PRÓ-REITORIA DE PESQUISA E PÓS-GRADUAÇÃO
MESTRADO EM CIÊNCIA ANIMAL

**ALINE DE OLIVEIRA SANTOS**

# EXPRESSÃO GÊNICA DE *GHR, IGF1* E *IGF2* NO OVÁRIO DE RATAS SUBMETIDAS AO TREINAMENTO RESISTIDO E/OU AO GH

Presidente Prudente - SP
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Dissertação apresentada Pró-Reitoria de Pesquisa e Pós-Graduação, Universidade do Oeste Paulista, como parte dos requisitos para obtenção do título de Mestre em Ciência Animal – Área de concentração: Fisiopatologia Animal.

Orientador:
Profa. Dra. Ines Cristina Giometti

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2019

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S237e

Santos, Aline de Oliveira.
Expressao Gênica de *Ghr*, *Igf1* e *Igf2* no Ovário de Ratas Submetidas ao Treinamento Resistido e/ou ao GH / Aline de Oliveira Santos. – Presidente Prudente, 2018.
50f.: il.

Dissertação (Mestrado em Ciência Aniamal) - Universidade do Oeste Paulista – Unoeste, Presidente Prudente, SP, 2019.
Bibliografia.
Orientador: Ines Cristina Giometti

1. Expressão gênica. 2. Hormônio do crescimento. 3. Ovário. I. Título.

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Presidente Prudente,01 de março de 2019

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## DEDICATÓRIA

À Deus, que é a inteligência suprema, causa primeira de todas as coisas.

À Jesus, meu mestre amado, que guia e ilumina meus caminhos e aos desígnios do meu Anjo protetor me acompanhando a cada dia dessa existência.

Aos meus pais Widnes e Silvana pelo apoio, em especial à minha querida mãe, que não mede esforços, me alicerçando em todos os momentos da minha vida, com tamanha serenidade e sabedoria.

À minha Vó, Idalcina, que demonstra muita admiração por mim, meu muito obrigada, porém, essa admiração sinto eu por ela, por ser uma estimada mulher.

Aos meus tios Davi Guarda e Geane de Deus Guarda, que me ajudaram quando mais precisei.

## AGRADECIMENTOS

À minha querida orientadora Profa. Dra. Ines Cristina Giometti, por todo conhecimento compartilhado, pela paciência e carinho na orientação e pela amizade, minha imensa admiração por essa pessoa maravilhosa.

Ao meu querido ex-orientador o Pesquisador Dr. Ricardo Firetti, de onde começou o meu interesse pela área acadêmica, pois me norteou e me orientou com esmero, abrindo portas para minha formação profissional.

À Profa. Dra. Sheila Merlo G. Firetti, que me ensinou com entusiasmo e transferiu seus conhecimentos laboratoriais, pessoa na qual possuo muita admiração.

À Profa. Dra. Caliê Castilho, pelo incentivo e pela indicação para que eu ingressasse no Mestrado.

Ao Prof. Dr. Anthony Castilho, também pelo apoio para meu ingresso no Mestrado.

A todos professores dos programas de Mestrado em Ciência Animal e Mestrado em Educação que contribuíram para meu conhecimento e para minha formação profissional e pessoal.

À minha amada amiga Francislaine Garcia, que foi meu amparo em diversos momentos, cedendo abrigo em São Paulo, para os experimentos na USP, aos seus cuidados, seus conselhos com muito carinho. Serei eternamente grata por tudo e espero em breve retribuir com o mesmo amor que recebi.

A todos os amigos que estiveram ao meu lado me proporcionando momentos alegres seja diretamente ou indiretamente; em especial a Gláucia Hernandez e Barbara Sena.

A todos funcionários da UNOESTE que contribuíram de alguma maneira para o desenvolvimento deste trabalho em especial a Profa. Luciana Machado Guaberto, como responsável do laboratório na disponibilização deste, também a

técnica do laboratório de Genética Molecular, Mayara Vidotto, que estava sempre pronta a nos atender, preparava tudo com muita eficiência, meu muito obrigada, também pela amizade.

À Universidade do Oeste Paulista e ao Programa de Mestrado em Ciência Animal, por me proporcionar todo conhecimento adquirido nesses anos.

À Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES), proporcionando as bolsas de auxílio financeiro.

*“Quando o mundo estiver unido na busca do conhecimento, e não mais lutando por dinheiro e poder, então nossa sociedade poderá enfim evoluir a um novo nível”*

Thomas Jefferson

## RESUMO

## EXPRESSÃO GÊNICA DE *GHR, IGF1* E *IGF2* NO OVÁRIO DE RATAS SUBMETIDAS AO TREINAMENTO RESISTIDO E/OU AO GH

O hormônio de crescimento (GH) é utilizado de forma indiscriminada por atletas e desconhece o efeito da administração do GH exógeno associado ao treinamento resistido no ovário de mulheres. O GH pode agir diretamente nos órgãos alvos por meio do seu receptor (GHR) ou indiretamente pelo aumento do fator do crescimento semelhante à insulina 1 (IGF1) produzido pelo fígado e liberado na corrente sanguínea ou pela produção local de IGF1 e IGF2. O objetivo foi verificar a expressão gênica de *Igf1*, *Igf2* e *Ghr*, e a população folicular e de corpo lúteo no ovário de ratas Wistar submetidas ao treinamento resistido associado ao tratamento com GH recombinante humano (rhGH). As 40 ratas do experimento foram distribuídas em 4 grupos (n=10): controle (CT); ratas com administração de 0,2 UI/Kg de rhGH (GH); ratas submetidas ao treinamento resistido (RT); e ratas submetidas a administração rhGH e ao treinamento resistido (RTGH). O treinamento resistido foi realizado em meio aquático com carga de 50% do peso do animal, em 4 séries de 10 saltos com intervalo de um minuto entre as séries. As ratas eram submetidas ao treinamento e ao rhGH 3 vezes/semana, em dias intercalados, por 30 dias. Após o período experimental, as ratas foram eutanasiadas em fase de diestro, foram colhidos os ovários para morfologia e para expressão gênica (RT-qPCR) dos genes *Ghr*, *Igf1* e *Igf2*. A análise estatística realizada foi ANOVA seguida do pós teste de Tukey (P<0,05). Não foram encontradas diferenças entre os grupos na expressão gênica de *Igf1* e Igf2, nem tampouco no número de folículos primordiais, primários, secundários, terciparios e corpos lúteos. A abundância de RNAm do Ghr foi maior (P<0,0194) no RT (2,24±0,53) que nos demais grupos; CT (1,06±0,14); GH (1,06±0,13) e RTGH (0,87±0,10). Conclui-se que o GH associado ou não ao treinamento resistido não altera o número de folículos e corpos lúteos, nem tampouco a abundância de RNAm de *Ghr, Igf1* e *Igf2* em ovários de ratas. Porém o treinamento resistido leva ao aumento na expressão gênica de *Ghr* em ovários de ratas Wistar.



