Comparison of one versus two doses of prostaglandin F2 alpha in a 5-day, progesterone-based synchronization protocol in Angus-cross beef cows

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The objective was to compare reproductive performance of Angus-cross beef cows synchronized with GnRH, a progesterone-releasing intravaginal device (CIDR) for 5 d, and one dose of either dinoprost tromethamine (PGF) or cloprostenol sodium (CLP), or two doses of PGF on the day of CIDR withdrawal. Angus-cross beef cows ($N = 830$) at six locations received 100 μg of GnRH and a CIDR on Day 0; the CIDR was subsequently removed on Day 5 (in the morning). Cows were fitted with a pressure-sensitive estrus detection device (Kamar) at CIDR withdrawal. Within farm, cows were randomly allocated to receive: 25 mg of PGF at CIDR removal (1XPGF; $n = 277$); or 25 mg of PGF at CIDR removal, and again 7 h later (2XPGF; $n = 282$); or 500 mg of CLP at CIDR removal (1XCLP; $N = 271$). On Day 8 (72 h after CIDR removal), all cows were given 100 μg of GnRH and concurrently inseminated.

Data were analyzed using a Glimmix model (SAS 9.1; SAS Institute, Cary, NC, USA). Accounting for other significant variables such as AI sire ($P < 0.05$) and cows that exhibited estrus at or prior to the time of AI ($P < 0.0001$), timed-AI pregnancy rates were greater ($P < 0.0001$) in the 2XPGF (69.0%) than the 1XPGF (52.0%) and 1XCLP (54.3%) treatments. Accounting for body condition score at the initiation of synchronization protocols ($P < 0.05$), breeding-season pregnancy rates were not different among 2XPGF, 1XPGF and 1XCLP treatment groups, 92.9, 87.0, and 87.5%, respectively ($P > 0.1$). The treatment × location interaction for pregnancy rate approached significance for timed-AI ($P = 0.09$) and was significant ($P < 0.01$) for breeding season pregnancy rate.

In conclusion, cows that received two doses of PGF on the day of CIDR removal in a 5-d CO-Synch + CIDR synchronization protocol had higher timed-AI pregnancy rates than cows receiving a single treatment with either PGF or CLP (at CIDR removal).

Keywords: Estrus synchronization; CIDR; $PGF2_a$; Pregnancy rate; Beef cattle

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Performance evaluation of the SQA-Vb automated sperm quality analyzer for bulls

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The objective was to compare the automated SQA-Vb (Medical Electronic Systems, Caesarea Industrial Park, Israel) test results to manual semen analysis data, to determine if the automated system is a viable alternative to the manual method. The SQA-Vb is an analytical sperm quality analyzer for bulls that performs a 45-s automated quantitative evaluation of fresh and frozen-thawed bull semen, including sperm concentration, motility, progressive motility, normal morphology, and velocity. The study was conducted at Sion AI laboratory (Israel). For fresh and frozen-thawed bull semen samples ($n = 101$ and $n = 30$, respectively), automated semen analysis results were compared to manual test data analyzed microscopically. Sperm concentration was measured using a Makler sperm counting chamber (Sefi-Medical Ltd., Haifa, Israel).

Table 1
Performance characteristics of SQA-Vb automated sperm quality analyzer

Semen sample	Semen parameter	CV (%)	Correlation to manual method (r)	Range
Fresh $n = 101$	Concentration ($\times 10^6$/mL)	2.4	0.93	205.7–1900.0
	Motility (%)	4.1	0.81	4.2–99.1
	Progressive motility (%)	8.0	0.75	0.1–97.2
	Velocity (μ/s)	9.9	0.81	0–70
Frozen $n = 30$	Concentration ($\times 10^6$/mL)	4.7	0.99	5.7–129.6
	Motility (%)	5.6	0.90	32.1–76.1
	Progressive motility (%)	6.0	0.71	15.2–42.9
	Velocity (μ/s)	3.6	0.86	0–70

Abbreviations: CV: coefficient of variation; r: correlation coefficient; n: no. samples.

Sperm motility and progressive motility were evaluated with B-Sperm™ computerized video software (Medical Electronic Systems, Ltd. Caesarea Industrial Park, Israel). Bull semen was visualized ''live'' on a PC monitor using the video image mode; non-motile and non-progressively motile spermatozoa were counted first, then the ''live'' image was ''frozen'' and all sperm were counted. Percentages of motility and progressive motility were calculated. Each semen sample was counted twice (minimum of 100 spermatozoa per replicate). For precision, coefficients of variation (CV) were calculated based on duplicate samples. For analytical accuracy, correlations were determined between the automated versus the manual method. Data are summarized below (Table 1).

