UNIVERSIDADE ESTADUAL PAULISTA – UNESP
CÂMPUS DE JABOTICABAL

Dayvid Vianêis Farias de Lucena
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UNIVERSIDADE ESTADUAL PAULISTA – UNESP

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AVALIAÇÃO BIOMECÂNICA DE UMA NOVA HASTE ORTOGONAL COM ÂNGULO ESTÁVEL EM UM MODELO DE FÊMUR CANINO

Discente: Dayvid Vianêis Farias de Lucena

Orientador: Prof. Dr. Bruno Watanabe Minto

Tese apresentada à Faculdade de Ciências Agrárias e Veterinárias – Unesp, Câmpus de Jaboticabal, como parte das exigências para a obtenção do título de doutor em Cirurgia Veterinária.

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CERTIFICADO DE APROVAÇÃO

TÍTULO DA TESE: AVALIAÇÃO BIOMECÂNICA DE UMA NOVA HASTE ORTOGONAL COM ÂNGULO ESTÁVEL EM UM MODELO DE FÊMUR CANINO

AUTOR: DAYVID VIANÊIS FARIAS DE LUCENA
ORIENTADOR: BRUNO WATANABE MINTO

Aprovado como parte das exigências para obtenção do Título de Doutor em CIRURGIA VETERINÁRIA, pela Comissão Examinadora:

Prof. Dr. BRUNO WATANABE MINTO (Participaçao Virtual)
Departamento de Clínica e Cirurgia Veterinária / FCAV / UNESP - Jaboticabal

Prof. Dr. LUÍS GUSTAVO GOSUEN GONÇALVES DIAS (Participaçao Virtual)
Departamento de Cliníca e Cirurgia Veterinária / FCAV / UNESP - Jaboticabal

Prof. Dr. PAULO VINICIUS TERTULIANO MARINHO (Participaçao Virtual)
Instituto Federal de Educação, Ciência e Tecnologia do Sul de Minas Gerais-Campus Muzambinho / Muzambinho/MG

Pesquisador Dr. ALEXANDRE SCHMAEDECKE (Participaçao Virtual)
Medico Veterinário / Centro Integrado de Especialidades Veterinárias / Curitiba/PR

Prof. Dr. BRUNO MARTINS ARAÚJO (Participaçao Virtual)
Universidade Federal do Piauí-UFPI / Teresina/PI

Jaboticabal, 11 de agosto de 2021

DADOS CURRICULARES DO AUTOR

Dayvid Vianêis Farias de Lucena, nascido no município de Patos, estado da Paraíba, aos 14 dias do mês de junho de 1987. Ingressou no curso de Medicina Veterinária na Universidade Federal de Campina Grande (UFCG) em 2008. Nessa instituição, realizou estágios na área de Clínica Médica e Cirúrgica de Pequenos Animais do primeiro ao décimo semestres da graduação, com ênfase em Ortopedia e Neurologia Clínica e Cirúrgica a partir do quinto semestre. Foi aluno monitor das disciplinas de Técnica Cirúrgica Veterinária e Patologia Cirúrgica. Obteve o título de Médico Veterinário em 2013. Entre os anos de 2014 e 2016, participou do Programa de Residência Integrada em Medicina Veterinária como residente em Clínica Cirúrgica de Pequenos Animais do Hospital Veterinário da Universidade Federal de Campina Grande (UFCG). Ingressou no mestrado em medicina veterinária com ênfase em ortopedia e neurologia veterinária de pequenos animais em 2016 na Universidade Federal Rural do Pernambuco (UFRPE), tendo concluído em 2018. Atualmente é aluno de doutorado do Programa de Pós-Graduação em Cirurgia Veterinária da Universidade Estadual Paulista “Júlio de Mesquita Filho” – Câmpus de Jaboticabal, ao qual ingressou em 2018.

DEDICATÓRIA

Dedico este trabalho as duas pessoas que nunca mediram esforços para que eu chegasse aqui, meus pais.

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CERTIFICADO

Certificamos que o projeto de pesquisa intitulado **"Haste intramedular bloqueada modificada com orifício cranial para tratamento de fraturas dos ossos longos em cães"**, protocolo nº 019201/17, sob a responsabilidade do Prof. Dr. Bruno Watanabe Minto, que envolve a produção, manutenção e/ou utilização de animais pertencentes ao Filo Chordata, subfilo Vertebrata (exceto o homem), para fins de pesquisa científica (ou ensino) - encontra-se de acordo com os preceitos da lei nº 11.794, de 08 de outubro de 2008, no decreto 6.899, de 15 de julho de 2009, e com as normas editadas pelo Conselho Nacional de Controle de Experimentação Animal (CONCEA), e foi aprovado pela COMISSÃO DE ÉTICA NO USO DE ANIMAIS (CEUA), da FACULDADE DE CIÊNCIAS AGRÁRIAS E VETERINÁRIAS, UNESP - CÂMPUS DE JABOTICABAL-SP, em reunião ordinária de 07 de dezembro de 2017.

Vigência do Projeto	01/03/2018 a 01/03/2021
Espécie / Linhagem	*Canis lupus familiaris*
Nº de animais	10
Peso / Idade	20 a 30 kg / 2 a 7 anos
Sexo	Ambos os sexos
Origem	Centro de Controle de Zoonoses

Jaboticabal, 07 de dezembro de 2017.

Prof. Dr. Everlon Cid Rigobelo
Vice Coordenador – CEUA

Faculdade de Ciências Agrárias e Veterinárias
Via de Acesso Prof. Paulo Donato Castellane, s/n - 14884-900 - Jaboticabal - SP - Brasil
tel 16 3209 2600 fax 3202 4275 www.fcav.unesp.br

AVALIAÇÃO BIOMECÂNICA DE UMA NOVA HASTE ORTOGONAL COM ÂNGULO ESTÁVEL EM MODELO DE FÊMUR CANINO

RESUMO - Vários procedimentos cirúrgicos estão disponíveis na medicina veterinária para estabilizar fraturas. Dentre estes têm-se as hastes intramedulares bloqueadas. No entanto, o uso de hastes na medicina veterinária ainda encontra-se bastante limitado devido à pouca disponibilidade de modelos efetivos. Objetivou-se com este estudo a desenvolvimento e avaliação biomecânica de hastes com interligação rígida entre parafusos posicionados de forma ortogonal em sua porção distal. Para os testes biomecânicos utilizaram-se vinte e um modelos ósseos sintéticos confeccionados em PLA, distribuídos em três grupos. Grupo 1 (hastes bloqueadas convencionais), Grupo 2 (hastes bloqueadas de ângulo estável uniplanar) e Grupo 3 (pelas hastes de ângulo estável ortogonais), sendo o último grupo o foco principal do estudo. Todos os grupos foram submetidos aos testes biomecânicos destrutivos com forças de compressão e torção. Não foram observadas diferenças estatísticas significativas em relação à resistência à torção entre os grupos em testes destrutivos. Diferença estatística nos valores de rigidez foi observada nos testes compressivos entre a haste ortogonal e bloqueada convencional (p=0.01) e também entre a haste uniplanar de ângulo estável e bloqueada (p=0.001). A nova haste ortogonal mostrou-se biomecanicamente similar ao modelo de ângulo estável e uniplanar, e superior à haste convencional.
Palavras-chave: caninos, cirurgia, fratura, haste intramedular

BIOMECHANICAL EVALUATION OF A NOVEL ORTHOGONAL ANGLE-STABLE INTERLOCKING NAIL IN A CANINE FEMUR MODEL

ABSTRACT - Various surgical procedures are available in veterinary medicine to stabilize fractures. Among these there are blocked intramedullary nails. However, some complications have been described, such as plastic deformation in screws and in the nail, with the possibility of breaking one or both items, due to the lack of rigid stabilization between the nail orifice and the screw. The present study aimed at making and evaluating rods with rigid interconnection between screws positioned orthogonally in their distal portion. For biomechanical tests, twenty-one synthetic bone models made from PLA were used, distributed in three groups. Group 1 using conventional blocked rods, group two composed of uniplanar stable angle blocked rods and group 3 formed by orthogonal stable angle rods, which are the main focus of the study. All groups, submitted to destructive biomechanical tests with compression and torsional forces. There were no statistically significant differences in relation to torsion resistance between groups in destructive tests. Statistical differences in stiffness values were observed in the compressive tests between the conventional orthogonal and blocked rod ($p = 0.01$) and also between the stable and locked uniplanar rod ($p = 0.001$). The new orthogonal nail proved to be biomechanically similar to the stable and uniplanar angle model, and superior to the conventional nail.
Keywords: canines, fracture, surgery

LISTA DE ABREVIATURAS

FCAV: Faculdade de Ciências Agrárias e Veterinárias

HI: Haste intramedular

mm: milímetro

N: Newton

Nm: Newton metro

PLA: ácido poli-lático

UNESP: Universidade Estadual Paulista

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Introdução

A maioria das fraturas nos cães é estabilizada de forma cirúrgica, utilizando implantes que permitam estabilização rígida, inibindo forças atuantes no foco de fratura (HORSTMAN et al., 2004). Inúmeros tipos são descritos para tal finalidade, tais como placas e parafusos ósseos, fixadores esqueléticos externos e as hastes intramedulares bloqueadas (HIB), desenvolvidas inicialmente para correção de fraturas em humanos, mas sendo adaptadas e introduzidas na Medicina Veterinária no início dos anos 90 (DÉJARDINI, 2012)

As hastes intramedulares bloqueadas são implantes ortopédicos metálicos utilizados para estabilização cirúrgica em fraturas de ossos longos, criadas com o intuito de promover estabilização elástica (BAKI, 2017). As hastes são implantadas no canal medular e fixadas preferencialmente à metáfise óssea por meio de parafusos que transfixam cânulas proximais e distais presentes na haste, sendo fixados nas corticais *cis e trans* do osso (BURNS et al., 2006; WHEELER, 2004).

Complicações estão descritas com o uso de hastes. Entre as principais estão as falhas por quebra ou deformação dos parafusos, (WHEELER, 2004). A ausência de interação rígida do parafuso com as hastes, pode promover excesso de movimento entre os fragmentos, desencadeando atraso na consolidação óssea e até mesmo casos de não união (LANSDOWNE et al., 2007).

Buscando melhores resultados com o uso da haste intramedular, modelos de ângulo estável surgiram. Por meio de ensaios biomecânicos foi possível confirmar maior estabilidade e interação mais rígida entre haste e parafusos em implantes que apresentam algum tipo de bloqueio entre haste e parafusos (DÉJARDINI, 2006, 2009). Uma redução no tempo de consolidação foi descrita com o uso desse modelo (DÉJARDINI, 2012).

Apesar das vantagens com os modelos de ângulo estável estarem provadas e o resultado clínico cirúrgico ser notório, uma série de tipos de fraturas ficam impossibilitadas de serem tratadas com as hastes, principalmente fraturas metafisárias ou supra articulares. Tal dificuldade ocorre devido à disposição dos parafusos nas hastes veterinárias. Fazendo comparativo com a medicina, hastes

com parafusos posicionados de forma divergente permitem a fixação em fraturas distais ou proximais ao osso, sendo capazes de resistir às forças atuantes. Neste contexto, é nítida a carência e necessidade de desenvolvimento de novos modelos ou aprimoramento dos já existentes e disponíveis.

Nessa perspectiva, este projeto buscou confeccionar um modelo de haste intramedular de ângulo estável com parafusos posicionados de forma ortogonal na extremidade da haste. Dessa forma, este estudo buscou contribuir com a prática cirúrgica, favorecendo e ampliando a interação entre pesquisa e prática profissional, com benefício para os cães com fraturas e seus tutores.

