SHORT COMMUNICATION

NEDLANDS, AUSTRALIA

The rapid detection of cytotoxicity using a modified human sperm survival assay*

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Sperm were cultured at room temperature in seminal plasma, in the presence or absence of a test item or solution. Progressive motility of the sperm was measured using a Sperm Quality Analyser IIB. A progressive decline in motility of sperm was seen under control conditions from 0 hr to 24 hrs. Culture medium did not cause a significant decline in sperm survival index during the first 6 hrs of culture but did after 24 hrs. A dose dependent reduction in sperm survival index was then seen with formaldehyde. Powder-free rubber gloves proved extremely toxic unlike the associated rubber plunger of a non-pyrogenic syringe. It is concluded that the use of unwashed human sperm in survival studies allows detection of toxicity within 6 hrs, a time much less than conventional methods using washed human sperm.

KEY WORDS: Sperm survival, toxicity.

INTRODUCTION

The review of pregnancy rates is important in monitoring the performance of an IVF program. Unfortunately, a decline in performance is usually only identified several weeks later when pregnancy rates fall thereby delaying the implementation of corrective action, and no indication is given as to the source of any problem. These major limitations have led to the introduction of a range of bioassays designed to detect toxicity, including the use of human sperm survival studies (1). Further

efforts have been made by some workers to optimize the human sperm survival bioassay by omitting protein from the medium and culturing at 37°C (2).

The present study describes a modification of the human sperm survival bioassay in order to simplify the protocol and obtain results more rapidly. Data are presented to demonstrate the continued effectiveness of the bioassay to detect toxicity.

MATERIALS AND METHODS

Semen Samples

Normozoospermic samples from a panel of 14 donors were used in this study.

Assessment of Sperm Motility

The Sperm Quality Analyzer SQA IIB (Medical Electronic Systems, Migdal-Haemek, Israel) together with the proprietary disposable capillary tubes was used to measure the motility of the sperm. The semen under investigation was thoroughly mixed for 20 s to ensure uniformity and then processed according to the instructions provided by the manufacturer of the SQA IIB.

General Toxicity Screen

Aliquots of 0.5 mL semen from one ejaculate were placed into a series of 60 mL wide mouthed universal containers. One container was set-up as the control by adding either no item for studies investigating the toxicity of materials, or an aliquot of unspiked medium for those studies investigating the toxicity of solutions. The initial motility of the sperm in the control container was then measured using the Sperm Quality Analyser, and the motility of sperm in the control and test containers measured after 2, 4, 6 and 24 h. A survival index was then calculated as:

$$\frac{\text{Percentage of progressive motility of test}}{\text{Percentage of progressive motility of control}}$$

Items were regarded as toxic if the survival index was <0.75.

HEPES Study

A 50 µL aliquot of HEPES-buffered Quinn's Advantage medium (Sage In Vitro Fertilisation Inc., Trumbull, USA) was added to the semen and the sperm survival was assessed as described above.

*Submitted in part by Melissa Iemmolo to the University of Western Australia towards her Postgraduate Diploma in Science (Zoology).

Formaldehyde Study

Experimental containers as described above were spiked with 10 μ L of one of the following solutions: HEPES-buffered Quinn's Advantage medium as a control, or 40% formaldehyde solution (Merck Pty Ltd., Kilsyth, Australia) diluted with HEPES-buffered medium to concentrations of 4×10^{-8} 4×10^{-7} g/mL, and 4×10^{-4} g/mL. Sperm survival was then calculated as described above.

Statistical Analysis

The profiles of sperm motility for the semen and sperm survival indices for the HEPES-buffered medium were assessed using either 1-factor or 2-factor repeated measures ANOVA, with a comparison of values at different time periods being made using Fisher's protected least significant difference. Differences were considered significant if $p < 0.05$.

RESULTS

There was a sustained and significant loss of progressive sperm motility under control conditions over time ($p < 0.0001$) as shown in Fig. 1, with all times being significantly lower than the preceding value (all $p < 0.05$). The time-dependent loss of motility confirms the need to run a control when testing any item.

