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UNIVERSIDADE ESTADUAL PAULISTA "JÚLIO DE MESQUITA FILHO"
FACULDADE DE MEDICINA VETERINÁRIA E ZOOTECNIA
CURSO DE PÓS-GRADUAÇÃO EM BIOTECNOLOGIA ANIMAL

POTENCIAIS BIOMARCADORES DE FERTILIDADE E PRODUÇÃO ESPERMÁTICA DE GARANHÕES TRATADOS COM ANÁLOGO DE GnRH (LECIRELINA)

Thaís Mendes Sanchez Cavalero

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Tese apresentada a Faculdade de Medicina Veterinária e Zootecnia da Universidade Estadual Paulista "Júlio de Mesquita Filho", Campus de Botucatu, para obtenção do título de Doutora em Medicina Veterinária.

Orientador: Prof. Dr. Frederico Ozanam Papa

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DEDICATÓRIA

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SUMÁRIO

CAVALERO, T.M.S. BIOMARCADORES DE FERTILIDADE E PRODUÇÃO ESPERMÁTICA DE GARANHÕES TRATADOS COM ANÁLOGO DE GnRH (LECIRELINA). Botucatu – SP. 2023. 80p. Tese (Doutorado) – Faculdade de Medicina Veterinária e Zootecnia, Campus Botucatu, Universidade Estadual Paulista.

Resumo

O uso de biotecnologias aplicadas ao sêmen é pouco eficaz em processos que reduzem drasticamente a produção espermática, como a senilidade e disfunções endócrinas. Nesses processos, o uso de GnRH tem ocasionado melhora da quantidade e qualidade espermática em algumas espécies. O objetivo desta pesquisa foi avaliar a eficácia e os efeitos adversos da aplicação de GnRH em garanhões na melhora da qualidade seminal. No experimento 01 foram utilizados garanhões com qualidade espermática normal, divididos em grupo controle (n=3), e tratado (n=4). O grupo tratado recebeu doses diárias de lecirelina (50 μg;im) durante 60 dias, ambos os grupos foram avaliados durante o tratamento e por mais 60 dias após o término do tratamento. Amostras de sêmen e soro sanguíneo foram semanalmente coletadas. No Exp. 02 foram utilizados garanhões subférteis (n=4), com histórico de ≤ 20% de taxa de concepção, baixa qualidade ou produção espermática. Os subférteis foram submetidos ao mesmo tratamento do Exp.01, porém com avaliações quinzenais dos parâmetros espermáticos e hormonais. Em ambos os experimentos a resposta testicular endócrina foi mensurada por dosagem sérica de inibina-B, AMH e sulfato de estrona, determinadas por ELISA; e testosterona por radioimunoensaio. No Exp. 02 também avaliou-se os níveis séricos de FSH e LH por radioimunoensaio. Os parâmetros espermáticos avaliados foram número de espermatozoides totais (NET); cinética espermática pelo sistema CASA (IVOS Version 12 Hamilton Thorne Research, MA, USA): motilidade total e progressiva, número de espermatozoides rápidos; e análise morfofuncional por citometria de fluxo: potencial de membrana mitocondrial, integridade de membrana plasmática e acrossomal, estabilidade de membrana plasmática, peroxidação lipídica e produção de espécies reativas de oxigênio. Os resultados hormonais e espermáticos foram comparados semanalmente com os valores pré-tratamento durante todo o período experimental. Os parâmetros avaliados foram analisados dentro de cada momento pelo teste t (Student) e dentro de cada grupo pela análise de medidas repetidas no tempo (One-way repeated measures). O perfil sérico de AMH, inibina-B e sulfato de estrona não alteraram com o tratamento em nenhum experimento. No Exp.02 não ocorreu redução do FSH, porém o LH diminuiu da semana