Revisão de literatura

1. Fraturas em cães

As fraturas podem ser definidas como uma ruptura parcial ou total do osso. Os pacientes caninos são frequentes no atendimento da clínica-cirúrgica ortopédica e, geralmente, por consequência de acidentes veiculares, quedas de locais altos e projeteis balísticos. Podendo ocorrer também em cães de trabalho por esforço repetitivo. Estas fraturas podem ser tratadas de forma cirúrgica, usando uma variedade de implantes como fixadores esqueléticos, placas e parafusos e haste intramedular bloqueada (DECAMP et al., 2016).

As fraturas que ocorrem em cães correspondem aproximadamente a um terço dos atendimentos, com destaque para as fraturas de ossos longos que correspondem a 45% de todos os tipos de fraturas, sendo a grande maioria decorrente de traumas de alta deformação (LUCAS et al., 2001). Dentre esses ossos, o fêmur representa cerca de 20 a 26% de todas as fraturas que acometem os cães e gatos (BOIANE, 2007; DALLABRIDA, 2004) e as fraturas tibiais representam cerca de 15 a 20% (POPE, 2005). Menos frequentes são fraturas de úmero que correspondem a 8,5% e rádio e ulna representando a 10,7% (BRIANZA et al., 2006).

As fraturas também podem decorrer do enfraquecimento ósseo (patológico ou secundário) e, na grande maioria, ocorrem por consequência de neoplasia, como o osteossarcoma e mieloma múltiplo, mas também podem ser de doenças geradas por osteopenia. Casos de osteomielite ou danos iatrogênicos ocorridos durante o reparo da fratura ou após a remoção do implante podem também predispor a fraturas. Hiperparatireoidismo em cães é um fator que predispõe ao surgimento de fraturas, pois o agravamento da doença causa reabsorção óssea subperiosteal comprometendo ossos longos e predispondo a fraturas. No osso enfraquecido, apenas a baixa energia é suficiente para causar a lesão, com isso geralmente essas fraturas apresentam traços simples (DECAMP et al., 2016).

As fraturas são classificadas quanto à sua exposição ao meio externo, a extensão do dano, deslocamento dos fragmentos ósseos, tipo da fratura, possibilidade de redução ou não e localização (WEERASOORIYA et al., 2016).

A classificação em fechada ou aberta deve ser levada em consideração para determinar o tratamento adequado. As fraturas abertas são classificadas de

acordo com a dimensão da lesão cutânea associada em: Grau I, quando apresenta ferimento menor que 1 cm, com lesão moderada de tecidos moles e em muitos casos o osso não está visível ao meio externo; Grau II, a ferida é maior que 1 cm, sem danos extensos ao tecido mole, com exposição óssea visível; a Grau III é subdividida em IIIa (apresenta lesão extensa de pele com exposição óssea), IIIb (apresenta lesão de pele semelhante a anterior com lesão ao periósteo podendo também ocorrer perda óssea) e IIIc (que apresenta todas lesões anteriores associados a danos vasculares e/ou ao tecido nervoso). VER TOBIAS

Fraturas também podem ser classificadas como completas ou incompletas em função do envolvimento cortical. Uma fratura em galho verde, mais comum em animais imaturos, é uma fratura incompleta na qual uma porção do córtex está intacta, estabilizando o osso em alguma porção. As fraturas classificadas como completas apresentam uma linha de fratura que envolve as duas corticais ósseas (DIRSKO; DECAMP, 2009).

Fraturas completas ainda são classificadas de acordo com sua linha de fratura em redutíveis, que normalmente são decorrentes de traumas de baixa energia e irredutíveis, que estão associadas a traumas de alta energia, como por exemplo acidente automobilístico. Fraturas redutíveis podem apresentar uma linha de fratura perpendicular ao eixo vertical longo do osso, caracterizando uma fratura transversa. Linhas de fraturas oblíquas também são consideradas redutíveis, envolvendo o eixo longitudinal do osso; elas são descritas como fraturas oblíquas curtas quando estão a 45 graus ou menos, ou fraturas oblíquas longas quando estão a mais de 45 graus em relação ao axis ósseo. Fraturas em espiral são similares às fraturas oblíquas longas, mas contornam o eixo longitudinal do osso (NICOL, 2002).

As fraturas irredutíveis são chamadas de cominutivas por apresentarem múltiplos fragmentos, variando desde fraturas com mínimo de três fragmentos como é caso das fraturas chamadas “asa de borboleta”, que apresentam duas linhas de fratura oblíqua formando uma silhueta que lembra o formato da borboleta, até fraturas altamente cominutivas com cinco ou mais fragmentos. Estas normalmente acompanham lesões em tecidos moles, de moderadas a graves, que são determinadas pelas forças de alta energia presentes no momento do trauma (DECAMP et al., 2016).

Um ponto importante na classificação das fraturas é quanto à sua localização. Estas podem ocorrer na diáfise, metáfise, fise ou superfície articular do osso. As fraturas fisárias possuem classificação particular chamada de Salter-Harris que identifica de forma numérica o tipo da fratura. Salter-Harris tipo I ocorrem ao longo de toda a fise, tipo II envolvem a fise e uma porção da metáfise, tipo III envolvem fise e epífise, essas por sua vez geralmente são fraturas articulares, tipo IV também são articulares envolvendo epífise, fise e metáfise, tipo V são lesões por compressão na físe, não visíveis em radiografias e a tipo VI são semelhantes ao tipo V, mas a compressão é parcial (DIRSKO; DECAMP, 2009).

2. Biomecânica das fraturas

O tecido ósseo sofre uma deformação durante a locomoção que varia de 0,04-0,3%, raramente excedendo 0,1%. O osso é um material considerado visco elástico e suas características de tensão-deformação e de resistência dependem da velocidade de deformação aplicada (DALMOLIN et al, 2013).

Uma taxa de deformação óssea pode ser descrita como baixa, como o que acontece durante a locomoção; moderada, que está associada à atividade esportiva extrema, por exemplo, corridas, saltos; e altas, que estão associadas a eventos não fisiológicos, como por exemplo, acidentes automobilísticos de baixa intensidade e quedas de edifícios. Já as consideradas extremamente altas são decorrentes de trauma de alta energia, como exemplo, os casos de ferimento a bala (ZIMMERMANN et al., 2014).

Quando uma força é aplicada ao osso, este se deforma. Se a deformação é baixa, quando removida, o osso reassume posição e conformação original, denominada de “deformação elástica”. Quando a carga é aplicada até o ponto em que não é mais possível reverter sua forma original, têm-se o “ponto de quiescência”. A deformação permanente é denominada “deformação plástica”, finalmente, com a continuidade da aplicação da carga, obtém-se a fratura (DALMOLIN et al, 2013).

Fisiologicamente, ossos e articulações são constantemente submetidos às cargas de flexão, compressão e torção, principalmente durante a locomoção, se mantendo apenas na deformação elástica. Forças inesperadas também

podem afeta-las como acidentes veiculares, quedas, ferimentos por balas, entre outros, saindo da deformação elástica e chegando a deformação plástica. Nesses casos, o trauma pode gerar a fratura do tecido ósseo, levando à perda da função musculoesquelética, como também danos a órgãos internos, predispondo a incapacitação do paciente em realizar certas atividades (ADHARAPURAPU; JIANG; VECCHIO, 2006).

A fratura ocorre quando uma carga particular excede a resistência óssea. Em deformações moderadas essa falha ocorre devido à repetição, já em deformações altas ou extremamente altas a fratura ocorrerá de imediato (PROT; CLOETE; SALETTI; LAPORTE, 2016).

3. Reparação de Fraturas

A maioria das fraturas é reparada sem necessidade de intervenção cirúrgica, entretanto, o osso pode não desenvolver sua função de forma eficaz devido ao mal alinhamento (má união) associado a contraturas musculares e tendíneas. Em fraturas articulares, é possível verificar a presença de quadros mais intensos de osteoartrose, bem como também impotência funcional do membro (GUIOT, DÉJARDIN, 2011).

O comprometimento do suprimento vascular do osso pode variar de acordo com a complexibilidade da fratura e um novo suprimento de sangue extra ósseo advindo em grande parte dos tecidos moles, é usado. Quando ocorre estabilização e formação de calo ósseo esta vascularização regride, permitindo que a vascularização medular continue o processo de consolidação (CIVITELLI, 2008).

As fraturas tratadas cirurgicamente podem consolidar de duas formas. Em fraturas transversas e oblíquas curtas, quando aplicada compressão interfragmentar e nas oblíquas longas e em espiral por meio do uso de parafusos com efeito *lag* ou cerclagem, estas podem desenvolver consolidação primária. Em fraturas cominutivas ou situações que não permitam a redução anatômica, a cicatrização secundária é a opção indicada (BOUDREAU et al, 2012).

A consolidação primária é alcançada pela compressão e estabilização rígida entre os fragmentos da fratura, permitindo apenas uma micro movimentação no foco de até 0,1mm. Neste caso, algumas etapas da fase de

cicatrização secundária não acontecem tendo a proliferação de tecido ósseo, em seguida, indo diretamente ao remodelamento cortical. A união é feita através do remodelamento dos canais haversianos, que ocorre reparo na superfície da fratura e ligeira perda de densidade na zona de solução de continuidade, que pode ser confundida com reabsorção óssea na avaliação radiográfica (GUIOT, DÉJARDIN, 2011).

A cicatrização secundária ocorre em três fases: 1 - Hemorrágica, que é caracterizado pelo extravasamento de sangue na área, formação de coágulos e edema: 2 - Da reparação, caracterizada pela liberação de células pluripotentes decorrentes do endósteo, periósteo e tecidos moles, que são diferenciadas em fibroblastos, condroblastos e osteoblastos, produzindo tecido fibroso, cartilagem e osso, respectivamente. 3 - Fase final de remodelação onde ocorre reabsorção de osso em excesso, e o que fica é lentamente convertido em osso lamelar através da remodelação dos canais haversianos (PORTER; CALVI, 2008).

4. Estabilização de Fraturas

O princípio básico para o tratamento de fraturas é manter os fragmentos principais fixos de forma rígida para que a cicatrização óssea aconteça, permitindo a locomoção do paciente após a estabilização. A integridade do membro, especificamente das articulações, é otimizada quando a musculatura continua a funcionar e o movimento das articulações permanece, mantendo a nutrição das cartilagens (BOLANDER, 1992).

A redução dos fragmentos pode ser complementada com estabilidade absoluta em fraturas que possuam traços simples (transversa, oblíqua curta). Para isto, é necessário que não ocorra micromovimentação superior a 0,1mm. Quando a movimentação no foco da fratura supera esse valor, o estresse no implante pode predispor a sua falha. Em estabilidade absoluta, busca-se reconstruções anatômicas, com alinhamento e aposição perfeitos, técnica indicada também em fraturas articulares, mas nesses casos, sucederá um tempo maior para a cicatrização óssea (SARRAU; MEIGE; AUTEFAGE, 2007).

Uma outra alternativa é o uso de estabilidade relativa, como em fraturas com cominuição. Nessa situação não há transmissão de carga pelo osso, toda a carga será suportada pelo implante. Assim a cicatrização óssea ocorrerá de

forma secundária, com formação de calo ósseo (BARNES et al., 1999). Atualmente, prefere-se a utilização de técnicas cirúrgicas menos invasivas, já que a lesão dos tecidos moles adjacentes é minimizada, preservando o foco de fratura. O implante escolhido deve ser rígido o suficiente para suportar as cargas que atuam no osso, sendo fixado nos fragmentos principais. Este tipo de estabilização é denominado de ponte, onde toda área da fratura é deixada livre e o implante suporta todo o peso e resiste a todas as forças aplicadas ao membro (CABASSU, 2001).

