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ACCEPTED MANUSCRIPT

Melatonin reduces oxidative damage and upregulates heat shock protein 90 expression in cryopreserved human semen

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Abstract

Sperm cells can be damaged during the semen cryopreservation process, decreasing their fertilizing ability. Physical damage and oxidative stress may occur during the freeze—thawing process. Antioxidants such as the native antioxidant melatonin can potentially improve cryopreservation outcomes. In this study, we added melatonin to cryoprotectant to examine its effect on frozen—thawed human sperm. We found that adding 0.1 mM melatonin to cryoprotectant significantly increased sperm viability $(24.80 \pm 0.46\% \text{ vs. } 20.97 \pm 1.27\%, P < 0.05)$ and membrane integrity (P < 0.05), and decreased intracellular reactive oxygen species and lipid peroxidation damage. Furthermore, mRNA levels of the transcription factor NF-E2-related factor-2 and its downstream genes were significantly increased. Resistance to oxidative stress was enhanced and expression of the antiapoptotic gene Bcl-2 was increased by inclusion of 0.1 mM melatonin in the cryoprotectant. Moreover, 0.1 mM melatonin upregulated

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