The clinical applications of stem cells

At present, stem cell therapies in veterinary patients are not rigorously supervised by regulatory agencies in any country [1]. Unfortunately, this has led to the implementation of some therapies that have not demonstrated efficacy in vitro or in preclinical animal studies. The same methods can be used to generate transgenic animals for production of pharmaceuticals or for use as biomedical models. Small and large animal species serve as valuable models for preclinical evaluation of stem cell applications in human beings and in veterinary patients in areas such as spinal cord injury and myocardial infarction. However, these applications have not been implemented in the clinical treatment of veterinary patients. Reviews on the use of animal models for stem cell research have been published recently. Therefore, in this review, animal model research will be reviewed only in the context of supporting the current clinical application of stem cells in veterinary medicine.

Stem cell products in clinical use

In veterinary patients, three MSC-based approaches are currently used for the treatment of tendon, ligament, or muscle injuries. These include direct injection of bone marrow aspirate, use of bone marrow aspirate-derived mesenchymal stem cells, and use of bone marrow aspirate-derived conditioned media. Direct injection of bone marrow aspirate is a common treatment for musculoskeletal injuries in veterinary patients. This approach involves the administration of a large volume of bone marrow aspirate into the injured tissue. The use of bone marrow aspirate-derived mesenchymal stem cells is a promising approach for the treatment of musculoskeletal injuries. However, the efficacy of this approach has not been fully established. The use of bone marrow aspirate-derived conditioned media is a novel approach for the treatment of musculoskeletal injuries. This approach involves the administration of conditioned media obtained from bone marrow aspirate into the injured tissue. The use of conditioned media is a promising approach for the treatment of musculoskeletal injuries. However, the efficacy of this approach has not been fully established.
cartilage/joint injuries in horses or dogs. As stated previously, there are research-based but no clinical reports that document the use of stem cells to enhance fracture repair, nor are there any reports in cardiovascular, gastrointestinal, or neuroendocrine body systems. The first MSC-based method relies on a culture-expanded cell population derived from bone marrow aspirate, the second is another bone marrow aspirate-based approach using a concentrated mixed cell population derived from bone marrow aspiration, and the third method employs a mixed nucleated cell population derived from adipose tissue. Each technique has its strengths and weaknesses. Embryonic stem (ES) cells, induced pluripotent stem (iPS) cells, and cord blood-derived cells are also beginning to be investigated in the laboratory but have not yet been applied to the clinical scenario.

**Culture-expanded bone marrow-derived mesenchymal stem cells**

Bone marrow-derived mesenchymal stem cells (BM-MSCs) have the advantages of being easily and relatively noninvasively obtained and have a greater capacity to differentiate into tissue types of the musculoskeletal system in comparison with other MSCs [8-10]. Furthermore, BM-MSCs have received the most scientific attention and hence are the best characterized. One disadvantage of culture-expanded BM-MSCs is the time lag of 3 to 6 weeks from bone marrow aspirate until treatment. This time lag is necessitated by the time required to grow the MSCs. Bone marrow is collected from the sternum or the tuber coxae of horses under sedation or can be collected intraoperatively if the horse is already anesthetized. The horse has seven marrow spaces in the sternum, and marrow spaces 3 to 5 are the largest (up to 5 cm in diameter). Ultrasonography can be used to isolate the marrow space but is not necessary if one is familiar with the regional anatomy. Bone marrow is typically aspirated from the proximal humerus, proximal femur, or tuber coxae in dogs.

