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# Effects of Thawing Procedure on Postthawed In Vitro Viability and In Vivo Fertility of Red Deer Epididymal Spermatozoa Cryopreserved at -196 °C

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In this study, we have determined the effects of individual factor and thawing procedure on in vitro viability and in vivo fertility of frozen-thawed red deer epididymal spermatozoa. The spermatozoa that were collected from 13 Iberian deer stags were diluted at room temperature in a Triladyl®-20% egg yolk medium and frozen in nitrogen vapors. In the first experimental series, sperm samples were collected from 10 mature stags. For thawing, the frozen straws were subjected to 3 different procedures: I (37°C for 20 seconds), II (60°C for 8 seconds) and III (70°C for 5 seconds). Sperm cryosurvival was judged in vitro by microscopic assessments of individual sperm motility (SM) and of plasma membrane and acrosome (NAR) integrities. Statistically significant variations were found ($P < .05$) between stags for most of the seminal parameters evaluated. The thawing procedure did not have an effect on the seminal characteristics evaluated after this process, except for SM ($P < .05$), with the best overall recovery rates after freezing and thawing found with the use of protocol I. Our results also show a differential resistance to return to isosmotic conditions of spermatozoa thawed using the different thawing protocols. In the second experimental series (insemination artificial trial), with spermatozoa from 3 stags, results of fertility were statistically higher (69.7% vs 42.4%, $P = .014$) when spermatozoa were thawed at 37°C for 20 seconds than were warmed at 60°C for 8 seconds. Therefore, thawing protocol I, which provides slow thawing rates, was the most beneficial for epididymal spermatozoa thawing of the cervid subspecies analyzed in this study. In summary, high in vitro survival and in vivo fertility of frozen-thawed deer epididymal spermatozoa were dependent on warming rates, but each stag exhibited its own sensitivity to cryopreservation.

Key words: *Cervus elaphus hispanicus*, freezing, membrane integrity, sperm motility, postmortem

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