**Palavras-chave:** Expressão gênica, GH, hormônio do crescimento, IGF, ovário.

## ABSTRACT

## GENE EXPRESSION OF *GHR, IGF1* AND *IGF2* IN OVARY OF RATS SUBMITTED TO RESISTED TRAINING AND /OR GH

Growth hormone (GH) is used indiscriminately by female athletes and those attending gyms and the effect of exogenous GH administration associated with resistance exercise on the ovaries of these women is unknown. GH can act directly on target organs through its receptor (GHR) or indirectly by increasing the insulin-like growth factor 1 (IGF1) produced by the liver and released into the bloodstream, or by local production of IGF1 and IGF2. The objective of this study was to verify the gene expression of *Igf1*, *Igf2*, and *Ghr* and the follicular and corpus luteum (CL) population in the ovaries of Wistar rats submitted to resistance exercise associated with recombinant human GH (rhGH). The 40 rats were divided into 4 groups (n = 10): control (CT); rats administered 0.2 IU/Kg rhGH (GH); rats submitted to resistance exercise (RT); and rats submitted to rhGH administration and resistance exercise (RTGH). The resistance exercise was performed in the aquatic environment with a load of 50% of the animal's weight, in 4 sets of 10 jumps, with an interval of one minute between sets. The rats underwent exercise and rhGH 3 times/week, on intercalary days, for 30 days. After the experimental period, the rats were euthanized in the diestrus phase, the ovaries were collected for morphology and for gene expression (RT-qPCR) of the Ghr, Igf1 and Igf2 genes. The statistical analysis performed used ANOVA followed by the Tukey test ($P < 0.05$). No differences were found between the groups in *Igf1* and *Igf2* gene expression, or in the number of primordial, primary, secundary, tertiary follicles and corpora lutea. The mRNA abundance of *Ghr* was higher ($P < 0.0194$) in the RT (2.24±0.53) than in the other groups; CT (1.06±0.14); GH (1.06±0.13); and RTGH (0.87±0.10). It is concluded that GH associated or not with resistance exercise does not alter the number of follicles and corpus luteum, or the mRNA abundance of *Ghr*, *Igf1,* and *Igf2* in the ovaries of rats. However, resistance exercise leads to an increase in gene expression of *Ghr* in Wistar rat ovaries.



**Key-words:** Gene expression, GH, growth hormone, IGF, ovary

## LISTA DE SIGLAS

| | |
|---|---|
| GH | Hormônio do Crescimento |
| *Igf1* | Fator de Crescimento Semelhante a Insulina tipo 1 |
| *Igf2* | Fator de Crescimento Semelhante a Insulina tipo 2 |
| *Ghr* | Hormônio receptor de GH |
| rhGH | GH recombinante humano |
| RNA | Ácido ribonucleico |
| IGFBP1 | Insulin-like growth factor binding protein 1 |
| IGFBP3 | Insulin-like growth factor binding protein 3 |
| CT | Grupo sem treinamento resistido e sem administrar GH |
| RT | Grupo com treinamento resistido e sem administração de GH |
| GH | Grupo sem treinamento resistido e com administração de GH |
| RTGH | Grupo com treinamento resistido e com administração de GH |
| RT-qPCR | Reação da Transcriptase Reversa e Reação em Cadeia da Polimerase |
| qPCR | PCR quantitativo em tempo real |
| RPS18 | Proteína ribossomal |
| HPRT1 | Hipoxantina-guanina fosforribosiltransferase |
| LH | Hormônio luteinizante |

**SUMÁRIO**

**ARTIGO PARA PUBLICAÇÃO NA REVISTA BIOLOGICALS**

**(Quails B1 na Medicina Veterinária)**

# Treinamento resistido eleva a abundância de RNAm do receptor de GH em ovários de ratas Wistar

Resistance training elevates abundance of mRNA of GH receptor in ovaries of Wistar rats

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## RESUMO

Os treinamentos físicos interferem no ciclo menstrual de mulheres dependendo da intensidade. Comumente ocorre a associação de tais treinamentos ao uso de hormônio de crescimento (GH) de forma indiscriminada. O objetivo deste estudo foi verificar o número de folículos primordiais, primários, secundários e corpos lúteos e a expressão gênica de *Igf1, Igf2 e Ghr* no ovário de ratas Wistar submetidas ao treinamento resistido e à aplicação de rhGH. As 40 ratas foram distribuídas em 4 grupos (n=10): controle (CT); ratas com administração de 0,2 UI/Kg de rhGH, 3 vezes/semana (GH); ratas submetidas ao treinamento resistido aquático de 4 séries de 10 saltos com incremento de peso, 3 vezes/semana (RT); e ratas submetidas ao rhGH e ao treinamento resistido (RTGH). A abundância de RNAm do Ghr (receptor de GH) foi maior (P<0,0194) no RT (2,24±0,53) que nos demais grupos; CT (1,06±0,14); GH (1,06±0,13) e RTGH (0,87±0,10) nos ovários. Não houve diferença entre os grupos no número de folículos e corpos lúteos, nem na abundância de RNAm de *Igf1* e *Igf2* nos ovários. Concui-se que o treinamento resistido leva ao aumento na expressão gênica de *Ghr* em ovários de ratas Wistar.

**Palavras-chave:** Expressão gênica, GH, hormônio do crescimento, IGF, ovário

## ABSTRACT

Depending on the intensity, physical exercise interferes with the menstrual cycle of women. The association of such training to the use of growth hormone (GH) usually occurs, in an indiscriminate way. The objective of this study was to verify the number of primordial, primary, secondary, tertiary follicles and corpora lutea; and the *Igf1*, *Igf2*, and *Ghr* expression in the ovaries of Wistar rats submitted to resistance training and rhGH application. The 40 rats were divided into 4 groups (n = 10): control (CT); rats administered with 0.2 IU/Kg rhGH, 3 times/week (GH); rats submitted to aquatic resistance training of 4 sets of 10 jumps with weight increases, 3 times/week (RT); and rhGH and resistance training (RTGH) rats. The mRNA abundance of *Ghr* (GH receptor) was higher (P <0.0194) in the RT (2.24±0.53) than in the other groups; CT (1.06±0.14); GH (1.06±0.13); and RTGH (0.87±0.10) in the ovaries. There were no differences between groups in the number of follicles and luteal bodies, or in the mRNA abundance of *Igf1* and *Igf2* in the ovaries. It is concluded that resistance taining leads to an increase in gene expression of *Ghr* in Wistar rat ovaries.