5. Osteossíntese Biológica

Durante a década de 50, a Fundação Internacional de Ortopedia (AO) criou as primeiras regras para o tratamento de fraturas, buscando maior rigidez com a estabilização, baseada em extensas abordagens para alcançar a redução anatômica. O resultado era trauma cirúrgico iatrogênico, incluindo a remoção do hematoma de fratura e a destruição periosteal, o que caracteriza uma técnica de redução aberta, com aplicação de implante interno (ORIF: open reduction internal fixation) (GERBER; MAST; GANZ, 1990). Apesar de apresentar melhores resultados, quando comparado com as técnicas anteriores de osteossíntese, surgiu uma nova série de complicações, incluindo atraso na união ou não união, falha no implante e osteomielites. Algumas dessas complicações estão em grande parte relacionadas à destruição do hematoma de fratura e dos tecidos moles circundantes, que por sua vez prejudica e atrasa a cicatrização óssea (HOHMANN; GLATT; TETSWORTH, 2016).

Devido a estas complicações, verificou-se o surgimento de algumas mudanças, desde uso de fixação rígida absoluta até a criação de um ambiente mais favorável para a cicatrização. No padrão atual, utiliza-se uma reconstrução menos precisa e fixação rígida, afim de reduzir o trauma iatrogênico e favorecer a cicatrização indireta, com formação de calo ósseo, apresentando consolidação mais rápida quando comparado a reconstrução anatômica (GUIOT; DÉJARDIN, 2011).

A osteossíntese biológica tem como objetivos a consolidação secundária com abordagem cirúrgica limitada, com mínimo ou nenhum trauma ao coágulo e foco da fratura. Esses princípios são conseguidos usando técnica de *Minimally*

invasive osteosynthesis (MIO) ou técnicas *open but not touch* (OBNT) (BUCKLEY; MOHANTY; MALISH, 2010).

O sucesso da MIO é a preservação do hematoma da fratura, bem como a manutenção do fornecimento de sangue ao local, sem acesso ao foco da fratura minimizando os riscos de infecções. A preservação do hematoma da fratura e o suprimento sanguíneo local são cruciais para melhorar a formação do calo e remodelação. Esta técnica é a que apresenta melhores resultados no tratamento de fraturas (GUIOT; DÉJARDIN, 2011).

A técnica OBNT é a forma mais simples de osteossíntese biológica. Embora esta técnica envolva uma abordagem aberta para observação direta, o local da fratura não é alterado e o alinhamento ósseo é conseguido através da manipulação dos principais fragmentos de osso, longe do foco da fratura. A distração e o alinhamento dos segmentos são facilitados pelo uso de pinças ósseas e/ou pinos intramedulares, como também haste intramedular bloqueada. No entanto, esta técnica "abra, mas não toque" pode resultar em danos à vascularização e outros tecidos moles, o que não acontece na MIO (BAUMGAERTEL; GOTZEN, 1994).

Com a MIO, a exposição aberta da fratura não é realizada e apenas pequenas incisões distantes do local de fratura são usadas para se conseguir a redução e fixação fechadas. Essa técnica permite a preservação do ambiente biológico para a cicatrização da fratura ocorrer de forma mais eficaz (DÉJARDIN; MARTURELLO GUIOT, 2016). No entanto, a falta de observação direta dos fragmentos da fratura e a implementação adequada de técnicas de osteossíntese minimamente invasivas estão associadas a uma curva de aprendizado maior. A presença de grande massa muscular envolvendo alguns ossos pode tornar a redução fechada particularmente desafiadora; nesses casos radiografias pré-operatórias do osso contralateral podem ser usadas como referência durante a cirurgia, evitando complicações que possam comprometer o resultado cirúrgico. Um meio mais eficaz é o uso de fluoroscopia intra-operatória sendo recomendada para obter a redução da fratura (alinhamento dos membros e aposição do fragmento da fratura), bem como para garantir o posicionamento adequado dos implantes (BISWAS et al., 2008; GUIOT; DÉJARDIN, 2012).

Para a execução das técnicas de MIO, os implantes mais indicados são os fixadores esqueléticos externos, as hastes intramedulares e as placas e parafusos. O uso de placas tem como desvantagem prejudicar o coágulo da fratura e o periósteo. Os implantes que minimizam esse trauma são os fixadores esqueléticos externos e as hastes bloqueadas, no entanto, os fixadores possuem rigidez inferior quando comparados a essas últimas (MOSES et al., 2002).

6. Haste Intramedular (HI)

História e modelos das HI

Em 1940 o alemão Gerhard Kuntscher, desenvolveu um implante para o tratamento de fraturas em humanos, sendo este precursor para o desenvolvimento das atuais HI (WEELER et al., 2004). O primeiro modelo de HI foi desenvolvido na Austrália, pelo médico Huckstep em 1970, tomando popularidade e se consolidando como um dos principais implantes para fixação de fraturas de ossos longos em humanos (DÉJARDINI, 2012).

O primeiro relato de uso da HI em cão foi apresentado por Johnson e Huckstep em 1986. Em seguida, diferentes modelos confeccionados para uso veterinário surgiram na década de 90. Muir (1993,1995); Durall (1996) e Duhautois (1996) foram alguns dos primeiros a relatar e utilizar modelos de HIB para o tratamento de fraturas em cães.

No Brasil, o modelo de fabricação inteiramente nacional ficou conhecido como Sistema Popak, em 2006. O principal diferencial deste modelo foi a modificação do segmento distal, o qual apresentava-se chanfrado para melhor adaptação à curvatura do fêmur canino (GIORDANO et al., 2006).

Muitos modelos de HI estão disponíveis em todo o mundo. Entre eles, os mais utilizados são fabricados nos Estados Unidos (DUELAND, 1997; DÉJARDIN, 2006; 2009; 2012).

A maioria das HI fabricadas para medicina veterinária é de aço inoxidável 316 L (DÉJARDIN, 2009), entretanto, os modelos de titânio foram desenvolvidos para animais de pequeno porte (SCOTTI et al., 2007). Esses implantes de aço estão normalmente disponíveis nos diâmetros de 4 a 4.7mm, com comprimento variando de 68 – 112mm, sendo para fraturas em felinos ou cães de porte pequeno. Hastes de 6 e 8mm em aço inoxidável com comprimento variando de

120 – 230mm são usadas para fraturas em cães de médio e grande porte. Hastes de 10mm estão disponíveis, porém de forma limitada, usadas para cães de grande porte. Possuem de dois a quatro orifícios localizados nas extremidades proximal e distal do implante, com uma distância entre orifícios variando de 11 a 22 mm, através dos quais são aplicados parafusos promovendo o bloqueio da haste ao osso (SCOTTI et al., 2007).

Um modelo de haste mais recente, desenvolvido para uso em felinos e cães de pequeno porte foi criado. Este apresenta algumas diferenças em relação aos modelos convencionais. Sua fixação é feita através de dois parafusos, um proximal e outro mais distal, sendo que ambos possuem um furo em seu centro, por onde passa um pino que é travado aos parafusos por uma bucha (MACEDO, 2017).

Componentes para aplicação

Para aplicação da haste, faz-se necessário um instrumental cirúrgico específico, tendo como itens básicos uma régua guia, introdutor de haste, guias de broca e medidor de profundidade adaptado. Pequenas diferenças ocorrem entre fabricantes (WEELER et al., 2004).

Os instrumentos necessários para a correta aplicação de uma haste bloqueada são disponibilizados pelo fabricante de cada sistema. Embora pequenas diferenças possam ocorrer entre um fabricante e outro, de uma forma geral, os instrumentos usados para a introdução e fixação de uma haste, são semelhantes. Os itens gerais para tal finalidade são: um cabo que é fixado a haste antes de sua passagem ao canal medular e régua de perfuração, que é fixada ao cabo após a introdução da haste, onde são colocados guias de perfuração através de orifícios presentes na régua que indicam de forma precisa o local exato da perfuração óssea para cada comprimento do implante. Os demais itens são semelhantes aos usados para osteossíntese com placas (DÉJARDIN et al., 2012).

Biomecânica das HI

As HI passaram a ser uma alternativa atraente para estabilização de fraturas em pacientes humanos e veterinários, pois mostram bons resultados ao serem aplicadas seguindo princípios minimamente invasivos (DÉJARDINI et al., 2014).

Diversas vantagens biomecânicas estão presentes ao comparar HIB com placas ósseas. As HI são colocadas no eixo central ósseo, conferindo suporte de peso direto, nas cargas de compressão, no entanto, placas são expostas a flexão durante aplicação desta força, pois são colocadas de forma excêntrica em relação ao eixo neutro do osso. (BERNARDE et al., 2001; BERNARDE et al., 2002; HULSE et al., 1997; HULSE et al., 2000; REEMS et al., 2003). Em fraturas cominutivas, a posição da HI permite melhor resistência às cargas de flexão, por se encontrar no eixo ósseo central. Os parafusos aplicados auxiliam, pois inibem as forças de torção e compressão (BERNARDE et al., 2001; BERNARDE et al., 2002; DÉJARDINI et al., 2006; BURNS et al., 2011).

Os furos presentes nas hastes atuam como concentradores de tensão. Este fato se dá quando os parafusos não interagem, de forma rígida, com a haste e assim não reduzem as tensões locais (DUELAND et al., 1996; 1997; DÉJARDINI et al., 2012)

Em uma fratura óssea a maior parte do suprimento sanguíneo ofertado para o foco fraturado ocorre de forma extra óssea (AGUILA et al., 2004). O uso de HIB através de abordagem limitada, aplicada de forma normógrada preserva o suprimento sanguíneo extra ósseo e causa pouco trauma aos tecidos moles, reduzindo assim a morbidade pós-operatória, promovendo a cura precoce. O uso de placas, mesmo de forma minimamente invasiva, é menos efetivo quanto a essa preservação devido a necessidade de alguma manipulação dos tecidos moles, afim de posicionar e fixar a placa ao osso (LANSDOWNE et al., 2007; MUIR; JOHNSON, 1995).

As primeiras gerações de HIB possuíam orifícios maiores que as atuais, o que tinha como consequência, a quebra da haste. Buscando diminuir esta complicação, diminuiu-se o tamanho desse orifício, passando a receber parafusos de menor diâmetro. Dessa forma, hastes de 6mm possuem orifícios para parafusos 2.7mm e hastes de 8mm possuem parafusos de 3.5mm. Esta mudança predispôs à falha dos parafusos, como quebra ou envergamento, devido a carga de compressão que são suportadas pelos mesmos, onde um parafuso de 3.5mm apresenta um centro medindo 2.5mm, havendo um espaço entre o orifício da haste e o parafuso. Com isso, há movimentação, deformação da rosca e, consequentemente, falha do parafuso (DÉJARDINI et al., 2012; DUELANDE et al. 1996; 1997).

O desenvolvimento de HIB com ângulo estável (possuindo bloqueio eficiente entre a haste e o parafuso) obteve resultados satisfatórios em testes biomecânicos com maior inibição das forças de flexão e torção sem apresentar folga na interface do orifício com parafuso (DÉJARDINI et al., 2006; 2009). A modificação no formato do implante, com uma diminuição de 25% no segmento central da haste (modelo ampulheta), proporcionou aumento da elasticidade do implante e da velocidade na consolidação óssea, sendo visível na 10ª semana de pós-operatório (DÉJARDINI et al., 2006; 2009; 2012; 2014).

