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Gene structure analysis of donkey oxytocin-neurophysin I (*OXT*) gene and genetic variability detection

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Oxytocin is a neurohypophysial peptide released into general circulation from posterior pituitary gland. It is involved in different physiological roles, including milk ejection from the mammary gland, stimulation of uterine smooth muscle contraction during labour and affects cognitive processes, tolerance, adaptation and complex sexual and maternal behaviour. The main objective of the present research was to determine the complete donkey oxytocin-neurophysin I encoding gene (*OXT*) sequences and to detect genetic diversity at this *locus*. Using the genomic DNA as template, we sequenced and compared the whole *OXT* gene of 5 Italian donkeys: 2 Amiatine, 2 Ragusana and 1 Grigio Siciliano donkeys. On average the gene extends over 946 bp, composed of 517 bp of exonic regions and 429 bp of intronic regions, with an A/T and G/C content of about 27% and 73%, respectively. Furthermore, the 5' flanking region (876 bp) was sequenced. The gene contains only three exons, ranging in size from 202 bp (exons 2) to 155 bp (exon 3), and two introns of 315 bp (intron 1) and 114 bp (intron 2). The first exon encodes a peptide leader (19 amino acids residues encoded by the nucleotides from 41 and 97), the nonapeptide hormone (from nucleotide 98 to 124), the tripeptide processing signal (GKR) (from nucleotides 125 and

133) and the first 9 of 94 residues of neurophysin I; the second exon encodes the central part of neurophysin I (67 aa), while the third exon encodes the COOH–terminal region of neurophysin I (18 aa). Among the different species of animals of species of livestock animals, the *OXT* donkey gene sequence showed the highest degree of similarity with the horse (98%) vs. ruminants (75%) and camelids (76%). The comparison of the sequences obtained allowed the identification of 5 SNPs: g.263A>G, g.334A>G, g.436T>C and g.304A>C in intron 1 and g.124G>A in promoter region. This last polymorphic site falls into a potential binding site for the transcription factor GATA-1 (www.generegulation.com). With this study, we provide the first contribution to the characterisation of the genomic sequence of the *OXT* gene and first examples of markers found at this *locus* in donkey. The detected polymorphisms represent a good opportunity to carry out studies focussed on the identification of significant association with the physiological processes controlled by this hormone as already performed in other species.

Acknowledgements
This work was financially supported by the University Research Projects (PRA), the University of Pisa (PRA project).