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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 β -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

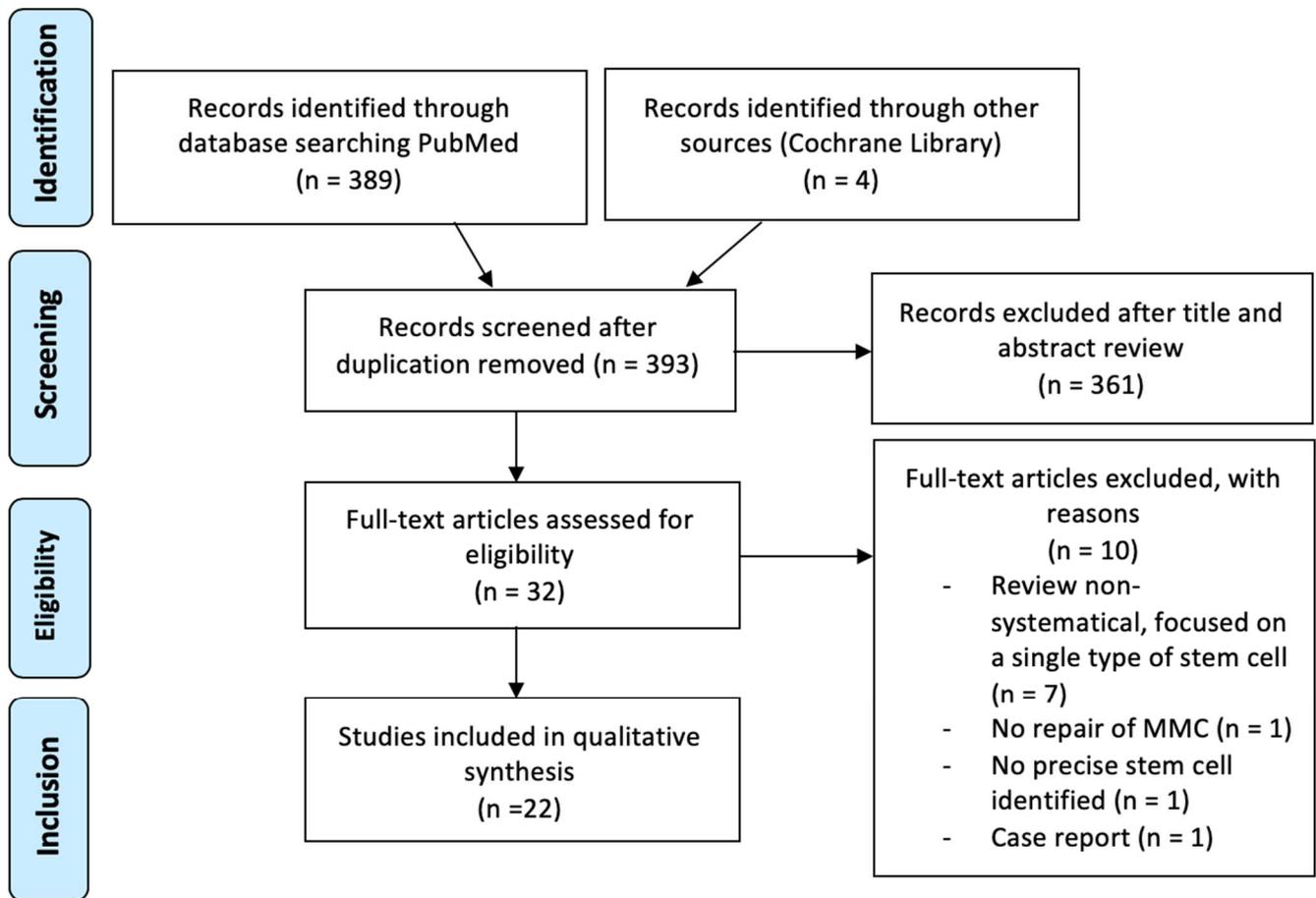
331 **References**

- 332 1. Bowman RM, Boshnjaku V, McLone DG. The changing incidence of myelomeningocele and its impact on
333 pediatric neurosurgery: a review from the Children’s Memorial Hospital. *Child’s Nervous System*. 2009
334 Jul;25(7):801–6.
- 335 2. Adzick NS. Fetal myelomeningocele: Natural history, pathophysiology, and in-utero intervention. *Seminars*
336 *in Fetal and Neonatal Medicine*. 2010 feb;15(1):9-14.
