UNIVERSIDADE ESTADUAL PAULISTA

"JÚLIO DE MESQUITA FILHO"

Campus de Botucatu

Faculdade de Medicina Veterinária e Zootecnia

EFEITO ANALGÉSICO DO PLASMA RICO EM PLAQUETAS E CÉLULAS-TRONCO NA DOR CRÔNICA DE CÃES COM DISPLASIA COXOFEMORAL

CELINA EMIKO OKAMOTO OKUBO

BOTUCATU – SP

Maio 2016

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CELINA EMIKO OKAMOTO OKUBO

Tese apresentada à Faculdade de Medicina Veterinária e Zootecnia, Universidade Estadual Paulista "Júlio de Mesquita Filho", Campus de Botucatu, para obtenção do título de mestre na área de Biotecnologia animal.

Orientador: Prof. Titular Stelio Pacca Loureiro Luna

Co-orientadora: Profa. Titular Fernanda da Cruz Landim

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Programa de Pós-Graduação em Biotecnologia Animal

Área de Cirurgia e Anestesiologia Veterinária

Pós Graduanda:

Celina Emiko Okamoto Okubo (Depto. Cirurgia e Anestesiologia Veterinária-FMVZ/ UNESP/ Botucatu)

Orientador:

Prof. Titular Stelio Pacca Loureiro Luna (Depto. Cirurgia e Anestesiologia Veterinária-FMVZ/ UNESP/ Botucatu)

Co-orientadora:

Profa. Titular Fernanda da Cruz Landim (Depto. Reprodução e Radiologia Veterinária. FMVZ/ UNESP/ Botucatu)

Equipe:

Profa. Titular Sheila Canevese Rahal (Depto. Cirurgia e Anestesiologia Veterinária-FMVZ/ UNESP/ Botucatu)

Dr. Leandro Maia (PG-Depto. Reprodução Animal-FMVZ/ UNESP/ Botucatu)

Dr. Jean G. F. Joaquim (Depto. Cirurgia e Anestesiologia Veterinária-FMVZ/ UNESP/ Botucatu)

M.V. Isadora Arruda (PG-Depto. Reprodução Animal. FMVZ/ UNESP/ Botucatu)

M.V. Luciane dos Reis Mesquita (PG-Depto. Cirurgia e Anestesiologia Veterinária-FMVZ/ UNESP/ Botucatu)

Profa. Dra. Regina K. Takahira (Depto. Clínica Veterinária- FMVZ/ UNESP/ Botucatu)

M.V. Washington T. Kano (PG-Depto. Cirurgia e Anestesiologia Veterinária-FMVZ/ UNESP/ Botucatu)

M.V. Hugo S. Oliveira (Depto. Reprodução e Radiologia Veterinária-FMVZ/ UNESP/ Botucatu)

Celina Emiko Okamoto Okubo

EFEITO ANALGÉSICO DO PLASMA RICO EM PLAQUETAS E CÉLULAS-TRONCO NA DOR CRÔNICA DE CÃES COM DISPLASIA COXOFEMORAL

Dissertação apresentada à Faculdade de Medicina Veterinária e Zootecnia, Universidade Estadual Paulista "Júlio de Mesquita Filho", Campus de Botucatu, para obtenção do título de Mestre.

_____

Orientador: Prof. Titular. Stelio Pacca Loureiro Luna

Comissão Examinadora

_____

Profa. Titular Sheila Canavese Rahal
Universidade Estadual Paulista

_____

Prof. Dr. Jean Guilherme Fernandes Joaquim
Instituto Bioethicus – Botucatu-SP

_____

Profa. Dra. Fabiana Ferreira de Souza
Universidade Estadual Paulista

_____

Profa. Dra. Bruna De Vita
Pesquisadora

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DEDICATÓRIA

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Carl Jung

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LISTA DE ANEXOS

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LISTA DE ABREVIATURAS, SIGLAS E SÍMBOLOS

p	Nível de significância
σ	Desvio padrão
BIDC	Breve Inventário de Dor Canina
BVS	Biblioteca Virtual em Saúde
CEUA	Comissão de Ética no Uso de Animais
CT	Célula-tronco
CTM-TAs	Células-tronco mesenquimais derivadas de tecido adiposo
DAD	Doença articular degenerativa
DCF	Displasia coxofemoral
DMSO	Dimetilsulfóxido
DP	Distribuição do Peso
EAV	Escala Analógica Visual
EAVdor	Escala Analógica Visual para dor
EAVloc	Escala Analógica Visual para claudicação
END	Escala Numérica Descritiva
FMVZ	Faculdade de Medicina Veterinária e Zootecnia
Kg	Quilogramas
IDCH	Indicador de Dor Crônica da Universidade de Helsink
IGP	Impressão Geral do Proprietário sobre a qualidade de vida do cão
µM	Micrômetros
mL	Mililitros
µL	Microlitros
n	Número de animais
PBS	Solução Tampão Fosfato-salino
PCR	Reação em Cadeia pela Polimerase
PDGF	Fator de Crescimento Derivado de Plaquetas
PRP	Plasma Rico em Plaquetas
SFB	Soro Fetal Bovino
TGF-beta	Fator de Crescimento Transformador beta
VEGF	Fator de Crescimento Vascular Endotelial

SUMÁRIO

RESUMO

OKAMOTO-OKUBO, C.E. Efeito analgésico do plasma rico em plaquetas e células-tronco na dor crônica de cães com displasia coxofemoral. 2016. 66p. Dissertação (Mestrado) - Faculdade de Medicina Veterinária e Zootecnia, Universidade Estadual Paulista "Júlio de Mesquita Filho", Botucatu, 2016.

Este estudo clínico, controlado, aleatório, duplo cego, investigou o efeito intra-articular do plasma rico em plaquetas (PRP) (n = 8) ou de células-tronco alogênicas (CT) (n = 8) na dor crônica de cães com displasia coxofemoral (DCF) bilateral. Os proprietários avaliaram o Breve Inventário de Dor Canina (BIDC), o Índice de Dor Crônica de Helsink (IDCH), a Escala Analógica Visual para dor (EAVdor) e claudicação (EAVloc), e a Qualidade de Vida (QV). Uma médica veterinária avaliou a Escala Visual Analógica da dor à palpação (EAVpalp), a Escala Numérica Descritiva para dor (END) e a porcentagem de distribuição de peso corporal. As avaliações foram realizadas antes, aos 30 e 60 dias após o tratamento. Depois de CT, IDCH, EAVdor e EAVpalp foram reduzidos, QV aumentou aos 60 dias e BIDC reduziu aos 30 e 60 dias. Após PRP, EAVloc e END reduziram em 60 dias e BIDC aos 30 e 60 dias. Não houve diferença entre os grupos. Ambos CT e PRP foram aparentemente benéficos para reduzir a dor crônica em cães que sofrem de DCF bilateral por 60 dias, mas CT foi superior nas variáveis de dor crônica em comparação com PRP. O benefício clínico de ambos os tratamentos é que eles produzem um alívio da dor a longo prazo em cães com DCF sem efeitos adversos aparentes.

Palavras chave: Células-tronco, injeções, intra-articular, coxofemoral, cães

ABSTRACT

Analgesic effect of platelet-rich plasma and stem cells in chronic pain in dogs with hip dysplasia. 2016. 66p. Faculdade de Medicina Veterinária e Zootecnia, Universidade Estadual Paulista “Júlio de Mesquita Filho”, Botucatu, 2015.

This randomized, controlled, double-blind clinical study investigated the effect of intra-articular platelet-rich plasma (PRP) (n=8) or allogeneic stem cells (SC) (n=8) in chronic pain of dogs with bilateral hip dysplasia (HD). The owners evaluated the Canine Brief Pain Inventory (CBPI), the Helsinki Chronic Pain Index (HCPI), a Visual Analogue Scale for pain (VASpain) and lamness (VASloc) and the quality of life (QL). A veterinarian evaluated the Visual Analogue Scale of pain in response to palpation (VASpalp), the descriptive numerical scale for pain (DNS) and the percentage of body weight distribution. All evaluations were performed before, at 30 and 60 days after treatment. After SC, HCPI, VASpain and VASpalp reduced and QL increased at 60 days and CBPI reduced at 30 and 60 days. After PRP, VASloc and DNS reduced at 60 days and CBPI at 30 and 60 days. There was no difference between groups. Both SCs and PRP were apparently beneficial to reduce chronic pain in dogs suffering from bilateral hip dysplasia for 60 days, but SCs improved more chronic pain variables compared to PRP. The clinical benefit of both treatments is that they produce a long term pain relief in dogs with hip dysplasia with no apparent adverse effects.

