Effects of administration of adipose-derived stromal vascular fraction and platelet-rich plasma to dogs with osteoarthritis of the hip joints

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OBJECTIVE
To evaluate effects of simultaneous intra-articular and IV injection of autologous adipose-derived stromal vascular fraction (SVF) and platelet-rich plasma (PRP) to dogs with osteoarthritis of the hip joints.

ANIMALS
22 client-owned dogs (12 placebo-treated [control] dogs and 10 treated dogs).

PROCEDURES
Dogs with osteoarthritis of the hip joints that caused signs of lameness or discomfort were characterized on the basis of results of orthopedic examination, goniometry, lameness score, the Canine Brief Pain Inventory (CBPI), a visual analogue scale, and results obtained by use of a pressure-sensing walkway at week 0 (baseline). Dogs received a simultaneous intra-articular and IV injection of SVF and PRP or a placebo. Dogs were examined again 4, 8, 12, and 24 weeks after injection.

RESULTS
CBPI scores were significantly lower for the treatment group at week 24, compared with scores for the control group. Mean visual analogue scale score for the treatment group was significantly higher at week 0 than at weeks 4, 8, or 24. Dogs with baseline peak vertical force (PVF) in the lowest 25th percentile were compared, and the treatment group had a significantly higher PVF than did the control group. After the SVF-PRP injection, fewer dogs in the treated group than in the control group had lameness confirmed during examination.

CONCLUSIONS AND CLINICAL RELEVANCE
For dogs with osteoarthritis of the hip joints treated with SVF and PRP, improvements in CBPI and PVF were evident at some time points, compared with results for the control group. (*Am J Vet Res* 2016;77:940–951)

Hip dysplasia is one of the most common orthopedic abnormalities in dogs, with an incidence of up to 40% in some breeds.[1–4] Current treatment strategies for osteoarthritis include weight loss and exercise to decrease the magnitude of forces on osteoarthritic joints, hip replacement to palliate joint discomfort, medications that modulate disease signs, physiotherapy exercises, cryotherapy, therapeutic ultrasound, low-level laser treatments, and electrical stimulation. Pharmaceutical agents commonly used to treat osteoarthritis include NSAIDs, which inhibit prostaglandin E_2 via cyclooxygenase inhibition, and nutraceuticals (eg, glucosamine and hyaluronan), which may function via anti-inflammatory activity.[5] These agents do not target most of the proinflammatory mediators, and they are unable to stop the catabolic state found in osteoarthritic joints.

ABBREVIATIONS

BCS	Body condition score
CBPI	Canine Brief Pain Inventory
IL	Interleukin
MSC	Mesenchymal stem cell
PRP	Platelet-rich plasma
PSW	Pressure-sensing walkway
PVF	Peak vertical force
SVF	Stromal vascular fraction
VAS	Visual analogue scale
VI	Vertical impulse

An ideal treatment modality would halt or reverse the inflammatory cascade that causes osteoarthritis, and treatment with MSCs can theoretically achieve that goal. Mesenchymal stem cells can engraft into host tissues and differentiate into target cells.[6] Proposed mechanisms of action of MSCs in the treatment of osteoarthritis include immunomodulation and reversal of the proinflammatory cascade, proangiogenic and antiapoptotic effects, and decreased production of scar tissue.[7] In vitro, the mechanism of action for MSCs is both contact mediated (smaller effect) via

an unknown mechanism and contact independent (larger effect), which is mediated by immune-modifying chemicals. For example, MSCs secrete IL-1–receptor antagonist, a potent inhibitor of IL-1.[8] It has been theorized that MSCs also inhibit tumor necrosis factor-α and produce the anti-inflammatory cytokine IL-10.[8–10] Mesenchymal stem cells inhibit activated T-cell, B-cell, and natural-killer cell proliferation and downregulate expression of major histocompatibility complex II on inflammatory cells.[9,10] In addition, MSCs can suppress dendritic cell maturation (which leads to generation of T-regulatory cells) and suppress macrophage activation.[11,12] Mesenchymal stem cells injected IV accumulate in areas of inflammation, including joints, where they can then exert local anti-inflammatory and immunomodulatory effects.[13]

Injection of MSCs into joints with experimentally induced osteoarthritis can decrease cartilage destruction, osteophyte formation, and subchondral sclerosis and even lead to regeneration of meniscal and articular cartilage.[14–17] Improved clinical outcomes have been reported when MSCs were injected intra-articularly into the joints of animals affected by naturally occurring osteoarthritis.[18–22] Subjective measures such as owner-perceived functional disability and lameness and pain evaluation performed by veterinarians have been used as outcome measures; improvement has been reported for some or all of these criteria for up to 7 months after treatment.[18–20] Quantitative outcomes measures (obtained by use of a PSW) have been used to evaluate dogs with hip joint osteoarthritis after MSC treatment.[21,22] Investigators of these studies[21,22] found an improvement in PVF of the more lame limb of dogs at 3 months after a single MSC treatment.

Two main concerns have prevented widespread clinical use of MSC treatment. First, MSC treatment requires in vitro expansion of cells to yield sufficient numbers of cells. In vitro expansion of cells requires Good Cell Manufacturing Processing protocols in a US FDA–inspected facility to comply with FDA manufacturing requirements.[23] Because of these restrictions, an alternative to expanded autologous MSCs must be considered because it would not be feasible or cost-effective to produce autologous MSCs in a Good Manufacturing Practices facility. Second, the safety of MSC treatment has been questioned, with concerns including neoplastic transformation of the cells, increased patient susceptibility to infection, embolism of the cells, and acute or chronic immune reactions to the cells.[24] Investigators of 1 meta-analysis[25] of human studies that involved the use of MSCs administered IV found that there was no increased risk of adverse effects with MSC treatment, except for an increased risk of transient fever. In a more recent study,[26] investigators found that mice had an 85% mortality rate (attributable to pulmonary thromboembolism) within 24 hours after receiving an IV injection of 1.5×10^5 adipose-derived MSCs. The authors of that study[26] speculated that the MSCs expressed tissue factor, which stimulated the extrinsic clotting cascade. Similar procoagulation effects were found in vitro when human adipose-derived MSCs were exposed to human blood or plasma.[26] A few cases of pulmonary thromboembolism associated with MSC treatment of humans have been reported[27,28]; 1 case was fatal. The safety of MSCs delivered via alternative routes and the safety of SVF have not yet been assessed, although studies[29–32] conducted in domestic animals revealed no adverse events. For these reasons, we believed that autologous SVF that can be harvested in a manner that meets the minimal manipulation criteria established by the US FDA should be evaluated as an alternative source of autologous MSCs for the treatment of osteoarthritis.

Adipose tissue can be easily harvested from most dogs and is a rich source of MSCs. Human adipose tissue contains 500 times as many MSCs per milliliter as does bone marrow, the other source commonly used to obtain autologous MSCs.[33] Although techniques differ for SVF isolation, adipose tissue is minced and then digested by enzymes to release SVF in a procedure that takes several hours and requires no in vitro culture or expansion.[34] It is important to mention that in addition to MSCs, SVF contains endothelial precursor cells, monocytes, macrophages, T-regulatory cells, pericytes, mast cells, preadipocytes, fibroblasts, and smooth muscle cells.[35,36] These other cell types may enhance the beneficial effects of MSCs. Endothelial precursor cells may secrete the proangiogenic mediator vascular endothelial growth factor, M2 macrophages may secrete IL-10 and IL-1–receptor antagonist, and T-regulatory cells may help to maintain the M2 phenotype of macrophages and act in an immunosuppressive capacity.[35,37–40]

Autologous PRP has been used as a treatment for osteoarthritis,[41,42] but it can also be coadministered with MSCs or SVF. Platelet-rich plasma contains several growth factors, including vascular endothelial growth factor, platelet-derived growth factor, fibroblast growth factor 2, and transforming growth factor-β, that can enhance regenerative processes and promote healing of intra-articular structures.[41,42] Coadministration of PRP and adipose-derived MSCs to mice with experimentally induced osteoarthritis led to enhanced proliferation of the MSCs as well as improved joint function and cartilage regeneration, compared with results after administration of MSCs alone.[42] A study[22] of dogs with osteoarthritis of the hip joints that were treated with intra-articular adipose-derived MSCs combined with PRP revealed improvement for kinetic outcome variables at 30 days, compared with kinetic variables measured before treatment, although the improvement was similar to that observed in a similar study[21] conducted with adipose-derived MSCs alone.

