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Novel polymorphisms in the equine FSH receptor (*eFSHR*) gene and their association with embryo production following artificial insemination in quarter horse mares

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Abstract

The quarter horse is a breed that stands out for its strength, explosiveness, versatility, and use in several equestrian sports. For equines, assisted reproduction presents challenges for artificial insemination and/or in vitro fertilization. This study aimed to characterize a portion of the *eFSHR* gene promoter in Quarter Horses and associate it with the number of embryos produced in the 2021 season. Peripheral blood samples were collected from 92 inseminated mares for several embryos produced, DNA extraction, followed by conventional PCR, purification, and sequencing using the Sanger method. Genetic population parameters and potential binding factor sites were determined. The association of haplotypes with the number of embryos produced was analyzed using the PROC MIXED of SAS (onDemand) with a significance level of 0.05. Three single nucleotide polymorphisms (SNPs) were identified: -217G>T, -195G>A, and 127A>G. Four haplotypes were determined: G/G/A, G/G/G, G/G/GA, and G/GA/G. The wild-type sequence had 74 binding sites, with Sp1 and TFAP-2alpha binding at position -217 and GR binding at position 195. The mutant sequence had 71 identified sites. The *eFSHR* gene promoter demonstrated polymorphism in Quarter Horses, but there was no association with the number of produced embryos. However, it could be used for other reproductive traits in mares.

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Introduction

Single-nucleotide polymorphisms (SNPs) are the most common in eukaryotic and prokaryotic genomes and can be found in genes, mainly in coding and non-coding regions for proteins (1). In the regulated gene regions, many SNPs are often identified and considered (2). However, mutations in the promoter gene region can cause significant alterations in gene expression by changing the binding sites of essential proteins for gene transcription, resulting in changes in traits associated with the gene (3). Follicle-stimulating hormone (FSH) plays an important role in gametogenesis, and its activity is mediated by a specific receptor (eFSHR), which is expressed in granulosa cells and Sertoli cells (4). eFSHR belongs to the superfamily of G protein-coupled receptors and has a crucial role in reproductive efficiency (5). Polymorphisms in the *eFSHR* gene may be associated with infertility, as observed in male patients, due to amino acid substitutions in the protein structure (6). In female patients, certain polymorphisms are associated with premature ovarian failure (7). Paschalidou *et al.* (8) evaluated a polymorphism in the *eFSHR* gene in patients undergoing in

vitro fertilization (IVF) and intracytoplasmic sperm injection (ICSI) treatments, associating it with clinical and biochemical characteristics, as well as characteristics related to the ovarian stimulation protocol and pregnancy rates. In animals, a study with sheep reported that a polymorphism in the 3' untranslated region (3'-UTR) plays a vital role in the regulation of transcription of the *eFSHR* gene (9). Polymorphisms in the 5' regulatory region were strongly associated with litter size in high and low-prolific sheep breeds (10). Another study identified polymorphisms in exon 10 of the *eFSHR* gene in pigs that are associated with the total number of births (TNB) and the number of live births (NBA) in litters (11). Polymorphisms in the promoter region of the *eFSHR* gene were identified in laying hens, and they are strongly associated with age at first egg laying and the number of eggs produced up to the age of 43 weeks (12). Kang *et al.* (13) also evaluated the characteristics associated with an INDEL (Insertion/Deletion) in the promoter region of the *eFSHR* gene and showed strong associations. Therefore, polymorphisms in the *eFSHR* gene can serve as excellent molecular markers in assisted reproduction (14). The search for animals with high genetic value and the possibility of utilizing individuals with acquired subfertility has stimulated the development of reproductive techniques in horses, such as artificial insemination, embryo transfer, oocyte transfer, and intracytoplasmic sperm injection (ICSI) (15,16). However, little is known about the genetic factors that affect equine fertility, so genomic studies have been primarily focused on stallion fertility (17,18). Implementing molecular biology techniques in identifying important genes related to economic traits in horses, particularly in selecting animals with high genetic value, has positively influenced the improvement of several horse breeds worldwide (19). Among the various horse breeds, the Quarter Horse (QH) stands out for its versatility and aptitude for various sports (20). It is an adaptable breed that excels in strength, transportation, and competitive performance in equestrian events while also serving as a genetic improver for the overall herd. However, compared to other species, horses still lag in advancements related to reproduction (20).

