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Equine Spermatozoa at Optimum Physiological State Are Selected by Chemotaxis Toward Progesterone

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The success of assisted reproduction techniques depends in part on sperm quality, which influences not only fertilization but also embryo development and implantation. In our laboratory, we designed the Sperm Selection Assay (SSA) based on chemotaxis towards progesterone, which selects human sperm at optimum physiological state (capacitated, with low levels of DNA fragmentation and reactive oxygen species). The aim of this study was to define the experimental conditions to apply the SSA in unsexed and sexed equine sperm samples. Cryopreserved sperm samples of three stallions were conventionally thawed, removing the seminal plasma and cryoprotectant by a modified swim up procedure. Spermatozoa were incubated in BWW media with or without capacitating conditions (25 mM $NaHCO_3$ and 0.3% BSA), at 38.5°C at an atmosphere of 5% CO_2 on air, for 45 minutes. The SSA device consists of two wells connected by a tube. Well 1 (W1) was filled with the sperm suspension and well 2 (W2) with the attractant solution, which diffused along the connecting tube as a gradient. The percentage of sperm accumulation in W2 was determined as the difference between with and without attractant. Firstly, we established the capacitation conditions in equine sperm samples by inducing the the acrosome reaction (AR) with A23187 calcium ionophore, and by the protein tyrosine phosphorylation pattern (PY). The level of capacitated spermatozoa was significantly increased at 45 minutes of incubation vs non-capacitated control. Next, we defined the experimental conditions to set up the SSA with frozen-thawed, unsexed and sexed equine spermatozoa, determining the percentage of accumulated spermatozoa in W2 under several dose response conditions and timing: by placing 2 million sperm per ml in W1 (16±2% and 19±2%, respectively), 10 pM progesterone in W2 as the attractant solution (13±2% and 17±2%, respectively), and running the SSA for 10 min (9±2% and 18±2%, respectively). We next verified whether the sperm selection in the SSA was indeed mediated by chemotaxis. Thus, sperm accumulation in W2 was only observed when capacitated spermatozoa were loaded in W1 and progesterone was displayed as an ascending gradient (10±2%). The quality of selected spermatozoa in W2 containing progesterone was better than that of spermatozoa without being selected by the SSA where a significant higher level of capacitated spermatozoa (PY) and lower level of DNA fragmentation (evaluated by the "Halo sperm test"), for sexed and unsexed samples, were observed. In conclusion, equine spermatozoa are selected by chemotaxis towards progesterone are at the optimum functional state, at a similar extent in sexed and unsexed samples. The results have potential application to improve current equine reproductive biotechnologies.