**Tendonitis**

The use of culture-expanded BM-MSCs for the treatment of tendon injuries is supported by experimental investigations in horses and laboratory animals in which MSCs were implanted in surgically or collagenase-induced tendon lesions. These studies have shown favorable effects on tissue organization, composition, and mechanics of MSC-implanted tendons and ligaments [11-14]. These studies vary in experimental design with respect to the number of BM-MSCs implanted (0.5 to 10 × 10⁶), vehicle for suspension (plasma, phosphate-buffered saline, bone marrow supernatant), and time post-injury to injection (up to 2 weeks). The clinical application of BM-MSCs was first reported in 2003 [15]. More recently, a small case control study (n = 11) demonstrated that, as a result of BM-MSCs, 90% of treated horses successfully returned to pre-injury athletic function and race horses suffered no re-injury of the superficial digital flexor tendon after 2 years whereas all of the horses of a control population suffered from re-injury [16]. In an unblinded, uncontrolled case series, Godwin and Smith [17] reported on 141 horses treated with cultured BM-MSCs with at least a 3-year follow-up. The authors reported a significant decrease in re-injury rate for National Hunt race horses but not flat-track Thoroughbred race horses treated with BM-MSCs when compared with conventionally treated historical controls (23% to 66%). To date, preclinical and clinical studies have focused on the ability of stem cells to enhance tissue regeneration and have not investigated the potential immunomodulatory roles of stem cells for tendon repair. This is most likely simply a matter of timing, with the concept of immunomodulation being more recent than the more traditional paradigm of stem cells differentiating and functioning as tissue-specific cells. Although the above-mentioned studies have documented stemness of the cells to varying degrees, tumor, ectopic bone, or cartilage formation has not been observed in either clinical or research investigations.

**Cartilage injury/osteoarthritis**

Culture-expanded BM-MSCs have been evaluated in an equine model of acute cartilage injury in which 15-mm-diameter full-thickness articular cartilage defects were created on the lateral trochlear ridge of the femur [18]. The BM-MSCs were implanted in autogenous fibrin as a scaffold in one limb, and the opposite limb was grafted with autogenous fibrin alone. At 30-day re-check arthroscopy, arthroscopy scores and biopsy assessments for the BM-MSCs lesions were significantly better than fibrin-only control grafts. However, at 8 months, no significant differences between the two groups in histologic or biochemical composition were observed. In an equine model of early osteoarthritis (OA), a direct comparison between BM-MSCs and adipose-derived stromal vascular fraction (AD-SVF) cells was made [19]. The two stem cell preparations were injected directly into affected joints 14 days after induction of OA. Joints treated with BM-MSCs showed significantly less synovial effusion and significantly lower prostaglandin E2 (PGE2) concentrations in comparison with those treated with AD-SVF cells. No differences in cartilage biochemistry or histology, synovial fluid analysis, or other clinical parameters were observed. It is interesting to note that synovial fluid PGE2 concentrations, though not directly investigated in the study, were decreased by BM-MSC treatment because PGE2 is one mechanism by which BM-MSCs modulate immune cells and exert...
anti-inflammatory/immunomodulatory effects, such as suppression of lymphocyte proliferation and T-cell activation [2,20]. Several other preclinical studies in OA models using goats, sheep, rabbits, and rats have demonstrated the capacity for BM-MSCs to enhance regeneration of cartilage and even meniscus [21,22]. Combined, these studies suggest that BM-MSCs have the dual function in an articular environment to modulate the local T cell-mediated immunological response and to enhance tissue regeneration. Long-term studies using BM-MSCs in naturally occurring articular cartilage injuries in veterinary and human patients are required to demonstrate restoration of joint function, decreased articular pain, and durability of BM-MSC-based therapies.

**Bone marrow concentrate**
Concentrated bone marrow aspirate was designed to increase the concentration of stem cells compared with naïve bone marrow aspirate and to avoid the lag time from diagnosis to treatment when culture-expanded BM-MSCs are used. In addition to the concentration of stem cells, the concentrations of platelets and therefore anabolic growth factors are increased [23]. When combined with thrombin, the fibrinogen present in BMC is converted to fibrin and a solid scaffold forms to retain the cells and growth factors in a given location.

**Tendonitis**
No peer-reviewed preclinical or clinical reports on the use of BMC for tendonitis have been published. BMC is being applied clinically for ligament and tendon injuries in horses, but sufficient data are not currently available to assess its therapeutic potential.

