	Allogenic – rodent AF and P	Intra-amniotic injection	Thicker defect coverage in the two groups with stem cells, without any difference between them
Shieh <sup>24</sup>	78	Rodent	MSCs	Allogenic – rodent AF	Intra-amniotic injection	Luminescence significantly higher in the defect and in the BM compared with all the other sites
Shieh <sup>25</sup>	35	Rabbit	MSCs	Allogenic – rabbit AF	Intra-amniotic injection	Thicker defect coverage
Wang <sup>26</sup>	6	Ovine	MSCs	Xenologous – human placenta	Surgically seeded, within a vehicle-only (hydrogel)	Significantly higher SLR score at birth, with 67% of them able to walk independently; MSCs had a paracrine activity <i>in vivo</i>

Kabagambe <sup>27</sup>	8	Ovine	MSCs	Xenologous – human placenta	Surgically seeded, within a SIS-ECM	No statistical difference on the SLR score, a clinically significant improvement of motor function with ability to walk over an obstacle
Chen <sup>28</sup>	78	Rodent	MSCs	Xenologous – human placenta	Surgically seeded, within a SIS-ECM	Significantly smaller degree of spinal cord compression and reduction of density of apoptotic cells in the spinal cords
Vanover <sup>29</sup>	23	Ovine	MSCs	Xenologous – human placenta	Surgically seeded, within a SIS-ECM	Significantly higher median SLR score and a higher neuron density, whatever the pMSCs' density
Galganski <sup>30</sup>	18	Ovine	MSCs	Xenologous – human placenta	Surgically seeded, within a SIS-ECM	An <i>in vitro</i> neuroprotection assay using a neuronal apoptosis model can be useful in selecting the most effective cell lines

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*hESCs : human embryonic stem cells*

*NSCs : neural stem cells*

*GDNF : glial cell line-derived neurotrophic factor*

*BDNF: brain-derived neurotrophic factor*

*NCSCs-iPSCs: neural crest stem cells derived from human induced pluripotent stem cells*

*hAFSCs: human amniotic fluid stem cells*

*EGF: Epidermal Growth Factor ; FGF: Fibroblast Growth Factor ;*

*NGF: neural growth factor*

*MSCs: mesenchymal stem cells*

*SIS-ECM: porcine small intestine submucosa derived extracellular matrix*

*BM: bone marrow*

*AF: amniotic fluid ; P: placental*