Treatment with SVF has become increasingly available to veterinarians in the United States via several commercially available platforms and procedures.[28] The efficacy of SVF for treatment of ani-

mals with osteoarthritis has been assessed.[29–32] In 3 of those studies,[29–31] a total of 27 dogs were evaluated and had subjective improvements in joint range of motion and owner-perceived functional outcome for 3 to 6 months after a single treatment. The other study[32] included radiographic and histopathologic data and concluded that SVF treatment had no benefit over MSC treatment or placebo treatment for horses with experimentally induced osteoarthritis of the carpal joints. However, none of those studies involved the use of objective kinetic data, such as that obtained with a PSW, for outcome measures.

Therefore, the study reported here was conducted to evaluate the safety and efficacy of autologous intra-articular and IV administration of SVF and PRP for treatment of dogs with naturally occurring osteoarthritis of the hip joints. Primary outcome measures were ground reaction force data obtained by use of a PSW, lameness examination score,[29] and a validated owner survey of pain.[43,44] Secondary outcome variables were results of goniometry,[45] a VAS completed by a veterinarian,[46] and radiographic analysis of affected joints. We hypothesized that dogs receiving SVF-PRP injections would have improvement in outcome measures, compared with results for outcome measures obtained before treatment. We also hypothesized that SVF-PRP injections would be safe as defined by a lack of adverse effects at the time of injection and no increase in the incidence of adverse events during the 6-month period after injection.

Materials and Methods

Animals

The study population consisted of client-owned dogs with osteoarthritis of the hip joints. Participants were recruited through email alerts as well as online, print, and radio advertisements. Costs associated with lameness evaluation and treatments were paid by grant funds, and owners received no financial incentive. Owners provided informed consent for participation of their dogs in the study. The study was approved by the Kansas State University Institutional Animal Care and Use Committee. The study was registered with the FDA for investigation of a new animal drug (INAD registry No. 12457).

Eligibility criteria for participation of dogs in the study included body weight > 15 kg, history of lameness or dysfunction attributable to osteoarthritis (decreased amount of activity, signs of pain, or inability to rise from a lying position) as reported by the owner, a difference in PVF (measured as a percentage of body weight) ≥ 5% between the 2 hind limbs (to establish one limb as clinically worse than the other) or PVF < 34% of the combined body weight on both hind limbs on a PSW,[a] and radiographic evidence of osteoarthritis of the hip joint of the lame limb. Dogs could be receiving NSAIDs, drugs that modified clinical signs of osteoarthritis, therapeutic diets, or analgesics (except for corticosteroids); no changes in medications or supplement-type products were permitted for 2 weeks prior to enrollment or during the 24-week study period. Exclusion criteria included dogs with an unsuitable temperament, historical or physical examination evidence of other pathological processes in the hind limbs (conditions affecting the stifle joint or tibiotarsal joint, or neurologic gait abnormalities), changes to analgesic medications within the 2 weeks preceding study enrollment or during the study, or evidence of systemic disease during physical or hematologic examinations.

Dogs were allocated to treatment or placebo (control) groups (1:1) at the time of evaluation for inclusion in the study (ie, prior to onset of the study). Allocations to experimental groups were performed with a random-number table.

Study design

A randomized, double-blind, placebo-controlled prospective trial was conducted. An initial evaluation of all candidate dogs was performed before onset of the study. Dogs also were evaluated at week 0 (baseline; which was prior to adipose tissue collection, SVF isolation, and joint injection) and at 4, 8, 12, and 24 weeks after joint injection.

Initial evaluation

Dogs were examined by one of the investigators (DAU) who was unaware of the experimental group allocation for each dog. Initial evaluation included determination of patient age, sex, breed, limb (or limbs) affected, duration of lameness, history of prior orthopedic surgery, and type and duration of current pain medications or supplement-type products. Body weight and BCS (scale, 1 [thin] to 5 [obese]) were recorded. Complete orthopedic and neurologic examinations and a visual lameness examination were performed. Lameness grade in accordance with a previously described scoring system[29] was assigned as follows: 1 = no lameness observed, 2 = intermittent weight-bearing lameness, 3 = persistent weight-bearing lameness, 4 = persistent non–weight-bearing lameness, 5 = ambulatory only with assistance, and 6 = nonambulatory.

Owners were unaware of experimental group allocation of their pets. Owners were required to complete the CBPI survey (0 = no pain or interference and 10 = extreme pain or interference) at each evaluation. The CBPI is a validated 2-part owner questionnaire that evaluates pain severity (questions 1 through 4) and interference of pain with daily activities (questions 5 through 10).[43,44]

Goniometry of the affected (lame) hip joint was performed by one of the investigators (DAU) using a 2-arm metal goniometer[b] with 1° increments, as described elsewhere.[45] Goniometry was also performed on the contralateral hip joint. After the orthopedic assessment was performed, the investigator (DAU) completed a VAS, as described elsewhere.[46]

After the initial examination and goniometry were completed, each dog was walked across the PSW by 1 of 2 handlers. Dogs were walked across the PSW until they appeared comfortable and acclimated to the testing room; data were then collected. A valid trial was defined as one in which a dog walked at a steady pace and in a straight line along the entire length of the PSW and in which each paw strike was within the recording area of the mat. Five valid trials were obtained for each dog. Data for these 5 trials were analyzed by use of system-specific software,[c] and stance time, stride velocity, PVF, VI, and maximum peak pressure were recorded.

After evaluation by use of the PSW was completed, dogs meeting inclusion criteria were sedated by IV administration of hydromorphone[d] (0.08 mg/kg) and acepromazine maleate[e] (0.02 mg/kg) and positioned for lateral and ventrodorsal extended-limb pelvic radiographs. A CBC and serum biochemical analysis were performed on all dogs. Dogs were included in the study if radiography confirmed osteoarthritis of the affected joint and no major abnormalities were detected during hematologic analysis.

Adipose tissue collection

Adipose tissue was harvested from each dog (regardless of treatment group) within 3 months after the initial evaluation. Dogs were sedated by IV administration of hydromorphone (0.08 to 0.1 mg/kg) and acepromazine (0.01 to 0.03 mg/kg). Anesthesia was induced by IV administration of propofol[f] (2 to 8 mg/kg), and dogs were placed in dorsal recumbency. A venous blood sample (18 mL) was collected, which was used for preparation of PRP. The ventral aspect of the abdomen was clipped and aseptically prepared for surgery. A 5-cm midline incision was made just cranial to the umbilicus. The incision was extended though the linea alba, and a minimum of 40 g of falciform adipose tissue was harvested and placed in a sterile plastic container. The incision was closed in a routine manner. Dogs were allowed to recover from anesthesia; later that day, dogs received the injections and then were released to their owners. Codeine sulfate[g] (1 to 2 mg/kg, PO, q 8 h for 72 hours) was administered for postoperative analgesia. Duration of anesthesia, duration of surgery, and postoperative complications were recorded.

Adipose tissue and PRP processing

A standardized quantity of adipose tissue (enough to fill a 40-mL container) was processed for each dog immediately after collection. Samples were processed by a trained technician, who used a commercial kit[h] to yield SVF pellets.

