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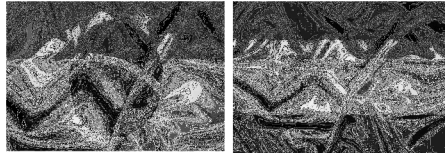
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THERAPEUTIC POTENTIAL OF MESENCHYMAL STEM CELLS TO TREAT ACHILLES TENDON INJURIES

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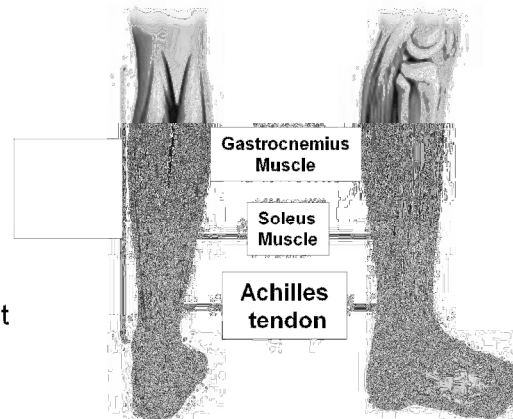
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Good morning, my name is Maria Helena and I first would like to thank the organizing committee and my advisor Doctor Andreia Antonioli for this opportunity. Well, the work theme is the therapeutic potential of mesenchymal stem cells to treat Achilles tendon injuries

Introduction

- **Achilles tendon** - mm. gastrocnemius and soleus
- Biarticular
- Longest and strongest tendon in the body:
 - 12 to 15 cm long
 - Race - 10x body weight



The Achilles tendon is formed by coalescing fibers of the gastrocnemius and soleus muscles.

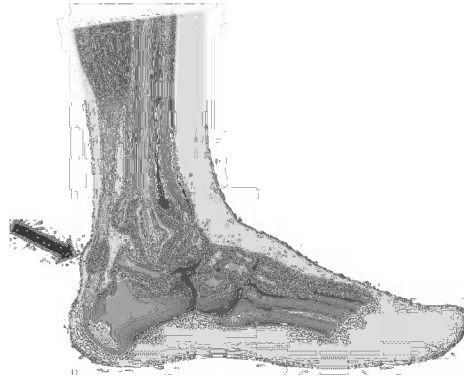
It is an biarticular muscle being fixed to the knee and ankle, making it more susceptible to damage than single joint muscles.

It is the longer and stronger tendon body, ranging from twelve do fifteen centimeters and on race it is subjected to forces greater than 10 times the body weight.

O tendão de Aquiles é formado por fibras coalescentes dos musculos gastrocnemio e soleo. É um musculo biarticular estando fixo no joelho e tornozelo, isso o torna mais susceptivel a lesões do que musculos uniarticular, É o tendão mais longo e forte do corpo, variando de 12 a 15cm e na corrida é submetido a forças superiores a 10x o peso corporal.

Introduction

- Blood supply segment - paratendon vessels
- Zone 2 to 6 cm above the calcaneal insertion – little vascularized
- Ruptures and tendinitis



The blood supply to the tendon is segmental and derives mainly from paratendon vessels. There is a zone from two to six centimeters above the calcaneal insertion that has a poor vascularization and correspond to the place of higher incidence of ruptures and tendinitis.

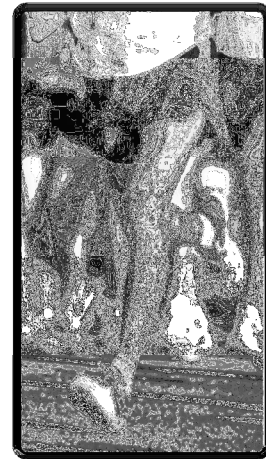
O suprimento sanguíneo do tendão é segmentar e deriva principalmente de vasos do paratendão.

Há uma zona hipovascularizada entre 2 a 6cm de sua inserção calcânea correspondendo ao local de maior ocorrência de rupturas e tendinites.

Introduction

- Ruptures
 - 30 to 55 years old
 - 35% tendon injuries
 - More than 75% - sports activity

- Annual incidence
 - 18-37 / 100,000



The ruptures of the Achilles tendon are common in patients between thirty to fifty-five years and accounts for thirty-five percent of all tendon injuries with more than seventy-five percent of injuries occurring during participation in sports activities.

The annual incidence is eighteen to thirty-seven for a hundred thousand cases.

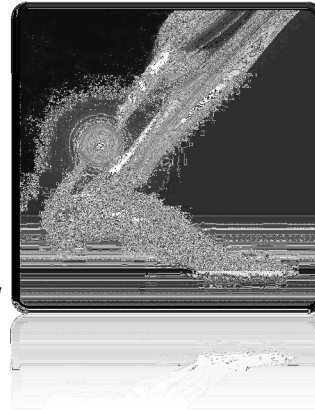
As rupturas do tendão de Aquiles são lesões comuns em pacientes de 30 a 55 anos e responde por 35% de todas as lesões tendíneas com mais de 75% das lesões ocorrendo durante a participação em atividades esportivas.

Apresenta incidência anual de 18 a 37 por cem mil casos e geralmente resultam de lesões indiretas ocasionadas por tração forçada do pé com o joelho em extensão, dorsiflexão súbita e inesperada do tornozelo e dorsiflexão violenta com o pé em flexão plantar.

Os fatores adicionais implicados nas rupturas incluem microtraumas de repetição, hipoxia, denervação mucoide, diminuição da perfusão decorrente de alterações degenerativas e infiltração local de corticosteroides

Introduction

- Indirect injuries:
 - Forced traction foot with the knee in extension
 - Sudden and unexpected ankle dorsiflexion
 - Violent dorsiflexion with the foot in plantar flexion
 - Microtrauma, hypoxia, mucoid degeneration, decreased vascularity and corticosteroids



The rupture usually result from indirect injuries caused by forced traction of the foot with the knee in extension, sudden and unexpected ankle dorsiflexion and violent dorsiflexion with the foot in plantar flexion.

