ABSTRACTS

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Intravaginal artificial insemination in bitches using frozen-thawed semen after dilution in powdered coconut water extender (ACP-106c)

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OBJECTIVES AND METHODS: The study aimed to evaluate the in vitro and in vivo efficacy of powdered coconut water extender (ACP-106c) on canine semen freezing. The experimental protocol was approved by the Animal Ethics Committee from State University of Ceará, Fortaleza, Ceará, Brazil (process number 08517678-8). Ejaculates (n = 5) were obtained by digital manipulation, with the second fraction evaluated for volume, spermatozoa concentration, total motility, vigor, morphological normal spermatozoa, functional of plasmatic membrane by hyposmotic test (HOST), alive spermatozoa and normal acrosome. For freezing, the semen was diluted in ACP-106c, 10% egg yolk and 6% glycerol (200 x 10⁶ sperm/mL) and subjected to cooling (15 °C/40 min and 4 °C/30 min) and freezing (liquid N₂). In step 1, samples were thawed (37 °C/1 min) and submitted to the same analysis of fresh semen and computer analysis (CASA). In step 2, samples were thawed and used for intravaginal artificial insemination of 10 bitches: Beagle (n = 1), Boxer (n = 3), French Bulldog (n = 4) and Brazilian Terrier (n = 2). After re-dilution in ACP-106c (total volume: 4 mL), the bitches were inseminated with frozen semen from their respective breeds (200 x 10⁶ sperm/mL). The same bitches had been subjected to natural mating in earlier estrous cycles. Natural mating and intravaginal artificial insemination was performed after 70% of vaginal cells keratinized on vaginal cytology. Pregnancy diagnosis was performed by ultrasonography. All data were expressed as mean ± standard error of means (SEM). Differences were considered to be significant when P<0.05. All percentual results were submitted to an angular transformation before being analyzed. In order to determine a group effect (NM x AI) ANOVA followed by PLSD Fisher test were used. Pregnancy and parturition rates were analyzed by X². It was used Stat View for Windows®, 5.0 and SAS, SAS Institute Inc., Cary, NC, USA.

RESULTS: Fresh semen presented a volume of 1.0 ± 0.1 mL and spermatozoa concentration of 10.5 ± 1.1 x 10⁶ sptz/mL. Fresh semen x frozen-thawed semen: total motility (97.0 ± 1.5 % x 77.0 ± 2.6 %); vigor (4.8 ± 0.1 x 3.8 ± 0.1); morphological normal spermatozoa (85.6 ± 1.1 % x 80.0 ± 1.9 %); HOST (87.2 ± 1.3 % x 49.5 ± 1.3 %); alive spermatozoa (97.4 ± 0.3 % x 47.1 ± 0.4 %) and normal acrosome (92.9 ± 0.9 % x 88.5 ± 2.0 %). All parameters decreased after frozen-thawing (P<0.05), except normal acrosome that remains the same (P>0.05). All parameters decreased after frozen-thawing were better than those described previously for sperm motility of semen frozen in tris-egg yolk, using glycerol (1) or ethylene glycol (2) and those using tris-egg yolk-glycerol added by Equex® 1% (2). The good results presented in our work can be due to the presence of natural anti-oxidants (vitamins and minerals) and sugars in the coconut water composition. The parameters evaluated by CASA were: total motility (77.3 ± 4.1 %), progressive motility (29.7 ± 2.4 %), VCL (32.6 ± 3.8 μm/sec), VSL (22.6 ± 2.4 μm/sec), VAP (20.4 ± 3.0 μm/sec), LIN (60.1 ± 1.31 %), STR (74.2 ± 1.9 %), ALH (1.8 ± 0.2 μm) and BCF (5.4 ± 0.9 Hz). The best results obtained here compared to those that our team obtained in the past (3) using the powder coconut water extender (ACP-106c) can be due to reduction of egg yolk concentration and a better dissolution that resulted in less granules and, consequently, in a best spermatozoa evaluation using the ACP-106c. The option to use only egg yolk at 10% comes from previous good results obtained with this egg concentration (4). Insemination with volumes between 4 and 6 mL are considered appropriated for intravaginal insemination (5). After natural mating, bitches achieved 100% of pregnancy and delivery rates and a prolificacy of 5.4 ± 0.3 puppies. After AI: 60%- pregnancy rate, 50%- delivery rate, 3.4 ± 0.6- prolificacy. All parameters decreased comparing natural mating x intravaginal artificial insemination (P < 0.05). A reduction in the prolificacy had already been observed after intravaginal artificial insemination using frozen-thawed semen (2,5).

CONCLUSION: We conclude that the ACP-106c can be used successfully for freezing canine semen, promoting good pregnancy rates after intravaginal artificial insemination.