Processing of PRP was conducted by use of a validated method that has been found to provide a platelet capture efficacy of 25%.[47] Each 18-mL venous blood sample was centrifuged (978 × *g* for 4 minutes), and plasma was harvested. The remaining cellular material was centrifuged at 978 × *g* for 8 minutes to yield a platelet pellet. The platelet pellet was resuspended in 4 mL of plasma, and platelets then were lysed by the addition of 0.7 mL of solution G contained in the commercial kit.[h] Samples were incubated for 25 minutes in a water bath at 37°C, which allowed the solution to gel. Samples were allowed to sit undisturbed for 1 hour, which allowed the gel to retract. The liquid phase then was removed, which yielded 3 to 4 mL of PRP.

The SVF pellet was suspended in 2 mL of autologous PRP and exposed to a light source for 20 minutes. A cell viability counter[i] was used to determine the number of live nucleated cells per milliliter and to determine a live-to-dead ratio. The SVF was plated in 20% fetal bovine serum containing Dulbecco modified Eagle medium at a density of 30,000 live cells/cm^2 and incubated (37°C, 90% humidity, and 5% CO_2) to allow determination of cell attachment and expansion consistent with the MSC phenotype, and an aliquot was used to confirm MSC content after in vitro expansion.

Injection of SVF-PRP or placebo treatments

The SVF-PRP or a placebo (sterile saline [0.9% NaCl] solution[j]) was administered the same day as harvest of adipose tissue. Data obtained from the baseline PSW trials were used to select the limb with the lower PVF, which would receive an intra-articular injection. When both limbs had the same PVF, 1 limb was arbitrarily selected for intra-articular injection. Dogs were sedated by IV administration of hydromorphone (0.08 to 0.10 mg/kg) and acepromazine (0.01 to 0.03 mg/kg). Hair was clipped from an area overlying the hip joint to be injected, and the area was aseptically prepared. Arthrocentesis of the hip joint was performed by one of the investigators (WCR) who was not aware of the treatment group for each dog. Synovial fluid was aspirated (0.2 to 5.0 mL was removed from the joint, if possible), after which 0.5 mL of SVF-PRP (treatment group) or 0.5 mL of sterile saline solution (control group) was injected into the joint. Simultaneously, 0.5 mL of SVF-PRP (treatment group) or 0.5 mL of sterile saline solution (control group) was injected into a cephalic vein.

Follow-up evaluation

All dogs were assessed by one of the investigators (DAU) at 4, 8, 12, and 24 weeks after injection. Dogs were evaluated as previously described, and data (body weight, BCS, lameness score, and range of motion for both hip joints) were recorded. At all time points, the investigator completed a VAS (score, 0 to 10 cm) after orthopedic and neurologic examinations were performed and ground reaction force data were collected. At each assessment, the owner who had completed the CBPI questionnaire previously was again requested to complete another CBPI questionnaire.

After examinations and PSW evaluation were completed at week 24, pelvic radiographs were obtained by use of the same protocols described previ-

ously. Radiographs from weeks 0 and 24 were scored for osteoarthritis by a board-certified veterinary radiologist (LJA) who was not aware of the treatment group for each dog. Radiographs were scored as follows: 0 = anatomically normal joint, 1 = joint with radiographic evidence of instability with no degenerative change, 2 = joint with mild degenerative change and a few osteophytes, 3 = joint with moderate degenerative change including osteophytes and subchondral sclerosis, and 4 = joint with severe degenerative change including osteophytes, subchondral sclerosis, and bone remodeling.[48]

Statistical analysis

Statistical analysis was performed with a commercially available software package.[k] Age, body weight, duration of lameness, data obtained from PSW analysis (stance time, stride velocity, PVF, VI, and maximum peak pressure), and goniometric data (maximum flexion, maximum extension, and range of motion) were compared between treatment groups by use of an independent group means test. Change in body weight, PSW data, and goniometric data was evaluated among time points within each treatment group by use of a repeated-measures ANOVA for main effects and interactions. Data for BCS, lameness examination scores, VAS, CBPI, and radiographic scores were compared between treatment groups at each time point by use of a nonparametric Mann-Whitney *U* test. Data for BCS, lameness examination scores, VAS, CBPI, and radiographic scores were compared among time points within each treatment group by use of the Friedman test for nonparametric repeated measures.

The PVF at baseline was used to stratify data, and a mixed-model ANCOVA was used to describe changes from baseline for the objective variables.[49] An unequal variance model for time and a first-degree autocorrelation were used to account for the correlation among the weeks within a dog. Dog within treatment group was used as a random effect. The baseline value and number of cells in the SVF were considered as covariates. Covariate variables with nonsignificant effects were deleted from the model. An ANCOVA was also used to compare changes between the treatment and control groups for variables at all postinjection time points, relative to the baseline value. A commercially available software package[l] was used for all computations.

A post hoc power analysis ($\alpha = 0.05$ and power = 0.80) was performed for each variable by use of an available program.[m] Data were reported as mean ± SD. Significance was set at values of $P \leq 0.05$. Standardized effect size was calculated by use of the Cohen d (the difference in the means of the treatment and control group for each variable divided by the pooled SD).

Results

Sixty-eight client-owned dogs were initially evaluated for inclusion in the study. Of these dogs, 46 were excluded. Reasons for exclusion included failure to meet PSW criteria for PVF abnormalities (n = 25 dogs), pathological changes to the stifle joint (16), hepatic disease (2), neurologic gait abnormalities (2), and respiratory tract disease (1).

Thus, 22 (32.3%) dogs (11 males and 11 females) were enrolled in the study. Dogs ranged from 1 to 14 years of age (mean, 8.0 years). Body weight ranged from 15.8 to 58.3 kg (mean, 35.0 kg), and BCS ranged from 2 to 5 (mean, 3.5). During the study, 3 dogs received NSAIDs, 7 dogs received glucosamine and hyaluronic acid products, 6 dogs received opioids, and 1 dog received acupuncture treatment. On the basis of results of PSW analysis, 11 dogs had a lower PVF in the right hind limb and 10 had a lower PVF in the left hind limb. One dog had the same PVF in both hind limbs with a combined 34% of body weight, and the right hind limb was arbitrarily selected to receive treatment. Nineteen dogs were visibly lame during initial assessment, and the remaining 3 dogs had signs consistent with hip dysplasia (difficulty rising and bunny-hopping gait) reported by the owners. Results for a CBC and serum biochemical analysis revealed no clinically important abnormalities. Twenty-one dogs had radiographic evidence of bilateral osteoarthritis of the hip joints, whereas the remaining dog had unilateral osteoarthritis.

Seventeen dogs completed the study. One dog in the control group was euthanized because of a splenic hemangiosarcoma approximately 2 weeks after placebo injection, and data for this dog were censored from the study. Two dogs in the control group were withdrawn from the study after cranial cruciate ligament rupture was diagnosed at the week 8 and week 12 evaluations (1 dog at each time point). One dog in the control group was lost to follow-up evaluations after week 4, and 1 dog in the treatment group was withdrawn from the study after the evaluation at week 12 because the owner perceived worsening signs of pain. Data for these dogs were included up to the time of removal from the study. Final follow-up evaluation for 2 dogs was performed at 32 weeks instead of 24 weeks.

Apart from the aforementioned cases, no dogs developed additional pathological conditions during the study. There were no acute toxic reactions associated with SVF-PRP infusion or any adverse reactions that could be attributed to SVF-PRP treatment. Two dogs (1 in the control group and 1 in the treatment group) had increased lameness and signs of pain on manipulation of the hip joint for up to 1 week after the injections. In both cases, signs of pain resolved without additional treatment.

Mean live cell count for the SVF injection as determined by use of a cell viability counter was 341 million cells/mL (range, 64 million to 584 million cells/mL). We observed MSC-like cells in all SVF isolates after culture for 7 to 10 days, and no bacterial contamination was detected. No significant differ-

ences were found in the viable cell count between the control and treatment groups.