Coefficients of variation $\leq$10% demonstrated high repeatability of the SQA-Vb. Furthermore, SQA-Vb and manual data were highly correlated. Therefore, we concluded that the SQA-Vb was a viable alternative to manual analysis of bull semen.

Keywords: SQA-Vb; Semen analysis; Motility; Sperm; Cattle

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Assessing cryocapacitation in frozen-thawed bovine sperm from a commercial bull stud

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Precocious sperm capacitation or 'cryocapacitation' may contribute to reduced fertility for cryopreserved semen, but there is no quantifiable, biochemical assay for this functional change. We hypothesized that if frozen-thawed bovine sperm undergo cryocapacitation, it would be detected by protein tyrosine phosphorylation, a quantifiable biochemical marker of fresh bovine sperm capacitation, and an increase in the rate of lysophosphatidylcholine-induced acrosome reaction (LC AR), a standard sperm capacitation assay. For this study, commercially processed cryopreserved semen (milk-glycerol extender) from single ejaculates of 19 Holstein bulls was evaluated twice for each bull. Samples were evaluated post-thaw (0 h) or after a 4-h incubation in TALP medium with: no additives (NA); or 10 μg/mL heparin (H); or 10 μg/mL heparin and 1 mM dibutyryl-cAMP plus 100 μM IBMX (HcA; known to maximize bovine sperm capacitation). Sperm were assessed for viability using CFDA/Propidium Iodide staining, for spontaneous (sAR) and LC AR (Coomassie G-250 stain), and for protein tyrosine phosphorylation, as assessed by both antiphosphotyrosine slot blot analysis and standard one-dimensional SDS-PAGE immunoblot analysis. Protein tyrosine phosphorylation of slots and one band (p48) were quantified by image analysis (Gel-Pro 4.5, Media Cybernetics, Silver Spring, MD, USA). Data are presented as means $\pm$ S.D. S.D. and were evaluated by three-way ANOVA, followed by Tukey's test for multiple comparisons, with significance set at $P < 0.05$ (Sigma-Stat 3.0; SPSS Inc., Chicago, IL, USA). Except for two bulls with low viability (A, B), there were no significant inter-bull differences in sperm viability, but viability declined in significant increments for NA, H, and HcA (0 h: 60.7 $\pm$ 8.9%; NA: 53.0 $\pm$ 8.2%; H: 47.8 $\pm$ 10.3; and HcA: 33.3 $\pm$ 8.9%). The rates of sAR at 0 h were lower than the 4 h incubated samples (0 h: 15.7 $\pm$ 6.6%; NA: 40.6 $\pm$ 21.3%; H: 42.7 $\pm$ 19.8; HcA: 39.6 $\pm$ 16.9%) and only two bulls (B, C) had significantly higher sAR rates. Rates of LC AR at 0 h were highly variable, did not differ significantly between bulls or between experiments for the same bull, and were consistent with some cryocapacitation (14.8 $\pm$ 17.1%). LC AR at 4 h was significantly reduced compared to 0 h, highly variable, and not consistently repeatable (NA: 1.7 $\pm$ 17.7%; H: $-$1.3 $\pm$ 13.8; HcA: 1.4 $\pm$ 10.2%). Slot blot analysis was consistently repeatable for 0 h samples, but not for incubated samples, and detected significantly higher protein tyrosine phosphorylation for one bull (A). Tyrosine phosphorylation of p48 relative to positive control (HcA), differed significantly among treatments (0 h: 17.8 $\pm$ 7.8%; NA: 27.5 $\pm$ 10.7%; H: 33.3 $\pm$ 13.5%), was consistently repeatable, and detected two bulls (D, E) with reduced, and one with increased (F) p48 tyrosine phosphorylation. The relative lack of inter-bull differences detected with these assays was not unexpected, as all bulls were proven sires. Tyrosine phosphorylation of p48 provided data similar to LC AR at 0 h, but not following incubation, perhaps due to the high rate of sAR. Protein tyrosine phosphorylation is therefore a good candidate biochemical marker for bovine sperm cryocapacitation, but should be characterized in bulls with more variable responses to freezing.

Keywords: Semen analysis; Sperm; Cryocapacitation; Tyrosine phosphorylation; Bovine

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