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**P-057 Development of a new sperm freezing medium containing serotonin to enhance sperm motility and viability**

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**Study question:** Is it possible to develop a new sperm freezing medium that improves sperm parameters after the freezing process in assisted reproductive techniques?

**Summary answer:** A new sperm freezing medium supplemented with Serotonin, L-Carnitine, and Coenzyme Q10 enhances sperm quality and improves sperm motility.

**What is known already:** Infertility affects 15% of couples worldwide, with 50% of infertility cases attributed solely to male factors. Reduced semen motility has been identified as a primary factor directly associated with infertility. The use of supplementation to enhance sperm quality has gained increasing popularity globally. The sperm freezing process in Assisted Reproductive Technology (ART) is critical for successful fertilization and can be performed in cases of severe oligozoospermia, as well as in conditions with potential future sperm count decline, such as spinal cord injury and multiple sclerosis. Sperm freezing media play a crucial role in enhancing the efficiency of the process.

**Study design, size, duration:** This study included 200 normozoospermic men, and their semen samples were analyzed over an 18-month period following the guidelines outlined by the World Health Organization (2010) to assess the efficacy of serotonin, selenium, zinc, L-Carnitine, and Coenzyme Q10 supplementation on sperm motility, viability, ROS, and DNA damage after freezing.

**Participants/materials, setting, methods:** Sperm samples obtained from 200 volunteer men undergoing infertility assessments were washed and subsequently subdivided into three groups. The study involved the examination of the samples in three distinct freezing media: (1) Origio, (2) 5HT-LK-CQ10 (serotonin, L-Carnitine, and Coenzyme Q10), and (3) a prototype (serotonin, selenium, zinc, L-Carnitine, and Coenzyme Q10). After being frozen in for 1-3 months, the samples were thawed. Post-washing, the samples were analyzed for motility, viability, apoptosis, ROS and DNA damage.

**Main results and the role of chance:** The general motility after freezing with Origio, 5HT-LK-CQ10, and Prototype was 57.6%, 39%, and 57.3%, respectively, while the progressive motility was 40.6%, 26%, and 40.2%, respectively. Annexin V staining was used to detect apoptosis in sperm samples. On average, early apoptosis over the total sperm count (TSC) per subgroup was 4.62% for Prototype, 3.51% for Origio, and 2.52% for 5HT-LK-CQ10, whereas late apoptosis ratios were significantly lower in Prototype compared to Origio and 5HT-LK-CQ10, at 59.71%, 69.9%, and 73.86%, respectively. The ratio of necrosis over TSC per subgroup was 22.85% for Prototype, 14.99% for Origio, and 15.25% for 5HT-LK-CQ10, while viability ratios were 12.82%, 11.50%, and 8.87%, respectively ( $p < 0.05$ ). ROS levels in semen after freezing were significantly lower for Prototype (8.92%, 11.32%, and 10.5%, respectively for Prototype, Origio, and 5HT-LK-CQ10) ( $p < 0.05$ ). Sperm DNA damage ratios in semen after freezing were significantly decreased for Prototype (4.1%, 5.8%, and 5.1%, respectively, for Prototype, Origio, and 5HT-LK-CQ10) ( $p < 0.05$ ).

**Limitations, reasons for caution:** Semen samples from males aged 28 to 45, who are unable to conceive, do not smoke, and have not undergone any surgical interventions, are collected from patients after a sexual abstinence period of 3-4 days.

**Wider implications of the findings:** We propose that our sperm freezing medium activated with serotonin and antioxidants could be a suitable option for selecting high-quality sperm after the freezing process in assisted reproductive technology programs. We have proven that these molecules enhance the success of assisted reproductive techniques when present in sperm preparation media.

**Trial registration number:** Yes