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108 Discriminating highly resilient spermatozoa during long-term chilled storage. Kristen M. Johnson¹, Serge L. Kameni¹, Notsile H. Dlamini¹, Shengfa F. Liao¹, Jean M. Feugang¹, ¹*Mississippi State University*

Abstract: Artificial insemination (AI) is the most important assisted reproductive technique devised to enhance the genetic potential of animals by enabling widespread utilization of elite males. In the swine industry, AI with extended semen is highly effective, with several extenders developed for long-term storage. Regardless, the gradual decline of sperm quality during storage remains a major concern for rapid genetic dissemination. We hypothesized that existing sire-to-sire variations could reflect on sperm survival during prolonged storage. Therefore, this study examined boar sperm motility and morphology profiles to determine potential disparities during long-term storage within a commercial extender. Freshly collected Duroc boar semen samples (n = 58 boars) were extended at a commercial boar stud (Prestage Farms, MS), and sample doses (n = 29) were appropriately transported to the laboratory for experiments. Semen samples were aliquoted in tubes (filled to the top) and immediately stored in an incubator set at 17°C for up to 10 d post-collection. Daily, samples were assessed for total motility (TM), progressive motility (PM), velocities [average path velocity (VAP), curvilinear velocity (VCL), and straight-line velocity (VSL)], and normal morphology (NM) using the computer-assisted sperm analyzer (CASA, CEROS II). The deviations of TM means were used for survival discrimination between so-called “good” and “bad” semen. Data were log-transformed and analyzed (repeated ANOVA and Bonferroni adjustment) to determine the effect of time during storage. Results are expressed as mean ± SEM and P < 0.05 indicates significant differences. As expected, all data demonstrated a consistent and significant decline over time (P < 0.05), from day-0 to day-10 (TM: 78.2 ± 1.0% to 42.8 ± 4.3%; PM: 46.1 ± 1.8% to 16.2 ± 2.3%, and NM: 85.1 ± 1.0% to 76.1 ± 1.9%). Notably, velocity parameters (VAP, VSL, and VCL) remained relatively stable during the storage period, while the distal droplets contributed the most to morphological defects (54.1 ± 1.5% vs. 28.3 ± 1.5%, 18.3 ± 0.5%, and 2.4 ± 0.2% for the proximal droplets, bent and coiled tailed, respectively; P < 0.05). Survival discrimination indicated the highest deviations from the overall averages on day-7. Consequently, good (n = 8) and bad (n = 7) semen samples had significantly different (P < 0.05) in TM (80.2 ± 1.4% and 21.7 ± 4.6%, respectively), PM (39.7 ± 2.7% and 4.7 ± 1.7%,

respectively), and NM (87.9 ± 1.9 and $66.4 \pm 2.6\%$, respectively). Similarly, the velocity parameters were significantly different ($P < 0.05$) between both sample groups, including VAP ($74.9 \pm 2.6 \mu\text{m/s}$ vs. $40.4 \pm 8.7 \mu\text{m/s}$), VCL ($135.2 \pm 6.0 \mu\text{m/s}$ vs. $81.9 \pm 17.3 \mu\text{m/s}$), and VSL ($59.0 \pm 1.8 \mu\text{m/s}$ vs. $31.3 \pm 6.8 \mu\text{m/s}$). These findings indicate that specific subsets of boar semen may be well-suited for survival during long-term chilled preservation. The development of tools for early discrimination of semen doses, immediately following collection, could significantly enhance their management and facilitate appropriate shipments to remote areas or during adverse weather conditions. Ongoing studies are dedicated to unraveling the cellular mechanisms underlying the resilience of such spermatozoa. - Research funded by USDA-ARS, grant#58-6066-3-038.

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