Mean ± SD age did not differ significantly (P = 0.457) between the treatment (8.60 ± 4.13 years) and control (7.46 ± 4.27 years) groups. Similarly, mean body weight at initial evaluation did not differ significantly (P = 0.175) between the treatment (32.74 ± 9.13 kg) and control (37.25 ± 12.39 kg) groups. Mean duration of lameness prior to enrollment in the study differed significantly (P = 0.035) between the treatment (36.4 ± 31.7 months) and control (49.9 ± 31.7 months) groups. There were no significant differences in body weight between the groups at any time point during the study, although the treatment group tended to lose weight over time, whereas the control group gained weight **(Table 1)**.

Goniometric data (maximum flexion, maximum extension, and range of motion), VAS score, and BCS were compared between treatment groups (Table 1). Similarly, lameness score and CBPI scores were also compared between treatment groups **(Table 2)**. Radiographic scores did not differ significantly between the treatment and control groups at week 0 (treated: median, 2 [range, 0 to 4]; control: median, 2 [range, 1 to 4]) or at week 24 (treated: median, 3 [range, 0 to 4]; control: median, 4 [range, 2 to 4]).

An ANOVA model was used to test main effects, and no significant differences were found between the treatment and control groups at any time point for PVF, VI, stance time, stride velocity, or maximum peak pressure when treated or untreated (contralateral) limbs were assessed **(Table 3)**. When an ANCOVA was used with the PVF value at baseline as a covariate, both treatment and baseline PVF values had a significant effect. When PVF data were stratified, a significant difference was detected between the treatment and control groups for dogs in the lowest 25th percentile for PVF value **(Figure 1)**. The standardized effect size of dogs in the PVF 10th percentile was 0.38. The standardized effect size of dogs in the PVF 25th percentile was 0.34.

The treatment group had a significantly (P = 0.036) lower BCS, compared with the BCS for the control group, at 24 weeks; BCS did not differ sig-

Table 1—Mean ± SD body weight, VAS score, and goniometric values at each time point for dogs that received simultaneous intra-articular and IV injections of SVF-PRP (treated group [n = 10]) or a placebo (saline [0.9% NaCl solution]; control group [12]).

		Time (wk)*				
Variable	**Group**	**0**	**4**	**8**	**12**	**24**
Body weight (kg)	Treated	32.74 ± 9.13	32.50 ± 9.17	32.40 ± 9.26	32.17 ± 9.00	30.42 ± 9.26
	Control	37.25 ± 12.39	36.91 ± 12.01	39.10 ± 11.53	39.96 ± 12.52	40.43 ± 12.09
VAS (cm)†	Treated	3.35 ± 1.09	1.96 ± 0.98	1.91 ± 1.09	2.08 ± 1.35	1.70 ± 1.14
	Control	4.23 ± 2.24	2.43 ± 1.51	2.81 ± 1.53	2.60 ± 0.99	2.14 ± 1.60
Maximum flexion (°)	Treated	43.36 ± 7.36	42.27 ± 6.47	40.91 ± 8.31	43.64 ± 9.24	43.50 ± 8.83
	Control	42.46 ± 8.38	44.55 ± 10.60	42.22 ± 8.70	46.25 ± 8.76	45.71 ± 4.50
Maximum extension (°)	Treated	137.27 ± 16.64	145.00 ± 7.75	146.82 ± 11.89	148.64 ± 9.77	143.30 ± 13.70
	Control	136.09 ± 15.07	142.27 ± 22.73	143.89 ± 12.44	138.75 ± 15.53	140.00 ± 19.58
Range of motion (°)	Treated	93.91 ± 19.18	102.73 ± 6.84	105.91 ± 14.97	105.00 ± 16.43	99.80 ± 16.27
	Control	93.64 ± 17.84	97.73 ± 24.53	101.67 ± 16.20	92.50 ± 20.53	94.29 ± 21.49

Values did not differ significantly ($P \leq 0.05$) between treatment groups at any time point.
*Injections were administered at week 0. †The VAS was scored on a scale of 0 to 10 cm.

Table 2—Median (range) BCS, lameness score, and CBPI score at each time point for dogs that received simultaneous intra-articular and IV injections of SVF-PRP (treated group [n = 10]) or saline solution (control group [12]).

		Time (wk)*				
Variable	**Group**	**0**	**4**	**8**	**12**	**24**
BCS†	Treated	4.0 (2.5–4.5)	4.0 (2.5–5.0)	3.0 (2.5–4.5)	3.5 (3.0–5.0)	3.0 (2.5–4.0)[a]
	Control	3.5 (2.0–5.0)	3.0 (2.0–5.0)	4.0 (2.5–5.0)	4.0 (2.5–5.0)	4.5 (3.0–5.0)[b]
Lameness score‡	Treated	3.0 (1.0–3.0)	3.0 (1.0–3.0)	1.0 (1.0–3.0)	1.0 (1.0–3.0)	1.5 (1.0–3.0)
	Control	3.0 (1.0–3.0)	3.0 (1.0–3.0)	3.0 (1.0–3.0)	3.0 (1.0-3.0)	3.0 (1.0–3.0)
CBPI§						
Pain severity‖	Treated	4.75 (0.25–7.25)	2.50 (0–6.25)	1.75 (0–7.00)	1.00 (0–6.75)	1.75 (0–7.00)[a]
	Control	5.00 (0–7.75)	4.38 (0– 6.35)	3.50 (0–5.35)	5.50 (0–6.25)	3.00 (0–8.75)[b]
Pain interference with activities¶	Treated	6.17 (0–8.67)	2.17 (0–7.50)	2.00 (0–8.00)	2.00 (0–8.83)	2.00 (0–8.00)
	Control	6.50 (0.17–9.67)	4.17 (0.17–9.50)	5.75 (0.17–8.17)	6.33 (0.50–8.33)	4.67 (0–9.83)

†The BCS was scored on a scale of 1 (thin) to 5 (obese). ‡Lameness was scored as follows: 1 = no lameness observed, 2 = intermittent weight-bearing lameness, 3 = persistent weight-bearing lameness, 4 = persistent non–weight-bearing lameness, 5 = ambulatory only with assistance, and 6 = nonambulatory. §The CBPI was scored on a scale of 0 = no pain or interference to 10 = extreme pain or interference. ‖Represents results for CBPI questions 1 through 4. ¶Represents results for CBPI questions 5 through 10.
[a,b]Within a variable, values with different superscript letters differed significantly ($P \leq 0.05$).
See Table 1 for remainder of key.

Table 3—Mean ± SD values for ground reaction force data obtained by use of a PSW at each time point for dogs that received simultaneous intra-articular and IV injections of SVF-PRP (treated group [n = 10]) or saline solution (control group [12]).

		Time (wk)*				
Variable	**Group**	**0**	**4**	**8**	**12**	**24**
PVF (% of body weight)	Treated	39.83 ± 6.11	38.66 ± 5.05	38.79 ± 5.51	40.17 ± 5.72	41.47 ± 4.38
	Control	41.18 ± 7.41	39.17 ± 9.32	34.26 ± 8.60	38.46 ± 6.45	40.86 ± 11.01
VI (% of body weight•s)	Treated	14.21 ± 3.15	13.56 ± 2.52	13.63 ± 2.82	13.78 ± 3.00	13.47 ± 4.00
	Control	14.61 ± 2.78	12.98 ± 4.60	12.10 ± 4.89	14.76 ± 2.87	14.70 ± 3.39
Stance time (s)	Treated	0.51 ± 0.11	0.49 ± 0.08	0.50 ± 0.09	0.49 ± 0.09	0.50 ± 0.08
	Control	0.52 ± 0.11	0.49 ± 0.12	0.53 ± 0.14	0.57 ± 0.12	0.56 ± 0.12
Stride velocity (cm/s)	Treated	91.41 ± 16.15	98.51 ± 19.65	95.15 ± 18.47	96.59 ± 12.16	96.66 ± 13.07
	Control	94.13 ± 14.98	100.61 ± 13.41	93.74 ± 21.79	85.70 ± 20.19	91.44 ± 21.32
Maximum peak pressure (kg/cm^2)	Treated	1.66 ± 0.54	1.54 ± 0.35	1.58 ± 0.36	1.59 ± 0.39	1.55 ± 0.42
	Control	1.71 ± 0.29	1.60 ± 0.41	1.48 ± 0.47	1.77 ± 0.24	1.71 ± 0.36

Values did not differ significantly ($P \leq 0.05$) between treatment groups at any time point.
See Table 1 for remainder of key.