**Key-words:** Gene expression, GH, growth hormone, IGF, ovary

## 1. Introdução

O treinamento físico afeta positivamente a fertilidade feminina, porém seu efeito depende do treinamento, periodização, frequência e intensidade [1]. Treinamentos voluntários em roda melhoram a qualidade do oócito em fêmeas obesas de camundongos [2]. Entretanto, treinamentos de alta intensidade levam a maiores problemas de fertilidade [1], enquanto treinamentos de moderada intensidade são positivos para a fertilidade [3].

Os treinamentos físicos levam a uma modulação na secreção de hormônio do crescimento (GH) e de fator de crescimento semelhante à insulina 1 (IGF1) séricos [4,5]. O treinamento resistido em mulheres, aumenta IGF1 e diminui a proteína ligadora de IGF do tipo 1 (IGFBP1) na circulação [6], além de aumentar o GH, o IGFBP3 e atrasar a puberdade [7].

O IGF1 é um potente estimulante da produção dos esteroides pelas células foliculares humanas e, sob certas condições, interage sinergicamente com as gonadotrofinas; e o sistema GH/IGF desempenha um papel na função ovariana humana, provavelmente pela modulação da ação das gonadotrofinas [8].

O GH atua no ovário de várias espécies de forma direta ou indireta por meio do IGF1 [8]. Segundo Martins et al. [9], a possibilidade de que o GH exerça controle nos folículos ovarianos é apoiada pela determinação da imunoreação do receptor do hormônio do crescimento (GHR) e do RNAm que codifica o GHR no tecido ovariano. Em ovário de ratas, o GHR é observado em oócito e células da granulosa e da teca [9]. A ligação do GH ao seu receptor resulta na ativação de diversos sinais intracelulares a ativação de muitos genes, que garantem a ação pleiotrópica do GH [10].

Quando o GH é administrado *in vivo* exerce ação gonadotrófica sobre a foliculogênese ovariana; este efeito é acompanhado, embora não necessariamente mediado por alterações no IGF1 circulante [8].

O GH tem sido utilizado de forma indiscriminada por mulheres atletas e frequentadoras de academias e se desconhece o efeito da administração do GH exógeno associado ao treinamento resistido no ovário dessas mulheres. Além disso o treinamento físico leva ao aumento do GH sérico e dependendo da intensidade provoca alteração da ciclicidade feminina. Portanto, mais pesquisas são necessárias

para elucidar os mecanismos moleculares de como o treinamento resistido e sua associação com o GH podem modular o sistema reprodutor feminino. E neste contexto, os ratos são indicados como modelo experimental de humanos em vários estudos envolvendo reprodução [11].

A hipótese deste estudo é de que o treinamento resistido associado ao rhGH altera a população folicular e a abundância de RNAm de *Ghr*, *Igf1* e *Igf2*. O objetivo foi verificar o efeito do treinamento resistido e sua associação com o rhGH no número de folículos primordiais, primários, secundários terciários e corpos lúteos e na expressão gênica de *Igf1*, *Igf2* e *Ghr* no ovário de ratas Wistar.

## 2. Material e métodos

### 2.1. Animais e local do experimento

Na presente pesquisa foram utilizadas 40 ratas Wistar (*Rattus norvegicus albinus*), sexualmente maduras, com 9 semanas de idade e média de peso corporal de 188,30±13,26 g.

As ratas foram mantidas em 12 caixas plásticas com dimensões de 41x34x17,5 cm, contendo 3 a 4 animais por caixa no Biotério de Experimentação da Universidade do Oeste Paulista (UNOESTE), em ambiente controlado, temperatura entre 20 - 30°C e luz e umidade (55±15%), em ciclo de luz de 12 horas (claro e escuro) com início do período claro as 7 horas da manhã e término as 19 horas. As ratas receberam água e ração da marca Supralab® (Supra, Empresa Alisul, Brasil) *ad libitum*.

### 2.2. Delineamento experimental

As ratas foram aleatoriamente divididas em quatro grupos (n=10): CT (administração de solução fisiológica); RT (treinamento resistido aquático e administração de solução fisiológica), GH (sem treinamento resistido e com administração de GH) e RTGH (treinamento resistido aquático e com administração de GH). Os procedimentos realizados foram aprovados pela Comissão de Ética no Uso de Animais (CEUA) da UNOESTE processo de número 4269 (Fig 1).

**FIGURA 1**

2.3. Aplicação de GH

As ratas que receberam GH foram submetidas ao uso de 0,2 UI/Kg de GH recombinante humano (Saizen® ; Merck, Brasil), seguindo o protocolo de Kaminsky et al. [12] e Junqueira et al. [13]. A administração foi realizada, por via subcutânea sempre no mesmo horário, 3 vezes/semana, em dias alternados, durante 30 dias. Os outros animais receberam solução fisiológica (0,9% de cloreto de sódio) em volume similar.

2.4. Treinamento resistido

O período de adaptação foi realizado uma semana antes do início do treinamento a cada dois dias e consistiu em uma série de 10 saltos verticais no primeiro dia, duas séries de 10 saltos no segundo dia e três séries de 10 saltos no terceiro dia com coletes acomodados na região anterior ao tórax que continham uma sobrecarga de metal de 50% do peso corporal; com intervalo de um minuto entre as séries. O treinamento ocorreu dentro de um tubo de policloreto de vinila de 25 cm de diâmetro com 38 cm de água aquecida (30°C) em seu interior. Os animais eram pesados, em uma balança de precisão (Modelo BT 8000, Marca Gehaka, Brasil), a cada dois dias de treinamento resistido a fim recalcular a sobrecarga do colete. Após o período de adaptação, os animais foram submetidos ao treinamento resistido que consistiu de 4 séries de 10 saltos com intervalo de 1 minuto entre cada série, por meio de um protocolo de saltos verticais na água, 3 vezes/semana, em dias alternados, por 30 dias consecutivos, como descrito por Malheiro et al. [14] e Castoldi et al. [15]. Após a prática do treinamento físico era realizada a secagem das ratas para retornarem as suas caixas (Fig. 1).

2.5. Colheita dos ovários

O experimento teve duração de um mês e ao final de 4 semanas, as ratas foram anestesiadas com éter etílico e induzidas à morte por exsanguinação, todas na fase de diestro, verificada por citologia vaginal diariamente seguindo protocolo de Marcondes [16].

Metade dos ovários foram armazenados em Solução de Davidson (ácido acético P.A. - 10 ml, formaldeído (37-40%) - 20 ml, etanol a 95% - 30 ml e água destilada -30 ml) por 24 horas, depois lavados em água corrente e então fixados

em álcool 70º para posterior confecção de lâminas de histologia e a outra metade dos ovários foi colocada em 1 mL de TRIzol® (Invitrogen®) em freezer a -80ºC.

