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CAN *ABELMOSCHUS ESCULENTUS* (OKRA FRUIT) MUCILAGE PROTECT HUMAN SPERMATOZOA FROM FROST BITE?

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Abstract: Human spermatozoa in semen sustain approximately 50% motility loss due to oxidative damage during the freezing/thaw process and as a consequence, reduces the effective number of spermatozoa needed for effective artificial insemination (EAI). We have shown that okra fruit mucilage (OKFM) has physical and diagnostic characteristics as human cervical mucus (HCM) collected during the follicular phase (FP) of the menstrual cycle when estrogen (E_2) is at peak secretion. Human semen contains antioxidants that neutralize the reactive oxygen species (ROS) produced by resident macrophages. Because OKFM contains significant levels of antioxidant(s), we hypothesize that freezing human semen in the presence of OKFM can improve the percentage yield of motile post-thaw spermatozoa for EAI. The objective of our research was to improve the freezing conditions for human semen with the ultimate goal of harvesting a higher percentage of motile cells for effective EAI. De-identified discarded freshly ejaculated human semen samples from the Nashville Fertility Center that met the W.H.O. criteria for normal semen, were used in this study. Each sample was divided into two groups to be frozen via slow cooling and freezing protocol, in the presence of commercial freezing buffer (TEST-Yolk freezing buffer [TFB]; Irvine Scientific, CA; Control) or TFB + OKFM (experimental freezing buffer, EFB). Pre-freeze sperm motility was determined for each sample prior to initiating the freezing procedure and subsequently, post-thaw sperm motion analysis, longevity, and acrosomal status were determined and compared between control and treatment group. EFB protected human spermatozoa in semen samples better ($P<0.05$) than its control counterpart. Similarly, a higher percentage ($P<0.02$) of post-thaw motile spermatozoa was harvested from samples frozen with EFB (60.0 + 12%) compared with its control counterparts (40.0 $\pm$ 9.0%). EFB also induced hyperactive motility and increased longevity among frozen-thawed motile spermatozoa compared with TFB ($P<0.05$). Staining of isolated motile cells from semen frozen in EFB and TFB with PSA-FITC revealed that acrosomal membrane was not compromised regardless of the type of freezing buffer. These data suggest that OKFM can reduce oxidative damage-related sperm death and consequently, increase the percentage yield of motile spermatozoa for EAI.

Key Words: Okra Mucus, Cervical Mucus, Semen, Human Spermatozoa, Freezing, Freezing Buffer.

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