Um ponto importante que deve ser levado em consideração, quanto às características biomecânicas das hastes é o momento de inércia deste implante, que caracteriza a capacidade que o implante tem para resistir a força de flexão. O momento de inércia é dado pelo raio da secção transversal do implante, quanto maior a massa do implante maior o seu momento de inércia. Quando se compara as hastes, com placas em ponte e placas associadas a pinos intramedulares, as hastes possuem um raio maior, com isso possuem uma maior resistência. Essa informação é importante quando decide usar a HIB em função ponte, pois as cargas aplicadas ao membro serão suportadas com maior resistência ao dobramento, se compararmos as placas ou no uso de placas associadas a pino intramedular (CHAO et al., 2012).

Indicações e técnicas de aplicação das HIB

As HIB são projetadas para o tratamento de fraturas diafisárias de úmero, tíbia e fêmur. Podendo ser utilizadas para fraturas cominutas ou de simples redução (BRUMBACK, 1996) e também para tratamento de fraturas metafisárias (SCOTTI et al., 2007). Elas são indicadas no tratamento de fraturas fechadas ou fraturas expostas, com resultados satisfatórios (LARIN et al., 2001). Outra indicação do uso de HIB ocorre em fraturas proximais de ulna nos cães de grande e gigante porte (GATINEAU; PLANTÉ, 2010).

A haste deve ocupar de 70% - 90% da porção mais estreita do canal medular, com uma distância mínima de 1cm dos parafusos mais centrais à linha de fratura. Esses implantes permitem a sua aplicação, muitas vezes, de forma fechada, sendo necessário em alguns casos a abertura e exposição do foco fraturado (WEELER et al., 2004).

Para Déjardini, (2012), a aplicação correta dessas técnicas, muitas vezes se faz necessário imagens de fluoroscopia, permitindo ao cirurgião condições precisas da correta aplicação do implante e de seus parafusos. As HIB são mais fáceis de aplicar em técnicas MIO, quando comparadas as placas e tem como vantagem a possibilidade de redução da fratura durante sua passagem no canal medular, causando efeito de ligamentotaxia (AGUILA et al., 2004).

A aplicação de HIB no fêmur é feita com paciente em decúbito lateral. Uma incisão crânio lateral ao trocanter e côndilo lateral femoral é realizada para se aplicar as pinças de fixação óssea para realizar a técnica MIO, no entanto, é necessário estender a incisão centralmente quando necessário (WEELER et al., 2004). A introdução normógrada da haste é feita pela fossa intertrocantérica (LARIN et al., 2001). Em fraturas cominutivas, que não permitem um alinhamento anatômico é de extrema importância um alinhamento correto do osso (MOSES et al., 2002).

A tíbia é o melhor osso para a execução de técnicas MIO, devido a pouca cobertura muscular em sua face medial. O animal disposto em decúbito dorsal com o membro apoiado sobre uma mesa de Mayo estará em uma boa posição para execução dessa técnica (AGUILA et al., 2004). Uma incisão medial dorsal é feita buscando acesso à face medial do ligamento patelar. Uma perfuração com broca deve ser feita na face medial dorsal da tibial com angulação de cerca de 20º para medial e caudal, buscando acesso a cavidade medular (AGUILA et al., 2004).

Pacientes com fratura em úmero devem ser posicionados em decúbito lateral com o membro fraturado para cima, com as incisões cutâneas na face lateral. A perfuração normógrada é feita no tubérculo maior. Alguns cuidados devem ser tomados para se evitar trauma ao nervo radial (MOSES et al., 2002).
Resultados clínicos e possíveis complicações das HIB

O tratamento de fraturas utilizando HIB, vem tendo altas taxas de sucesso, com uma média variando de 83% a 96% (LARIN et al., 2001; MOSES et al., 2002). Resultados parecidos vêm acontecendo no tratamento de fraturas na medicina humana. O tempo mínimo de consolidação com a utilização de hastes varia em torno de seis semanas, ao passo que no tratamento de fraturas com placas ósseas dura cerca de oito semanas (AGUILA et al., 2005).

Um menor tempo de consolidação vem ocorrendo também com os implantes mais recentes, como é o caso dos resultados obtidos com uso da haste com ângulo estável, onde parafusos cônicos com rosca são usados no lugar de parafusos convencionais, promovendo melhor bloqueio da haste (DÉJARDINI, 2012).

A maioria das complicações relatadas com a utilização de hastes está relacionada com decisões erradas. A literatura aponta uma média de 4% a 23% de falha de consolidação, a depender do estudo e do tipo de implante utilizado. As complicações que ocorrem durante a aplicação incluem: flexão ou quebra de broca, haste ou parafuso, fratura do osso durante perfuração com broca e fratura/fissura óssea próximo ao parafuso; osteomielite, lesão do nervo ciático ou radial, contratura do músculo quadríceps, formação de granulomas na ponta da haste, um "efeito limpa para-brisas" na cavidade medular em torno da ponta da haste distal devido ao movimento do implante, dor no joelho devido a parafusos excessivamente longos ou sequestro ósseo; infecção da ferida superficial, seromas e falhas no posicionar do bloqueio no furo mais distal, que podem resultar em prematura dinamização, instabilidade e atraso da união ou não união (DUHAUTOIS, 1996, 2003).

Objetivos

1. Objetivo geral

Testar mecanicamente um novo modelo de haste intramedular de ângulo estável com interação rígida entre parafuso, haste e corticais ósseas, com a presença do orifício mais distal posicionado a 90 graus em relação aos demais, com a utilização de corpos de provas sintéticos, por meio de estabilização relativa.

2. Objetivos específicos

• Avaliar a estabilidade do modelo proposto, em testes biomecânicos, em cargas de torção e compressão;
• Comparar, em testes biomecânicos, um modelo com parafusos de ângulo

estável posicionados paralelos e outro modelo a 90 graus entre eles.

• Constatar possíveis complicações com a utilização de cada implante.

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CAPÍTULO 2 - - Biomechanical evaluation of a novel orthogonal angle-stable interlocking nail in a canine femur model

Normas do periódico “Plos One” (Anexo 1)

Abstract

Interlockings nails (IN) are orthopedic implants with superior mechanical and, potentially, biological qualities. Despite the countless and indisputable advantages of current angle-stable models, there are still limitations for their use in certain scenarios. The objective of the present study was to describe and biomechanically test a new orthogonal angle-stable intramedullary nail model for veterinary use. The proposed orthogonal angle-stable nail has two 3.8-mm threaded cylindrical holes in each of its portions: in the proximal portion, the holes are 11 mm apart; in the distal portion, the penultimate orifice is positioned at 90 degrees in relation to the last one, with a distance of 5.5 mm between them. The novel orthogonal nail (Group 3 – G3) was evaluated and compared biomechanically with the conventional interlocking nail (Group 1 – G1) and the uniplanar angle-stable nail (Group 2 – G2) by means of destructive torsion and axial compression tests. No statistically significant differences were observed in torsion resistance between the groups in the destructive tests. However, statistical diferences were found in stiffness values in the compression tests between the orthogonal (G3) and conventional interlocking (G1) nails ($p=0.01$) and also between the uniplanar (G2) and interlocking (G1) nails ($p=0.001$). The new orthogonal nail proved to be biomechanically similar to the uniplanar angle-stable model and superior to the conventional nail. This new arrangement of interlocking screws (orthogonal and closer to each other) potentially enables the fixation of small fragments and at the extremities of long bones in dogs. Nonetheless, further clinical studies are necessary to validate such hypotheses.

Keywords: dog, internal fixation, fracture, orthogonal, treatment

Introduction

A wide range of orthopedic implants are available for fixing long bone fractures in companion animals, and many of them show adequate results as long as they have been applied correctly and following the mechanical and biological principles for decision making and approach [1]. Among them, the intramedullary nail (IN) can be highlighted, which stands out mechanically because it is applied to the neutral axis of force (medular canal of the bone) and presents significant resistance to flexion [2]. Due to its potentially minimally invasive application, the method also presents relevant advantages from a biological point of view [3].

INs are considered the implant of choice in many types of fractures in humans [4,5] and they have been progressively gaining space in medicine of small animals [1, 3], especially in the fixation of diaphyseal fractures of the femur, tibia, and humerus [6]. In spite of the qualities and excellent results reported with the use of IINs in dogs and cats [7], some limitations have been found over time, leading to several modifications and improvements since the first description made by Küntscher in 1940 [8] and in models designed and used in subsequent decades [1, 3, 9 - 11].

The initially described defect of this model was related to the slack between the nail orifice and the interlocking screws when submitted to compression and torsional force [12, 13]. The lack of a rigid interaction between conventional screws and the holes in the nails creates an unstable environment, predisposing the occurrence of complications [1]. In an attempt to mitigate such risk, systems with rigid interactions between the nail orifice and the screw (angle-stable nail) were developed, which present greater stiffness and resistance than conventional models [1, 10, 11, 14].

Despite the countless and indisputable advantages of the available angle-stable models and their use in juxta-articular fractures [1, 3], there are still some limitations for their use in specific clinical settings or in animals that are too heavy [15]. Improvements in this sense have been observed in IIN systems for use in humans, with an emphasis in multiplanar interlocking nails [16 – 18]. However, these systems remain unavailable or have not been sufficiently tested for clinical application in dogs, cats, and other animals. Orthopedic implants applied in multiple planes are used in challenging scenarios, such as, for example, patients who are considerably heavy or robust, large animals, wild animals, and in

metaphyseal or epiphyseal fractures with fragments that are too small [19,20]. The main advantages of this configuration are correlated with increases in the área moment of inertia and the consequent marked expansion of the system's mechanical resistance, in addition to the potential capacity for fixing fragments to bone extremities with limited area for fixation [21, 22]. Multiplanar external skeletal fixators and double or triple plates are commonly used for these purposes, with excellent results [23]. However, interlocking intramedullary nails for veterinary use, despite some reports [3], were not designed specifically for this function. Improved nail systems for humans present significant variations in the arrangement of interlocking screws: orthogonal, multiplanar, or in a varied angle [24, 25], which can be used on different fractures.

Inspired by the existence of a gap in the arsenal of options for fixing these fractures in veterinary patients, the objective of this study was to describe and biomechanically test a new orthogonal angle-stable interlocking nail model for veterinary use. It is hypothetically believed that this implant is feasible for application in canine bones and is potentially superior regarding mechanical resistance, thus rendering it capable of minimizing interfragmentary movements.

Material and Methods

All protocols were approved by the Animal Care and Use Committee of the Júlio Mesquita Filho State University (UNESP).

Substitute bone models

Substitute bone models were designed using a CAD program (SOLIDWORKS ®2016) to replace and standardize the cortical bone to be used as a test specimen. The bone substitutes were manufactured with polylactic acid (PLA) and had specific dimensions to simulate proximal and distal fracture fragments (19 mm external diameter, 5-mm thick corticals, 9 mm medullary canal diameter, and 98 mm of total length of each segment). They also had pre-defined holes for fixing the screws and a square base measuring 2.5mm (20 mm X 20 mm X 20 mm) for fixation to the mechanical testing machines. The spacing between the proximal and distal fragments was 25 mm in order to simulate the gap of a comminuted fracture. The substitute bone models were designed in two

parts: one proximal and one distal. Each group had a specific model (Figure 1). After developing the synthetic models with the CAD program, they were saved in STL format and printed three-dimensionally (3D) in polylactic acid (PLA).

Figure 1: Substitute models of three-dimensionally printed bones with predetermined orifices for the passage of screws. Figure (A) illustrates the proximal model (test specimen) that mimicked the proximal bone fragment (Groups 1, 2, and 3). The model (specimen) shown in Figure (B) was used to mimic the distal bone fragment in Groups 1 and 2. Figure (C) represents the model used in the distal base of Group 3. Note the holes positioned at 90-degree angles from each other. All models had a square base to facilitate their attachment to the universal testing machines.