- 337 3. Adzick NS, Thom EA, Spong CY, Brock JW, Burrows PK, Johnson MP, et al. A Randomized Trial of Prenatal
338 versus Postnatal Repair of Myelomeningocele. *New England Journal of Medicine*. 2011 mar;364(11):993-
339 1004.
- 340 4. Farmer DL, Thom EA, Brock JW 3rd, Burrows PK, Johnson MP, Howell LJ, et al; Management of
341 Myelomeningocele Study Investigators. The Management of Myelomeningocele Study: full cohort 30-
342 month pediatric outcomes. *Am J Obstet Gynecol*. 2018 Feb;218(2):256.e1-256.e13.
- 343 5. Rama P, Matuska S, Paganoni G, Spinelli A, De Luca M, Pellegrini G. Limbal Stem-Cell Therapy and Long-
344 Term Corneal Regeneration. *New England Journal of Medicine*. 2010 Jul 8;363(2):147–55.
- 345 6. Ronfard V, Rives JM, Neveux Y, Carsin H, Barrandon Y. Long-term regeneration of human epidermis on
346 third degree burns transplanted with autologous cultured epithelium grown on a fibrin matrix.
347 *Transplantation*. 2000 Dec 15;70(11):1588–98.
- 348 7. Liberati A, Altman DG, Tetzlaff J, Mulrow C, Gøtzsche PC, Ioannidis JPA, et al. The PRISMA statement for
349 reporting systematic reviews and meta-analyses of studies that evaluate health care interventions:
350 explanation and elaboration. *PLoS Med*. 2009 Jul 21;6(7):e1000100.
- 351 8. Lee D-H, Park S, Kim EY, Kim S-K, Chung Y-N, Cho B-K, et al. Enhancement of re-closure capacity by the
352 intra-amniotic injection of human embryonic stem cells in surgically induced spinal open neural tube
353 defects in chick embryos. *Neuroscience Letters*. 2004 july;364(2):98-100.
- 354 9. Lee D-H, Kim EY, Park S, Phi JH, Kim S-K, Cho B-K, et al. Reclosure of surgically induced spinal open neural
355 tube defects by the intraamniotic injection of human embryonic stem cells in chick embryos 24 hours after
356 lesion induction. *Journal of Neurosurgery: Pediatrics*. 2006 aug;105(2):127-33.
- 357 10. Fauza DO, Jennings RW, Teng YD, Snyder EY. Neural stem cell delivery to the spinal cord in an ovine model
358 of fetal surgery for spina bifida. *Surgery*. 2008 sept;144(3):367-73.
- 359 11. Meuli M, Meuli-Simmen C, Yingling CD, Hutchins GM, Hoffman KM, Harrison MR, et al. Creation of
360 myelomeningocele in utero: a model of functional damage from spinal cord exposure in fetal sheep. *J*
361 *Pediatr Surg*. 1995 Jul;30(7):1028–32; discussion 1032-1033.
- 362 12. Turner CG, Pennington EC, Gray FL, Ahmed A, Teng YD, Fauza DO. Intra-Amniotic Delivery of Amniotic-
363 Derived Neural Stem Cells in a Syngeneic Model of Spina Bifida. *Fetal Diagn Ther*. 2013;34(1):38–43.
- 364 13. Saadai P, Wang A, Nout YS, Downing TL, Lofberg K, Beattie MS, et al. Human induced pluripotent stem cell-
365 derived neural crest stem cells integrate into the injured spinal cord in the fetal lamb model of
366 myelomeningocele. *Journal of Pediatric Surgery*. 2013 jan;48(1):158-63.
- 367 14. Kajiwara K, Tanemoto T, Wada S, Karibe J, Ihara N, Ikemoto Y, et al. Fetal Therapy Model of
368 Myelomeningocele with Three-Dimensional Skin Using Amniotic Fluid Cell-Derived Induced Pluripotent
369 Stem Cells. *Stem Cell Reports*. 2017 Jun;8(6):1701–13.
- 370 15. Abe Y, Ochiai D, Masuda H, Sato Y, Otani T, Fukutake M, et al. In Utero Amniotic Fluid Stem Cell Therapy
371 Protects Against Myelomeningocele via Spinal Cord Coverage and Hepatocyte Growth Factor Secretion.
372 *Stem Cells Translational Medicine*. 2019 Nov;8(11):1170–9.