**Cartilage injury/osteoarthritis**
In the equine model of acute cartilage injury discussed above (15-mm-diameter lesions), one limb was treated with BMC and microfracture and the other was treated with microfracture alone [23]. Re-check arthroscopy at 3 months demonstrated significantly improved repair tissue in BMC-grafted defects compared with microfracture tissue with increased volume and greater integration of repair tissue with surrounding host cartilage. At 8 months, all macroscopic, histologic, and magnetic resonance imaging data indicated sustained improvement in BMC-grafted repair tissue in comparison with microfracture. Like many other stem cell-based technologies, BMC is being applied in clinical veterinary and human patients, but no peer-reviewed results have been published.

**Adipose-derived stromal vascular fraction cells**
The currently available technique uses a mixture of cells derived from adipose tissue surgically excised from horses or dogs. The AD-SVF cells are simply isolated and injected into the patient without a cell culture step. Compared with cultured BM-MSCs, this technique has the advantage of supplying cells in a short time period (48 hours), and it should be remembered that although there are a large number of nucleated cells retrieved from the adipose digest, only a small percentage of nucleated cells are stem cells. In humans, 0.7% to 5% of nucleated cells in the stromal vascular fraction are stem cells [24].

**Tendonitis**
No references regarding the clinical application of AD-SVF cells in equine tendonitis are currently available. Results of a pilot study demonstrated significant improvement in histologic score in AD-SVF cell-treated tendons over phosphate buffered saline-treated control tendons [25]. Although AD-SVF cells have been available for nearly 8 years and have been used to treat several thousand horses, no reports documenting their use in clinical cases of equine tendonitis have been published. AD-SVF cells are not approved by the US Food and Drug Administration for human application at this time.

**Cartilage injury/osteoarthritis**
As mentioned above, AD-SVF cell application in an equine model of early OA failed to result in any detectable improvement in articular health [19]. In fact, AD-SVF cells led to an increase in synovial fluid concentration of the proinflammatory cytokine tumor necrosis factor-alpha. In dogs, two reports of improved clinical signs of OA after treatment have been published. In a double-blinded study assessing the use of AD-SVF cells in the hip joint of dogs, examining veterinarians (but not the dog owners) reported signs of clinical improvement [26]. In a second, uncontrolled study using AD-SVF cells for elbow OA, veterinarians and, to a lesser extent, owners both reported improvements in clinical signs [27]. The disparity in the clinical benefits noted by owners in these studies investigating the use of AD-SVF cells in OA is unclear but perhaps suggests that any benefit of AD-SVF cell application can be seen only in more advanced cases of OA or that changes in lameness associated with elbow OA in comparison with those of hip OA are more easily perceived by owners.

**Debated hypothesis and the future of clinical stem cell therapy**
Irrespective of the type of stem cell being investigated, the nature of the target tissue, or the species that is being treated, the fundamental questions underlying the clinical application of stem cells are the same and include the following: (a) What is the optimal tissue source of stem cells for each clinical application? In the current clinical applications of adult-derived stem cells, it is unlikely that a single stem cell source will be best for
regeneration of tissues from the three different embryonic germ layers (endoderm, mesoderm, and ectoderm).

(b) How many stem cells are needed to effectuate regeneration? Very few dose-response studies have been performed to date, and the available data suggest that ‘more is not better’. (c) What is the best means to deliver the cells? Should they be administered locally to the site of damage or intravenously? Is a scaffold necessary, and if so, which scaffold is optimal for each tissue type? (d) Is there a requirement for co-delivery of growth factors to direct the function of the implanted cells? Many of these questions are intricately linked, and carefully designed research studies will be required to answer the debated theories.

Several avenues of stem cell therapy for tendon/ligament pathologies are currently under investigation. Several types of stem cells not discussed herein, including ES cells, umbilical cord blood-derived stem cells, and iPS cells, show promise for regenerative applications. Finally, genetically modified stem cells have been investigated in vitro and in vivo and show tremendous promise for enhancing organized repair of tendons and other musculoskeletal tissues.