In this context, the objective of this study was to genetically characterize and identify polymorphisms in the promoter and 5'-UTR regions of the *eFSHR* gene in QM mares that donate embryos, identify SNPs at binding sites, and associate haplotypes with the number of embryos produced during the 2021 breeding season.

Materials and methods

Ethical approve

The Ethics Committee on the Use of Animals of the Federal Rural University of Amazon (CEUA/UFRA) approved the study under the number CEUA 6957170620 ID000213.

Animals

Ninety-two quarter horses (*Equus caballus*) mares from farms in the states of Pará (n=30), Pernambuco (n=16), and Rio Grande do Norte (n=46) were used. All mares were approximately eight years old and weighed between 400 and 500 kg. The mares were under the technical supervision of veterinarians who managed their reproduction, health, nutrition, and welfare. They were kept under the same sanitary conditions, including multiple annual vaccines, deworming, and ectoparasite control.

Reproductive management

Throughout 2021, all these mares were of the high-performance index in short-distance equestrian events in the regions. Therefore, they underwent assisted reproduction protocols for embryo recovery. Initially, they were evaluated for estrus using transrectal ultrasound to monitor follicular development (references of 35 mm follicular diameter and grade 2 endometrial edema), according to Segabinazzi *et al.* (21), followed by the induction of ovulation through an intramuscular injection of 1.0 mg of deslorelin acetate per animal. After 24 hours from induction, these embryo donors were inseminated with cooled semen ($0,1\text{-}0,2x10^9$ spermatozoa/mL) from stallions with proven fertility according to the andrology manual of the Brazilian College of Animal Reproduction (22). Embryo collections were conducted 8 days after AI through a uterine flush using 900 to 1000 mL of Ringer's lactate solution, utilizing probes for embryo collection (CH 28 Minitube Brazil) and a collection filter for embryo transfer (Vitrocell Brazil). After the flush, the filter was sent to the laboratory to be evaluated for the presence or absence of an embryo. The recovered embryos were classified by their developmental stage (Morula, Early Blastocyst, or Expanded Blastocyst) and quality (poor, fair, good, and excellent) according to McCue *et al.* (23) to then be transferred to the recipient mares.

Blood collection

During flushing for embryo collection and counting, vacuum blood sampling was performed from the jugular vein using a 4 mL tube containing EDTA. The samples were labeled, stored in a refrigerated thermal box, and transferred to a freezer at -20°C for further laboratory analysis.

DNA extraction

Blood samples were subjected to genomic DNA extraction using the Phenol-chloroform method developed by Sambrook *et al.* (24). DNA samples were analyzed for integrity by electrophoresis in a 1.5% agarose gel.

Conventional polymerase chain reactions (cPCR)

cPCR reactions were performed using a pair of primers designed to flank the 5' UTR region and part of the promoter region of the equine *eFSHR* receptor gene (Equus caballus), based on the sequence deposited in Genbank ID: 100052957.

The primers were designed using the Primer3 program available on the website (http://bioinfo.ut.ee/primer3-0.4.0). The primer sequences were as follows: Forward (For): TCCCCACTGAAAACATAGCC position: -319:-300, and Reverse (Rev): AAGGAGACCAGGAGCAAGG position: 365:388. The size of the amplification product was 707 bp. Polymerase Chain Reactions were performed in a final volume of 25 µl using the Master mix kit (Cellco Biotec do Brasil Ltda, São Carlos, SP, Brazil) according to the manufacturer's recommendations, being: 1X of 2X Taq Pol Master Mix, ten pmol/µL of each primer (forward and reverse), 100 ng/µL of genomic DNA. The reactions were carried out in a CFX 96 Thermocycler (BIO-RAD, Hercules, CA, USA) with the following temperature and time conditions: initial denaturation at 95°C for 3 minutes, followed by 30 cycles of denaturation at 95°C for 30 seconds, annealing at 62.5°C for 30 seconds, extension at 72°C for 1 minute, and final extension at 72°C for 5 minutes.