- 373 16. Lee D-H, Phi JH, Kim S-K, Cho B-K, Kim SU, Wang K-C. Enhanced Reclosure of Surgically Induced Spinal Open
374 Neural Tube Defects in Chick Embryos by Injecting Human Bone Marrow Stem Cells Into the Amniotic
375 Cavity. *Neurosurgery*. 2010 Jul 1;67(1):129–35.

- 376 17. Li H, Gao F, Ma L, Jiang J, Miao J, Jiang M, et al. Therapeutic potential of in utero mesenchymal stem cell
377 (MSCs) transplantation in rat fetuses with spina bifida aperta. *Journal of Cellular and Molecular Medicine*.
378 2012 July;16(7):1606-17.
- 379 18. Li H, Miao J, Zhao G, Wu D, Liu B, Wei X, et al. Different expression patterns of growth factors in rat fetuses
380 with spina bifida aperta after in utero mesenchymal stromal cell transplantation. *Cytotherapy*. 2014
381 Mar;16(3):319-30.
- 382 19. Ma W, Wei X, Gu H, Li H, Guan K, Liu D, et al. Sensory neuron differentiation potential of in utero
383 mesenchymal stem cell transplantation in rat fetuses with spina bifida aperta: Sensory Neuron
384 Differentiation of in utero MSCs. *Birth Defects Research Part A: Clinical and Molecular Teratology*. 2015
385 Sep;103(9):772-9.
- 386 20. Li X, Yuan Z, Wei X, Li H, Zhao G, Miao J, et al. Li X, Yuan Z, Wei X, Li H, Zhao G, Miao J, Wu D, Liu B, Cao S,
387 An D, Ma W, Zhang H, Wang W, Wang Q, Gu H. Application potential of bone marrow mesenchymal stem
388 cell (BMSCs) based tissue-engineering for spinal cord defect repair in rat fetuses with spina bifida aperta. *J*
389 *Mater Sci Mater Med*. 2016 Apr;27(4):77.
- 390 21. Dionigi B, Ahmed A, Brazzo J, Connors JP, Zurakowski D, Fauza DO. Partial or complete coverage of
391 experimental spina bifida by simple intra-amniotic injection of concentrated amniotic mesenchymal stem
392 cells. *Journal of Pediatric Surgery*. 2015 Jan;50(1):69-73.
- 393 22. Dionigi B, Brazzo JA, Ahmed A, Feng C, Wu Y, Zurakowski D, et al. Trans-amniotic stem cell therapy
394 (TRASCET) minimizes Chiari-II malformation in experimental spina bifida. *Journal of Pediatric Surgery*. 2015
395 June;50(6):1037-41.
- 396 23. Feng C, D. Graham C, Connors JP, Brazzo J, Zurakowski D, Fauza DO. A comparison between placental and
397 amniotic mesenchymal stem cells for transamniotic stem cell therapy (TRASCET) in experimental spina
398 bifida. *Journal of Pediatric Surgery*. 2016 June;51(6):1010-3.
- 399 24. Shieh HF, Ahmed A, Rohrer L, Zurakowski D, Fauza DO. Donor mesenchymal stem cell kinetics after
400 transamniotic stem cell therapy (TRASCET) for experimental spina bifida. *Journal of Pediatric Surgery*. 2018
401 June;53(6):1134-6.
- 402 25. Shieh HF, Tracy SA, Hong CR, Chalpin AV, Ahmed A, Rohrer L, Zurakowski D, Fauza DO. Transamniotic
403 stem cell therapy (TRASCET) in a rabbit model of spina bifida. *J Pediatr Surg*. 2019 Feb;54(2):293-296.
- 404 26. Wang A, Brown EG, Lankford L, Keller BA, Pivetti CD, Sitkin NA, et al. Placental Mesenchymal Stromal Cells
405 Rescue Ambulation in Ovine Myelomeningocele: PMSCs Rescue Ambulation in Ovine Myelomeningocele.
406 *Stem Cells Translational Medicine*. 2015 June;4(6):659-69.