Clinical uses of stem cells in reproductive medicine

Currently, there are no widespread uses of stem cell-based therapies in reproductive medicine. However, the potential utility of such approaches makes them subjects of intensive research. Broadly speaking, two stem cell types are the primary topics of investigation: ES/iPS cells and spermatogonial stem cells (SSCs). Unfortunately, despite great effort, there are no completely characterized ES or iPS cells derived from species other than primates or mice [28]. For this reason, we focus here on SSCs, which are used in the techniques of testis xenografting and spermatogonial stem cell transplantation (SSCT).

Testis xenografting

The primary clinical application for testis xenografting would be as a means to preserve the breeding potential of a genetically valuable pre-pubertal male animal [29]. For example, in the captive management of threatened or endangered species, specific individuals often have high genetic value. If adult males die before contributing their genes to the population, mature sperm can be collected and cryopreserved for future use in artificial insemination or a form of in vitro fertilization (IVF). If neonatal or juvenile males die, testis xenografting offers a means to develop sperm from their gonocytes or SSCs, which are present from parturition. In this procedure, small pieces (1 to 2 mm³) of donor testes are surgically grafted into immunodeficient mice. In the absence of a functioning immune system, the recipient mice nurture the foreign testis tissue, which supports spermatogenesis [30]. By means of this approach, morphologically mature sperm have been produced in xenografts from a number of species, including rabbits [31], pigs and goats [30], hamsters [32], rhesus macaques [33], sheep [34], cats [35], and dogs [36]. However, the efficiency of spermatogenesis in xenografts differs among species, with the bull [37-39], cats [35,40], and dogs [36] being less efficient. One common finding across species is that if the donor testis tissue has germ cells actively undergoing meiosis (as in puberty or adulthood), then the xenografts lose the ability to support spermatogenesis [40,41]. The fertilizing ability of graft-derived sperm has been verified by the production of viable offspring in allografted mouse [42] and xenografted rabbit [31] and pig [43]. Because there is no epididymis in this system, the functionally immature sperm can help generate offspring only through intracytoplasmic sperm injection (ICSI), a procedure in which sperm are injected directly into an oocyte. Thus, although banking of material from genetically valuable individuals of multiple species might begin now, the ultimate production of offspring is restricted until ICSI is optimized for that species.

Spermatogonial stem cell transplantation

The primary clinical uses of SSCT would be to preserve or manipulate the male germline or both [44]. Briefly, the technique involves isolation of a mixed germ cell population from a donor testis (preferably enriched in SSC if markers are known for that species). The isolated cells are then injected in a retrograde fashion into the testes of a recipient animal. To increase the SSC niches that might be open for colonization, the recipients are often treated with focal testicular irradiation [45,46] or systemic busulfan [47,48] to reduce their endogenous SSC. After time is allowed for colonization, proliferation, and spermatogenesis, semen is collected and assessed for the relative percentage that is of donor origin. Although it has been performed successfully in several species, this technique has multiple steps that are technically challenging and time- and labor-intensive. Therefore, it is likely to be used in the future primarily as a clinical tool to develop transgenic biomedical research models or for the production of transgenic farm animals that produce tissues/organs genetically engineered to be compatible across species or to produce pharmaceutical proteins [49]. Xenogeneic transplantation has been attempted with various donor and recipient species. Unless the donor and recipient are closely taxonomically related (for example, rat and mouse [50] as opposed to dog and mouse [51]), the recipient testes do not support spermatogenesis. Therefore, utilization for the conservation of threatened species would require not only the use of a suitable domestic animal recipient that would support spermatogenesis of the donor but also some method of sorting the sperm of donor origin from that of recipient origin.
Debated hypothesis and the future of stem cell technologies in clinical reproduction

Several questions need to be addressed in order to enhance the clinical utility of both testicular xenografting and SSCT approaches: Can markers that will label the SSC of various species be identified? Can cryopreservation methods for individualized SSC, pieces of testis tissue, and sperm be optimized? Can ‘downstream’ technologies such as classical IVF and ICSI be developed for different species? Other questions are specific to one or the other technique: Why are there differences among species in the efficiency of xenograft spermatogenesis? Why do xenografts from meiotic testes fail? Can we determine the critical parameters that define the taxonomic gulf between SSC donor species and the species that might be able to function as recipients?