Nail models

The nails used in the three studied groups were made of 316L solid stainless steel and measured 8 mm in external diameter and 175 mm in length. The angle-stable nails (Groups 2 and 3) had two threaded cylindrical holes, measuring 3.8 mm in diameter (3.2 mm diameter core) in each portion (proximal and distal). The nails in Group 1 (Control Group), on the other hand, had cylindrical (smooth) holes measuring 3.5 mm in diameter and a 3.0 mm diameter core). The two proximal holes in the nails of all the analyzed groups were 11 mm apart. The distal holes in Groups 1 and 2 were also 11 mm apart (Figure 2A). The orthogonal angle-stable nails (Group 3) presented modifications in the first hole of the distal portion, *i.e*., it was positioned at 90 degrees in relation to the others (two orifices in the proximal portion and the distal orifice in the distal portion); the distance between the two distal holes was 5.5 mm (Figure 2B).

Figure 2: Designs of the angle-stable nails used in the study, measuring a total length of 175 mm and a uniform diameter of 8 mm. Uniplanar nail with 3.8 mm proximal and distal holes distanced 11 mm apart (A). Orthogonal model nail developed for the study, with distal holes measuring 5.5 mm apart and an upper orifice positioned at 90 degrees in relation to the others (B).

In order to perform the mechanical tests, the nails were locked to the models (synthetic test specimens) that mimicked the bone fragments. For the accurate and consistent placement of the locking implants, two pilot holes were made in all the proximal (Groups 1, 2, and 3) and distal (Groups 1 and 2) test specimens, with their centers separated from each other by exactly 11 mm, to coincide with the uniplanar nail holes. Meanwhile, in the distal specimens of Group 3, the two pilot holes (orthogonal) were 5.5 mm apart. The orifices in the synthetic specimens of Groups 2 and 3 measured 2.5 mm in diameter and were previously countersunk (3.2 mm in diameter) regarding the central portion (solid-core) of the screw. Each studied group included 14 test specimens.

Group configurations

A total of 42 test specimens (comprised of proximal and distal portions, mimicking fractured bone fragments) were used in this study. Each of the three groups had 14 sets of specimens, stabilized with specific metallic implants (intramedullary nails and interlocking implants), denominated constructs. The 14 constructs of each group were divided equally (n=7) to be used in the torsion and axial compression tests. Four bicortical interlocking implants (screws) were used in each construct, two proximally and two distally. All screws were obligatorily fixed to the two corticals of the test specimens. In Group 1, a conventional intramedullary nail was used, *i.e*., without locking between the hole and the screw. In this group, the used cortical type screws measured 3.5 mm in diameter and were arranged on a single plane. In Group 2 (G2), the angle-stable nail model (threaded) was used, as well as uniplanar locking, with screws with an external diameter of 3.8 mm. Finally, in Group 3 (G3), the screws had an outer diameter of 3.8 mm and were arranged on the same plane in the proximal portion and orthogonally in the distal portion of the specimen.

Biomechanical test

The biomechanical properties of the constructs (synthetic specimens-nail) were evaluated by means of destructive axial compression and torsion tests. The obtained data were recorded in Newtons (N) in the axial compression assessment and Newtons-meter (Nm) in the torsion test.

For the compression tests, a universal testing machine (UTM – model DL 10000) was used, with a standardized displacement of 0.05 mm per second. The tested constructs were positioned in the upright position so that the machine could exert an eccentric force of compression in relation to the specimen, simulating the main load applied to appendicular bones during locomotion (Figure 3).

Figure 3: Photographic image of the construct positioned vertically within the servo-hydraulic testing machine. The solid block on the distal portion of the specimen was fixed to a specific device of the testing machine, remaining immobile. The load cell exerted force at the base of the proximal portion of the test specimen. When the test started, the proximal portion of the specimen was pressed towards its distal portion.

The torsion tests were carried out using the Instron torsion testing machine (model 55MT), which consisted of two pulleys that enabled the fixation of the constructs in horizontal position. One side of the construct was maintained stable (stationary), while the other side (proximal portion) was subjected to torsion. The three groups were loaded until failure, with a displacement rate of one degree per second.

Figure 4: Photographic image of the construct coupled to the servo-hydraulic testing machine, which was attached by two pulleys to the square base of the synthetic bone model. When the test started, the pulley located on the right in the picture was responsible for promoting rotation.

Statistical analysis

All statistical procedures were conducted with the SPSS software, version 20.0, for Windows. The analysis of the normal distribution of data was performed using the Shapiro-Wilk test, which resulted in non-parametric distribution. Three variables were evaluated in this study: the peak torque when torsion was carried out, stiffness, and the maximum force registered during the compression tests. All variables were analyzed using the Kruskal-Wallis test, in addition to Dunn’s

post-hoc test, performed in order to establish the p-value between the G1, G2, and G3 nail groups. A 5% ($p<0.05$) level of significance was adopted in all the statistical analyses.

Results

Among the three groups, G2 obtained the highest maximum torque value (maximum force of 29.47 Nm), as well as the highest mean (23.39 Nm), in the torsion test. During the assessment, the specimens of this group exhibited plastic deformation when reaching the maximum load. Since it was impossible to detect deformation or breakage of the screws or nails, the failure occurred in the synthetic specimens themselves. In G3, the maximum recorded force was 27.16 Nm, with a mean of 21.76 Nm. Similar to G2, the plastic deformation occurred only in the synthetic specimens, without macroscopic alterations in the screws or nails. As for G1, the maximum and mean force values of this group were 24.72 Nm and 21.27 Nm, respectively, representing the lowest values found in the three groups. After the end of the test, it was possible to observe plastic deformations in the screws in that group. As in the other groups, the test was also interrupted when the synthetic specimen displayed failure.

In the compression test, two factors were evaluated: the stiffness of the constructs and the maximum force they sustained. The maximum and mean resistance observed in G1 corresponded to 12,128 N and 11,254 N, respectively. In G2, the maximum and mean resistance values were 12,944 N and 11,648 N, respectively. G3 obtained the highest values of maximum force, reaching 13,053 N in maximum resistance.

The stiffness values found in G1 were 1,873 N of mean stiffness and a standard deviation of 153.1 N. In G2, a mean stiffness of 2,522 N was observed, as well as a standard deviation of 153.1 N. Meanwhile, in G3, the mean value found was 2,466 N, with a standard deviation of 95.94 N.

At the end of the compression tests, all constructs underwent macroscopic evaluation to identify possible plastic deformations in the screws. In G1, all constructs showed evident screw deformation, and in some specimens, the deformation was such that the ends of the screws (head and tip) entered the medullary cavity of the synthetic specimens. In G2, all specimens underwent

plastic deformation in the distal screws, although to a lesser extent when compared to G1. The same alteration in G2 was observed in three specimens from G3. One of the constructs exhibited nail deformation (flexion), although the screws did not show any apparent alterations.

According to the obtained results, it can be noted that only the stiffness values were statistically significant (Kruskal-Wallis p-value of $p<0.001$). Dunn's *post-hoc* test revealed a significant difference between the orthogonal and interlocking nail ($p=0.01$) and also between the uniplanar and interlocking nail ($p=0.001$). The other analyses were not statistically different; their p-values were: peak torque ($p=0.339$) and maximum force ($p=0.739$) (Figure 5).

Figure 5: Image showing the graphs in Boxplot format. The mean and median are represented by the plus symbol (+) and the line inside the boxes, respectively. Lines above the boxes indicate the maximum values, while those below them indicate the minimum values.

Discussion

A vast arsenal of techniques and implants are currently available to veterinary surgeons, allowing them to make more effective and appropriate decisions in various scenarios. Despite this reality, certain fractures remain major challenges, and the complication rates are higher than expected [26]. Periarticular fractures, with tiny fragments, or those in patients who are too heavy or large, are noteworthy since they still challenge surgeons and the most modern implants [27]. The present study demonstrated the mechanical characteristics of a orthogonal angle-stable interlocking intramedullary nail implant (Group ASO) and compared it with models classically used in veterinary patients. The initial hypothesis that it could exhibit good mechanical behavior and be superior to the tested models was not entirely confirmed, given that the new device significantly increases the stiffness of the construct under axial load. However, it was not significantly superior to Group AS in the axial test. In addition, the change in the positioning of one of the bolts, which created a right angle between the two interlocking implants in the distal specimen, combined with the more distal location of these two implants on the nail, significantly increased the stiffness of the construct under axial load and potentially allowed its fixation to fragments closer to the adjacent joint in a hypothetical clinical scenario. The hypothesis still

needs to be evaluated in more studies because it presents resistance to supraphysiological loads, which will not be used in vivo.

The torsion tests applied to the constructs in Groups AS and ASO were interrupted at the time of failure of the synthetic specimens, with a load of 2,466 Nm and 2,522 Nm, respectively. In contrast, some sets from IC exhibited plastic deformation of the screws before specimen failure and with less load (1,873 Nm), indicating this group's inferiority in relation to the others regarding resistance to the applied torsional loads, despite their being undoubtedly supraphysiological [28]. The differences between the internal diameters of the screws/bolts used in Groups AS and ASO (3.2 mm) compared to Group 1 (2.4 mm) infer a marked increase in the area moment of inertia (AMI) of the screws, justifying such an event [28]. Plenert [29] demonstrated greater resistance of the sets formed by nails interlocked with screws of increased diameter in compression and torsion tests. Despite the methodological differences, both studies used supraphysiological loads, which does not invalidate the sets' ability to be resistant enough in a hypothetical clinical scenario.

In the absence of statistically significant differences between the maximum loads in the destructive mechanical torsion test between Groups 2 and 3, the initial hypothesis stated in the introduction of this study that the orthogonal nail would be superior also in the torsion tests was annulled. It has already been proven that angle-stable models have superior resistance in mechanical tests when compared to traditional models since they promote greater stability [10, 11], a fact that, once again, was made evident in our study. However, despite the orthogonal model having shown lower values than the uniplanar model, these values are representative.

The addition of oblique screws significantly increases the mechanical stability of constructs with intramedullary nails [16]. The cross-sectional geometry of oblique screws also adds stiffness to the construct, opposing torsional movement symmetrically around the longitudinal axis [28]. The authors of the present study suggest that future cyclic tests with this nail model will help to better understand the mechanical behavior of this type of implant.

Orthogonal fixation is often used in other fixation methods, such as external skeletal fixators [30]. Pins positioned at 60 or 90 degrees of angulation enable an increase in resistance, thus improving stiffness against torsional

deformation [31]. Favorable mechanical and clinical results with the use of circular fixators using orthogonal pins have brought popularity to this stabilization method that is widely used in veterinary medicine to correct several types of fractures [32, 33]. In the same way that satisfactory results have emerged with the use of orthogonal fixators, the authors of the present study believe that they may be similar to orthogonal intramedullary nails.

A material's stiffness is directly influenced by the load volume necessary to promote its deformation [28]. The stiffness recorded in the axial compression test in the orthogonal nail group was superior to the other groups and statistically relevant in relation to the interlocking nails (IC) ($p = 0.01$). Even though there were no statistically significant differences between the groups of angle-stable intramedullary nails, the values were numerically higher in ASO. Implants positioned to allow the orthogonal placement of their screws have proven to be mechanically superior in other studies involving several types of implants [19, 20]. This fact may have been responsible for the differences found between the AS and ASO groups. In the study by Baseri [34] where the authors compared the use of angle-stable nails and locking plates in the treatment of distal tibial fractures in humans, the authors stated that nails are mechanically more efficient than locking plates; however, the use of uniplanar nails requires the transverse fixation of Poller screws to avoid shear. Since orthogonal nails used to stabilize proximal or distal metaphyseal fractures in humans promote fixation in multiple planes, this method has shown to be superior to other models [18]. Their surgical applicability confirms the results obtained in mechanical tests, and minimizes complications with the use of fixed uniplanar mid-lateral screws that can result in nail translation, causing misalignment in bone valgus or varus [18].