Purification and sequencing of the PCR product

PCR products (25 µL) were purified using the DNA purification kit on an agarose gel band (Ludwig Biotech LTDA, Alvora, RS, BR) following the manufacturer's recommendations. The purified products were subjected to sequencing reactions using the Sanger method with the BigDye kit (Invitrogen, Carlsbad, CA, USA), according to the manufacturer's instructions, and run on the ABI3500XL DNA sequencer (Applied Biosystem, Foster City, CA, USA).

Alignment of sequences and identification of polymorphisms

The generated sequences were edited using the FinchTV program (Version 1.4.0) developed by the Geospiza Research Team (USA). They were compared with the reference sequence deposited in Genbank ID: 100052957 and aligned for comparison among the animals to detect possible polymorphisms using the Bioedit program (25). The sequence under study was submitted to identify segments as potential binding sites using the AliBaba 2.1 program.

Statistical analysis

The detected SNPs were tabulated and analyzed using the GENEPOP (v.5) program (26). This analysis determined the allelic and genotypic frequencies, the inbreeding coefficients (F_{IS}), and the probabilities for the Hardy-Weinberg equilibrium. Haplotypes with a frequency equal to or greater than 0.05 were tested using the ANOVA test, assuming a model with the haplotype effect and the effects of management (three different farms) and the age of the animals. The PROC MIXED application of the SAS statistics package (OnDemand) was used for the analysis, with a significance level of 0.05.

Results

Three polymorphisms were identified along the studied sequence, two in the regulatory region and one in the 5' UTR region (Figure 1). The first polymorphism (SNP 1) was found at position -217G>T, with the G allele and the GG genotype being the most frequent. SNP 2 was identified at position -195G>A, with the G allele and the GG genotype being the most frequent. SNP 3 was identified at position 127 A>G, with the G allele and the GG genotype being the most frequent. All SNPs' inbreeding coefficients (FIS) values were positive, indicating possible inbreeding relationships. All SNPs showed deviations from the Hardy-Weinberg equilibrium ($p < 0.05$) (Table 1). Twelve haplotypes were identified, but only 4 had frequencies greater than 0.05. These haplotypes were evaluated for the number of embryos produced in the 2021 season. The G/G/A haplotype had the highest number of produced embryos, while the G/G/G haplotype had the lowest number. However, there was no significant difference in the number of produced embryos between the haplotypes ($P>0.05$) (Table 2). Seventy-four segments were identified as potential binding sites along the 707 bp sequence, including the positions of the identified SNPs. At position -217G>T, two binding segments for Sp1 (Specific Protein 1) and TFAP-2alpha (Transcription Factor AP-2 Alpha) were identified, while at position -195G>A, a binding site segment for GR (Glucocorticoid Receptor) was identified. When evaluating the mutant sequences, the total number of segments was reduced to 71, and some identified sites were lost.

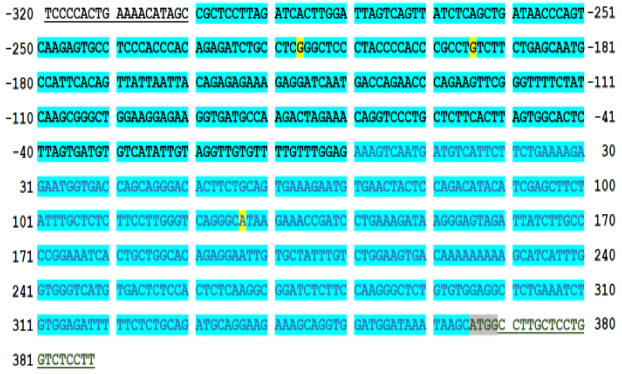

Figure 1: Equine eFSHR Promoter and 5' UTR Sequences. Letters in black represent the promoter region, and letters in blue represent the 5' UTR. Nucleotides highlighted in yellow represent novel polymorphisms. Sequences underlined represent the primers for PCR.

