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ABSTRACT

Peripheral nerve injury and regeneration is a challenging scientific field with relevant clinical implications. Most peripheral nerve regeneration studies have been mainly carried out on rodents. However, it is important to note that the validity of the rodent as a model to study nerve injury and regeneration and translate these results into clinical practice has been questioned by several researchers. To overcome this problem, some investigators have used companion animals and large animal species as models for experimental peripheral nerve regeneration studies. Live sheep are often used in biomedical research because of availability, simplicity of care and housing, cost and body weight similar to humans and acceptance by society as a research animal. Despite these advantages, studies on nerve regeneration and repair in sheep have only been undertaken a few decades ago and compared to rat and mice experimental studies, there are much fewer investigations. The authors have compiled and sorted the available literature on experimental ovine nerve studies in order to guide the peripheral nerve investigator in choosing clinically relevant and interpretable models for studies on neural regeneration that are much needed in order to make progress towards new surgical and medical treatment of peripheral nerves.

Introduction

Peripheral nerve injury may result in partial or total loss of the sensory and motor functions [1–3]. The main causes of peripheral nerve lesion are penetrating injury, crush injury, traction injury, ischemia, laceration, com-pression and thermal injury [4–7]. Experimental animal models have been used to study peripheral nerve injury, and therefore, it is important to mimic the mechanical, physiological and pathologic response of human condi-tions [8,9]. The development of new clinical approaches to promote the regeneration of peripheral nerve injury, includes pre-clinical animal testing prior to human clin-ical trials and use in patients [10,11].

The adequate selection of an animal model is a crit-ical point for the success of an experimental study on nerve regeneration [12]. Important factors concerning the animal model must be considered during design of the experimental study, including the cost for acquisition and maintenance of the animals, availability, accepta-bility by society, tolerance to captivity, ease of housing, resistance to infection, inter-animal uniformity, lifespan and biological information must be considered [13–15].

The rat is by far the most commonly employed ani-mal model in experimental nerve research [16], with the mouse being the second most popular model [11]. There are three putative advantages at utilising a rodent model for regeneration studies: these species are small, their nerves regenerate well, and there are many inbred strains available [17–21]. Additionally, the rodent model offer the possibility of conducting experimental studies using large numbers of animals, thus, allowing experiments to be sufficiently well powered statistically.

Despite the fact that anatomy of rodent nerves has been well studied and is similar to that of humans [22], peripheral nerve regeneration is much faster in these species than in humans, a limitation to their use as a model that is accentuated due to the relative short nerve gaps that can be produced [23,24].

To overcome this problem, some researchers have used large animals as models for experimental peripheral nerve regeneration studies [25–27] because the distances that regenerating axons must travel are similar to those in humans [9,28]. Therefore, studies conducted in large animal models are likely to advance this field of study and produce results that are more clinically relevant [25,29,30].

Sheep models have been established as one of the most relevant animal models for experimental and pre-clinical human studies [8,25,26]. Nerves in sheep and in human have similar size and regeneration behav-iour [25,29,31,32]. Median nerve size is comparable to ulnar and median nerves size in humans [27]. The femo-ral nerve in sheep has the same length as the ulnar nerve in humans [33].

Furthermore, one-year-old sheep are the closest equivalent to humans around 15–20 years of age [9]. At one week of age, lambs correspond well to young humans. The possibility of determining an age relation between these species is important because it is better to study nerve regeneration according to age and some dis-eases occur in certain age groups [31]. Comparing with other large animals, sheep are an appropriate experimen-tal model for biomedical research because of availability, simplicity of care and housing, cost and acceptance by society as a research animal [34,35].

Despite these advantages, studies on nerve regener-ation and repair in sheep have only been undertaken a few decades ago [33]. When compared to rat and mice experimental studies, there are fewer investigations on this subject in sheep [22,23]. Other specialities, such as anaes-thesiology, cardiology, and orthopaedics, have also used sheep model to perform experimental designs [36–45].

In our review, we included experimental studies about peripheral nerve regeneration in sheep. PubMed was used to search the published literature for the fol-lowing terms: ‘sheep’ or ‘ovine’ or ‘lamb’ or ‘ewe’ or ‘ram’ combined with other terms using AND, ‘nerve repair’ or ‘nerve regeneration’ or ‘nerve injury’ or ‘peripheral nerve’ or ‘cranial nerve’ or ‘spinal nerve’ or ‘neural regen-eration’ or ‘reinnervation’. The abstracts of retrieved cita-tions were reviewed, and prioritised according to the relevance of their content. Full articles were obtained for all selected articles and reference lists were checked for additional material where appropriate.

