Unraveling the Role of Seminal Fluid Exosomes Within the Male Reproductive Tract

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BACKGROUND

Exosomes are small membrane vesicles containing functional biomolecules such as proteins, lipids, RNA and DNA. They are shed from most cells and are present in various body fluids. Although they have been previously identified in seminal fluid, their precise source or role in the male reproductive tract remains puzzling. It is known that exosomes can mediate immunomodulatory influences on recipient cells and may indeed exert an effect on the female reproductive organs during the conception and implantation period. Exosomes may also play a role in the communication between Sertoli cells among seminiferous tubules and be responsible for modulating spermatogenic waves.

OBJECTIVE

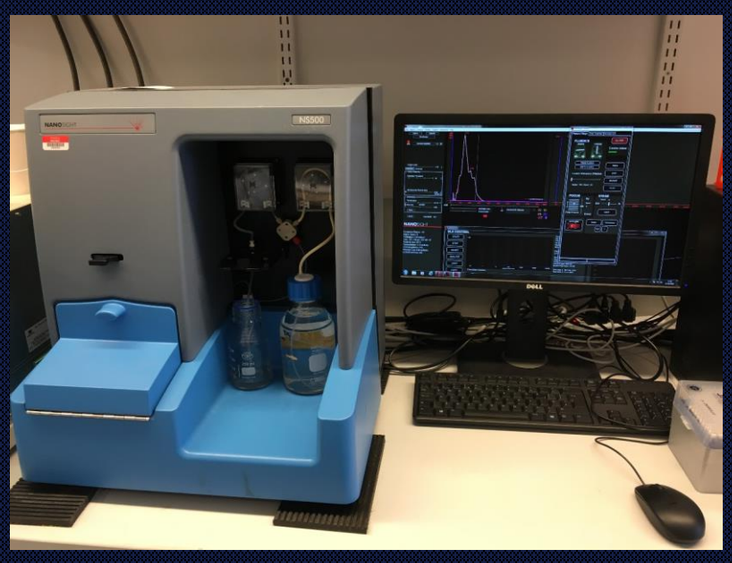
To identify exosomes in the male reproductive tract and suggest putative signaling mechanisms in human ejaculates, epididymal and testicular tissue.

METHODS

From January to December 2016, 85 consenting men screened for infertility donated their 99 ejaculated, 2 testicular and 1 epididymal tissue specimens. Epididymal and testicular tissue specimens were surgically retrieved from men with obstructive azoospermia (OA) or non-obstructive azoospermia (NOA).

Sperm samples were centrifuged 500x*g* for 10 min followed by 3,000x*g* for 20min on the supernatant to remove cell debris. The supernatant was preserved at 4˚ C until exosome isolation. Seminal fluid was centrifuged at 12,000x*g* for 20 min to ensure removal of residual debris. The resulting supernatant was centrifuged at 100,000xg for 70 min for exosomal isolation. Exosomes were washed in 3ml PBS and pelleted again by ultracentrifugation. The final exosome pellet was re-suspended in PBS and protein concentration was measured by BCA. The NanoSight LM10 nanoparticle analysis system was used for characterization of exosomes (Figure 1).

Figure 3: NanoSight LM10 used for exosome characterization



RESULTS

A total of 99 semen samples in 74 men had the following semen parameters: 69.6 ±22 x 106/mL (concentration) and 46.1 ±5% (motility). The number of seminal fluid exosomes isolated was 4.1 ±3 x 108/ µL, with a mean size of 171 ±16 nm in the samples that went through NanoSight analysis (Figure 2).

The exosome protein content of the 100 µL seminal fluid sample was 96.8 ±119 µg. The exosome protein content in the ejaculate of men showed a negative correlation of exosome protein per spermatozoon (R^2 0.14; *P*<0.001) and exosome protein per motile spermatozoon (R^2 0.13; *P*<0.001; Fig. 2) in relation to the sperm concentration in the initial ejaculate.

The exosome protein content in the ejaculate of oligoasthenozoospermic men (101.9 µg per 100 µL) was comparable to normozoospermic men (111.9 µg per 100 µL). Interestingly, men with OA due to vasectomy retained some exosome protein content, while men with congenital absence of the vas deferens (CBAVD) did not. Exosomal protein content in epididymal tissue of men with OA was 1.29 µg/100 µL and in testicular tissue of men with NOA was 25.0 µg/100 µL, possibly representing a proportional sourcing of exosomes from the different levels of the male genital tract (Table 1).

