Capacitation Signatures in Fresh and Cryopreserved Bovine Sperm

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Capacitation is an irreversible process sperm must go through prior to fertilization. Our hypothesis was that variation in capacitation status between bulls serves as an indicator of their fertility. Recently, it was discovered that zinc (Zn) signatures can be used to quantify capacitation status in bovine sperm as measured by flow cytometry. *In vitro* capacitation (IVC) and flow cytometry are long (5+ hours) processes, and sample collection can require extensive travel to a laboratory. Storing samples overnight and evaluating the following day would facilitate the workflow of sample analysis, but may provide different capacitation signatures than those of same day and cryopreserved samples. The objective of this study was to characterize Zn capacitation signatures on the day of collection (D0), stored overnight in OptiXcell extender (D1) or cryopreserved (FT) samples. Comparisons of capacitation signatures during IVC between D1 and FT samples were also made. Bulls (n=24) were collected by artificial vagina, and semen was either cryopreserved in a commercial egg-yolk based extender or diluted in OptiXcell extender and transported to the lab for analyses. Day 0 samples were analyzed for Zn signatures, viability, and acrosome integrity via flow cytometry approximately 7 hours after collection without IVC. Day 1 samples were stored at 4^0C overnight and analyzed approximately

20 hours after collection. Day 1 and FT samples were *in vitro* capacitated for 3 hours and evaluated for Zn signatures, acrosome integrity, and viability via flow cytometry at times (T) 0, 1.5, and 3 hours. We observed that D0 samples had at least 21% more ($P < 0.001$) viable sperm cells than D1 and FT samples at T0, which did not differ ($P > 0.10$). An estimated 20% of sperm appeared to advance through capacitation signatures when stored in OptiXcell overnight and these cells also appeared to progress through the acrosome reaction. Freezing and thawing resulted in approximately 30% of sperm being cryocapacitated and not progressing through capacitation signatures. The capacitation signatures also differed during IVC in samples stored overnight compared to cryopreserved. Viable sperm cells that had not yet capacitated or were early in capacitation (signatures 1 and 2) were greater in FT compared to D1 ($P < 0.01$) and greater at T0 than T1.5 or T3 ($P \leq 0.01$). Cryopreserved samples had more sperm cells in a capacitated state (signature 3) during IVC when compared to D1 ($P < 0.0001$) and these cells did not progress further through capacitation. Dead cells with no Zn present (signature 4) were substantial in D1 samples and increased in FT and D1 samples from T0 to T1.5 ($P < 0.0001$). Both FT and D1 samples had fewer live sperm with an intact acrosome from T0 to T1.5 ($P = 0.002$). We conclude that storing cells overnight in OptiXcell extender does not prevent the progression of sperm from going through the process of capacitation. Cryocapacitation has been previously reported to occur in FT samples. These cells die without progressing through the final stages of capacitation. Capacitation signatures observed in bulls were highly variable during IVC for cryopreserved samples and samples stored overnight in extender. Further research is needed to understand how these signatures relate to bull field fertility.

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