Conclusions

The clinical use of stem cells in veterinary medicine is clearly in its early stages. Applications for BM-MSC and AD-SVF cells in the treatment of musculoskeletal pathologies are currently in use in several species, although the differential efficacies of various approaches are still being investigated. Optimization of these stem cell-based therapies will focus on cellular origin, isolation, enrichment, and processing as well as on the timing, route of administration, formulation, and dosing of those therapies. Development of confirmed ES or iPS cells in domestic species would greatly facilitate the development of a wider range of clinical applications. Use of stem cell-based approaches in attempts to preserve the germ plasm of threatened species could begin on an opportunistic basis in the form of xenografting of testis tissue obtained quickly after the death of pre-pubertal individuals. However, this must still be considered a research endeavor given the largely unknown causes of species differences in the success of spermatogenesis as well as the need to perform subsequent techniques of assisted reproduction which have themselves not yet been determined for most species.

Abbreviations

AD-SVF, adipose-derived stromal vascular fraction; BM-MSC, bone marrow-derived mesenchymal stem cell; ES, embryonic stem; ICSI, intra-cytoplasmic sperm injection; IVF, in vitro fertilization; MSC, mesenchymal stem cell; OA, osteoarthritis; PGE2, prostaglandin E2; SSC, spermatogonial stem cell; SSCT, spermatogonial stem cell transplantation.

Competing interests

The authors declare that they have no competing interests.

Authors’ contributions

LAF generated the review on the clinical use of stem cells. AJT provided information regarding the use of stem cells in reproductive medicine. Both authors read and approved the final manuscript.

Authors’ information

LAF is a DVM, PhD, board-certified surgeon, and associate professor of surgery at Cornell University Hospital for Animals. She maintains an active role as an orthopedic surgeon, and her laboratory focuses on translational investigation of biological methods to enhance cartilage and tendon repair. She is president of the International Cartilage Repair Society and vice president of the International Veterinary Regenerative Medicine Society. AJT is a VMD, PhD, and associate professor of reproductive biology and wildlife conservation at the Baker Institute for Animal Health at Cornell University College of Veterinary Medicine. His laboratory focuses on the functional maturation of mammalian spermatozoa, the development of reproductive technologies, and holistic approaches to wildlife conservation. He is director of the Cornell Center for Wildlife Conservation and a recipient of an NIH Pioneer Award.

Acknowledgments

The authors thank Paula Sharp for her assistance in the acquisition of data in preparation of this review. The authors acknowledge the support of the Grayson Jockey Club Research Foundation (LAF), the New York State Department of Health (N08T-64) (LAF and AJT), the Morris Animal Foundation (D07Z0-097) (AJT), and the National Institutes of Health (1RC1-1HL-100270 and 5D1 OD-006431) (AJT).

Author details

1Department of Clinical Sciences, Cornell University, VMC C3-181, Ithaca, NY 14850, USA. 2Baker Institute for Animal Health, Cornell University, VMC C3-181, Ithaca, NY 14850, USA.