The objective of the present review is to offer a helpful tool for the investigator in order to make progress towards new therapies for the benefit of the peripheral nerve injured patients. Therefore, the authors have compiled and sorted the available literature on experimental ovine nerve studies. Data on peripheral nerve regeneration and repair studies using sheep is summarised in Table 1.

Forelimb

Median nerve

In experimental regeneration research, the median nerve is the most studied nerve in sheep, while in rodents, the sciatic nerve is the one most frequently used [11,16]. In

sheep, experimental studies with median nerves have the advantage that a high injury does not affect limb extension necessary for locomotion and weight bearing. Furthermore, hoof desensitisation, trauma and sepsis are uncommon problems [28,46,47].

The sheep forelimb is innervated by nerve branches originating from the brachial plexus [48]. The median nerve originates from the eighth cervical and first tho-racic nerves. It trails along the ulnar nerve within the same sheath until the middle of the forelimb [49]. The median nerve is a large mixed nerve whose size in sheep is analogous to the size of the median and ulnar nerves at the human wrist, being a good experimental model for applied to humans [27,46,50].

Neurotmesis has been performed in all studies about repair and regeneration of the sheep median nerve. For surgery, it is necessary to perform a straight skin incision on the anteromedial aspect of the forelimb between the elbow and the wrist [35,51] or a medial incision between the axilla and the elbow [46,47,52]. The use of micro-surgical instruments under an operating microscope is mandatory when performing a careful dissection and separation of the median nerve from the brachial or median arteries [35,53,54]. This care regarding the brachial artery and soft tissues is relevant, since it has been proved that the presence of a complex arterial injury added towards a poorer outcome for recovery of nerve function and maturation. Glasby and collabora-tors promoted a brachial artery injury using a Castafeda clamp and a DeBakey bulldog clamp, next to the site of median nerve injury. The artery was excised between the clamps and a vein autograft used to replace this gap with a superficial vein accessible through the same incision [35]. Other complications such as cavitation, fibrosis and haematoma damage were shown to negatively influence regeneration of a simple nerve injury. Experimentally, these lesions were produced with Mayo cholecystectomy clamps to crush the superficial muscle and soft tissue around the neurovascular bundle of the median nerve; the soft tissue were thoroughly abraded and painted to result in an inflammatory response [47].

After isolation of the median nerve, the injury may be achieved [35] using a Meyer neurotome, and neurotme-sis may be obtained in one of two ways: by doing only an incision in or removing a gap of said nerve [46,55,56]. In published studies on median nerve regeneration, the length of the gap varied between 5 mm and 5 cm. In addition, study times regarding nerve regeneration was 35–51 days, 6, 7, 9, 10 and 12 months [27,28,46,47,50–58].

To evaluate nerve regeneration after median nerve transection, the recording of nerve blood flow, electro-physiological studies, nerve morphometry and muscle weights have mainly been used. The wet muscle mass of the flexor carpi radialis is used to measure median nerve regeneration. It is a specific test because only the median nerve supplies the flexor carpi radialis muscle [58]. The nerve blood flow of the repaired median nerve may be performed distal to the graft and at the normal nerve, simultaneously [52]. This method measures tissue blood flow, detecting changes even in areas of low flow, without damaging the nerve [28]. The compound nerve action potential may be obtained with a stimulating elec-trode placed under the median nerve proximal to the graft, high in the axilla, and a second bipolar recording electrode placed below the median nerve in the mid forearm, distal to the graft [47]. Latency, conduction velocity, amplitude and area of the waveform have been determined. A stimulating current of 1–2 mA delivered to the median nerve proximal to the nerve graft has been used to observe the presence or absence of a hoof twitch after electric stimulation [50].

Another electrophysiological technique is transcu-taneous stimulated jitter, which has also been used to evaluate median nerve regeneration. This technique measures the variability in the latency between the stim-ulation of a motor axon and the contraction of the sup-plied muscle fibre [58]. The muscle used to study median nerve regeneration in this test is the flexor carpi radia-lis, in which terminal branches of the median nerve are stimulated with two monopolar needle electrodes [54]. Isometric twitch and tetanic tension are two methods for assessing muscle function where the median nerve is stimulated proximal to the location of nerve repair, and the twitch and tetanic tension are measured [56].

