



A026E Physiology of Reproduction in Male and Semen Technology

### Expression of $\beta$ - nerve growth factor in rabbit male tract and seminal plasma

R.M. Garcia-Garcia<sup>1</sup>, M. Masdeu<sup>1</sup>, A. Sánchez-Rodríguez<sup>1</sup>, J.M. Bautista<sup>2</sup>, M. Arias-Alvarez<sup>3</sup>, P.L. Lorenzo<sup>1</sup>, P.G. Rebollar<sup>4</sup>

<sup>1</sup>Dpto. Fisiología (Fisiología Animal); <sup>2</sup>Dpto. Bioquímica y Biol. Mol. IV; <sup>3</sup>Dpto. Producción Animal. Facultad de Veterinaria. Universidad Complutense de Madrid. Madrid, Spain; <sup>4</sup>Dpto. Producción Animal. ETSI Agrónomos. Universidad Politécnica de Madrid. Madrid, Spain.

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Nerve growth factor (NGF) has been recently identified as an ovulation inductor factor (OIF) in the seminal plasma (SP) (Ratto et al. PNAS 2012; 109:15042-7). The presence of OIF in rabbit has been suggested but this protein has not yet been identified. Our aim was to study the mRNA expression in the rabbit male reproductive tract and to identify the protein  $\beta$ -NGF in the SP. Total RNA was extracted from prostate, testicles and seminal glands of 3 male rabbits (TRIzol® Plus RNA Purification Kit, Life Technologies) to subsequently isolate mRNA (FastTrack® MAG mRNA Isolation Kit, Ambion, Life Technologies,) for retrotranscription to generate cDNA. Specific primers were designed on the mRNA sequence deposited in GenBank (XM\_008264614.1) to target a highly conserved region of NGF among species (5'-AGCCCACTGGACTAAACTGCA-3'; 5'-TCGCACACCGAGAAGCTCTCC-3'; product size: 305 nucleotides). PCR was performed on cDNA to obtain the expected 300 pb fragment that was sequenced confirming the presence of NGF-mRNA in seminal plasma, testicle and prostate. To determine the expression of mature NGF protein in SP, an aliquot was prepared from collected semen, centrifuged at 3000xg for 30 min at 4°C and stored at -20°C. For Western blot (WB) analysis, samples were loaded in 12% SDS-PAGE and electrotransferred onto nitrocellulose membranes. The membranes were probed with mouse  $\beta$ -NGF antibody (Promega) using donkey anti-mouse as secondary antibody (Li.Cor Biotechnology). Blots were scanned in an Odyssey Infrared imaging system. In addition, NGF was purified by offgel technique with the 3100 OFFGEL Kit pH 3-10 (Agilent Technologies Inc) and the recovered fraction recognized with the mouse  $\beta$ -NGF antibody was used for mass spectrometry analysis (MS) (4800 Plus Proteomics Analyzer Applied Biosystems,). MS was operated in positive reflector mode with an accelerating voltage of 20,000 V. For protein identification NCBIInr was used. Database without taxonomy restriction and a home-made database with the sequence of NGF (gi|655847230) downloaded from NCBIInr was searched using MASCOT v 2.3. The probability scores of NGF sequences from several species were greater than the score fixed by MASCOT as significant with a p-value < 0.05. Our results show that expression of NGF-mRNA were clearly identified in the rabbit male tract organs above described and the corresponding mature protein band with a mass of ~60 kDa was also identified by WB whereas a ~13 kDa band was detected in the basic fraction (pH=8.24-8.83) obtained when offgel electrophoresis was performed. Furthermore, protein identification by mass spectrometry revealed the existence of NGF in the SP. In conclusion, mRNA and protein NGF are present in rabbit male reproductive tissue and SP respectively, providing the basis to undertake further functional analysis for its potential role in rabbit reproduction.

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