PRÓ-REITORIA DE PESQUISA E PÓS-GRADUAÇÃO
MESTRADO EM CIÊNCIAS DA SAÚDE

GUSTAVO ESTEVAM NÓBREGA THOMAZ

EFEITOS NO TECIDO ÓSSEO DE RATOS SUBMETIDOS À EXPOSIÇÃO DE DESREGULADORES ENDÓCRINOS DURANTE A VIDA FETAL E A LACTAÇÃO

Presidente Prudente - SP
2025

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Orientador:
Prof. Dr. Wilson Romero Nakagaki

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Aos quinze dias do mês de maio do ano de dois mil e vinte e cinco, às oito horas, o(a) Prof(a). Dr(a). Wilson Romero Nakagaki, orientador(a) do(a) mestrando(a) **GUSTAVO ESTEVAM NÓBREGA THOMAZ**, fez a abertura da sessão de arguição da Defesa Pública de Dissertação de Mestrado do Programa de Pós-Graduação em Ciências da Saúde - Área de Concentração: Ciências da Saúde, na sala 210, bloco B2, Campus II. Na condição de Presidente da Banca Examinadora, procedeu a chamada dos membros indicados e aprovados pelo Colegiado do Programa de Pós-Graduação em Ciências da Saúde, para compor a mesa, com os seguintes doutores: Eliana Peresi Lordelo – Unoeste/Universidade do Oeste Paulista e Gustavo Ferreira Simões - Faculdade de Ciências Médicas da Santa Casa de São Paulo. Iniciados os trabalhos, a Presidência declarou para o conhecimento dos membros da Banca e do(a) Candidato(a), as normas que regem a defesa pública e definiu a ordem a ser seguida pelos examinadores para a arguição. A seguir o(a) candidato(a) passou a apresentação de sua dissertação intitulada: **"EFEITOS NO TECIDO ÓSSEO DE RATOS SUBMETIDOS À EXPOSIÇÃO DE DESREGULADORES ENDÓCRINOS DURANTE A VIDA FETAL E A LACTAÇÃO"**. Encerrada a defesa, procedeu-se ao julgamento, cujo resultado foi:

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wilromero@unoeste.br
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elianaperesi@unoeste.br
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GUSTAVO FERREIRA SIMÕES (SIGNATÁRIO EXTERNO)
gfsimoes2@gmail.com
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DEDICATÓRIA

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“Não fui eu que ordei a você? Seja forte e corajoso! Não se apavore nem desanime, pois o SENHOR, o seu Deus, estará com você por onde você andar.”

Josué 1:9

RESUMO

Efeitos no tecido ósseo de ratos submetidos à exposição de desreguladores endócrinos durante a vida fetal e a lactação

A homeostasia óssea pode ser comprometida por substâncias denominadas desreguladores endócrinos (DEs). Elas atuam mimetizando ou bloqueando a ação de hormônios naturais, interferindo em aspectos de síntese, secreção e nos processos a elas associados, como a regulação do desenvolvimento celular para a formação dos tecidos do organismo. O objetivo deste estudo foi avaliar ossos de ratos com 420 dias de vida, descendentes de ratas alimentadas com uma mistura desses compostos, desde o 7º dia de gestação até o 21º dia de lactação, com consequente desmame das proles e o fim da exposição a esses alimentos. Os animais foram distribuídos em dois grupos: Grupo Controle e Grupo Desreguladores Endócrinos. As ratas prenhes ou lactantes receberam o tratamento no 7º dia de gestação até o 21º dia pós-natal. Os filhotes machos foram desmamados no vigésimo segundo dia. Assim, passaram a receber somente água e ração até serem eutanasiados aos 14 meses. Então, os fêmures foram avaliados por análise mecânica e de Raman com as razões de bandas espectrais (430/1272, 960/1660 e 1070/1660). A força máxima de resistência e a energia absorvida foram similares entre os dois grupos. Porém, o grupo Desreguladores Endócrinos demonstrou ser 16,4% mais deformável e 22,1% menos rígido comparado ao grupo Controle. Para a espectroscopia Raman, as três razões de bandas analisadas foram diferentes entre os grupos, sendo maiores no Desreguladores Endócrinos, indicando alterações na matriz óssea. A exposição aos DEs durante a gestação e na lactação provocou comprometimento da qualidade óssea na vida adulta.

Palavras-chave: Desreguladores endócrinos; Força óssea; Gestação; Lactação; Raman.

ABSTRACT

Effects on bone tissue of rats exposed to endocrine disruptors during fetal life and lactation

Substances called endocrine disruptors (EDs) can compromise bone homeostasis. They mimic or block the action of natural hormones, interfering with aspects of their synthesis, secretion, and associated processes, such as the regulation of cell development for the formation of the body's tissues. The aim was to evaluate the bones of 420-day-old rats, offspring of rats fed a mixture of these compounds, from the 7th day of gestation to the 21st day of lactation, with consequent weaning of the offspring and the end of exposure to these foods. The animals were divided into two groups: the Control Group and the Endocrine Disruptors (EDs) Group. The pregnant or lactating rats received the treatment on the 7th day of gestation until the 21st postnatal day. The male pups were weaned on the 22nd day. They were then given only water and food until they were euthanized at 14 months. The femurs were then evaluated by mechanical and Raman analysis (spectral band ratios of 430/1272, 960/1660 and 1070/1660). The maximum resistance force and absorbed energy were similar between the two groups. However, the Endocrine Disruptors group proved to be 16.4% more deformable and 22.1% less rigid compared to the Control group. For Raman spectroscopy, the three band ratios analyzed were different between the groups. These bands were higher in the EDs group, indicating alterations in the bone matrix. Exposure to EDs during pregnancy and lactation led to impaired bone quality in adulthood.

Keywords: Endocrine disruptors; Bone strength; Pregnancy; Lactation; Raman.