Regarding median nerve morphometry analyses, parameters such as total number of axons, total number of myelinated axons, axon diameter, fibre diameter, mye-lin thickness, g-ratio (axon diameter/fibre diameter) and percentage of neural tissue (amount of the total fibre area divided by the total area sampled 100 times) have been used [27,46,50,57]. The g-ratio has been used to meas-ure the maturity of reinnervated nerves [58]. Histology and immunohistochemistry have been used to evaluate median nerve regeneration in sheep [50].

Alternatively to microsurgical epineural suturing, sheep median nerve has been repaired with biodegrad-able tubes, mostly to avoid tension in the suture site or when there is a critical defect that is not possible to reconstruct using an end-to-end suture [27,58]. This technique consists in positioning the end of the tran-sected nerve on the top of wrap with no gap between their ends and applying tissue glue on the nerve, careful to avoid the end of the stump [27]. Therefore, the associ-ation of wrap with tissue glue has the advantage of being a simple technique, faster than other methods, and that requires only a basic surgical knowledge [58]. Even when the wrap was applied with polyglactin, they were quickly applied when compared with the conventional epineural suture [27]. Additionally, the porous nature of the wrap allows macromolecules access to the site of repair and promotes a beneficial local microenvironment for nerve regeneration [58]. The use of polycaprolactone glue was not recommended because the wrap, when used with polycaprolactone, was not absorbed at 7 months and stimulated more fibrosis, which was not observed with tissue glue nor with polyglactin sutures [58].

A flexible biodegradable glass wrap with tissue glue or suture and epineural suture to repair median nerve sec-

tion in ovine showed a g-ratio similar to normal nerve. In spite of that, axon diameter, fibre diameter and myelin thickness were lower than in the uninjured nerve. This suggests that myelination was finished in treated nerves, but the mean size and thickness of the myelin were not ideal [27].

Kelleher et al. [54] observed after 10 months that the use of biodegradable glass tube to repair the median nerve was not influenced by an exogenous static magnetic field. Morphometry outcomes observed in this study were dif-ferent from the research that used biodegradable wrap; the values were higher. However, the studies observed nerve regeneration at different times. In addition, the use of ciliary neurotrophic factor (CNTF) to promote nerve regeneration showed at 12 months a similar morphom-etry outcome to a study that used a static magnetic field [55]. These authors have applied CNTF with an osmotic pump catheter placed under the dura mater to produce a concentration of CNTF at the level of the cell body of 1.5 ng/ml. When the CNTF group was compared to a group that had saline solution introduced in the same manner, there was no difference in morphometry results, suggesting that CNTF, applied at the level of the cell, does not improve nerve regeneration [55].

Another study compared conventional methods of median nerve repair with end-to-side repair, in which an oval window was created in the epineurium of the intact ulnar nerve and the transected median nerve was sutured there [56]. Although this method led to regen-eration, results were inferior to the other experimental groups and its clinical application is questionable [56]. Traditional techniques consisted in end-to-end epineu-ral nerve suture, entubulation with biodegradable glass tube, and autologous nerve grafting. There were no sig-nificant differences in their outcome [56], which sup-ported the benefits offered by entubulation repair in the ovine median nerve described by other authors [27,58].

Efficacy of the freeze-thawed muscle graft to pro-mote median nerve regeneration in a sheep model at six months was studied after a 2–3 cm median nerve resection. [28,46,47,52,57]. The outcomes of immediate and delayed (after four weeks) repair with freeze-thawed muscle graft showed that nerve reconstruction is possi-ble in these two situations and with minimal resulting damage after delayed repair. Blood nerve flow and nerve conduction velocities have not been significantly differ-ence in those two conditions, however, morphometry studies revealed a smaller axon and fibre diameter after delayed reconstruction [46].

The presence of bone fracture [57], cavity, fibrosis, haematoma [47] and artery injury [52] in the area of nerve repair contributed negatively to median nerve regeneration even using freeze-thawed muscle grafts.

