Functional Capacitation of Stallion Sperm Promotes Interspecies Embryo Chimerism and Equine In Vitro Blastocyst Development from Cryopreserved Sperm

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ABSTRACT

In vitro fertilization (IVF) is highly optimized in many species but has historically failed in horses. Equine IVF failures are often attributed to incomplete functional capacitation of stallion sperm, despite the efficacy of similar techniques in other species. Lack of data describing conditions that promote capacitation while maintaining sperm function remains a significant obstacle to the success of equine IVF. Herein, we report the first equine embryo produced by IVF from cryopreserved stallion sperm, achieved through a thorough characterization of conditions that promote sperm capacitation conducive to interspecies and equine fertilization. The capacitation status of stallion sperm was characterized using fresh, cooled, and frozen sperm (2-4 ejaculates; 5 stallions). Sperm isolation was achieved by Percoll centrifugation, Swim-up, or microfluidics (VetMotl), depending on experiment. Four incubation conditions were tested: 1) fresh sperm maintained at 38°C and 6% CO_2, 2) fresh sperm incubated at room temperature (RT), 3) cooled sperm incubated at RT (5°C for 24 and 48 hr), and 4) frozen-thawed sperm incubated at RT. Sperm were diluted to 20 x 10^6 total sperm/mL in capacitating media and evaluated at 0, 4, 8, 12 and 22 hr (fresh and cooled sperm) or at 0, 1 and 2 hr (frozen-thawed sperm) for kinematics (CASA system), acrosome integrity (FITC-PSA) and capacitation status (tyrosine phosphorylation). No significant differences across time in all sperm parameters were identified for Percoll vs. Swim-up. For fresh sperm, total motility (TM) dropped quickly over time (0-22 hr) when samples were incubated at 38°C (87%, 43%, 24%, 21%, 15%). Therefore, experiments were repeated with fresh sperm maintained at RT, where TM remained constant for the first eight hours (0 hr = 80%, 4 hr = 69%, and 8hr =65%). When sperm were cooled and then held at RT for 22 hr, no interaction was detected between cooling time (24 and 48 hr) and incubation time for all variables. The motility of previously cooled sperm remained above 50% for 8 hr (70%, 55%, 51%, 47%, 39%). For all sperm holding conditions except cryopreserved sperm, maximum capacitation status was reached at 4 hr of incubation (45-73%), while acrosome integrity remained above 75% for fresh and 57% for cooled samples. Frozen-thawed sperm isolated by microfluidics exhibited maximum motility (73%) and capacitation status (80%) immediately after

thawing. To challenge sperm fertilizing ability and discern reasons for failed equine IVF success that may falsely be attributed to stallion sperm factors confounded by suboptimal culture systems, IVF was conducted in a highly optimized bovine embryo culture system to generate interspecies embryo chimeras. Fresh stallion sperm maintained at RT for 4 hr prior to gamete co-incubation resulted in 41% cleavage (171/380) and 7% blastocyst development (22/380; n = 4 reps). Equine IVF was then performed using pooled frozen-thawed sperm from two stallions selected by microfluidics. Sperm were diluted in capacitating media and added immediately to fertilization drops containing 6 oocytes aspirated from a 19-year-old mare and matured for ~28 hr. Two embryos developed to the expanded blastocyst stage. In summary, the temporal characterization of capacitation suggests that stallion sperm do not require prolonged pre-incubation for successful fertilization. Optimization of sperm conditions for in vitro production of blastocysts by interspecies and equine IVF indicates a requirement for motility maintenance while promoting capacitation status and preserving acrosome integrity.