Published: 23 February 2011

References

1. Yingling GL, Nobert KM. Regulatory considerations related to stem cell treatment in horses. J Am Vet Med Assoc 2008, 232:1657-1661.
2. Yagi H, Soto-Gutierrez A, Parekkadan B, Kitagawa Y, Tompkins RG, Kobayashi N, Yarmush ML. Mesenchymal stem cells: mechanisms of immunomodulation and homing. Cell Transplant 2010, 19:667-679.
3. Henkel DJ. Suspensory desmitis therapies. Proc 12th ACVS Symp 2002, 165-167.
4. Pittenger MF, Mackay AM, Beck SC, Jaiswal RK: Multilineage potential of adult human mesenchymal stem cells. Science 1999, 284:143-147.
5. Martin DR, Cox NR, Harchcock TL, Niemeyer GP, Baker HJ. Isolation and characterization of multipotential mesenchymal stem cells from feline bone marrow. Exp Hemotol 2002, 30:879-885.
6. Schnabel LV, Mohammed HO, Miller BJ, McDermott WG, Jacobson MS, Santangelo KS, Fortier LA: Platelet rich plasma (PRP) enhances anabolic gene expression patterns in flexor digitorum superficialis tendons. J Orthop Res 2007, 25:230-240.
7. Schnabel LV, Mohammed HO, Jacobson MS, Fortier LA. Effects of platelet rich plasma and acellular bone marrow on gene expression patterns and DNA content of equine suspensory ligament explant cultures. Equine Vet J 2008, 40:260-265.
8. Kissiday JD, Kopesky PW, Evans CH, Godzinsky AJ, McIvor LH, Frisbee DD. Evaluation of adult equine bone marrow- and adipose-derived progenitor cell chondrogenesis in hydrogel cultures. J Orthop Res 2008, 26:522-31.
9. Vidal MA, Kilroy GE, Lopez MJ, Johnson JR, Moore RM, Gimble JM. Characterization of equine adipose tissue-derived stromal cells: adipogetic and osteogenic capacity and comparison with bone marrow-derived mesenchymal stromal cells. Vet Surg 2007, 36:613-622.
10. Vidal MA, Robinson SO, Lopez MJ, Paulsen DB, Borkhousen O, Johnson JR, Moore RM, Gimble JM. Comparison of chorondrogenic potential in equine mesenchymal stromal cells derived from adipose tissue and bone marrow. Vet Surg 2008, 37:713-724.
11. Schnabel LV, Lynch ME, van der Meulen MC, Yaeger AE, Konstatovski MA, Nivon AJ. Mesenchymal stem cells and insulin-like growth factor-I gene-enhanced mesenchymal stem cells improve structural aspects of healing in equine flexor digitorum superficialis tendons. J Orthop Res 2009, 27:1392-1398.
12. Crowace A, Lafragola L, Rossi G, Francisco E. Histological and immunohistochemical evaluation of autologous cultured bone marrow mesenchymal stem cells and bone marrow mononucleated cells in collagenase-induced tendinitis of equine superficial digital flexor tendon. Vet Med Int 2010, 2010:250978.
13. Kovacevic D, Rodeo SA. Biological augmentation of rotator cuff tendon repair. Clin Orthop Relat Res 2008, 466:622-633.
14. Butler DL, Juncosa-Melvin N, Buvin GP, Galloway MT, Sheam JT, Gouch C, Awad H. Functional tissue engineering for tendon repair.
a multidisciplinary strategy using mesenchymal stem cells, bioscaffolds, and mechanical stimulation. J Orthop Res 2008, 26:1-9.

15. Smith RK, Korda M, Blunn GW, Goodship AE: Isolation and implantation of autologous equine mesenchymal stem cells from bone marrow into the superficial digital flexor tendon as a potential novel treatment. Equine Vet J 2003, 35:99-102.

16. Pacini S, Spinabellini S, Tormel L, Fazzi R, Galimberti S, Dini F, Carlucci F, Petrini M: Suspension of bone marrow-derived undifferentiated mesenchymal stromal cells for repair of superficial digital flexor tendon in race horses. Tissue Eng 2007, 13:2949-2956.

17. Godwin EE, Smith RW: Implantation of bone marrow-derived mesenchymal progenitor cells demonstrates improved outcome in horses with over-strain injury of the superficial digital flexor tendon. Equine Vet J, in press.

18. Wilke MM, Nydam DV, Nixon AJ: Enhanced early chondrogenesis in articular defects following arthroscopic mesenchymal stem cell implantation in an equine model. J Orthop Res 2007, 25:913-925.