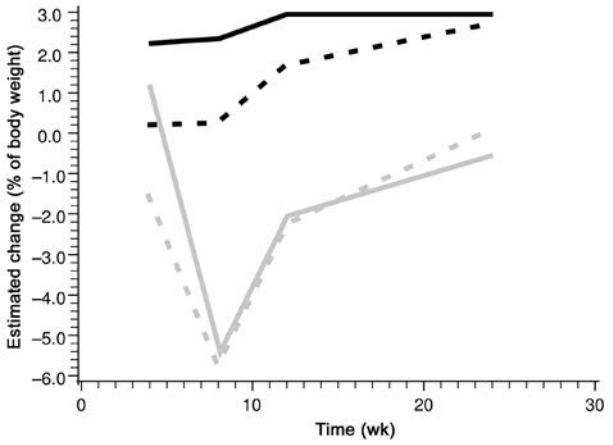

Figure 1—Change in PVF from the value at week 0 (baseline) for dogs with the lowest 25% of PVF. Dogs received simultaneous intra-articular and IV injections of SFV-PRP (treated group) or a placebo (0.9% NaCl solution; control group). Results are reported for the untreated (contralateral) limb from the control group (solid gray line), treated limb from the control group (dashed gray line), untreated (contralateral) limb from the treatment group (solid black line), and treated limb from the treatment group (dashed black line).

nificantly between groups at any other time point. The treatment group had a significantly ($P = 0.042$) lower score for the CBPI pain severity scores at week 24 than did the control group; these scores did not differ between groups at any other time point. The change from baseline values for the treatment group was significantly greater for both the CBPI scores on pain severity and interference of pain with activities at all time points, compared with values for the control group; these values decreased significantly for the treatment group. The standardized effect size for CBPI scores for pain severity was 1.0 to 1.1. Similarly, the standardized effect size for CBPI scores for interference of pain with activities was 1.0 to 1.3.

Assessment of dogs as lame versus nonlame resulted in a significant difference between treatment and control groups at all time points after joint injection, with a greater number of lame dogs in the control group. No significant differences were found in VAS score, goniometric data, radiographic scores, or CBPI scores for interference of pain with activities at any time points.

No significant differences over time were detected for either group with respect to body weight, BCS, lameness score, goniometric data, radiographic score, or CBPI score for interference of pain with activities. The VAS score for the treatment group was significantly greater at week 0 than at weeks 4, 8, or 24 but not at week 12. No significant differences were detected over time for VAS scores of the control group. The CBPI pain severity score for the treatment group was significantly ($P = 0.011$) greater at week 0 than at week 12 but not at any other time point. No significant differences were detected over time for CBPI pain severity scores of the control group.

Results of an ANOVA revealed no significant differences within groups at any time point with regard to PVF, VI, stance time, stride velocity, or maximum peak pressure of the treated limb. When evaluating the untreated (contralateral) limb, PVF for the control group was significantly ($P = 0.009$) higher at week 0 than at week 8. The VI for the control group was significantly ($P = 0.026$) higher at weeks 0, 12, and 24 than at week 8. No significant differences for any of these variables were detected for the treatment group at any time point.

Post hoc power analysis ($\alpha = 0.05$ and power = 0.8) was performed on the PSW data. It revealed that the minimum number of dogs needed to detect a significant difference between groups for PVF at 8 weeks was 41 dogs/group. Post hoc power analysis performed on subjective criteria (VAS, CBPI, and lameness score) revealed that the minimum number of dogs needed to detect a significant difference between groups for lameness score at 12 weeks was 15 dogs/group. For a fully powered clinical study when stratification of PVF data was used and only the lowest 25th percentile was enrolled (eg, dogs with a baseline PVF value < 38% of body weight), the mini-

mum number of dogs needed to detect a significant difference ($\alpha = 0.025$ and power = 0.90) was 25 dogs/group.

Discussion

In the study reported here, a combination of SVF and PRP was evaluated for treating naturally occurring osteoarthritis of the hip joint in dogs. One aim of the study was to evaluate safety of the SVF-PRP injection for hip joint osteoarthritis. In the present study, the only adverse effect noted after joint injection was transient lameness and signs of pain that resolved within 1 week. Because 1 dog each in the control and treatment groups had this complication, it was most likely an effect of joint distention or inflammation caused by arthrocentesis and injection of liquid into the joint and not an effect of the SVF-PRP combination. No specific diagnostic tests were pursued in dogs to rule out de novo neoplasia, pulmonary embolism, or other potential complications, but no dogs had any unexpected clinical signs during the 6-month course of the study. One dog identified with hemangiosarcoma after inclusion in this study was in the control group. On the basis of results for these 22 dogs, a combination of SVF and PRP apparently was safe for intra-articular and IV injection. Our safety end point observations were similar to those reported in previous studies[29,30] of treatment of osteoarthritis by use of SVF.

Objective gait analysis by use of force plates or a PSW has become an accepted technique in both human and veterinary medicine.[50,51] The most commonly used PSW variables are PVF (maximum force applied by a limb perpendicular to the PSW during stance phase of the gait) and VI (force applied perpendicular to the PSW during stance phase over the stance time). Although objective gait analysis may have a theoretical advantage over subjective measures, the ability to determine the sensitivity or specificity of any method used to detect lameness is hampered by the lack of a criterion-referenced test for comparison.[52] Nevertheless, force plate or PSW data have been compared to subjective criteria, but there have been poor correlations between PSW variables and subjective outcome measures.[53–55] Although the lack of correlation does not indicate superiority of one method over another, the authors believe that data collected objectively for a variable and generated by the patient should be superior to data collected subjectively for a variable and generated by an observer. Objective variables lend themselves to more powerful statistical approaches.

Results of an ANOVA indicated no significant difference in PSW variables of the treated limb between the treatment and control groups. In contrast, use of an ANCOVA that included the baseline PVF as a covariate revealed significant treatment effects. This finding underscores the importance of objective variables and the need to include baseline measures for linear modeling in osteoarthritis, as has been mentioned elsewhere.[56]

Changes in body weight can significantly influence ground reaction forces in lame dogs. Kinetic measurements decrease with decreasing body weight in dogs.[57,58] Investigators of another study[59] found that dogs treated surgically or nonsurgically for cranial cruciate ligament disease did not have a significant decrease in weight but did have a significant decrease in body fat percentage. This correlates to results for a study[60] in humans in which it was found that loss of body fat, but not weight, was related to symptomatic relief of osteoarthritis. In the study reported here, dogs in the treatment group with the greatest improvement in PVF were also the dogs that lost the most weight. Whether the improvements in PVF detected in the present study were attributable to decreased body weight is unknown. Because only body weight and BCS were recorded at each time point, but not a quantification of body fat percentage, the reason for loss of body weight in the treatment group is also unknown. It is possible that weight loss was an unknown effect of SVF-PRP treatment, that dogs in the treatment group had a decrease in pain and were more active and thus lost body fat, or that dogs in the treatment group did not improve and, in fact, lost lean body mass. It is also possible that the observation that dogs in the treatment group lost more weight was a type I error, especially because body weight was never significantly different between the groups.