Furthermore, these complications led to a worse out-come in recovery of nerve function and maturation when there was a delayed repair performed after 30 days [47,52,57].

Comparing freeze-thawed muscle graft with fascicular nerve grafting in median nerve reconstruction revealed that both treatments resulted in regeneration of a 3-cm nerve injury after immediate repair. The mean nerve conduction velocity and the mean myelin sheath thick-ness were greater in nerves with freeze-thawed muscle graft repair, suggesting this method could be better than conventional nerve grafts [28]. Despite the nerve auto-graft being the gold standard treatment in many species, including humans, when it is not possible to perform end-to-end suture in the nerve gap [24,59,60], the use of muscle grafts (freeze-thawed) have been shown to be a good alternative for nerve regeneration in sheep [28]. In addition to the satisfactory outcomes, there is an abun-dant supply of autologous muscle and this technique has less site morbidity [28].

The vast majority of work in sheep has included nerve gap segments that ranged in length until 3 cm, as pre-viously mentioned. Strasberg et al. [53] studied median nerve regeneration after injury with a gap of 5 cm in length. They tested the repair, at 6 and 10 months, of a segment of 8 cm of fresh allograft and autograft nerve, and of 8 cm of preserved cold allograft and autograft nerve. The method of graft conservation (fresh or cold) did not seem to influence the outcome. Both allograft groups showed no regeneration at 6 months and indi-cations of poor regeneration at 10 months, different from what was observed in the autograft groups, which demonstrated histological and morphometric signs of great regeneration [53]. Another study also investigated 5-cm lesions in the sheep median nerve at 6 months and 9 months after surgery, but a 7-cm autograft segment was used [50]. They showed good nerve regeneration with conduction velocity, amplitude, myelinated axon density and g-ratio similar to normal nerve, with no difference at either times of evaluation.

Regarding to nerve allograft rejection in sheep, associ-ation of cold preservation and immunosuppressive agents was proposed to contribute to reduce the antigenicity in the allograft [53]. Daily subcutaneous administration of Cyclosporin A (15 mg/kg) was be able to prevent rejection of allograft repair (8 cm of cable) and permit regeneration in the proximal segment without axonal regeneration in distal segment. Nevertheless, immunosuppression had a negative effect on sheep health, causing severe lung complications which reduced the duration of the study to 35–51 days, initially intended to be 6 months [51].

Radial nerve

The radial nerve is formed exclusively by the ven-tral ramus of the seventh and eighth cervical nerves and first thoracic nerve [49]. It innervates the triceps muscle, tensor fascie antebrachii, anconeus, carpal and digital extensor muscles (in the middle of the arm), and has a cutaneous branch that provides rami to the digits [61].

The radial nerve has been poorly investigated in terms of nerve regeneration in sheep. Recently, an experimental

research was performed on radial nerve regeneration, testing bone marrow mesenchymal stem cells (BM-MSCs), platelet-rich plasma (PRP) with a bio-degradable scaffold 3 weeks after nerve transection with a 1-cm gap [62].

At 3 months, treatments with PRP or 50 BM-MSC had not resulted in regeneration of the radial nerve when evaluated by morphological and neurophysio-logical studies [62]. However, at 6 months, important improvement in morphometric and neural conduction outcomes were observed, showing myelinated nerve fibres both in the distal and proximal segments of the radial nerve when treated with an association of PRP with 30 BM-MSCs in a biodegradable scaffold [62].

Hindlimb

Femoral nerve

The femoral nerve emerges from the cranial part of the lumbosacral plexus [61], originated from the ventral branch of the fifth lumbar nerve with occasional contri-butions from the ventral fibres of the sixth lumbar nerve [49]. In sheep, femoral nerve regeneration has been stud-ied using some of its branches, such as the saphenous nerve, rectus nerve [63], vastus nerve [64,65] or the fem-oral nerve itself [33]. A surgical approach to the femoral nerve may be made by dissecting the area lateral to the femoral artery and isolating the nerve below the inguinal ligament. Glasby et al. [33] performed neurotmesis (gap of 1 cm) at this point in the nerve, where the saphenous nerve originates.