Additional factors involved in injuries include repeated microtraumas, hypoxia, mucoid degeneretaion, decreased perfusion due to degenerative changes and local infiltration of corticosteroids.

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Introduction

- Conservative treatment
 - High risk of re-rupture - 13-30%
 - Leg muscle atrophy
 - Advantage - no complications of the surgical wound
- Surgical treatment
 - Lower percentage of rerupture - 0-6%
 - Early mobilization
 - Disadvantage - wound complications (0-21%)



The treatment can be conservative or surgical with the first at high risk for rerupture ranging from thirteen to thirty percent, atrophy of the leg muscles but with the advantage of not presenting complications of the surgical wound.

Since the surgery has a lower percentage of rerupture ranging from zero to six percent with the possibility of early mobilization but with the disadvantage of surgical complications such as dehiscence of points and infection which can reach twenty one percent.

As opções de tratamento são o tto conservador e cirurgico sendo que o primeiro apresenta alto risco de reruptura variando de 13 a 30%, atrofia da musculatura da perna mas com a vantagem de não apresentar as complicações da ferida cirurgica.

Já o tto cirurgico apresenta menor porcentagem de reruptura variando de 0 a 6%, possibilidade de mobilização precoce mas com a desvantagem das complicações cirurgicas como deiscencia dos pontos, infecção e que podem chegar a 21%.

Introduction

- Healed tendon
 - High cell density
 - Decreased collagen organization
 - Re-rupture
 - Adhesions
 - Low function

- Re-rupture - methods - tendon healing
 - Stem Cells
 - Growth Factors
 - Materials to support

Studies show that healed tendon has a high cell density and reduced collagen organization compared to the pre-injured tissue. This altered cellular organization serves as the basis for poor results as rerupture, adhesions and low function.

Re-rupture high rates stimulated research methods to modulate tendon healing promoting better repair with more consistency with the original tendon architecture.

Current research has focused on new tissue engineering techniques including the use of mesenchymal stem cells, growth factors and support materials such as collagen.

Estudos mostram que o tecido tendineo cicatrizado apresenta uma densidade celular alta e diminuição da cicatrização do colágeno em comparação ao tecido pré-lesado. Esta organização celular alterada serve como base para resultados ruins como reruptura, aderências e baixa função.

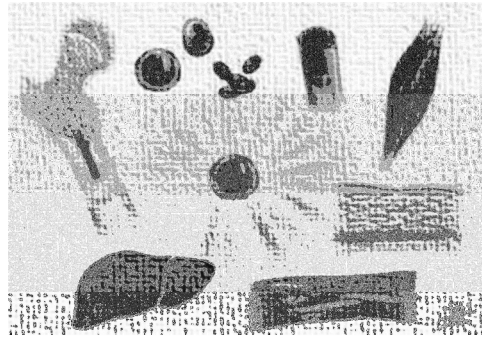
Altos índices de reruptura estimularam a investigação de métodos para modular a cicatrização tendinea promovendo melhor reparo com mais consistência com a arquitetura original do tendão.

Pesquisas atuais tem se concentrado em novas técnicas de engenharia de tecidos incluindo o uso de células-tronco mesenquimais, fatores de crescimento e materiais como colágeno para suporte

Introduction

➤ Mesenchymal stem cells (MSC) originate:

- Bone marrow
- Perivascular tissues
- Blood
- Muscle
- *** Fat



Mesenchymal stem cells originate from various tissues such as bone marrow, perivascular tissue, blood, tendon, muscle and adipose tissue.

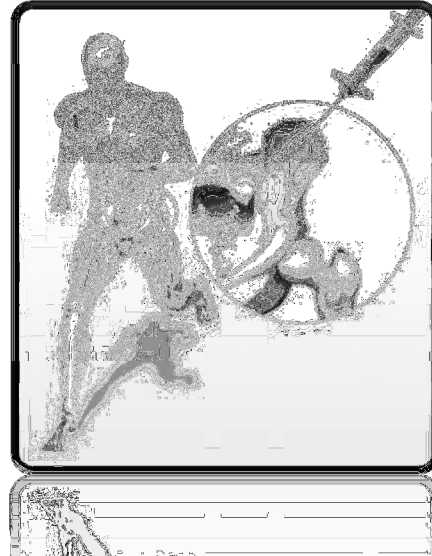
The adipose-derived tissue have multipotent properties and easy isolation. They are abundant in man and animals and can be easily extracted subcutaneously with limited incision techniques.

As células tronco mesenquimais originam-se de diversos tecidos como a medula óssea, tecidos perivascularares, sangue e tecido adiposo.

As derivadas do tecido adiposo apresentam propriedades multipotentes, alta resposta a estímulos ambientais distintos e fácil isolamento. São abundantes no homem e animais e pode ser facilmente extraídas do subcutâneo de forma percutânea e com técnicas de incisão limitadas.

Literature review

- Starnes *et al.* (2007)
 - Procedures – bone marrow
 - Invasive
 - Painful
 - Complications (> 30%)
 - Low number of cells



Juncosa-Melvin in two thousand and six used mesenchymal stem cells derived from bone marrow of rabbits in the injury of the patellar tendon and noted improvement in biomechanics and tissue histology after 12 weeks.

According Starnes, procedures with bone marrow use are very invasive, painful, with over than thirty percent of omplications and provide low number of cells.

Juncosa-Melvin em 2006 utilizou CTM derivadas da medula óssea de coelhos na lesão do tendão patelar e observou melhora biomecanica e histologica do tecido após 12 semanas.