2.6. Análise histológica da população folicular e de corpo lúteo

O material foi incluso em parafina e obtidos cortes de 5µm de espessura. As lâminas foram confeccionadas e submetidas à coloração de hematoxilina-eosina. As estruturas ovarianas foram examinadas em duplo-cego, avaliou - se número de folículos primordiais, primários, secundários, terciários e corpos lúteos. Considerou-se os folículos primordiais aqueles que contêm apenas uma camada de células achatadas de tamanho muito pequeno; os primários aqueles que apresentam uma única camada de células da granulosa de aspecto cuboide; secundários aqueles que apresentam mais de uma camada da granulosa, neste estádio já se evidencia a zona pelúcida; terciários aqueles em que a coroa radiata envolve a zona pelúcida e apresentam o antro; e o corpo lúteo [17]. As lâminas contendo um corte de ambos ovários foram observadas em toda a extensão em aumento de 40x, 100x e 400x em microscópio óptico binocular e todas as estruturas ovarianas observadas foram contadas (Fig 2).

**FIGURA 2**

2.7. Análise de expressão gênica (RT-qPCR)

A outra metade dos ovários armazenados foram triturados em homogeneizador de tecidos (Homomix®) e submetidos ao protocolo de extração do TRIzol® de extração total.

A concentração do RNA total recuperado foi mensurada por espectrofotometria. Todas as amostras de RNA total foram tratadas com DNAse antes de serem submetidas à transcrição reversa seguida de reação em cadeia da polimerase quantitativa (RT-qPCR), conforme as instruções do protocolo DNAse I – Amplification Grade (Invitrogen®). A transcrição reversa foi realizada utilizando o protocolo da SuperScript III (Invitrogen®) utilizando OligoDT como oligunocleotídeo iniciador.

A qPCR foi realizada para a análise da expressão gênica relativa, a média dos genes endógenos, proteína ribossomal S18 (*Rps18*) e hipoxantina-guanina fosforribosiltransferase (*Hprt1*) foi utilizada como controle interno da PCR, a fim de normalizar os resultados obtidos para o gene-alvo, já que para ovários de ratas, esses foram os endógenos mais estáveis entre os estudados [18,19]. Os oligonucleotídeos iniciadores (“primers”) para os genes-alvo: *Igf1, Igf2 e Ghr* e para os endógenos foram obtidos a partir de sequências de ratos previamente publicadas (Tab 1). As reações foram realizadas utilizando o sistema TaqMan® (Applied Biosystems, Foster, USA).

As qPCR foram conduzidas em duplicatas para cada amostra e a expressão foi determinada pela quantificação em relação ao gene endógeno. O cálculo das eficiências para os genes alvo e controle foi feita por meio do programa “LinRegPCR”. Para isso, considerou a eficiência média com base na curva de amplificação individual de cada amostra. Para quantificação relativa das amplificações foi empregado o método de Pfaffl [20].

**Tab 1.** Oligonucleotídeos iniciadores (“primers”) do genes-alvos, fator de crescimento semelhante à insulina 1 e 2 (*Igf1 e Igf2*) e receptor do hormônio do crescimento (*Ghr*) e dos genes endógenos, proteína ribossomal S18 (*Rps18*) e hipoxantina-guanina fosforribosiltransferase (*Hprt1*) utilizados na RT-qPCR

| **Primers** | **GeneBanK** | **Produto** |
|---|---|---|
| Primer Taq-Man IGF1 | NM_001082477.2 | 69pb |
| Primer Taq-Man IGF2 | NM_001190162.1 | 74pb |
| Primer Taq-Man GHR | NM_017094.1 | 92pb |
| Primer Taq-Man RPS18 | NM_213557.1 | 62pb |
| Primer Taq-Man HPRT-1 | NM_012583.2 | 64pb |

2.8. Análise estatística

Todos os resultados foram analisados quanto ao pressuposto de normalidade foram empregando-se o teste de Shapiro-Wilk. As variáveis foram submetidas ao teste análise de variância (ANOVA) para comparar as médias dos quatro grupos, seguido do teste de Tukey. O nível de significância adotado para todas as comparações foi de 5%.

3. Resultados

Não houve diferença significativa na média de número de estruturas ovarianas, folículos e corpos lúteos, nos ovários das ratas dos diferentes grupos estudados, (Fig 3).

**FIGURA 3**

Quando comparados os grupos em relação à expressão gênica relativa, observou-se que não houve diferença significativa entre os grupos na expressão de *Igf1* (Fig 4), também não foram encontradas diferenças significativas entre os grupos na expressão de *Igf2* (Fig 5), porém o grupo RT demonstrou uma maior abundância

de RNAm para *Ghr* (P<0,0194) que os demais grupos (Fig 6).

**FIGURAS 4, 5 e 6**

4. Discussão

O treinamento resistido aumentou (P<0,05) a expressão gênica do *Ghr* em ovários de ratas Wistar, apesar disso, esse aumento na abundância de RNAm não resultou em alterações nas estruturas ovarianas, indicando que o treinamento resistido não interferiu na foliculogênese ou ovulação dos (P>0,05).

Os treinamentos de alta intensidade provocam irregularidades menstruais, ausência de ovulação e deficiência da fase luteal em mulheres atletas quando comparadas às sedentárias [21]. Os níveis de hormônio luteinizante (LH) são diminuídos na fase luteínica e no final da fase folicular em mulheres sedentárias que praticam treinamento físico [22], e são menores na fase folicular em mulheres atletas do que em mulheres sedentárias [23]. Sugere-se que o balanço energético negativo devido ao treinamento físico levaria a essa disfunção [24,25]. Porém, no presente estudo não foram observadas diferenças significativas (P>0,05) no ganho de peso, nem na circunferência abdominal entre os grupos (dados não apresentados), o que poderia justificar a ausência de alteração nas estruturas ovarianas apesar dos resultados indicarem uma maior ação do GH nos ovários.

O treinamento físico excessivo também provoca irregularidades na secreção do GH [3]. Estudos têm demonstrado que a secreção de hormônios anabólicos está relacionada com treinamentos de curta duração e alta intensidade [26,27]. Em mulheres de meia idade, o treinamento resistido associado a caminhadas por 12 semanas foi mais efetivo que o treinamento aeróbico associado a caminhadas para aumentar o GH sérico [28]. Em compensação treinamento concorrente não apresentou alteração no GH sérico de mulheres idosas, somente houve aumento no IGF1 sérico, podendo ser explicado pelo fato de mulheres de meia idade apresentarem alterações no GH sérico diferentemente das mulheres idosas [29].

Em outro estudo, observou-se que treinamento físico aumentou os níveis séricos de IGF1 em homens [30], mas não alterou os níveis séricos em mulheres [31]. Em ratos, resultados semelhantes foram encontrados, o treinamento físico em esteira por 6 semanas aumentou os níveis de IGF1 somente em ratos machos, não alterando nas fêmeas [32]. Provavelmente, os treinamentos resistido sejam diferentes dos treinamentos aeróbicos na secreção dos hormônios.

