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Cryoprotective Effects of Natural Honey Supplementation on Post-Thaw Sperm Quality of Nili-Ravi Buffalo (*Bubalus bubalis*)

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KEYWORDS	ABSTRACT
Cryoprotective, Natural Honey, Sperm Quality, Nili-Ravi Buffalo	Cryopreservation is essential for buffalo breeding and artificial insemination (AI) programs. Freezing-thawing process often induces cryo-damage, compromising sperm quality. Conventional cryoprotectants can also exert toxic effects, creating the need for safer, natural alternatives. This study evaluated the cryoprotective potential of natural honey supplementation in a Tris–Fructose–Egg-Yolk (TFEY) extender for Nili-Ravi buffalo (<i>Bubalus bubalis</i>) spermatozoa. Semen from healthy Nili-Ravi bulls was diluted with TFEY extender containing 0% (control), 2.5%, 5.0%, 7.5%, or 10% (v/v) natural honey, equilibrated, and cryopreserved in 0.5 mL straws. Sperm quality parameters: progressive motility, plasma membrane integrity (PMI; via hypo-osmotic swelling test), and livability were evaluated at both post-dilution and post-thaw stages. Data were analyzed using one-way ANOVA followed by Tukey's HSD test ($p < 0.05$). The findings demonstrate honey's potential as a natural and biocompatible cryoprotectant, offering a promising approach to improve AI success rates and support sustainable buffalo production.
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