Segundo Starnes, os procedimentos com uso de medula óssea são muito invasivos, dolorosos, com complicações acima de 30% e fornecem baixo numero de celulas

Literature review

- Vishnubalaji *et al.* (2012)
 - Differentiation capacity - MSC
 - Bone marrow and adipose tissue
 - Colony higher – adipose tissue
 - Differentiation capacity

- Dressler *et al.* (2004)
 - MSC does not loose the ability with increasing age
 - Cells stored for 3 years

Vishnubalaji two thousand and twelve compares the differentiation capacity of MSCs derived from bone marrow and adipose tissue of humans and concluded that the colony of fat cells is larger but both have the same cellular differentiation capacity depending on where they are deposited .

Dressler in two thousand and four used MSC of elderly rabbits and noted that the MSC does not loose the capacity to cell therapy repair with increasing age and when stored for a three year period continue to promote effective repair.

Vishnubalaji em 2012 compara a capacidade de diferenciação de CT derivadas da medula óssea e do tecido adiposo de humanos e conclui que a colonia de células adiposas é maior mas que ambas as colonias tem a mesma capacidade de diferenciação celular dependendo do local onde são depositadas.

Dressler em 2004 utilizou CTM de coelhos idosos e observou que as CTM não perdem a capacidade necessaria para terapia celular de reparo com aumento da idade e quando estocadas por um periodo de 3 anos continuam a promover reparo efetivo.

Goals

To study, by histological analysis, the therapeutic potential of adipose-derived MSC to treat Achilles tendon injuries.

Then based on the literature presented there was a need and curiosity in studying in general the therapeutic potential of adipose-derived MSC to treat Achilles tendon injuries and specifically to examine, by histology, the repair of the Achilles tendon submitted to treatment with use of MSC.

Baseado então na literatura apresentada houve a necessidade e a curiosidade em estudar de forma geral o potencial terapêutico das CTM de gordura na lesão do tendão de Aquiles em coelhos e especificamente a analisar, por histologia, o reparo do tendão de Aquiles em coelhos submetidos a tto com uso de CTM.

Methodology

➤ Sample

- 30 rabbits, New Zealand, male, adult , 2.5kg
- Housed in cages
- Commercial feed (Guabi®) and water ad libitum
- Guidelines of the Universal Declaration of Animal Rights
- Approval of the UFMS Ethics Committee on the use of animals

In the methodology, as a sample we used 30 New Zealand rabbits, males, adults with an average weight of 2.5kg.

The animals were individually housed in cages with at least a minimum degree of freedom of movement. It was provided commercial feed and water ad libitum.

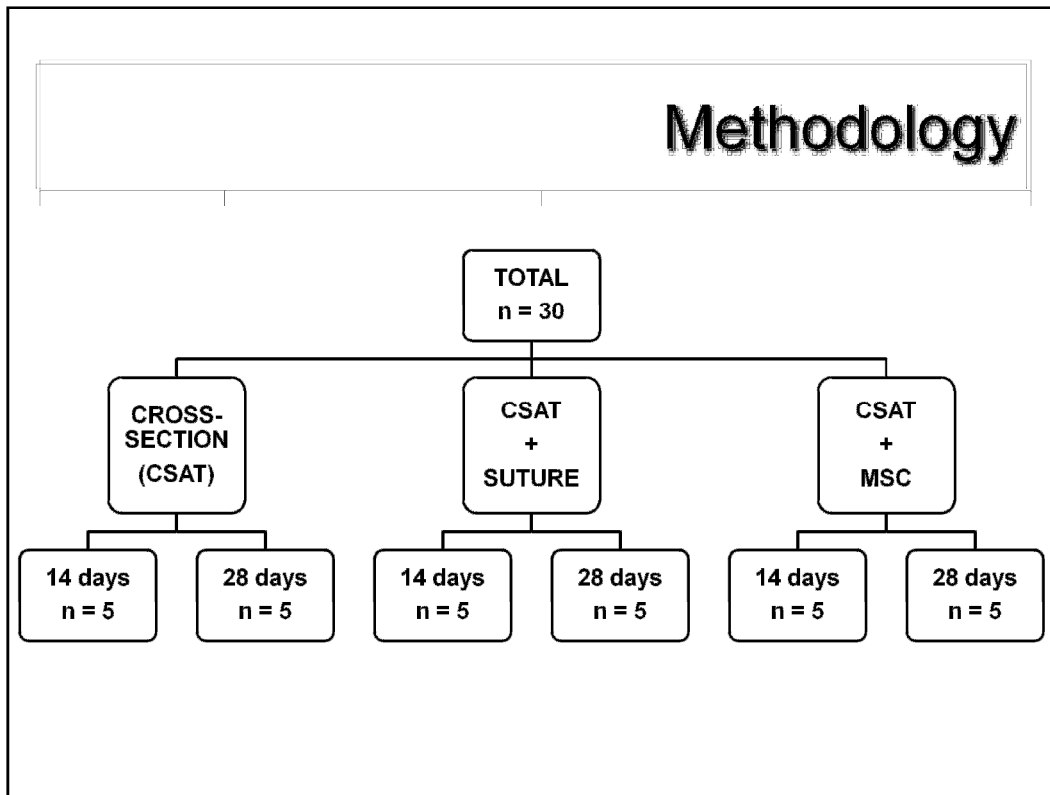
The experiment was conducted according to the guidelines of the Universal Declaration of Animal Rights and with the approval of the ethics committee in the use of animals of our university.

Na metodologia, como amostra utilizou-se de 30 coelhos da raça nova zelandia, machos, adultos com peso medio de 2.5kg

Os animais foram alojados individualmente em gaiolas recobertas com cepilho autoclavado, trocado semanalmente com pelo menos minimo grau de liberdade de movimentos. Foi fornecida ração comercial e agua ad libitum.