Key words: Stem cells, injections, intra-articular, hip joint, dogs

CAPÍTULO I

1. INTRODUÇÃO

A medicina regenerativa é uma nova área das medicinas humana e veterinária e é foco de estudos recentes, principalmente voltados para o uso da terapia de células-tronco mesenquimais derivadas de tecido adiposo (CTM-TAs), com o objetivo de tratar osteoartroses [1-20]. Com esse mesmo foco, a aplicação de Plasma Rico em Plaquetas (PRP) também é amplamente utilizada para tratar problemas osteoarticulares não apenas no homem, mas também em diversas espécies como nos cães [7,8,21-33].

A dispasia coxofemoral (DCF) é uma alteração não infecciosa e progressiva que ocorre na cartilagem das articulações acometidas[34]. Caracteriza-se por uma sinovite e degeneração da cartilagem articular com perda da matriz, e que pode resultar na completa perda da superfície cartilaginosa[35].

A hipótese deste estudo é que as terapias com CTM-TAs alogênicas ou PRP são alternativas terapêuticas eficazes e similares para controlar a dor e melhorar a qualidade de vida em cães com DCF bilateral [3,4].

2. REVISÃO DE LITERATURA

2.1- Displasia coxofemoral (DCF) bilateral em cães

A displasia coxofemoral (DCF) é uma enfermidade crônica, conhecida também como doença articular degenerativa (DAD)[36]. Estudos mostram a alta prevalência da DAD em cães, com 20% da população canina acima de 1 ano de idade apresentando esta enfermidade em diferentes graus[37]. A DCF está relacionada com dor crônica, claudicação, incapacidade funcional e redução da

qualidade de vida, levando finalmente à perda da função articular e mobilidade[38]. O diagnóstico é comumente realizado por meio da avaliação radiográfica, com a classificação em graus diferentes de DCF de acordo com a severidade das alterações encontradas no exame [39].

A DCF é um processo decorrente à falha em reparar danos causados à articulação por excesso de estresse mecânico, com consequente dor e perda da função articular[40]. Embora possa ser desencadeada por múltiplos fatores, a DCF é resultado de eventos mecânicos e biológicos sucessivos que desestabilizam o acoplamento normal da articulação[39]. A evolução da doença não envolve apenas a cartilagem da articulação, mas todos os seus tecidos, que inclui o osso subcondral, a cápsula articular, a membrana sinovial, os ligamentos e músculos periarticulares[41].

A DCF é uma enfermidade que tem início com a instabilidade da articulação coxofemoral em cães jovens, alterando a concentração de forças sobre a cabeça femoral e sobre o acetábulo que ainda estão em desenvolvimento[42]. Estas alterações afetam o crescimento ósseo e a remodelação, resultando em conformação articular anormal[41]. Estas forças de pressão anormais sobre as articulações causam microfraturas no osso subcondral da borda acetabular e da cabeça femoral, levando à inflamação, com maiores áreas de degeneração, futura eburneação, esclerose do osso subcondral e formação de osteófitos periarticulares [43]. A etiologia envolve vários fatores além da biomecânica da articulação coxofemoral, como a taxa de crescimento, a interação da musculatura pélvica com o tecido ósseo, a lassitude articular, influências genéticas, metabólicas e hormonais[39].

Na DCF, ocorre a superprodução de mediadores pró-inflamatórios em relação aos inibitórios, o que intensifica o catabolismo em relação ao anabolismo, levando à destruição progressiva da cartilagem articular[35,39,44-46]. Todas estas alterações causam diferentes graus de dor crônica [47-49]. A cartilagem articular hialina é avascular, por este motivo possui uma baixa propensão à regeneração[50,51].

O tratamento da DCF bilateral em cães envolve uma abordagem multimodal[37]. A principal recomendação é o controle dos sinais clínicos, reduzindo a dor, melhorando a mobilidade e consequentemente a qualidade de vida dos animais[52]. Mas além do alívio da dor, impedir a degradação da cartilagem é um desafio importante no tratamento, validando a importância da prevenção da DCF e manutenção máxima da função articular [53].

2.2- Células-Tronco Mesenquimais derivadas de tecido adiposo

As células-tronco (CT) são células com grande capacidade de proliferação e auto-renovação, que respondem a estímulos externos e dão origem a diferentes linhagens celulares mais especializadas [54].

Quanto à origem, as CTs podem ser classificadas em células-tronco embrionárias (CTEs) e adultas (CTAs). As CTAs ou somáticas são responsáveis pelo reabastecimento tecidual ao longo da vida e estão presentes na maioria dos tecidos [55]. As CTAs têm uma capacidade muito menor do que as CTEs de auto-renovação e diferenciação ao longo de múltiplas vias de linhagem[4]. No entanto, as CTAs são imunocompatíveis[56], e a sua utilização não é limitada pelas preocupações de ordem ética associados com as células derivadas de embriões. Além disso, células embrionárias têm sido

demonstradas com capacidade de crescimento descontrolado[57,58].

Uma fonte acessível, e abundante de células-tronco adultas mesenquimais é o tecido adiposo, onde uma pequena amostra pode gerar um grande número de células-tronco mesenquimais (CTM-TAs)[59]. Estudos também mostram a utilização da fração vascular estromal como fonte de células-tronco mesenquimais para o tratamento de doença articular degenerativa em cães[7]. A fração vascular do estroma é composta por uma população heterogênea de células, contendo as células-tronco mesenquimais, linfócitos T, macrófagos, células precursoras endoteliais e pré-adipócitos[60]. No entanto para que haja uma amostra com composição homogênea de células-tronco mesenquimais, é necessária a realização do cultivo *in vitro* [61].

Um estudo demonstrou que a célula-tronco mesenquimal interage com linfócitos CD4 e CD8 e, uma vez ativada, na presença de mediadores pró-inflamatórios, ocorre a liberação de substâncias por estas células que regulam a supressão da inflamação[62]. Autores sugerem que as células-tronco mesenquimais autólogas ou alogênicas são não-imunogênicas e não induzem proliferação de linfócitos[63] na ativação *in vitro*[64-66] *e in vivo*[67]. A habilidade das CTMs de inibir a proliferação de linfócitos T estimulados *in vitro* tem sido descrita em primatas não humanos[68,69], cães[70-72], aves[73], coelhos[74,75], suínos[66,76], ovinos[64] e equinos[65,77], assim como também as CTMs diminuem a expressão da ativação de marcadores de superfície (CD25, CD38, CD69) de linfócitos T, prevenindo sua ativação[78]. A produção ou a expressão de fatores solúveis imunossupressores também foram demonstradas pelas CTMs em diversas espécies, incluindo TGF-beta1[65,66,69,72,75], prostaglandina E2[65,70,72], óxido nítrico[65,73], fator de crescimento endotelial vascular[69,72] e IL-6[65,69,70].

A capacidade de reparação tecidual das CTM-TAs ocorre devido às múltiplas interações que incluem secreção de fatores parácrinos, como as citocinas, estimulação da proliferação e diferenciação de células progenitoras que podem diminuir as reações inflamatórias[79].

A eficácia clínica da aplicação intra-articular das CTM-TAs autólogas em cães já foi demonstrada anteriormente no tratamento de osteoartrites crônicas da articulação coxofemoral[2]. Estudos recentes também mostraram bons resultados clínicos no tratamento de doenças articulares degenerativas de cães com a aplicação intra-articular de CTM-TAs autólogas [1,5,6].

2.3- Plasma Rico em Plaquetas (PRP)

Plaquetas são células sanguíneas pequenas, anucleadas, achatadas, derivadas da fragmentação de megacariócitos precursores, com vida média de 5 a 9 dias[80]. Componentes chave na hemostasia, estimulam a formação de novos tecidos conectivos e a revascularização[29]. Estas células são ativadas no organismo quando em contato com o fator de von Willebrand, colágeno ou pela ação da trombina [82]. Uma vez ativadas, as plaquetas secretam o conteúdo de seus grânulos alpha e grânulos densos, onde diversos fatores de crescimento são liberados, atuando sobre a reparação tecidual nos estágios de inflamação, proliferação e remodelação [80].