O aumento da expressão gênica do *Ghr* não foi acompanhado por aumento na abundância de RNAm de *Igf1* no ovário de ratas Wistar. Pode ser que o GH tenha

uma ação direta no ovário, sem alterar a expressão gênica de *Igf1* tópico no ovário. Não foi dosado o IGF1 sérico neste estudo e muitos tecidos do corpo também produzem IGF1 [32], apesar do fígado produzir aproximadamente 70% do IGF1 sérico total em resposta ao GH da hipófise [33]. Cruzat et al. [34] relatam que a síntese e a liberação do IGF1 podem ser influenciadas pela concentração do GH, a composição corporal e a concentração de hormônios. Porém, esse estudo refere-se a outros órgãos como fígado, músculo e osso. Não foram encontrados na literatura trabalhos que demonstrassem a expressão gênica de *Igf1* em ovários de ratas submetidas à administração de GH e ao treinamento físico.

Nesse sentido, o presente estudo revelou que, os grupos experimentais não apresentaram alterações quanto à expressão gênica relativa do *Igf1* e do *Igf2* ($p>0,05$) nos ovários das fêmeas submetidas ao treinamento físico e a aplicação do GH, provavelmente isso se deva ao sexo dos animais e à interferência hormonal. É relatado que quando o GH liga-se ao GHR em células do fígado e na maioria das células orgânicas induz uma série de eventos que acabam resultando na produção do IGF1 e IGF2 [35]. Os fisiológicos efeitos de sobrevivência, proliferação, diferenciação celular do IGF1 e IGF2 são mediados via ativação de seu receptor do tipo tirosina quinase [36]. No geral, IGF1 tem um significativo papel no crescimento e IFG2 é relacionado ao desenvolvimento embrionário, mas isso não é bem verdade em se tratando de órgãos reprodutivos que apresentam diferenciação e proliferação celular por toda a vida reprodutiva [36].

O controle da liberação do GH parece ser diferente em machos e fêmeas, pois os hormônios sexuais esteroides provocam diferentes respostas no eixo hipotâmico-hipófise [37]. A expressão gênica do hormônio liberador de GH (GHRH) é de 2 a 3 vezes maior no núcleo arqueado de ratos machos do que de fêmeas [38,39]. Enquanto, em fêmeas, uma situação de hipersecreção de GH provocada por implantação de tumor diminui em duas vezes a expressão hipotalâmica de GHRH, em machos diminui em 7 vezes [40]. Foi demonstrado em estudo que ovariectomia em ratas aumenta a expressão do receptor do GHRH, e a expressão desse receptor é diminuída pela subsequente suplementação de estradiol [41].

Outro possível motivo para nenhuma alteração ser observada no ovário das fêmeas é que aparentemente as fêmeas são menos responsivas ao GH. O tratamento com GH em humanos com deficiência de GH é capaz de aumentar as concentrações plasmáticas de IGF1 em ambos os sexos, mas as mulheres são menos responsivas ao tratamento que os homens [42], especialmente em pacientes que recebem terapia

com estrógeno [43]. A administração de estrógeno em mulheres após a menopausa atenua o efeito do GH exógeno (rhGH) em aumentar o IGF1 plasmático [43]. Além disso, em algumas espécies, o estrógeno e o GH tem habilidade de retroalimentação negativa dos receptores de GH do fígado impedindo a ação do GH [37], o que justificaria nenhuma alteração na expressão gênica de *Igf1*, de *Igf2* e de *Ghr* e das estruturas ovarianas (folículos e corpo lúteo) nos grupos tratados com GH, pois as fêmeas estavam ciclando normalmente, com a presença fisiológica dos hormônios gonadais.

Apesar de estudos demonstrando o envolvimento do GH no crescimento folicular, na ovulação, na função luteínica, na esteroidogênese e na gametogênese [44,45], a atuação do GH com IGFI e gonadotrofinas na reprodução não está clara, pois aparentemente cada espécie apresenta uma interdependência hormonal na foliculogênese diferente [46]. O mecanismo pelo qual o GH estimula o desenvolvimento folicular parece ser espécie-específica e variar durante o ciclo ovariano [46].

Os trabalhos encontrados na literatura com ratas são com fêmeas ovariectomizadas, ou hiofisectomizadas, sem influência hormonal, neste estudo utilizou-se ratas que estavam com todas as fases fisiológicas de ciclicidade reprodutiva, pois a intenção era verificar os efeitos do GH em fêmeas ainda em fase reprodutiva, sob a influência hormonal. E nas condições analisadas de dose, tempo de tratamento, treinamento e fase do ciclo estral (diestro) não houve alteração nos parâmetros verificados, porém não se pode descartar a possibilidade de outros parâmetros reprodutivos que não foram analisados serem alterados com o uso indiscriminado de GH por atletas.

## 5. Conclusão

O treinamento resistido leva ao aumento na expressão gênica de *Ghr* em ovários de ratas Wistar sem alterar o número de estruturas foliculares ou de corpos lúteos e sem interferência na expressão gênica local de *Igf1* ou *Igf2*. O uso associado de GH ao treinamento resistido inibe o incremento na expressão gênica de *Ghr*.

## Conflitos de interesse

Os autores declaram não haver conflitos de interesse.

## Agradecimentos

O presente trabalho foi realizado com apoio da Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) - Código de Financiamento 001.