Figure 1: Exosome protein amount per million of motile spermatozoa

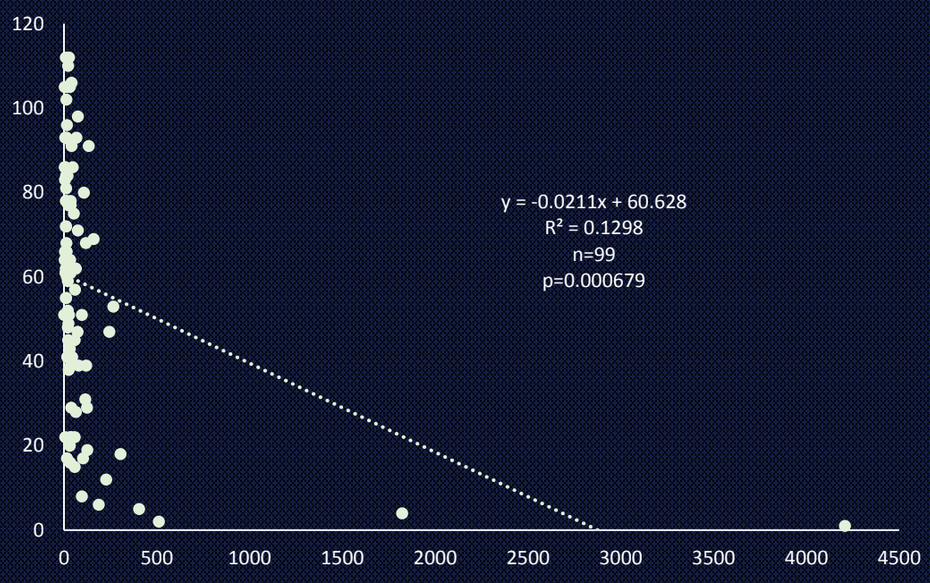
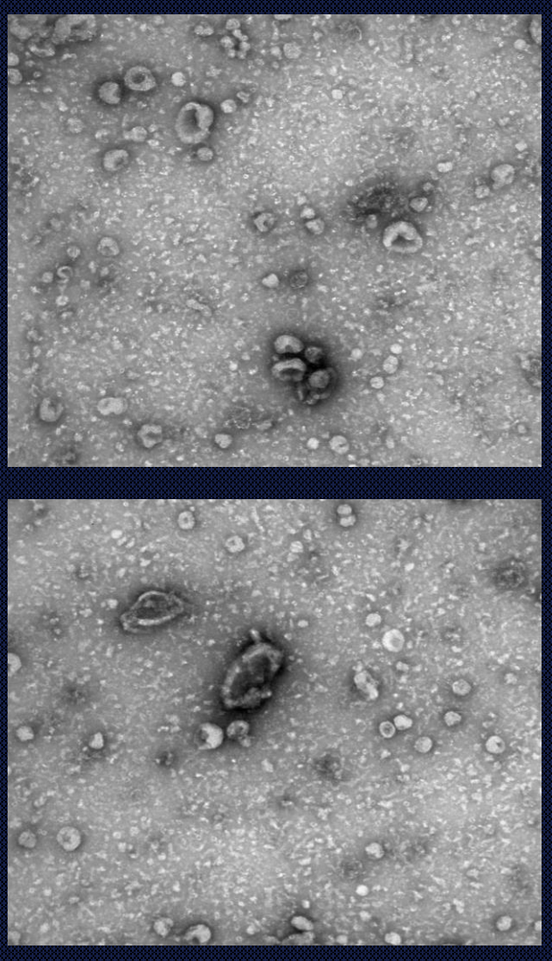
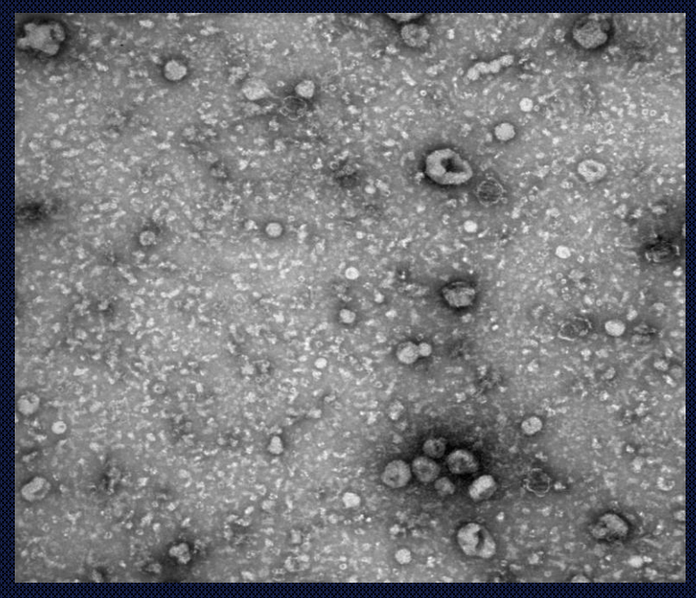


Figure 2: Exosomes isolated from human ejaculates





LIMITATIONS

The proportional contribution of exosomes in the testis, epididymis and seminal fluid in the same individual was not assessed. The current study only included men undergoing infertility screening. Thus, whether the profile in exosomal number, size, and protein content is comparable to normozoospermic men with proven fertility remains unanswered.

Table 1: Comparison of exosomal protein content based on source of sample (n=85)

Parameters	Ejaculated Samples				Surgical Samples	
	Normo-zoospermic	Oligoastheno-zoospermic	CBAVD	Vasectomy	Obstructive Azoospermia	Non-obstructive Azoospermia
Number of Men	71	10	1	1	1	1
Number of Samples	85	11	2	1	2	1
Source	Ejaculated	Ejaculated	Ejaculated	Ejaculated	Epididymis	Testicle
Total exosomal protein content (µg)	318.8 ±32	289.3 ±29	0	157.3±13	6.98	59.5 ±20
Exosomal protein/100 µL	111.9	101.9	0	55.9	1.29	25.0

CONCLUSION

Analysis of seminal fluid exosomes may elucidate their role in immunomodulation within the genital tract during the peri-conception period. Exosomes may be involved in ordaining waves of spermatogenesis within the seminiferous tubule network. Exosomes are differentially produced at various levels of the male genital tract. Exosomal vesicles are produced at different levels in the male genital tract and appear to control the seminiferous tubules' spermatogenic function.