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UNIVERSIDADE ESTADUAL PAULISTA “JÚLIO DE MESQUITA FILHO”

FACULDADE DE MEDICINA VETERINÁRIA E ZOOTECNIA

DEPARTAMENTO DE REPRODUÇÃO ANIMAL E RADIOLOGIA VETERINÁRIA

PERFIL PROTEICO DO PLASMA SEMINAL DE BÚFALOS (*Bubalus bubalis*) E BOVINOS (*Bos taurus indicus*)

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Dissertação apresentada á Faculdade de Medicina Veterinária e Zootecnia da Universidade Estadual Paulista "Júlio de Mesquita Filho", Câmpus de Botucatu, para Exame Geral de Defesa de Mestrado do Programa de Pós-graduação em Biotecnologia Animal

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-Eleanor Roosevelt.

LISTA DE ABREVIATURAS E SÍMBOLOS

aSFP	Proteína ácida do fluido seminal
BSP	Proteína do plasma seminal bovino
GAG	Glicosaminoglicanos
HBP	Proteínas de ligação a heparina
HDL	Lipoproteína de alta densidade
kDa	Kilodalton
LDL	Lipoproteínas de baixa densidade
NHBP	Proteínas não ligadoras de heparina
OPN	Osteopontina
PDGS	Prostaglandina D-sintetase tipo Lipocalina
pI	Ponto isoelétrico
PLA-2	Fosfolipase A2
PGs	Prostaglandinas

Sumário

RESUMO

CODOGNOTO, V. M. (2018) **Perfil proteico do plasma seminal de búfalos (*Bubalus bubalis*) e bovinos (*Bos taurus indicus*)**. Botucatu – SP. 2018, 87 p. Defesa (Mestrado) – Faculdade de Medicina Veterinária e Zootecnia, Campus Botucatu, Universidade Estadual Paulista "Júlio de Mesquita Filho", Departamento de Reprodução Animal e Radiologia Veterinária.

O presente estudo teve como objetivo descrever o perfil proteico do plasma seminal de búfalos e bovinos e identificar proteínas que funcionem como marcadores de funções espermáticas. Foram utilizados 16 búfalos e 16 bovinos sadios, de 2,5 a 5 anos de idade. A colheita do sêmen foi realizada por eletroejaculação e analisado segundo características macroscópicas e microscópicas. Após a análise, as amostras foram centrifugadas a 800g durante 10 minutos para a separação do plasma seminal, o qual foi imediatamente congelado. No laboratório, as amostras foram descongeladas em banho de gelo e recentrifugadas a 10.000g durante 30 minutos, a 4° C. A concentração de proteína total foi realizada pelo método Bradford utilizando nanoespectrofotômetro As amostras foarm digeridas *in solution*, seguida da análise de espectrometria de massas. No primeiro experimento, foram encontradas 48 proteínas no plasma seminal de búfalos, sendo que 10 destas apresentaram diferenças estatísticas significativas entre os grupos. As proteínas spermadhesin, ribonuclease, 14-3-3 protein zeta/delta, acrosin inhibitor, epididymal secretory protein E, serum albumin e clusterin foram encontradas em maior abundância no plasma seminal de búfalos de alta motilidade espermática, sendo as demais prosaposin, peptide YY, cystatn-C e secretoglobin family 1D member encontradas em animais de baixa motilidade espermática. No experimento 2 foram identificados um total de 25 proteínas relevantes em bovinos e búfalos, sendo comuns para ambos os grupos apenas 5: secretoglobin family 1D member, serum albumin, clusterin, spermadhesin e epididymal secretory protein E1. No grupo búfalos foram encontradas 4 proteínas: peptide YY, prosaposin, cystatin C e keratin. Já no grupo bovinos 15 proteínas foram relevantes: osteopontin, nucleobidin, acrosin, ribonuclease 4, seminal plasma protein PDC-109, seminal plasma protein A3, seminal plasma protein BSP-30 kDa, C-type natriuretic peptide, serine protease inhibitor Kazal-type 6, C-C motif chemokine 2, angiogenin, metalloproteinase inhibitor 2, seminal ribonuclease, ephrin-A1 e caltrin. O experimento 1 identificou e correlacionou proteínas do plasma seminal de búfalos com a motilidade espermática do sêmen, sendo estas proteínas possíveis marcadores de qualidade espermática na espécie. O experimento 2 comparou o perfil proteico do plasma seminal de búfalos e bovinos e encontrou diferenças proteicas entre as espécies.

Palavras-chaves: proteínas, biomarcador, touro, espectrometria de massas

ABSTRACT

CODOGNOTO, V. M. (2018). **Proteins in seminal plasma of buffalo (*Bubalus bubalis*) and bovine (*Bos taurus indicus*).** Botucatu – SP. 2018, 87 p. Defesa (Mestrado) – Faculdade de Medicina Veterinária e Zootecnia, Campus Botucatu, Universidade Estadual Paulista "Júlio de Mesquita Filho", Departamento de Reprodução Animal e Radiologia Veterinária.