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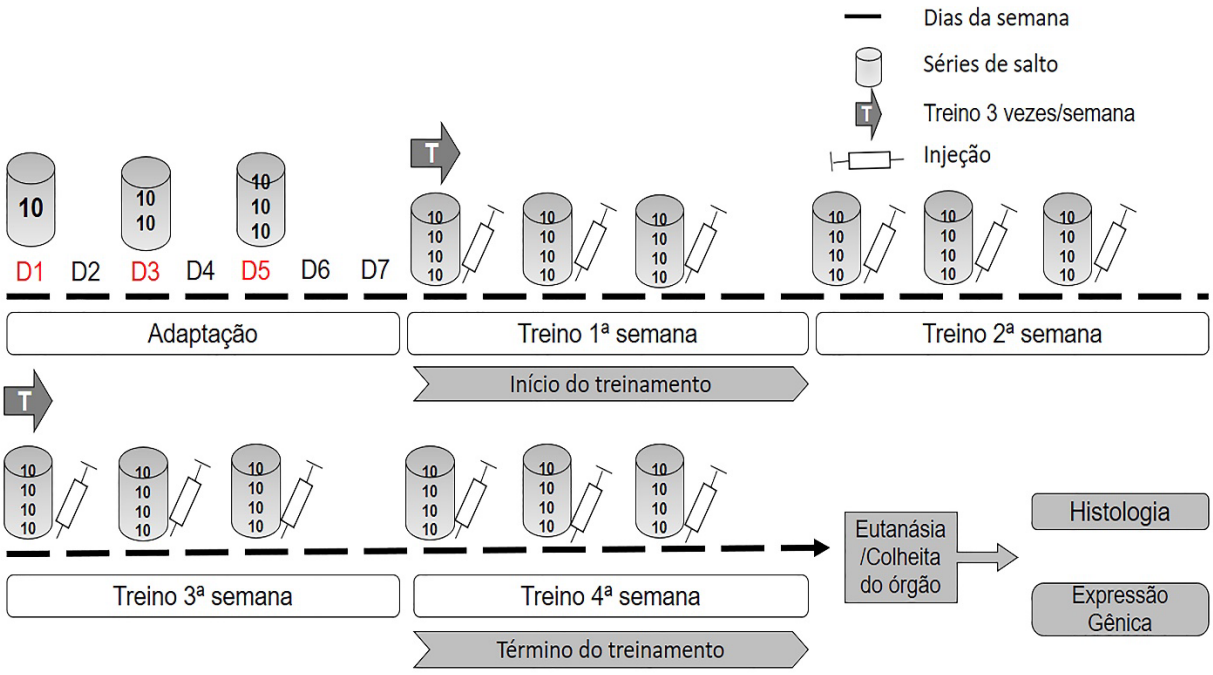


Fig.1 - Desenho experimental do presente estudo com ratas Wistar com 4 grupos experimentais: CT: receberam aplicação de injeção com solução fisiológica nos momentos indicados no desenho; GH: receberam aplicação de injeção com 0,2 UI/Kg de rhGH nos momentos indicados no desenho; TR: receberam aplicação de injeção com solução fisiológica e participaram da adaptação e do treinamento resistido em meio aquático com sobrecarga de 50% do peso do animal nos momentos indicados pelo desenho; TRGH: receberam aplicação de injeção com 0,2 UI/Kg de rhGH e participaram da adaptação e do treinamento resistido em meio aquático com sobrecarga de 50% do peso do animal nos momentos indicados pelo desenho. D=dias

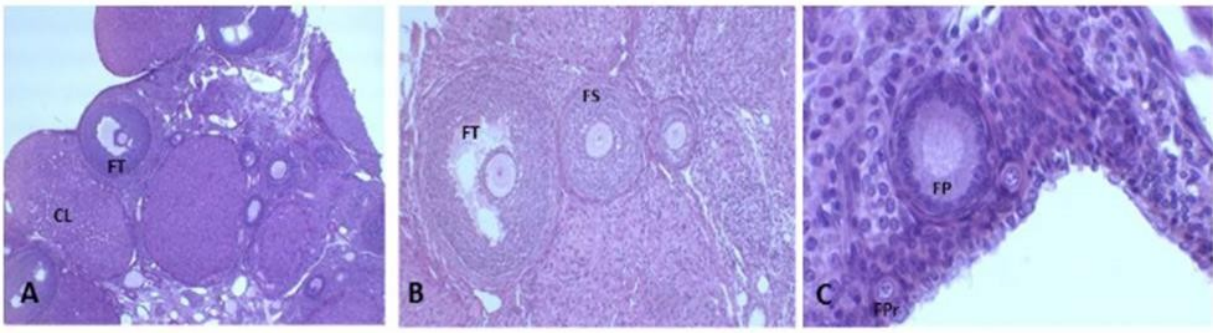


Fig 2. Fotomicrografia de ovário de rata Wistar. **A:** Ovário de rata com hematoxilina-eosina em aumento de 100x em que é possível visualizar as estruturas ovarianas: FT (folículo terciário), CL (Corpo lúteo), **B:** Ovário de rata corado com hematoxilina-eosina em aumento de 400x, em que é possível visualizar as estruturas ovarianas: FT (folículo terciário), FS (folículo secundário), **C:** Ovário de rata corado com hematoxilina-eosina em aumento de 400x, em que é possível visualizar as estruturas ovarianas: FP (folículo primário), FPr (folículo primordial).

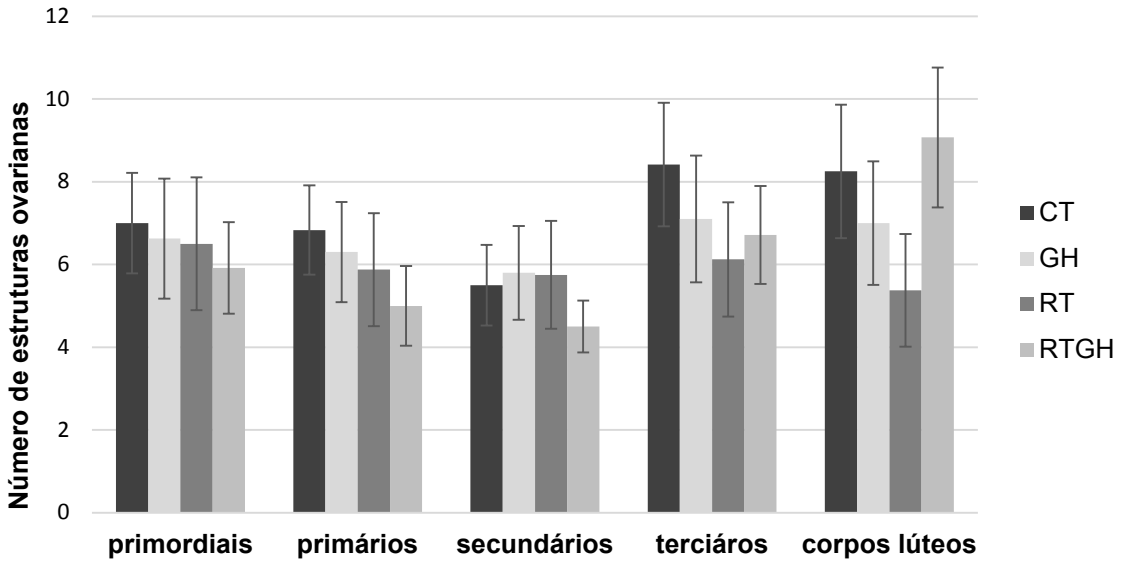


Fig 3. Médias e erro padrão do número de estruturas ovarianas (folículos primordial, primário, secundário, terciário e corpos lúteos) nos ovários de ratas dos diferentes grupos (n=10): CT (controle), GH (tratadas com 0,2UI/Kg de hrGH a cada dois dias), RT (submetidas ao treinamento resistido a cada dois dias) e RTGH (tratadas com hrGH e submetidas ao treinamento resistido). ANOVA seguida de Tukey. Não foram encontradas diferenças entre os grupos nas estruturas ovarianas ($p>0,05$).