O experimento foi conduzido segundo as diretrizes da Declaração Universal dos Direitos dos Animais e com a aprovação da Com Etica no uso de animais da ufms cujo protocolo é o 307 de 2011.

Methodology

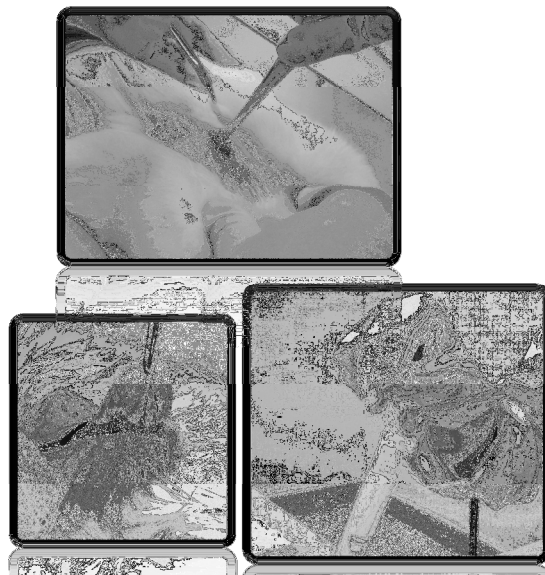


The animals were randomly divided into 6 groups of 5 animals each. The groups were cross-section of Achilles Tendon, cross-section plus suture and cross-section plus injection of mesenchymal stem cells and histological analysis was performed with fourteen and twenty-eight days.

Os animais foram distribuídos aleatoriamente em 6 grupos de 5 animais cada sendo os grupos: ruptura, ruptura mais sutura e ruptura mais CTM; e A análise histológica foi realizada com 14 e 28 dias. No grupo ruptura foi realizada a secção transversa do tendão, no grupo ruptura mais sutura foi realizada secção transversa do tendão e sutura e no grupo ruptura + celular foi realizada secção transversa mais a injeção local das CTM.

Methodology

- **Surgical procedure**
 - **General anesthesia**
 - **Adipose tissue collection**
 - 5cm of occipito
 - Incision - 2 cm
 - 1cm³ fat
 - Wash PBS 1%
 - Skin suture: polyamide 4-0
 - Refrigerated environment 4° C
 - Explant and cultivate in 24h



To collect the adipose tissue was conducted longitudinal incision at the larger animal fat accumulation located five centimeter from occipito. The incision in the dorsal line with two centimeters with the use of the scalpel blade was performed. Then held lipectomy block with removal of 1 cubic centimeter of fat using the same scalpel blade.

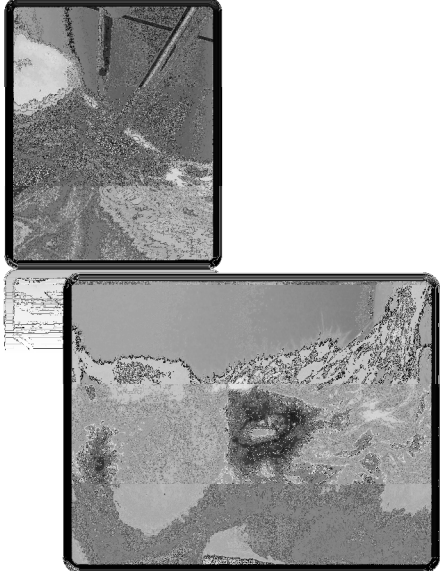
The material was washed with phosphate buffer solution supplemented with 1% penicillin / streptomycin, PBS 1% by conical tube sterile until the removal of excess blood. Skin suture was performed with 4-0 polyamide suture and the material was stored refrigerated for up to 24 hours before the explant and cell culture procedures.

Para a coleta do tecido adipo após os cuidados de assepsia e antisspsia, foi realizada incisão cirurgica longituinal no local de maior acumulo de gordura do animal, localizado a 5cm do occipito. Foi realizada a incisão na linha dorsal com 2cm de comprimento com uso de lamina de bisturi no. 24 . Realizada então lipectomia em bloco com retirada de 1 centimetro cubico de gordura com o uso da mesma lamina de bisturi.

O material foi lavado com solução tampão fosfato suplementado com 1% de penicilina/estreptolisina, o PBS a 1%, em tubo conico, esteril ate a retirada do excesso de sangue. Foi realizada sutura da pele com fio de poliamida 4-0 e o material foi estocado em ambiente refrigerado a 4°C por ate 24h antes dos procedimentos de explante e cultivo celular.

Methodology

- **Tendon Injury**
 - Epilation and longitudinal incision lateral to the Achilles tendon
 - Cross-section of the tendon - 10mm calcaneal insertion



After specific aseptic and antiseptic care, hair removal and longitudinal and lateral incision to the Achilles tendon of the animal's right hind leg was made of approximately 1.5 cm, with scalpel blade and exposure of the tendon.

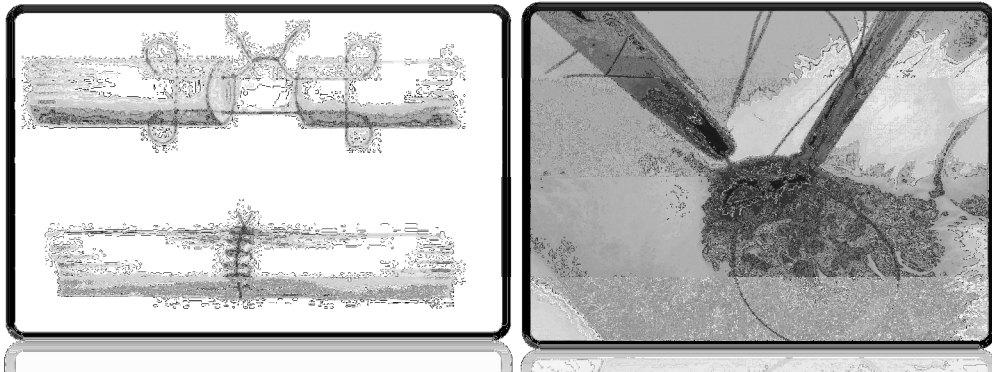
Cross section of the Achilles tendon was performed at 10 mm from its insertion on the calcaneus.