LISTA DE SIGLAS

BMP1	– Proteína morfogenética óssea 1
BMP4	– Proteína morfogenética óssea 4
BPA	– Bisfenol A
CEMIB	– Centro Multidisciplinar para Investigação Biológica
CPDI	– Coordenadoria de Pesquisa, Desenvolvimento e Inovação
CEUA	– Comissão de Ética em Utilização Animal
DDT	– Dicloro-Difenil-Tricloroetano
DG7	– Dia gestacional 7
DPN21	– Dia pós-natal 21
DPN22	– Dia pós-natal 22
DEs	– Desreguladores endócrinos
HA	– Hidroxiapatita
RUNX2	– Fator de transcrição relacionado ao Runt 2
TGF-β	– Fator de crescimento transformador beta

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EFFECTS ON BONE TISSUE OF RATS EXPOSED TO ENDOCRINE DISRUPTORS DURING FETAL LIFE AND LACTATION

Gustavo Estevam Nóbrega Thomaz[1], Vanessa Camargo Patussi[2], Thainá Cavalleri Sousa[3], Leonardo de Oliveira Mendes[1,2,3], Carlos Jose Leopoldo Constantino[4], Wilson Romero Nakagaki[1,2,,3]

[1]Programa de Pós-Graduação em Ciências da Saúde, Universidade do Oeste Paulista (UNOESTE), Presidente Prudente, SP, Brasil.
[2]Faculdade de Ciências da Saúde, Universidade do Oeste Paulista (UNOESTE), Presidente Prudente, SP, Brasil.
[3]Programa de Pós-Graduação em Ciências da Saúde, Universidade do Oeste Paulista (UNOESTE), Presidente Prudente, SP, Brasil.
[4]Departamento de Física, Universidade Estadual Paulista (UNESP), Presidente Prudente, São Paulo, Brasil.

Autor para correspondência: Prof. Dr. Wilson Romero Nakagaki Universidade do Oeste Paulista (UNOESTE)
Pró-Reitoria de Pesquisa e Pós-Graduação em Ciências da Saúde Rodovia Raposo Tavares, km 572 - Bairro do Limoeiro, Campus II Presidente Prudente - SP, Brasil
CEP: 19067-175

E-mail: wilromero@unoeste.br

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INTRODUÇÃO

O hormônio do crescimento (somatotrofina), secretado pela hipófise, promove não só o aumento do tamanho das células, do número de mitoses, como também sua multiplicação e a diferenciação específica de alguns tipos celulares, por exemplo, as células de crescimento ósseo e células musculares iniciais (Dixit, Poudel, e Yakar 2021). A calcitonina é um peptídeo produzido pelas células C da tireoide e liberada quando as concentrações plasmáticas de Ca^{2+} aumentam. Experimentos realizados em animais mostram que a calcitonina diminui a reabsorção óssea e aumenta a excreção renal de cálcio (Xie et al. 2020). Já o paratormônio sintetizado pelas glândulas paratireoides, atua no osso, no rim e no intestino para aumentar as concentrações plasmáticas de Ca^{2+}, sendo que um dos mecanismos realizados para isso seja elevando a reabsorção óssea pelos osteoclastos (Khan, Jose, e Sharma 2024). Também envolvidos no metabolismo do tecido ósseo estão os hormônios sexuais por serem um fator-chave na aquisição de massa óssea, ajudando na atividade de remodelação e de formação de novo tecido (Guebeli et al. 2020).

Estudos apontam que o osso sofre, ainda, a influência dos exercícios físicos. A vibração mecânica proveniente do exercício provou ser benéfica para a microarquitetura óssea, melhorando a densidade e a resistência óssea, além de aumentar a função física (Moreira et al. 2014). Além disso, diversos outros fatores como idade, doenças e medicamentos também interferem no metabolismo ósseo. A avaliação *in vivo* dos índices de densidade mineral óssea e conteúdo mineral ósseo fornece uma descrição objetiva das possíveis taxas de perda óssea durante o envelhecimento e várias doenças, bem como a resposta ao tratamento por várias drogas, afetando o metabolismo ósseo (Grygorieva et al. 2017), além da dieta nutricional adotada ao longo da vida (Miggiano e Gagliardi 2005). Além disso, os ossos são capazes de absorver toxinas e metais pesados, minimizando seus efeitos adversos em outros tecidos (Pizzorno e Pizzorno 2021).

Analisando-se sob o viés do atual cenário ambiental brasileiro e mundial, observa-se a forte influência do uso em larga escala de substâncias químicas deletérias, muitas delas sintéticas, em diversos itens e sobre a alimentação dos indivíduos, cujos efeitos estão associados à desordem no funcionamento do sistema endócrino. Tais produtos são conhecidos como desreguladores endócrinos (DEs) e referem-se a compostos que alteram o funcionamento normal do sistema endócrino de humanos e animais selvagens e que podem modificar a biossíntese hormonal e provocar instabilidades na saúde humana, incluindo alterações genéticas (Kabir, Rahman, e Rahman 2015).

Classicamente, um hormônio é uma substância química secretada na corrente sanguínea e que atua em tecidos distantes, geralmente de forma regulatória (Bahadoran et al. 2019). Ao alcançarem os tecidos-alvo, farão sua ligação a receptores celulares a eles associados, resultando em uma resposta biológica específica, logo, coordenam o desenvolvimento dos tecidos, suas funções e promovem a adaptação e a manutenção dos processos corporais em vista dos estímulos ambientais experienciados. Sendo assim, os DEs são definidos pela *Endocrine Society* como “substância química exógena (não natural), ou mistura de substâncias químicas, que interferem em qualquer aspecto da ação hormonal” (Gore et al. 2014).

Estima-se que existam cerca de 1.000 produtos químicos com propriedades de ação endócrina. Os DEs compreendem pesticidas, fungicidas, produtos químicos industriais, plastificantes, nonilfenóis, metais, agentes farmacêuticos e fitoestrógenos (Yilmaz et al. 2020). Podem passar diretamente para a cadeia alimentar quando são usados como pesticidas, ou podem ser liberados de embalagens de alimentos contendo metais, bisfenol A ou ftalatos. (Gálvez-Ontiveros et al. 2020). Alguns exemplos comuns de DEs incluem o Dicloro-Difenil-Tricloroetano (DDT) e outros pesticidas; bisfenol A (BPA) e ftalatos, utilizados em produtos infantis, produtos de higiene pessoal e em recipientes para alimentos, retardadores de chamas utilizados em móveis e pavimentos. Além dos DEs conhecidos, suspeita-se da existência de inúmeros DEs ou de substâncias químicas que nunca foram testadas (Gore et al. 2014).

