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Reproductive System and Sexual Disorders: Current Research

Opinion Article

Fertility Prognosis in Males with Virtual Azoospermia for Ejaculated Spermatozoa

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ABOUT THE STUDY

Spermatozoons are a unique cell type in the human body, and glycosylation plays critical roles in their development, maturation, capacitation, sperm-egg recognition, and fertilization. In this study, we show that spermatozoa contain a number of distinct glycoproteins that are primarily involved in spermatogenesis, acrosome reaction and sperm: oocyte membrane binding and fertilization by mapping the most comprehensive N-glycoproteome of human spermatozoa using our recently developed site-specific glycoproteome approaches. Heavy fucosylation is seen on 14 glycoproteins in spermatozoa, particularly at extracellular and cell surface areas, but not in other organs. In the biological process of immune response in spermatozoa, sialylation and Lewis epitopes are enriched, but bisected core structures and LacdiNAc structures are highly expressed in acrosomes. These findings add to our understanding of glycosylation in spermatozoa and set the groundwork for a functional investigation of glycosylation and glycan structures in male infertility. It is believed that 10%-15%of couples attempting to conceive without medical aid fail to do so after one year. Male infertility is present in around 60% of infertile couples, as evidenced by reduced spermatozoa quantities (oligozoospermia or azoospermia) or an increased prevalence of spermatozoa with aberrant morphology (teratozoospermia) or lower motility (asthenozoospermia). Because artificial reproductive technology now permits many of these men to father their own children, there is an urgent need to understand the reasons of male infertility and the implications for gamete quality. This will allow for the advancement and expansion of infertility therapy, as well as the development of educated risk assessments suited to each couple. It will also provide a plethora of knowledge about gametogenesis biology and the proteins required for human conception. Flagella and motile cilia have a microtubule-doublet axoneme structure, and 76% of men with Primary Ciliary Dyskinesia had asthenozoospermia (reduced spermatozoa motility) (PCD). Nonetheless, in cases of isolated asthenozoospermia, causative genetic variations in a conserved axonemal component have been discovered: 30% of males with Multiple Morphological Abnormalities of sperm Flagella (MMAF) have bi-allelic mutations in DNAH, which codes for one of the axoneme's seven inner-arm dynein heavy chains. We combined whole-exome and Sanger sequencing to analyse two siblings and two independent males with MMAF to better understand the causes of isolated asthenozoospermia. We discovered bi-allelic loss-of-function mutations in WDR66, which encodes cilia- and flagella-associated protein 251 (CFAP251): two brothers were homozygous for the frameshift chr12: g.122359334delA (p.Asp42Metfs4), and the third individual was compound heterozygous for chr12: g. 122359542G>T (p.Glu111) and we show that CFAP251 is typically found along the flagellum but is missing in men with WDR66 mutations, and we discover a spermatozoa-specific isoform that is most likely synthesised during spermatozoon maturation. CFAP251 is a component of the calmodulin- and radial-spoke-associated complex, which is found on the inner surface of the axoneme's peripheral microtubule doublets, next to DNAH1. The CFAP251 ortholog is required for successful coordinated ciliary beating in Tetrahymena. We show that the deletion of CFAP251 impacts the development of the mitochondrial sheath using immunofluorescent and transmission electron microscopy. We postulate that CFAP251 has a structural function in vertebrate spermatozoon flagellum formation.

During the initial evaluation of male fertility, males who were recommended to the ejaculate therapy had their ejaculated semen examined with an extended search for 120-240 minutes (regardless of OPU date). If spermatozoa were not found during this extensive search, the males were sent to microTESE and were not included in the analysis. If spermatozoa were found during the prolonged search, these men were diagnosed with virtual azoospermia, a condition characterised as spermatozoa missing from fresh preparation and not found in a centrifuged pellet but evident after the extended search of the full ejaculate. These men were then offered the option of continuing with ejaculated sperm retrieval on OPU day, which was done an average of 3 hours before the OPU procedure. The second extended search on OPU day was typically undertaken 1-2 months following the first extended search. If no spermatozoa were found following a thorough search on OPU day, oocyte

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cryopreservation (available since 2009) was performed, and the males were sent to microTESE. As previously stated, several patients with nonconstructive azoospermia were referred to microTESE with no extensive search prior to the treatment. Men in the microTESE group who did not have sperm found during the treatment were advised to utilise donor sperm. It is critical to note that all of the males in our group underwent only one operation, either an extended search or a microTESE.

Furthermore, if no spermatozoa were detected in the extended search or subsequently in the microTESE technique, the vitrified oocytes were employed just for sperm donors.