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Andrology Lab Corner*

Human Sperm Survival Assay as a Bioassay for the Assisted Reproductive Technologies Laboratory

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Recently, Carrell and Cartmill (2002) provided a chronology of government oversight and intervention in the provision of clinical reproductive medicine laboratory services. They also outlined the advent and development of proficiency testing as a tool by which all clinical andrology laboratories and in vitro fertilization laboratories (herein collectively referred to as assisted reproductive technology [ART] laboratories), are able to assess themselves internally and externally. In order for ART laboratories to be deemed certified, clearly defined objectives must be met, including mechanisms for monitoring quality (Clinical Laboratory Improvement Amendments of 1988). This progression toward standardized methods for monitoring quality is not unique to the United States. The same dedication to ensuring the delivery of quality ART laboratory service and care is practiced to varying degrees throughout the world.

Rationale for Quality Control and Assurance

All products used in ART laboratories that directly or indirectly contact gametes and embryos must have no negative influence on organism viability or function (Parinaud et al, 1987). In addition, all products that directly contact gametes and embryos must be tested for toxicity before clinical implementation. In order for a laboratory to detect toxicity it must first have a reliable method for making that determination. The most appropriate mechanism for quality control/quality assurance

(QC/QA) testing is to use a bioassay. The most appropriate bioassay is one that provides a sensitive and robust monitoring system that approximates the conditions that will be experienced by gametes and embryos during their in vitro lifetime. To ensure that the method being used performs optimally a laboratory should participate in an external proficiency-testing program.

Current Methods for QC/QA

Three bioassays are in common use for determining the acceptability of media and materials. The first and with perhaps the longest history of use is the mouse embryo assay (Ackerman et al, 1984a,b). This assay involves the culture of frozen or nonfrozen, 1- or 2-cell mouse embryos to the blastocyst stage. The mouse embryo assay is easy to perform simply by virtue of the commercial availability of biological materials. However, the use of mouse embryos has several downsides, such as the reporting system for toxicity (eg, Fleming et al, 1987; Scott et al, 1993), and different mouse strains vary in their sensitivity to the same test conditions (Fleetham et al, 1993; Scott et al, 1993). Further, there is no apparent consensus on which mouse embryonic cell stage (eg, 1- or 2-cell) is best for use in testing (Davidson et al, 1988). There are cost issues as well, related both to the purchase of embryos on a routine basis and liquid nitrogen dewars for the storage of separate human cells and tissues. Finally, animal rights issues may also be a concern with the use of this assay.

The second most commonly used bioassay for QC and proficiency testing is the human sperm survival assay (Critchlow et al, 1989; Claassens et al, 2000). This assay is convenient to perform and materials are readily available. Further, in the context of an ART laboratory, the cell type in question is familiar to testing personnel and is compatible with the clinical laboratory environment.

A less commonly used but still relevant bioassay is the hamster sperm motility assay (Bavister and Andrews, 1988; Rinehart et al, 1988). This assay has a consensus in use of materials and methods alike. Similar to the mouse assay, the hamster assay can be somewhat expensive, cumbersome, and its use may also be offensive to animal rights groups.

The purpose of the present study was to determine whether the human sperm survival assay, based on the work by Claassens et al (2000), could be used effectively in a multicenter trial using a consensus protocol and whether it would yield consistent results between laboratories.

Materials and Methods

This was a multicenter (n = 5) controlled study.

Sperm Processing

Aliquots of frozen donor semen (multiple specimens from each donor) and in-house fresh specimens were tested. Fresh semen was allowed to liquefy and cryopreserved semen was allowed to thaw prior to assessing semen parameters following World Health Organization criteria (WHO, 1999). Semen was layered over a discontinuous, two-layer (47%/90% v:v) PureCeption (SAGE BioPharma, San Clemente, Calif) density gradient and centrifuged at 300 x *g* for 25 minutes. Sperm pellets were resuspended in 1 mL of Quinns Hepes human tubal fluid (HTF) medium (SAGE BioPharma) containing 5 mg/mL of human serum albumin (HSA) and centrifuged at 300 x *g* for 5 minutes. Sperm pellets were resuspended in 37 °C CO_2-equilibrated bicarbonate-buffered HTF (SAGE BioPharma) and sperm parameters were assessed. Aliquots were removed and added to tubes containing HTF to achieve a

final motile sperm concentration of 5 x 10^6/mL. All media, including HSA, were from SAGE BioPharma.

