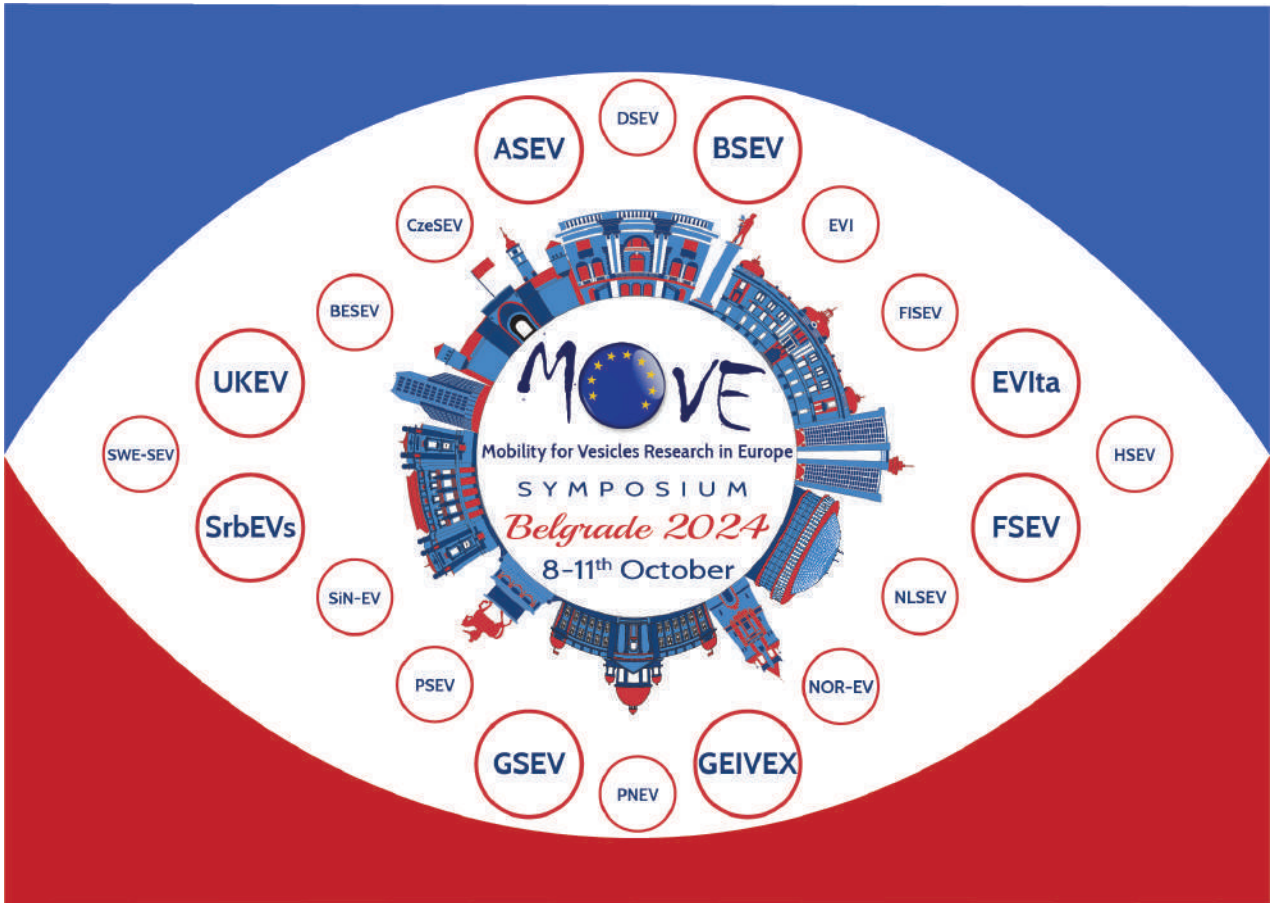


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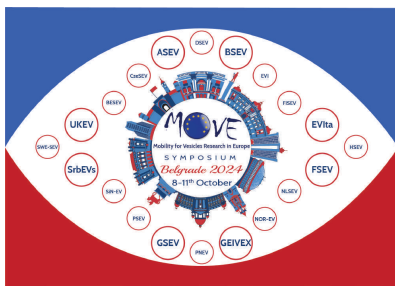


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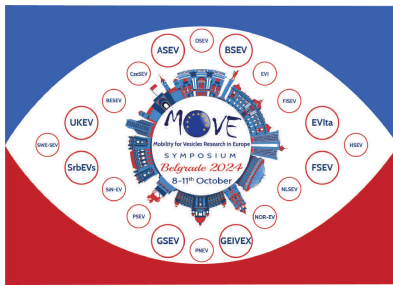


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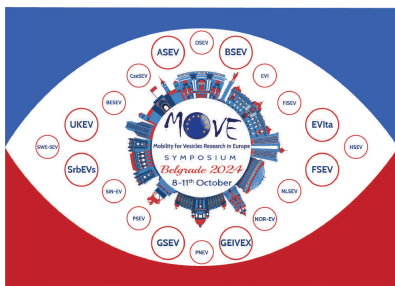
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Comparative analysis of surface glycosylation of prostasomes from human seminal plasma of normozoospermic and asthenozoospermic men

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Introduction: Seminal prostasomes are extracellular vesicles secreted by prostate epithelial cells. Using lectin affinity-chromatography, previous studies showed that mannosylated and sialylated glycans contribute to the prostasomal surface. Taking advantage of nanoparticle tracking analysis (NTA), we further investigated the difference of surface glycosylation and size distribution of prostasomes isolated from seminal plasma of normozoospermic (sProN) and asthenozoospermic (sProA) men.

Methods: A panel of fluorescently labeled plant lectins with different sugar specificity, along with antibodies for the tetraspanins CD9 and CD63 were used to analyze sProN and sProA by NTA.

Results: NTA analysis showed that majority of CD9/CD63 positive populations of both sProN and sProA were within the range of 100-200 nm. Wheat germ agglutinin (WGA), recognizing sialic acid and N-acetylglucosamine, and Ricinus communis agglutinin I (RCA I), that binds to terminal galactose or N-acetylgalactosamine, both exhibited high reactivity to sProN and sProA. Concentration (particles/mL) of WGA-positive prostasomes was equal in sProN ($3.55E+11$) and sProA ($3.50E+11$). Their size distribution matched that of tetraspanin-positive populations. Concentration of RCA-positive prostasomes was slightly higher in sProN ($4.05E+11$) compared to sProA ($3.4E+11$). Their size distribution shifted in relation to tetraspanin-positive populations towards sizes larger than 200 nm, most notably in sProA. Concanavalin A (ConA), specific for mannose and glucose, as well as peanut agglutinin (PNA), specific for galactosyl (β -1,3) N-acetylgalactosamine, showed low reactivity to both sProN and sProA, hindering number and size distribution analysis. Ulex europaeus agglutinin specific for α -linked fucose, Dolichos biflorus agglutinin specific for α -linked N-acetylgalactosamine and soybean agglutinin specific for terminal N-acetylgalactosamine showed no reactivity to both sProN and sProA.

Conclusion: Unlike RCA-, the size distribution of WGA-positive populations of sProN and sProA corresponded to that of tetraspanin-positive populations. RCA-positive populations of sProN and sProA showed slight differences in their size distribution.

Funding information: The study was supported by the Ministry of Science, Technological Development and Innovation of the Republic of Serbia - Grant No. 451-03-66/2024-03/200019.