The aim of the present study was to describe the protein profile of bovine and buffalo seminal plasma and to identify proteins that function as markers of sperm function. Sixteen buffalo and 16 healthy cattle, 2.5 to 5 years old, were used. The semen collection was performed by electroejaculation and analyzed according to macroscopic and microscopic characteristics. After the analysis, the samples were centrifuged at 800g for 10 minutes for separation of the seminal plasma, which was immediately frozen. In the laboratory, the samples were thawed in an ice bath and recentrifuged at 10,000g for 30 minutes at 4øC. Total protein concentration was performed by the Bradford method using nanospectrophotometer. The samples were digested in solution, followed by analysis of spectrophotometry pastas. In the first experiment, 48 proteins were found in the seminal plasma of buffaloes, 10 of which showed significant statistical differences between the groups. The proteins spermadhesin, ribonuclease, 14-3-3 protein zeta / delta, acrosin inhibitor, epididymal secretory protein E, serum albumin and clusterin were found in greater abundance in seminal plasma of buffaloes with high sperm motility, being the other prosaposin, peptide YY , cystatn-C and secretoglobin family 1D member found in animals of low sperm motility. In the experiment 2, a total of 25 relevant proteins were identified in bovines and buffaloes, with only 5 groups: secretaglobin family 1D member, serum albumin, clusterin, spermadhesin and epididymal secretory protein E1. In the buffalo group were found 4 proteins: peptide YY, prosaposin, cystatin C and keratin. In the bovine group 15 proteins were relevant: osteopontin, nucleobidin, acrosin, ribonuclease 4, seminal plasma protein PDC-109, seminal plasma protein A3, seminal plasma protein BSP-30 kDa, C-type natriuretic peptide, serine protease inhibitor Kazal-type 6, CC motif chemokine 2, angiogenin, metalloproteinase inhibitor 2, seminal ribonuclease, ephrin-A1 and caltrin. Experiment 1 identified and correlated

buffalo seminal plasma proteins with sperm motile semen, these proteins being possible markers of sperm quality in the species. Experiment 2 compared the protein profile of the seminal plasma of buffaloes and cattle and found protein differences between species.

Key-words: proteins, biomarker, bull, mass spectrometry

celular (COULTHARD et al., 2012). Foi descrita pela primeira vez no plasma seminal de touros por Rego et al. (2014) sendo sua real função no trato reprodutor masculino ainda desconhecida.

2.4. Considerações finais

As proteínas do plasma seminal exercem funções essenciais nos processos de capacitação espermática, reação do acrossomo e fecundação, tornando importante o conhecimento de suas funções em bovinos o e búfalos, o que pode permitir, futuramente, uma classificação de touros férteis e subférteis.

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Authors' contributions

Substantial contributions to conception and design (V.M.C, P.H.Y., F.F.S and E.O); acquisition of data (V.M.C., P.H.Y., R.A.S., F.R.R., P.F.L., C.S. and E.O.); analysis and interpretation of data (V.M.C, P.H.Y., F.F.S., R.A.S., C.P.F.D. and E.O.); statistical analyses (C.P.F.D, F.F.S and V.M.C.), drafting the manuscript (V.M.C., F.F.S., C.S., R.A.S. and E.O.); critically revising the manuscript for important intellectual content (V.M.C, F.F.S. and E.O.); and final approval of the manuscript for publication (all authors).

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ARTIGO II: Perfil proteico do plasma seminal de bovinos e búfalos

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RESUMO

O objetivo deste estudo foi avaliar o perfil proteico do plasma seminal de bovinos e búfalos e compará-los, com o intuito de avaliar diferenças proteicas entre as espécies correlatas. Foram utilizados 16 búfalos e 16 bovinos entre 30 a 60 meses de idade. A colheita do sêmen foi realizada por eletroejaculação, seguida de análises subjetivas macroscópicas e microscópicas. Após a análise do ejaculado, as amostras foram centrifugadas a 800g por 10 minutos e o sobrenadante (plasma seminal) imediatamente congelado. No laboratório, as amostras foram descongeladas em banho de gelo e recentrifugadas a 10.000g durante 30 minutos a 4º C. A concentração proteica total foi determinada pelo método Bradford e as proteínas digeridas *in solution* para a espectrometria de massas. A análise estatística multivariada dos resultados da proteômica foi realizada no software livre on-line MetaboAnalyst 3. A análise proteômica identificou 25 proteínas relevantes, sendo 4 delas em búfalos, 15 em bovinos e 5 em ambos os grupos. Dentre as proteínas relevantes em ambos os grupos, a secretoglobin family 1D member, serum albumin e epididymal secretory protein E1 apresentaram maior abundância no grupo dos búfalos e a clusterin e spermadhesin encontradas em maior abundância nos bovinos. Foi clara a distinção baseada no perfil proteico do plasma seminal entre búfalos e bovinos, apesar de serem espécies correlatas, sendo encontrada menor variedade e quantidade de proteínas nos búfalos.

Palavras:chave: espectrometria-massas, proteômica, sêmen, macho

ABSTRACT

The objective of this study was to evaluate the protein profile of bovine and buffalo seminal plasma and to compare them, in order to evaluate protein differences between related species. Sixteen buffaloes and 16 cattle between 30 and 60 months of age were used. Semen collection was performed by electroejaculation, followed by macroscopic and microscopic subjective analyzes. After the ejaculate analysis, the samples were centrifuged at 800g for 10 minutes and the supernatant (seminal plasma) immediately frozen. In the laboratory, the samples were thawed in an ice bath and recentrifuged at 10,000g for 30 minutes at 4 ° C. Total protein concentration was determined by the Bradford method and the proteins digested in solution for mass spectrometry. The multivariate statistical analysis of proteomic results was performed in MetaboAnalyst 3 online free software. Proteomic analysis identified 25 relevant proteins, 4 of which were in buffaloes, 15 in cattle and 5 in both groups. Among the relevant proteins in both groups, the secretaglobin family 1D member, serum albumin and epididymal secretory protein E1 showed greater abundance in the group of buffaloes and the clusterin and spermadhesin found in greater abundance in cattle. The distinction was based on the protein profile of the seminal plasma between buffaloes and cattle, although they are related species, being less variety and quantity of proteins in the buffaloes.

Key words: spectrometry-masses, proteomics, semen, male

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