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Clinical and Functional Analyses of Frozen Porcine Sperm that Indicate IVF Success

Daigneault BW[1,2] **and Miller DJ**[1*]

[1]*Department of Animal Sciences, University of Illinois, Urbana-Champaign, Illinois, USA*

[2]*Department of Animal Science, Michigan State University, East Lansing, Michigan, USA*

Corresponding author: Miller DJ, Department of Animal Sciences, University of Illinois at Urbana-Champaign, USA, Tel: 217-333-3408; Fax 217-333-8286; E-mail: djmille@illinois.edu

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Cryopreserved sperm are often used for in vitro fertilization (IVF) because having frozen sperm available simplifies IVF logistics when dealing with unpredictable female reproductive cycles and oocyte availability. Despite the advantages of using frozen sperm, a significant disadvantage is that IVF success rates are typically decreased compared to fresh semen [1]. Identifying traits of frozen sperm that are related to IVF success would help to screen sperm prior to use for improved fertilization and development rates. Our objective was to identify sperm traits that are routinely evaluated in a clinical setting to determine if any traits were related to IVF success [2]. In addition, we also sought to test a more functional measure of sperm by determining if sperm binding to oviduct, or Fallopian tube cell aggregates, was related to IVF measures or other sperm traits.

A single semen collection from sixteen boars was frozen and each was independently evaluated for post-thaw motility, acrosome integrity, IVF fertilization and development and the number of sperm bound to oviduct epithelial cell aggregates. Although we expected to reduce IVF variability with frozen sperm, there remained a considerable amount of variation in IVF between replicates despite using a consistent number of sperm from the same semen collection. Additionally, there was much variation between males when comparing IVF results regardless of pre-freeze characteristics.

Traditional analyses of post-thaw motility immediately after thawing sperm indicated that higher motility samples tended to have higher rates of normal monospermic fertilization (P=0.06). As expected, samples with higher motility also contained fewer acrosome compromised sperm. However, because neither motility or acrosome integrity were related to IVF success, we used a sperm-to-oviduct binding assay as a measure of sperm binding function. The oviduct, or Fallopian tube, plays a critical role in storing sperm within the female mammalian reproductive tract prior to fertilization so that sperm are available near the time of ovulation [3]. Sperm storage is particularly important for maintaining viable sperm if semen deposition significantly precedes ovulation. To assess sperm binding in vitro, we used a sperm-to-oviduct binding assay by collecting epithelial cells from the lower portion of the oviduct or isthmus, and allowed them to form spherical aggregates in culture. Equal numbers of sperm from each boar were added to droplets containing oviduct aggregates and the number of sperm bound to the periphery of each aggregate was counted. We found that ejaculates containing higher numbers of sperm bound to oviduct aggregates were related to IVF polyspermy rates (r^2=0.62). Polyspermy is common when using porcine gametes and is particularly frequent when using high quality semen samples. These results are consistent with the principle that oviduct-bound sperm are likely more functional because they have not undergone capacitation and have less cryo-induced damage and are therefore, more effective at fertilization [4-7].

In conclusion, sperm traits that are often used for clinical assessment, such as motility and acrosome integrity, were not strongly predictive of IVF success. However, a binding assay that determined the number of sperm bound to oviduct epithelial aggregates was predictive of IVF polyspermy rates. Our observations indicate that the oviduct has an important role in contributing to in vitro fertilization and support a need for the development of assays that assess sperm function as a more powerful tool to screen samples that are compatible for technologies such as IVF.

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