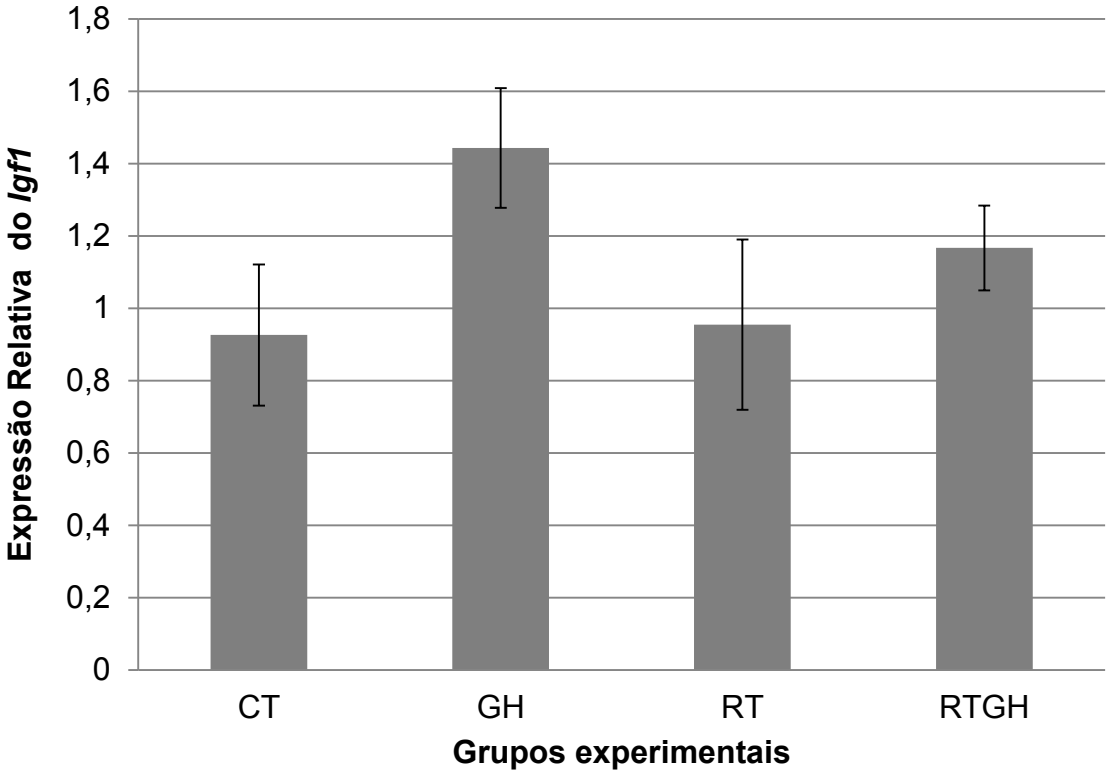


Fig 4. Expressão gênica relativa do *Igf1* em ovários de ratas que foram submetidas aos grupos experimentais (n=10): CT (grupo sem treinamento resistido e sem administrar GH), RT (grupo com treinamento resistido e sem administração de GH), GH (grupo sem treinamento resistido e com administração de GH) e RTGH (grupo com treinamento resistido e com administração de GH). Dois endógenos (*Rps18* e *Hprt1*) foram utilizados para normalização dos dados. ANOVA seguida de Tukey. Não foram encontradas diferenças entre os grupos nas estruturas ovarianas ($p>0,05$).

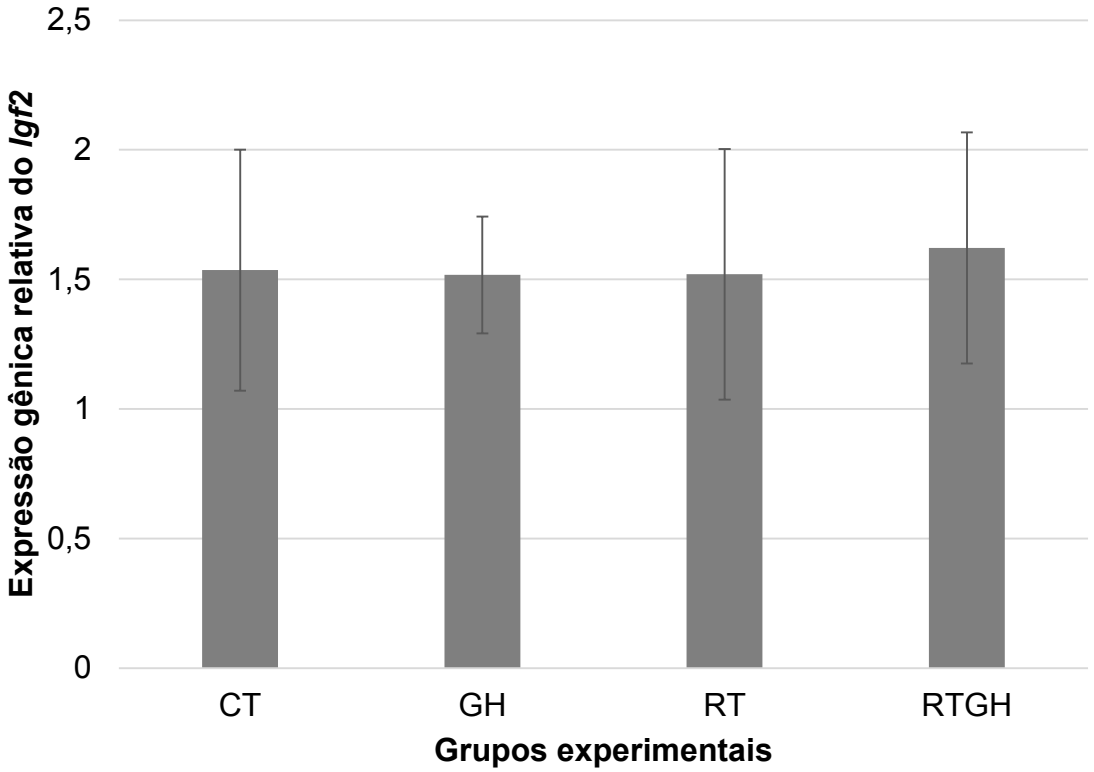


Fig 5. Expressão gênica realtiva do *Igf2* em ovários de ratas que foram submetidas aos grupos experimentais (n=10): CT (grupo sem treinamento resistido e sem administrar GH), RT (grupo com treinamento resistido e sem administração de GH), GH (grupo sem treinamento resistido e com administração de GH) e RTGH (grupo com treinamento resistido e com administração de GH). Dois endógenos (*Rps18* e *Hprt1*) foram utilizados para normalização dos dados. ANOVA sequida de Tukey. Não foram encontradas diferenças entre os grupos nas estruturas ovarianas ($p>0,05$).