Esses compostos têm a capacidade de causar desregulação endócrina através da interrupção da atividade, tempo de liberação ou imitando hormônios do sistema endócrino (Medeiros, Acayaba, e Montagner 2021). Ademais, foi proposto que os DEs podem aumentar a suscetibilidade a distúrbios metabólicos, alterando o tecido adiposo, pâncreas, fígado, trato gastrointestinal, músculo e os caminhos homeostático e hedônico do cérebro (Gálvez-Ontiveros et al. 2020).

É difícil avaliar o impacto total da exposição humana aos DEs porque os efeitos adversos se desenvolvem de forma latente e se manifestam em idades mais avançadas (Yilmaz et al. 2020). Segundo o estudo de (Predieri et al. 2022), o tempo entre a exposição aos DEs e a expressão clínica de uma doença pode levar anos para o surgimento de suas consequências, causando efeitos negativos durante a vida adulta.

Sabe-se que fetos e recém-nascidos em desenvolvimento são os mais vulneráveis à desregulação endócrina (Yilmaz et al. 2020). Ambos os estágios de desenvolvimento são considerados períodos críticos, pois englobam todo o desenvolvimento e a formação do organismo, bem como os processos a eles associados. Por isso, a interferência de DEs na ação hormonal durante fases cruciais do ciclo de vida pode gerar consequências irreparáveis (Yilmaz et al. 2020).

O tempo de exposição é fundamental para entender quais órgãos ou tecidos podem ser afetados, uma vez que o desenvolvimento de diferentes partes do corpo ocorre de maneiras distintas (Gore et al. 2014). Logo, o presente projeto basear-se-á em uma pesquisa que durou cerca de um ano, na qual as fêmeas prenhes foram alimentadas pelos referidos compostos químicos até seu 21º dia de lactação, gerando descendentes expostos a essas substâncias desde sua vida intrauterina. Findado esse período, cessou-se também essa exposição, e o desenvolvimento das proles foi acompanhado até sua fase de senescência, sucedida de eutanásia.

Considerando o que foi apresentado anteriormente, aos ossos é conferida também uma importante função de regulação sobre as toxinas e metais pesados que adentrem ao organismo, com o fim de minimizar seus possíveis efeitos danosos nos demais tecidos. Neste contexto, o presente estudo avaliou ossos de ratos com 420 dias de vida, descendentes de ratas alimentadas com uma mistura desses compostos, desde o 7º dia de gestação até o 21º dia de lactação, com consequente desmame das proles e o fim da exposição a esses alimentos.

MATERIAL E MÉTODOS

Animais

Neste estudo foram utilizadas ratas fêmeas prenhes da linhagem de ratos Sprague-Dawley (120 dias de idade, pesando aproximadamente 300g), aleatoriamente separadas e obtidas no Centro Multidisciplinar para Investigação Biológica na Área de Ciência de Animais de Laboratório (CEMIB/UNICAMP). Os animais foram mantidos em gaiolas individuais, com livre acesso à água e à alimentação (ração comercial), onde permaneceram em ambiente com temperatura

(24±1ºC) controlada, em ciclo claro/escuro de 12 horas, durante todo o experimento. O presente estudo foi aprovado pela Comissão de Ética em Utilização Animal (CEUA) da Universidade do Oeste Paulista (UNOESTE) e protocolado na Coordenadoria de Pesquisa, Desenvolvimento e Inovação (CPDI) sob número 8709.

Divisão dos grupos

Os animais foram distribuídos em 2 grupos experimentais (n=8 por grupo) denominados de Grupo Controle (veículo: óleo de milho, por gavagem) e Grupo Desreguladores Endócrinos (receberam 168 mg/kg/dia da mistura de DEs, incluindo sintéticos ftalatos, pesticidas, filtros U.V., além do bisfenol A e butilparabeno, diluídos em óleo de milho (2ml/kg) por gavagem). Os animais do grupo Desreguladores Endócrinos receberam uma mistura adaptada de DEs desenvolvida por (Christiansen et al. 2012) e reproduzida por (Axelstad et al. 2014), (Isling et al. 2014) e (Boberg et al. 2015). A composição da mistura é descrita detalhadamente na Tabela 1, adaptada de Christiansen et al. (2012).

As ratas prenhes ou lactentes receberam o tratamento no dia gestacional 7 (DG7) até o dia pós-natal 21 (DPN21), sempre no mesmo horário (entre as 8h e 10h). Foram mantidas em gaiolas individuais e pesadas em dias alternados para permitir o cálculo do volume da mistura de DEs a ser administrado, assim como para a verificação de eventuais sinais clínicos de toxicidade.

Os filhotes machos foram desmamados no vigésimo segundo dia (DPN22) e alojados em caixas com 2 animais cada. A partir desse momento, passaram a receber somente água e ração *ad libitum*. Assim, foram mantidos no Biotério Experimental da UNOESTE, em condições controladas já descritas, até completarem 14 meses (cerca de 420 dias) e foram, então, eutanasiados.

Eutanásia dos animais e coleta do material biológico

Ao fim do período experimental, os animais foram sacrificados aos 420 dias de vida. Para a eutanásia, foram utilizados cetamina (10g/100mL de Cloridrato de Cetamina 10%) e xilazina (20g/100mL de Cloridrato Xilazina 2%), sendo administrados por via intraperitoneal uma solução destes fármacos na proporção de 0,2mL de cetamina e 0,1mL de xilazina. Foram verificados os indicativos de morte, tais como ausência de movimentos respiratórios, batimentos cardíacos e perda dos reflexos. Posteriormente, foi realizada dissecação e remoção dos fêmures de ambos os membros posteriores.

Tabela 1. Composição da mistura, consumo humano individual justado dos compostos químicos e misturas 100x

Compostos químicos	**Consumo humano ajustado e escolhido como base para estudo das misturas (mg/kg de peso corpóreo ao dia) [a]**	**Mistura 100x (mg/kg de peso corpóreo ao dia)**
DBP	0,01	1
DEHP	0,02	2
Vinclozin	0,009	0,9
Procloraz	0,014	1,4
Procimidona	0,015	1,5
Linuron	0,0006	0,06
Epoxiconazol	0,01	1
p,p'-DDE	0,001	0,1
4-MBC	0,06	6
OMC	0,12	12
Bisfenol A	0,0015	0,15
Butilparabeno	0,06	6
Total (mg/kg)	0,32	32,11

[a] Ver Christiansen et al. (2012) para informações detalhadas sobre as estimativas do consumo humano e para as concentrações ajustadas que foram escolhidas como base para a mistura. Tabela reproduzida com permissão de (Cavalleri Sousa et al. 2024).