Após tbem cuidados especificos de assepsia e antissepaia, foi realizada epilação e incisão longitudinal lateral ao tendão de aquiles direito do animal , de aproximadamente 1,5cm, com lamina de bisturi no 24 e exposição do tendão

Foi realizada secção transversa do tendão de Aquiles a 10 mm da sua inserção no calcaneo.

Methodology

- In Group CSAT + suture
 - Polyamide 3-0 and 4-0
 - *Kessler* technique modified by *Masson e Allen*

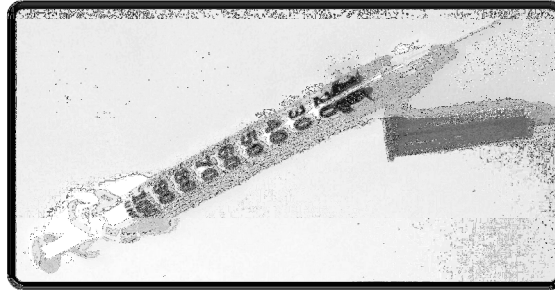


In CSAT group, the tendon was sutured with polyamide thread 3-0 and 4-0 respectively by modified Kessler technique by Masson and Allen. In the original technique Kessler suture is made as seen in this figure and by modifying the peritendon is also sutured.

No grupo ruptura mais sutura o tendão foi suturado com fio de poliamida 3-0 e 4-0 respectivamente pela técnica de Kessler modificada por Masson e Allen. Na técnica original de Kessler faz-se a sutura como vista nesta figura e com a modificação o peritendão tbem é suturado. E aqui ao lado a foto da realização do procedimento.

Methodology

- Application of the MSC
 - Cell culture – **$3,46 \times 10^6$** ($3,20 \times 10^6$ to $3,65 \times 10^6$)
- Group CSAT+ MSC
 - Tendon injury
 - 0.1ml cell culture



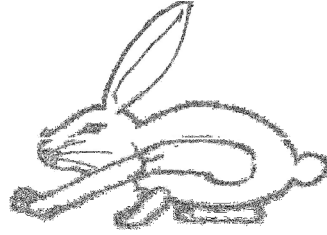
The cell culture was obtained with this average number of cells.

In group CSTA plus MSC, was injected at the site of tendon damage the volume of zero point one millimeters of cell culture.

O cultivo de células obteve um número médio de $3,46 \times 10^6$ a sexta. No grupo ruptura mais CTM Foi injetado no local da lesão tendínea o volume de 0.1ml em seringa de 1ml e agulha 13 x 4,5, ou seja, a seringa geralmente utilizada para aplicação de insulina.

Methodology

- Immobilization
 - Plaster cast– equine position
 - Groups: CSAT and CSAT+ MSC
 - Control approach of the stumps by palpation and not identifying the gap between them



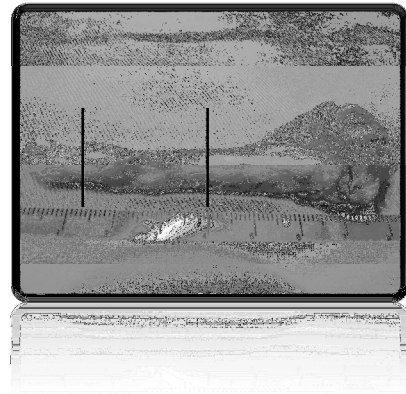
All the animals were immobilized in plaster cast keeping the operated ankle at equine position.

In groups CSTA and CSAT plus MSC the control approach of the stumps was performed by palpation and not identifying the gap between them prior to making the plaster.

Todos os animais foram imobilizados em aparelho gessado mantendo o tornozelo operado em posição equina sendo que nos grupos ruptura e ruptura mais CTM o controle da aproximação dos cotos foi realizado pela palpação e não identificação do afastamento entre eles previamente a confecção do gesso.

Methodology

- **Collection of biological samples**
- **Euthanasia**
 - 14 or 28 days
- **Dissection hind legs – tendon integrity**
 - Fragment - 10mm proximal and distal to cross-sectional area



To obtain the samples, the animals were euthanized by intraperitoneal injection of pentobarbital after 14 or 28 days the surgical procedure. The hind legs were dissected preserving the integrity of the tendon and tendon fragment was removed with 10mm proximal and distal to the site of cross-section.

Para a obtenção das amostras os animais foram submetidos a eutanásia por injeção intraperitoneal de pentobarbital na dose de 100mg/kg após 14 ou 28 dias do procedimento operatorio. As patas traseiras foram dissecadas preservando a integridade do tendão e foi retirado fragmento do tendão com margem de 10mm proximal e distal ao local da secção transversa

Methodology

- Histological analysis
 - Hematoxylin-eosin - study of vascularization and proliferation of fibroblasts.
 - Gömöri Trichrome - collagen I and III study
- Histologic evaluation
 - Brightfield microscopy
 - Double-blind
- Statistical analysis

For the histological analysis the tendon fragment was fixed in formaldehyde 4% for 5 days, processed and included in paraffin and cut transversely into slices of 5 microns thickness and stained with hematoxylin-eosin techniques to study the vascularization and proliferation of fibroblasts and trichrome Gomori for the study of collagen type I and III. The slices were subjected to histological evaluation with brightfield microscopy and double blind system.