The rectus nerve (motor nerve) was sutured distally to the proximal section of the vastus nerve (motor nerve) directly or with an ipsilateral saphenous nerve (sensory nerve) graft or with a contralateral cross-over nerve graft [63]. A saphenous nerve graft (in a reversed position) was also used to connect the vastus nerve with fascia to the hindlimb, in the same side or using a contralateral cross-over nerve, without fixing it in a target muscle [64,65]. The median diameter of myelinated fibres was similar in both sides. On the ipsilateral side, median fibres diameter reached a maximum at 6 months, but with a longer interval after repair, number of fibres and myelinated fibres decreased with time on the contralat-eral side [64,65].

Frey et al. [63] verified that, on the ipsilateral side using a long graft, the number of fibres increased from proximal to distal and mean fibre diameter decreased. There was a sign of regeneration, namely the sprouting of nerve fibres, mainly immediately and behind proximal nerve suture. However, with a cross-over nerve graft, the number of regenerated nerve fibres was lower in the distal graft when compared with ipsilateral side. When muscle (freeze-thawed) was used as graft in the femoral nerve, there was an increase in number of axons at each time, with the best number of axons and diameter of the myelinated fibres after 10 months [33].

Sciatic and tibial nerves

The sciatic nerve is the largest nerve in the body. It orig-inates from the ventral branches of the L6, S1, S2 and S3 spinal nerves [66]. The tibial nerve is a branch of the sciatic nerve that innervates the lateral portion of the gastrocnemius, popliteus, soleus and deep digital flexor muscles [49].

Both the sciatic and tibial nerves have been studied after nerve transection in utero in foetal sheep [67–69]. The tibial nerve was used to compare intrauterine and mature nerve tissue regeneration in adults. After sheep hysterotomy, this nerve was divided and end stumps were immediately sutured in the foetuses. The same pro-cedure was performed in adult sheep [67]. In the sciatic nerve, transection was done at the trunk level in sheep foetuses, immediately followed by coaptation [68,69]. A guiding material constructed from decellularised vein grafts filled with spider silk fibres was applied to repair a 6.0 cm gap of the tibial nerve in adult sheep at 6 and 10 months. [30]

In the tibial nerve, conduction velocity and amplitude from intra uterine repaired nerves was measured only after 6 and 8 weeks in both foetal and maternal nerves. However, after 4 weeks, it was possible to observe indi-cation of axonal growth in foetuses. After 6 weeks, there was evidence in both foetuses and adults [67]. Following the sciatic nerve procedure, there was gait dysfunction and absence of sensory function distal to the stifle, his-tologic signs of degeneration and absence of or small somatosensory evoked potentials at 70–75 days after nerve repair [68,69]. The use of constructed graft with spider silk showed motor nerve conduction velocity and amplitude of the compound motor action with no statis-tical differences when compared with autologous nerve graft at 6 and 10 months [30].

As with the radial nerve, regeneration of the tibial nerve has been evaluated using mesenchymal stem cells and PRP in a biodegradable scaffold at 3 and 6 months after 3 weeks of nerve transection. Similar to what was observed in the radial nerve, outcomes for tibial nerve regeneration was poor at 3 months in both morpho-logic and electrophysiological studies, but improved at 6 months when treated with different concentrations of BM-MSCs (30×10^6 or 50×10^6) associated or not with PRP [62]. At 10 months, spider silk construct, used in the tibial nerve, resulted in axonal regeneration and myelination similar to what occurred in autologous transplantation [30].

Cranial nerves

Facial nerve

The facial nerve (cranial nerve VII) is composed by a motor branch, responsible for innervation of facial expression muscles, and a sensitive branch, responsi-ble for gustatory functions [61]. This nerve follows the vestibulocochlear nerve in the acoustic meatus of the petrous portion of the temporal bone, enters the facial canal within the temporal bone, and arises through the stylomastoid foramen [70].

The facial nerve is the second most studied nerve for regeneration in sheep. Neurotmesis was performed, creat-ing a 5 cm [25] or 3 mm gap [71], or only transecting the nerve [8,29,72,73]. The approach to the facial nerve can be performed several ways. For repair of the facial nerve in the cerebellopontine angle, a craniectomy of the occip-ital bone was necessary, with retraction of the cerebellum medially. Microdissection between the facial and vestibu-locochlear nerves was then performed, and correct identi-fication of the facial nerve confirmed by facial movement when the nerve rootlets were manipulated [70]. Another alternative approach to the facial nerve can be performed after its emergence at the stylomastoid foramen and before the posterior border of the parotid gland, which are about 6 to 8 cm apart in an adult sheep [25,29,72]. In some sit-uations, the buccal branch of the facial nerve was studied and, in these cases, excision of a segment of the nerve was done some centimetres after its emergence from the parotid gland [8,73]. The buccal nerve may be identified using a transcutaneous supramaximal constant current stimulating the depressor labii maxillaris muscle, which is innervated only by the buccal branch [8].