- 407 27. Kabagambe S, Keller B, Becker J, Goodman L, Pivetti C, Lankford L, et al. Placental mesenchymal stromal
408 cells seeded on clinical grade extracellular matrix improve ambulation in ovine myelomeningocele. *Journal*
409 *of Pediatric Surgery*. 2018 Jan;53(1):178-82.
- 410 28. Chen YJ, Chung K, Pivetti C, Lankford L, Kabagambe SK, Vanover M, et al. Fetal surgical repair with
411 placenta-derived mesenchymal stromal cell engineered patch in a rodent model of myelomeningocele.
412 *Journal of Pediatric Surgery*. Janv 2018;53(1):183-8.
- 413 29. Vanover M, Pivetti C, Lankford L, Kumar P, Galganski L, Kabagambe S, Keller B, Becker J, Chen YJ, Chung K,
414 Lee C, Paxton Z, Deal B, Goodman L, Anderson J, Jensen G, Wang A, Farmer D. High density placental
415 mesenchymal stromal cells provide neuronal preservation and improve motor function following in utero
416 treatment of ovine myelomeningocele. *J Pediatr Surg*. 2019 Jan;54(1):75-79.
- 417 30. Galganski LA, Kumar P, Vanover MA, Pivetti CD, Anderson JE, Lankford L, et al. In Utero Treatment of
418 Myelomeningocele with Placental Mesenchymal Stromal Cells - Selection of an Optimal Cell Line in
419 Preparation for Clinical Trials. *J Pediatr Surg*. 2019 Oct 21. pii: S0022-3468(19)30681-5.
- 420 31. Brown EG, Keller BA, Pivetti CD, Sitkin NA, Wang A, Farmer DL, et al. Development of a Locomotor Rating
421 Scale for Testing Motor Function in Sheep. *Journal of Pediatric Surgery*. 2015 Apr;50(4):617-21.
- 422 32. Hentze H, Soong PL, Wang ST, Phillips BW, Putti TC, Dunn NR. Teratoma formation by human embryonic
423 stem cells: evaluation of essential parameters for future safety studies. *Stem Cell Res*. 2009 May;2(3):198-
424 210.
- 425 33. Stenudd M, Sabelström H, Frisén J. Role of Endogenous Neural Stem Cells in Spinal Cord Injury and Repair.
426 *JAMA Neurology*. 2015 Feb 1;72(2):235.
- 427 34. Medvedev SP, Shevchenko AI, Zakian SM. Induced Pluripotent Stem Cells: Problems and Advantages when
428 Applying them in Regenerative Medicine. *Acta Naturae*. 2010 Jul;2(2):18-28.
- 429 35. Young MA, Larson DE, Sun C-W, George DR, Ding L, Miller CA, et al. Background Mutations in Parental Cells
430 Account for Most of the Genetic Heterogeneity of Induced Pluripotent Stem Cells. *Cell Stem Cell*. 2012
431 May;10(5):570-82.

- 432 36. Ohi Y, Qin H, Hong C, Blouin L, Polo JM, Guo T, et al. Incomplete DNA methylation underlies a
433 transcriptional memory of somatic cells in human iPS cells. *Nature Cell Biology*. 2011 May;13(5):541–9.
- 434 37. Mitchell, K.E., Weiss, M.L., Mitchell, B.M. et al. (2003) Matrix cells from Wharton’s jelly form neurons and
435 glia. *Stem Cells*, 21, 50–60.
- 436 38. Cízková D, Rosocha J, Vanický I, Jergová S, Cízek M. Transplants of human mesenchymal stem cells improve
437 functional recovery after spinal cord injury in the rat. *Cell Mol Neurobiol*. 2006 Nov;26(7–8):1167–80.
- 438 39. Erdogan B, Bavbek M, Sahin IF, et al. Fetal allogeneic umbilical cord cell transplantation improves motor
439 function in spinal cord-injured rats. *Turk Neurosurg* 2010;20(3):286-94
- 440 40. Kuroda Y, Kitada M, Wakao S, Dezawa M. Mesenchymal Stem Cells and Umbilical Cord as Sources for
441 Schwann Cell Differentiation: their Potential in Peripheral Nerve Repair. *The Open Tissue Engineering and*
442 *Regenerative Medicine Journal*. 2011; 4:54–63
- 443 41. Liu J, Han D, Wang Z, et al. Clinical analysis of the treatment of spinal cord injury with umbilical cord
444 mesenchymal stem cells. *Cytotherapy* 2013;15(2):185-91
- 445 42. Liang X, Ding Y, Zhang Y et al. Paracrine mechanisms of mesenchymal stem cell-based therapy: Current
446 status and perspectives. *Cell Transplant* 2014;23:1045–1059.
