A Rapid QC Testing Platform Using Frozen Semen

S.D. Prien[1,2], and L.L. Penrose[1,2]

Department of Obstetrics and Gynecology, Texas Tech University Health Sciences Center; Lubbock, Texas 79430[1]
and Department of Animal and Food Sciences, Texas Tech University; Lubbock, Texas 79409[2]

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 stretched laboratories to their limits in an attempt to maintain regular and required activities. One area of concern in the laboratory has been the lack of available fresh semen (FS) samples at the proper times for quality control (QC) and proficiency testing (PT). Cryopreserved semen (CS) would appear a reasonable 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, given the limitations 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 bank-specific protocols. The thawed sample was split in half and prepared using an IUI 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. Starting at 0 hrs, 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.

Result: As expected semen parameters decreased over time regardless of treatment ($P < 0.001$). No pair of samples lasted more than seven hours of incubation. While sperm in the non-tainted media maintained at least 60% of its initial motility at 3 hrs (range 64-91%), 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 tainted media had 0% motility (range 0-4%), while six of seven samples in the non-tainted media still maintained a minimum of 25% of their initial motility at thaw (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 that unexpected and uncontrollable events can disrupt routine procedures. 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 CS 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 become a fact of life in the Assisted Reproductive Laboratory (ART), as they are in all clinical laboratory. However, unlike other QC/PT, ART labs are often dependent on a fresh semen samples; especially to determine the biocompatibility of media and cultureware within the laboratory. While other testing exists (mice embryos, animal semen, etc.), the fresh human semen bioassay has proven both reliable and inexpensive compared to other techniques and fresh semen is thought of as always available. However, the last 18 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 almost 15 months, limiting access to fresh semen sample for PT/QC testing. An obvious alternative is 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 materials, the goal of the present study was to determine if frozen semen could be substituted in the QC assay, if time of observations we altered to shorter periods.

Presented at the 77th Annual Scientific Congress of the American Society for Reproductive Medicine - October 2021

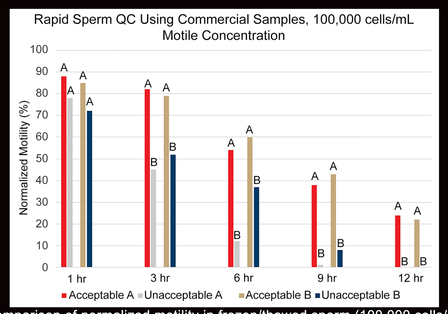

Figure 1. A comparison of normalized motility in frozen/thawed sperm (100,000 cells/mL) exposed to media deemed acceptable or unacceptable for use in assisted reproductive procedures. Paired means with different superscripts are significantly different ($P<0.05$)

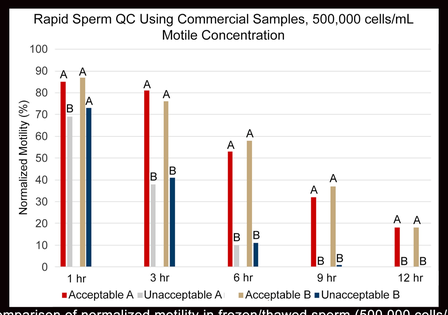

Figure 2. A comparison of normalized motility in frozen/thawed sperm (500,000 cells/mL) exposed to media deemed acceptable or unacceptable for use in assisted reproductive procedures. Paired means with different superscripts are significantly different ($P<0.05$)

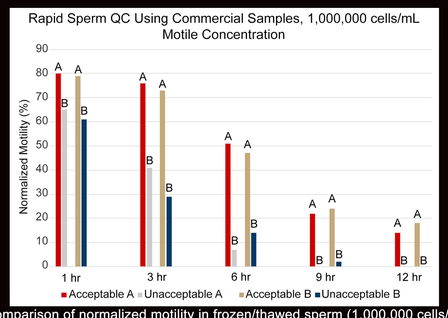

Figure 3. A comparison of normalized motility in frozen/thawed sperm (1,000,000 cells/mL) exposed to media deemed acceptable or unacceptable for use in assisted reproductive procedures. Paired means with different superscripts are significantly different ($P<0.05$)

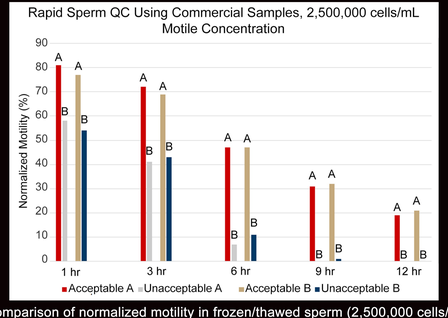

Figure 4. A comparison of normalized motility in frozen/thawed sperm (2,500,000 cells/mL) exposed to media deemed acceptable or unacceptable for use in assisted reproductive procedures. Paired means with different superscripts are significantly different ($P<0.05$)

Materials and Methods

Semen Samples

Frozen donor semen samples from commercial banks projected to yield a between 10- 20 million motile cells donated from patient who had completed their course of reproductive treatment (N=7).

Testing Materials

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

Quality Control Modification

1. Tests were conducted in a standard 24 well culture plate.
2. For each media to be test 4 (labelled A-D), .5 mL wells were prepared using the AAB media with 5% serum supplementation.
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 cellular concentrations can affect assay out come, prepared motile sperm cells were added in 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, 12, 24 Hr. However, no group exhibited >50% recover 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 being expressed as a percentage of the 0 hr time point. This allowed direct comparison of the various frozen samples regardless of their observed initial motility (ranged from 82-97% after preparation) and at what time points they had 75% and 50% of the initial motility.
8. Resulting data paired data at a time point (same donor, media from one AAB challenge) were analyzed using student's-T, comparisons across time were made using ANOVA.

Results

1. As expected, motility decreased rapid, causing a truncation of data collection to 12 hrs.
2. As also 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; $P < 0.001$).
3. However, motility in untainted samples (acceptable) seemed to show consistent declines to 75% and 50% of the original normalized motility at 3 and 6 hrs respectively (Figures 1-4).
4. Further, tainted (unacceptable) media demonstrated significantly lower motility rates at all time points > 1 hr when compared to their untainted control (Figures 1-4: $P < 0.05$).
5. 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/PT source when fresh materials are unavailable.
2. However, as frozen sperm lose motility rapidly after thaw, time points for observation must be altered.
3. Alterations 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 serum supplement affects the rapid decline in motility seen 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.