Main diagnostic methods used to evaluate facial nerve repair are electrophysiological studies measuring indices such as jitter of that muscle, amplitude of com-pound muscle action potential, maximum conduction velocity and minimum conduction velocity [8,29,73], and morphometric evaluation in which axon diameters, fibre diameters, myelin sheath thickness and g-ratio were the most estimated parameters [25,70,72]. In some cases, clinical observation was done immediately after surgery and at the end of the study, evaluating corneal reflex, facial symmetry and snout movement [25,29,70,72]. Other assessments made less often in facial nerve studies were blood flow recordings [72] and electron micros-copy analysis [8].

The use of a tube-guide in sheep was performed for the first time by Gilchrist et al. [73]. They connected biodegradable glass tubes to nerve stumps with two epineurium sutures. At 10 months, there was complete dissolution of the glass tube and significant nerve regen-eration with the following outcomes: 6.75 μm fibre diam-eter, 4.14 μm axon diameter, 2.17 μm myelin thickness and 42.93 m/s maximum conduction velocity. Another study, using a biodegradable glass fabric with fibrin glue, found similar results after 6 months [29]

Simple epineural suturing associated with injection of CNTF over the depressor labii maxillaris was tested for repair of sheep facial nerve. After 9 months, CNTF did not improve nerve regeneration [8]. Comparing end-to-end epineural suture findings to entubulation in facial nerve repair, outcomes were numerically sim-ilar; corroborating the idea that entubulation is a great alternative to traditional suture repair [29,73].

End-to-end epineural suture and entubulation repair after facial nerve transection showed similar results [29], supporting the idea that a guide tube is a great alternative to traditional suture repair [29,73].

Freeze-thawed muscle autografts have also been used to repair facial nerve [25,70,72]. After creating a 5-cm nerve gap, the use of freeze-thawed muscle auto-graft showed reduced conduction velocity, and axon and fibre diameter in comparison to the normal nerve. Nevertheless, the level of regeneration was considered great and in accordance to what was expected with the created gap length [25].

The cerebellopontine angle approach has been stud-ied to access the facial nerve when a gap of 3 mm was performed and then repaired with freeze-thawed muscle autograft. There was regeneration after 12 months, show-ing 34.6 m/s conduction velocity, 2.78 μm axon diameter, 4.27 μm fibre diameter, 0.75 μm myelin thickness and 0.65 g-ratio [70].

Freeze-thawed skeletal muscle autograft was used as jump-grafts associating the hypoglossal nerve and the facial nerve, after creation of a 5-cm facial nerve gap. The hypoglossal nerve was divided longitudinally to make an arm that would connect to the distal stump of the facial nerve with a freeze-thawed muscle autograft. At 8 months, conduction velocity, axon diameter, and fibre diameter were reduced when compared with nor-mal facial nerve, but g-ratio was unchanged, reflecting normal myelination [72].

Inferior alveolar nerve

The inferior alveolar nerve is a cutaneous ramus of the trigeminal nerve [71], formed from a sensitive root and a motor one [48]. In sheep, regeneration of the inferior alveolar nerve has been studied associated to mandib-ular osteotomy [74,75]. Nerve injury was performed by neutoromy and irradiation [75] and by distraction oste-ogenesis in which there

is consequently a nerve lesion [74]. The inferior alveolar nerve has also been evaluated by histologic studies [74,75].

In sheep, nerve growth factor (NGF) and brain-- derived neurotrophic factor (BDNF) participate in inferior alveolar nerve regeneration. After mandibular osteotomy and distraction osteogenesis, the inferior alveolar nerve is injured. Myelin sheath debris may stimulate higher expression of NGF and BDNF, help-ing Schwann cell multiplication and remyelination of the injured nerve [74]. Furthermore, conjunctive tissue proliferation seems to cause more damage to nerve regeneration than irradiation [75].