Although the initial hypothesis that the orthogonal angle-stable nail model would improve mechanical performance has not yet been statistically proven, we can state that the new nail model represents a construct capable of resisting supraphysiological loads. This study evaluated three models, where two load cases were applied, one in torsion and the other one in axial load. The load was applied in one cycle only until failure of the models was detected. However, it was no information obtained, how the models would have performed, when cyclic loading would be applied.

Limitations

The present study performed a purely mechanical controlled ex vivo test model of destructive testing. Future ex vivo studies of cyclic assays will be critical to assess fatigue-induced implant failure.

Acknowledgment

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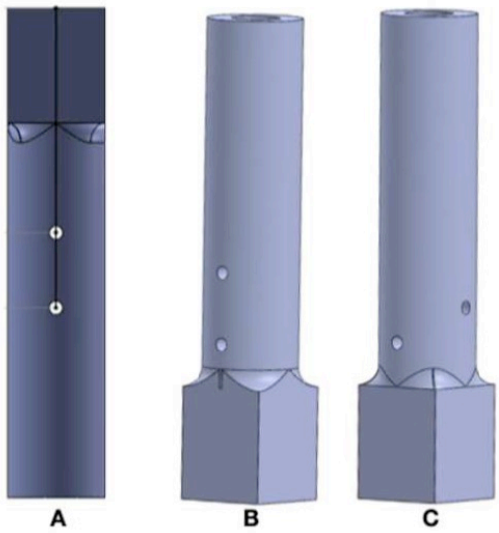


Fig. 1

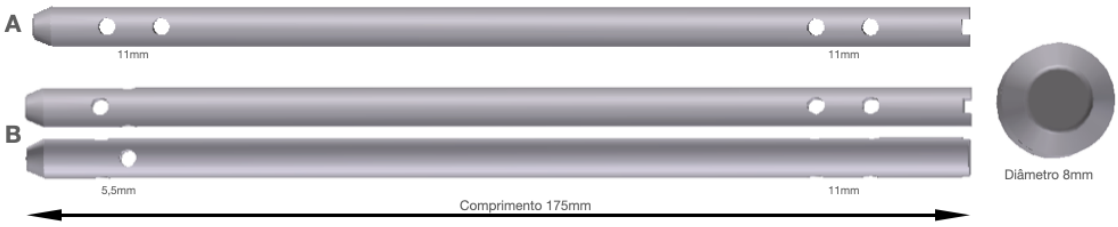


Fig. 2

Fig. 3

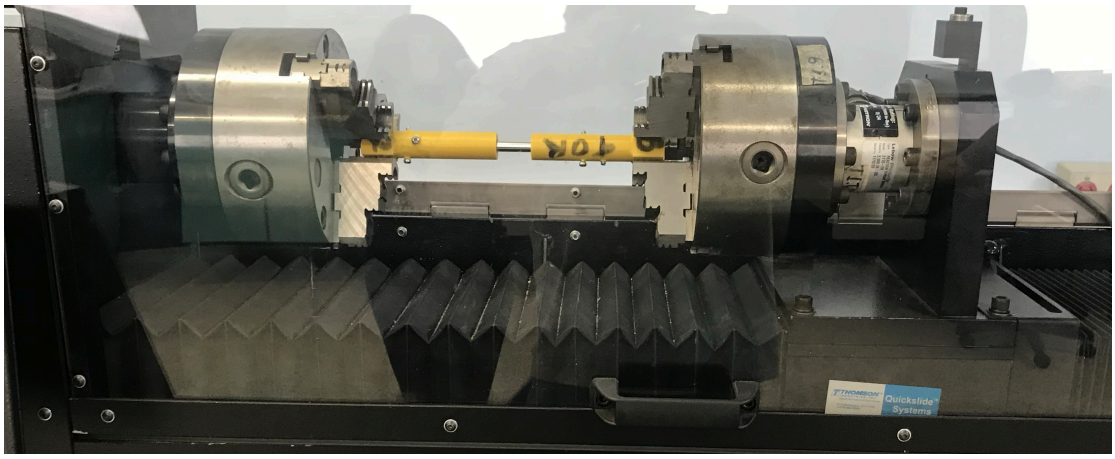
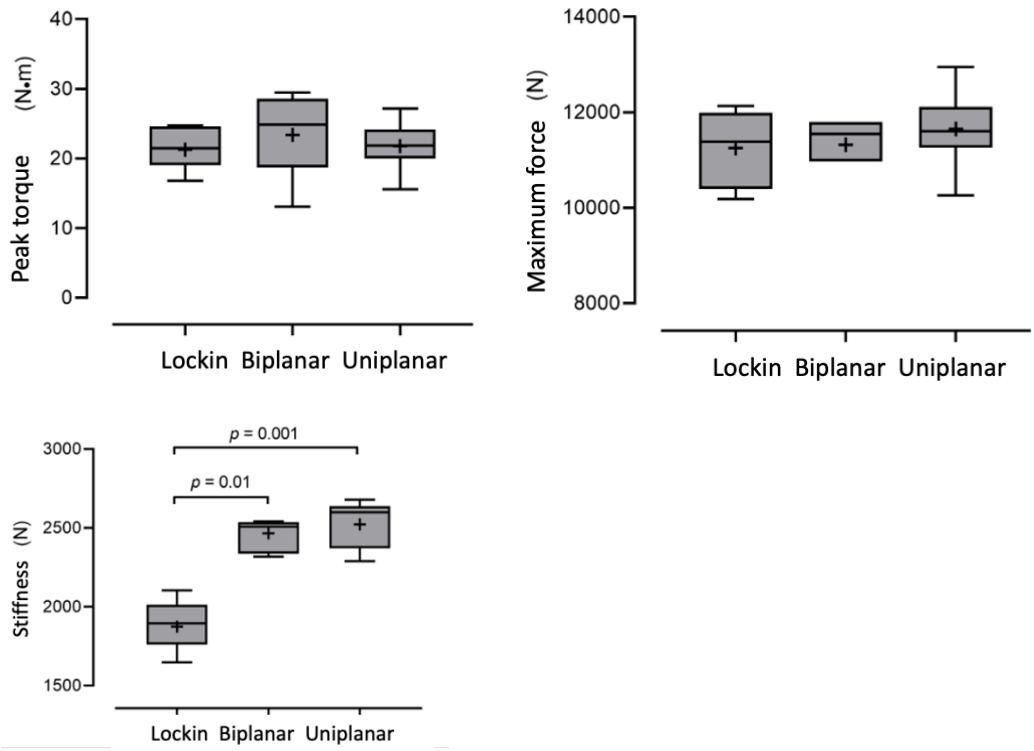


Fig. 4


Peak torque (N•m)
40
30
20
10
0
Lockin Biplanar Uniplanar
Maximum force (N)
14000
12000
10000
8000
Lockin Biplanar Uniplanar

p = 0.001
p = 0.01
Stiffness (N)
3000
2500
2000
1500
Lockin Biplanar Uniplanar

Fig. 5

ANEXO 1 – Normas do periódico "Plos One"

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Manuscripts disputing published work

For manuscripts disputing previously published work, it is *PLOS ONE* policy to invite a signed review by the disputed author during the peer review process. This procedure is aimed at ensuring a thorough, transparent, and productive review process.

If the disputed author chooses to submit a review, it must be returned in a timely fashion and contain a full declaration of all competing interests. The Academic Editor will consider any such reviews in light of the competing interest.

Authors submitting manuscripts disputing previous work should explain the relationship between the manuscripts in their cover letter, and will be required to confirm that they accept the conditions of this review policy before the manuscript is considered further.

Related manuscripts

Upon submission, authors must confirm that the manuscript, or any related manuscript, is not currently under consideration or accepted elsewhere. If related work has been submitted to *PLOS ONE* or elsewhere, authors must include a copy with the submitted article. Reviewers will be asked to comment on the overlap between related submissions.

We strongly discourage the unnecessary division of related work into separate manuscripts, and we will not consider manuscripts that are divided into “parts.” Each submission to *PLOS ONE* must be written as an independent unit and should not rely on any work that has not already been accepted for publication. If related manuscripts are submitted to *PLOS ONE*, the authors may be advised to combine them into a single manuscript at the editor's discretion.

Preprints

PLOS encourages authors to post preprints as a way to accelerate the dissemination of research and supports authors who wish to share their work early and receive feedback before formal peer review. Deposition of manuscripts with preprint servers does not impact consideration of the manuscript at any PLOS journal.

Authors posting on bioRxiv or medRxiv may submit directly to relevant PLOS journals through the direct transfer to journal service.

Authors submitting manuscripts in the life sciences to *PLOS ONE* may opt-in to post their work on bioRxiv during the *PLOS ONE* initial submission process.

Guidelines for Specific Study Types

Registered Reports

Submission and format requirements for Registered Report Protocols and Registered Reports are similar to those for a regular submission and may be specific to your study type. For instance, if your Registered Report Protocol submission is about a Clinical Trial or a Systematic Review, follow the appropriate guidelines.

For Registered Report Protocols:

- Provide enough methodological detail to make the study reproducible and replicable
- Confirm that data will be made available upon study completion in keeping with the PLOS Data policy
- Include ethical approval or waivers, if applicable
- Preliminary or pilot data may be included, but only if necessary to support the feasibility of the study or as a proof of principle
- For meta-analyses or Clinical Trials, use the protocol-specific reporting guidelines PRISMA-P or SPIRIT respectively

For more guidance on format and presentation of a protocol, consult the sample template hosted by the Open Science Framework. Discipline-specific and study-specific templates are also available.

If data need to be collected, modified or processed specifically for your study, or if participants need to be recruited specifically for your study, then it should occur only after your Registered Report Protocol is accepted for publication.

For Registered Report Research Articles:

- Report the results of all planned analyses and, if relevant, detail and justify all deviations from the protocol.
- The manuscript may also contain exploratory, unplanned analyses.

Read more about Registered Report framework.

Human subjects research

All research involving human participants must have been approved by the authors' Institutional Review Board (IRB) or by equivalent ethics committee(s), and must have been conducted according to the principles expressed in the Declaration of Helsinki. Authors should be able to submit, upon request, a statement from the IRB or ethics committee indicating approval of the research. We reserve the right to reject work that we believe has not been conducted to a high ethical standard, even when formal approval has been obtained.

Subjects must have been properly instructed and have indicated that they consent to participate by signing the appropriate informed consent paperwork. Authors may be asked to submit a blank, sample copy of a subject consent form. If consent was verbal instead of written, or if consent could not be obtained, the authors must explain the reason in the manuscript, and the use of verbal consent or the lack of consent must have been approved by the IRB or ethics committee.

All efforts should be made to protect patient privacy and anonymity. Identifying information, including photos, should not be included in the manuscript unless the information is crucial and the individual has provided written consent by completing the Consent Form for Publication in a PLOS Journal (PDF). Download additional translations of the form from the Downloads and Translations page. More information about patient privacy, anonymity, and informed consent can be found in the International Committee of Medical Journal Editors (ICMJE) Privacy and Confidentiality guidelines.

