

P-102 Exosomes, a bio-compatible delivery platform for mammalian sperm cells: A non-invasive approach for the transfer of therapeutic compounds

T. Vilanova<sup>1</sup>, C. Plow<sup>2</sup>, C. Jones<sup>3</sup>, R. Dragovic<sup>2</sup>, K. Coward<sup>2</sup>

<sup>1</sup>PhD Student, Nuffield Department of Women's and Reproductive Health, Oxford, United Kingdom <sup>2</sup>University of Oxford, Nuffield Department of Women's and Reproductive Health, Oxford, United Kingdom

<sup>3</sup>University of Oxford, Nuffield Department of Women's and Reproductive Health, Oxford, United Kingdom

**Study question:** Can naturally-synthesised exosomes rival the efficacy of engineered nanoparticles to mediate the delivery of compounds into gametes for potential clinical use? Summary answer: In vitro exposure of sperm to in vivo-synthesised exosomes did not significantly affect motility. Thus, exosomes may represent an effective delivery-tool for compounds into sperm.

**What is known already:** Over recent years, exosomes have become widely utilised as an efficient tool to mediate directed delivery of nucleic acids, peptides, antibodies, fluorescent compounds and other small molecules into cells and tissue in a non-invasive manner. However, the potential application of exosomes for the delivery of therapeutic compounds to mammalian gametes has not yet been elucidated.

**Study design, size, duration:** This study aimed to develop a mammalian cell line (HEK293T cells) from which exosomes can be synthesised, isolated and characterised for their ability to deliver compounds to mammalian sperm. A fluorescent dye (BODIPY) was used to test the efficiency of association between exosomes and sperm and to evaluate internalisation and safety aspects.

**Participants/materials, setting, methods:** BODIPY-labelled exosomes were synthesised and characterised by Nanoparticle Tracking Analysis (NTA) and Western blotting. Different exposure times (1 h, 2 h and 4 h) were tested by incubation with pre-activated boar sperm (n = 3). Sperm motility was then assessed by Computer Assisted Sperm Analysis (CASA), sperm-exosome association was quantified by Metafer-4 analysis and localisation/internalisation was assessed by confocal microscopy.

**Main results and the role of chance:** NTA showed that synthesised exosomes had a mean diameter of  $152.5 \pm 5$  nm and concentration of  $4.55 \times 10^8$ /mL. Furthermore, Western blotting showed bands of the expected sizes for the characteristic exosome markers: Alix, syntenin and CD9. The association rate of BODIPY-exosomes incubated with boar sperm increased in a time dependent manner (1 h, 19.0%; 2 h, 24.9%; 4 h, 26.8%). Moreover, a consistent trend was observed for both internalisation and multiple association to the sperm with increased incubation (internalisation: 1 h, 12.5%; 2 h, 18.0%; 4 h, 55.0%; multiple association: 1 h, 0.83%; 4 h, 1.63%). Association to the tail declined progressively (tail association: 1 h, 50%; 2 h, 46%; 4 h, 35%). Interestingly, no exosome-surface head attachment was observed after 4 h. Metafer-4 analysis indicated a relationship between association and the length of incubation (1 h, 5.33%; 2 h, 6.6%; 4 h, 7.38%). Finally, exposure to labelled-exosomes did not significantly influence sperm motility after 2 h of incubation (p = 0.244).

**Limitations, reasons for caution:** The data presented here is preliminary in nature and derived from only a small sample size, thus limiting statistical analysis. Furthermore, Metafer-4 analysis is unable to quantify sperm tail association, and association rates may have been under-represented.

**Wider implications of the findings:** Our preliminary data demonstrated the successful synthesis, labelling and characterisation of exosomes. In vitro exposure of labelled-exosomes to mammalian sperm showed encouraging patterns of association and did not affect sperm motility. An exosome delivery platform represents a multifaceted tool for reproductive biology with which to investigate gamete structure, physiology and preservation.

**Trial registration number:** not applicable.