O PRP é o plasma sanguíneo autólogo com alta concentração de plaquetas [81], obtido normalmente por centrifugações seriadas, com diferentes protocolos para cães [29,82-85]. Embora alguns autores relatem que o PRP com a inclusão da zona de névoa (incluindo os leucócitos) junto ao plasma preparado após primeira centrifugação sanguínea, apresenta uma maior quantidade de

plaquetas (de 3 a 5 vezes mais que o plasma original) [86], outros autores citam que o PRP proveniente de amostra sanguínea de cães sem a inclusão da zona de névoa apresenta superioridade na função das plaquetas e menor contaminação com hemácias [29].

Diversos estudos descrevem o uso do PRP autólogo como tratamento efetivo para o controle da dor em osteoartroses, justificado principalmente pela presença da grande quantidade de fatores de crescimento em seus grânulos alpha, PDFG, TGF-beta, VEGF e outros fatores[7,88]. O PRP estimula a síntese de colágeno do tipo I e aumenta a expressão dos reguladores de progressão do ciclo celular para acelerar a recuperação tecidual [89].

Segundo alguns autores, o PRP estimula a formação do ácido hialurônico endógeno e a diminuição do catabolismo da cartilagem[30]. Outros estudos sugerem que o PRP isolado de sangue autólogo seja uma fonte de fatores anabólicos de crescimento para a estimulação de condrócitos, atuando na recuperação do tecido cartilaginoso[90] com ação inibitória sobre o processo inflamatório dos condrócitos danificados pelas osteoartroses[91].

Autores de um estudo com a aplicação intra-articular de PRP em joelho de ovelhas com DAD induzidas experimentalmente concluíram que os fatores de crescimento contidos no PRP estimula a formação de tecido fibrocartilaginoso[81]. A eficácia da terapia intra-articular de PRP também foi demonstrada por Wei et al. (2012), mostrando bons resultados clínicos após o tratamento de lesões em menisco em pacientes humanos[92].

Cães submetidos a correção cirúrgica do ligamento cruzado cranial foram tratados com uma única injeção intra-articular de PRP produzidos com

redução de leucócitos em sua composição. Os animais do grupo PRP apresentaram menor claudicação, menor índice de dor e efusão intra-articular, e melhor função e amplitude de movimento da articulação do joelho ao comparar com o grupo tratado com antiinflamatórios não esteroidais[93]. Outro estudo foi realizado com única aplicação de PRP em joelho de cães, avaliando o escore de alterações histológicas e macroscópicas, sugerindo que o PRP promove de forma eficaz a recuperação de defeitos da cartilagem de joelhos de cães[32].

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ANEXO 1- Escala Analógica Visual (EAV) da dor (EAVdor) e Escala Analógica Visual (EAV) de claudicação (EAVloc)

Animal_____data_____

Avaliação da dor

Faça um X na seguinte linha imaginando que ela vai de zero a 10 (lado esquerdo indica ausência de dor e o lado direito indica a maior dor possível)

Semana ( ) _____	_____

Avaliação da dificuldade de locomoção

Faça um X na seguinte linha imaginando que ela vai de zero a 10 (O lado esquerdo indica ausência de dificuldade de locomoção e lado direito representa a maior dificuldade de locomoção possível)

Semana ( ) _____	_____

ANEXO 2- Breve Inventário de dor canina (BIDC)

Data de Hoje: ☐☐ / ☐☐ / ☐☐ **Identificação do Paciente/Estudo:** _____

Breve Inventário de dor canina (BIDC)

Descrição da dor:

Classifique a dor do seu cachorro.

1. Preencha o espaço oval do lado do número que melhor descreve a **pior** dor nos últimos sete dias

Sem dor — Dor extrema

○0 ○1 ○2 ○3 ○4 ○5 ○6 ○7 ○8 ○9 ○10

2. Preencha o espaço oval do lado do número que melhor descreve a **menor** dor nos últimos sete dias

Sem dor — Dor extrema

○0 ○1 ○2 ○3 ○4 ○5 ○6 ○7 ○8 ○9 ○10

3. Preencha o espaço oval do lado do número que melhor descreve a **média** de dor nos últimos sete dias

Sem dor — Dor extrema

○0 ○1 ○2 ○3 ○4 ○5 ○6 ○7 ○8 ○9 ○10

4. Preencha o espaço oval do lado do número que melhor descreve como está **agora**

Sem dor — Dor extrema

○0 ○1 ○2 ○3 ○4 ○5 ○6 ○7 ○8 ○9 ○10

Descrição da função:

Preencha o espaço oval do lado do número que melhor descreve como, durante os últimos sete dias, a **dor interferiu** no seu cachorro com relação a:

5. **Atividades em geral:**

○0 ○1 ○2 ○3 ○4 ○5 ○6 ○7 ○8 ○9 ○10

Não interfere — Interfere Completamente

6. **Prazer da Vida**

○0 ○1 ○2 ○3 ○4 ○5 ○6 ○7 ○8 ○9 ○10

Não Interfere — Interfere Completamente

Descrição da função (Continuação):

Preencha o espaço oval do lado do número que melhor descreve como, durante os últimos sete dias, a **dor interferiu** no seu cachorro com relação a:

7. **Capacidade de se levantar de quando estava deitado:**

○0 ○1 ○2 ○3 ○4 ○5 ○6 ○7 ○8 ○9 ○10

Não Interfere — Interfere Completamente

8. **Capacidade de andar:**

○0 ○1 ○2 ○3 ○4 ○5 ○6 ○7 ○8 ○9 ○10

Não Interfere — Interfere Completamente

9. **Capacidade de correr**

○0 ○1 ○2 ○3 ○4 ○5 ○6 ○7 ○8 ○9 ○10

Não interfere — Interfere completamente

10. **Capacidade de subir (por exemplo, escada, passeio "calçada")**

○0 ○1 ○2 ○3 ○4 ○5 ○6 ○7 ○8 ○9 ○10

Não interfere — Interfere completamente

ANEXO 3- Indicador de Dor Crônica da Universidade de Helsink (IDCH)

Data_____ Questionário número 1 2 3 4 5 MV _____

Nome do/a proprietário/a_____

Número do/a paciente_____

Nome do/a cachorro/cadela _____Idade _____

Raça do/a cachorro/cadela_____

Diagnóstico_____

Assinatura da pessoa que completa o questionário_____

Estado geral do/a paciente agora

Marque com um "X" apenas uma resposta para cada pergunta: aquela que melhor explica o estado de seu/sua cachorro/cadela na semana passada.

1. Estado de ânimo está:

muito ativo	ativo	nem ativo, nem abatido	abatido	muito abatido
☒	☒	☒	☒	☒

2. O cachorro/a cadela brinca com:

muita vontade	vontade	má vontade	muita má vontade	não brinca
☒	☒	☒	☒	☒

3. O cachorro/a cadela chora de dor:

nunca	raramente	às vezes	frequentemente	muito frequentemente
☒	☒	☒	☒	☒

4. O cachorro/a cadela anda :

com muita vontade	com vontade	com dificuldade	com muita dificuldade	não anda
☒	☒	☒	☒	☒

5. O cachorro/a cadela trota:

com muita vontade	com vontade	com dificuldade	com muita dificuldade	não trota
☒	☒	☒	☒	☒

6. O cachorro/a cadela galopa:

com muita vontade	com vontade	com dificuldade	com muita dificuldade	não galopa
☒	☒	☒	☒	☒

7. O cachorro/ a cadela pula (por exemplo no sofá, no carro):

com muita vontade	com vontade	com dificuldade	com muita dificuldade	não pula
☒	☒	☒	☒	☒

8. O cachorro/ a cadela se deita:

muito facilmente	facilmente	razoavelmente	dificilmente	muito dificilmente
☒	☒	☒	☒	☒

9. O cachorro/a cadela se levanta de uma posição deitada:

muito facilmente	facilmente	razoavelmente	dificilmente	muito dificilmente
☒	☒	☒	☒	☒

10. Após um longo descanso, o cachorro/a cadela se move:

muito facilmente	facilmente	razoavelmente	dificilmente	muito dificilmente
☒	☒	☒	☒	☒