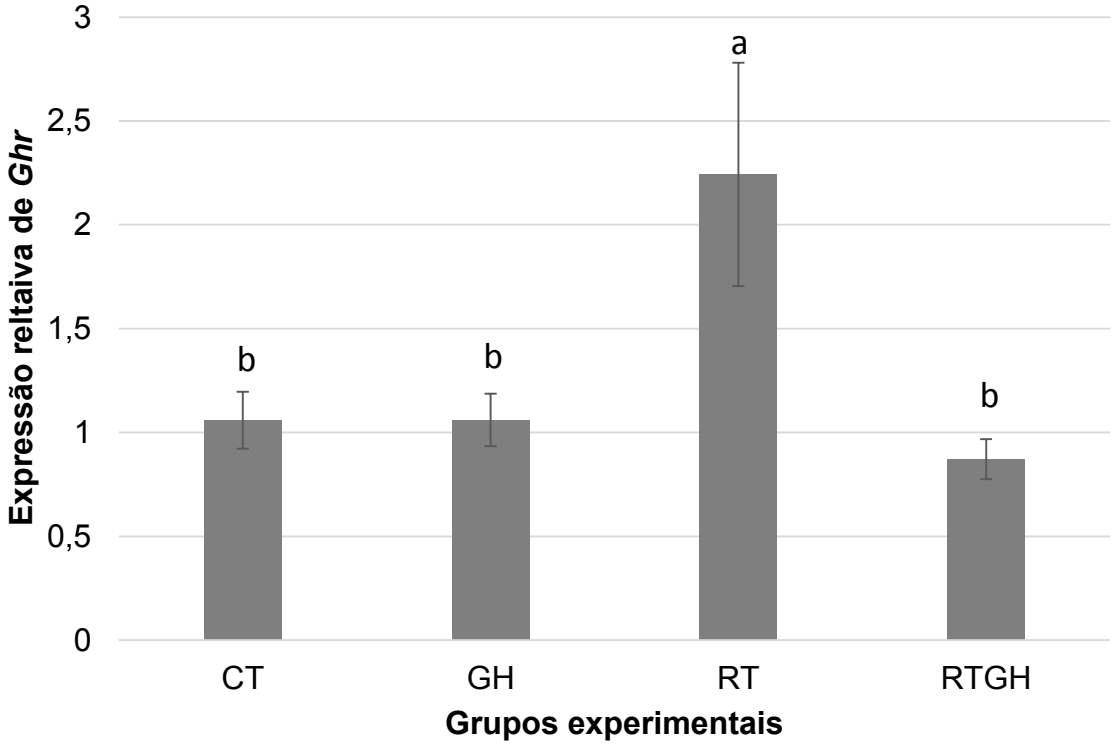


Fig 6. Expressão gênica relativa do *Ghr* em ovários de ratas que foram submetidas aos grupos experimentais (n=10): CT (grupo sem treinamento resistido e sem administrar GH), RT (grupo com treinamento resistido e sem administração de GH), GH (grupo sem treinamento resistido e com administração de GH) e RTGH (grupo com treinamento resistido e com administração de GH). Dois endógenos (*Rps-18* e *Hprt-1*) foram utilizados para normalização dos dados. ANOVA seguida de Tukey. Letras diferentes representam diferença significativa entre grupos em cada momento ($p<0,05$)

**ANEXO 1**



# UNOESTE - Universidade do Oeste Paulista

PRÓ-REITORIA DE PESQUISA E PÓS-GRADUAÇÃO

**PPG - Programa de Pesquisa de Pós-Graduação**
**PROBIC - Programa de Bolsas de Iniciação Científica**

# Parecer Final

Declaramos para os devidos fins que o Projeto de Pesquisa intitulado **"EXPRESSÃO GÊNICA DO GHR NOS OVÁRIOS DE RATAS WISTAR SUBMETIDAS AO HORMÔNIO DO CRESCIMENTO E AO EXERCÍCIO FÍSICO"**, cadastrado na Coordenadoria de Pesquisa, Desenvolvimento e Inovação (CPDI) sob o número nº **4269** e tendo como participante(s) **ALINE DE OLIVEIRA SANTOS (discente), GISLAINE DA SILVA RODRIGUES (discente), ANDREA SATIKO MURATA (discente), PAULO HENRIQUE GUILHERME BORGES (discente), REGINA RAFAEL TEIXEIRA (participante externo), CALIE CASTILHO SILVESTRE (docente), LUCIANA MACHADO GUABERTO (docente), INES CRISTINA GIOMETTI CEDA (orientador responsável)**, foi avaliado e **APROVADO** pelo **COMITÊ ASSESSOR DE PESQUISA INSTITUCIONAL (CAPI) e 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 13/12/2017.

Vigência do projeto: 01/2018 a 07/2019.

| Espécie/Linhagem | Nº de Animais | Peso | Idade | Sexo | Origem |
|---|---|---|---|---|---|
| Ratos Wistar | 40 | 200 gramas | 90 dias | F | Biotério |

Presidente Prudente, 24 de Janeiro de 2018.

Prof. Dr. Jair Rodrigues Garcia Jr.
Coordenador Científico da CPDI

Profª Ms. Adriana Falco de Brito
Coordenadora da CEUA - UNOESTE

**ANEXO 2**

# BIOLOGICALS

**FACTOR**

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## ABSTRACTING AND INDEXING

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•BCG Bacille Calmette-Guerin
•CCID50 median cell culture infective dose
•CCTD50 median cell culture toxic dose
•CF complement fixation
•cfu colony forming units
•cpe cytopatahic effect
•cpm counts per minute
•DEAE-cellulose diethylaminoethyl-cellulose
•DF degrees of freedom
•DNA deoxyribonucleic acid
•eop efficiency of plating

- HA haemagglutination
- HAI haemagglutination inhibition
- IgA immunoglobulin A
- ID50 median infective dose
- IgE immunoglobulin E
- IgG immunoglobulin G
- IgM immunoglobulin M
- ImD50 median immunizing dose
- IU International Unit
- Lf Flocculation unit
- LD50 median lethal dose
- MIC minimum inhibiting concentration

•NCTC National Collection of Type Cultures

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- PAGE polyacrylamide gel electrophoresis
- P probability
- PD50 median paralytic dose
- pfu plaque forming unit
- RBC erythrocyte
- RNA ribonucleic acid
- SDS sodium dodecyl sulphate
- SD standard deviation
- SEM Standard error of the mean
- WBC leucoyte

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[5] Cancer Research UK. Cancer statistics reports for the UK, http://www.cancerresearchuk.org/aboutcancer/statistics/cancerstatsreport/; 2003 [accessed 13 March 2003].

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