Induction of hyperactivation in stallion sperm using 4-amynopyridine

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Hyperactivation is a change in the pattern of sperm motility required for fertility. Procaine-treated stallion sperm evaluated by computerized-assisted sperm motility analysis (CASA) became hyperactivated as defined by decreases in straight line velocity (VSL) and linearity (LIN), and increases in amplitude of lateral head displacement (ALH) and curvilinear velocity (VCL). This also yielded high rates (~60%) of homologous in vitro fertilization (IVF). Because procaine may be toxic to oocytes, alternative hyperactivation-inducing treatments are desirable. Thus, our hypothesis was that 4-aminopyridine (4-AP), which raised calcium levels in human and mouse sperm and hyperactivated mouse sperm, would induce stallion sperm hyperactivation. Sperm (3 stallions x 2 ejaculates) were diluted in modified Whitten’s (37°C) and treated with: 5 mM procaine; 2, 4, 6, 8 or 10 mM 4-AP; or, untreated. Sperm motility measures obtained via CASA were analyzed by ANOVA with Fisher LSD test applied for mean separation (P<0.05). Notably, 4 mM 4-AP yielded motility changes similar to procaine (P>0.05) and consistent with hyperactivation as compared to untreated controls (P<0.05): VSL = 52.3±23.9 vs. 87.9±12.1 μm/sec; LIN = 21.6±5.1 vs. 52.4±8.4 %; ALH 9.1±2.8 vs. 6.3±0.8 μm/sec; and, VCL = 256.7±74.1 vs. 192.6±31.3 μm/sec, respectively. We conclude that inducing hyperactivation with 4-AP may be an alternative to procaine for supporting equine IVF.

Keywords: Stallion sperm, hyperactivation, 4-amynopyridine, procaine, CASA.