Ensaio mecânico

Para cada grupo, foram utilizadas 8 amostras. O fêmur direito de cada animal foi testado mecanicamente, até sua fratura completa, em teste de flexão em 3 pontos a uma velocidade de 3 mm/min no equipamento mecânico INSTRON (modelo EMIC 23-2S), com célula de carga de 100 Kgf (Rocha et al. 2020). A distância entre os 2 suportes inferiores foi de 5 mm.

Os dados de força (carga ou *load*) e deslocamento (deformação absoluta ou *displacement*) foram obtidos diretamente da máquina de ensaio que foram mesurados pelo *software* do computador acoplado à máquina de ensaio. Os registros destes dados foram utilizados para aquisição e cálculo das propriedades estruturais: força máxima *(maximum load*), deformação absoluta na força máxima (*displacement at maximum load*), rigidez extrínseca ou estrutural *(stiffness*) e a energia absorvida na ruptura (*energy at failure*). A rigidez extrínseca foi calculada como sendo a inclinação da porção mais linear da região elástica da curva força-deslocamento, enquanto a energia foi calculada com a área sob o gráfico da referida curva (Akhter et al. 2001; Nakagaki et al. 2011; de Oliveira et al. 2022; Rocha et al. 2020).

Espectroscopia de espalhamento Raman

A análise foi obtida a partir do osso cortical (n=8) não descalcificado na região da diáfise do fêmur. As medidas de espectroscopia Raman foram obtidas com um espectrógrafo micro-Raman, modelo inVia da marca Renishaw, sendo o Raman equipado com microscópio óptico da marca Leyca. O comprimento de onda do laser utilizado foi de 785 nm e com rede de difração de 1200 linhas por mm. O tempo de exposição adotado foi de 10 segundos, com 5 acumulações e intervalo espectral foi de 200 cm^{-1} a 1800 cm^{-1}. Os espectros foram coletados utilizando uma lente objetiva de 50X.

Nesta análise, foram usados picos de intensidade relativos de alguns pares de banda (430, 960, 1070, 1272, 1660) do espectro de Raman. Assim, as seguintes razões foram usadas: $v^2PO_4^{3-}$/amide III (430/1272) (Roschger et al. 2014), $v^1PO_4^{3}$/amide I (960/1660) (Ciubuc et al. 2018) e $v^1CO_3^{2-}$/amide I (1070/1660) (Morris e Mandair 2011). A razão 430/1272 está relacionada com o conteúdo de cálcio (Roschger et al. 2014), a razão 960/1660 indica a quantidade de mineralização e a razão 1070/1660 pode indicar o *turnover* e a atividade de remodelamento ósseo (Ciubuc et al. 2018; Morris e Mandair 2011) (Tabela 2)

Tabela 2. Bandas de espectros Raman atribuídas ao tecido ósseo.

Banda	Atribuição da banda
430	Alongamento simétrico de $v_2PO_4^{3-}$ (fosfato HA)
872	Principalmente hidroxiprolina de colágeno
960	Alongamento simétrico de $v_1PO_4^{3-}$ (fosfato HA)
1070	$v_1CO_2^{3-}$ (sobrepõe-se aos componentes de $v_3PO_4^{3-}$)
1272	Amida III (proteína α-hélice)
1660	Amida I (componente amida I v(C=O) mais forte)

HA - hidroxiapatita

Índice Seedor

Com auxílio de um paquímetro de precisão, foi mensurado o comprimento do fêmur em milímetros. Esta medida foi entre o ponto mais próximo do fêmur até o seu ponto mais distal, mais próximo da articulação do joelho (Lammers, German, e Lightfoot 1998). Os fêmures também foram pesados (miligramas) em balança analítica. Então, foi calculado o índice Seedor que representa um indicativo para densidade óssea (Ferreira Junior et al. 2018; Seedor, Quartuccio, e Thompson 1991) e quanto menor for o valor obtido sugere-se uma menor densidade mineral óssea. Para calculá-lo, usou-se a seguinte equação (Seedor et al. 1991): índice Seedor (mg/mm) = massa do osso (mg) / comprimento do osso (mm).

Análise estatística

A análise estatística dos dados foi realizada pelo teste t de *Student* para comparar duas amostras independentes. Os resultados foram expressos em média ± desvio padrão. Todos os testes foram feitos com 5% de nível de significância ($p<0,05$).

RESULTADOS

Os pesos e os comprimentos dos fêmures não demonstraram diferenças estatísticas entre os grupos Controle e Desreguladores com valores de *p*, respectivamente de 0,0744 e de 0,2303. De modo semelhante o índice Seedor também foi similar estatisticamente entre os referidos grupos (*p*=0,0674) (Figura 1).

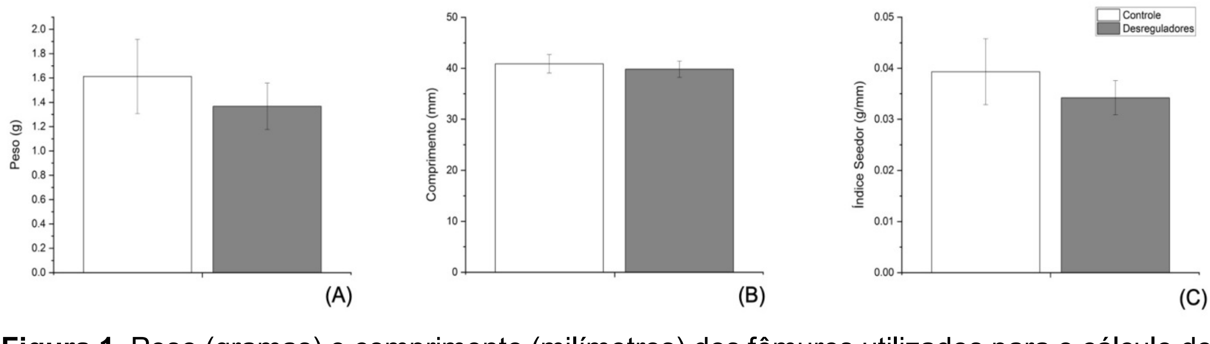

Figura 1. Peso (gramas) e comprimento (milímetros) dos fêmures utilizados para o cálculo do índice Seedor (gramas/milímetros). Os resultados foram expressos em média ± desvio-padrão.