Manuscripts should conform to the following reporting guidelines:

- Studies of diagnostic accuracy: STARD
- Observational studies: STROBE
- Microarray experiments: MIAME
- Other types of health-related research: Consult the EQUATOR web site for appropriate reporting guidelines

Methods sections of papers on research using human subjects or samples must include ethics statements that specify:

- **The name of the approving institutional review board or equivalent committee(s)**. If approval was not obtained, the authors must provide a detailed statement explaining why it was not needed
- **Whether informed consent was written or oral**. If informed consent was oral, it must be stated in the manuscript:
    - Why written consent could not be obtained
    - That the Institutional Review Board (IRB) approved use of oral consent
    - How oral consent was documented

For studies involving humans categorized by race/ethnicity, age, disease/disabilities, religion, sex/gender, sexual orientation, or other socially constructed groupings, authors should:

- Explicitly describe their methods of categorizing human populations
- Define categories in as much detail as the study protocol allows
- Justify their choices of definitions and categories, including for example whether any rules of human categorization were required by their funding agency
- Explain whether (and if so, how) they controlled for confounding variables such as socioeconomic status, nutrition, environmental exposures, or similar factors in their analysis

In addition, outmoded terms and potentially stigmatizing labels should be changed to more current, acceptable terminology. Examples: “Caucasian” should be changed to “white” or “of [Western] European descent” (as appropriate); “cancer victims” should be changed to “patients with cancer.”

For papers that include identifying, or potentially identifying, information, authors must download the Consent Form for Publication in a PLOS Journal, which the individual, parent, or guardian must sign once they have read the paper and been informed about the terms of PLOS open-access license. The signed consent form should not be submitted with the manuscript, but authors should securely file it in the individual's case notes and the methods section of the manuscript should explicitly state that consent authorization for publication is on file, using wording like:

The individual in this manuscript has given written informed consent (as outlined in PLOS consent form) to publish these case details.

For more information about *PLOS ONE* policies regarding human subjects research, see the Publication Criteria and Editorial Policies.

Clinical trials

Clinical trials are subject to all policies regarding human research. *PLOS ONE* follows the World Health Organization's (WHO) definition of a clinical trial:

A clinical trial is any research study that prospectively assigns human participants or groups of humans to one or more health-related interventions to evaluate the effects on health outcomes [...] Interventions include but are not restricted to drugs, cells and other biological products, surgical procedures, radiologic procedures, devices, behavioural treatments, process-of-care changes, preventive care, etc.
All clinical trials must be registered in one of the publicly-accessible registries approved by the WHO or ICMJE (International Committee of Medical Journal Editors). Authors must provide the trial registration number. Prior disclosure of results on a clinical trial registry site will not affect consideration for publication.

We reserve the right to inform authors' institutions or ethics committees, and to reject the manuscript, if we become aware of unregistered trials.

PLOS ONE supports prospective trial registration (i.e. before participant recruitment has begun) as recommended by the ICMJE's clinical trial registration policy. **Where trials were not publicly registered before participant recruitment began**, authors must:

- Register all related clinical trials and confirm they have done so in the Methods section
- Explain in the Methods the reason for failing to register before participant recruitment

Clinical trials must be reported according to the relevant reporting guidelines, i.e. CONSORT for randomized controlled trials, TREND for non-randomized trials, and other specialized guidelines as appropriate. The intervention should be described according to the requirements of the TIDieR checklist and guide. Submissions must also include the study protocol as supporting information, which will be published with the manuscript if accepted.

Authors of manuscripts describing the results of clinical trials must adhere to the CONSORT reporting guidelines appropriate to their trial design, available on the CONSORT Statement web site. Before the paper can enter peer review, authors must:

- The name of the registry and the registration number must be included in the Abstract.
- Provide a copy of the trial protocol as approved by the ethics committee and a completed CONSORT checklist as supporting information (which will be published alongside the paper, if accepted). This should be named S1 CONSORT Checklist.
- Include the CONSORT flow diagram as the manuscript's “Fig 1”

Any deviation from the trial protocol must be explained in the paper. Authors must explicitly discuss informed consent in their paper, and we reserve the right to ask for a copy of the patient consent form.

The name of the registry and the registry number must be provided in the Abstract. If the trial is registered in more than one location, please provide all relevant registry names and numbers.

Lab Protocols

Lab Protocols consist of two interlinked components: a protocol hosted on the protocols.io platform and a peer-reviewed article on *PLOS ONE* that contextualises the protocol.
protocols.io is a secure open access platform that specializes in laboratory protocols. It allows scientists to share, discover and reuse up-to-date protocol knowledge. The platform provides specialist tools and guidance on how to add

each element of the protocol, including the title, abstract, steps, files, links, reagents, measurements, formulae, videos, charts and more.

The *PLOS ONE* article component must comply with the general submission guidelines (detailed above in this article).

The *PLOS ONE* article component must also comply with the general *PLOS ONE* criteria for publication and in addition it should:

- Present a step-by-step protocol that adds value to the published literature.
- Link, in the Introduction section, to at least one supporting peer-reviewed publication in which the protocol was applied to generate data.
- Link, in the Materials and Methods section, to the protocol.io component, using the digital object identifier (DOI) and format provided by protocols.io, for example *https://dx.doi.org/10.17504/protocols.io[....]*.
- Describe the appropriate controls, sample sizes and replication needed to ensure that the data are robust and reproducible.
- Provide the protocol as a supporting information (S1) file for printing purposes. You can download a PDF from protocols.io for this purpose.
- Optionally, provide minimal new data relevant to the development of the protocol e.g., for additional benchmarking, validation or troubleshooting purposes.

We encourage you to post your protocol to the protocols.io platform before submitting your manuscript to *PLOS ONE*, or at the latest, before the editorial and peer review process. This approach is optional, but beneficial, because:

- Your DOI is assigned on the protocols.io platform. You need this identifier to link out from the Material and Methods section of your manuscript.
- You can keep your protocol private on the protocols.io platform (until you are satisfied that it is ready for publication), but still assign a DOI.
- The protocol will be accessible to editors and reviewers during the editorial and peer review process.

If you prefer to submit your manuscript to *PLOS ONE* before uploading your protocol to protocols.io, please provide your protocol as a supporting information (S1) file. You can use protocols.io's editorial service at no cost: they will check and publish your protocol for you t. As part of *PLOS ONE*'s partnership with protocols.io, your waiver code for this purpose will be provided in the first decision letter.

Study Protocols

Study Protocols describe plans for conducting research projects and consist of a single article on *PLOS ONE*.

Study Protocols must comply with the *PLOS ONE* general submission guidelines (detailed above in this article) and any guidelines specific to the related research study type. In addition, the protocol must:

- Relate to a research study that has not yet generated results.
- Be submitted before recruitment of participants or collection of data for the study is complete.
- Meet the same standards for ethics of experimentation and research integrity as the research study. If it involves human or animal subjects, cell lines or field sampling, or has potential biosafety implications, prior approval from the relevant ethics body must be obtained prior to submission. Please contact us if you have a valid reason for not obtaining approval.

Additional prerequisites apply for these study types:

- Clinical trials:
    - The trial must be registered prior to submission of your protocol in one of the publicly accessible registries approved by the WHO or ICMJE (Internation Committee of Medical Journal Editors).
    - The name of the registry and the trial or study registration number must be included in the Abstract.
    - A copy of the protocol that was approved by the ethics committee must be submitted as a supplementary information file. Please provide an addtional English translation if the original document is not in English.
    - A SPIRIT schedule of enrollment, interventions, and assessments must be included as the manuscript's Figure 1, and a completed SPIRIT checklist must be uploaded as Supporting Information file S1.
- Systematic reviews and meta-analyses:
    - A completed PRISMA-P checklist must be provided as a supporting information (SI) file. See PRISMA-P Explanation and Elaboration for more information on completing your checklist.

Study Protocols must also comply with general *PLOS ONE* criteria for publication and in addition you should:

- include the word "Protocol" in your Title.
- include a detailed description of the planned study in the Materials and Methods section.This should provide sufficient methodological detail for the protocol to be reproducible and replicable. Your description should cover all relevant and applicable facts and hypothesis, including:

    - the aim, design, and settling
    - the sample size calculation
    - how data saturation will be determined (for qualitative studies)
    - the characteristics of participants e.g., inclusion and exclusion criteria, sample selection criteria, variables to be measured, randomization and blinding criteria (where applicable), and how informed consent will be obtained
    - how materials will be selected and used e.g., where and how they will be sourced, the processes, interventions, or comparisons to be used, the outcomes to be measured, and when and how they will be measured
    - the data management plan
    - safety considerations
    - the type of data and statistical analyses to be used
    - the status and timeline of the study, including whether participant recruitment or data collection has begun
    - where and when the data will be made available. See our Data Availability policy for more.
- include an analysis of preliminary or pilot data, only if it is necessary to support the feasibility of the study or as a proof of principle. This is optional.
- we encourage authors you to register with OSF and provide the your registration number in the Materials and Methods section. This is optional.
- optionally add any other SI files, figures or tables that elaborate or authenticate the protocol: e.g., any reporting checklists applicable to your study type.

Read the supporting information guidelines for more details about adding SI files.

Study Protocols are subject to the same editorial and peer review process as all other articles, and are eligible for both signed and published peer review.

You can expedite the review process by providing:

- proof of external funding. This is typically your funding approval letter and a list of the names and credentials of the funders who conducted the external peer review of the protocol. Include an English translation if needed.
- proof of ethics approval (if required). This is typically the approval or waiver letter from the relevant ethics body and a copy of the protocol approved Animal research

All research involving vertebrates or cephalopods must have approval from the authors' Institutional Animal Care and Use Committee (IACUC) or equivalent ethics committee(s), and must have been conducted according to applicable national and international guidelines. Approval must be received prior to beginning research.

Manuscripts reporting animal research must state in the Methods section:

- The full name of the relevant ethics committee that approved the work, and the associated permit number(s).
- Where ethical approval is not required, the manuscript should include a clear statement of this and the reason why. Provide any relevant regulations under which the study is exempt from the requirement for approval.
- Relevant details of steps taken to ameliorate animal suffering.

Observational and field studies

Methods sections for submissions reporting on any type of field study must include ethics statements that specify:

- Permits and approvals obtained for the work, including the full name of the authority that approved the study; if none were required, authors should explain why
- Whether the land accessed is privately owned or protected
- Whether any protected species were sampled
- Full details of animal husbandry, experimentation, and care/welfare, where relevant

Paleontology and archaeology research

Manuscripts reporting paleontology and archaeology research must include descriptions of methods and specimens in sufficient detail to allow the work to be reproduced. Data sets supporting statistical and phylogenetic analyses should be provided, preferably in a format that allows easy re-use. Read the policy.

Specimen numbers and complete repository information, including museum name and geographic location, are required for publication. Locality information should be provided in the manuscript as legally allowable, or a statement should be included giving details of the availability of such information to qualified researchers.

If permits were required for any aspect of the work, details should be given of all permits that were obtained, including the full name of the issuing authority. This should be accompanied by the following statement:

All necessary permits were obtained for the described study, which complied with all relevant regulations.
If no permits were required, please include the following statement:

No permits were required for the described study, which complied with all relevant regulations.

Systematic reviews and meta-analyses

A systematic review paper, as defined by The Cochrane Collaboration, is a review of a clearly formulated question that uses explicit, systematic methods to identify, select, and critically appraise relevant research, and to collect and analyze data from the studies that are included in the review. These reviews differ substantially from narrative-based reviews or synthesis articles. Statistical methods (meta-analysis) may or may not be used to analyze and summarize the results of the included studies.

