REPRODUCTIVE BIOLOGY

SHORT NOTE

Maximum rates of cooling by three programmable freezers, and the potential relevance to sperm cryopreservation

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SUMMARY

Maximum rates of cooling for the Asymptote EF 100 and the Cryologic CL8800 temperature controller with either a standard or fast chamber were determined and viewed in the context of sperm cryopreservation. All three systems use liquid nitrogen to cool the plate or chamber which would hold the sample, opposed by a variable amount of heat from an internal heater. Maximum rates of cooling for all systems were a function of the temperature gradient between the liquid nitrogen and the plate/chamber and at a plate/chamber temperature of 15°C were 16.5°C/min, 13.3°C/min and 8.0°C/min for the Asymptote EF100, Cryologic fast and slow chambers respectively. These machines are not suited to the freezing of sperm from species requiring rapid rates of cooling, an important consideration when planning to purchase a piece of equipment for this application, and scientists are advised to discuss specifically their requirements with prospective suppliers. *Reproductive Biology 2008* ***8*** *1: 69-73.*

Key words: sperm, cryopreservation, maximal cooling rates, Cryologic, Asymptote

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INTRODUCTION

Programmable freezers are proving useful to many reproductive biology laboratories for the cryopreservation of gametes, gonadal tissue, and embryos of a number of species despite the increased cost associated with the purchase of equipment [7]. Embryos and ovarian tissue are often frozen at slow rates in the order of 0.3°C/min [5] making the degree of control offered by such machines ideal. Whilst sperm have been frozen successfully with a number of manual techniques including the dropping of semen on to dry ice to form pellets and the holding of semen in the vapour phase of liquid nitrogen [12], reproducibility with the manual systems can be a problem and so programmable freezers have proven valuable in reducing variability between freezes [6]. Effective cooling rates for sperm of the horse [11], silk worm [10] and cane toad [4] are around 5°C/min or less. However, quicker rates of cooling are required for some other species with human sperm being about 10°C/min [8], and optimal rates of cooling of 20°C/min or more for sperm of dogs [1], sheep [3], pigs [2] and mice [9].

Many Reproductive Biology Units in zoological institutions around the world are buying programmable cryopreservation machines for the purposes of freezing sperm from a range of genetically valuable animals. This use can be applied to material collected electively or opportunistically following post-mortem. However, the prospective purchaser of such equipment must discover in advance whether the machines are capable of achieving the rates of cooling required for the species likely to be encountered. The aim of the present study was therefore to determine the maximum cooling rates of three programmable freezers and determine whether they could achieve the rates of cooling that are reportedly required to successfully freeze sperm from a wide range of species. All three machines work on a similar principle, namely the cooling of a block or plate by liquid nitrogen being opposed by a heating element to a variable degree. The maximum rate of cooling is therefore achieved by the liquid nitrogen when no heat is provided by the heater.

MATERIALS AND METHODS

The maximum rates of cooling from 20°C to -80°C of unloaded systems were determined for the Asymptote Embryo freezer EF100 (Asymptote Ltd, Cambridge, UK) and the Cryologic CL-8800 temperature controller (Cryologic Pty Ltd, Mulgrave, Victoria, Australia) with either the standard (CC20S) or fast (CC20F) chamber designed for use with security straws. Temperatures were measured using the in-built thermometers for each system. Runs were made in triplicate and the mean rate of cooling calculated.

The Asymptote freezer uses a plate to hold straws or ampoules and this is cooled by liquid nitrogen held in a storage tank under the plate. Liquid nitrogen was poured into the tank and the machine programmed using the Asymptote data logging software and set to cool at a rate of 50°C/min, beyond the capacity of the machine, to a final temperature of -100°C. The result was that the internal heater provided no heat and the plate cooled maximally over the temperature range under investigation. The rate was calculated by observing the temperature fall over a 10 second period at 5°C intervals. The Cryologic CL8800 temperature controller was programmed to "freefall" to a final temperature of -100°C, first with the standard chamber and then the fast chamber. The chamber under investigation was held in liquid nitrogen within a large Cryobath. The rate of cooling was then calculated at 5°C intervals by timing the fall in temperature from 1 degree above the nominated temperature to 1 degree below.

RESULTS AND DISCUSSION

Programmable freezers are proving attractive as they are simple to use, enable a combination of cooling ramps without continued operator intervention, and have been used to increase reproducibility between freezes [6]. Whilst there are several makes available with different underlying principles of operation, it is important that the expectations of the operator are met especially when using the machine in a facility that will be expected to deal with sperm from a variety of species. The maximal

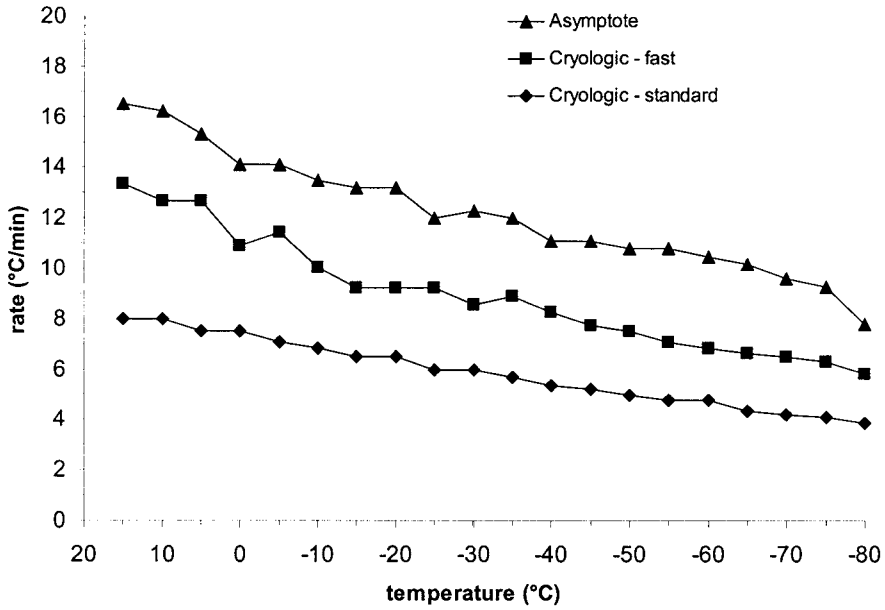

Figure 1. The maximum rates of cooling achieved with three programmable freezing systems, namely the Asymptote EF100 freezer, and the Cryologic CL8800 temperature controller with either a standard or fast chamber.

rates of cooling shown in Figure 1 for all three freezers were not constant but were a function of the temperature gradient between the liquid nitrogen and the plate/chamber. The rates of cooling at a plate/chamber temperature of 15°C was 16.5°C/min for the Asymptote EF100, and 13.3°C and 8.0°C for the Cryologic fast and slow chambers respectively; these rates came down at a plate/chamber temperature of -80°C to 7.8°C/min for the Asymptote EF100, and 5.9°C/min and 3.9°C/min for the Cryologic fast and slow chambers. The present study clearly showed that all three systems tested had maximum rates of cooling that may be inadequate for some freeze protocols, and consideration should therefore be given to the suitability of each machine for the proposed application prior to purchase.

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