The sperm survival index with HEPES-buffered Quinn's Advantage medium is shown in Fig. 2. There was an overall effect of time upon the sperm survival index ($p < 0.0002$). Whilst there were no significant changes over the first 6 h, the survival index at 24 h was significantly reduced ($p < 0.01$).

The low progressive sperm motility seen after 24 h (Fig. 1), coupled with the significant reduction in sperm survival index following the addition of innocuous culture medium, cast significant doubt upon the reliability of the 24 h culture period for use in a routine test. Accordingly, the 6 h culture period was adopted as the standard time, which avoided excessive loss of motility under control conditions and enabled the result to be obtained within the working day.

The ability of the sperm survival bioassay to detect different concentrations of formaldehyde is shown in Table I. There was a significant effect of time upon the survival indices ($p < 0.0001$), as there was with concentration of formaldehyde ($p < 0.0001$). Given that a survival index of <0.75 after 6 h was arbitrarily regarded as indicating a significant level of toxicity, it can be seen that the addition of the lower concentration of formaldehyde was not detected by the assay. Conversely, a reduction below the threshold of 0.75 was seen within 2 h for the two higher formaldehyde concentrations of 4×10^{-7} and 4×10^{-4} g/mL.

Fig. 1. The progressive motility (%; mean $\pm$ SEM) of sperm kept in seminal plasma at room temperature for up to 24 h ($n = 15$ replicates). *Times with different letters are significantly different from each other.

Fig. 2. The sperm survival index for saline ($n = 4$ replicates). The arbitrary cut-off for the detection of toxicity of an index of 0.75 is shown by (—). *Times with different letters are significantly different from each other.

DISCUSSION

Given the ease of sperm washing and that seminal plasma affects sperm motility adversely, it is not surprising that IVF laboratories use washed sperm to maximize sperm longevity in survival studies (1). Washed sperm have been used elsewhere but results were obtained more rapidly by the exclusion of albumin from the culture system, (i.e. from 48 h to within 8 h), (2). However, the present study has taken a very different approach in modifying the human sperm survival assay, and used whole semen to stress the sperm and shorten the time required to obtain information on toxicity.

Knowing at what point a loss of motility becomes clinically significant is an impossible task. However, the basic survival of sperm was initially assessed in

two ways, namely (i) by reviewing the loss of progressive motility under control conditions, and (ii) testing a minute sample of culture medium that should be innocuous (i.e. designed for use in an IVF laboratory and tested by the manufacturer with the 1-cell mouse embryo assay). Once viewed in this light, it became apparent that the culture of sperm in seminal plasma for 24 h caused too much of a decline in progressive motility with or without the medium, and so a standard culture period of 6 h was recommended. The clinical relevance of the sperm survival studies was then checked using formaldehyde. The lower concentration used was not detected after 6 h, but this is consistent with the findings of an external quality assurance (EQA) programme where the rate of detection of toxicity at this concentration by laboratories using a range of protocols for human sperm survival studies was no different to the control sample tested (3). However, the medium and higher concentrations were detected after 6 h, and this is again in line with the majority of laboratories within the Australian EQA scheme (3).

In summary, the use of unwashed sperm cultured at room temperature for 6 h, and the measurement of progressive sperm motility with an automated analyzer, is suitable as a rapid test of toxicity for items that may be used in an IVF laboratory.

Table 1. The Survival Index (mean ± SEM) of Sperm Exposed to Varying Concentrations of Formaldehyde and Maintained at Room Temperature for 2, 4, 6 and 24 h ($n = 5$ replicates)

Time (h)	Formaldehyde concentration (g/mL)		
	4×10^{-8}	4×10^{-7}	4×10^{-4}
2	0.95 ± 0.04	0.50 ± 0.18	0
4	0.91 ± 0.04	0.33 ± 0.17	0
6	0.84 ± 0.10	0.23 ± 0.17	0
24	0.29 ± 0.18	0	

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