Manuscript tittle: **Autophagy-related protein LC3 is processed in stallion spermatozoa during biotechnology practices and the related stressful conditions**

Authors: Aparicio IM[1], Martin Muñoz P[2] Salido GM[1], Peña FJ[2], Tapia JA[1]

Author's affiliations: Cell Physiology Research Group, Department of Physiology[1], and Veterinary Teaching Hospital, Laboratory of Spermatology[2], University of Extremadura, Caceres, Spain

References (Before 2010)

Aboagla EM and Terada T 2003. Trehalose-enhanced fluidity of the goat sperm membrane and its protection during freezing. Biology of reproduction 69, 1245-1250.

Aurich C 2008. Recent advances in cooled-semen technology. Animal Reproduction Science 107, 268-275.

Ball BA and Vo A 2001. Osmotic tolerance of equine spermatozoa and the effects of soluble cryoprotectants on equine sperm motility, viability, and mitochondrial membrane potential. Journal of andrology 22, 1061-1069.

Ball BA, Medina V, Gravance CG and Baumbe J 2001. Effect of antioxidants on preservation of motility,viability and acrosomal integrity of equine spermatozoa during storage at 5 degrees C. Theriogenology 56, 577-589.

Bursch W, Ellinger A, Gerner C, Frohwein U and Schulte-Hermann R 2000. Programmed cell death (PCD). Apoptosis, autophagic PCD, or others? Annals of the New York Academy of Sciences 926, 1-12.

Chaveiro A, Santos P and da Silva FM 2007. Assessment of sperm apoptosis in cryopreserved bull semen after swim-up treatment: a flow cytometric study. Reproduction in domestic animals 42, 17-21.

Dietrich GJ, Szpyrka A, Wojtczak M, Dobosz S, Goryczko K, Zakowski L and Ciereszko A 2005. Effects of UV irradiation and hydrogen peroxide on DNA fragmentation, motility and fertilizing ability of rainbow trout (Oncorhynchus mykiss) spermatozoa. Theriogenology 64, 1809-1822.

Dondero F, Rossi T, Delfino M, Imbrogno N, Cannistra S and Mazzilli F 2006. Human semen refrigeration at + 4 degrees C: bio-kinetic characteristics. Cell and tissue banking 7, 61-64.

Eisenberg-Lerner A, Bialik S, Simon HU and Kimchi A 2009. Life and death partners: apoptosis, autophagy and the cross-talk between them. Cell Death and Differentiation 16, 966-975.

Glazar AI, Mullen SF, Liu J, Benson JD, Critser JK, Squires EL and Graham JK 2009. Osmotic tolerance limits and membrane permeability characteristics of stallion spermatozoa treated with cholesterol. Cryobiology 59, 201-206.

Guthrie HD, Liu J and Critser JK 2002. Osmotic tolerance limits and effects of cryoprotectants on motility of bovine spermatozoa. Biology of reproduction 67, 1811-1816.

Januskauskas A, Johannisson A and Rodriguez-Martinez H 2003. Subtle membrane changes in cryopreserved bull semen in relation with sperm viability, chromatin structure, and field fertility. Theriogenology 60, 743-758.

Linfor JJ and Meyers SA 2002. Detection of DNA damage in response to cooling injury in equine spermatozoa using single-cell gel electrophoresis. Journal of andrology 23, 107-113.

Loomis PR and Graham JK 2008. Commercial semen freezing: individual male variation in cryosurvival and the response of stallion sperm to customized freezing protocols. Animal Reproduction Science 105, 119-128.

Martin G, Sabido O, Durand P and Levy R 2004. Cryopreservation induces an apoptosis-like mechanism in bull sperm. Biology of reproduction 71, 28-37.

Medeiros CM, Forell F, Oliveira AT and Rodrigues JL 2002. Current status of sperm cryopreservation: why isn't it better? Theriogenology 57, 327-344.

Neild DM, Brouwers JF, Colenbrander B, Aguero A and Gadella BM 2005. Lipid peroxide formation in relation to membrane stability of fresh and frozen thawed stallion spermatozoa. Molecular reproduction and development 72, 230-238.

Ortega-Ferrusola C, Sotillo-Galan Y, Varela-Fernandez E, Gallardo-Bolanos JM, Muriel A, Gonzalez-Fernandez L, Tapia JA and Pena FJ 2008. Detection of "apoptosis-like" changes during the cryopreservation process in equine sperm. Journal of andrology 29, 213-221.

Ortega Ferrusola C, Gonzalez Fernandez L, Macias Garcia B, Salazar-Sandoval C, Morillo Rodriguez A, Rodriguez Martinez H, Tapia JA and Pena FJ 2009a. Effect of cryopreservation on nitric oxide production by stallion spermatozoa. Biology of reproduction 81, 1106-1111.

Ortega Ferrusola C, Gonzalez Fernandez L, Morrell JM, Salazar Sandoval C, Macias Garcia B, Rodriguez-Martinez H, Tapia JA and Pena FJ 2009b. Lipid peroxidation, assessed with BODIPY-C11, increases after cryopreservation of stallion spermatozoa, is stallion-dependent and is related to apoptotic-like changes. Reproduction 138, 55-63.

Pruski AM, Nahon S, Escande ML and Charles F 2009. Ultraviolet radiation induces structural and chromatin damage in Mediterranean sea-urchin spermatozoa. Mutation research 673, 67-73.

Rovegno M, Feitosa WB, Rocha AM, Mendes CM, Visintin JA and D'Avila Assumpcao ME 2013. Assessment of post-thawed ram sperm viability after incubation with seminal plasma. Cell and tissue banking 14, 333-339.

Sokolowska A, Garcia BM, Fernandez LG, Ortega-Ferrusola C, Tapia JA and Pena FJ 2009. Activated caspases are present in frozen-thawed canine sperm and may be related to post thaw sperm quality. Zygote 17, 297-305.

Tanida I, Ueno T and Kominami E 2004. LC3 conjugation system in mammalian autophagy. The International Journal of Biochemistry and Cell Biology 36, 2503-2518.

Watson PF 2000. The causes of reduced fertility with cryopreserved semen. Animal Reproduction Science 60-61, 481-492.