Enhanced Identification and Autophagic Clearance of Aging Spermatozoa Through a Dual-Response Logic Gate Probe

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Background: With increasing paternal age, concerns regarding the repercussions of sperm aging have grown. These encompass diminished male fertility, declined sperm quality, heightened spermatic mosaicism, and an elevated susceptibility to hereditary diseases in offspring. Notably, the manifestations and issues stemming from sperm aging extend beyond older populations to include men of reproductive age diagnosed with infertility. Therefore, the identification and rational intervention to eradicate aging spermatozoa within sperm populations emerge as a significant issue warranting exploration.

Results: To accurately discern aging spermatozoa, we utilized β-galactosidase as the primary marker and hypochlorous acid accumulation during aging as the secondary marker, devising an "And" type dual-response logic gate probe, L5. Laser confocal microscopy revealed L5's effective recognition of H_2O_2-induced aging-like spermatozoa, with β-galactosidase predominantly localized in the sperm head and hypochlorous acid in the

neck region. Assessing samples from 20 clinically diagnosed infertile men across 20–60-year ages and contrasting them with 20 fertile men of ages of same range, L5 achieved a recognition accuracy of 69.84% (AUC value from the ROC curve). Furthermore, flow cytometry analysis indicated significant clustering in sperm post-L5 staining. Subsequently segregating sperm subgroups post-L5 staining into positive and negative populations, and proteomic and metabolomic analyses suggested aberrant glycerophospholipid metabolism in the L5 positive group, indicative of compromised cell membrane function. Propidium iodide (PI) and DIR (a long-chain carbocyanine dye) staining confirmed a decrease in lipid affinity and an increase in permeability in the L5 positive sperm group. Consequently, we leveraged differential cell membrane permeability to introduce a toxic fatty acid in aging cells for "self-clearance." This led to the development of L5-OA, designed to introduce oleic acid into aging spermatozoa, thereby inducing autophagic clearance. Compared to sperm samples from men under 35 ages with normal fertility outcomes, L5-OA exhibited enhanced clearance of H_2O_2-induced aging spermatozoa and those from infertile men of varying ages. Computer-aidedspermanalysis (CASA) results demonstrated that treatment with L5-OA at 10 μM for 1.5 hours significantly improved forward motility ($p < 0.05$), indicating the potential of L5-OA to significantly eliminate aging spermatozoa and enhance their fertilizing ability.

Future Directions: Building on these promising outcomes, future research will focus on optimizing L5-OA to further restore sperm motility and increase the success rate of assisted reproductive technologies. This innovative approach holds the potential to significantly improve reproductive health and outcomes in the context of increasing paternal ages, ultimately benefiting individuals facing fertility problem.

Conclusion: This study presents a novel strategy to identify aging sperm and target autophagic clearance of aging spermatozoa, providing insights into addressing male infertility and potentially improving reproductive outcomes amidst increasing paternal ages.