Reports of systematic reviews and meta-analyses must include a completed PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) checklist and flow diagram to accompany the main text. Blank templates are available here:

- Checklist: PDF or Word document
- Flow diagram: PDF or Word document

Authors must also state in their “Methods” section whether a protocol exists for their systematic review, and if so, provide a copy of the protocol as supporting information and provide the registry number in the abstract.

If your article is a systematic review or a meta-analysis you should:

- State this in your cover letter
- Select “Research Article” as your article type when submitting
- Include the PRISMA flow diagram as Fig 1 (required where applicable)
- Include the PRISMA checklist as supporting information

Meta-analysis of genetic association studies

Manuscripts reporting a meta-analysis of genetic association studies must report results of value to the field and should be reported according to the guidelines presented in *Systematic Reviews of Genetic Association Studies* by Sagoo *et al.*

On submission, authors will be asked to justify the rationale for the meta-analysis and how it contributes to the base of scientific knowledge in the light of previously published results. Authors will also be asked to complete a checklist (DOCX) outlining information about the justification for the study and the methodology employed. Meta-analyses that replicate published studies will be rejected if the authors do not provide adequate justification.

Personal data from third-party sources

For all studies using personal data from internet-based and other third-party sources (e.g., social media, blogs, other internet sources, mobile phone companies), data must be collected and used according to company/website

Terms and Conditions, with appropriate permissions. All data sources must be acknowledged clearly in the Materials and Methods section.

In the Ethics Statement, authors should declare any potential risks to individuals or individual privacy, or affirm that in their assessment, the study posed no such risks. In addition, the following Ethics and Data Protection requirements must be met.

For interventional studies, which impact participants' experiences or data, the study design must have been prospectively approved by an Ethics Committee, and informed consent is required. The Ethics Committee may waive the requirement for approval and/or consent.

For observational studies in which personal experiences and accounts are not manipulated, consultation with an Ethics or Data Protection Committee is recommended. Additional requirements apply in the following circumstances:

- If information used could threaten personal privacy or damage the reputation of individuals whose data are used, an Ethics Committee should be consulted and informed consent obtained or specifically addressed.
- If authors accessed any personal identifying information, an Ethics or Data Protection Committee should oversee data anonymization. If data were anonymized and/or aggregated before access and analysis, informed consent is generally not required.

Cell lines

Authors reporting research using cell lines should state when and where they obtained the cells, giving the date and the name of the researcher, cell line repository, or commercial source (company) who provided the cells, as appropriate.

Authors must also include the following information for each cell line:

For *de novo* (new) cell lines, including those given to the researchers as a gift, authors must follow our policies for human subjects research or animal research, as appropriate. The ethics statement must include:

- Details of institutional review board or ethics committee approval; AND
- For human cells, confirmation of written informed consent from the donor, guardian, or next of kin

For established cell lines, the Methods section should include:

- A reference to the published article that first described the cell line; AND/OR
- The cell line repository or company the cell line was obtained from, the catalogue number, and whether the cell line was obtained directly from the repository/company or from another laboratory

Authors should check established cell lines using the ICLAC Database of Cross-contaminated or Misidentified Cell Lines to confirm they are not misidentified or contaminated. Cell line authentication is recommended – e.g., by karyotyping, isozyme analysis, or short tandem repeats (STR) analysis – and may be required during peer review or after publication.

Blots and gels

Please review *PLOS ONE's* requirements for reporting blot and gel results and providing the underlying raw images.

Antibodies

Manuscripts reporting experiments using antibodies should include the following information:

- The name of each antibody, a description of whether it is monoclonal or polyclonal, and the host species.
- The commercial supplier or source laboratory.
- The catalogue or clone number and, if known, the batch number.
- The antigen(s) used to raise the antibody.
- For established antibodies, a stable public identifier from the Antibody Registry.

The manuscript should also report the following experimental details:

- The final antibody concentration or dilution.
- A reference to the validation study if the antibody was previously validated. If not, provide details of how the authors validated the antibody for the applications and species used.

Manuscripts reporting new and unpublished three-dimensional structures must include sufficient supporting data and detailed descriptions of the methodologies used to allow the reproduction and validation of the structures. All novel structures must have been deposited in a community endorsed database prior to submission (please see our list of recommended repositories).

Small molecule single crystal data

Authors reporting X-Ray crystallographic structures of small organic, metal-organic, and inorganic molecules must deposit their data with the Cambridge Crystallographic Data Centre (CCDC), the Inorganic Crystal Structure Database (ICSD), or similar community databases providing a recognized validation functionality. Authors are also required to include the relevant structure reference numbers within the main text (e.g. the CCDC ID number), as well as the crystallographic information files (.cif format) as Supplementary Information, along with the checkCIF validation reports that can be obtained via the International Union of Crystallography (IUCr).

Macromolecular structures

Authors reporting novel macromolecular structures must have deposited their data prior to initial submission with the Worldwide Protein Data Bank (wwPDB), the Biological Magnetic Resonance Data Bank (BMRB), the Electron Microscopy Data Bank (EMDB), or other community databases providing a recognized validation functionality. Authors must include the structure reference numbers within the main text and submit as Supplementary Information the official validation reports from these databases.

Methods, software, databases, and tools

PLOS ONE will consider submissions that present new methods, software, databases, or tools as the primary focus of the manuscript if they meet the following criteria:

Software submissions

Manuscripts whose primary purpose is the description of new software must provide full details of the algorithms designed. Describe any dependencies on commercial products or operating system. Include details of the supplied test data and explain how to install and run the software. A brief description of enhancements made in the major releases of the software may also be given. Authors should provide a direct link to the deposited software from within the paper.

Database submissions

For descriptions of databases, provide details about how the data were curated, as well as plans for long-term database maintenance, growth, and stability. Authors should provide a direct link to the database hosting site from within the paper.

New taxon names

Zoological names

When publishing papers that describe a new zoological taxon name, PLOS aims to comply with the requirements of the International Commission on Zoological Nomenclature (ICZN). Effective 1 January 2012, the ICZN considers an online-only publication to be legitimate if it meets the criteria of archiving and is registered in ZooBank, the ICZN's official registry.

For proper registration of a new zoological taxon, we require two specific statements to be included in your manuscript.

In the **Results** section, the globally unique identifier (GUID), currently in the form of a Life Science Identifier (LSID), should be listed under the new species name, for example:

Anochetus boltoni *Fisher* ***sp. nov.*** *urn:lsid:zoobank.org:act:B6C072CF-1CA6-40C7-8396-534E91EF7FBB*
You will need to contact Zoobank to obtain a GUID (LSID). Please do this as early as possible to avoid delay of publication upon acceptance of your manuscript. It is your responsibility to provide us with this information so we can include it in the final published paper.

Please also insert the following text into the **Methods** section, in a sub-section to be called “Nomenclatural Acts”:

All PLOS articles are deposited in PubMed Central and LOCKSS. If your institute, or those of your co-authors, has its own repository, we recommend that you also deposit the published online article there and include the name in your article.

Botanical names

When publishing papers that describe a new botanical taxon, PLOS aims to comply with the requirements of the International Code of Nomenclature for algae, fungi, and plants (ICN). The following guidelines for publication in an online-only journal have been agreed such that any scientific botanical name published by us is considered effectively published under the rules of the Code. Please note that these guidelines differ from those for zoological nomenclature, and apply only to seed plants, ferns, and lycophytes.

Effective January 2012, the description or diagnosis of a new taxon can be in either Latin or English. This does not affect the requirements for scientific names, which are still to be Latin.

Also effective January 2012, the electronic PDF represents a published work according to the ICN for algae, fungi, and plants. Therefore the new names contained in the electronic publication of PLOS article are effectively published under that Code from the electronic edition alone, so there is no longer any need to provide printed copies.

Additional information describing recent changes to the Code can be found here.

For proper registration of the new taxon, we require two specific statements to be included in your manuscript.

In the **Results** section, the globally unique identifier (GUID), currently in the form of a Life Science Identifier (LSID), should be listed under the new species name, for example:

Solanum aspersum S.Knapp, sp. nov. [urn:lsid:ipni.org:names:77103633-1] Type: Colombia. Putumayo: vertiente oriental de la Cordillera, entre Sachamates y San Francisco de Sibundoy, 1600-1750 m, 30 Dec 1940, J. Cuatrecasas 11471 (holotype, COL; isotypes, F [F-1335119], US [US-1799731]).
Journal staff will contact IPNI to obtain the GUID (LSID) after your manuscript is accepted for publication, and this information will then be added to the manuscript during the production phase

In the **Methods** section, include a sub-section called “Nomenclature” using the following wording:

All PLOS articles are deposited in PubMed Central and LOCKSS. If your institute, or those of your co-authors, has its own repository, we recommend that you also deposit the published online article there and include the name in your article.

Fungal names

When publishing papers that describe a new botanical taxon, PLOS aims to comply with the requirements of the International Code of Nomenclature for algae, fungi, and plants (ICN). The following guidelines for publication in an online-only journal have been agreed such that any scientific botanical name published by us is considered effectively published under the rules of the Code. Please note that these guidelines differ from those for zoological nomenclature.

Effective January 2012, the description or diagnosis of a new taxon can be in either Latin or English. This does not affect the requirements for scientific names, which are still to be Latin.

Also effective January 2012, the electronic PDF represents a published work according to the ICN for algae, fungi, and plants. Therefore the new names contained in the electronic publication of PLOS article are effectively published under that Code from the electronic edition alone, so there is no longer any need to provide printed copies.

Additional information describing recent changes to the Code can be found here.

For proper registration of the new taxon, we require two specific statements to be included in your manuscript.

In the **Results** section, the globally unique identifier (GUID), currently in the form of a Life Science Identifier (LSID), should be listed under the new species name, for example:

Hymenogaster huthii. Stielow et al. 2010, sp. nov. [urn:lsid:indexfungorum.org:names:518624]
You will need to contact either Mycobank or Index Fungorum to obtain the GUID (LSID). Please do this as early as possible to avoid delay of publication upon acceptance of your manuscript. It is your responsibility to provide us with this information so we can include it in the final published paper. Effective January 2013, all papers describing new fungal species must reference the identifier issued by a recognized repository in the protologue in order to be considered effectively published.

In the **Methods** section, include a sub-section called “Nomenclature” using the following wording. Note that this example is for taxon names submitted to MycoBank; please substitute appropriately if you have submitted to Index Fungorum using the prefix http://www.indexfungorum.org/Names/NamesRecord.asp?RecordID=.

All PLOS articles are deposited in PubMed Central and LOCKSS. If your institute, or those of your co-authors, has its own repository, we recommend that you also deposit the published online article there and include the name in your article.

Qualitative research

Qualitative research studies use non-quantitative methods to address a defined research question that may not be accessible by quantitative methods, such as people's interpretations, experiences, and perspectives. The analysis methods are explicit, systematic, and reproducible, but the results do not involve numerical values or use statistics. Examples of qualitative data sources include, but are not limited to, interviews, text documents, audio/video recordings, and free-form answers to questionnaires and surveys.

Qualitative research studies should be reported in accordance to the Consolidated criteria for reporting qualitative research (COREQ) checklist or Standards for reporting qualitative research (SRQR) checklist. Further reporting guidelines can be found in the Equator Network's Guidelines for reporting qualitative research.

You may be eligible for APC support

Many institutional partners globally have publishing agreements with PLOS to allow their corresponding authors to publish with reduced or no APCs. To determine if your corresponding author is eligible, please visit our institutional partners page to determine what kind of agreement your institution has with PLOS.

If your corresponding author is affiliated with a participating institution, she or he must follow the instructions below to demonstrate eligibility.