1 (P>0.001) até o final do experimento. A testosterona sérica apresentou diferença entre as semanas 6 e 16 (302 vs 977 pg/ml, respectivamente P<0,033) e um garanhão subfértil aumentou o NET em 100% na semana 4. No Exp.01 o NET do grupo tratado aumentou em 50% após 4 semanas de tratamento e se mantiveram elevados até o final da avaliação. A estabilidade de membrana e o potencial de membrana mitocondrial aumentaram uma semana após o início do tratamento (P<0,001) no grupo tratado, retornando aos valores pré-tratamento 60d após o fim da aplicação. Em conclusão, embora o tratamento tenha ocasionado queda da testosterona sérica em alguns animais, outros hormônios indicadores da funcionalidade testicular não foram afetados. Já o aumento da produção espermática, da estabilidade e potencial de membrana mitocondrial demonstram que a terapia com lecirelina pode ser benéfica em determinados casos de subfertilidade.

Palavras-chave: subfertilidade, sêmen, equinos, função testicular, neuroendocrinologia

CAVALERO, T.M.S. POTENCIAL FERTILITY BIOMARKERS AND SPERM PRODUCTION IN STALLIONS TREATED WITH GnRH ANALOGUE (LECIRELIN). Botucatu – SP. 2023. 80p. Tese (Doutorado) – Faculdade de Medicina Veterinária e Zootecnia, Campus Botucatu, Universidade Estadual Paulista.

ABSTRACT

The use of biotechnology in semen manipulation is not effective in processes that drastically reduce sperm production, such as senility and endocrine disorders. In these cases, the use of GnRH has led to an improvement in sperm quantity and quality in some species. Thus, the aim of this research was to evaluate the effectiveness and adverse effects of GnRH application in stallions in improving seminal quality. In experiment 01, stallions with normal sperm quality were divided into a control (n=3) and treated group (n=4). The treated group received daily doses of lecirelin (50 µg;im) for 60 days and were evaluated for another 60 days after the end of treatment. Semen and blood serum samples were analyzed weekly during the evaluation period. In experiment 02, subfertile stallions (n=4) with a history of ≤ 20% conception rate with fresh and cooled semen, low quality or sperm production were selected. The subfertile stallions were submitted to the same treatment as Exp.01, but with biweekly evaluations of sperm and hormonal parameters. In experiments 1 and 2, the testicular endocrine response was measured by serum levels of inhibin-B, AMH, testosterone and estrone sulfate. Exp. 02 also assesses serum levels of FSH and LH by radioimmunoassay. The sperm parameters evaluated were total sperm number, total and progressive motility, rapid sperm number, mitochondrial membrane potential, plasma and acrosomal membrane integrity and stability, lipid peroxidation and sperm production of reactive oxygen species (ROS). Hormone and sperm results were compared with pre-treatment values up to 60 days after treatment. Serum concentrations of inhibin-B, AMH and estrone sulfate were determined by ELISA and testosterone by radioimmunoassay. Sperm kinetics were analyzed by the CASA system (IVOS Version

12 Hamilton Thorne Research, MA, USA). Plasma membrane integrity and stability, mitochondrial membrane potential, lipid peroxidation and ROS formation by flow cytometry, only in Exp 01. The evaluated parameters were analyzed within each moment by the t test (Student) and within each group by repeated measures over time analysis (One-way repeated measures). Serum levels of AMH, inhibin-B and estrone sulfate did not change with treatment in any experiment. In Exp.02, there was no reduction in FSH, but LH decreased from week 1 ($P>0.001$) until the end of the experiment. Serum testosterone showed a difference between weeks 6 and 16 (302 vs 977 pg/ml, respectively $P<0.033$) and one subfertile stallion (1/4) increased sperm production by 100% in week 4. In Exp.01 the total sperm number in the treated group increased by 50% after 4 weeks of treatment and remained high until the end of the evaluation. Membrane stability and mitochondrial membrane potential increased one week after the start of treatment ($P<0.001$) in the treated group, returning to pre-treatment values 60d after the end of the application. In conclusion, although the treatment decreased serum testosterone in some stallions, other hormones that indicate testicular function were not affected. Thus, the increase in sperm production, stability and mitochondrial membrane potential demonstrate that the treatment may be interesting in certain cases of subfertility.

Keywords: subfertility, semen, equine, testicular function, neuroendocrinology.

