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The Effect of Two Cooling Protocols on Quality of Liquid Boar Semen

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Introduction: After collection, extension and packaging of liquid boar semen into tubes or bottles, artificial insemination doses are routinely cooled to 15-18°C for storage up to 7 days. Laboratories typically place the doses directly into an incubator at the storage temperature immediately after processing, or place the doses on the benchtop at room temperature for approximately 2 hours prior to moving them into the storage incubator for final cooling. The purpose of the current investigation was to evaluate the quality of extended semen following cooling by these two procedures.

Materials and Methods: In experiment 1 and 2, ejaculates (samples) were collected (day = 0) in the routine manner using the gloved-hand technique and were extended using Androhep® extender at approximately 45 x 10^6 total sperm per ml (n= 41, experiment 1) or 12 x 10^6 total sperm per ml (n = 15, experiment 2). Extended semen was packaged into Minitube polyethylene tubes or bottles. For experiment 1, one tube per sample was immediately placed in a 15°C cool room (treatment 1). A second tube per sample was placed on the laboratory bench at room temperature (approximately 23°C, treatment 2). After 2 hours, this second tube was placed in the 15°C cool room. On day 1, samples were each aliquoted into five precooled (15°C) glass culture tubes. Then on days 1, 2, 3, 4 and 7, one culture tube per sample per treatment was warmed in a block heater for 20 minutes at 36°C. Semen samples were then evaluated microscopically (200x, phase contrast) in the routine manner for percent normal motility. For experiment 2, one tube or bottle per sample was placed immediately in a storage incubator at 17°C, while a second tube or bottle was placed in an incubator at 25°C for 2 hours prior to transfer to the storage incubator at 17°C. Two to 3 ml aliquots were removed from each tube or bottle on days 1, 3, 5, and 7 and placed immediately in a glass culture tube for subsequent warming in a water bath at 35°C for 10 minutes prior to microscopic evaluation of motility (200x, phase contrast). In both experiments, prewarmed microscope slides and cover glasses were used and the microscope stages were prewarmed to the same temperature (37°C) using Minitube® HT-200 stage warming systems. Paired means of motility data within day were analyzed by the Student's T-test.

Results and Discussion: In both experiments, within day, spermatozoa motility was not significantly different ($P > 0.05$) between treatments. In experiment 1, mean ± S.D. percent sperm motility for treatments 1 and 2 on days 1,2,3,4 and 7 were 81.8±3.8, 81.3±4.6; 80.0±6.4, 78.3±5.7; 79.4±7.0, 78.8±4.2; 76.8±9.7, 76.0±12.6; and 74.5±8.8, 70.2±17.3, respectively. In experiment 2, data for days 1,3,5 and 7 were 85.3±10.4, 85.7±9.0; 85.0±9.1, 82.7±9.8; 82.0±10.0, 81.0±8.5, and 75.3±10.1, 75.3±10.1, respectively. Data from these experiments provides support that both procedures for cooling semen result in semen of similar quality. However, depending upon individual laboratory conditions during routine semen processing, the temperature of doses placed on bench tops may not be stable or consistent. Variation in temperature may result in inconsistent seminal quality. Therefore, for decreased variation of cooling conditions and increased standardization and optimization of semen cooling, it is suggested that doses be placed directly in the storage incubator.