19. Frisbie DD, Ksdaay JD, Kawcak CE, Werpy WM, McIwrath CW: Evaluation of adipose-derived stromal vascular fraction or bone marrow-derived mesenchymal stem cells for treatment of osteoarthritis. J Orthop Res 2009, 27:1675-1680.

20. Najar M, Raicevic G, Bouker H, Kazan H, Bruyn CD, Meuleman N, Bron D, Toungouz M, Lagneaux L: Mesenchymal stromal cells use PGE2 to modulate activation and proliferation of lymphocyte subsets: combined comparison of adipose tissue, Whatton's Jersey and bone marrow sources. Cell Immunol 2010, 264:117-129.

21. Murphy JM, Fink DJ, Hunziker EB, Barry FP: Stem cell therapy in a caprine model of osteoarthritis. Arthritis Rheum 2003, 48:3464-3474.

22. Izuta Y, Ochi M, Adachi N, Deie M, Yasamaki T, Shimomura Y: Meniscal repair using bone marrow-derived mesenchymal stem cells: experimental study using green fluorescent protein transgenic rats. Knee 2005, 12:217-223.

23. Fortier LA, Potter HG, Rickett EJ, Schnabel LV, Foo LF, Chong LR, Stokol T, Cheetham J, Nixon AJ: Concentrated bone marrow aspirate improves full-thickness cartilage repair compared to microfracture in an equine model of extensive cartilage loss. J Bone Joint Surg 2010, 92:1927-1937.

24. Jurgens WJ, Oedayasrings-Varma MJ, Helder MN, Zandehdosouli B, Schouten TE, Kuik DJ, Ritt MJ, van Milligen FJ: Effect of tissue-harvesting site on yield of stem cells derived from adipose tissue: implications for cell-based therapies. Cell Tissue Res 2008, 332:415-426.

25. Nixon AJ, Dahlgren LA, Haupt JL, Yeager AE, Ward DL: Effect of adipose-derived nucleated cell fractions on tendon repair in horses with collagenase-induced tendinosis. Am J Vet Res 2008, 69:928-937.

26. Black LL, Gaynor J, Gahring D, Adams C, Aron D, Harman S, Gingerich DA, Harman R: Effect of adipose-derived mesenchymal stem and regenerative cells on lamenesis in dogs with chronic osteoarthritis of the coxofemoral joints: a randomized, double-blinded, multicenter, controlled trial. Vet Ther 2007, 8:272-284.

27. Black LL, Gaynor J, Adams C, Dhupa S, Sarns AE, Taylor R, Harman S, Gingerich DA, Harman R: Effect of intrarticular injection of adipose autologous adipose-derived mesenchymal stem and regenerative cells on clinical signs of chronic osteoarthritis of the elbow joint in dogs. Vet Ther 2008, 9:192-200.

28. Munoz M, Trigal B, Molina I, Diez C, Caamano JN, Gomez E: Constraints to progress in embryonic stem cells from domestic species. Stem Cell Rev 2009, 5:6-9.

29. Pukazhenthi B, Comizzoli P, Travis AJ, Wildt DE: Applications of emerging technologies to the study and conservation of threatened and endangered species. Reprod Fertil Dev 2006, 18:77-90.

30. Honaramooz A, Snedaker A, Boiani M, Scholler H, Dobrinski I, Schlatt S: Sperm from neonatal mammalian testes grafted in mice. Nature 2002, 418:778-781.

31. Shinohara T, Inoue K, Ogonuki N, Kanatsu-Shinohara M, Miki H, Nakata K, Kurose M, Nagashima H, Toyokuni S, Kogishi K, Hono T, Osga A: Birth of offspring following transplantation of cryopreserved immature testicular pieces and in-vitro microinsemination. Hum Reprod 2002, 17:3039-3045.

32. Schlatt S, Kim SS, Gosen R: Spermatogenesis and steroidogenesis in mouse, hamster and monkey testicular tissue after cryopreservation and heterotopic grafting to castrated hosts. Reproduction 2002, 124:339-346.

