Metabolic Characterization of Sperm after Early-life Exposure to Malnutrition

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Nutrition is one of the most significant environmental factors influencing both health and fertility. Experimental and epidemiological studies have highlighted the profound influence of dietary factors on male reproductive function. Furthermore, the maternal diet, spanning from pre-conception through pregnancy and lactation, plays a pivotal role in shaping offspring fitness, impacting body composition and the development of various organs, including the reproductive tract, ultimately affecting optimal performance. This study aimed to evaluate the impact of malnutrition during the early postnatal period on the metabolic status of sperm in sexually mature male mice.

Newborn pups (F1) were assigned to either the control group (CON), where mothers were fed *ad libitum*, or the lactation undernutrition group (LUN), where mothers were provided with 50% of the daily portion of chow consumed by control dams. After weaning (postnatal day 21), F1 offspring was fed *ad libitum*. Sperm was collected from the cauda epididymis of sexually mature F1 males (> 6 months) and suspended in EmbryoMax HTF medium. Flow cytometry was then utilized to assess mitochondrial potential (using JC-1 staining) and reactive oxygen species (ROS) production (using MitoSOX Red staining). High-resolution confocal microscopy was employed to visualize stained spermatozoa. Additionally, the Seahorse XF Cell Mito Stress test kit was used to evaluate the metabolic status of the collected sperm in the presence of either 5.6 mM glucose and 20 mM lactate or 5.6 mM glucose alone.

The maternal diet during lactation had a mild effect on the sperm metabolic status of F1 male progeny. Although mitochondrial membrane potential was comparable in CON and LUN sperm, lower susceptibility to depolarization by 50 µM carbonyl cyanide m-chlorophenyl hydrazone (CCCP), a typical mitochondrial uncoupler, was observed in LUN males (vs. CON, $p < 0.04$; n=4). Additionally, the basal metabolic parameters of F1 offspring sperm (e.g., respiration, ECAR, OCR) were not affected by the maternal diet during lactation (n=6). However, in both CON and LUN spermatozoa, baseline respiration and OCR were higher in the presence of 20 mM lactate ($p < 0.0001$). Notably, basal ATP production in the presence of 20 mM lactate was increased in sperm collected from LUN males (vs. CON, $p < 0.0001$; n=4). MitoSOX staining (n=7), a superoxide indicator, did not reveal changes in ROS+ sperm counts under normal and heat stress conditions.

In summary, it seems likely that early-life malnutrition in the F1 generation impairs the metabolic status of sperm. Further studies are necessary to elucidate the impact of maternal undernutrition on the reproductive performance of male offspring and their capacity to conceive healthy progeny.

Research supported by Polish National Science Centre [2018/31/B/NZ4/03527].