The queens of the social ants, bees and wasps mate only at the beginning of their life, and afterwards store sperm in their spermatheca. As queens are ultimately sperm limited, they use very few sperm per egg fertilisation, selecting for sperm of maximal quality and viability. However, little is known about the physiological processes that allow social insect sperm to achieve these traits. For example, it remains unclear how chemical energy is generated to sustain sperm function during the very different timeframes governing post ejaculation traits or long-term storage traits. We have conducted a detailed metabolite profiling of seminal fluid and combined it with sperm proteome data and measurements of metabolic rate to enhance our understanding of honeybee sperm metabolism. We have identified the specific pathways of energy metabolism that are abundant in the sperm proteome and the chemical components of seminal fluid that change after ejaculation to determine which are most important. Measurements of metabolic rates (glycolysis and respiration) within single bee ejaculates provide quantitative assessment of ATP synthesis rates in sperm. This has pinpointed the subset of metabolites in seminal fluid that are ideal substrates for energy generation in honeybee sperm, and are key in determining sperm activity.