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Effects of copaiba oil-resin (*Copaifera pubiflora*) on the quality of cooled and frozen/thawed canine semen

Otávio Luís de Oliveira Henriques Paulo\(^a\), Maricy Apparicio\(^a\), Beatrice Ingrid Macente\(^b\), Fabiana Ferreira de Souza\(^c\), Laiza Sartori de Camargo\(^c\), Luiz Roberto Pena de Andrade Junior\(^c\), Frederico Ozanam Papa\(^c\), Maria Anita Vasconcelos Ambrósio\(^d\), Carlos Henrique Gomes Martins\(^d\), Valéria Amorim Conforti\(^a\)

\(^a\)Animal Reproduction Laboratory, Animal Science Graduate Program, University of Franca, Franca, São Paulo, Brazil

\(^b\)Department of Preventive Veterinary Medicine and Animal Reproduction, São Paulo State University, Jaboticabal, São Paulo, Brazil

\(^c\)Department of Animal Reproduction and Veterinary Radiology, College of Veterinary Medicine and Animal Science, São Paulo State University, Botucatu, São Paulo, Brazil

\(^d\)Research Laboratory of Applied Microbiology, University of Franca, Franca, São Paulo, Brazil

valeria.conforti@unifran.edu.br

The objective of this study was to investigate the effects of the oil-resin obtained from the Amazonian tree *Copaifera pubiflora* on canine semen. The oil-resin from *Copaiba* sp is a naturally-derived product whose antibacterial and anti-inflammatory properties have been researched for pharmaceutical applications \(^1\). In this study, four different media were used to determine the oil-resin’s effects as an ingredient in semen extenders, as follows: 1) standard tris-egg yolk medium (control); and tris-egg yolk-based media containing the following concentrations of *C. pubiflora* oil-resin: 2) 0.1% (OIL-0.1); 3) 0.08% (OIL-0.08); and 4) 0.0% (OIL-0). The control medium (1) contained penicillin and streptomycin but no antibiotics were added to any medium containing the oil-resin. The effects of the 4 extenders on bacterial growth after culture and longevity of cooled semen were evaluated. Additionally, sperm membrane integrity of frozen/thawed semen was assessed through both fluorescent and eosin-nigrosin staining techniques. Lastly, computer-assisted sperm analysis – CASA was employed to evaluate post-thaw motility parameters. The effects of the addition of the oil-resin on motility parameters and membrane integrity were assessed via ANOVA and Tukey’s test. Results of the microbiological culture of semen diluted in each of the media showed that only the control medium was able to inhibit proliferation of *Escherichia coli* and *Staphylococcus aureus*. Longevity of spermatozoa in cooled semen, as indicated by motility and vigor after 96 hours at 4°C, was comparable (\(P > 0.05\)) across all media. Likewise, sperm membrane integrity after thawing did not differ (\(P > 0.05\)) between the treatment groups. CASA revealed that frozen/thawed semen in both the control and the OIL-0.08 groups were comparable (\(P > 0.05\)) in terms of total motility, progressive motility, path velocity, progressive velocity, and percentage of rapid sperm cells; and that both groups were superior (\(P < 0.05\)) to the other treatments. Compared to the oil-resin-free media, both OIL-0.08 and OIL-0.1 had superior (\(P < 0.05\)) amplitude of lateral head displacement and inferior (\(P < 0.05\)) linearity, which might be related to hyperactivation of sperm cells in the oil-resin-containing groups \(^2\). Future studies should confirm whether or not the addition of *C. pubiflora* oil-resin causes hyperactivation in canine spermatozoa, which could potentially have implications on *in vitro* fertilization results.