The use of MSCs as a treatment for osteoarthritis in laboratory or companion animals has been reported.[16–22,42] Canine adipose-derived MSCs have been harvested from retroperitoneal tissue; subcutaneous tissue of the lateral thoracic area, gluteal area, and inguinal area; and the falciform ligament.[21] In the present study, we chose to use the falciform ligament because of the ease of access and concerns that other areas may have led to transient lameness because of their proximity to the thoracic or pelvic limbs. To the authors' knowledge, there have been no studies in veterinary medicine conducted to compare these sources of adipose tissue for MSC yield or differentiation potential.

Previous studies[29,30] of the use of SVF for the treatment of osteoarthritis in dogs involved the use of only subjective outcome measures, including score for a lameness evaluation, signs of pain on manipulation of the limb, subjective range of motion of the joint, and functional disability score provided by the owner. In an effort to compare results of the study reported here with results of previous studies, several subjective outcome measures (including lameness score, VAS completed by a veterinarian, CBPI completed by the owner, goniometric analysis, and radiographic assessment of the pelvis [at weeks 0 and 24]) were evaluated.

Both the lameness scale[29] and VAS[46] have been validated for use in assessing lameness and signs of

pain in dogs. Although these evaluations can be performed by owners, it has been found that the VAS lacks validity when performed by individuals untrained in recognizing clinical signs of pain.[61] In an effort to avoid this complication, as well as to avoid interobserver variability, all VAS and lameness scores were provided by 1 investigator (DAU). Results of an ANOVA indicated no significant differences in lameness scores between groups. However, results of an ANCOVA with the baseline PVF as a covariate indicted a significant treatment effect for lameness score. This underscores the importance of accounting for the pretreatment baseline value during analysis of osteoarthritis data. The VAS for the treatment group was significantly greater at week 0 than at weeks 4, 8, or 24, but not at any other time points. This most likely represented an effect of the treatment on signs of pain in the treated dogs. The VAS was completed after the investigator performed a physical examination and took into account lameness as well as signs of pain during manipulation of the limb. Although lameness severity may not have changed over time, it is possible that the subjective amount of pain during manipulation of the limb decreased in the treatment group, which accounted for the significant difference in VAS score. If this were the case, the SVF-PRP injection was associated with a rapid reduction in VAS that was evident by 4 weeks after treatment and that persisted for 6 months after treatment. A similar pattern was not found for the control group. Lack of a significant difference between the baseline value and the value at week 12 may have been a result of a type II error.

In a previous study[29] of SVF treatment for osteoarthritis of the hip joint in dogs, a nonvalidated owner survey indicated a greater percentage of improvement for treated dogs, compared with improvement for control dogs. In that study,[29] mean effect sizes were significant (> 0.8) for pain (1.57), lameness at a trot (1.36), range of motion (1.45), and composite score (1.34). In the present study, a CBPI was used. The CBPI has been validated as an owner assessment of pain associated with chronic osteoarthritis.[43,44] It is divided into a pain severity score that assesses the magnitude of pain of an animal and a pain interference score that assesses the degree to which pain affects daily activities. The treatment group had a significantly lower pain severity score at week 24, compared with the score for the control group at week 24, and the pain severity score for the treatment group was significantly greater at week 0 than at week 12. Change from baseline values was found to be significantly different at all postinjection time points for both pain severity score and pain interference score, with the treatment group having a greater decrease in these values. The standardized effect size for pain severity score was 1.0 to 1.1, and the standardized effect size for pain interference score was 1.0 to 1.3. These effect sizes are lower than those of the aforementioned study,[29] but this likely reflected the use of different outcome measures (validated owner survey vs lameness examination performed by a veterinarian) and different measures of effect size (mean scores vs Cohen d).

Recent evaluation of the ability of the CBPI to detect significant improvement in osteoarthritic dogs treated with carprofen found that a decrease in pain severity score > 1 and a decrease in pain interference score > 2 resulted in the most statistical power to predict whether a treatment would lead to a response in an individual dog.[44] In the present study, the mean pain severity score decreased > 2.0 and the mean pain interference score decreased > 2.8 for the treatment group, compared with baseline scores. For the control group, the pain severity score decreased > 1 at week 8, compared with the baseline score, but was not significantly different at any other time points. On the basis of the results of that previous report,[44] the changes detected in the present study may have indicated a positive response to treatment.

For comparison of the untreated (contralateral) limb of the control group, PVF was significantly greater at week 0 than at week 8, and VI was significantly greater at weeks 0, 12, and 24 than at week 8. We cannot account for this observation, but it was readily evident in the stratified PVF data (Figure 1). Dogs in the study were predominantly affected bilaterally by osteoarthritis in the hip joints. Because osteoarthritis can have waxing and waning periods of severity, it is possible that at week 8 the untreated limb caused clinical lameness worse than that of the treated limb within the control group. However, this phenomenon should have been equally likely to affect the treatment group, which had no significant differences over time. It was more likely the result of a type I error attributable to the small sample size. It was found that dogs in the control group typically had a lower PVF at week 8 than at all other time points. Although these values did not differ significantly, the pattern was consistently observed when dogs were stratified on the basis of baseline PVF. The reason for this observation is unclear. It would be expected that pain attributable to injection of saline solution would resolve by week 8, and the PVF at week 4 was higher than that at week 8. No specific event occurred between weeks 4 and 8 that would enable us to explain this observation.

When the data were stratified into quartiles on the basis of PVF, it was found that treated dogs in the lowest 25th percentile (corresponding to a baseline PVF < 38% of body weight) had a significant increase in PVF at all time points, compared with values for the control group. No significant differences were seen for dogs in the 50th or 75th percentiles. This may indicate that dogs with the lowest PVF, and presumably the worst osteoarthritis, had a more clinically substantial response to treatment with SVF-PRP injections. It is possible, however, that this phenomenon represented regression toward the mean, where the most extreme values tend to become closer to the mean with subsequent measurements.

Dogs enrolled in the study were of multiple breeds, ages, body weights, and BCSs. On the basis of radiographic and clinical lameness evaluations, the dogs had various degrees of severity of osteoarthritis of the hip joints. A more homogenous population of dogs would be ideal to decrease variance of PSW analysis. Future studies will optimally involve a cohort of dogs of similar age, sex, body weight, breed, and severity of osteoarthritis.

One goal for the study reported here was to determine the size of effect necessary to design a fully powered study for the primary outcome variables. Enrollment of 22 dogs in the present study was not sufficient to reduce type I error to the level expected for fully powered clinical trials, but it was effective for estimating effects and estimating the sample size needed for such a trial. Results of a power analysis ($P < 0.025$; power > 0.9) indicated that 25 dogs/group were needed to detect significant differences between PVF, CBPI scores, and VAS scores for dogs with < 38% PVF at baseline. These estimates should be used in the design of future studies.

The low number of dogs enrolled in the present study also precluded analysis of the effect of sex, age, or severity of osteoarthritis on response to treatment. It is possible that dogs would respond differently or not at all to SVF-PRP treatment depending on these variables, but further studies with a larger sample size are necessary to assess effects of those variables.

Other limitations of the study reported here included IV injection of the SVF-PRP treatment in addition to intra-articular injection. Mesenchymal stem cells may express receptors that allow them to mediate their response and migration to tissue damage and inflammation.[35] It is possible that the IV-injected SVF cells migrated to both hip joints in bilaterally affected dogs and caused treatment effects in both joints, which led to a symmetric improvement in both joints that may have been responsible for the lack of improvement detected by use of PSW analysis. However, if this were the case, it would be expected that both hind limbs would have an increase in PVF and the forelimbs would have a decrease in PVF as weight shifted from the forelimbs to the hind limbs. This was not observed. A study[41] conducted to evaluate the effect of PRP injected intra-articularly into osteoarthritic joints in dogs found a significant increase in PVF at 12 weeks after injection, compared with pretreatment values. Similar significant findings were not observed in the present study without data stratification, although it is possible that significant differences for VAS and CBPI values were attributable to PRP and not to SVF. To the authors' knowledge, this study was the first in which IV injection of PRP to dogs has been reported. Because of the study protocol, it was not possible to know whether IV administration of PRP had an effect on osteoarthritis in this population of dogs. The use of both SVF and PRP was chosen because the commercially available protocol[h] involved use of both. Further studies with a larger sample population would be needed to assess whether SVF enhances or inhibits the effects of PRP and whether PRP administered IV has any therapeutic benefit for dogs with osteoarthritis.

