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## Validation-verification of a highly effective, practical human testicular tissue *in vitro* culture-cryopreservation procedure optimizing pre-freeze and post-thaw motility

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For the past two decades, fresh and cryopreserved testicular sperm has been effectively used by sperm injection in the production of healthy IVF children. However, for many ART laboratories, the processing of testicular biopsy (TBx) tissue and isolation of viable sperm remains laborious and unreliable. Our goal was to simplify TBx handling, processing and cryopreservation procedures, while optimizing cryopreserved sperm motility by integrating pre-freeze *in vitro* culture (IVC) and whole tissue freezing procedures. Comparative testicular tissue IVC and evaluation was performed as part of the standard processing of whole TBx tissue (i.e., intact mass of tubules) for cryopreservation and/or for fresh use in an ICSI cycle. Two prospective studies were conducted to validate, optimize and understand the virtues of testicular tissue IVC at different temperatures (21, 30 or 37°C). Concurrently, the effectiveness of IVC-cryopreserved TBx sperm was documented with fertilization rates, clinical pregnancies and live birth data. Reliable post-thaw motility of testicular sperm was achieved by promoting pre-freeze total and progressive motility through IVC (24-96 h) post-biopsy at an intermediate temperature of 30°C. Furthermore, it was determined that whole tissue cryopreservation effectively maintained post-thaw motility of IVC TBx tissue (up to 85% viability retention), with no differences in ICSI-fertilization rates or pregnancy outcomes compared to fresh TBx sperm used for women under 38 years old. Over the past 17 years, intact whole testicular biopsy cryopreservation has proven highly effective without laborious pre-freeze processing, by simply adopting IVC of TBx tissue into clinical practice. Today, the technology is also being applied to freeze preservation efforts for men undergoing cancer or vasovasotomy-related surgeries.

### Biography

Mitchel C Schiewe attained a BS/MS at UC Davis (1981)/LSU (1983) focused on Animal Reproductive Physiology. Working with the Smithsonian Institution/National Zoological Park and the NIH, he completed his PhD in Human Physiology in 1989 at the Uniformed Services University of the Health Sciences (Bethesda, MD). Subsequently, he performed his Post-doctoral studies at NIH/NCRR as an NSF Associate. He is currently a Scientific/Technical Lab Director for Ovagen Fertility and the California Cryobank. He considers himself to be a Comparative Reproductive Physiologist, specializing in Embryology, and has published more than 35 peer-reviewed papers and 110 scientific abstracts, including several research award presentations.

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