Spinal nerves

Spinal nerves are formed by a dorsal and ventral root that originate from spinal cord segments. These nerves divide into dorsal and ventral rami, i.e. branches [48]. Dorsal and ventral branches join with their neighbours to form plexuses, the most important of which are the brachial and the lumbosacral plexus [61].

Some spinal nerve branches have been studied using histologic and morphometric evaluation [9,31,32,76,77], electrophysiological diagnostics [9,26,31,32,76,77] and functional record [26,32,77].

Obstetric brachial plexus palsy, common in humans, has been studied in a sheep model with brachial plexus injury [9,31,76]. Crush injury and distraction were used to produce a Sunderland type IV nerve injury and to simulate brachial plexus avulsion [9,76].

In lambs, treatment with freeze-thawed muscle auto-grafts showed similar results when compared to treat-ment with cable grafts [76]. The use of cable grafts to repair cervical (C6) roots in adult and new-born sheep resulted in the same level of regeneration [9], and delayed repair (30 days) in lambs using cable grafts had similar results to immediate repair [76]. However, delayed repair in lambs treated with freeze-thawed muscle autografts revealed a decrease in g-ratio [76]. The use of freeze-thawed muscle autografts had a great potential to pro-mote cervical root regeneration in lambs after avulsion injuries, but the same did not occur in adult sheep [31].

Transection of the cervical (C6) nerve root has been performed to study regeneration [26,32]. The nerve tran-section was done close to the spinal cord [26] or in the dorsal rootlets with avulsion of ventral rootlets [32]. In the latter situation, there was reinnervation of muscle and functioning motor fibres from the spinal cord due to ventral root repair with freeze-thawed muscle autograft [32]. There was a gradual improvement in the proximal forelimb weakness and a positive EMG recording from the brachiocephalicus muscle when repair of the C6 root was stimulated [32]. After ventral and dorsal roots were repaired with freeze-thawed muscle autograft [26], morphometric outcome was worse than that observed by Hems et al. [32], but, probably, this difference existed because evaluations were done at 8 months [26] and 12 months [32]. The use of freeze-thawed muscle auto-graft was also able to support nerve regeneration after neurotmesis injury of the sacral root [77].

Final considerations

Median and facial nerves were the most investigated in sheep models. It is possible to imagine clinical trans-lational applicability since these nerves may improve understanding of maxilla-facial [22], hand, and fingers repair [78]. Studies on nerve antigenicity [79,80] provide a scientific basis for evaluation of nerve allograft regen-eration in sheep models [53].

Regeneration studies duration for nerve repair and regeneration in sheep ranged from 5 days to 18 months after different types of treatments. Furthermore, six months was the most commonly used study duration among existing research. Results after delayed repair in experimental models that are closer to human [47,62,76] are significant because repair of human nerve injury is not always immediate. Therefore, various research pos-sibilities could be explored within this scope.

Aside from the use of autograft and allograft to repair peripheral nerve damage in sheep [9,51,64], tubulisation has been used to reconstruct injured nerves [54,56,62]. The use of an artificial biodegradable scaffold in sheep was performed for the first time in 1998 [73]. This method revealed itself to be a quicker and easier alter-native to microsurgical repair than traditional suture [27,58]. The use of tissue glue for nerve repair in sheep was not the main objective of these studies, but they were considered efficient in connecting nerve stumps and guide tubes, which facilitated the surgery and decreased surgical time [27,29].

Most of studies on nerve regeneration in a sheep model used histomorphometric and electrophysiologi-cal analysis to evaluate outcome. Other kinds of meth-ods have also been applied, such as muscle weight, nerve blood flow, immunohistochemistry and electron microscopy. Despite functional assessments being the best method to evaluate nerve regeneration and cor-rect connection with the target organ [81], tests using functional gait analysis are rarely used and limited in ovine models when compared with small laboratory animals.

In general, experimental peripheral nerve injury in sheep does not affect animal health and wellness, though sometimes reversible complications occurred after sur-gery [32,70]. Sheep models were shown to be effective and

adequate to study peripheral nerve regeneration and repair, mainly because of the similar nerve size and rate of regeneration when compared to humans. Thus, the study of neural regeneration in sheep may represent an important step to pre-clinical application in humans. Nevertheless, few investigations have been performed with this purpose, which implies the necessity for new studies in the near future.

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