One other limitation was the concurrent use of NSAIDs and other disease-modifying treatments in some dogs during the study. It is possible that use of these treatments could have affected results. Ideally, administration of analgesic medications would have ceased, and there would have been a washout period prior to study enrollment. We elected to allow dogs to be continued on previous medical management to avoid changes in lameness.

For the study reported here, intra-articular and IV administration of SVF-PRP treatment to dogs with osteoarthritis of the hip joints did not cause adverse reactions during a 24-week observation period. Subjective improvements in lameness and CBPI were detected in dogs in the treatment group. There was a significant treatment effect that was consistent with results in previous reports. Use of an ANOVA failed to reveal significant improvements in PSW data in dogs treated with SVF-PRP for osteoarthritis of the hip joints. However, use of an ANCOVA with baseline PVF as a covariate revealed a treatment effect for SVF-PRP injection. Specifically, for dogs with < 38% PVF at baseline, SVF-PRP injection resulted in significant improvement in PVF. Future studies with at least 25 osteoarthritic dogs with < 38% PVF at baseline/group would be needed to evaluate the effect of SVF on PVF in a fully powered randomized clinical trial.

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Footnotes

a. Hi-Rez Versatek walkway, Tekscan Inc, South Boston, Mass.
b. Robinson metal goniometer, 180° range, 6-inch legs, Lafayette Instrument Co, Lafayette, Ind.
c. Tekscan pressure measurement system walkway software, version 7.02, Tekscan Inc, South Boston, Mass.
d. Westword, Eatontown, NJ.
e. Vedco, St Joseph, Mo.
f. PropoFlo, Abbott Laboratories, North Chicago, Ill.
g. Roxane Laboratories Inc, Columbus, Ohio.
h. Provided by Medivet Biologics LLC, Nicholasville, Ky.
i. Nexcelom Auto 2000, Nexelcom Biosciences, Lawrence, Mass.
j. Veterinary 0.9% sodium chloride injection USP, Abbott Laboratories, North Chicago, Ill.
k. WINKS SDA 6, version 6.0.93, Texasoft Inc, Cedar Hill, Tex.
l. PROC MIXED, SAS, version 9.4, SAS Institute Inc, Cary, NC.
m. G*Power, version 3.1.9.2, Franz Faul, Universitat Kiel, Germany.

References

1. Johnson JA, Austin C, Breur GJ. Incidence of canine appendicular musculoskeletal disorders in 16 veterinary teaching hospitals from 1980 through 1989. *Vet Comp Orthop Traumatol* 1994;7:5–18.
2. Smith GK, Karbe GT, Agnello KA, et al. Pathogenesis, diagnosis, and control of canine hip dysplasia. In: Tobias K, Johnston SA, eds. *Veterinary surgery: small animal*. Vol 1. St Louis: WB Saunders Co, 2012;824–848.
3. Corley EA. Hip dysplasia: a report from the Orthopedic Foundation for Animals. *Semin Vet Med Surg (Small Anim)* 1987;2:141–151.
4. Coopman F, Verhoeven G, Saunders J, et al. Prevalence of hip dysplasia, elbow dysplasia and humeral head osteochondrosis in dog breeds in Belgium. *Vet Rec* 2008;163:654–658.
5. Renberg WC. Pathophysiology and management of arthritis. *Vet Clin North Am Small Anim Pract* 2005;35:1073–1091.
6. Jack GS, Almeida FG, Zhang R, et al. Processed lipoaspirate cells for tissue engineering of the lower urinary tract: implications for the treatment of stress urinary incontinence and bladder reconstruction. *J Urol* 2005;174:2041–2045.
7. Gimble JM, Katz AJ, Bunnell BA. Adipose-derived stem cells for regenerative medicine. *Circ Res* 2007;100:1249–1260.
8. Chen FH, Tuan RS. Mesenchymal stem cells in arthritic diseases. *Arthritis Res Ther* 2008;10:223–234.
9. Fang B, Song Y, Zhao RC, et al. Using human adipose tissue-derived mesenchymal stem cells as salvage therapy for hepatic graft-vs-host disease resembling acute hepatitis. *Transplant Proc* 2007;39:1710–1713.
10. Wood JA, Chung D, Park SA, et al. Periocular and intra-articular injection of canine adipose-derived mesenchymal stem cells—an in vivo imaging and migration study. *J Ocul Pharmacol Ther* 2012;28:307–317.
11. Djouad F, Charbonnier LM, Bouffi C, et al. Mesenchymal stem cells inhibit the differentiation of dendritic cells through an interleukin-6-dependent mechanism. *Stem Cells* 2007;25:2025–2032.
12. Németh K, Leelahavanichkul A, Yuen PS, et al. Bone marrow stromal cells attenuate sepsis via prostaglandin E2–dependent reprogramming of host macrophages to increase their interleukin-10 production. *Nat Med* 2009;15:42–49.
13. Sullivan C, Barry F, Ritter T, et al. Allogeneic murine mesenchymal stem cells: migration to inflamed joints in vivo and amelioration of collagen induced arthritis when transduced to express CTLA4Ig. *Stem Cells Dev* 2013;22:3203–3213.
14. Murphy JM, Fink DJ, Hunziker EB, et al. Stem cell therapy in a caprine model of osteoarthritis. *Arthritis Rheum* 2003;48:3464–3474.
15. Horie M, Sekiya I, Muneta T, et al. Intra-articular injected synovial stem cells differentiate into meniscal cells directly and promote meniscal regeneration without mobilization to distant organs in rat massive meniscal defect. *Stem Cells* 2009;27:878–887.
16. Lee KB, Hui JH, Song IC, et al. Injectable mesenchymal stem cell therapy for large cartilage defects—a porcine model. *Stem Cells* 2007;25:2964–2971.
17. Augello A, Tasso R, Negrini SM, et al. Cell therapy using allogeneic bone marrow mesenchymal stem cells prevents tissue damage in collagen-induced arthritis. *Arthritis Rheum* 2007;56:1175–1186.
18. Guercio A, Marco P, Casella S, et al. Production of canine mesenchymal stem cells from adipose tissue and their application in dogs with chronic osteoarthritis of the humeroradial joints. *Cell Biol Int* 2012;36:189–194.
19. Ferris DJ, Frisbie DD, Kisiday JD, et al. Clinical outcome after intra-articular administration of bone marrow derived mesenchymal stem cells in 33 horses with stifle injury. *Vet Surg* 2014;43:255–265.
20. Cuervo B, Rubio M, Sopena J, et al. Hip osteoarthritis in dogs: a randomized study using mesenchymal stem cells from adipose tissue and plasma rich in growth factors. *Int J Mol Sci* 2014;15:13437–13460.
21. Vilar JM, Batista M, Morales M, et al. Assessment of the effect of intraarticular injection of autologous adipose-derived mesenchymal stem cells in osteoarthritic dogs using a double blinded force platform analysis. *BMC Vet Res* 2014;10:143.
22. Vilar JM, Morales M, Santana A, et al. Controlled, blinded force platform analysis of the effect of intraarticular injection of autologous adipose-derived mesenchymal stem cells associated to PRGF-Endoret in osteoarthritic dogs. *BMC Vet Res* 2013;9:131.
23. US Department of Health and Human Services FDA Center for Veterinary Medicine. *Cell-based products for animal use*. Industry guidance #218. Rockville, Md: FDA Center for Veterinary Medicine, 2015;1–14.
24. Prockop DJ, Brenner M, Fibbe WE, et al. Defining the risks of mesenchymal stromal cell therapy. *Cytotherapy* 2010;12:576–578.
25. Lalu MM, McIntyre L, Pugliese C, et al. Safety of cell therapy with mesenchymal stromal cells (SafeCell): a systematic review and meta-analysis of clinical trials. *PLoS ONE* 2012;7:e47559 .
26. Tatsumi K, Ohashi K, Matsubara Y, et al. Tissue factor triggers procoagulation in transplanted mesenchymal stem cells leading to thromboembolism. *Biochem Biophys Res Commun* 2013;431:203–209.
27. Jung JW, Kwon M, Choi JC, et al. Familial occurrence of pulmonary embolism after intravenous, adipose tissue-derived stem cell therapy. *Yonsei Med J* 2013;54:1293–1296.
28. Cyranoski D. Stem cells boom in vet clinic. *Nature* 2013;496:148–149.
29. Black LL, Gaynor J, Gehring D, et al. Effect of adipose-derived mesenchymal stem and regenerative cells on lameness in dogs with chronic osteoarthritis of the coxofemoral joints: a randomized, double-blinded, multicenter controlled trial. *Vet Ther* 2007;8:272–284.
30. Black LL, Gaynor J, Adams C, et al. Effect of intraarticular injection of 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.
31. Marx C, Silveira MD, Selbach I, et al. Acupoint injection of autologous stromal vascular fraction and allogeneic adipose-derived stem cells to treat hip dysplasia in dogs. *Stem Cells Int* 2014;2014:391274.
32. Frisbie DD, Kisiday JD, Kawcak CE, et al. 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.
33. Qi Y, Feng G, Yan W. Mesenchymal stem cell-based treatment for cartilage defects in osteoarthritis. *Mol Biol Rep* 2012;39:5683–5689.
34. Bourin P, Bunnell BA, Casteilla L, et al. Stromal cells from the adipose tissue-derived stromal vascular fraction and culture expanded adipose tissue-derived stromal/stem cells: a joint statement of the International Federation for Adipose Therapeutics and Science (IFATS) and the International Society for Cellular Therapy (ISCT). *Cytotherapy* 2013;15:641–648.
35. Webster RA, Blabber SP, Herbert BR, et al. The role of mesenchymal stem cells in veterinary therapeutics—a review. *N Z Vet J* 2012;60:265–272.
36. Riordan NH, Ichim TE, Min WP, et al. Non-expanded adipose stromal vascular fraction cell therapy for multiple sclerosis. *J Transl Med* 2009;7:29.
37. Miranville A, Heeschen C, Sengenes C, et al. Improvement of postnatal neovascularization by human adipose tissue-derived stem cells. *Circulation* 2004;110:349–355.
38. Zeyda M, Stulnig TM. Adipose tissue macrophages. *Immunol Lett* 2007;112:61–67.
39. Odegaard JI, Ricardo-Gonzalez RR, Goforth MH, et al. Macrophage-specific PPARγ; controls alternative activation and improves insulin resistance. *Nature* 2007;447:1116–1120.
40. Zeyda M, Farmer D, Todoric J, et al. Human adipose tissue macrophages are of an anti-inflammatory phenotype but capable of excessive pro-inflammatory mediator production. *Int J Obes (Lond)* 2007;31:1420–1428.
41. Fahie MA, Ortolano GA, Guercio V, et al. A randomized controlled trial of the efficacy of autologous platelet therapy for