11. Após um esforço físico ou esforço intenso, o cachorro/a cadela se move:

muito facilmente	facilmente	razoavelmente	dificilmente	muito dificilmente
☒	☒	☒	☒	☒

ANEXO 4- Escala Numérica Descritiva (END)

ESCALA NUMÉRICA DESCRITIVA (END) (página 1-2)

Data:_____ Questionário número 1 2 3 4 5 MV_____

Nome do/a proprietário(a)_____

Nome do paciente_____ Número do paciente_____

Raça do/a cachorro (a)_____

Diagnóstico_____

Assinatura da pessoa que completa o questionário_____

Marque com um "X"apenas uma resposta para cada pergunta:

1- DOR À PALPAÇÃO

(0) Sem sinais de dor à palpação da articulação afetada

(1) Sinais moderados de dor à palpação da articulação afetada, o cão vira a cabeça em reconhecimento

(2) Sinais de dor moderada à palpação da articulação afetada, cão puxa o membro em reação de defesa

(3) Sinais de dor severa à palpação da articulação afetada, o cão vocaliza ou se torna agressivo

(4) O cão não permite a palpação

ESCALA NUMÉRICA DESCRITIVA (END) (página 2-2)

2- GRAU DE CLAUDICAÇÃO

(0) Totalmente norma, não claudica

(1) Claudicação leve, não é tão difícil se mover

(2) Claudicação clara, não se move livremente

(3) Claudicação evidente

(4) Claudicação total, evita sustentar o peso do membro acometido

3- HABILIDADE EM PULAR EM UMA MESA

(0) Pula normalmente, bem

(1) Pula com leve cuidado

(2) Pula com um pouco de dificuldade

(3) Pula ou sobe com grande dificuldade

(4) Nem irá tentar devido à grande dificuldade/dor

4- HABILIDADE EM SUBIR ESCADAS

(0) Sobe e desce as escadas normalmente

(1) Levemente cuidadoso, utiliza ambas as patas sucessivamente

(2) Algumas vezes utiliza ambas as patas ao mesmo tempo, não se move evidentemente de maneira livre

(3) Sobe a escada como coelho durante todo o tempo, sobe a escada com grande dificuldade

(4) Não irá tentar devido à dificuldade/ dor

ANEXO 5- Escala Analógica Visual (EAV) de dor à palpação (EAVpalp dor)

Animal_____**data**_____

Escala analógica visual da dor à palpação
(EAVdor palp)

Faça um X na seguinte linha imaginando que ela vai de zero a 10 (lado esquerdo indica ausência de dor e o lado direito indica a maior dor possível)

Semana ( ) _____	_____

ANEXO 6- Impressão geral do proprietário sobre a qualidade de vida do cão (QV)

Impressão geral:

11. Preencha o espaço oval do lado da resposta que melhor descreve a qualidade de vida em geral do seu cachorro **nos últimos sete dias**?

○ Ruim ○ Razoável ○ Boa ○ Muito boa ○ Excelente

CAPÍTULO II

Chronic pain and gait analysis of dogs with degenerative hip joint disease treated either with platelet-rich plasma or allogeneic stem cells

Celina Emiko Okamoto-Okubo, DVM; Stelio Pacca L. Luna, PhD; Jean Guilherme Joaquim, PhD; Luciane dos Reis Mesquita, PhD; Sheila Canevese Rahal, PhD; Hugo Salvador Oliveira, MSc; Regina Takahira, PhD; Isadora Arruda, MSc; Leandro Maia, PhD; Fernanda da Cruz Landim, PhD.

From the Department of Veterinary Anesthesiology and Surgery , Department of Veterinary Clinics and Department of Veterinary Radiology and Reproduction, School of Veterinary Medicine and Animal Science, São Paulo State University UNESP, Rubião Júnior, Botucatu, São Paulo, Brazil.

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Address correspondence to Celina at celinaokamoto@hotmail.com

Chronic pain and gait analysis of dogs with hip dysplasia treated either with platelet-rich plasma or allogeneic stem cells

This randomized, controlled, double-blind clinical study investigated the effect of intra-articular platelet-rich plasma (PRP) (n=8) or allogeneic stem cells (SC) (n=8) in chronic pain of dogs with bilateral hip dysplasia. The owners evaluated the Canine Brief Pain Inventory (CBPI), the Helsinki Chronic Pain Index (HCPI), a Visual Analogue Scale for pain (VASpain) and lamness (VASloc) and the quality of life (QL). A veterinarian evaluated the Visual Analogue Scale of pain in response to palpation (VASpalp), the descriptive numerical scale for pain (DNS) and the percentage of body weight distribution. All evaluations were performed before, at 30 and 60 days after treatment. After SC, HCPI, VASpain and VASpalp reduced and QL increased at 60 days and CBPI reduced at 30 and 60 days. After PRP, VASloc and DNS reduced at 60 days and CBPI at 30 and 60 days. There was no difference between groups. Both SCs and PRP were apparently beneficial to reduce chronic pain in dogs suffering from bilateral hip dysplasia for 60 days, but SCs improved more chronic pain variables compared to PRP. The clinical benefit of both treatments is that they produce a long term pain relief in dogs with hip dysplasia with no apparent adverse effects.

Key words: Stem cells, injections, intra-articular, hip joint, dogs

1. INTRODUCTION

Hip dysplasia (HD) is a noninfectious and progressive disease affecting synovial joints[1]. The increased production of pro-inflammatory mediators and catabolism lead to synovitis and progressive degeneration of the joint cartilage, which can evolve loss of the cartilage surface[10].

Regenerative medicine based on adipose mesenchymal stem cell therapy (SC) and platelet-rich plasma (PRP) have been increasingly used to treat HD[2-8]. Stem cells have great capacity of proliferation and self-renovation and originate specialized cell strains[9]. They can be classified as embryonic or adult SC[8]. Adult or somatic SC are responsible for tissue replenishment and are found in most tissues[11]. The adipose tissue is a rich and accessible source of mesenchymal adult SC[4]. Intra-articular administration of autologous SC improved lameness and showed positive effects in dogs with chronic hip dysplasia [3,9,10,11].

Platelets are small blood cells without nucleus, derived from megakaryocytes and they live for 5 to 9 days and are activated by the von Willebrand factor, collagen or trombine[12]. Once activated, they secrete the content of their alpha and dense granules, followed by release of growth factors which act on tissue reparation at the stages of inflammation, proliferation and remodeling [16].

PRP is the autologous plasma with a high concentration of platelets[13]. It is usually obtained by seriated centrifugation according to different protocols[13-17]. Although some reports suggest that the inclusion of buffy coat, including leucocytes, in the plasma formed after the first centrifugation, has a three or five fold concentration of platelets compared to the original plasma [15], others report

that there are more platelets and less contamination with red blood cells when buffy coat is not included [13].

Autologous SC and PRP have been successfully used to control pain in osteoarthrosis[3,4,6,8,9,12,15,16,18], however to our knowledge, there is no reports investigating the effect of allogeneic SC to treat hip dysplasia in dogs. When allogeneic source of SC is used, only one source is required, a more refined characterization protocol may be performed, the procedure is less expensive and requires less time, several samples are produced and are promptly available for injection and it is not necessary to perform biopsy of the treated animal. Otherwise, considering that PRP also showed good results in alleviating pain derived from DJD, this might be a good alternative compared to SC, as it is less expensive and easier and faster to produce.

The hypothesis of this study is that the intra-articular injection of allogeneic SC or PRP would equally minimize pain and improve quality of life for 60 days in dogs suffering from bilateral hip dysplasia[4,8]. In order to test the hypothesis, this study aimed to compare the effect of intra-articular administration of SC or PRP in controlling pain and evaluate the influence on quality of life in dogs with hip dysplasia.

2. MATERIAL AND METHODS

The study was approved by the Institutional Ethical Committee for the Use of Animals in Research under the protocol 35/2013 and written informed consent was obtained from all dog owners before recruitment to the study.

