SHORT COMMUNICATION

GREENVILLE, SOUTH CAROLINA

Longevity of Donor Serum¹

Submitted July 18, 2003; accepted January 14, 2004

Purpose: To determine the viability of long-term, stored serum, which is used in human in vitro production as a source of energy substrates, amino acids, vitamins, growth factors, and other nutrients.

Method: Two-cell mouse embryos were used in this prospective, cohort study. Serum stored for 1-, 5-, 10-, and 12-year intervals was subjected to four replications of mouse-embryo testing.

Result(s): There were no significant differences in blastocyst rates between any of the replicates by year (80–100% blastocyst rate; $P > 0.01$) or between pooled replicate means by time period.

Conclusion(s): Serum may be frozen up to 12 years.

KEY WORDS: Donor serum; in vitro production; mouse embryo assay.

INTRODUCTION

Serum has been added to culture media for many years. While its indefinable nature lends itself to much criticism, especially by those who wish to have a defined culture medium for the sake of repeatability, serum still has an important role in today's media.

Because serum is proteinaceous in nature and because it has the ability to add a protective coating around gametes and embryos that helps prevent sticking to cells and culture vessels, this product remains a vital part of the culturing mystique. Additionally, serum brings undefined additives to the culture media that often aid in the performance of the cells being cultured.

Although serum substitutes are commercially available (Synthetic Serum Substitute [SSS], plasmatein, plasmanate, and albuminar) (1–4), we have discovered that at least one of these (SSS) causes a great deal of variability when added to our cryopreservation solutions. Serum seems to provide a more stable osmolality and pH than does SSS. Because of serum's stability in these two areas, we have elected to continue the use of serum for our freezing and thawing solutions. Unfortunately, donor serum is costly. Therefore, the purpose of this study is to assess the ability of long-term, stored, frozen-thawed donor serum to support development of mammalian embryos.

MATERIAL AND METHODS

Serum was collected during the follicular phase of the menstrual cycle (day 9–11) from a healthy, naturally menstruating woman. Two-hundred-forty milliliters of whole blood were collected into 10-mL red-top tubes. The blood was centrifuged at 1200 g for 20 min. The serum was removed, sterile filtered, heat inactivated at 56°C for 30 min and frozen at –70°C. A negative or nonreactive result for the following tests was required before serum was utilized: HIV, Hepatitis B, Hepatitis C, rapid plasma reagin, and antisperm antibody. Serum was tested initially for toxicity using the two-cell mouse embryo assay (5). A blastocyst rate of 80% or greater was required in two separate testing trials before the serum was used as an additive in our cryopreservation and thawing media.

Donor serum tested in this experiment was stored at –70°C for 1, 5, 10, and 12 years. Serum from each time period was assayed four times with approximately 10 mouse embryos per assay.

To compare developmental rates of embryos exposed to different ages of serum, different batches of media were prepared with different ages of frozen-thawed serum (15% donor serum in Human Tubal Fluid; HTF; Irvine Scientific, CA. Controls did not contain donor serum. Media were sterile filtered using a 0.22- μ m filter (Pall Corporation, Ann Arbor, MI) into a Falcon 2001 tube (Becton Dickinson, Franklin Lakes, NJ). Media were incubated for 24 h at 37°C and 5% CO₂ to allow for equilibration. After incubation, two 50- μ L drops of media were placed into a Falcon 3001 dish. The drops were overlaid with washed mineral oil. The dishes containing the media were placed into the incubator until needed.

¹This paper was Presented at South Carolina Academy of Science 75th Annual Meeting, University of South Carolina, Aiken, April 12, 2002.

Table I. Blastocyst Rates Associated with Two-Cell Mouse Embryo Testing of Donor Serum Across Years and Replications

Years of storage ^a	Replications				<i>P</i> value ^b
	1	2	3	4	
1	9/10 (90%)	9/10 (90%)	11/11 (100%)	8/10 (80%)	0.50
5	8/10 (80%)	9/10 (90%)	9/10 (90%)	10/10 (100%)	0.53
10	10/10 (100%)	8/10 (80%)	9/10 (90%)	10/10 (100%)	0.26
12	7/8 (88%)	9/10 (90%)	9/11 (82%)	11/11 (100%)	0.55
Controls	10/10 (100%)	10/10 (100%)	10/10 (100%)	10/10 (100%)	

^a Controls consisting of 10 two-cell mouse embryos were assayed with each replicate. Control results were not compared; they were run solely for quality control.