Table 1: SNPs positions and respective statistical descriptions in the *eFSHR* gene promoter and 5'UTR in quarter horse embryo donor mares

SNPs	Alleles	Freq	Genotypes	Freq	F_{IS}	HWP
SNP1	G	0.939	GG	0.902	0.0009	0.025
-217G→T	T	0.061	GT	0.073		
			TT	0.024		
SNP2	A	0.104	AA	0.049	0.0003	0.003
-195G→A	G	0.896	AG	0.110		
			GG	0.841		
SNP3	A	0.271	AA	0.145	0.0002	0.002
127A→G	G	0.729	AG	0.253		
			GG	0.602		
Total						0.00001

SNPs: Single Nucleotide Polymorphisms; Freq: Frequencies; FIS: inbreeding coefficients; HWP: Hardy-Weinberg Probability.

Table 2: Identification of haplotypes from 3 SNPs of the *eFSHR* gene

Haplotypes	Frequencies	PEN2021 (Average±SD)
G/A/G	0.01	
G/G/A	0.14	0.827±0.541
G/G/G	0.46	0.664±0.464
G/G/GA	0.23	0.761±0.380
G/GA/G	0.05	0.741±0.354
G/GA/A	0.01	
G/GA/GA	0.01	
GT/A/G	0.01	
GT/G/G	0.02	
GT/GA/G	0.02	
GT/GA/GA	0.01	
T/A/G	0.01	

PEN2021: Produced embryos number in 2021; SD: Standard Deviation.

Discussion

SNPs in the eFSHR gene are commonly observed in various gene regions, including the regulatory region (promoter and 5'-UTR), coding regions, and the 3'-UTR region. This pattern has been observed in different organisms, including humans (8) and production animals such as sheep (9,10), swine (11), and chickens (12,13). This study identifies the first SNPs in the *eFSHR* gene, mainly in the QM breed. The promoter region exhibited variability, similar to the promoter regions of other genes of reproductive interest, such as the melatonin receptor in buffaloes (27) and the leptin gene in cattle (28).

The high allele frequencies observed in all SNPs indicate a high degree of inbreeding in the mares, as evidenced by the inbreeding coefficients and deviations from the Hardy-Weinberg equilibrium ($P<0.05$). These high frequencies are directly associated with the selection process employed by the breeders, who prioritize traits of high economic value. Silva-Filho *et al.* (29) also reported significant levels of inbreeding in six horse breeds, including the QM breed, using neutral markers (microsatellite DNA), leading to deviations from the Hardy-Weinberg equilibrium.

The average production of embryos after insemination is closely related to an interfollicular biochemical complex that activates inhibin, which controls the synthesis and release of FSH. On the other hand, Estradiol decreases the expression of eFSHR and LHR in the follicles. This leads to an increase in IGF-1 in the fluids of the dominant follicle, altering the blood flow in the other follicles and ultimately releasing only one follicle (30). This explanation justifies donors' low rate of embryo production during the 2021 breeding season, which was not significantly different between haplotypes.

Regarding identifying more than 70 segments as potential binding sites for factors that regulate gene expression, SNP1 stands out with the presence of the transcription factor SP1 (Specific Protein 1). SP1 regulates numerous genes by binding to GT or GC boxes in promoters, playing a role in cell differentiation and embryonic development (31). TFAP-2alpha, another factor identified, binds to GC-rich regulatory regions in the 5' regulatory region and is directly associated with embryonic development in various species when interacting with other genes (32). The GR (Glucocorticoid Receptor) binding site was found in SNP2, and its absence is directly associated with glucocorticoid resistance (33).

Conclusion

The regulatory region of the *eFSHR* gene exhibits significant polymorphic variations suitable for population analysis. These variations may play a role in regulating gene expression in primordial gametic cells, making the *eFSHR* gene a potential candidate for selection processes assisted by molecular markers targeting traits related to follicular formation. However, it should be noted that the number of maturing follicles is not directly associated with follicular release or the production of embryos in horses. Nevertheless, these findings can be utilized in studies that evaluate the follicular wave in mares.