O ensaio mecânico de flexão em três pontos foi analisado sob a perspectiva das propriedades estruturais. Tanto a força máxima de resistência (*p*=0,2815) quanto a energia absorvida na falha (*p*=0,5684) não apresentaram diferenças (Figura 2, A e

D). Porém, o grupo Desreguladores demonstrou maior deformação (*p*=0,0485) e menor rigidez extrínseca (*p*=0,0208) comparado ao grupo Controle (Figura 2, B e C). Também foi plotado o gráfico de curva aparente obtido pelas médias de força e de deformação absoluta (Figura 3).

A espectroscopia de espalhamento Raman foi estudada ao se analisar cinco bandas espectrais (430, 872, 960, 1070, 1272 e 1660). Quatro delas foram apresentaram diferença estatística, tais como 872 (*p*=0,0458), 1070 (*p*=0,0070), 1272 (*p*=0,0079) e 1660 (*p*=0,0126), sendo 872 e 1070 maiores no grupo Controle e as outras duas maiores no grupo Desreguladores (Figura 4). Além disso, foram encontradas diferenças para todas as razões de bandas analisadas com *p*=0,0079 para 430/1272 (Figura 5A), *p*=0,0032 para 960/1660 (Figura 5B) e com *p*<0,0001 para 1070/1660 (Figura 5C).

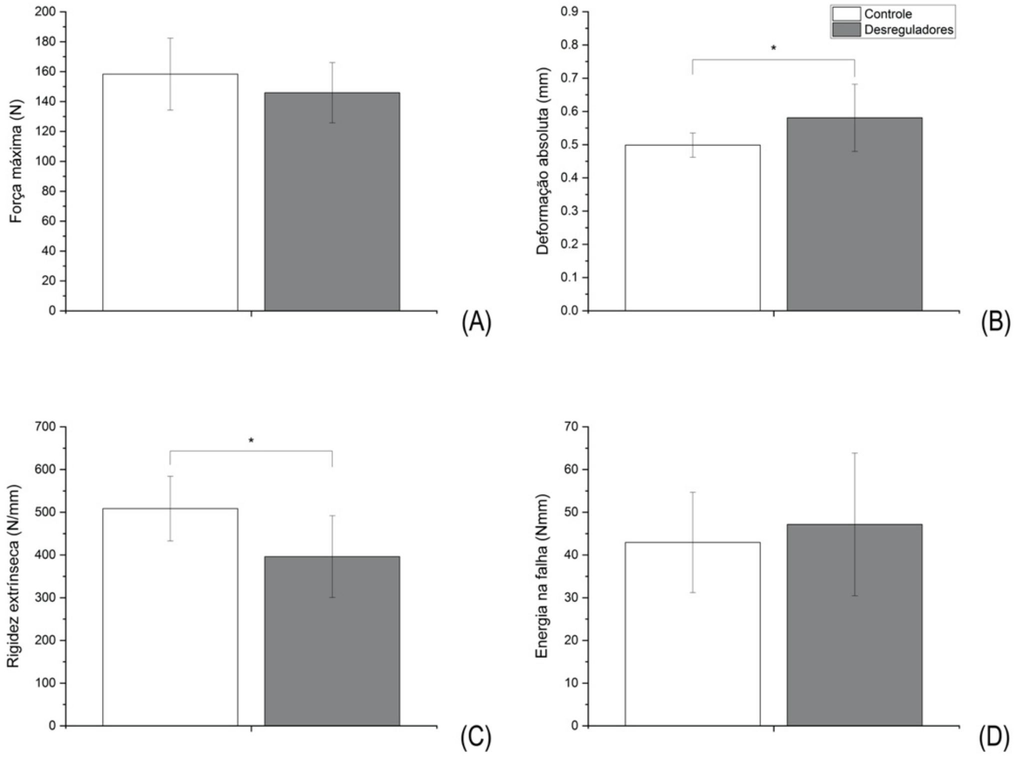

Figura 2. Propriedades estruturais (força máxima, deformação absoluta na força máxima, rigidez extrínseca e energia na falha) dos grupos Grupo Controle e Grupo Desreguladores Endócrinos. O asterisco indica diferença estatística entre os grupos (p<0,05). Resultados expressos em média ± desvio-padrão.

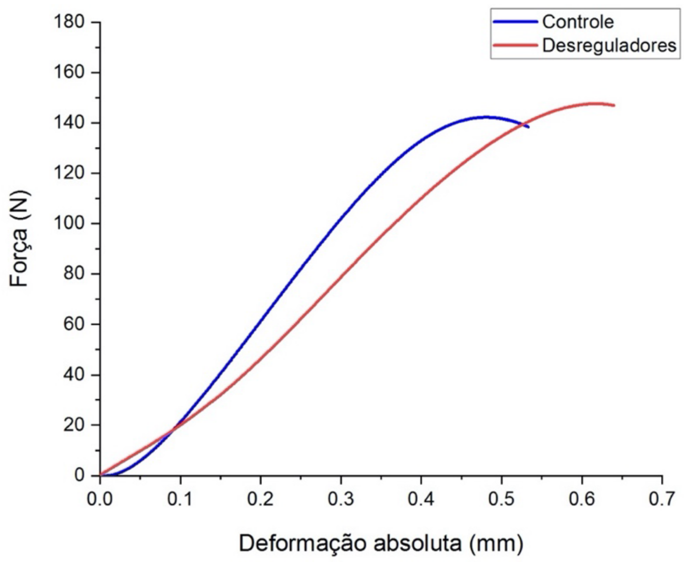

Figura 3. Curva Força (Newton) *vs*. Deformação Absoluta (milímetros) aparente obtidas do grupo Controle e do grupo Desreguladores Endócrinos.