33. Honaramooz A, Li MW, Penedo MC, Meyers S, Dobrinski I: Accelerated maturation of primate testis by xenografting into mice. Biol Reprod 2004, 70:1500-1503.

34. Zeng W, Awler G, Rath R, Franca LR, Dobrinski I: The length of the spermatogenic cycle is conserved in porcine and ovine testis xenografts. J Androl 2006, 27:527-533.

35. Snedaker AK, Honaramooz A, Dobrinski I: A game of cat and mouse: xenografting of testis tissue from domestic kittens results in complete cat spermatogenesis in a mouse host. J Androl 2004, 25:936-939.

36. Abnishi M, Abbasi S, Honaramooz A: The effect of donor age on progression of spermatogenesis in canine testicular tissue after xenografting into immunodeficient mice. Theriogenology 2010, 73:512-522.

37. Rath R, Honaramooz A, Zeng W, Turner R, Dobrinski I: Germ cell development in equine testis tissue xenografted into mice. Reproduction 2006, 131:1091-1098.

38. Rath R, Honaramooz A, Zeng W, Schlatt S, Dobrinski I: Germ cell fate and seminiferous tubule development in bovine testis xenografts. Reproduction 2005, 130:923-929.

39. Oatley JM, de Avila CM, Reeves JJ, McLean DJ: Spermatogenesis and germ cell transgene expression in xenografted bovine testicular tissue. Biol Reprod 2004, 71:494-501.

40. Kim Y, Selvaraj V, Pukazhenthi B, Travis AJ: Effect of donor age on success of spermatogenesis in feline testis xenografts. Reprod Fertil Dev 2007, 19:869-876.

41. Areegui L, Rath R, Zeng W, Honaramooz A, Gomendio M, Roldan ER, Dobrinski I: Xenografting of adult mammalian testis tissue. Anim Reprod Sci 2008, 106:65-76.

42. Schlatt S, Honaramooz A, Boiani M, Scholer H, Dobrinski I: Prenency from sperm obtained after ectopic grafting of neonatal mouse testes. Biol Reprod 2003, 68:2331-2335.

43. Nakai M, Kaneko H, Somfai T, Maedomi N, Ozawa M, Noguchi J, Ito J, Kashiwazaki N, Kikuchi K: Production of viable piglets for the first time using sperm derived from ectopic testicular xenografts. Reproduction 2010, 139:331-335.

44. Dobrinski I, Travis AJ: Germ cell transplantation for the propagation of companion animals, non-domestic and endangered species. Reprod Fertil Dev 2007, 19:322-329.

45. Honaramooz A, Behboudi E, Hausler CL, Blash S, Ayres S, Azuma C, Echelard Y, Dobrinski I: Depletion of endogenous germ cells in male pigs and goats in preparation for germ cell transplantation. J Androl 2005, 26:696-705.

46. Kim Y, Turner D, Nelson J, Dobrinski I, McIntee M, Travis AJ: Production of donor-derived sperm after spermatozonal stem cell transplantation in the dog. Reproduction 2008, 136:823-831.

47. Brinster RL, Zimmermann JW: Spermatogenesis following male germ-cell transplantation. Proc Natl Acad Sci U S A 1994, 91:11298-11302.

48. Hill JR, Dobrinski I: Male germ cell transplantation in livestock. Reprod Fertil Dev 2006, 18:13-18.

49. Houdebin LM: Production of pharmaceutical proteins by transgenic animals. Comp Immunol Microbiol Infect Dis 2009, 32:107-121.

50. Clouthier DE, Avarbock MR, Maika SD, Hammer RE, Brinster RL: Rat spermatogenesis in mouse testis. Nature 1996, 381:418-421.

51. Dobrinski I, Avarbock MR, Brinster RL: Transplantation of germ cells from rabbits and dogs into mouse testes. Biol Reprod 1999, 61:1331-1339.