2.1- Animals

2.1.1- SC Source

A 1.5 years old female Pit Bull dog was selected as a donor of allogeneic SC. Healthy status was based on complete blood analysis, kidney and liver biochemical profile (blood urea nitrogen, serum creatinine, alanine aminotransferase - ALT, bilirubin, total protein, albumin and globulin) and negative polymerase chain reaction for *Brucella canis,* distemper*,* canine Herpesvirus*, Erlichia canis, Leishmania sp., Leptospira sp. and Toxoplasma sp.*

2.1.2- Selection of treated dogs

2.1.2.1- Inclusion criteria

The treated groups included dogs with bilateral hip dysplasia confirmed by digital radiography and classified according to OFA - Orthopedic Foundation for Animals[19], normal blood count, kidney and liver biochemical profile (blood urea nitrogen, serum creatinine, ALT, bilirubin, total protein, albumin and globulin). The animals should not have been treated by steroid or non-steroid anti-inflammatory drugs, chondroprotectors (like chondroitin or glucosamine sulfate), phytotherapy, physiotherapy or acupuncture for at least 30 days before enrolment at the study. Animals would be excluded if they suffered from any clinical intercurrence or hematological changes. Sixteen dogs met these inclusion criteria from twenty-five dogs that were previously selected. These dogs were randomly distributed in two groups (www.randomizer.org).

2.2 – Laboratorial evaluation

Complete blood count and kidney and liver biochemical function (blood urea nitrogen, serum creatinine, ALT, bilirubin, total protein, albumin and

globulin) were performed before and 30 and 60 days after treatments.

2.3 – Radiography of the hip joint

Ventrodorsal hip-extend view digital radiography was performed to confirm the diagnosis and degree of bilateral hip dysplasia. The score of HD ranged from 0 to 4 [10,19]: **0** = Excellent, good or fair OFA classification; **1** = Borderline hip dysplasia OFA classification; **2** = Mild hip dysplasia OFA classification; **3** = Moderate hip dysplasia OFA classification and **4** = Severe hip dysplasia.

2.4- Obtention of stem cells

2.4.1- Isolation and culture of stem cells (*in vitro*)

Adipose tissue (4 g) of the donor dog was removed, under general anesthesia, from the subcutaneous region next to the gluteus muscle. The adipose tissue sample was washed with phosphate-buffered saline (PBS) solution (GIBCO®, USA) with penicillin-streptomycin (GIBCO®, USA) and processed in collagenase solution (GIBCO®, USA), filtrated and washed twice by centrifugation for 5 minutes at 2000 rpm in culture medium of 20% bovine fetal serum (GIBCO®, USA), combined to 77.8% low glucose Dulbecco's Modified Eagle Medium (DMEN) (GIBCO®, USA), 1% penicillin and 1.2% amphotericin B. The final pellet was homogenized in culture medium and divided in plastic culture flasks of 75 cm^2 (SARSTED®).

The bottles were identified, incubated at 37ºC in a humidified atmosphere

containing 5% CO2. The culture medium was changed every four days. Cell replication was monitored thought the level of confluence observed by microscopy. Once confluence was achieved (about 80%), trypsin was used to disrupt adherence (Trypsin GIBCO®, USA).

2.4.2- *In vitro* Characterization of stem cells

SCs were characterized by their morphology, adherent quality, and osteogenic, chondrogenic and adipogenic *in vitro* differentiation (STEMPRO-Invitrogen GIBCO®, USA).

2.4.3- Cell viability, stem cells phenotypes and karyotyping

Cell viability was investigated by flow cytometry (fresh samples: 96.5% of whole cells and after cryopreservation 95.5% of whole cells).

The following surface monoclonal conjugated antibodies were used to evaluate the sample immunogenicity: CD 44 (RatantiDog: AlexaFluor® 488-MCA1041A488- AbDSerotec) (Pre-cryopreservation: 88%, pos-cryopreservation: 50.4%), CD34 (FITC- BD®) (Pré-cryopreservation: 2.4%, pós-cryopreservation: 2.8%), MHC class II – (RatantiDog: FITC – MCA1044F - AbDSerotec® (Pre-cryopreservation: 3.8%, pos- cryopreservation: 3.4%), CD90 (Mouse anti human – FITC- BD®) (Pre-cryopreservation: 99.4%, pos-cryopreservation: 96.5%) and control (only cells) (Pre-cryopreservation: 1.9%, pos-cryopreservation: 2.1%) .

The karyotype of SCs was studied in second and fifth passage. All analysis showed normal characteristics of mitosis, without nucleus morphological changes.

2.4.4- Storage of stem cells bank

The cells were cryopreserved in second passage and diluted in cryoprotector medium [10% of Dimethyl Sulfoxide (GIBCO®, USA) and 90% of fetal bovine serum (GIBCO®, USA)] with about 500.000 cells/cryotube. The crytubes were submitted to slow cryopreservation with the Mr. Freeze container (Nalgene®, USA), kept for 24 hours in a ultra-freezer at -80°C (Thermo Scientific – Forma 8800 Series), cooled gradually for approximately 1 °C per minute. They were transferred to liquid nitrogen (-196°C) and maintained in this state until the completion of the cultivation in the third passage.

2.4.5- Stem cells culture and intra-articular injection preparation

The microtubes were thawed in a water bath and the SC introduced in the middle of pre-prepared culture (37ºC). Each sample underwent two sequential washings (centrifugation for 5 minutes, 2000 rpm), the final pellet was homogenized in the middle of pre-prepared culture and divided into four plastic culture flasks 75 cm^2 (37ºC, 95% air and 5% CO2, 7 days). The culture medium was changed on the third day. For the preparation of the SCs for intra-articular injections, trypsinization and two washes were performed (5 minutes, 2000 rpm). The final pellet was homogenized in PBS solution (GIBCO®, USA). Volumes of 0.4 ml (for dogs up to 10 kg body weight) and 0.8 mL (for dogs over 10 kg body weight) were divided into two samples averaging 18.08 ± 0.65 million SCs (cell count in Neubauer chamber and inverted microscope - LEICA Microsystems ®, Germany) for each joint subjected to treatment.

2.5- Obtainment of autologous platelet-rich plasm (PRP)

2.5.1- Samples of autologous blood for preparation of PRP

From 10 to 20 mL (dogs until and above 10 kg of weight respectively) of

blood was collected from the jugular vein of each dog treated with PRP. Blood was mixture with 3.2% sodium citrate. Samples were left without moving, at 25^{o}C for 30 minutes before the first centrifugation.

2.5.2- Processing of autologous PRP

PRP was prepared without the inclusion of the buffy coat[14]. The mean platelet concentration was 3.45 ± 0.21 times compared to the initial plasma platelet concentration [(mean count performed in Neubauer chamber and diluted in Rees - Renylab®). Mean baseline plasma platelet concentration was 270.20 ± 14.51 platelets/µL and final mean PRP was 932.60 ± 31.50platelets/µL. Two centrifugations were carried out, the first was performed with 1200 rpm for 10 minutes. The erythrocytes and the coat were discarded for the second centrifugation and the plasma was centrifuged at 1600 rpm for 10 minutes. About 80% of the supernatant plasma was discarded and PRP was obtained. The final application volume of PRP was 0.4 mL (for dogs up to 10 kg body weight) and 0.8 mL (for dogs up to 10kg body weight) for each joint subjected to the treatment.

2.6- Intra-articular administration

Dogs were sedated with 0.02 mg.kg^{-1} of acepromazine (Acepran® 0,2% Vetnil®) combined to 4 mg.kg^{-1} of tramadol IM (Tramal® 50mg/ml- Searle®). Subcutaneous local anesthesia was performed with 1 mL of 1% lidocaine (Xylestesin® 2%- Cristália® + Sodium Chloride 0,9% Equiplex®). Ultrasound-guided bilateral hip intra-articular SC and PRP injection was performed caudal-cranial and dorsal to the greater trochanter, at 0 and 30 days using a 22G

intravenous catheter mandrel (Angiocath® - BD®) Tramadol 3 $mg.kg^{-1}$ BID (Tramal® Retard 100mg - Searle®) was administered orally for three days.

2.7- Assessments

All assessments were performed before and 30 and 60 days after treatments.

2.7.1- Clinical evaluation

2.7.1.1- Owner assessments

The Canine Brief Pain Inventory (CBPI) [20], the Helsinki Chronic Pain Index (HCPI) [21], a Visual Analogue Scale for pain (VASpain) [22] and lamness (VASloc) [22] and the quality of life (QL) according to bad=0, reasonable=1, good=2, very good=3 and excellent=4, was assessed by the owners blinded to the treatment.

