A Rapid QC Testing Platform Using Frozen Semen

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Abstract

Objective: The last twelve months have presented significant challenges for the ART laboratory. Mandatory shutdowns, lack of patient access, supply chain issues, and changing rules and recommendations brought on by COVID-19 have had a significant impact on ART laboratories’ ability to maintain quality and procedures. One area where concern has been the lack of available fresh semen (PS) samples at the appropriate times for quality control (QC) and proficiency testing (PT). Cryopreserved semen (CS) would appear reasonable as an alternative. However, the quality of CS is known to deteriorate much faster than FS, even in favorable culture conditions. The goal of the present study was to determine the quality of CS samples, if a protocol could be developed for QC testing using CS.

Materials and Methods: Using materials of known quality from previous PT challenges, seven commercial donor semen samples thawed and prepared for quality control monitoring as follows. Samples were thawed using standard methods. The thawed sample was loaded into a full wash protocol with the assigned PT challenge media, either tainted or un-tainted. Once prepared, samples were maintained at 37°C, room air, and 95% relative humidity. Steating of 0 hr, the samples underwent a semen analysis hourly using an IVOS semen analyzer for a minimum of 6 hrs or until one sample in the pair reached 0% motility after the 6 hr time-point. The resulting data were compared using a paired student’s T test. Further, results were compared with reports from laboratory PT to verify the efficacy of using frozen semen.

Results: As expected semen parameters decreased over time regardless of treatment (P < 0.001). No pair of samples lasted more than 100 hrs of incubation. While sperm in the non-tainted media remained at 60% of its initial motility at 3 hrs (range 0-69%), none of the cells in the tainted media had more than 50% motility at that time point (range 12-43%; P < 0.001). Further by six hours, all but one of the seven samples in the non-tainted media had 0% motility (range 0-3%) whereas six of seven samples in the non-tainted media maintained a minimum of 25% of their initial motility at 0 hr (22-37%; P = 0.001). Further, all samples correlated with previous PT results.

Discussion: The data suggest it is possible to perform a rapid sperm QC assay using CS. Having a secondary QC protocol would not only provide an alternative when fresh semen, mice embryos, or other methods are unavailable, it would also potentially allow for more standardized methods of QC and PT testing.

Impact: The past twelve months have taught us that unexpected and uncontrollable events can disrupt even the best laid plans. Sperm QC assays, which are the mainstay for QC in many andrology laboratories, are dependent on the availability of fresh semen. However, if a standardized QC method can be created, QC could be done at the convenience of the lab without sacrificing quality or patient safety.

Introduction

Quality control (QC) and proficiency testing (PT) has been a part of life in the Assisted Reproductive Laboratory (ART), as they are in all clinical laboratory. However, unlike other QIPT, ART labs are often dependent on a fresh semen supply given the biocompatibility of media and cultureware within the laboratory. While other testing exists (mice embryos, animal semen, etc.), the fresh human semen biopsy has proven both reliable and inexpensive compared to other techniques and fresh semen is thought of as always available. However, the last 16 months have proven that even things thought of as readily available can, in a relatively short period of time, become unavailable. The COVID lockdown, and institutional rules caused this lab to abandon all in lab collections for a period of at least 15 months, limiting access to fresh semen samples for PT/QC testing. As an alternative the potential use of cryopreserved samples. However, it is well documented that cryo samples rapidly lose cellular function, especially progressive motility after thaw and would therefore prove useless in the current test model that measures motility at 24 and 48 hrs.

Using a modification of a previously described human sperm QC method (S.D. Prien. An improved quality control protocol for ART. Fertility and Sterility, Volume 80, Supplement 3, September 2003, Pages 293-294), donated commercially available semen samples, and previously tested PT material, the goal of the present study was to determine if frozen semen could be substituted in the QC assay, if time of observations were altered to shorter periods.

Materials and Methods

Semen Samples

Frozen donor semen samples from commercial banks projected to yield a between 10– 20 million mobile sperm per donor patient who had completed their course of reproductive treatment (N=7).

Teasing Materials

Media remaining from the two previous AAB proficiency challenges (two identified as acceptable and two as unacceptable), without semen supplementation.

Quality Control Methodology

1. Tests were conducted in a standard 24 well culture plate.

2. For each media to be tested each 4-labeled A-D, 5 wells were prepared using the AAB media with the rapid assay in rotation.

3. Once thawed, the semen samples were subject to a wash and swimup procedure using standard lab techniques to remove cryopreservation reagents and isolate motile cells.

4. As previous research (Prien 2003) demonstrated cellcultures can be grown from all samples. After the following approximate concentrations:

   - a. 100,000 cells per mL – A
   - b. 500,000 cells per mL – B
   - c. 1,000,000 cells per mL – C
   - d. 2,500,000 cells per mL – D

5. Once prepared the samples were analyzed for motility and rapid cells were determined using an IVOS Computer Assisted Semen Analyzer and the plate culture at 37°C and 95% relative humidity.

6. Analysis were planned and data collection was repeated at times 1, 3, 6, 9, and 12 hr. However, no group exhibited >50% recovery of the initial motility at times beyond 12 hrs so the experiment only included data for 0, 1, 3, 6, 9, and 12 hr.

7. Once all data was collected for each donor sample it was normalized with the 0 hr time point set to 100% for that treatment and all subsequent measurements were expressed as a percentage of the 0 hr time point. The changed motility of this particular (without frozen samples) regardless of their observed initial motility (ranged from 82-97% after preparation) and at what time points they had 75% and 90% of the initial motility.

8. Results data point (same donor media from one AAB challenge) were analyzed using student’s T, comparisons across time were made using ANOVA.

Results

As expected, motility decreased rapidly, causing a truncation of data collection to 12 hrs.

As expected from previous observations, sperm concentration appeared to influence the rate at which motility declined, with treatments containing higher motile sperm concentrations declining faster (Figures 1-4, P < 0.001).

Hence, motility at 12 h post thaw in all samples (acceptably) seemed to show consistent declines to 75% and 50% of the original normalized motility at 3 and 6 hrs respectively (Figures 1-4).

Further, tained (unacceptable) media demonstrated significantly lower motility rates at all time points by 24 hrs (P < 0.001).

No correlation was found between rapid cell numbers media source (P = 0.621).

Conclusions

1. Data suggest frozen/thawed sperm might be useful as a QC/QP source when fresh materials are unavailable.

2. However, as frozen semen lose motility rapidly after thaw, time points for observation must be altered.

3. Standards in observation times may allow the technique to be used as a rapid technique, taking hours instead of days.

4. Further study will be needed to determine how the addition of sperm supplement affects motility in frozen semen in supplemented media.

5. Further study is also needed to determine if the assay is sensitive enough to detect materials determined unacceptable by conventional QC.