Animal Reproduction

Abstracts - 37th Annual Meeting of the Association of Embryo Technology in Europe (AETE)

Physiology of reproduction in male and semen technology

Characterization of extracellular vesicles in seminal plasma of fertile and subfertile rabbit bucks

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Extracellular vesicles (EVs) 40-120 nm in diameter are secreted particles present in all biological fluids, transporting molecular components that can affect gamete maturation, fertilization, early embryonic development, and embryo-maternal communication. The study of the composition of the EVs present in the seminal plasma (SP) and its correlation with the fertilizing capacity can help us modulate or enhance the productive results of the rabbit bucks. This study aimed to establish a standardized procedure for isolating and characterizing SP-EVs from rabbit bucks of different semen qualities. Fourteen sexual mature rabbit bucks Cal x NZW (10-12 months old) were used. During a month, 2 ejaculates/week were obtained with an artificial vagina to determine their semen quality (CASA, Microptic S.L., Barcelone, Spain), choosing 3 rabbits of high (HSQ) and 3 of low (LSQ) semen quality. A total of 25 rabbit females were artificially inseminated (seminal dose: $5x10^6$ spermatozoa/ml) with the diluted ejaculate of each male, confirming their high or low fertilizing capacity (fertility: 71.9 and 40.9%; prolificacy: 11.6± 0.3 and 8.1±0.4 liveborn, 0.1±0.1 and 0.7±0.1 stillborn in HSQ and LSQ, respectively; $p<0.001$). Then, 6 ejaculates of each animal obtained in 3 weeks (2 ejaculates/week) were centrifugated (800 × g 20 min, 2000 × g 20 min, and 16000 × g 60 min), and the resulting SP was pooled and frozen at −80 °C. The isolation of SP-EVs from ejaculates of each male was based on size exclusion chromatography analysis PURE-EV® (Hansa BioMed Life Sciences). SP-EVs were quantitative and qualitatively characterized by transmission electron microscopy (Jeol, Ltd Tokyo, Japan), nanoparticle tracking analysis (Nanosight NS500: Marven, INC) using software NTA 3.1, and western blot to confirm the expression of the classical exosome markers (HSP70, CD9 y ALIX). A correlation analysis between seminal parameters and the concentration and size of SP-EVs from the two groups of males was made (SAS, 2001). Different SP-EVs concentrations ($8.53x10^{11}$±$1.0x10^{11}$ and $1.84x10^{12}$±$1.75x10^{11}$ particles/ml of SP; p=0,008) with a similar average size (143.9 ± 11.9 and 115.5 ± 2.4 nm; p=0.7422) in HSQ and LSQ males, respectively were observed. The concentration of SP-EVs was positively correlated with the percentage of abnormal forms ($r=0.94$; $p<0.05$) and with the percentage of immotile spermatozoa ($r=0.88$; $p<0.05$). Particle size was not correlated with any kinetic parameter. We can conclude that the methodology used for the extraction and characterization of the SP-EVs is valid by confirming their existence in the SP of rabbits and the SP-EVs concentration depends on semen quality and its fertilizing ability. *Supported by projects RTI 2018-094404-B-C-21 and PID2019-111641RB-I00.*

Keywords: extracellular vesicles, semen quality