2.7.1.2- Orthopedist assessment

A veterinarian orthopedist, blinded to the treatment, evaluated the Visual Analogue Scale for pain in response to palpation (VASpalp) [22] and the descriptive numerical scale for pain (DNS) [23].

2.7.2 – Gait Analysis

A pressure sensory walkway was used to measure the percentage of body weight distribution of the forelimbs and hind limbs (Walkway High Resolution HRV4 Teckscan ®, South Boston, Massachusetts, USA). Data acquisition and processing were performed by Walkway 7.0 software. Dogs were guided in straight line with the conductor positioned in the right side. The

velocity was maintained between 0.9 and 1.1 $m.s^{-1}$ and the acceleration between -0.15 and 0.15$m.s^{-2}$. For each dog, an average of 15 trials was obtained, and first five valid trials were selected. A trial was considered valid if the four limbs contacted the walkway surface during each gait cycle without the dog turning the head or pulling on the leash. The percentage of body weight distribution among the four limbs was calculated as follows: (peak vertical force of the limb/ total peak vertical force of the four limbs) x 100.

2.8- Statistical analysis

Friedman test followed by Dunn's post-test for multiple comparisons were used to evaluate the different scores over time (before, 30 and 60 days) within each treatment.

Except for QL, the improvement for each score was calculated for each animal by subtracting the scores at day 30 minus day 0; day 60 minus day 0 and day 60 minus day 30. The QL initial (day 0) was subtracted from the final score (day 60).

Mann Whitney test was used to compare differences between groups, and for grouped observations (right + left) in each group (PRP or SC). The paired Wilcoxon test compared the degree of hip dysplasia between the right and left side within groups, and also the sides matched to each group.

3. RESULTS

Demographic data are presented in Table 1. There was no significant difference in body mass and age between groups and there was no post intra-articular injection complications.

Table 1- Mean, standard deviation (SD), maximum and minimum values of body weight and age of dogs with bilateral hip dysplasia treated with mesenchymal stem cell (SC, n=8) or platelet rich plasm (PRP, n=8)

SC Group	Body mass (kg)	Age (years)	PRP Group	Body mass (kg)	Age (years)
SC 1	28	11	**PRP 1**	38	2
SC 2	50	9	**PRP 2**	33	7
SC 3	25	5	**PRP 3**	48	4
SC 4	20	5	**PRP 4**	82	2
SC 5	40	7	**PRP 5**	25	3
SC 6	62	7	**PRP 6**	9	4
SC 7	40	3	**PRP 7**	32	12
SC 8	39	3	**PRP 8**	38	8
Mean	38	6	**Mean**	38	5
SD	14	3	**SD**	21	3
Maximum value	62	11	**Maximum value**	82	12
Minimum Value	20	3	**Minimum Value**	9	2

There were no significant differences in blood count and biochemical variables either in time within groups or between groups. There was no difference in the degree of hip dysplasia intra and between groups (Table 2).

Table 2- Mean and standard deviation (SD) of the degree of right and left hip dysplasia (HD) in dogs treated with stem cell (SC group, n=8) or platelet rich plasma (PRP group, n=8).

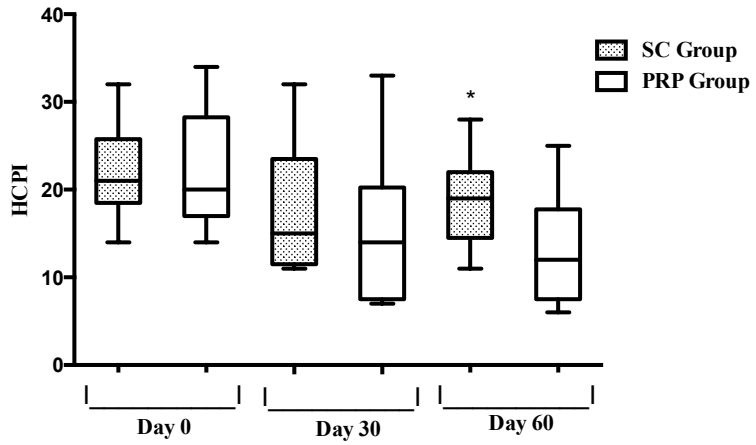
SC Group	Level of HD Right	Level of HD Left	PRP Group	Level of HD Right	Level of HD Left
SC 1	3	3	**PRP 1**	4	4
SC 2	3	3	**PRP 2**	3	3
SC 3	4	3	**PRP 3**	2	3
SC 4	3	3	**PRP 4**	3	4
SC 5	4	3	**PRP 5**	4	4
SC 6	3	3	**PRP 6**	3	3
SC 7	2	2	**PRP 7**	3	3
SC 8	2	1	**PRP 8**	3	3
Mean	3	3	**Mean**	3	3
SD	0,7	0,7	**SD**	0,6	0,5

There was no changes in the radiographic findings before *versus* 60 days after treatment.

HCPI improved 40.9% at 60 days after SCs intra-articular injection only ($P<0,05$). CBPI reduced significantly in 29% at 30 days in both groups, 48% in SC and 41% in PRP group at 60 days.

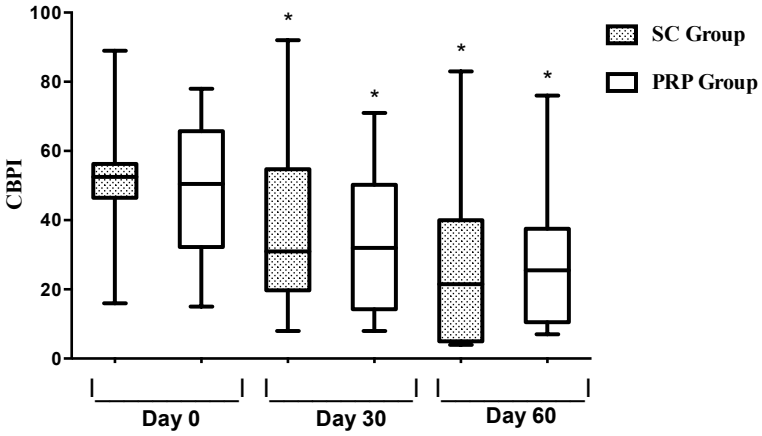
VASpain and VASpalp reduced significantly by 48% and QL increased only in animals treated with SC at 60 days. VASloc and DNS reduced significantly by 43% and 33% only in dogs treated with PRP at day 60.

Figure 1 – Boxplots (median and interquartile interval) of Helsinki chronic pain index (HCPI)[21] scores, before and 30 and 60 days, after intra-articular injection of stem cells (SC Group, n = 8) or platelet rich plasm (PRP Group, n = 8) in dogs with bilateral hip dysplasia.



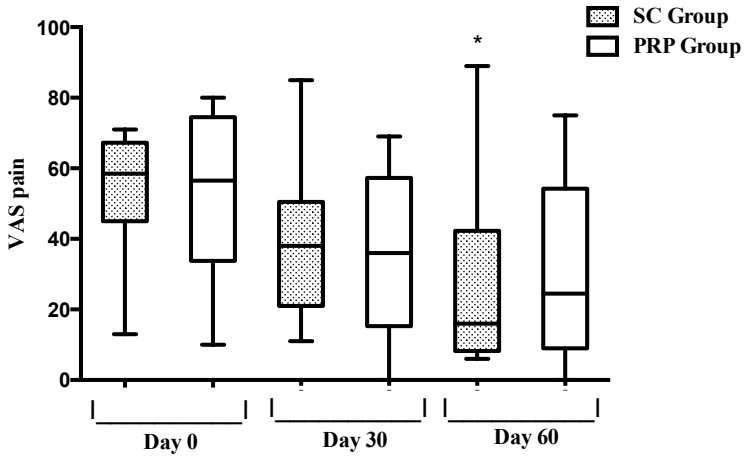
Legend: * indicates differences within group compared to day 0.

Figure 2 – Boxplots (median and interquartile interval) of canine brief pain inventory (CBPI) scores, before and 30 and 60 days after intra-articular injection of stem cells (SC Group, n = 8) or platelet rich plasm (PRP Group, n = 8) in dogs with bilateral hip dysplasia.