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References

1. Ramírez-Bello J, Jiménez-Morales M. Functional implications of single nucleotide polymorphisms (SNPs) in protein-coding and non-coding RNA genes in multifactorial diseases. Gac Med Mex. 2017;153(2):238-50. [available at]
2. Liang S, Su YQ, Liang YL, Wu F, Zhang H, Hong WX. Allele frequency and distribution of single nucleotide polymorphisms in promoter region of gene encoding the Kidd blood group antigens. Zhongguo Shi Yan Xue Ye Xue Za Zhi. 2020;28(1):300-6. DOI: 10.19746/j.cnki.issn.1009-2137.2020.01.050
3. Broniarczyk JK, Kędzia A, Nowak W, Kościński Ł, Lewandowski M, Goździcka-Józefiak A. Polymorphisms in the P1 promoter of the IGF-1 gene in children with growth disorders. Pediat Endocrinol Diabetes Metab. 2015;20(4). DOI: 10.18544/PEDM-20.04.0013
4. Szymańska K, Kałafut J, Przybyszewska A, Paziewska B, Adamczuk G, Kiełbus M, Rivero-Müller A. FSHR trans-activation and oligomerization. Front Endocrinol. 2018;9:760. DOI: 10.3389/fendo.2018.00760
5. Ulloa-Aguirre A, Zariñán T, Jardón-Valadez E, Gutiérrez-Sagal R, Dias JA. Structure-function relationships of the follicle-stimulating hormone receptor. Front Endocrinol. 2018;9:707. DOI: 10.3389/fendo.2018.00707
6. Luo J, Wang L, Shi HC, Zeng QQ, Wu QY, Li WW. Relationship between the FSHR Thr307Ala-Asn680Ser gene polymorphism and male infertility: A meta-analysis. Nat J Androl. 2017;23(12):1121-6. DOI: 10.1109/5.771073
7. Liu, H., Guo, T., Gong, Z., Yu, Y., Zhang, Y., Zhao, S., Qin, Y. 2019. Novel FSHR mutations in Han Chinese women with sporadic premature ovarian insufficiency. Mol. Cell. Endocrinol. 492:110446. DOI:10.1016/j.mce.2019.05.005
8. Prodromidou A, Dimitroulia E, Mavrogianni D, Kathopoulis N, Pappa KI, Loutradis D. The effect of the allelics of Ser680Asn polymorphisms of follicle-stimulating hormone receptor gene in IVF/ICSI cycles: a systematic review and meta-analysis. Reprod Sci. 2023;30(2):428-41. DOI: 10.1007/s43032-022-00996-x
9. Wang D, Du X, Li Y, Li Q. A polymorphism in the transcriptional regulatory region strongly influences ovine FSHR mRNA decay. Reprod Domest Anim. 2019;54(1):83-90. DOI: 10.1111/rda.13316
10. Chu MX, Guo XH, Feng CJ, Li Y, Huang DW, Feng T, Cao GL, Fang L, Di R, Tang QQ, Ma YH. Polymorphism of 5′ regulatory region of ovine FSHR gene and its association with litter size in Small Tail Han sheep. Mol Biol Rep. 2012;39:3721-5. DOI: 10.1007/s11033-011-1147-x
11. Zhang XD, Zhu HY, Zhou J, Wang N, Zhou N, Huang L, Wu T, Feng YF, Ding YY, Yin ZJ. Relationship between polymorphisms in exon 10 of FSHR gene and litter size in swine. Genet Mol Res. 2015;14(3):8252-61. DOI: 10.4238/2015.July.27.13
12. Li X, Lu Y, Liu X, Xie X, Wang K, Yu D. Identification of chicken FSHR gene promoter and the correlations between polymorphisms and egg production in Chinese native hens. Reprod Domest Anim. 2019;54(4):702-11. DOI: 10.1111/rda.13412
13. Kang L, Zhang N, Zhang Y, Yan H, Tang H, Yang C, Wang H, Jiang Y. Molecular characterization and identification of a novel polymorphism of 200 bp indel associated with age at first egg of the promoter region in chicken follicle-stimulating hormone receptor (FSHR) gene. Mol Biol Rep. 2012;39:2967-73. DOI: 10.1007/s11033-011-1058-x
14. Papanikolaou IG, Giannelou P, Anagnostou E, Mavrogianni D, Drakakis P, Loutradis D. Combined study on the single nucleotide polymorphisms in the follicle-stimulating hormone receptor (Ser680Asn) and anti-Müllerian hormone receptor type II (− 482A> G) as genetic markers in assisted reproduction. Horm Mol Biol Clin Investig. 2019;38(1). DOI: 10.1515/hmbci-2018-0077
15. Bertozzo BR, Sampaio BF, Bender ÉS, Pagnoncelli RR, Silva EV, Zuccari CE. Vantagens e desafios das biotécnicas avançadas utilizadas na reprodução equina assistida. Bol Ind Anim. 2014:84-93. DOI: 10.17523/bia.v71n1p84