Incubation Conditions

Sperm were incubated (0.5 mL) at 37 °C in a humidified 5.0% CO_2 incubator under the following conditions: 1) HTF, 2) HTF with 5 mg/mL HSA, 3) adulterated HTF, and 4) adulterated HTF with 5 mg/mL HSA. At 2, 4, 6, 8, 24, and 48 hours aliquots were removed and added to the same volume of Hepes HTF for sperm motility assessment.

Statistical Analysis

Data were collated and analyzed using a mixed model ANOVA and linear regression. Slopes from regression lines were used for comparison. The percentage change from baseline (time = 0) was the dependent variable.

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Mean motility regression lines were calculated, the slopes of which are reflective of motility change over time. For example, a slope of 0 means that motility was unchanged during the course of the experiment. A slope less than 0 means that motility decreased during the course of the experiment.

Four laboratories had similar slope of motility patterns: control + HSA slope of motility > control - HSA slope of motility > treatment + HSA slope of motility > treatment - HSA slope of motility. The fifth laboratory had a slope of motility pattern as follows: control + HSA > treatment + HSA > control - HSA > treatment - HSA. The incubation conditions in laboratory 5 were not consistent (CO_2 = 6.0) with the other four laboratories, and therefore the remaining data consist of only the four laboratories that used identical incubation conditions. It is important to indicate that for control and treatment conditions, when HSA was present the regression lines for laboratory 5 were comparable to the other four laboratories.

Table 1 shows the mean motility regression line slopes for the four laboratories. A negative regression line slope value reflects a decline in motility over the 48-hour incubation time. Precipitous declines in sperm motility over the 48-hour incubation time are reflected by elevated negative mean regression line slope values. When HSA was included in control and adulterated medium, the latter had a threefold steeper mean motility regression line slope compared to that of the control. When HSA was excluded from control and adulterated medium, the latter had a fivefold steeper mean motility regression line slope compared to that of the control. If the data for treatment without HSA from laboratory 1 are excluded, then the apparent difference in mean motility regression line slopes between media with or without HSA is negated.

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Table 1. *Mean motility linear regression lines per lab*

Table 2 reflects the mean regression values for the same donor tested at different laboratory locations. These data are consistent with those in Table 1, in which mean regression line slope

values are greater (reflective of a more precipitous rate of decline in motility) for sperm incubated in adulterated medium compared with sperm incubated in control medium. In addition, there appears to be greater sensitivity to the adulterant, as indicated by the regression line slope values, when HSA is excluded from the medium. There is also an apparent greater variation in the rate at which sperm lost motility under these conditions as suggested by the large standard deviations.

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Table 2. *Mean motility linear regression lines per cryopreserved donor specimen*

Conclusions

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The present data concur with those published by Claassens et al (2000) in that sperm motility is influenced by the presence or absence of protein in incubation medium adulterated by a potential toxicant. In order to more readily interpret the data in our study, mean regression lines were calculated, the slope of which is reflective of an increase, a decrease, or no change in sperm motility over time. In the present study a deleterious effect by adulterant on sperm motility was detected, as evidenced by negative slope of regression line values <-1, regardless of whether or not the media contained protein. In contrast, sperm motility changed only minimally after incubation in nonadulterated media and regardless of whether or not protein was added. Motility appeared to be more negatively influenced when the medium contained adulterant and lacked protein, suggesting a possible protective effect by protein on sperm from cytotoxic substances in the test media. However, more testing is required to validate this hypothesis.

Quality control and proficiency testing for ART laboratories has historically been problematic. The commonly used mouse embryo assay suffers from strain dependence, high cost, and other negative factors. As demonstrated by the results herein, the sperm survival assay, when performed under controlled conditions, can be used effectively as part of standard quality monitoring and proficiency testing programs. The inherent problems found with use of other bioassays currently in use are minimized or eliminated with the use of the human sperm survival assay.

The human sperm survival assay performs well as a bioassay for testing gamete toxicity by contact materials, reagents, and media, which are essential for ART laboratories. However, it is important to caution that the sensitivity of the sperm survival assay remains to be established. These results also show that performance data from the sperm survival assay between different laboratories is comparable, and thus can be not only compared, but used in proficiency testing regimens, and other inter-laboratory tests of competence.

Footnotes

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