Para a análise histológica o fragmento de tendão foi fixado em solução de formaldeído a 4% por 5 dias, processado e incluído em parafina e cortado transversalmente em fatias de 5 micrometers de espessuras e corados pelas técnicas de Hematoxilina-eosina para o estudo da vascularização e proliferação de fibroblastos e tricomico de gomori para o estudo do colageno tipo I e III . As laminas foram submetida a avaliação histologica com microscopia de campo claro e sistema duplo cego.

Table 1. Classification of histological findings reported at the site of the cross section of the Achilles tendon.			
Histological findings		Classification	Score
Inflammatory process		Absence	0
	+	1 focus	1
	++	2 collections of lymphocytes and histiocytes with presence of lymphoid follicles	2
	+++	Diffuse inflammatory process	3
Adipose tissue		Absence	0
	+	1 to 2 collections of adipocytes	1
	++	3 to 5 collections of adipocytes*	2
	+++	More than 5 collections of adipocytes*	3
Capillaries		Absence	0
	+	Up to 5*	1
	++	From 5 to 10*	2
	+++	More than 10*	3
Arterioles		Absence	0
	+	From 1 to 3 per field**	1
	++	From 3 to 5 per field**	2
	+++	More than 5 per field**	3
Structural disorganization		Absence	0
	+	Low	1
	++	Moderate	2
	+++	High	3

**40X magnification; **10X magnification.*

On the table are the analyzed variables: inflammation, presence of local adipose tissue, capillaries, arterioles and the structural disorganization.

Na tabela encontram-se as variáveis analisadas: processo inflamatório, presença de tecido adiposo local, capilares, arteríolas e a desorganização estrutural

Results

Table 2. Means \pm SD of scores obtained from the variables.

Variable	CSAT	CSAT + Suture	CSAT + MSC	p ⁽¹⁾
	Means \pm SD	Means \pm SD	Means \pm SD	
Inflammatory process:				
14 days	1.3 \pm 0.5*	1.3 \pm 0.6*	1.4 \pm 0.5*	0.902
28 days	0.0 \pm 0.0**	0.6 \pm 0.5*	0.2 \pm 0.4**	0.230
p ⁽²⁾	0.011	0.100	0.014	
Adipose tissue:				
14 days	1.5 \pm 0.0*	1.7 \pm 0.0*	1.0 \pm 0.0*	0.328
28 days	0.5 \pm 0.1*	1.0 \pm 1.0*	0.7 \pm 1.2*	0.500
p ⁽²⁾	0.266	0.273	0.315	
Capillaries:				
14 days	2.0 \pm 0.0*	3.0 \pm 0.0*	3.0 \pm 0.0*	0.004
28 days	2.0 \pm 0.0*	2.4 \pm 0.2*	2.7 \pm 0.5*	0.002
p ⁽²⁾	1.000	0.100	0.361	
Arterioles:				
14 days	0.8 \pm 0.5*	2.0 \pm 0.0*	1.0 \pm 0.0*	0.009
28 days	1.0 \pm 0.0*	1.6 \pm 0.5*	1.2 \pm 0.4*	0.068
p ⁽²⁾	0.522	0.273	0.648	
Structural disorganization:				
14 days	3.0 \pm 0.0*	2.3 \pm 0.0*	2.0 \pm 0.0*	0.014
28 days	2.0 \pm 0.0*	1.6 \pm 0.5*	1.2 \pm 0.4**	0.064
p ⁽²⁾	0.033	0.100	0.022	

It was obtained as follows: reduction of inflammation between the periods on groups CSTA and CSTA + MSC. Among the groups there was no significant difference in the degree of inflammation in both periods. Regarding adipose tissue there was no difference between the periods. As for the capillaries, the CSTA group had fewer than the rest within 14 days. There was no difference between groups at 28 days and between periods for each group. There was a higher number of arterioles on the CSTA + suture group than the others in the 14-day period and no difference between the groups at 28 days and between periods for each group.

Obteve-se o seguinte: diminuição do processo inflamatório entre os períodos nos grupos ruptura e ruptura + CTM. Entre os grupos não houve diferença significativa no grau de processo inflamatório nos dois períodos.

Com relação ao tecido adiposo não houve diferença entre os períodos. Quanto as capilares o grupo ruptura obteve menor número do que os demais no período de 14 dias. Não houve diferença entre os grupos no período de 28 dias e entre os períodos para cada grupo.

Houve maior número de arteríolas no grupo ruptura + sutura do que os demais no período de 14 dias e não houve diferença entre os grupos no período de 28 dias e entre os períodos para cada grupo.

Discussion

- Therapeutic potential
 - Adipose-derived MSC
 - Achilles tendon regeneration – not sutured tendon ruptures
- Tendon ruptures
 - Tendinopathy
 - Fraying of the tendon
- Thein *et al.* (2004) Chong *et al.* (2007) Daher *et al.* (2011)
 - Cross-section – 10mm calcaneal insertion
 - Resistent area - tensile forces

Well, this study evaluated the therapeutic potential of adipose-derived mesenchymal stem cells in the Achilles tendon injury of rabbits focused employment of this method in not sutured tendon since most studies in the literature evaluating its use in tendons sutured.

Despite the tendon ruptures usually occur by prior tendinopathy and be a fraying of fibers works in the literature as the Thein, Chong and Daher has submitted a cross-section of the tendon above 10 mm of tendon insertion on the calcaneus to be the location that corresponds to the more resistant area to shear forces so the same technique was used in this work.

Bom , este trabalho avaliou o potencial terapeutico das CTM de gordura na lesao do tendão de aquiles em coelhos com foco do emprego deste metodo em tendões não suturados pois a maioria dos trabalho na liberatura avaliam seu emprego em tendões suturados

Apesar das rupturas do tendão ocorrerem geralmente por tendinopatia previa e haver um esgarçamento de suas fibras os trabalhos na literatura como o de Thein Chong e Daher tem submetido o tendão a ruptura transversa a 10mm da inserção do tendão no calcaneo por ser o local que corresponde a area mais resistente as forças de tensão por isso foi utilizada a mesma tecnica neste trabalho.