16. Hinrichs K. Assisted reproductive techniques in mares. Reprod Domest Anim. 2018;53:4-13. DOI:10.1111/rda.13259
17. Giesecke K, Sieme H, Distl O. Infertility and candidate gene markers for fertility in stallions: a review. Vet J. 2010;185(3):265-71. DOI: 10.1016/j.tvjl.2009.07.024
18. Hinrichs K. Assisted reproduction techniques in the horse. Reprod Fertil Dev. 2012;25(1):80-93. DOI:10.1071/RD12263
19. Pereira GL, Chud TC, Bernardes PA, Venturini GC, Chardulo LA, Curi RA. Genotype imputation and accuracy evaluation in racing quarter horses genotyped using different commercial SNP panels. J Equine Vet Sci. 2017;58:89-96. DOI: 10.1016/j.jevs.2017.07.012
20. Kasper DT, Gandy RF. Ranking quarter horse sires via models of offspring performance. J Equine Sci. 2018;29(3):67-74. DOI: 10.1294/jes.29.67
21. Segabinazzi LG, Friso AM, Correal SB, Crespilho AM, Dell'Aqua Jr JA, Miró J, Papa FO, Alvarenga MA. Uterine clinical findings, fertility rate, leucocyte migration, and COX-2 protein levels in the endometrial tissue of susceptible mares treated with platelet-rich plasma before and after AI. Theriogenol. 2017;104:120-6. DOI: 10.1016/j.theriogenology.2017.08.007
22. Brazilian College of Animal Reproduction-CBRA. Manual for Andrological Examination and Animal Semen Evaluation. Belo Brazil: Horizonte, MG; 2013. 104 p. [available at]
23. McCue PM, DeLuca CA, Ferris RA, Wall JJ. How to evaluate equine embryos. Proc 55th Am Assoc Equine Practit. USA: CABI Databases; 2009. 252–256 p. [available at]
24. Sambrook J, Maniatis T, Fritsch EF. Molecular Cloning: A Laboratory Manual. 2nd ed. USA: Cold Spring Harbor Laboratory; 1989.
25. Hall JM, McDonnell DP. The estrogen receptor β-isoform (ERβ) of the human estrogen receptor modulates ERα transcriptional activity and is a key regulator of the cellular response to estrogens and antiestrogens. Endocrinol. 1999;140(12):5566-78. DOI: 10.1210/endo.140.12.7179
26. Raymond M, Rousset F. GENEPOP (version 1.2): population genetics software for exact tests and ecumenicism. J Hered. 1995;86:248-249. DOI: 10.1093/oxfordjournals.jhered.a111573
27. Barbosa EM, Souza BB, Guimarães RC, Silva LK, Azevedo JS, Gonçalves EC, Ribeiro HF, Rolim Filho ST, Silva Filho E. Polymorphisms in the melatonin receptor gene promoter and their associations with fertility characteristics in buffalo herd in Eastern Amazon. Genet Mol Res. 2017;16(2):1-1. DOI: 10.4238/gmr16029610
28. Guimarães RC, Azevedo JS, Corrêa SC, Campelo JE, Barbosa EM, Gonçalves EC, Filho ES. Polymorphisms in the leptin gene promoter in Brazilian beef herds. Genet Mol Res. 2016;15(4):1-6. DOI: 10.4238/gmr15048981
29. da Silva Filho E, Schneider MP, da Silva AL. Variabilidade Genética de Cavalos baseada em DA Microssatélites. Rev Tróp Ciên Agrár Biol. 2007;1(1):76. [available at]
30. Chinait J, Cabral C, Gualtieri de Andrade E, Nichi M. Dinâmica folicular em éguas: aspectos intrafoliculares. Rev Bras Reprod Anim. 2008;32(2):122-32. [available at]
31. Thomas K, Wu J, Sung DY, Thompson W, Powell M, McCarrey J, Gibbs R, Walker W. SP1 transcription factors in male germ cell development and differentiation. Mol Cell Endocrinol. 2007;270(1-2):1-7. DOI: 10.1016/j.mce.2007.03.001
32. Praetorius C, Grill C, Stacey SN, Metcalf AM, Gorkin DU, Robinson KC, Van Otterloo E, Kim RS, Bergsteinsdottir K, Ogmundsdottir MH, Magnusdottir E. A polymorphism in IRF4 affects human pigmentation through a tyrosinase-dependent MITF/TFAP2A pathway. Cell. 2013;155(5):1022-33. DOI: 10.1016/j.cell.2013.10.022
33. Charmandari E, Kino T, Ichijo T, Jubiz W, Mejia L, Zachman K, Chrousos GP. A novel point mutation in helix 11 of the ligand-binding domain of the human glucocorticoid receptor gene causing generalized glucocorticoid resistance. J Clin Endocrinol Metab. 2007;92(10):3986-90. DOI: 10.1210/jc.2006-2830

