Isolation, Purification and Initial RNA Sequence Analysis of Seminal Fluid Exosomes between Pregnant and Non-Pregnant Intrauterine Insemination Pregnancies

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Objective: To compare the RNA content in exosomes purified from seminal fluid associated with intrauterine insemination (IUI) pregnancies with known pregnancy status.

Design: Retrospective analysis of exosome RNA from seminal fluid from patients undergoing IUI and pregnancy outcomes.

Materials and methods: Seminal fluid was collected following semen washing for IUI. Exosomes were isolated and purified by hydrophobic interaction chromatography using a polyester, capillary-channeled polymer phase. Exosome purification was verified by TEM. Small RNAs were isolated from purified exosomes and libraries prepared. Following Illumina NextSeq500 library sequencing, sequences were aligned to the human genome, RNA copy numbers determined, and profiled based on type (i.e. miRNA, lincRNA, RefSeq, exons). RNA copy numbers were compared by analysis of variance (ANOVA) and Students' *t*-test.

Results: Exosomes were successfully purified from seminal fluid samples using hydrophobic interaction chromatography and confirmed with TEM. ANOVA revealed a significant difference ($P<0.001$) in RNA type within each of the pregnant and non-pregnant IUI groups. All pairwise comparisons (e.g. lincRNA vs. protein coding) were found to be significantly different ($P<0.05$) except for between miRNA and the other RNA groups (e.g. miRNA vs. lincRNA). A greater number of RNA copy numbers were found associated with the non-pregnant IUI group than the pregnant groups as follows: non-pregnant miRNA (n= 738,950.67) vs. pregnant miRNA (543,930.60); and non-pregnant lincRNA (n= 3,494,765.83) vs. pregnant lincRNA (2,171,745.40). A greater number of RNA copy numbers were found associated with the pregnant IUI protein coding RNA group (42,658,001.83) than the non-pregnant IUI protein coding RNA group (n=30,796,607.40).

Conclusions: This study provides further evidence that exosomes are present in seminal fluid and the RNA content is different in those that successfully enabled a pregnancy versus those that did not.

Identifying the molecules within seminal fluid provides a snapshot into the males' spermatogenesis past. Specific proteins must be expressed at the correct level, time and location for spermatogenetic processes to precisely occur. Therefore, any pathway disruption may render spermatozoa as unsuitable for fertilization. Further analysis of specific gene expression differences between the two groups may reveal signaling pathways associated with positive or negative pregnancy outcomes.

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