Discussion

- Young *et al.* (1998); Chong *et al.* (2007); Daher *et al.* (2011)
 - Do not immobilize - complications
- Gossman *et al.* (2013)
 - Stretching and circumference
 - Muscles soleus and plantar – rabbit
 - After cast immobilization
 - No decrease of both – fixed with stretching
- Present study
 - Immobilization
 - Shortening of the tendon

Young, Chong and Daher do not use immobilization as postoperative method by the aforementioned complications.

Gossman assessed the stretching and circumference of the soleus and plantar muscle in rabbits after cast immobilization and noted that there was no change in both when the muscle was immobilized in stretched position. In this work it was necessary to maintain muscle shortening to approximate the proximal and distal tendon stumps after injury on groups CSTA and CSTA plus MSC. Then to standardize the method it was used in all groups.

Young, Chong e Daher não utilizam a imobilização como método pos-operatório pelas complicações já mencionadas.

Gossman avaliou o alongamento e circunferência dos músculos soleo e plantar em coelhos após imobilização gessada e observou que não houve alteração em ambos quando o músculo foi imobilizado em posição alongada. Já neste trabalho houve a necessidade de manutenção do músculo com encurtamento para aproximar os cotos proximal e distal após a lesão tendínea nos grupos ruptura e ruptura + CTM. Então para padronizar, o método foi utilizado em todos os grupos

Discussion

- Histological analysis
 - 14 and 28 days
 - Critical phase of tendon healing - 14 and 16 days
 - Carlstedet et al. (1987)
 - Study - tendon healing
 - Exudation and fibrin union - 5th after surgery
 - After - fibroplasia and increased tensile strength
 - 14-16 days after surgery
 - Tenorrhaphy depends on the suture
 - Maturation, differentiation and organization - undetermined increase tensile strength

With respect to histological analysis, this study was performed at fourteen and twenty eight days since the critical phase of tendon healing occurs between fourteen and sixteen days.

Carlstedet studied the tendon healing and determined that the phase of exudation and fibrin Union begins around the fifth Postoperative day. After this phase begins the fibroplasia phase and increased tensile strength stabilizing between the fourteenth to sixteenth days postoperatively.

During this period the suture still depends of the suture. From this phase begins the maturation phase, organization and differentiation and tendon tensile strength increases indefinitely.

Com relação a análise histológica, neste trabalho foi realizada com 14 e 28 dias pois a fase crítica da cicatrização tendinea esta entre 14 e 16 dias.

Carlstedet estudou a cicatrização tendinea e determinou que a fase de exsudação e união da fibrina inicia-se por volta do 5º. Dia de PO. Após esta fase inicia-se a fase de fibroplasia e aumento da força de tensão estabilizando por volta do 14 ao 16º dia de PO.

Neste periodo a sutura ainda depende do fio da sutura. A partir desse periodo inicia-se a fase de maturação, organização e diferenciação e a força de tensão do tendão vai aumentando gradativamente por periodo de tempo indeterminado.

Discussion

- Inflammatory process
 - Increased CSAT + suture - 14 and 28 days
 - Suture
 - Used Wire
 - Surgical technique

Increased tissue damage

- Klein et al. (2003) and Lee et al. (2010)

In the study it was observed that the inflammation was higher in the group CSTA plus suture in both periods which may mean that the use of suture, wire type used and the technique employed may increase tissue damage as observed in studies in literature as Klein and Lee.

No trabalho foi observado que o processo inflamatório foi maior no grupo ruptura + sutura em ambos os períodos o que pode significar que o uso de sutura, tipo de fio utilizado e técnica empregada podem aumentar o dano ao tecido como observado em trabalhos na literatura como os de Klein e Lee.

Discussion

- Capillary
 - Lower - CSAT group 14 days
 - No difference between groups at 28 days and between periods
- Okamoto et al. (2010)
 - MSC
 - Extrinsic factor
 - Release growth factors and cytokines
 - Suture - foreign body reaction

Increases vascularization

With regard to capillaries, was lower in the CSTA group with 14 days and there was no difference between groups in 28 days and between periods for each group.

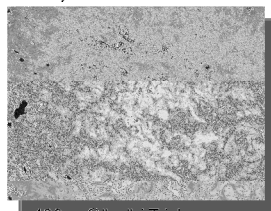
According to the study of Okamoto, the cells act as extrinsic factor for the healing tendon and promote the release of growth factors and cytokines increasing local vascularization. The use of suture because of its foreign body reaction also increases vascularization therefore the number of capillaries may have been lower in the group where there was no extrinsic stimuli.

Com relação aos capilares, foi menor no grupo ruptura com 14 dias e não houve diferença entre os grupos no período de 28 dias e entre os períodos para cada grupo.

Conforme o trabalho de Okamoto em 2010, as CTM agirem como fator extrínseco para a cicatrização tendínea, promovem tbem a liberação de fatores de crescimento e citocinas aumentando as vascularização local. O uso da sutura devido a sua reação de corpo estranho, também aumenta a vascularização por isso o número de capilares possa ter sido menor no grupo em que não houve estímulo extrínseco.

Discussion

- Structural Disorganization
 - More - CSAT group - 14 days
 - Between periods - more CSAT and CSAT + MSC groups
 - Suture - facilitating the orientation of collagen fibers
 - Smith and Evans (2001) Groth et al. (2005) Park et al. (2010)



400x - Gomori Trichrome
CSAT + suture 14 days



400x - Gomori Trichrome
CSAT + suture 28 days

The structural disorganization was greater on CSTA group with 14 days and between periods was higher in CSTA and CSTA plus MSC groups. The suture facilitated the orientation of the collagen fibers which is in agreement with the works of Smith and Evans, Groth and Park which even emphasize early mobilization.