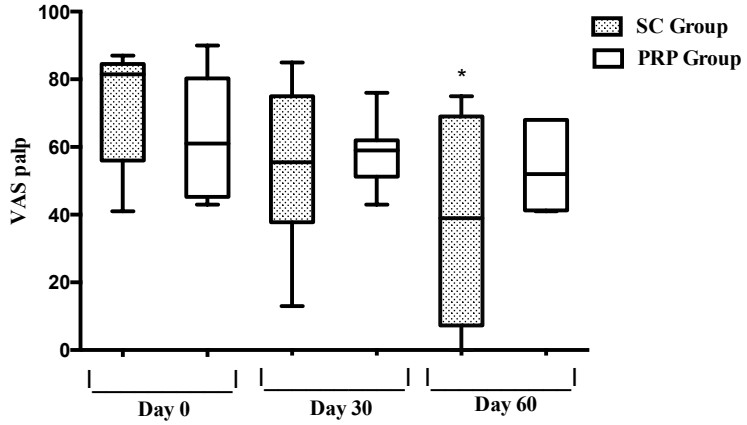
Legend: * indicates differences within group compared to day 0.

Figure 3 - Boxplots (median and interquartile interval) of visual analogue scale of pain (VASpain)[22] scores, before and 30 and 60 days after intra-articular injection of stem cells (SC Group, n = 8) or platelet rich plasm (PRP Group, n = 8) in dogs with bilateral hip dysplasia.



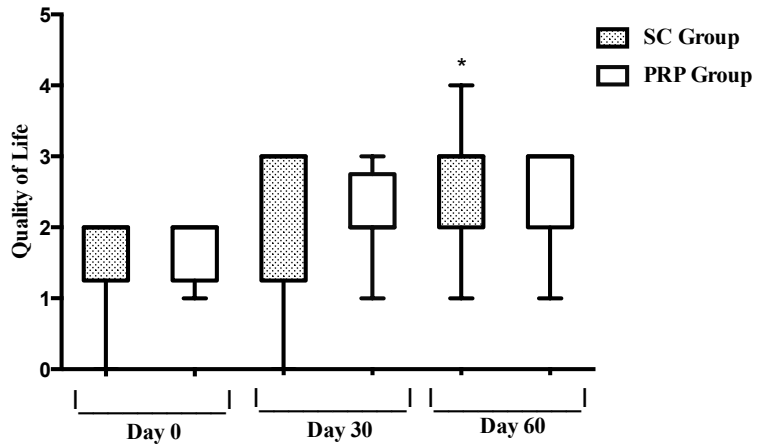
Legend: * indicates differences within group compared to day 0.

Figure 4 - Boxplots (median and interquartile interval) of visual analogue scale of pain on palpation (VASpalp)[22] scores, before and 30 and 60 days after intra-articular injection of stem cells (SC Group, n = 8) or platelet rich plasm (PRP Group, n = 8) in dogs with bilateral hip dysplasia.



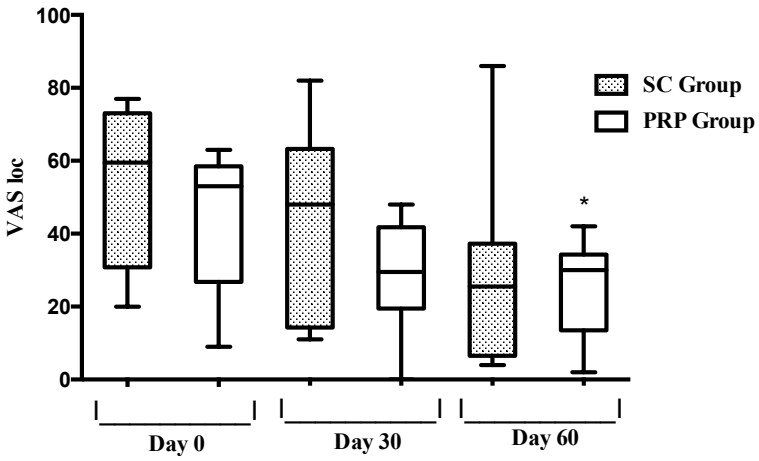
Legend: * indicates differences within group compared to day 0.

Figure 5 - Boxplots (median and interquartil interval) of quality of life scores, before and 30 and 60 days after intra-articular injection of stem cells (SC Group, n = 8) or platelet rich plasm (PRP Group, n = 8) in dogs with bilateral hip dysplasia.



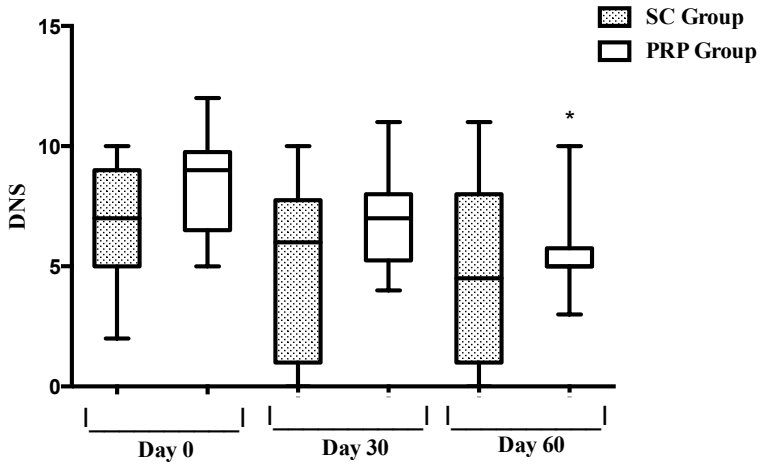
Legend: * indicates differences within group compared to day 0.

Figure 6 - Boxplots (median and interquartil interval) of visual analogue scale (VAS)[22] of lamness (VASloc) scores, before and 30 and 60 days after intra-articular injection of stem cells (SC Group, n = 8) or platelet rich plasm (PRP Group, n = 8) in dogs with bilateral hip dysplasia.



Legend: * indicates differences within group compared to day 0.

Figure 7 - Boxplots (median and interquartil interval) of descriptive numeric scale (DNS)[22] scores, before and 30 and 60 days after intra-articular injection of stem cells (SC Group, n = 8) or platelet rich plasm (PRP Group, n = 8) in dogs with bilateral hip dysplasia.



Legend: * indicates differences within group compared to day 0.

There were no differences in the percentage of body weight distribution data within or between groups and between right and left hind limbs within each group (Table 3 and 4).

Table 3- Percentage (%) of body mass distribution of forelimbs (FL) and hind limbs (HL) in dogs with bilateral hip dysplasia, before and 30 and 60 days after intra-articular injection of stem cell (SC Group, n = 8).

Animal	Day 0				Day 30				Day 60			
SC Group	FL		HL		FL		HL		FL		HL	
	R	L	R	L	R	L	R	L	R	L	R	L
1- SC	33.6	31.0	18.5	16.1	32.2	35.2	15.9	16.7	30.6	37.2	15.3	16.9
2- SC	32.0	32.1	16.8	19.1	33.3	30.7	19.4	16.6	35.3	33.2	16.7	14.8
3- SC	35.2	36.8	14.1	13.9	32.6	37.0	14.8	15.6	35.3	35.4	14.9	14.4
4- SC	28.8	32.6	18.6	20.0	27.7	30.6	20.7	21.0	29.5	29.8	20.6	20.1
5- SC	31.6	32.4	18.1	17.9	33.3	32.6	17.6	16.5	33.8	32.5	18.3	15.4
6- SC	32.2	32.4	15.3	20.1	35.7	33.2	13.5	17.6	33.9	34.3	13.9	17.9
7- SC	34.3	34.1	15.8	15.8	35.5	32.6	16.5	15.4	31.7	33.8	16.9	17.6
8- SC	31.5	33.7	16.9	17.9	31.8	32.3	16.3	19.6	34.0	34.9	14.6	16.5
Mean	32.4	33.1	16.8	17.6	32.8	33.0	16.8	17.4	33.0	33.9	16.4	16.7
SD	2.0	1.8	1.6	2.2	2.5	2.2	2.3	2.0	2.2	2.2	2.2	1.9

Table 4- Percentage of body weight distribution of forelimbs (FL) and hind limbs (HL) in dogs with bilateral hip dysplasia, before and 30 and 60 days after intra-articular injection of platelet rich plasma (PRP Group, n =8).