CAPÍTULO 01

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CAPÍTULO 02

ARTIGO I

PERFIL HORMONAL E QUALIDADE ESPERMÁTICA DE GARANHÕES TRATADOS COM LECIRELINA

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RESUMO

O objetivo desta pesquisa foi avaliar o efeito da aplicação de lecirelina na qualidade espermática e funcionalidade testicular. Os garanhões foram divididos em grupos controle (GC, n=3), fértil (GF, n=4) e subfértil (GSF, n=4). Os GF e GSF receberam doses diárias de lecirelina (50 µg;im) durante 60 dias e foram avaliados por mais 60 dias. A resposta testicular endócrina foi mensurada pelos níveis séricos de inibina-B, AMH, sulfato de estrona, testosterona, LH e FSH. Os níveis séricos de AMH, inibina-B e sulfato de estrona não alteraram com o tratamento, mas a testosterona sérica diminuiu entre as semanas 6 e 16 ($P<0,05$) no GSF. O LH reduziu nos grupos GF e GSF após a semana 1 ($P<0,001$) enquanto o FSH não apresentou alteração significativa. O número de espermatozoides totais no GF aumentou em 50% na semana 4 e se mantiveram altos até o final da avaliação. Um garanhão subfértil aumentou em 100% o número de espermatozoides totais após a semana 4, apresentando queda após o final do tratamento. O potencial de membrana mitocondrial, a estabilidade de membrana plasmática e a produção de peróxido de hidrogênio (H_2O_2) aumentaram uma semana após o início do tratamento ($P<0,001$), retornando aos valores pré-tratamento 60 dias após o fim da aplicação no GF. Já a peroxidação lipídica, produção do ânion superóxido ($^{-}O_2$) e integridade de membrana plasmática e acrossomal (MPAI) não apresentaram alterações no GF, porém o GC apresentou queda da MPAI e aumento da produção de $^{-}O_2$ e H_2O_2. A MP reduziu nos grupos GF e GC e o RAP no GC. Em conclusão, embora o tratamento tenha ocasionado queda da testosterona sérica, outros hormônios indicadores da funcionalidade testicular não foram afetados. O aumento da produção espermática, da MMS e da MMP demonstram que o tratamento pode ser interessante em determinados casos de subfertilidade.

Palavras-chave: GnRH, subfertilidade, inibina-B, AMH, testosterona.

ABSTRACT

The aim of this research was to evaluate the effect of lecirelin application on sperm quality and testicular function. The stallions were divided into control (GC, n=3), fertile (GF, n=4) and subfertile (GSF, n=4) groups. The GF and GSF received daily lecirelin (50 µg;im) for 60 days and were evaluated for another 60 days. Endocrine testicular response was assessed by serum levels of inhibin-B, AMH, estrone sulfate, testosterone, LH and FSH. Serum levels of AMH, inhibin-B, and estrone sulfate did not change over time whereas serum testosterone decreased between weeks 0 and 6 ($P<0.05$) in the GSF. LH dropped in the GF and GSF groups after week 1 ($P<0.001$) while FSH showed no significant change. The total sperm number in the GF increased by 50% at week 4 and remained high until the end of the experiment. One subfertile stallion also increased the total sperm number after week 4. Mitochondrial membrane potential, plasma membrane stability and hydrogen peroxide (H_2O_2) production increased one week after the beginning of treatment ($P<0.001$), returning to pre-treatment values 60 days at week 16 in the FG . Lipid peroxidation, production of superoxide anion ($^{-}O_2$) and integrity of the plasmatic and acrosomal membrane (MPAI) did not change in the GF, but the GC showed a decrease in MPAI and an increase in the production of $^{-}O_2$ and H_2O_2. Progressive motility decreased in the GF and GC groups and the RAP in the GC. In conclusion, although the treatment caused a decline in serum testosterone, other indicators of testicular functionality were not affected. The increase in sperm production, membrane stability and higher mitochondrial potential showed that the treatment can be interesting in certain cases of subfertility.

Keywords: GnRH, subfertility, inhibin-B, AMH, testosterone.

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