A desorganização estrutural foi maior no grupo ruptura com 14 dias e entre os períodos foi maior nos grupos ruptura e ruptura + CTM. A sutura facilitou a orientação das fibras de colágeno o que está de acordo com os trabalhos de Smith e Evans, Groth e Park os quais enfatizam inclusive a mobilização precoce.

Discussion

- Daher *et al.* (2011)
 - Histological analysis Achilles tendon rats
 - CSAT + suture and MSC injection
 - 2,4,6 weeks
 - 2 weeks largest cellular organization in the first group
 - 4, 6 weeks - similar

Daher to perform the histological analysis of the Achilles tendon of rats submitted to cross-section plus suture and injection of MSC during 2, 4 and 6 weeks as compared to cross-section plus suture noted that in 2 weeks, there was increased cell organization in the experimental group and already with 4 and 6 weeks tissue organization was similar to the control group.

Daher ao realizar a análise histológica do tendão de aquiles de ratos submetidos a ruptura + sutura e injeção de CTM nos periodos de 2, 4 e 6 semanas quando comparados com grupo Ruptura e sutura observou que com 2 semanas houve maior organização celular no grupo experimento e já com 4 e 6 semanas a organização tecidual foir similar ao grupo controle.

Discussion

- Results achieved
- Literature studies
- Study with three groups + (CSAT + suture + MSC)
 - Assess improvement in cellular organization and early healing

With the results obtained and the works presented here, we observed that there is the need for a study to assess the three groups of this research added a fourth group that performs the cross-section, suture and injection of MSC to assess whether there is improvement of cellular organization and early healing as we have the suture promoting the targeting of collagen and MSC anticipating healing.

Com os resultados obtidos e os trabalhos na literatura apresentados, observamos que há a necessidade de estudo que avalie os tres grupos desta pesquisa somados a um quarto grupo em que se realize a ruptura + sutura + a injeção de CTM para avaliar se há melhora da organização celular e cicatrização precoce já que teremos a sutura promovendo o direcionamento do colágeno e as CTM antecipando a cicatrização

Conclusion

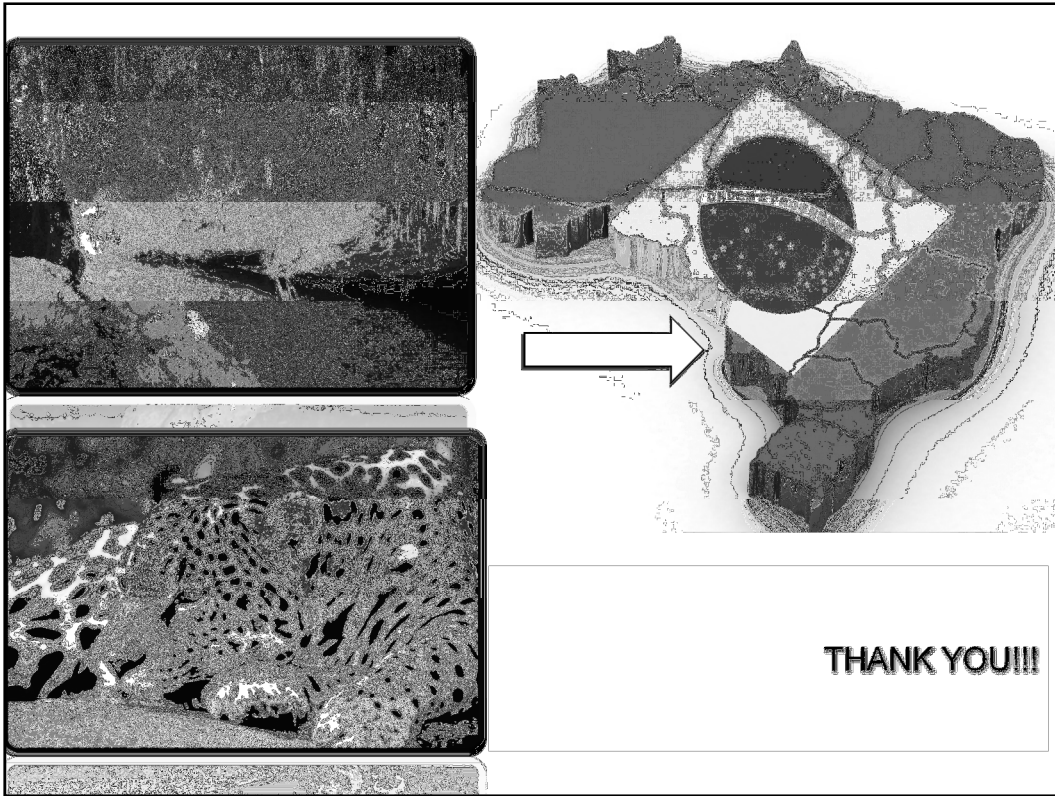
- Mesenchymal Stem Cells
 - Rabbits's Achilles tendon injury not sutured
 - Similar to suture the tendon
 - Biomechanical Testing
 - Complement
 - Function assessment
 - Clinical application

it is concluded that the use of mesenchymal stem cells in the lesion of the Achilles tendon not sutured in rabbits was similar to using the gold standard method, so, suture of the tendon.

There is need for biomechanical testing to complement the study asr the evaluation function is critical for clinical application.

Conclui-se que o uso de CTM em lesão do tendão de Aquiles não suturados de coelhos foi similar ao uso do metodo padrao-ouro, ou seja, a sutura do tendão.

Há a necessidade de teste biomecanico para complementação do estudo pois a avaliação da função é fundamental para aplicação clinica.



And I would like to seize the moment and make an invitation to come e visit Brazil, thank you!!!

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