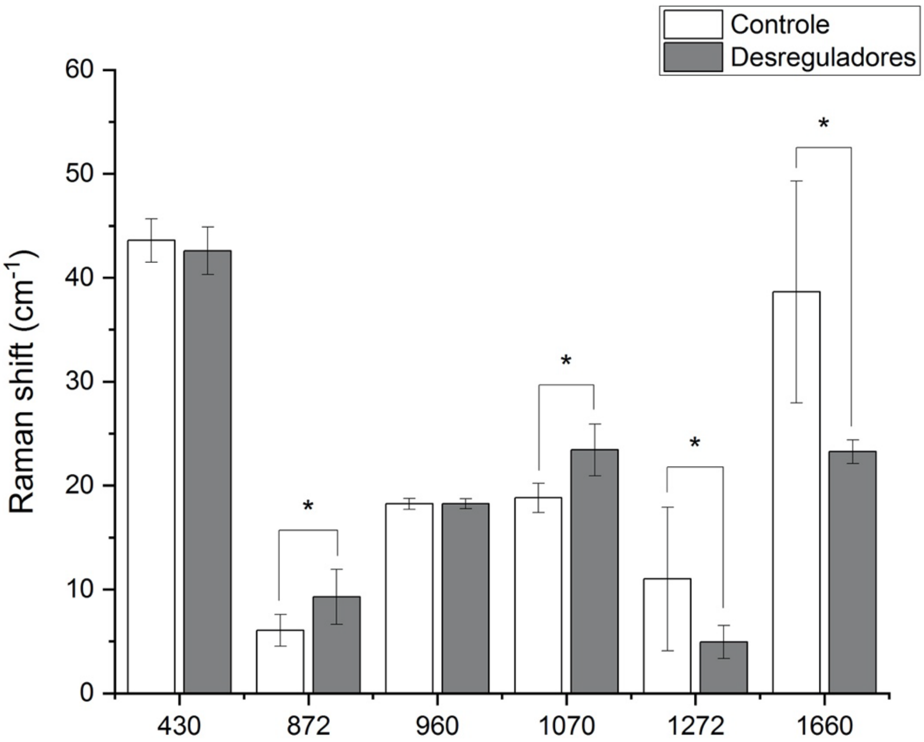

Figura 4. Bandas do espectro de Raman (430, 872, 960, 1070, 1272 e 1660). O asterisco demonstra que houve diferença estatística entre o grupo controle e o grupo desreguladores endócrinos ($p<0,05$). Os resultados foram expressos em média ± desvio-padrão.

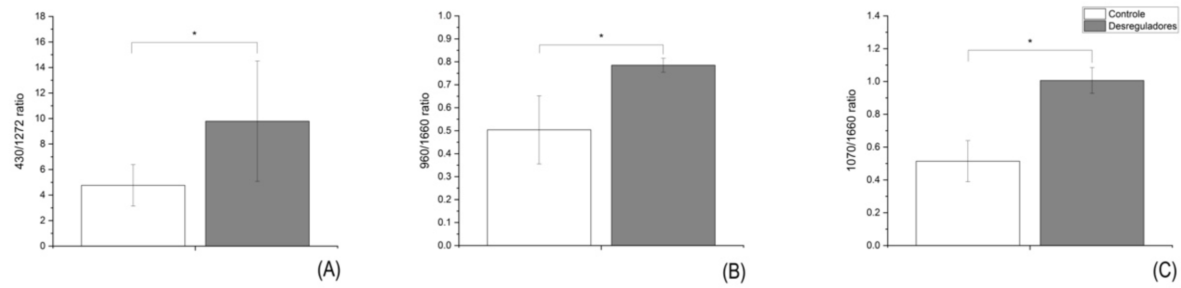

Figura 5. Razões 430/1272, 960/1660 e 1070/1660. O asterisco demonstra que houve diferença estatística entre o grupo controle e o grupo desreguladores endócrinos (p<0,05). Os resultados foram expressos em média ± desvio-padrão.

DISCUSSÃO

Este estudo apresentou como proposta avaliar a resposta óssea mediante à exposição de substâncias químicas, como os desreguladores endócrinos (DEs), em ratos durante o ciclo vital. Desta forma, o tecido ósseo foi estudado que concerne ao peso e comprimento do fêmur, da sua composição, da sua estimativa de densidade mineral óssea e da sua resistência mecânica pressupondo-se que a ocorrência de possíveis efeitos adversos.

De acordo com os resultados obtidos, a exposição aos DEs não foi capaz de causar alterações significativas no peso e no comprimento dos óssos quando comparado ao grupo controle. Além disso, a densidade óssea avaliada através do Índice de Seedor também não demonstrou alterações. De acordo com Yaglova and Yaglov (2021) demonstra que a exposição aos DEs pode perturbar o metabolismo ósseo e a principal razão é a interrupção dos efeitos que o estrogêno desempenha na mineralização e desenvolvimento dos ossos. Segundo os autores, o bisfenol A (BPA), uma das substâncias químicas utilizadas neste estudo, tem a capacidade de promover alterações no metabolismo ósseo ainda durante o desenvolvimento intrauterino.

Contudo, é necessário considerar que fêmeas podem apresentar uma maior suscetibilidade a alterações no tecido ósseo em comparação aos machos, devido à influência do estrogênio, hormônio essencial para a manutenção da densidade mineral óssea. Em situações de deficiência do estrogênio, a exposição aos DEs pode intensificar a perda óssea e comprometer a resistência mecânica (Motlani et al. 2023; Wang et al. 2021). A exposição ao BPA inibe a função osteogênica e regula negativamente a via de sinalização do fator de crescimento transformador beta (TGF-β) nos tecidos ósseos, especialmente em fêmeas (Wang et al. 2021). Esta via é crucial para a formação e manutenção óssea. Além disso, o BPA pode alterar a expressão de genes relacionados à formação óssea, como *BMP1, BMP4, SMAD1, SMAD5*, e *RUNX2*, que são mediadores importantes na mineralização óssea (Varma et al. 2024).

