

SPERM PROCESSING AFTER CONVENTIONAL VS. PIEZO-DRIVEN ICSI IN EQUINE ASSISTED REPRODUCTION

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Abstract Body

Intracytoplasmic sperm injection (ICSI) is conducted using two main techniques, conventional (Conv) and Piezo-driven (Piezo). Use of the Piezo increases activation rates after sperm injection in the mouse; however, because Piezo ICSI is associated with added equipment and expertise, and the use of mercury, many human laboratories perform only Conv ICSI. We compared results between Conv and Piezo ICSI in an equine system, using a non-toxic ballast (Fluorinert) in the Piezo pipette. In a preliminary study, there was no difference in blastocyst rates between Conv and Piezo (39% and 40% per injected oocyte, respectively; n = 169). However, there was a tendency for earlier blastocyst formation in the Piezo treatment (74% at Days 7-8; vs 59% for Conv). Therefore, we evaluated the kinetics of sperm processing after ICSI in the two treatments. In vitro-matured equine oocytes were randomly assigned to Conv or Piezo, performed concurrently by separate operators. Sperm tail mitochondria were labeled before injection. The oocytes were fixed immediately after injection (0H) or after 6 or 18 h culture. Fixed oocytes (n=123) were stained with DAPI and PNA. Median relative values for sperm head area at 0, 6 and 18H were 1289, 1340 and 62328, respectively for Conv and 1210, 4079, and 72953 for Piezo; these differed significantly (Mann-Whitney U test) at 6H ($P < 0.01$). At 6H, more sperm had an attached acrosome in Conv than in Piezo (80% vs. 14%, respectively; $P < 0.0001$, Fisher's Exact); also more sperm had attached tails (75% vs. 29%; $P < 0.01$). Moreover, at 6H, more oocytes remained in metaphase II in Conv (80%) vs. Piezo (9.5%; $P < 0.0001$). These results show that although there was no difference in overall blastocyst rates between Conv and Piezo, the kinetics of development after sperm injection were significantly faster in Piezo ICSI.