- 447 43. Götherström C, Ringdén O, Tammik C, Zetterberg E, Westgren M, Le Blanc K. Immunologic properties of
448 human fetal mesenchymal stem cells. *American Journal of Obstetrics and Gynecology*. 2004
449 Jan;190(1):239–45.
- 450 44. McClain LE, Flake AW. In utero stem cell transplantation and gene therapy: Recent progress and the
451 potential for clinical application. *Best Practice & Research Clinical Obstetrics & Gynaecology*. 2016
452 Feb;31:88–98.
- 453 45. Silvestro S, Bramanti P, Trubiani O, Mazzon E. Stem Cells Therapy for Spinal Cord Injury: An Overview of
454 Clinical Trials. *IJMS*. 2020 Jan 19;21(2):659.
- 455 46. Assinck P, Duncan GJ, Hilton BJ, Plemel JR, Tetzlaff W. Cell transplantation therapy for spinal cord injury.
456 *Nat Neurosci*. 2017 May;20(5):637–47.
- 457 47. Danzer E, Schwarz U, Wehrli S, Radu A, Adzick NS, Flake AW. Retinoic acid induced myelomeningocele in
458 fetal rats: characterization by histopathological analysis and magnetic resonance imaging. *Exp Neurol*.
459 2005 Aug;194(2):467-75.
- 460 48. Paek BW, Farmer DL, Wilkinson CC, Albanese CT, Peacock W, Harrison MR, et al. Hindbrain herniation
461 develops in surgically created myelomeningocele but is absent after repair in fetal lambs. *Am J Obstet*
462 *Gynecol*. 2000 Nov;183(5):1119–23.
- 463 49. Cohrs G, Drucks B, Sürie J-P, Vokuhl C, Synowitz M, Held-Feindt J, et al. Expression profiles of pro-
464 inflammatory and pro-apoptotic mediators in secondary tethered cord syndrome after myelomeningocele
465 repair surgery. *Childs Nerv Syst*. 2019;35(2):315–28.
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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 ^{8,9}	15 / 63	Chick	hESCs	Xenologous – human blastocysts	Intra-amniotic injection	Lengths of the MMC defect were significantly shorter in the hES group
Fauza ¹⁰	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 ¹²	15	Rodent	NSCs	Allogenic – rodent AF	Intra-amniotic injection	NSCs located on the neural placode, predominantly located on exposed neural surfaces
Saadai ¹³	2	Ovine	NCSCs - iPSCs	Xenologous – human fibroblasts	Surgically seeded, within a biocompatible scaffold	Survival and integration of NCSCs-iPSC in the spinal cord
Kajiwara ¹⁴	20	Rodent	Keratinocytes - iPSCs	Xenologous – human AF	Surgically seeded	20% of complete coverage at birth
Abe ¹⁵	116	Rodent	hAFSCs CD117-positive cells	Xenologous – human AF	Intra-amniotic injection	Promote skin coverage of the MMC lesion Induced neurogenesis
Lee ¹⁶	30	Chick	MSCs and NSCs	Xenologous – MSCs: human BM NSCs: human	Intra-amniotic injection	Better reclosure indice
Li ¹⁷⁻¹⁹	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 ²⁰	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 ²¹	87	Rodent	MSCs	Allogenic – rodent AF	Intra-amniotic injection	Thicker defect coverage, with a paucity of adnexa
Dionigi ²²	28	Rodent	MSCs	Allogenic – rodent AF	Intra-amniotic injection	Smaller brainstem displacement
Feng ²³	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 ²⁴	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 ²⁵	35	Rabbit	MSCs	Allogenic – rabbit AF	Intra-amniotic injection	Thicker defect coverage
Wang ²⁶	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 ²⁷	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 ²⁸	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 ²⁹	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 ³⁰	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