the treatment of osteoarthritis in dogs. *J Am Vet Med Assoc* 2013;243:1291–1297.
42. Van Pham P, Bui KH, Ngo DQ, et al. Activated platelet-rich plasma improves adipose-derived stem cell transplantation efficiency in injured articular cartilage. *Stem Cell Res Ther* 2013;4:91–102.
43. Brown DC, Boston RC, Coyne JC, et al. Ability of the canine brief pain inventory to detect response to treatment in dogs with osteoarthritis. *J Am Vet Med Assoc* 2008;233:1278–1283.
44. Brown DC, Bell M, Rhodes L. Power of treatment success definitions when the Canine Brief Pain Inventory is used to evaluate carprofen treatment for the control of pain and inflammation in dogs with osteoarthritis. *Am J Vet Res* 2013;74:1467–1473.
45. Jaegger G, Marcellin-Little DJ, Levine D. Reliability of goniometry in Labrador Retrievers. *Am J Vet Res* 2002;63:979–986.
46. Hudson JT, Slater MR, Taylor L, et al. Assessing repeatability and validity of a visual analogue scale questionnaire for use in assessing pain and lameness in dogs. *Am J Vet Res* 2004;65:1634–1643.
47. Franklin SP, Garner BC, Cook JL. Characteristics of canine platelet-rich plasma prepared with five commercially available systems. *Am J Vet Res* 2015;76:822–827.
48. Smith GK, Paster ER, Powers MY, et al. Lifelong diet restriction and radiographic evidence of osteoarthritis of the hip joint in dogs. *J Am Vet Med Assoc* 2006;229:690–693.
49. Milliken GA, Johnson DE. One-way analysis of covariance—one covariate in a completely randomized design structure. In: Milliken GA, Johnson DE, eds. *Analysis of messy data, volume 3: analysis of covariance*. New York: Chapman & Hall/CRC, 2001;11–39.
50. Clayton HM. The force plate: established technology, new application. *Vet J* 2005;169:15–16.
51. Cordova ML, Armstrong CW. Reliability of ground reaction forces during a vertical jump: implications for functional strength assessment. *J Athl Train* 1996;31:342–345.
52. Besancon MF, Conzemius MG, Derrick TR, et al. Comparison of vertical forces in normal Greyhounds between force platform and pressure walkway measurement systems. *Vet Comp Orthop Traumatol* 2003;16:153–157.
53. Quinn MM, Keuler NS, Lu YAN, et al. Evaluation of agreement between numerical rating scales, visual analogue scoring scales, and force plate gait analysis in dogs. *Vet Surg* 2007;36:360–367.
54. Horstman CL, Conzemius MG, Evans R, et al. Assessing the efficacy of perioperative oral carprofen after cranial cruciate surgery using noninvasive, objective pressure platform gait analysis. *Vet Surg* 2004;33:286–292.
55. Oosterlinck M, Bosmans T, Gasthuys F, et al. Accuracy of pressure plate kinetic asymmetry indices and their correlation with visual gait assessment scores in lame and nonlame dogs. *Am J Vet Res* 2011;72:820–825.
56. Weiss E. Knee osteoarthritis, body mass index and pain: data from the Osteoarthritis Initiative. *Rheumatology* 2014;53:2095–2099.
57. Moreau M, Troncy E, Bichot S, et al. Influence of changes in body weight on peak vertical force in osteoarthritic dogs: a possible bias in study outcome. *Vet Surg* 2010;39:43–47.
58. Marshall WG, Hazewinkel HA, Mullen D, et al. The effect of weight loss on lameness in obese dogs with osteoarthritis. *Vet Res Commun* 2010;34:241–253.
59. Wucherer KL, Conzemius MG, Evans R, et al. Short-term and long-term outcomes for overweight dogs with cranial cruciate ligament rupture treated surgically or nonsurgically. *J Am Vet Med Assoc* 2013;242:1364–1372.
60. Toda Y, Toda T, Takemura S, et al. Change in body fat, but not body weight or metabolic correlates of obesity, is related to symptomatic relief of obese patients with knee osteoarthritis after a weight control program. *J Rheumatol* 1998;25:2181–2186.
61. Hielm-Björkman AK, Kapatkin AS, Rita HJ. Reliability and validity of a visual analogue scale used by owners to measure chronic pain attributable to osteoarthritis in their dogs. *Am J Vet Res* 2011;72:601–607.