Updating Quality Control Assays in the Assisted Reproductive Technologies Laboratory with a Cryopreserved Hamster Oocyte DNA Cytogenotoxic Assay

PHILIP J. CHAN,1,2,3 J. HAZEL CALINISAN,1 JOHANNAH U. CORSELLI,1 WILLIAM C. PATTON,1 and ALAN KING1

Submitted: June 20, 2000
Accepted: October 13, 2000

Purpose: Despite advances in assisted reproduction, there is no progress in quality control bioassays. The objectives were to develop a comet assay to measure DNA fragmentation in thawed cryopreserved oocytes and compare this assay with one-cell mouse embryo bioassay.

Methods: Thawed hamster oocytes from a commercial source were incubated in culture media with either 0-, 50-, or 100-μM hydrogen peroxide, or, in media exposed to different contact materials and unknown proficiency analytes. Incubation time was 1.5 h at 37°C. The oocytes were dried, fixed, stained with acridine orange, embedded in a mini-agarose layer and electrophoresis was carried out. Fluorescent images were analyzed. The results were compared with standard one-cell mouse assay data.

Results: The 100-μM hydrogen peroxide treatment caused greatest DNA fragmentation in the hamster oocytes at Hours 1 and 2. A dose response was observed. Intrassay coefficient of variation was 5.7%. Only one of the five materials tested passed both assays. The data for the unknown proficiency analytes were similar for both assays.

Conclusions: The oocyte comet assay demonstrated DNA fragmentation in the presence of toxic substances. The detection of toxicity in two materials that passed the mouse bioassay suggested increased sensitivity in the new assay. The oocyte comet assay and the mouse bioassay results matched in the proficiency test. However, more studies are still needed to determine optimal sensitivity.

KEY WORDS: Single-cell gel electrophoresis; comet assay; quality control; hamster ova; in vitro fertilization.

INTRODUCTION

Testing of water, culture media, protein supplements, and contact materials at the centers of assisted reproductive technologies are presently performed using either the one- or two-cell mouse embryo bioassay (1–8), the hamster epididymal sperm motility bioassay (9,10), the human sperm survival bioassay (11,12) or the hybridoma cell culture assay (13). Thus, the centers routinely perform bioassays and have become proficient in cytogenotoxic testing. Despite advances in assisted reproduction, there has been no progress in quality control bioassays since 1989. Present quality control bioassays are complicated, time-consuming (13), expensive, lack sensitivity (3,14,15) and require several days. Also, the bioassays do not directly measure DNA damage. The comet assay, or single-cell gel electrophoresis, is a simple, rapid, and inexpensive assay for the determination of DNA damage or fragmentation (16–18). The comet assay involves measuring the streaks of DNA strands (“comet” tail moment) unraveling from the individual nucleus of the white blood cell (19,20). The challenge was to adapt the assay for low numbers of oocytes, unlike the easily obtainable quantities of white blood cells.

The hypothesis was that toxic substances would cause DNA fragmentation in metaphase II oocytes. The objectives were (a) to develop a comet assay to measure DNA fragmentation based on oocytes,
and 24 at 37°C in room air. Slides were prepared in the meantime. Self-adhesive sticker labels (Catalog No. 43-540, Preaply, Dennison Co., Framingham, MA) were cut into strips and pasted to form a rectangular frame around diamond-pen–etched circles made on each glass slide. The type of stickers was important and they served to anchor the mini-agarose gel layer. It was essential to make sure that the stickers were stuck to the glass slide by pressing down hard on the stickers. The etched circles helped to locate the oocytes.

Two oocytes were pipetted out on to each glass slide and air-dried. The entire procedure was carried out with the room lights off or in diffuse lighting. As soon as the oocytes were dried on the glass slides, they were fixed by flooding with methanol solution (Fixative Solution, Diff-Quik, Dade Behring Inc., Newark, DE) for 15 s and air-dried again. At this point, the fixed oocytes could be batched and kept for a few days before continuing with the next step in the oocyte comet assay process as described later.

In a separate experiment involving the testing of contact material, zona-intact oocytes were pipetted into either control medium or media that had been exposed to new laboratory materials. The contact material media were prepared by placing aliquots of 1 ml of modified HTF medium for 1.5 h into either a new lot of Falcon 3037 petri dish (Becton Dickinson Labware, Franklin Lanes, NJ), Falcon 7575 sterile 3-ml transfer pipet, Henke-Sass Wolf 10-ml Fortuna syringe (Tutlingen, Germany), Pure Advantage powder free gloves (THC66TP-1, Tillotson Healthcare Corp., Bedford, NH) or Sempermed supreme gloves (Semperit Technische Produkte Ges.m.b.H., Vienna, Austria). The contact material media were also used for testing in the one-cell mouse biological assay system. A description of the mouse assay system is presented later.

Finally, a third section of this study involved using the oocyte comet assay on two unknown laboratory proficiency analytes supplied by the American Association of Bioanalysis. The assay was carried out alongside the standard mouse one-cell biological quality control assay system with the exception that this assay was completed the same day, whereas the mouse assay required several days to obtain the results. Sometimes the oocytes exposed to toxic media would appear darkened and lack the shiny “glass marble” structure, but this was inconsistent and we did not find a good correlation between appearance in culture and DNA fragmentation. Hence, the appearance parameter was not studied further.

Preparation of the Mouse Embryo Assay System

Mature female mice were stimulated with 5-IU pregnant mares serum gonadotropin (PMSG; Sigma Chemical Co., St. Louis, MO) followed by 5-IU human chorionic gonadotropin (hCG; Sigma Chemical Co., St; Louis, MO). Each female mouse was mated with a proven male 2 days after hCG treatment (21).