A rigidez extrínseca e na deformação absoluta no grupo exposto aos DEs foram prejudicadas quando comparadas às do grupo controle. A rigidez óssea está ligada diretamente com a sua boa mineralização e quando isso ocorre, o osso é capaz de suportar tensões de cargas maiores (Hernandez e van der Meulen 2017). Além disso, o colágeno tipo I é uma proteína que compõem abundantemente a matriz óssea e concede a resistência e a flexibilidade aos ossos. Deste modo, haverá uma maior resistência as tensões mecânicas e diminuindo as chances de ocorrer fraturas (Hung, Hutton, e Grayson 2013). Entretanto, o estudo de (Sy et al. 2024) afirma que a exposição à essas substancias químicas pode propiciar um desequilíbrio durante o processo de formação e remodelação óssea, diminuindo a densidade óssea e resistência mecânica.

O estudo de (Lind et al. 2017) demonstrou que após ratas prenhas serem submetidas a baixas doses ao BPA (diluído em água) não tiveram suas propriedades biomecânicas alteradas. Segundo uma análise mecânica feita no estudo de (Massing et al. 2022), ao comparar grupos de camundongos ovariectomizados que foram submetidos a diferentes modalidades de treinamento físico e sem treinamento, foi possível constar que para ambos houve redução da resistência mecânica e elasticidade óssea. Neste caso, o exercício físico não foi capaz de preservar a perda óssea. O uso de glicocorticoides de forma prolongada também pode provocar alterações na resistência mecânica e com isso (Bozzini et al. 2015) propôs avaliar o efeito prolongado do uso de dexametasona em ratas fêmeas com 23 dias de idade durante 4 semanas. Após a realização do teste mecânico, nota-se um enfraquecimento significativo, nas perspectivas de força e rigidez do osso quando comparado ao grupo controle.

Para a análise da espectroscopia de espalhamento Raman, cinco bandas espectrais foram estudadas (430, 872, 960, 1070 e 1660). Em destaque para a banda 872, que está intimamente ligada ao aumento da hidroxiprolina de colágeno. O estudo de (Russell 1997) nos proporciona entender que o colágeno tipo I é a principal proteína no osso para fornecer resistência. Sua sequência de aminoácidos é formada principalmente por glicina e prolina, deste modo a hidroxiprolina é produto da hidroxilação pós-traducional da prolina. A hidroxiprolina é considerada um marcador bioquímico e através da sua dosagem sérica é possível compreender os efeitos adversos que o aumento na sua produção pode gerar na homeostase do tecido ósseo. (Rai et al. 2021) em seu estudo, analisou os biomarcadores rina osfatase alcalina total e hidroxiprolina urinária de mulheres antes e após-menopausa em amostras de sangue e urina e em seguida avaliou a densidade mineral óssea através da técnica de ultrassom. Constatou-se que a combinação dessas dosagens foi útil para à predição da densidade mineral óssea, além de prever a osteoporose no período pós-menopausa. (Engelen, Com, e Deutz 2014) corrobora afirmando em seu estudo que, ao avaliar a produção de hidroxiprolina no corpo inteiro através da metodologia de isótopos estáveis em pacientes com fibrose cística, o aumento no nível sérico está associado à perda mineral óssea grave.

Contudo, foi possível notar diferenças significativas nos resultados das razões das bandas. Em destaque para a razão 430/1272 que está ligada ao aumento no conteúdo de cálcio. O Cálcio Iônico (Ca^{2+}) está presente em compartimentos intra e extracelulares no organismo vivo. Em uma das suas funções, o Ca^{2+} junto à ions de fosfato é responsável pela regulação e mineralização óssea. Entretanto, o aumento e a diminuição desses íons podem ocasionar desequilíbrios no metabolismo e restringir a reabsorção óssea (Reid e Bristow 2020). Observou-se no estudo de GODA et al., 1998 que após a indução de maltitol (um álcool dissacarídeo) na dieta de ratos durante 21 dias, houve uma maior retenção e absorção intestinal de cálcio, consequentemente levando a um aumento da resistência à ruptura no ossos. VITKU et al., 2018 propuseram avaliar o impacto da exposição dos DEs das famílias dos Bifenóis e Parabenos no metabolismo do cálcio-fosfato e na densidade mineral óssea em mulheres pós-menopausa. Através da regressão linear múltipla, foi possível notar uma associação positiva no aumento do nível de cálcio no plasma associada aos níveis de Bisfenol A, porém não possível encontrar associação da densidade mineral óssea com os níveis de Bisfenol A, descartando a associação com os níveis elevados de cálcio.

Entretanto, considerando que a exposição aos DEs representa um risco considerável para o tecido ósseo, a saúde humana também pode estar suscetível às alterações de forma sistêmica. Além de interferir diretamente na regulação hormonal, substâncias como os ftalatos podem afetar de maneira significativa a função reprodutiva, ocasionar distúrbios neurológicos durante o desenvolvimento e aumentar o risco de parto prematuro (Ahn e Jeung 2023). Ademais, estudos indicam que o impacto dos DEs pode estar relacionado até mesmo com processos carciogênicos. Calaf et al. (2020) afirma que os altos níveis de BPA aumentam o risco de câncer de mama, induzindo a proliferação celular por meio da redução da taxa de apoptose. Além disso, a exposição crônica a essa substância tem sido associada à alterações no metabolismo da glicose, no aumento da resistência à insulina e maior predisposição a doenças cardiovasculares (Keskesiadou et al. 2024).

Considerando os fatos apresentados, é possível concluir que a exposição aos DEs pode afetar de maneira significativa a saúde óssea na vida adulta, incluindo alterações nas propriedades biomecânicas e na mineralização do tecido ósseo. Embora a interação dos DEs com o metabolismo ósseo seja complexa, os estudos analisados sugerem que o aumento dos níveis de cálcio pode afetar negativamente o equilíbrio no processo de formação e reabsorção óssea, comprometendo a resistência e qualidade do osso. Entretanto, novos estudos são necessários para aprofundar a compreensão dos efeitos da exposição aos DEs nos demais ciclos da vida.