^b *P* value based on χ^2 test with three degrees of freedom.

Two-cell mouse embryos were thawed according to manufacturer's specifications (Conception Technologies, San Diego, CA). The media dishes were removed from the incubator and 5 two-cell mouse embryos were placed into each drop of media.

Embryos were incubated for 72 h after which a blastocyst rate was determined. Blastocyst rate (%) was determined by the number of embryos at the blastocyst stage divided by the total number of embryos placed in the media and multiplied by 100. The blastocyst stage was defined as a collection of cells having a blastocoele cavity that were expanded, hatching, or hatched. The chi-square test for homogeneity was used to test for a difference in pooled (four replicates) blastocyst rates by length of time in storage (1, 5, 10, and 12 years). *P* values <0.05 were considered indicative of statistical significance. Ninety-five percent confidence intervals were calculated for the pooled blastocyst rates (Fig. 1).

RESULTS

No significant differences were noted between the four replicates within time period ($P > 0.01$); therefore, replicates were averaged. Pooled blastocyst rates for 1, 5, 10, and 12 years were 90, 90, 92, and 90%, respectively. There was no apparent trend of decreasing viability with longer term storage and there was no significant difference in the four rates ($P = 0.26$).

DISCUSSION

Serum variation can occur depending on when it is collected during the menstrual cycle and can vary by changes in diet, exercise, and medication (6). Furthermore, the process of collecting and testing serum can be time consuming and expensive. Because of

these factors, long-term storage of high-quality donor serum would be beneficial. These results indicate that donor serum can be stored at -70°C for up to 12 years and still support development of the two-cell mouse embryo to the blastocyst stage (Table I).

There is controversy even as to the method of choice for testing culture media and the additives associated with the media. Bavister (7) notes that quality control of serum necessitates the use of an "...expensive, time-consuming [mouse] embryo culture assay that is insensitive and unlikely to yield useful information." However, the two-cell mouse assay does provide some insight as to whether an item is toxic or not. The American Association of Bioanalysts (AAB Proficiency Testing Service, Brownsville, TX) considers the mouse assay a valid test of medium acceptability. It accepts the assay for proficiency testing of media supplied to participating laboratories. For example, 62% (1106/1792) of the results reported by participating laboratories used some kind of a mouse embryo assay during the years 2000–02. Of those laboratories reporting mouse embryo assay results, 68% (750/1106) were using the two-cell mouse assay. The remaining participating laboratories

Fig. 1. Exact 95% confidence intervals for control and pooled replicates of two-cell mouse embryo assays performed on donor serum.

used human sperm survival [38% (678/1792)] or hamster sperm motility [0.4% (8/1792)] to determine if a media supplemented with protein was toxic or not.

Khan *et al.* (8) noted that embryos and oocytes do not come in contact with serum *in vivo*; therefore, a more chemically defined media would be ideal. While we agree with this idea, our laboratory has used the serum protocol with great success. Our laboratory has been able to achieve a 37% (17/46) live birth rate for frozen embryo transfers (years 1996–99) compared to results from the American Society for Reproductive Medicine/Society for Assisted Reproductive Technology Registry which reported an overall live birth rate for the same years of 18% (7073/38909) (9–12). Once serum-free protocols are developed which rival our current frozen embryo transfer pregnancy rate, we will discontinue the use of serum-supplemented media.

Future research should include other more sensitive bioassays (i.e., a one-cell mouse embryo assay) (13). Another potential weakness of this study was the low number of replicates and mouse embryos per replicate. Thus, other ART centers that use serum, or serum-based products, should repeat this study.

In conclusion, this study indicated that donor serum stored at -70°C for up to 12 years does not have adverse effects on embryo culture; therefore, it can be thawed and used in cryopreservation media with no detriment to future embryo development.

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