Animal	Day 0				Day 30				Day 60			
PRP Group	FL		HL		FL		HL		FL		HL	
	R	L	R	L	R	L	R	L	R	L	R	L
1- PRP	34.2	38.7	14.9	12.2	22.1	48.1	17.3	12.5	32.8	35.7	18.3	13.2
2- PRP	31.7	29.7	18.7	19.9	31,1	31.0	18.4	19.5	34.3	32.4	16.4	16.9
3- PRP	35.0	32.7	17.4	14.9	36.1	32.7	15.9	15.3	36.9	32.8	16.8	13.5
4- PRP	28.1	32.2	19.4	20.3	29.8	30.9	18.4	20.9	29.0	30.8	17.1	23.1
5- PRP	27.1	28.7	21.8	22.4	28.4	30.8	18.6	22.2	28.9	30.4	19.8	20.9
6- PRP	29.4	31.9	18.9	19.9	28.0	32.3	19.5	20.2	29.0	32.9	17.7	20.4
7- PRP	44.2	36.7	10.5	8.6	36.3	31.3	17.8	14.6	37.5	31.38	16.4	14.3
8- PRP	31.3	32.9	19.7	16.1	31.6	30.6	19.9	17.9	31.7	33.3	18.7	16.3
Mean	32.6	32.9	17.7	16.8	30.4	33.5	18.2	17.9	32.5	32.5	17.7	17.3
SD	5.4	3.3	3.5	4.7	4.6	6.0	1.3	3.4	3.5	1.7	1.2	3.7

4. DISCUSSION

Considering that the hip dysplasia cannot be cured, the goal of the treatment is to reduce pain and improve locomotion, in order to reduce muscle atrophy [4,8,10,11,23]. This was achieved in this study as both SC and PRP progressively reduced chronic pain in dogs with bilateral hip dysplasia. Although there was no significant differences between treatments, SC improved the majority of the variables, specially related to owner assessments. Our results met the outcomes of previous studies that recommended intra-articular injection of SC and PRP to minimize pain derived from HD. [3,4,6,8-12,16,18,24,25].

Although previous studies showed promising results with autologous adipose SC to treat HD in dogs[3,4,8,10], to our knowledge there are no reports about allogeneic SC to treat HD in dogs, as used in this study. The advantage of allogeneic source of SC, compared to the autologous one is that only one source is required, it is less time-consuming, less expensive and the bank is promptly available when treatment is necessary, which provides a great amount of samples to be used. Restrict inclusion criteria were considered, such as cell viability, characterization, differentiation, immunogenicity and karyotyping, which improved safety for the receptor[9,24]. This procedure has not always been executed when autologous transplantation is performed, as it is expensive and there are technical limitations[3,4,9]. To our knowledge there is no previous clinical studies that performed karyotyping before injecting SC. It is important to do this procedure to guarantee absence of nucleus morphological changes and assure normal characteristics of mitosis. This is another advantage of the bank.

Otherwise, compared to SC, production of PRP is technically easier, faster and less expensive. According to our results PRP would be a considerable option to treat HD, as reported previously in dogs and other species[2,18,26]. Several protocols may be used to obtain PRP[13-17], like different times and speed of centrifugation for sample manipulation. Although discrepant protocols have been suggested, most of them estipulate two centrifugations to provide a greater platelet concentration[14,17].

The PRP protocol should be chosen according to the required constitution of the final product, such as whether the buffy coat is included or not. The buffy coat is the leukocyte zone formed after the first centrifugation. Although beneficial histological findings were observed in dogs with acute cartilaginous injuries after PRP therapy, including the buffy coat [25], in our study we did not use the buffy coat, as according to a previous study[13], when the buffy coat was not included, there was less contamination with red and white blood cells, and platelet function was superior compared to the PRP where buffy coat was included[13]. Another study reported that five intra-articular applications of PRP, without the inclusion of buffy coat, improved range of motion, decreased pain and improved limb function for up to 6 months, compared to a control group, in dogs with cranial cruciate ligament[27].

As reported previously, pressure sensory walkway did not reveal changes on percentage of body weight distribution in dogs with bilateral hip dysplasia after treatments [23]. This might be related to the location of HD, as gait analysis of hip joint is complex [23]. One of the limitations related to the fact that pressure sensory walkway was not a sensitive indicator of improvement of pain, after treatment of bilateral hip dysplasia in this study, was that a short period of

evaluation of 60 days may be insufficient to demonstrate significant changes, because biomechanical changes are slow. Muscle atrophy is present in 58% of dogs with bilateral HD [27], therefore in such a short period it is unlikely that there is sufficient biomechanical compensation to change the percentage of body weight distribution. A period longer than 6 months should be considered for evaluation of the percentage of body weight distribution in dogs with hip dysplasia[3].

Hip dysplasia may be treated pharmacologically, however the effect is palliative and drugs may produce considerable adverse effects[28]. NSAIDs are usually the first choice to treat HD [29], either combined or not to analgesic adjuvants like gabaergic[30], antidepressives[30] and NMDA antagonists[31].

The possible mechanisms involved in the effect of intra-articular injection of SC and PRP have been discussed elsewhere[2,9,11,16,18]. In summary the reparation capacity of SCs are related to multiple interaction, including secretion of paracrine factors, such as cytokines, stimulation of progenitor cell proliferation and differentiation and, as a result, reduction of inflammation [32]. The PRP beneficial effect is produced by a great concentration of growth factors in alpha granules, like platelet-derived growth factor, transforming growth factor beta, vascular endothelial growth factor and others[16,33]. PRP stimulates type I collagen synthesis and increases the expression of cell cycle progression regulators to accelerate tissue recovery[18]. PRP stimulates the formation of hialuronic acid and reduce the cartilage catabolism [26].

Apparently the effect of SCs and PRP is maintained for at least 6 months[6,25]. However, longer term follow up studies are necessary to define duration of effect after SC or PRP intra-articular injection. The combined intra-

articular injection of PRP and SC may show synergism to treat HD via stimulation of extracellular matrix synthesis joint, chondrocyte proliferation and inhibition of inflammation[5].

The CBPI and HCPI scales used in this study have been previously validated, and therefore, are reliable instruments to evaluate chronic pain in dogs[20-22]. The CBPI score reduced after 30 and 60 days following treatment with SC and PRP. It is difficult to know if a second intra-articular injection of SC or PRP performed at 30 days was beneficial or not, because although there was an apparent progressive beneficial effect, there was no differences between 30 and 60 days in any variable. Previous studies also reported that CBPI scores were reduced for 24 weeks after bilateral hip intra-articular single application of autologous vascular stromal fraction derived from adipose tissue (fraction composed of heterogeneous cell population containing mesenchymal stem cells) added to PRP compare with placebo[6]. Like in our study the VAS pain score was reduced four weeks following treatment and there was no further improvement afterwards.

Other support therapies, such as physiotherapy, hydrotherapy, acupuncture and gold implants have been successfully used as non pharmacological treatments for HD[23,34,35]. However, except gold implants which have a prolonged effect, these therapies require continuous treatment, which might be a long term limitation in terms of cost and owner availability to bring the animals to the facility.

Inherent to any clinical study, this study had some limitations. Clinical response is individual according to the previous severity of the disease and

distinct response to treatment. There was no difference in demographic data and degree of hip dysplasia between groups, showing that groups were apparently homogeneous in terms of severity of the disease.

The short time of evaluation might be considered another limitation of this study. A long term study showed that hip intra-articular injection of autologous vascular stromal fraction derived from adipose tissue reduced BCPI scores only after 2 years, when compared to placebo[6]. Otherwise hip intra-articular injection of both PRP[8] and SC[3,4,8,11] reduced VASpain[8], peak vertical force and vertical impulse[11] and orthopedic scores (lameness, pain, range of motion and functional disability)[10] at the first month, but scores were stable for the remaining three [10] or six [8,11] months. Therefore it appears that the beneficial effect of SC or PRP will be observed in the first 60 days, and usually there is not a progressive improvement after this period, as the clinical signs remain stable.

5. CONCLUSION

Both SCs and PRP were apparently beneficial to reduce chronic pain in dogs suffering from bilateral hip dysplasia for 60 days, but SCs improved most of chronic pain variables compared to PRP. The clinical benefit of both treatments is that they produce a long term pain relief in dogs with hip dysplasia, with no apparent adverse effects.

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(Vancouver Citation Style)

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