تعدد الأشكال الجديدة في جين مستقبلات الـ FSH للخيول (*eFSHR*) وارتباطها بإنتاج الأجنة بعد التلقيح الاصطناعي في أفراس الربع

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الخلاصة

خيول الربع هي سلالة تتميز بقوتها وتعدد استخداماتها في العديد من رياضات الفروسية. بالنسبة للخيول، يمثل التناسل المساعد تحديات للتلقيح الاصطناعي والإخصاب في المختبر. هدفت هذه الدراسة إلى توصيف جزء من محفز جين *eFSHR* في خيول الربع وربطه بعدد الأجنة المنتجة في موسم ٢٠٢١. تم جمع عينات الدم المحيطية من ٩٢ فرسا ملقحا لانتاج العديد من الأجنة، واستخراج الحمض النووي، متبوعا بتفاعل البوليميراز المتسلسل التقليدي، والتنقية، والتسلسل باستخدام طريقة سانجر. تم تحديد المعلمات الجينية للمجموعة ومواقع عوامل الربط المحتملة. تم تحليل ارتباط الأنماط المفردة بعدد الأجنة المنتجة باستخدام PROC MIXED of SAS (onDemand) بمستوى دلالة ٠,٠٥. تم تحديد ثلاثة أشكال متعددة النوكليوتيدات المفردة - :(SNPs) 217G>T و -195G>A و 127A>G. تم تحديد أربعة أنماط فردية: G / G / A ،/ G / G / G ،G / G / GA ، G / GA / G. يحتوي التسلسل من النوع البري على ٧٤ موقعا للربط، مع ربط Sp1 و TFAP-2alpha في الموضع -٢١٧ وارتباط GR في الموضع ١٩٥. كان التسلسل المتحور يحتوي على ٧١ موقعا محددا. أظهر محفز الجينات *eFSHR* تعدد الأشكال في خيول الربع، ولكن لم يكن هناك ارتباط بعدد الأجنة المنتجة. ومع ذلك، يمكن استخدام هذه النتائج للسمات التناسلية الأخرى في الأفراس.