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ANEXOS

ANEXO A
Normas do periódico *Environmental Science and Pollution Research*

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Manuscript Submission

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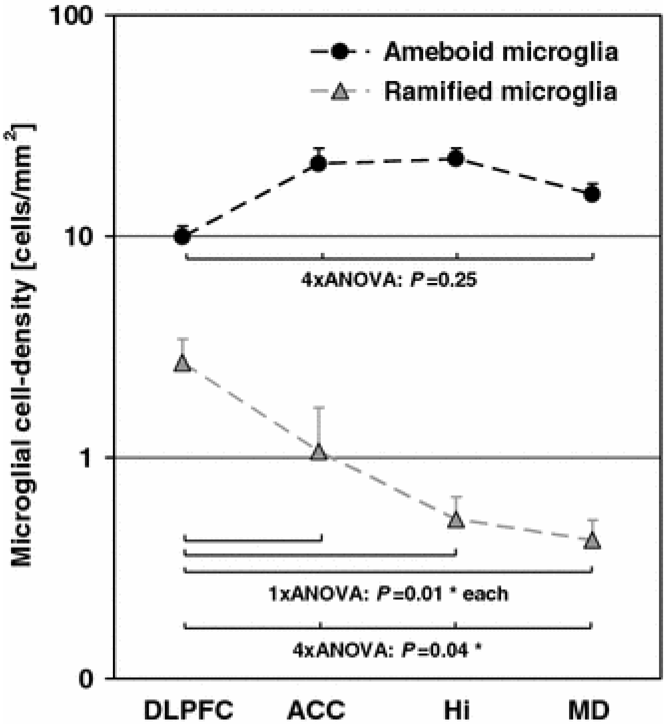

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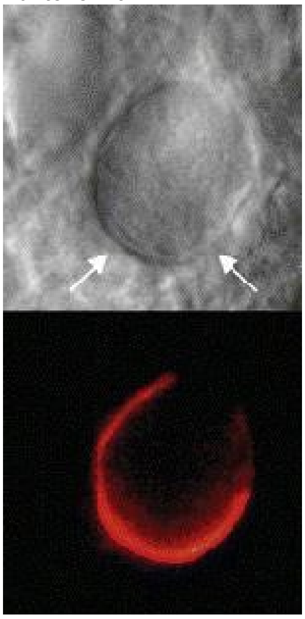

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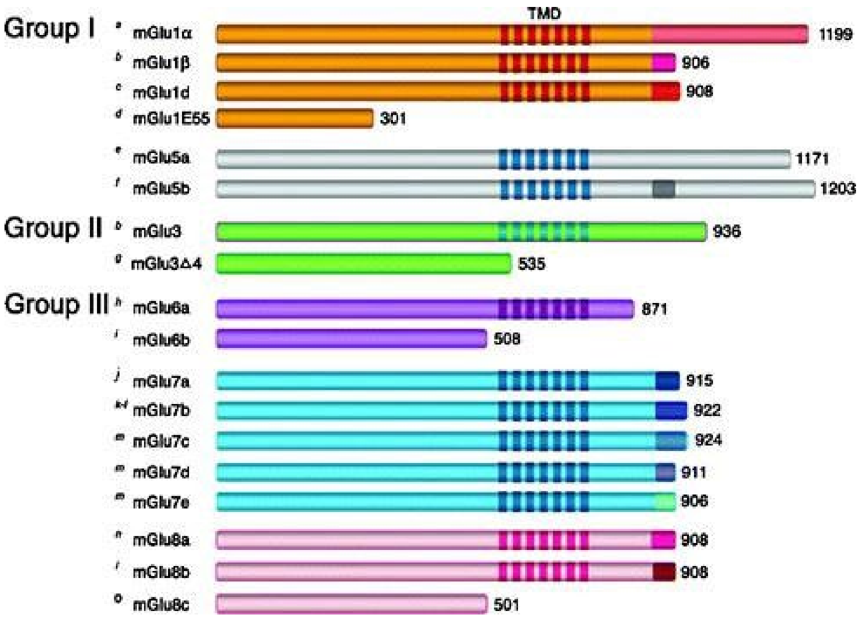

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Sample statements for **"Consent to participate"**:

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Summary of requirements

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PPG - Programa de Pesquisa de Pós-Graduação

Parecer Final

Declaramos para os devidos fins que o Projeto de Pesquisa intitulado **"Efeitos no tecido ósseo de ratos submetidos à exposição de desreguladores endócrinos durante a vida fetal e a lactação"**, cadastrado na Coordenadoria de Pesquisa, Desenvolvimento e Inovação (CPDI) sob o nº **8709** e tendo como participante(s) **GUSTAVO ESTEVAM NOBREGA THOMAZ (discente), WILSON ROMERO NAKAGAKI (docente responsável)**, foi avaliado e **Aprovado** pelo **Comitê Assessor de Pesquisa Institucional (CAPI), Comissão de Ética Uso de Animais (CEUA)** da Universidade do Oeste Paulista - UNOESTE de Presidente Prudente/SP.

Este Projeto de Pesquisa, que envolve a produção, manutenção e/ou utilização de animais pertencentes ao filo Chordata, subfilo Vertebrata (exceto o homem), para fins de pesquisa científica, encontra-se de acordo com os preceitos da Lei nº 11.794, de 8 de Outubro de 2008, do Decreto nº 6.899, de 15 de Julho de 2009, e com as normas editadas pelo Conselho Nacional de Controle da Experimentação Animal (CONCEA), tendo sido Aprovado em reunião realizada em 08/05/2024 00:00:00.

MATERIAL ARMAZENADO/DOADO COM ORIGEM EM PESQUISA

Protocolo(s)	Data Aprovação	Armazenado (local)	É doação	Detalhes armazenamento
6034	11/03/2020 00:00:00	Unoeste	SIM	Biotério experimental da Unoeste

Presidente Prudente, quinta-feira, 28 de novembro de 2024.

Prof. Dr. Jair Rodrigues Garcia Jr.
Docente Responsável pela CPDI

Prof. Dr. Felipe Rydygier de Ruedige
Coordenador da CEUA - UNOESTE

Coordenadoria de Pesquisa, Desenvolvimento e Inovação – CPDI – 18 3229-2079 – cpdi@unoeste.br
Comitê de Ética em Pesquisa – CEP – 18 3229-2079 – cep@unoeste.br
Comissão de Ética no Uso de Animais – CEUA – 183229-2079 – ceua@unoeste.br

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